frontiers | Research Topics

Advances in the diagnosis and treatment in kidney transplantation, volume II

Edited by
Kathrin Eller, Miriam Banas, Georg Böhmig and Ondrej Viklicky

Published in
Frontiers in Medicine

ISSN 1664-8714
ISBN 978-2-83251-271-5
DOI 10.3389/978-2-83251-271-5

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Advances in the diagnosis and treatment in kidney transplantation, volume II

Topic editors

Kathrin Eller — Medical University of Graz, Austria
Miriam Banas — University Hospital Regensburg, Germany
Georg Böhmig — Medical University of Vienna, Austria
Ondrej Viklicky — Institute for Clinical and Experimental Medicine (IKEM), Czechia

Citation

Eller, K., Banas, M., Böhmig, G., Viklicky, O., eds. (2023). *Advances in the diagnosis and treatment in kidney transplantation, volume II*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83251-271-5

Table of contents

frontiers in Medicine

ORIGINAL RESEARCH
published: 28 March 2022
doi: 10.3389/fmed.2022.864865

SARS-CoV-2 in Kidney Transplant Patients: A Real-Life Experience

Biagio Pinchera[1], Lorenzo Spirito[2], Lucia Ferreri[3], Roberto La Rocca[2], Giuseppe Celentano[2], Antonio Riccardo Buonomo[1], Maria Foggia[1], Riccardo Scotto[1], Stefano Federico[3], Ivan Gentile[1], Rosa Carrano[3] and "Federico II" COVID-19 Team*

[1] Department of Clinical Medicine and Surgery, University of Naples "Federico II", Naples, Italy, [2] Department of Neurosciences, Reproductive and Odontostomatological Sciences, Section of Urology, University of Naples "Federico II", Naples, Italy, [3] Department of Public Health, University of Naples "Federico II", Naples, Italy

Background: The COVID-19 pandemic has significantly impacted the management of solid organ transplant recipients and on clinical evolution in post-transplantation. Little is known on the impact of SARS-CoV-2 infection in these patients. The severity and lethality of this disease in solid organ transplant patients are higher thanin the general population. This study aims to describe clinical characteristics of SARS-CoV-2 infection in solid organ transplant recipients followed in our center.

Methods: In this observational study, we enrolled all kidney transplant recipientsattending the A.O.U. Federico II of Naples from March 2020 to January 2021. For each patient we evaluated the epidemiological and clinical characteristics as well as outcome.

Results: We enrolled 369 kidney transplant patients (229, male, 62%). Of these, 51 (13.8%) acquired SARS-CoV-2 infection and 29 showed symptomatic disease. Of the 51 patients with the infection, 48 (94.11%) had at least one comorbidity and such comorbidities did not constitute a risk factor for a more severe disease. Hospitalization was necessary for 7 (13.7%) patients. Of these, 2 required low-flow oxygen supplementation, 3 non-invasive/high flow ventilation and 2 invasive ventilation. Finally, 2 patients died.

Conclusions: Our study shows a lower mortality and hospitalization rate compared to figures available in the literature (4% vs. 13–30% and 14% vs. 32–100%, respectively). Furthermore, the comorbidities examined (hypertension, dyslipidemia, and diabetes) did not constitute a risk factor for a more severe disease condition in this patient category. Further studies with larger sample size are necessary to confirm these data.

Keywords: kidney transplant, SARS-CoV-2, COVID-19, transplant, immunosuppression

OPEN ACCESS

Edited by:
Kathrin Eller,
Medical University of Graz, Austria

Reviewed by:
Julia Kerschbaum,
Innsbruck Medical University, Austria
Michael Kolland,
Medical University of Graz, Austria

****Correspondence:***
Biagio Pinchera
biapin89@virgilio.it

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *28 January 2022*
Accepted: *07 March 2022*
Published: *28 March 2022*

Citation:
Pinchera B, Spirito L, Ferreri L, Rocca RL, Celentano G, Buonomo AR, Foggia M, Scotto R, Federico S, Gentile I, Carrano R and "Federico II" COVID-19 Team (2022) SARS-CoV-2 in Kidney Transplant Patients: A Real-Life Experience. Front. Med. 9:864865. doi: 10.3389/fmed.2022.864865

INTRODUCTION

The COVID-19 (CoronaVIrus Disease-19) pandemic has significantly impacted the management of solid organ transplant patients and the clinical evolution in post-transplantation, notably by reducing the activity of transplant centers. Currently, this category of patients is considered to be at greater risk for developing a severe course of COVID-19 disease (1). Most studies

show a high risk of developing a severe form of the disease and a high lethality in solid organ transplant patients (2). In fact, in SOT (Solid Organ Transplant) recipients, the reported lethality for COVID-19 ranges from 13 to 30% (3) and the hospitalization rates range from 32 to 100% (4–6). Several studies showed a high hospitalization rate among kidney transplant patients, about 70% undergoing hospitalization and of hospitalized patients, about 25% requiring mechanical ventilation (7, 8). Therefore, also in Europe, data on the COVID-19 mortality rate among kidney transplant patients range between 19 and 50% (9, 10). Several studies evaluated the clinical characteristics of immunocompromised patients with SARS-CoV-2 (Severe Acute Respiratory Syndrome-CoronaVirus-2) infection, comparing them with the general population affected by this infection. From these studies it emerged that SOTs infected with SARS-CoV-2 had more frequently diabetes, cardiovascular disease, hypertension, respiratory disorders and more often needed hospitalization and intensive care, showing a higher lethality (11). Moreover, a systematic review and meta-analysis of SOT recipients with SARS-CoV-2 infection, enrolling 2,772 SOT recipients, showed that the majority (81%) needed hospitalization (12–14). However, the impact of SARS-CoV-2 infection in solid organ transplant patients is not fully understood and data on this topic are still scarce and scanty. This study aims to describe clinical characteristics of SARS-CoV-2 infection in solid organ transplant recipients followed in our center.

MATERIALS AND METHODS

We conducted an observational retrospective cohort study. We enrolled kidney transplant patients attending the A.O.U. Federico II of Naples and followed up from March 2020 to January 2021. Patients underwent regular rhino-oropharyngeal swabs for health surveillance or for suspected COVID-19 symptoms. In these patients, we evaluated rate of SARS-CoV-2 and of COVID-19 disease. Diagnosis of SARS-CoV-2 infection was defined as positivity to the rhino-oropharyngeal swab for SARS-CoV-2 RNA research by reverse transcription - polymerase chain reaction (RT-PCR). To describe the clinical status of SARS-CoV-2 infected patients we used the NIAID ACTT-1 (National Institute of Allergy and Infectious Diseases Adaptive COVID-19 Treatment Trial-1) Clinical Status Ordinal Scale (15). Based on this score, we classified each patient with the infection into one of eight categories: (1) Not hospitalized, no limitations on activities; (2) Not hospitalized, limitation on activities, and/or requiring home oxygen; (3) Hospitalized, not requiring supplemental oxygen and no longer requires ongoing medical care (if hospitalization extended for infection-control purposes); (4) Hospitalized, not requiring supplemental oxygen; requiring ongoing medical care (COVID-19 related or otherwise); (5) Hospitalized, requiring supplemental oxygen; (6) Hospitalized, on noninvasive ventilation or high-flow oxygen devices; (7) Hospitalized, on invasive mechanical ventilation or ECMO; (8) Death (15). In addition, for patients with COVID-19 disease, we also used the Henry Ford Hospital (HFH) COVID-19 severity scoring system to distinguish mild, moderate, and severe forms of the disease (16). Mild disease was defined as patients who had normal chest radiography and SpO2 of ≥94% without the need for supplemental oxygen. Moderate disease patients were those who had abnormal chest radiography, SpO2 of <94% and needing between 1 and 5 liters/min supplemental O2. Patients with severe disease were defined by abnormal chest radiography, SpO2 of <94% and requiring ≥6 liters/min of O2 (16). For each patient we evaluated epidemiological and clinical characteristics, laboratory and radiological data, the need for hospitalization and access to the ICU (Intensive Care Unit), the type of immunosuppressive treatment and the changes of immunosuppression during SARS-CoV-2 infection, the treatment for SARS infection-CoV-2 and the outcome. For each patient we evaluated SARS-CoV-2 IgG (Roche Diagnostics GmbH, Mannheim, positive threshold > 15 BAU/ml). Furthermore, we assessed the risk of co-infections. Data are presented as mean and SD or median and interquartile range (IQR), in case of Gaussian or non-Gaussian distribution, respectively. For correlation analysis, Pearson or Spearman tests were used for data distributed in Gaussian or non-Gaussian fashion, respectively. Continuous variables are compared by Student's *t*-test or Mann-Whitney *U*-Test, as parametric or non-parametric test, respectively. The p-value for statistical significance was set at <0.05 for all the tests. The odds ratio analysis was conducted to evaluate and measure possible risk factors for more severe disease evolution. In particular, age, sex, comorbidities and immunosuppressive therapy were assessed and compared, it did not adjust for confounders. The study was conducted in compliance with the Declaration of Helsinki and the principles of good clinical practice. The study was exempt from approval from an ethics' board.

RESULTS

We enrolled 369 kidney transplant patients (229, male, 62%) with a median age of 49 years (IQR, 18–86). Of these, 51 (13.8%) became infected with SARS-CoV-2 during the period of the study. Anagraphic and clinical features of these patients are reported in **Tables 1**, **2**. Only 17/51 (33.3%) SARS-CoV-2 infected patients had positive anti-SARS-CoV-2 IgG antibodies performed 14–21 days after the onset of symptoms. Of the 51 SARS-CoV-2 infected patients, 29 (56.9%) showed COVID-19 (**Tables 1**, **2**). The most frequent symptoms were fever and cough (**Table 1**). Seven of the 29 (13.7%) patients were admitted to hospital. Of these seven patients, two required low-flow oxygen supplementation, three non-invasive/high flow ventilation and two invasive ventilation. In relation to the Henry Ford Hospital (HFH) COVID-19 severity scoring system, we distinguished 22 mild (75%), two moderate (7%) and five severe (18%) forms in the 29 patients. Of the 51 patients with the infection, 48 (94.11%) had at least one comorbidity. However, comorbidities did not constitute a risk factor for a more severe disease condition [OR: 1.1, 95 CI (0.40–2.2); *p*: 0.480] (**Tables 1**, **2**). We compared and evaluated SARS-CoV-2 infected patients

TABLE 1 | Anagraphic and clinical features of enrolled kidney transplant patients with SARS-CoV-2 infection ($n = 51$).

Age (median, IQR)	**50 (18–71)**
Gender:	
M	41 (80.4%)
F	10 (19.6%)
Patients with infection:	
Asymptomatic with infection	22 (43.14%)
COVID-19	29 (56.86%)
Asymptomatic	22 (43.14%)
M	20 (90.9%)
F	2 (9.1%)
Symptoms:	
Fever	19 (65.5%)
Cough	12 (41.3%)
Asthenia	8 (27.6%)
Dyspnea	10 (34.5%)
Anti SARS-CoV-2 antibodies	17 (33.3%)
Comorbidities in patients with SARS-CoV-2 infection:	51
Hypertension	48 (94.1%)
Dyslipidemia	26 (50.9%)
Diabetes	7 (13.7%)
Anemia	13 (23.7%)
Ischemic heart disease	1 (1.96%)
Therapy for COVID-19:	29
Modifications of immunosuppressive therapy	20 (69%)
Steroid therapy	19 (65.5%)
Low molecular weight heparin	16 (55.1%)
Remdesivir	2 (6.8%)

TABLE 2 | Anagraphic clinical features of patients with SARS-CoV-2 infection: asymptomatic vs. COVID-19.

	Patients with SARS-CoV-2 infection		***p*-value**
	Asymptomatic $n = 22$ (43.14%)	**COVID-19 $n = 29$ (56.86%)**	
Age, years (median, IQR)	49 (26–70)	52 (18–71)	0.657
Gender:			
M	20 (90.9%)	21 (72.4%)	0.748
F	2 (9.1%)	8 (27.6%)	
Comorbidities:			
Hypertension	20 (90.9%)	28 (96.5%)	0.284
Dyslipidemia	10 (45.5%)	16 (55.2%)	0.310
Diabetes	3 (13.6%)	4 (13.7%)	0.540
Anemia	6 (27.3%)	7 (24.1%)	0.620
Ischemic heart disease	0	1 (3.4%)	0.218
Immunosuppressive therapy:			
Corticosteroids	21 (95%)	28 (96.5%)	0.620
Calcineurin inhibitor	20 (90.9%)	27 (93%)	0.244
Antimetabolite	9 (40.9%)	15 (51.7%)	0.186
mTOR inhibitors	8 (36.3%)	5 (17.2%)	0.112
Time from transplant to diagnosis of SARS-CoV-2 infection in months (median, IQR)	132 (7–420)	84 (7–264)	0.120

with diabetes vs. non-diabetic patients, assessing the risk of evolving to a severe form of COVID-19 related disease [OR: 1.2, 95 CI (0.85–1.7); *p*: 0.240]. We also compared and evaluated patients with SARS-CoV-2 infection with cardiovascular disease vs. patients not affected by this condition, evaluating the risk of evolution toward a severe form of COVID-19 related disease [OR: 1.1, 95 CI (0.70–1.4); *p*: 0.290]. Twenty patients received therapy for COVID-19. In details, 19 received steroid therapy, 16 low molecular weight heparin, two Remdesivir. All patients with symptoms underwent modifications of immunosuppressive therapy (**Table 1**). In detail, at baseline, most patients were receiving calcineurin inhibitor (CNI) (92%) and corticosteroids (96%) at the time of the diagnosis of the infection. Antimetabolite (azathioprine and mycophenolate mofetil) were used in 49%, while mTOR (mammalian Target Of Rapamycin) inhibitors were in 18% of cases. With respect to patients with a moderate-severe form of the disease, calcineurin inhibitors (CNI), corticosteroids and antimetabolite were used in 100, 85, and 57%, respectively, while mTOR inhibitors were used by no patient with a moderate—severe form of the disease [OR: 1.27, 95 CI (0.60–1.8); *p*: 0.097]. Regarding the therapeutic management of the infection, the first step was the reduction of immunosuppressive therapy, which consisted in the reduction or suspension of antimetabolites in the case of moderate forms. In the case of severe forms of the disease, all immunosuppressive therapy was suspended, except for the steroid therapy. We observed 9/51 (17.6%) bacterial co-infections among patients with COVID-19: four urinary tract infections, three pneumonia and two sepsis. Only one patient experienced acute organ rejection. Finally, two patients died.

DISCUSSION

In our study we showed that the rate of SARS-CoV-2 infection was higher than that of the general population (13 vs. 2.6%) (1). In addition, we noted that the most majority of our patients were males while no risk factor for infection was identified.

Moreover, it was observed that only 33% of patients with infection had an anti-SARS-CoV-2 IgG serology. This finding is probably related to the characteristic immunosuppression of solid organ transplant patients which could suppress the production of an effective antibody response. However, no correlation was observed between the time from transplantation and the risk of infection [OR: 1.2, 95 CI (0.90–1.4); *p*: 0.190]. In this way, it could have been hypothesized that patients with a more recent transplant were more at risk of contracting the infection, given the more pronounced immunosuppression in the first months after transplantation. However, among infected patients, those ones with a symptomatic disease showed a trend toward a shorter time from transplantation to symptoms than

those with an asymptomatic infection [OR: 1.1, 95 CI (0.50–1.7); *p*: 0.090].

Regarding the symptoms, in our cohort, kidney transplant patients with SARS-CoV-2 infection showed a high rate of symptomatic disease (56.9%). However, symptoms were mild in most cases (75%) and similar to those observed in non-transplant patients with SARS-CoV-2 infection. Moreover, our study shows a lower rate of admission to hospital compared to the data in the literature (14% vs. 32–100%). We also observed a lack of correlation between comorbidities and the risk of developing COVID-19 [OR: 1.1, 95 CI (0.40–2.2); *p*: 0.480] (17, 18). In particular, in our study having type 2 diabetes mellitus as a comorbidity did not constitute a risk factor for a more severe evolution of COVID-19 related disease [OR: 1.2, 95 CI (0.85–1.7); *p*: 0.240]. Furthermore, our study also highlighted that patients with cardiovascular pathologies did not present an increased risk of evolution toward a severe form of COVID-19 related disease [OR: 1.1, 95 CI (0.70–1.4); *p*: 0.290]. Our results are in contrast with those reported in the literature (19, 20). However, due to the relatively small sample size, our observation needs to be confirmed.

In relation to the immunosuppressive therapy, it was observed that no patient who presented a moderate-severe form of the disease, received immunosuppressive therapy which includes an mTOR inhibitor at the time of the diagnosis of infection. This result might be interpreted at the light of the potential antiviral effects of mTOR inhibitors (21), although an antiviral effect against SARS-CoV-2 has never been demonstrated. The small number of patients enrolled, and the design of our study prevent to draw a definitive conclusion but do generate a hypothesis that should be tested in an *ad hoc* study.

We underline that we observed only nine bacterial co-infections (17.6%). This confirms once again, even in a subset of immunocompromised patients, that there is an excessive use of antibiotic therapy during COVID-19 (22–24).

Finally, in our study, the rate of episodes of acute organ rejection during SARS-CoV-2 infection was similar to that found in the literature (1.9 vs. 1%) (25) while the mortality rate was lower than that reported in the literature (4% vs. 13–30%). Indeed, while in Jager's study et al. there was a morality rate in kidney transplant patients equal to 19%, in our case the mortality rate was much lower, in particular equal to 4% in our case. Probably this data is to be considered within the age of the population considered, in fact in our case the median age was much lower than that of the population considered by Jager (49 vs. 71.7) (26). Probably also the reduced hospitalization rate found in our experience is to be attributed to the younger age of the transplanted population considered at our Center (26). Furthermore, the reduced mortality and hospitalization rates found in our experience could also be partly attributable to the type of immunosuppressive therapy found in our case. In fact, in our experience, only 49% of SARS-CoV-2 infected patients practiced immunosuppressive therapy with antimetabolites. As evidenced by the study by Goffin et al., the intensity of immunosuppressive therapy, in particular triple therapy vs. dual immunosuppressive therapy, significantly impacted the severe evolution of the disease and the risk of mortality (27, 28).

We acknowledge that our study presents several limitations: the small sample size, the retrospective and monocentric design. Furthermore, we did not correct for multiple testing and we did not adjust for confounders. The strength of our study is the real-life setting and the availability of several weapons that were not available at the time of the previous reports on the topic, such as the use of corticosteroids, antivirals or anticoagulants.

CONCLUSION

In our real-life study conducted in kidney transplant patients with SARS-CoV-2 infection, we showed a lower mortality and admission rate compared to those available in the literature (4% vs. 13–30% and 14% vs. 32–100%, respectively). The potential role of mTOR inhibitors in the management of SARS-CoV-2 infection needs to be further investigated in future studies.

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 Federico II University Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

FEDERICO II COVID-19 TEAM

Amicone Maria, Borrelli Francesco, Buonomo Antonio Riccardo, Cattaneo Letizia, Conte Maria Carmela Domenica, Cotugno Mariarosaria, Di Filippo Giovanni, Foggia Maria, Gallicchio Antonella, Gentile Ivan, Giaccone Agnese, Lanzardo Amedeo, Mercinelli Simona, Minervini Fulvio, Piccione Amerigo, Pinchera Biagio, Reynaud Laura, Salemi Fabrizio, Sardanelli Alessia, Schiano Moriello Nicola, Scordino Fabrizio, Scotto Riccardo, Stagnaro Francesca, Tosone Grazia, Viceconte Giulio, Zappulo Emanuela, and Zotta Irene.

AUTHOR CONTRIBUTIONS

BP: conceptualization and writing—original draft preparation. LS and AB: writing—review and editing. LF and MF: investigation. RR: data curation. GC: software. RS: methodology and formal analysis. SF: validation. IG: writing—original draft preparation and supervision. RC: conceptualization and supervision. All authors contributed to the article and approved the submitted version.

REFERENCES

1. *Coronavirus Disease 2019 - People of Any Age with Underlying Medical Conditions*. Atlanta, GA: Centers for Disease Control and Prevention (2020).
2. Fung M, Babik JM. COVID-19 in immunocompromised hosts: what we know so far. *Clin Infect Dis.* (2021) 72:340–50. doi: 10.1093/cid/ciaa863
3. Azzi Y, Bartash R, Scalea J, Loarte-Campos P, Akalin E. COVID-19 and solid organ transplantation: a review article. *Transplantation.* (2021) 105:37–55. doi: 10.1097/TP.0000000000003523
4. Columbia University Kidney Transplant P. Early description of coronavirus 2019 disease in kidney transplant recipients in New York. *JASN.* (2020) 31:1150–6. doi: 10.1681/ASN.2020030375
5. Nair V, Jandovitz N, Hirsch JS, Nair G, Abate M, Bhaskaran M, et al. COVID-19 in kidney transplant recipients. *Am J Transplant.* (2020) 20:1819–25. doi: 10.1111/ajt.15967
6. McKechnie JL, Blish CA. The innate immune system: fighting on the front lines or fanning the flames of COVID-19? *Cell Host Microbe.* (2020) 27:863–9. doi: 10.1016/j.chom.2020.05.009
7. Akalin E, Azzi Y, Bartash R, Seethamraju H, Parides M, Hemmige V, et al. Covid-19 and kidney transplantation. *N Engl J Med.* (2020) 382:2475–7. doi: 10.1056/NEJMc2011117
8. Bossini N, Alberici F, Delbarba E, Valerio F, Manenti C, Possenti S, et al. Kidney transplant patients with SARS-CoV-2 infection: The Brescia Renal COVID task force experience. *Am J Transplant.* (2020) 20:3019–29. doi: 10.1111/ajt.16176
9. Elias M, Pievani D, Randoux C, Louis K, Denis B, Delion A, et al. COVID-19 infection in kidney transplant recipients: disease incidence and clinical outcomes. *J Am Soc Nephrol.* (2020) 31:2413–23. doi: 10.1681/ASN.2020050639
10. Favà A, Cucchiari D, Montero N, Toapanta N, Centellas FJ, Vila-Santandreu A, et al. Clinical characteristics and risk factors for severe COVID-19 in hospitalized kidney transplant recipients: a multicentric cohort study. *Am J Transplant.* (2020) 20(11):3030–41. doi: 10.1111/ajt.16246
11. Belsky JA, Tullius BP, Lamb MG, Sayegh R, Stanek JR, Auletta JJ. COVID-19 in immunocompromised patients: a systematic review of cancer, hematopoietic cell and solid organ transplant patients. *J Infect.* (2021) 82:329–338. doi: 10.1016/j.jinf.2021.01.022
12. Raja MA, Mendoza MA, Villavicencio A, Anjan S, Reynolds JM, Kittipibul V, et al. COVID-19 in solid organ transplant recipients: a systematic review and meta-analysis of current literature. *Transpl Rev.* (2020) 35:100588. doi: 10.1016/j.trre.2020.100588
13. Kates OS, Haydel BM, Florman SS et al. COVID-19 in solid organ transplant: a multi-center cohort study. *Clin Infect Dis.* (2020) 73:e4090–e4099. doi: 10.1093/cid/ciaa1097
14. Laguna-Goya R Utrero-Rico A Talayero P et al. IL-6-based mortality risk model for hospitalized patients with COVID-19. *J Allergy Clin Immunol.* (2020) 146:799–807.e9. doi: 10.1016/j.jaci.2020.07.009
15. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the treatment of Covid-19 - final report. *N Engl J Med.* (2020) 383:1813–26. doi: 10.1056/NEJMoa2007764
16. Chaudhry ZS, Williams JD, Vahia A, Fadel R, Acosta TP, Prashar R, et al. Clinical characteristics and outcomes of COVID-19 in solid organ transplant recipients: a case-control study. *Am J Transplant.* (2020) 20:3051–60. doi: 10.1111/ajt.16188
17. Spirito L, Manfredi C, Carrano R, Trinchieri A, Salemi F, Sciorio C, et al. Impact of kidney transplantation on male sexual function: results from a ten-year retrospective study. *J Sex Med.* (2020) 17:2191–97. doi: 10.1016/j.jsxm.2020.07.014
18. COVID-19 RISk and Treatments (CORIST) Collaboration. RAAS inhibitors are not associated with mortality in COVID-19 patients: Findings from an observational multicenter study in Italy and a meta-analysis of 19 studies. *Vascul Pharmacol.* (2020) 135:106805. doi: 10.1016/j.vph.2020.106805
19. Di Castelnuovo A, Bonaccio M, Costanzo S, Gialluisi A, Antinori A, Berselli N. Common cardiovascular risk factors and in-hospital mortality in 3,894 patients with COVID-19: survival analysis and machine learning-based findings from the multicentre Italian CORIST Study. *Nutr Metab Cardiovasc Dis.* (2020) 30:1899–913. doi: 10.1016/j.numecd.2020.07.031
20. Mancusi C, Grassi G, Borghi C, Ferri C, Muiesan ML, Volpe M, et al. Clinical characteristics and outcomes of patients with COVID-19 infection: the results of the SARS-RAS Study of the Italian Society of Hypertension. *High Blood Press Cardiovasc Prev.* (2021) 28:5–11. doi: 10.1007/s40292-020-00429-3
21. Bowman LJ, Brueckner AJ, Doligalski CT. The role of mTOR inhibitors in the management of viral infections: a review of current literature. *Transplantation.* (2018) 102(2S Suppl. 1):S50–9. doi: 10.1097/TP.0000000000001777
22. Tonziello G, Caraffa E, Pinchera B, Granata G, Petrosillo N. Present and future of siderophore-based therapeutic and diagnostic approaches in infectious diseases. *Infect Dis Rep.* (2019) 11:8208. doi: 10.4081/idr.2019.8208
23. Spirito L, Pinchera B, Patrì A, Delfino M, Imbimbo C, Salvatore P, et al. No detection of SARS-CoV-2 RNA on urethral swab in patients with positive nasopharyngeal swab. *Adv Virol.* (2020) 2020:8826943. doi: 10.1155/2020/8826943
24. Di Castelnuovo A, Costanzo S, Antinori A, Berselli N, Blandi L, Bonaccio M. Heparin in COVID-19 patients is associated with reduced in-hospital mortality: the multicenter Italian CORIST study. *Thromb Haemost.* (2021) 121:1054–65. doi: 10.1055/a-1347-6070
25. Angelico R, Blasi F, Manzia TM, Toti L, Tisone G, Cacciola R. The management of immunosuppression in kidney transplant recipients with COVID-19 disease: an update and systematic review of the literature. Medicina. (2021) 57:435. doi: 10.3390/medicina57050435
26. Jager KJ, Kramer A, Chesnaye NC, Couchoud C, Sánchez-Álvarez JE, Garneata L. Results from the ERA-EDTA Registry indicate a high mortality due to COVID-19 in dialysis patients and kidney transplant recipients across Europe. *Kidney Int.* (2020) 98:1540–8. doi: 10.1016/j.kint.2020.09.006
27. Goffin E, Candellier A, Vart P, Noordzij M, Arnol M, Covic A, et al. COVID-19-related mortality in kidney transplant and haemodialysis patients: a comparative, prospective registry-based study. *Nephrol Dial Transplant.* (2021) 36:2094–105. doi: 10.1093/ndt/gfab200
28. Kumar N, Rana R, Rana DS, Gupta A, Sachdeva MP. SARS-CoV-2 in kidney transplant recipients: a systematic review. *Transplantology.* (2022) 3:33–48. doi: 10.3390/transplantology3010004

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.

frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 29 April 2022
doi: 10.3389/fmed.2022.875206

The Kidney Donor Profile Index (KDPI) Correlates With Histopathologic Findings in Post-reperfusion Baseline Biopsies and Predicts Kidney Transplant Outcome

Quirin Bachmann[1‡], Flora Haberfellner[1‡], Maike Büttner-Herold[2], Carlos Torrez[1], Bernhard Haller[3], Volker Assfalg[4], Lutz Renders[1], Kerstin Amann[2], Uwe Heemann[1], Christoph Schmaderer[1] and Stephan Kemmner[1†]*

[1] Department of Nephrology, School of Medicine, Klinikum Rechts der Isar, Technical University of Munich, Munich, Germany, [2] Department of Nephropathology, Institute of Pathology, Friedrich-Alexander University (FAU) Erlangen-Nürnberg, Erlangen, Germany, [3] School of Medicine, Institute of AI and Informatics in Medicine, Klinikum Rechts der Isar, Technical University of Munich, Munich, Germany, [4] Department of Surgery, School of Medicine, Klinikum Rechts der Isar, Technical University of Munich, Munich, Germany

Background: The increasing organ shortage in kidney transplantation leads to the necessity to use kidneys previously considered unsuitable for transplantation. Numerous studies illustrate the need for a better decision guidance rather than only the classification into kidneys from standard or expanded criteria donors referred to as SCD/ECD-classification. The kidney donor profile index (KDPI) exhibits a score utilizing a much higher number of donor characteristics. Moreover, graft biopsies provide an opportunity to assess organ quality.

Methods: In a single center analysis 383 kidney transplantations (277 after deceased and 106 after living donation) performed between January 1st, 2006, and December 31st, 2016, retrospectively underwent SCD/ECD and KDPI scoring. Thereby, the quality of deceased donor kidneys was assessed by using the KDPI and the living donor kidneys by using the living KDPI, in the further analysis merged as (L)KDPI. Baseline biopsies taken 10 min after the onset of reperfusion were reviewed for chronic and acute lesions. Survival analyses were performed using Kaplan-Meier analysis and Cox proportional hazards analysis within a 5-year follow-up.

Results: The (L)KDPI correlated with glomerulosclerosis ($r = 0.30$, $p < 0.001$), arteriosclerosis ($r = 0.33$, $p < 0.001$), interstitial fibrosis, and tubular atrophy ($r = 0.28$, $p < 0.001$) as well as the extent of acute tubular injury ($r = 0.20$, $p < 0.001$). The C-statistic of the (L)KDPI concerning 5-year death censored graft survival was 0.692. Around 48% of ECD-kidneys were classified as (L)KDPI<85%. In a multivariate Cox proportional hazard analysis including (preformed) panel reactive antibodies, cold ischemia time, (L)KDPI, and SCD/ECD-classification, the (L)KDPI was significantly associated with risk of graft loss

OPEN ACCESS

Edited by:
Ondrej Viklicky,
Institute for Clinical and Experimental Medicine (IKEM), Czechia

Reviewed by:
Karl Martin Wissing,
University Hospital Brussels, Belgium
Gaurav Gupta,
Virginia Commonwealth University, United States

****Correspondence:***
Stephan Kemmner
stephan.kemmner@tum.de

†Present address:
Stephan Kemmner,
Transplant Center, University Hospital Munich,
Ludwig-Maximilians-University (LMU),
Munich, Germany

‡These authors have contributed equally to this work

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *13 February 2022*
Accepted: *28 March 2022*
Published: *29 April 2022*

Citation:
Bachmann Q, Haberfellner F, Büttner-Herold M, Torrez C, Haller B, Assfalg V, Renders L, Amann K, Heemann U, Schmaderer C and Kemmner S (2022) The Kidney Donor Profile Index (KDPI) Correlates With Histopathologic Findings in Post-reperfusion Baseline Biopsies and Predicts Kidney Transplant Outcome. Front. Med. 9:875206. doi: 10.3389/fmed.2022.875206

(hazard ratio per 10% increase in (L)KDPI: 1.185, 95% confidence interval: 1.033–1.360, $p = 0.025$). Survival analysis revealed decreased death censored ($p < 0.001$) and non-death censored ($p < 0.001$) graft survival in kidneys with an increasing (L)KDPI divided into groups of <35, 35–85, and >85%, respectively.

Conclusion: With a higher granularity compared to the SCD/ECD-classification the (L)KDPI is a promising tool to judge graft quality. The correlation with chronic and acute histological lesions in post-reperfusion kidney biopsies underlines the descriptive value of the (L)KDPI. However, its prognostic value is limited and underlines the urgent need for a more precise prognostic tool adopted to European kidney transplant conditions.

Keywords: kidney biopsies, living kidney donor profile index (LKDPI), ischemia/reperfusion injury, kidney transplant outcomes, expanded criteria donor (ECD), standard criteria donor (SCD), kidney donor profile index (KDPI), kidney transplantation

INTRODUCTION

There is a worldwide shortage of organs suitable for kidney transplantation and especially in Germany the demand clearly exceeds the allocable organ numbers (1, 2). Therefore, rising donor age and an increased use of organs from expanded criteria donors (ECD) is recorded (3, 4).

The distinction between standard criteria donors (SCD) and ECD was introduced to grade graft quality, identifying 4 simple characteristics (age, kidney function, hypertension, and cerebrovascular death) (5). ECDs are donors who are either older than 60 years, or 50 to 59 years old and meet at least two of the following criteria: cerebrovascular death, history of hypertension, or last serum creatinine >1.5 mg/dl (**Table 1**). Although ECD-kidneys perform worse survival than SCD-kidneys, it could also be shown that transplantation of ECD-kidneys can be live saving compared to maintenance of hemodialysis (6–8). However, recipient's age and the increasing number of ECD-kidneys due to older donor age affects the prognostic value of the standard and expanded criteria donor classification (9).

The KDPI (kidney donor profile index) is an index displayed as a cumulative percentage scale representing the risk for kidney transplant failure. For example, the graft of a donor with a KDPI of 70% has a higher predictive risk of graft failure than 70% of the grafts transplanted in the precedent year (10, 11). The KDPI is calculated from the KDRI (kidney donor risk index) which considers 10 donor-related factors including age, height, weight, history of diabetes and hypertension, serum creatinine, hepatitis C status, ethnicity, cause of death, and donation after cardiac death (**Table 1**). Its predictive power for transplant outcome and patient survival as well as eGFR in long term follow up after kidney transplantation has been demonstrated in several studies (12–14).

Abbreviations: ATI, acute tubular injury; CIT, cold ischemia time; DBD, donation after brainstem death; DGF, delayed graft function; ECD, expanded criteria donor; IF/TA, interstitial fibrosis, and tubular atrophy; IRI, ischemia-reperfusion injury; KDPI, kidney donor profile index; KDRI, kidney donor risk index; LKDPI, living kidney donor profile index; (L)KDPI, (living)KDPI; OPTN, organ procurement and transplantation network; PRA, panel reactive antibodies; SCD, standard criteria donor.

Increasing donor age is not limited to cadaveric kidneys but also affects kidneys from living donors. Furthermore, donor age is a predictor of graft function in kidney transplantation after living donation (15). The living KDPI (LKDPI) is based on the same scale as the KDPI and thus allows for graft comparison from living and deceased donors (16). Compared to the KDPI not only donor-specific parameters but also recipient- and transplant-specific variables are used in the calculation of the LKDPI such as gender, AB0 incompatibility, relationship ratio, HLA mismatches, and weight ratio (**Table 1**). For example, a LKDPI of 20% corresponds to the same expected graft survival as a KDPI of 20%. At the same time, the LKDPI may yield negative values, indicating a lower risk as compared to all deceased donor kidneys (16).

Here we investigated on the additional prognostic value of the (L)KDPI in SCD and ECD kidneys from a single center cohort by use of routinely taken baseline-biopsies 10 min after the onset of reperfusion. This allows for the consideration of histological graft quality including tubular injury following transplantation in the evaluation of the (L)KDPI as prognosis score in renal allografts from SCD and ECD.

METHODS

Inclusion and Exclusion Criteria

All kidney transplantations with baseline biopsy during transplant surgery after deceased or living donation at Klinikum rechts der Isar, Munich, Germany between January 1st, 2006, and December 31st, 2016, were included in this retrospective analysis. These baseline biopsies were taken routinely 10 min after the onset of graft reperfusion by core needle (18G) biopsy as part of the clinic's internal standard of care protocol to allow for initial assessment of graft quality by baseline histology.

All patients included into this study were at least 18 years old at time of transplantation. Informed consent was obtained for using the kidney specimens retrospectively for further investigation. The local ethics committee of the Technical University of Munich, Germany had approved this retrospective analysis of the cohort (No. 178/21s). For data collection the hospital's information system, patient records, routine clinical

TABLE 1 | Donor and recipient characteristics used to calculate the SCD/ECD-classification, the KDPI and the LKDPI.

	ECD	KDPI	LKDPI
Donor associated	Age > 60 y	Age	Age
	Or	Height	BMI
	Age 50–59 y and 2 of the following:	Weight	
	Death from CVA	Arterial hypertension	Systolic blood pressure
	Arterial hypertension	Diabetes	Cigarette use
	SCr > 1.5 mg/dl	Hepatitis C	
		Cause of death	
		DBD/DCD	
		Last SCr	eGFR
		Ethnicity	Ethnicity
Transplant associated			AB0 incompatibility
			HLA-mismatches
			Weight ratio
			Biological relationship
			Sex

BMI, Body Mass Index; CVA, cerebro-vascular accident; DBD, donation after brainstem death; DCD, donation after cardiac death; eGFR, estimated glomerular filtration rate; HLA, Human leukocyte antigen; (L)KDPI, (Living) Kidney Donor Profile Index; SCr, Serum creatinine.

follow-up from external nephrologists, and the Eurotransplant Network Information System (ENIS) for donor and recipient data were used. Patients were followed up until June 30th, 2017 (data lock).

Recipients with early graft failure due to perioperative (surgical and obviously non-immunological) complications were excluded from further statistical analyses.

Recipients were subclassified whether they received an organ from SCDs or ECDs according to the definition by Port et al. as written above (5).

Classification According to (L)KDPI

For the calculation of the KDRI, ten donor characteristics (age, height, weight, ethnicity, history of hypertension and diabetes, last serum creatinine, cause of death, hepatitis C status, and donation after cardiac death) were used as guided by the Organ Procurement and Transplantation Network (OPTN) (17). In case of missing information about hypertension or diabetes, the average prevalence reported by the OPTN was used (18). Since there is no information about donor's ethnicity in the Eurotransplant system, all donors were classified as "Caucasian" according to the current German epidemiology. Using the OPTN mapping table with the scaling factor of 2017, the KDRI was translated into the KDPI score (%) (18).

The LKDPI was calculated by using donor and recipient factors such as age, eGFR, BMI, ethnicity, history of cigarette use, systolic blood pressure, sex, AB0 incompatible transplantation, relation, HLA status, and donor/recipient weight ratio (16).

Both KDPI and LKDPI were divided in groups (<35%, low risk; 35–85%, medium risk; > 85%, high risk), inspired by the OPTN. Delayed graft function (DGF) was defined as proposed by the OPTN: need for dialysis during the first week after transplantation (19).

Primary and Secondary Endpoints

The primary endpoint was death censored transplant failure, comprising permanent need for dialysis after transplantation, including both primary non-function (apart from surgical complications) and follow-up end-stage transplant failure requiring reinstitution of dialysis. In the event of death with a functioning graft, the follow-up period was censored at date of death (20). Graft failure was assessed within 5 years after transplantation. Transplantations were censored at 5 years or at the last day of detected kidney function in follow-up examination within 5 years.

Primary non-function was defined as an initially non-working allograft with need for intermittent dialysis after transplantation, without accountable perioperative complications, and with proven organ perfusion confirmed by ultrasound examination.

The secondary endpoint was non-death censored transplant failure, which is a composite of primary non-function, follow-up end-stage transplant failure requiring the reinstitution of dialysis, and recipient death with a functioning allograft. Furthermore, we hypothesized that the (L)KDPI is associated with factors representing limited organ quality and prolonged transport, ECD, increased cold ischemia time, and histological findings in the baseline biopsy such as the histological extent of (acute) tubular injury (ATI), interstitial fibrosis and tubular atrophy (IF/TA), arteriosclerosis and glomerulosclerosis.

Assessment of Allograft Biopsies

All biopsy specimens included in this study were retrospectively reviewed by the same experienced renal pathologist (M.B.-H.), who was blinded for clinical data. The biopsy specimens were core-needle biopsies prepared on slides containing paraffin sections (2–4 μm) that were stained with hematoxylin and eosin (HE) and periodic acid–Schiff (PAS).

Chronic lesions in the biopsies were assessed. The severity of arteriosclerosis was scored semi-quantitatively according to revised Banff Classification. The severity of IF/TA was reported as a percentage concerning the proportion of the affected cortical area in the biopsy sample. Glomerulosclerosis was expressed as a percentage of the total number of glomeruli in the biopsy (21).

ATI was scored as previously described (22) and the assessment involved apical blebbing, epithelial hydropic swelling with lucency of the cytoplasm, loss of brush border, luminal dilatation with flattening of the epithelium, cytoplasmatic vacuolization, and sloughing of tubular cells and was diagnosed whenever one or more of these histologic features were present. Thereby, the extent of ATI was categorized as "none" (0%), "mild" (<25%), "moderate" (25–50%), or "severe" (>50%) tubular injury (23).

Statistical Analysis

Normally distributed data was summarized by mean ± standard deviation, for skewed data median and interquartile range (IQR), represented as first quartile to third quartile, are shown. Categorical data is displayed as absolute number (n) and percentage of the total number (%). Comparisons between groups of the baseline characteristics was performed by using Kruskal-Wallis and Mann-Whitney U test for non-normally distributed data, univariate ANOVA and *t*-test for normally distributed data and chi-square (χ^2) tests for categorical data. Pearson's correlation was used to assess associations between metric, normally distributed data, Spearman rank correlation between metric and ordinal, Eta coefficient (η) between metric and nominal data, and the χ^2 -test (ϕ) between ordinal and nominal scaled variables.

Kaplan-Meier analysis, univariate and multivariate Cox proportional-hazards analysis, and log-rank tests were used to examine the association between the SCD/ECD-classification as well as the (L)KDPI and the primary and secondary endpoint. Univariate and multivariate Cox proportional-hazards analyses were calculated with the 5-year follow-up values.

For estimation of hazard ratios, Cox proportional-hazards models were fitted to the data. Those multivariate models included recipient and donor associated risk factors from univariate analysis for the primary endpoint (death censored transplant failure). The (L)KDPI score was included in a Cox proportional-hazards analysis as a continuous variable. All tests were performed two-sided using a significance level of $\alpha = 0.05$. C-statistics (24) were estimated using the concordance() function provided in the survival package of R (25, 26).

Statistical elaboration was performed using the software programs "IBM SPSS Statistics" version 25 (IBM Corp., NY, USA) and "R" version 3.4.4 (R development team, Vienna, Austria). In addition, GraphPad Prism, version 7.0 (Graph-Pad Software) was used for data presentation.

RESULTS

Patients

In total, 406 potential kidney transplantations (**Figure 1**) which underwent baseline biopsy were performed between January 1st, 2006, and December 31st, 2016, at Klinikum rechts der Isar, Munich. Of these, 14 underwent combined kidney-pancreas transplantation and were therefore excluded from statistical analysis, as well as nine transplantations with early graft loss due to perioperative (surgical) complications.

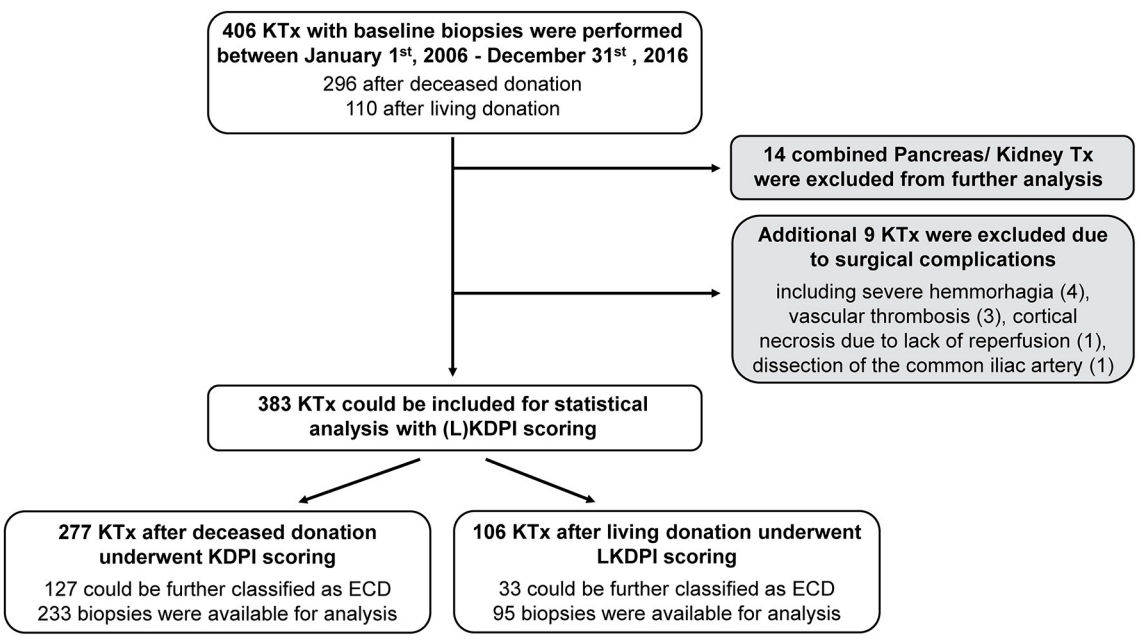


FIGURE 1 | Study population. Flowchart representing the evaluation process of kidney transplantations for statistical analysis and histological judgement of baseline biopsies. Expanded Criteria Donor (ECD); (Living) Kidney Donor Profile Index (L)KDPI; kidney transplantation KTx.

Of these 383 remaining transplantations, 277 underwent KDPI scoring according to deceased donation and 106 transplantations underwent LKDPI scoring after living donation. During the observation period five patients were transplanted twice due to early failure of first kidney transplant. Further, of the 277 deceased donations 150 (54%) were classified as SCD and 127 (46%) as ECD. In living donations 73 (69%) were classified as SCD and 33 (31%) as ECD. Detailed baseline demographics are presented in **Table 2**.

Since in Germany non-heart-beating kidney donation is not possible all deceased donations in this cohort were donations after brainstem death (DBD) and will be referred to as such.

Of 383 initially taken biopsy samples, 54 specimens were not available for further analysis due to poor specimen quality, e.g., insufficient cortical tissue or autolysis. The median follow-up time for recipients at the time of data extraction from the clinical follow-up database (data lock: June 30th, 2017) was 4.8 (0.1–11.4) years. During observation, three patients were lost to follow-up and censored: one patient after deceased donation after 54 days and two patients after living donation (after 342 and 428 days).

Renal Graft Outcomes

Within 5 years after transplantation 47 patients suffered from transplant failure and 34 patients died with a functioning allograft. Of these, 8 patients with transplant failure had a transplant with a KDPI or LKDPI [(L)KDPI] of <35%, 17 patients of 35–85%, and 22 patients of more than 85%. Primary non-function occurred in 1 kidney transplant with a (L)KDPI of <35%, in 6 transplants of 35–85% and in 7 transplants of more than 85% ($p = 0.018$). Of these, only one patient received a living donation (LKDPI 35–85%). There also were significant differences in the eGFR of renal grafts 3 years after transplantation in the three categories (L)KDPI <35, 35–85, and >85% with eGFRs of 61 ml/min/1.73 m^2, 45 ml/min/1.73 m^2, and 39 ml/min/1.73 m^2 ($p < 0.001$), respectively. Focusing on transplantation after deceased donation only, the eGFR after 3 years showed comparable values in the three (L)KDPI categories: 65 ml/min/1.73 m^2, 45 ml/min/1.73 m^2, and 38 ml/min/1.73 m^2 ($p < 0.001$). No significant differences were present in the number of rejections 1 year after transplantation between the (L)KDPI groups, neither for all transplantations nor within the recipients after deceased donation. Average dialysis vintage was not different between the 3 KDPI-groups of kidney transplantations after DBD. Significant differences were also present in 3 year-eGFR and PNF for transplants divided by the SCD/ECD-criteria, whereas there was no significant difference for biopsy proven rejections (BPRs) as shown in **Table 3**.

Transplant failure and death with functioning graft increased significantly with a (L)KDPI >85% compared to (L)KDPI <35% and 35–85% (**Figures 2E,F**). Graft loss at 5 years was 8/127 (Kaplan-Meyer estimator 0.92) for (L)KDPI <35%, 17/171 (Kaplan-Meyer estimator 0.88) for (L)KDPI 35–85%, and 22/85 (Kaplan-Meyer estimator 0.65) for (L)KDPI >85% respectively ($p < 0.001$). Nonetheless, average death censored graft survival in the high-risk group was still 7.5 years (±1.2 years). Mean death censored graft survival time was 9.3 years (±0.7 years) in the medium- and 10.3 years (±1.2 years) in the low-risk group. In transplantation from DBDs it was 9.1 (±0.8 years) and 10.6 years (±0.6 years), respectively. The median LKDPI in transplantations after living donation was 28 (IQR: 8, 59) whereas the median KDPI after deceased donation was 67 (IQR: 37, 89; $p < 0.001$, **Figure 2B**). The eGFR of kidneys with (L)KDPI >85% 3 years after transplantation was only 9 ml/min/1.73 m^2 below the overall average. DGF occurred in 24/127 (19%) transplanted kidneys with (L)KDPI of <35%, in 66/171 (39%) transplanted kidneys with (L)KDPI of 35–85%, and in 34/85 (40%) transplanted kidneys with (L)KDPI of >85% ($p < 0.001$). The recipient's Charlson Comorbidity Index correlated with the (L)KDPI of all recipients irrespective of living or deceased donation ($r = 0.18$, $p < 0.001$, **Figure 4A**).

Predictive Value of the (L)KDPI

The (L)KDPI as a continuous variable was significantly associated with death censored graft survival (HR per 10% increase in (L)KDPI: 1.197, 95% CI: 1.085–1.320, $p < 0.001$) and non-death censored graft survival (HR per 10% increase in (L)KDPI: 1.221, 95% CI: 1.129–1.231, $p < 0.001$). Likewise, this was applicable to the KDPI after deceased donation for death censored graft survival (HR per 10% increase in (L)KDPI: 1.297, 95% CI: 1.153–1.459, $p < 0.001$) and non-death censored graft survival (HR per 10% increase in (L)KDPI: 1.259, 95% CI: 1.164–1.361, $p < 0.001$) but not for the LKDPI in living donation (**Table 4**). As dichotomous variable the SCD/ECD-classification reaches a greater association compared to the continuous (L)KDPI for death censored graft survival (HR 2.223, 95% CI: 1.509–3.275, $p < 0.001$) and non-death censored graft survival (HR 2.602, 95% CI: 1.539–4.397, $p < 0.001$), respectively. **Table 4** shows the HR for previously identified factors influencing kidney transplantation outcomes for death censored and non-death censored graft survival.

The estimated C-statistics of long-term death censored graft survival (5 years) was 0.692 (±0.042) for the (L)KDPI alone and 0.714 (±0.05) if IF/TA in the post reperfusion biopsy was included into the model. On the other hand, donor age alone also yielded a C-statistic of 0.662 (±0.043). The C-statistic of 1-year prediction of death censored graft survival was 0.775 (±0.046) for the (L)KDPI alone and 0.772 (±0.056) for (L)KDPI + IF/TA. In comparison, the C-statistics of donor age considering events within 1 year was 0.715 (±0.053).

Nonetheless, in a multivariate Cox-regression model the (L)KDPI was significantly associated with death censored graft survival if ECD-status, panel reactive antibodies (PRA) and cold ischemia time (CIT) were considered in the model (HR per 10% increase in (L)KDPI: 1.185, 95% CI: 1.033–1.360, $p = 0.025$, **Table 5**). If the same model was applied to DBD kidneys only, the significant association with the KDPI was increased (HR per 10% increase in KDPI: 1.323, 95% CI: 1.088–1.610, $p = 0.006$). To exclude the bias of assignment of better kidney grafts to younger patients, we also fitted a Cox-regression for death censored graft survival to the data with the KDPI (only DBD), ECD-status, PRA, and the recipient's age as independent variables. Here also the KDPI showed a significant association (HR per 10% increase in KDPI: 1.336, 95% CI: 1.077–1.658, $p = 0.008$). Interestingly, in all models including PRA, PRA also had a HR statistically

TABLE 2 | Demographic and clinical characteristics of donors and recipients in the total cohort and in kidney transplantations after living or deceased donation.

Characteristics	Total	Living	Deceased	*p*-value
Number, *n* (%)	383 (100)	106 (28)	277 (72)	
Living donors, *n* (%)	106 (28)	106 (100)	0 (0)	<0.001
Donor associated				
(L)KDPI	54 (27; 83)	28 (8; 49)	67 (38; 89)	**<0.001**
Female, *n* (%)	172 (45)	62 (59)	110 (40)	**0.001**
Age (years)	53 ± 15	54 ± 11	53 ± 16	0.313
BMI (kg/m^2)	27 ± 5	27 ± 4	27 ± 5	0.451
Cause of death (*n*)	277	0	277	
Trauma	63 (23)		63 (23)	
CVA	160 (58)		160 (58)	
Other	54 (20)		54 (20)	
History of				
hypertension	154 (41)	38 (36)	116 (42)	0.217
diabetes	38 (10)	0 (0)	38 (14)	**<0.001**
last SCr (mg/dl)	0.9 (0.7; 1.1)	0.8 (0.7; 0.9)	0.9 (0.7; 1.3)	**0.004**
Transplant associated				
HLA-mismatch	4 (3; 5)	4 (3; 5)	4 (3; 5)	0.154
CIT (h)	8 (2; 13)	2 (2; 2)	11 (8; 15)	**<0.001**
WIT (min)	20 (20; 22)	20 (20; 20)	20 (18; 30)	0.726
Recipient associated				
Female, *n* (%)	134 (35)	37 (35)	97 (35)	0.984
Age (years)	52 ± 13	47 ± 13	55 ± 12	**<0.001**
BMI (kg/m^2)	25 ± 5	25 ± 5	25 ± 5	0.952
Caucasian	377(98)	105 (99)	272 (98)	0.362
First transplantation	318 (83)	97 (92)	221 (80)	**0.006**
Induction therapy	89 (23)	25 (24)	64 (23)	0.171
Reason for ESKD				
Glomerulonephritis	117 (31)	34 (32)	83 (30)	0.688
Diabetes	37 (10)	9 (9)	28 (10)	0.632
Hypertension	57 (15)	15 (14)	42 (15)	0,803
Other	172 (45)	48 (45)	124 (45)	0.734
Duration of dialysis (months)	51 (19; 86)	5 (0; 17)	70 (43; 93)	**<0.001**
Immunosuppression				
Glucocorticoids	382 (100)	106 (100)	277 (100)	
CNI	382 (100)	106 (100)	277 (100)	
Tacrolimus	296 (77)	99 (93)	197 (71)	**<0.001**
CCI Score	2 (2,4)	2 (2,3)	3 (2,4)	**0.012**
Results				
Transplant failure				
After 1 year	25 (7)	1 (1)	24 (9)	**0.006**
After 3 years	38 (10)	5 (5)	33 (12)	**0.035**
After 5 years	47 (12)	7 (7)	40 (14)	**0.037**
Death with functioning transplant				
After 1 year	16 (4)	1 (1)	15 (5)	0.050
After 3 years	30 (8)	2 (2)	28 (10)	**0.007**
After 5 years	34 (9)	2 (2)	32 (12)	**0.003**
Delayed graft function	124 (32)	16 (15)	108 (41)	**<0.001**
Primary non function	14 (4)	1 (1)	13 (5)	0.080
Patients with rejections within 1 year	102 (27)	34 (32)	68 (25)	0.136
eGFR (ml/min/1,73 m^2)				
After 3 years	48 (36; 64)	58 (42; 71)	44 (35; 61)	**0.002**

n (%) for categorical data, mean ± standard deviation for normally distributed data, median [interquartile range] for non-parametric data. Comparison between living and deceased groups by $\chi 2$ for categorical data, independent t-test for normally distributed and Mann-Whitney U test for non-parametric data. BMI, Body Mass Index; CCI Score, Charlson Comorbidity Score; CIT, cold ischemia time; eGFR, estimated glomerular filtration rate; ESKD, end stage kidney disease; HLA, Human leukocyte antigen; (L)KDPI, (Living) Kidney Donor Profile Index; SCr, Serum creatinine; WIT, warm ischemia time; CVA, cerebro-vascular accident.

Statistically significant p-values are printed in bold.

TABLE 3 | Demographic and clinical characteristics of donors and recipients, divided in SCD/ECD and (L)KDPI groups.

Characteristics	SCD	ECD	*p*-value	(L)KDPI-score			*p*-value
				<35	35–85	>85	
Number, *n* (%)	223 (58)	160 (42)		127 (33)	171 (45)	85 (22)	
Living donors, *n* (%)	73 (33)	33 (21)		61 (48)	44 (26)	1 (1)	
Donor associated							
(L)KDPI (%)	31 (14; 53)	87 (70; 95)	**<0.001**	16 (3; 27)	58 (50; 72)	95 (90; 98)	**<0.001**
ECD				7 (6)	70 (41)	83 (98)	**<0.001**
Female, *n* (%)	99 (44)	73 (46)	0.811	46 (36)	94 (55)	32 (38)	**0.002**
Age (years)	44 ± 12	66 ± 7	**<0.001**	41 ± 13	55 ± 9	69 ± 10	**<0.001**
BMI (kg/m^2)	27 ± 5	28 ± 4	**0.014**	26 ± 5	27 ± 5	28 ± 4	0.095
Cause of death (*n*)	150	127		66	127	84	
Trauma	52 (35)	11 (9)	**<0.001**	39 (31)	17 (10)	7 (8)	**<0.001**
CVA	63 (42)	97 (76)	**<0.001**	6 (5)	87 (51)	67 (80)	**<0.001**
Other	35 (23)	19 (15)	0.289	21 (17)	23 (13)	10 (12)	0.587
History of							
Hypertension	55 (25)	99 (62)	**<0.001**	18 (14)	80 (47)	56 (66)	**<0.001**
Diabetes	14 (6)	24 (15)	**0.005**	0 (0)	18 (11)	20 (24)	**<0.001**
Last SCr (mg/dl)	0.8 (0.7; 1.1)	0.9 (0.7; 1.2)	0.076	0.8 (0.7; 1.0)	0.8 (0.7; 1.1)	1.0 (0.8; 1.3)	**0.002**
Transplant associated							
HLA-mismatch	3 (3; 4)	4 (3; 5)	**<0.001**	3 (2; 4)	4 (3; 5)	5 (4; 5)	**<0.001**
CIT (h)	8 (2; 13)	8 (4; 14)	0.231	4 (2; 12)	8 (3; 14)	10 (6; 16)	**<0.001**
WIT (min)	20 (20; 20)	20 (20; 30)	0.782	20 (20; 20)	20 (20; 20)	20 (20; 30)	0.062
Recipient associated							
Female, *n* (%)	78 (35)	56 (35)	0.996	51 (40)	55 (32)	28 (33)	0.325
Age (years)	48 ± 12	59 ± 12	**<0.001**	46 ± 13	52 ± 11	63 ± 10	**<0.001**
BMI (kg/m^2)	25 ± 5	26 ± 5	0.039	24 ± 5	26 ± 5	25 ± 4	**0.048**
Reason for ESKD							
Glomerulonephritis	68 (30)	49 (31)	0.978	45 (35)	47 (27)	25 (29)	0.327
Diabetes	19 (9)	18 (11)	0.372	8 (6)	21 (12)	8 (9)	0.224
Hypertension	35 (16)	22 (14)	0.598	17 (13)	24 (14)	16 (19)	0.506
Other	101 (45)	71 (44)	0.859	57 (45)	79 46)	49 (58)	0.222
Duration of dialysis (months)	51 (13; 87)	50 (28; 86)	0.563	25 (4; 80)	68 (26; 92)	49 (33; 69)	**<0.001**
CCI Score	2 (2; 3)	3 (2; 4)	**0.004**	2 (2; 3)	2 (2; 3)	3 (2; 4)	**0.001**
Results							
Transplant failure							
After 5 years	16 (7)	31 (19)	**<0.001**	8 (7)	17 (10)	22 (26)	**<0.001**
Death with functioning transplant							
After 5 years	192 (86)	110 (69)	0.081	3 (2)	15 (9)	16 (19)	**<0.001**
Delayed graft function	67 (30)	57 (36)	0.139	24 (19)	66 (39)	34 (40)	**<0.001**
Primary non function	4 (2)	10 (6)	**0.022**	1 (1)	6 (4)	7 (8)	**0.018**
Patients with rejections within 1 year	52 (23)	50 (31)	0.097	29 (23)	48 (28)	25 (29)	0.483
eGFR (ml/min/1,73 m^2)							
After 3 years	54 (40; 71)	41 (31; 51)	**<0.001**	57 (45; 74)	48 (37; 61)	35 (30; 45)	**<0.001**

n (%) for categorical data, mean ± standard deviation for normally distributed data, median [interquartile range] for non-parametric data. Comparison between SCD and ECD by $\chi 2$ for categorical data, independent t-test for normally distributed and Mann-Whitney U test for non-parametric data. Comparison of (L)KDPI groups by $\chi 2$ for categorical data, ANOVA for normally distributed or Kruskal-Wallis test for non-parametric data. BMI, Body Mass Index; CCI Score, Charlson Comorbidity Score; CIT, cold ischemia time; CVA, cerebro-vascular accident; ECD, Expanded Criteria Donor; eGFR, estimated glomerular filtration rate; ESKD, endstage kidney disease; HLA, Human leukocyte antigen; (L)KDPI, (Living) Kidney Donor Profile Index; SCD, Standard Criteria Donor; SCr, Serum creatinine; WIT, warm ischemia time.

Statistically significant p-values are printed in bold.

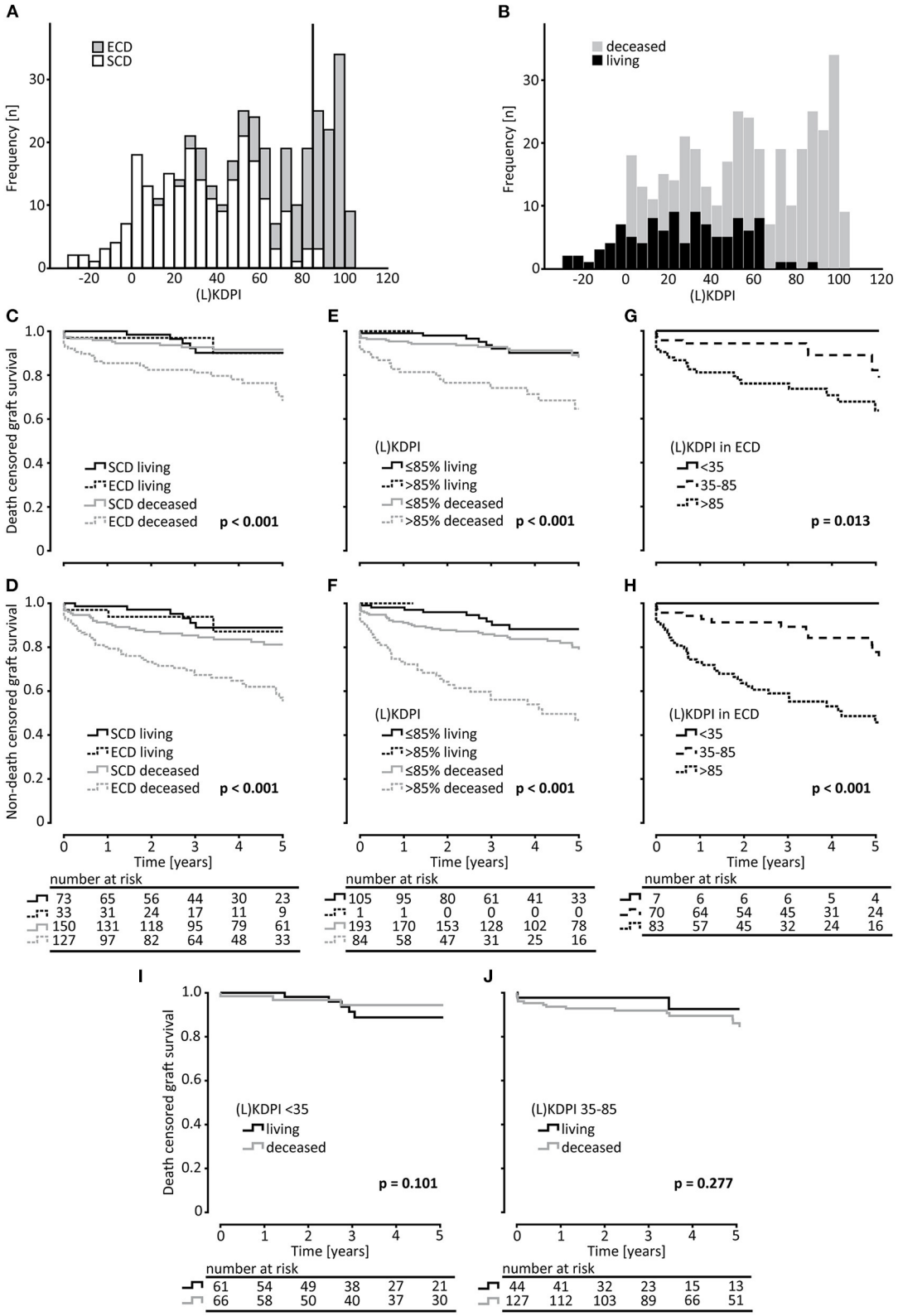


FIGURE 2 | Survival analysis of kidney transplantations rated by the ECD-criteria and the (L)KDPI. **(A)** Histogram of the distribution of Standard Criteria Donor (SCD) and Expanded Criteria Donors (ECD) in (Living) Kidney Donor Profile Index [(L)KDPI] rated transplantations. **(B)** Histogram of the distribution of living and deceased transplantations in (Living) Kidney Donor Profile Index [(L)KDPI] rated transplantations. On the x-axis the transplantations are divided into groups of (L)KDPI-increase = 5. **(C–F)** Kaplan-Meier estimates for death censored graft survival and non-death censored graft survival for SCD vs. ECD and (L)KDPI ≤85% and >85% of living and

(Continued)

FIGURE 2 | deceased donation. **(G,H)** Kaplan-Meier estimates for death censored graft survival and non-death censored graft survival of ECD-kidneys for survival of (L)KDPI groups of <35, 35–85, and >85. Living and deceased donation was pooled for this analysis. **(I,J)** Kaplan-Meier estimates for death censored graft survival comparing living and deceased donation for (L)KDPI <35% and 35–85%. Log-rank testing was used for calculation of each *p*-value.

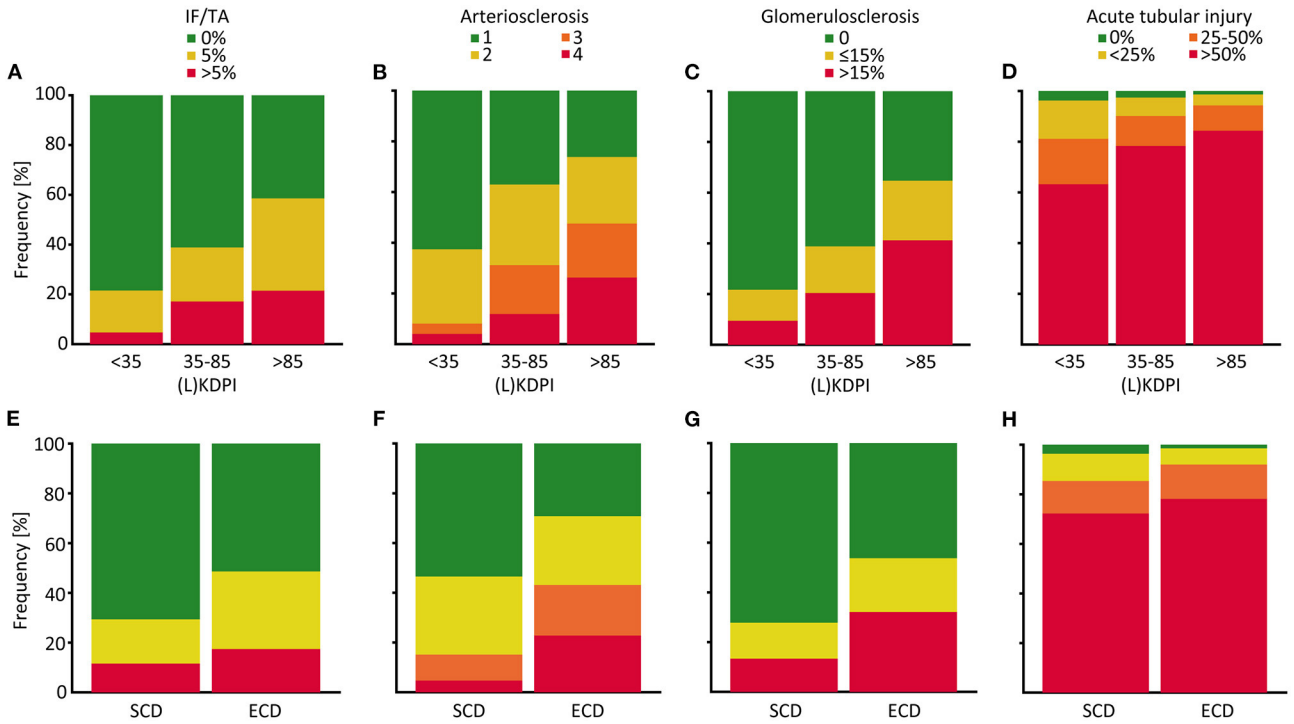


FIGURE 3 | Distribution of histological properties of post-reperfusion biopsies depending on the (L)KDPI of kidney transplantations after living and deceased donation. Percent stacked column chart of the amount of interstitial fibrosis and tubular atrophy (IF/TA), arteriosclerosis, glomerulosclerosis and acute tubular injury subdivided into (Living) Kidney Donor Profile Index [(L)KDPI] <35, 35–85, and >85% **(A–D)** or Standard Criteria Donor (SCD) and Expanded Criteria Donor (ECD) **(E–H)**. Kidney graft tissue was taken 10 min after the onset of reperfusion by 18G core needle biopsy. Histological evaluation was performed by one renal pathologist blinded for clinical data. A semi-quantitative score according to the Banff Classification was used to assess arteriosclerosis. IF/TA, glomerulosclerosis, and acute tubular injury are shown as percentage of the entire area used for histological investigation.

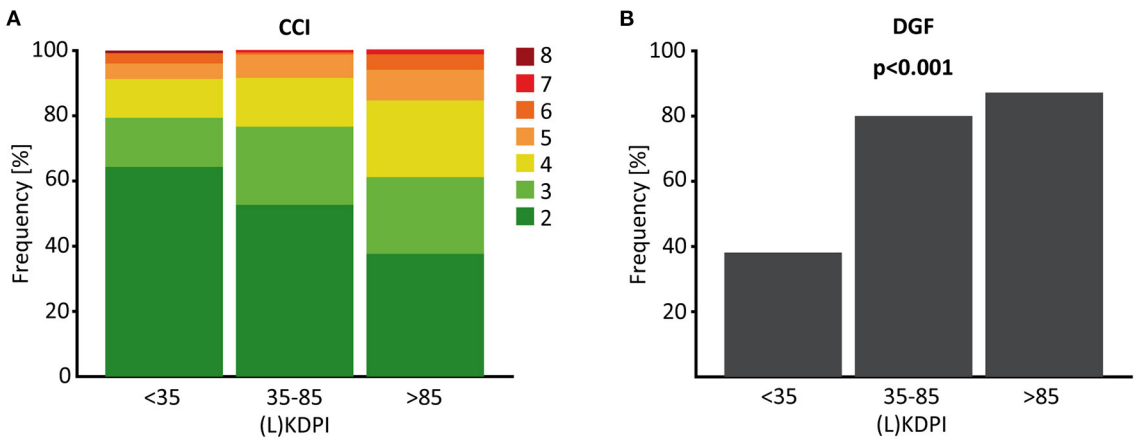


FIGURE 4 | CCI and DGF depended on the (L)KDPI in living and deceased donation. **(A)** Percent stacked column chart of the Charlson Comorbidity Index (CCI) of living and deceased kidney transplantation with the (Living) Kidney Donor Profile Index [(L)KDPI] divided into <35, 35–85 and >85%. **(B)** Boxplot showing the percentage of delayed graft function (DGF) of kidney transplantations after living or deceased donation divided into (L)KDPI <35, 35–85, and >85%. Kruskal-Wallis test was used for calculation of *p*-value.

TABLE 4 | Univariate Cox proportional hazards models for 5-year death censored and non-death censored graft survival with hazard ratios (HR) and 95% confidence intervals (CI) for donor, recipient and transplant associated factors.

	Death censored graft survival HR (95% CI)	*p*-value	Non-death censored graft survival HR (95% CI)	*p*-value
Donor associated				
(L)KDPI	1.197 (1.085–1.320)	**<0.001**	1.221 (1.129–1.231)	**<0.001**
KDPI	1.297 (1.153–1.459)	**<0.001**	1.259 (1.164–1.361)	**<0.001**
LKDPI	0.852 (0.660–1.099)	0.229	0.951 (0.782–1.157)	0.659
ECD	2.602 (1.539–4.397)	**<0.001**	2.223 (1.509–3.275)	**<0.001**
Age	1.038 (1.018–1.059)	**<0.001**	1.039 (1.024–1.055)	**<0.001**
Gender (f)	1.019 (0.610–1.702)	0.943	1.271 (0.866–1.864)	0.221
Height	0.995 (0.973–1.017)	0.660	0.989 (0.975–1.003)	0.115
Weight	1.008 (0.993–1.024)	0.308	1.000 (0.988–1.013)	0.949
History of				
Hypertension	2.347 (1.381–3.988)	**0.002**	1.656 (0.114–2.459)	**0.012**
Diabetes	4.471 (2.462–8.119)	**<0.001**	2.973 (1.818–4.863)	**<0.001**
Smoking	0.508 (0.264–0.979)	**0.043**	0.400 (0.243–0.659)	**<0.001**
Cause of death: CVA	1.888 (1.026–3.474)	**0.041**	1.950 (1.240–3.067)	**0.004**
Last SCr	0.820 (0.492–1.366)	0.445	0.818 (0.557–1.201)	0.305
Recipient associated				
Age	1.023 (1.001–1.046)	**0.036**	1.047 (1.028–1.066)	**<0.001**
BMI	1.050 (0.999–1.104)	0.057	1.015 (0.976–1.056)	0.458
Gender (f)	0.929 (0.545–1.585)	0.787	0.732 (0.483–1.111)	0.143
CCI	1.047 (0.832–1.318)	0.696	1.326 (1.142–1.540)	**<0.001**
Reason for ESKD				
Glomerulonephritis	0.717 (0.399–1.288)	0.266	0.618 (0.392–0.975)	**0.039**
Diabetes	1.639 (0.778–3.456)	0.194	2.014 (1.198–3.386)	**0.008**
Hypertension	0.511 (0.204–1.279)	0.152	1.137 (0.684–1.890)	0.621
Duration of dialysis	1.005 (0.998–1.011)	0.145	1.003 (0.998–1.008)	0.259
Transplant associated				
Living vs. deceased donation	1.745 (0.882–3.452)	0.109	2.150 (1.243–3.719)	**0.006**
CIT	1.022 (0.981–1.065)	0.297	1.042 (1.011–1.074)	**0.007**
WIT	1.011 (1.002–1.019)	**0.011**	1.009 (1.002–1.017)	**0.012**
Number of HLA-mismatches	1.318 (1.090–1.595)	**0.004**	1.263 (1.099–1.452)	**0.001**
PRA	1.013 (1.006–1.021)	**0.001**	1.007 (1.000–1.014)	**0.039**
DGF	2.138 (1.191–3.839)	**0.011**	1.514 (0.996–2.302)	0.052
Number of BPR in first year	2.021 (1.607–2.541)	**<0.001**	1.802 (1.483–2.190)	**<0.001**
Number of all BPR	0.613 (0.421–0.894)	**0.011**	0.670 (0.506–0.886)	**0.005**
eGFR after 3 years	0.957 (0.931–0.983)	**0.001**	0.967 (0.949–0.986)	**0.001**

HR and CI were calculated per 10% increase of the (L)KDPI. BMI, Body Mass Index; BPR, biopsy-proven rejection; CCI, Charlson Comorbidity Index; CIT, cold ischemia time; CVA, cerebro-vascular accident; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; ESKD, end stage kidney disease; HLA, Human leukocyte antigen; (L)KDPI, (Living) Kidney Donor Profile Index; PRA, panel-reactive antibody; SCr, Serum creatinine; TX, transplantation; WIT, warm ischemia time.
Statistically significant p-values are printed in bold.

significant from one, indicating its independent association from all other factors investigated on death censored graft survival. To investigate the known highly important association between the primary outcome and donor age, a Cox-regression was calculated for the (L)KDPI and donor age. In this model neither parameters could prove a significant association.

Accuracy of (L)KDPI and the SCD/ECD-Classification

Although there was a statistically significant association between the LKDPI graft survival in living donation, it was possible to judge survival of living and DBD grafts with the KDPI (**Figures 2E,F**). Comparing death censored graft survival of all living and all DBD grafts in our cohort, both KDPI and LKDPI showed no significant differences in the two superior categories <35% and 35–85% (**Figures 2I,J**). Thus, we investigated the influence of (L)KDPI >85% on death censored graft survival compared to transplantations of (L)KDPI of ≤85% (<35% and 35–85% combined). As categorical variable (L)KDPI ≤85% and >85% the HR of death censored graft survival was 3.205 (95% CI 1.888–5.442, $p < 0.001$) for all transplantations and 2.981 (95% CI 1.682–5.283, $p < 0.001$) after DBD, respectively. Since there was only one living donation with an LKDPI >85%, we were not able to perform statistical analyses in this group. Thus,

TABLE 5 | Multivariate Cox-regression model for death censored graft survival with hazard ratios (HR) and 95% confidence intervals (CI) including prognostic factors for reduced graft survival.

Variables	Model 1		Model 2		Model 3		Model 4	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
(L)KDPI	1.139 (0.993; 1.306)	0.066	1.185 (1.033; 1.360)	**0.025**	1.323 (1.088; 1.610)	**0.006**	1.336 (1.077; 1.658)	**0.008**
ECD			1.564 (0.700; 3.497)	0.276	1.118 (0.436; 2.865)	0.817	1.091 (0.430; 2.764)	0.855
PRA			1.015 (1.007; 1.023)	**<0.001**	1.015 (1.007; 1.023)	**<0.001**	1.015 (1.006; 1.024)	**0.001**
Recipient age							0.998 (0.970; 1.027)	0.896
Donor age	1.016 (0.986; 1.047)	0.293						
CIT			1.007 (0.959; 1.057)	0.787	1.009 (0.955; 1.066)	0.751		

Models 1 and 2 include all 383 kidney transplantations and models 3 and 4 only transplantations after deceased donation. HR and CI were calculated per 10% increase of the (L)KDPI. CIT, cold ischemia time; ECD, Expanded Criteria Donor; (L)KDPI, (Living) Kidney Donor Profile Index; PRA, panel reactive antibodies.
Statistically significant p-values are printed in bold.

we decided to pool living and deceased donor kidneys into one analysis. Although 31% of the living donations were classified as ECD, there was no relevant difference between survival of ECD and SCD kidneys in living donations (**Figures 2C,D**).

In total, 77 kidney grafts with a (L)KDPI of ≤85% were classified as ECD whereas only 2 renal grafts >85% were classified as SCD (**Figure 2A**). Survival of ECD-kidneys divided into the 3 (L)KDPI groups showed significant differences for death censored and non-death censored graft survival (**Figures 2G,H**).

Correlation of (L)KDPI With the Histology of Post-reperfusion Graft Baseline Biopsies and Ischemia-Reperfusion Injury

To assess correlation of the (L)KDPI with the quality of the transplanted kidneys more accurately we included the histopathological findings in post-reperfusion biopsies into the statistical analyses as described above. Glomerulosclerosis, arteriosclerosis, and IF/TA as chronic lesions and ATI as renal hallmark of acute injury were histologically evaluated. Naturally, the extent of the chronic parameters increases with an increasing (L)KDPI (**Figures 3A–C**). Fittingly, we found a significant correlation between these parameters and the (L)KDPI (glomerulosclerosis $r = 0.30$, $p < 0.001$; arteriosclerosis $r = 0.33$, $p < 0.001$; IF/TA $r = 0.28$, $p < 0{,}001$). This was most likely due to the high number of deceased donations as only arteriosclerosis turned out to significantly correlate in living donations (glomerulosclerosis $r = 0.03$, $p = 0.8$; arteriosclerosis $r = 0.34$, $p = 0.001$; IF/TA $r = 0.08$, $p = 0.4$).

Furthermore, we found a moderate, but highly significant correlation between the (L)KDPI and the extent of ATI ($r = 0.198$, $p < 0.001$, **Figure 3D**). In line with this observation, higher rates of delayed graft function (DGF) could be revealed as clinical counterpart of severe ischemia-reperfusion injury (IRI) in transplants with a higher (L)KDPI (>35%) in contrast to a lower KDPI (<35%), as shown in **Figure 4B**.

Likewise, associations between the histopathological characteristics and the SCD/ECD-classification were apparent (**Figures 3E–H**). Glomerulosclerosis ($\eta = 0.245$, $p < 0.001$), arteriosclerosis ($\phi = 0.340$, $p < 0.001$), and IF/TA ($\eta = 0.161$, $p = 0.003$) were significantly associated with the ECD status whereas ATI was not ($\phi = 0.104$, $p = 0.318$). In living donations no statistically significant associations between chronic lesions except arteriosclerosis and no association between ATI and the ECD status existed (glomerulosclerosis $\eta = 0.113$, $p = 0.277$; arteriosclerosis $\phi = 0.395$, $p = 0.003$; IF/TA $\eta = 0.161$, $p = 0.119$; ATI $\phi = 0.221$, $p = 0.205$).

DISCUSSION

In this retrospective single center analysis, we evaluated the (L)KDPI against the background of the SCD/ECD-classification, which is more commonly used in Europe. We further compared this classification with baseline biopsies, which are routinely taken 10 min after the onset of reperfusion in our transplant center. The present study revealed the following major findings: *First*, the application of the KDPI and the LKDPI turned out to be a useful tool in this European single center analysis to assess the quality of donor kidneys all in line with earlier reports (12, 27). Furthermore, it was possible to demonstrate the comparability of living donation and DBD with KDPI and LKDPI. Most important, the (L)KDPI showed a distinct correlation with histopathological findings in baseline biopsies.

Interestingly, 48% of all ECD-kidneys in this study had a KDPI <85%. This underlines the usefulness of the 85% cutoff and together with the predictive value (C = 0.69) this suggests a better assessment of ECD kidneys by use of the (L)KDPI with regards to the further probable course after transplantation. Therefore, it can be assumed that the more complex and gradient (L)KDPI, which is based on a bigger range of information, reduces the risk to discard a valuable organ as compared to the dichotomous SCD/ECD-classification. On the other hand, the estimated overall graft survival of KDPI kidneys >85% of only about 60% after 5 years emphasizes the question if these organs should be used for transplantation. *Second*, this trial at hand proved that KDPI and LKDPI enable transplant physicians to compare graft quality between living and deceased donation in a non-US transplant cohort, the way the LKDPI classification was originally defined for (16). Hence, these data suggest an advantage of using the highly granular (L)KDPI to stratify the prognosis of donor kidneys origin as compared

to the SCD/ECD-classification. However, several American and European validation studies on the discriminative ability of the KDPI it never exceeded a Harrell's C of 0.62–0.66 (10, 12, 13, 27, 28), which means that only 66% of the predictions hit the correct outcome. Accordingly, SCD-kidneys may be labeled by a KDPI >85% and be discarded. The rather high impact of donor age on transplant outcomes compared with the KDPI also underlines its additional predictive limits (29). Noteworthy, Bae et al. cautioned against an increasing mortality amongst patients remaining on dialysis and waiting for a kidney offer with a lower KDPI instead of transplantation of these kidneys (30). Fittingly, in our cohort, Assfalg et al. were able to demonstrate similar 5-year graft and patient survival after standard and rescue allocation (31). Interestingly, in the multivariate Cox-regression models of this analysis the KDPI and LKDPI turned out to have a predictive value whereas the ECD status, and cold ischemia time and recipient's age which are not included into the ECD-classification did not. On the other hand, a retrospective analysis of 5,667 patients older than 70 years showed a decreased relative risk of death of 0.75 in patients transplanted with ECD-kidneys as compared to patients remaining on the waiting list (32). ECD kidneys display a significant predictor of mortality in all age groups except for patients older than 70 years (33).

Third, the (L)KDPI in the investigated cohort correlated well with chronic lesions such as arteriosclerosis, glomerulosclerosis and IF/TA giving reason to expected lower graft quality of marginal donor kidneys, which was shown previously (34). This correlation was also observed in a study on pre-implantation biopsies (35). Kidney grafts can also be evaluated by pre-transplant donor biopsies, but due to a high heterogeneity in biopsy-technique, histological evaluation, and study design no valuable recommendation can be derived to include pre-transplant donor biopsies into daily routine (36). Nonetheless, Gandolfi et al. were able to show that pre-transplant donor biopsies allowed for save transplantation of high KDPI-kidneys provided that a specifically trained pathologist is available (37, 38). However, pre-transplant biopsies do not map renal ischemia reperfusion injury (IRI).

Fourth, a correlation between the (L)KDPI and the degree of ATI as histological hallmark of renal IRI is present in this cohort. Earlier studies demonstrated that marginal and especially ECD kidneys are significantly more vulnerable to cold ischemia time, which is part of the transplantation process after deceased donation and the subsequent tubular injury (39–41). Severe acute tubular injury becomes clinically apparent in delayed graft function (DGF) defined as dialysis in the first week after kidney transplantation (42). Gill et al. showed a decreased graft survival benefit of kidney transplantation with high KDPI grafts followed by DGF as compared to recipients of a higher quality graft followed by DGF but still better than in patients remaining on dialysis (43). DGF is a well-known independent risk factors of 1-year graft survival (44). Our data strongly underlines the approach that ATI could be a therapeutic target to improve graft quality of kidneys with high KDPI (45). The use of hypo- or normothermic machine perfusion may be an option here (46). Using *ex vivo* normothermic perfusion, Kabagambe et al. prompted increasing blood flow and urine output and histologically less ATI in 7 marginal kidneys with a mean KDPI of 79%, which were initially discarded for kidney transplantation based on a combination of clinical findings, suboptimal biopsies, long CIT, and/or poor hypothermic perfusion parameters (47).

Our study has several points for critical discussion. We investigated on a single center cohort with a moderate number of cases including kidney transplantation after deceased as well after living donation. Comparison of DBD to living donors bears a risk for bias due to big differences in organ quality (48). However, the LKDPI was developed to take these issues into account e.g., by negative values and was explicitly created for comparison of living to deceased donor grafts (16). Concerning DBD kidney grafts, outcomes might be biased by our selection policy accepting grafts from older donors with presumably lower quality for older recipients. Although the KDPI was predictive in a multivariate Cox-regression model including the recipients age, patients who received kidneys with a low KDPI were significantly younger than patients who received kidneys with a high KDPI ($p < 0.001$). Finally, the retrospective design of this study cannot reach the quality of a prospective observation study.

In conclusion, also in a European single center cohort the (L)KDPI for kidney transplants living donation and DBD is useful to assess organ quality more accurate than SCD/ECD-classification and to stratify their risk for later graft loss. Until this study there was no certainty, if the predictive value of the (L)KDPI translates into histopathological findings of baseline kidney biopsies. Additionally, the increase in Harrell's C after inclusion of IF/TA suggests an even better judgement of organ quality utilizing a biopsy. Thus, a prospective, multicenter study with a higher number of patients performing baseline biopsies is required to clarify, if the combination of the (L)KDPI and histopathological findings can improve the predicted outcome of kidney grafts. This might give clinicians the missing tool to better judge the value of grafts rendered as bad quality by current scores, since evidence proving the need to transplant these organs to improve patient survival accumulates (31, 49, 50). The overall small predictive value of the currently available tools illustrates the necessity for comprehensive international databases, further research on the predictive value of donor, graft, and transplant specific properties including more variables, and transplant physicians' courage to even accept marginal organs for distinct subgroups in times of growing organ shortage.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article are available from corresponding author upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Technical University of Munich, Germany. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

QB and SK wrote the manuscript. SK and CS designed the concept of the study and profile index of the study. QB, FH, CT, and SK collected clinical data. QB, FH, BH, and SK performed and reviewed statistical analysis. VA is responsible for the routinely performance of surgical biopsies used in this study. MB-H analyzed the biopsies. VA, KA, LR, and UH oversaw the study and critically discussed the manuscript. All authors approved the submitted version of the manuscript.

FUNDING

This work was supported by the Klinikum Rechts der Isar of the Technical University of Munich.

REFERENCES

1. Hart A, Lentine KL, Smith JM, Miller JM, Skeans MA, Prentice M, et al. OPTN/SRTR 2019 annual data report: kidney. *Am J Transplant.* (2021) 21(Suppl. 2):21–137. doi: 10.1111/ajt.16502
2. Nelson MJ, Bahl JS, Buckley JD, Thomson RL, Davison K. Evidence of altered cardiac autonomic regulation in myalgic encephalomyelitis/chronic fatigue syndrome: a systematic review and meta-analysis. *Medicine.* (2019) 98:e17600. doi: 10.1097/MD.0000000000017600
3. Coemans M, Susal C, Dohler B, Anglicheau D, Giral M, Bestard O, et al. Analyses of the short- and long-term graft survival after kidney transplantation in Europe between 1986 and 2015. *Kidney Int.* (2018) 94:964–73. doi: 10.1016/j.kint.2018.05.018
4. Aubert O, Reese PP, Audry B, Bouatou Y, Raynaud M, Viglietti D, et al. Disparities in acceptance of deceased donor kidneys between the United States and france and estimated effects of increased US acceptance. *JAMA Intern Med.* (2019) 179:1365–74. doi: 10.1001/jamainternmed.2019.2322
5. Port FK, Bragg-Gresham JL, Metzger RA, Dykstra DM, Gillespie BW, Young EW, et al. Donor characteristics associated with reduced graft survival: an approach to expanding the pool of kidney donors. *Transplantation.* (2002) 74:1281–6. doi: 10.1097/00007890-200211150-00014
6. Ojo AO, Hanson JA, Meier-Kriesche H, Okechukwu CN, Wolfe RA, Leichtman AB, et al. Survival in recipients of marginal cadaveric donor kidneys compared with other recipients and wait-listed transplant candidates. *J Am Soc Nephrol.* (2001) 12:589–97. doi: 10.1681/ASN.V123589
7. Merion RM, Ashby VB, Wolfe RA, Distant DA, Hulbert-Shearon TE, Metzger RA, et al. Deceased-donor characteristics and the survival benefit of kidney transplantation. *JAMA.* (2005) 294:2726–33. doi: 10.1001/jama.294.21.2726
8. Querard AH, Le Borgne F, Dion A, Giral M, Mourad G, Garrigue V, et al. Propensity score-based comparison of the graft failure risk between kidney transplant recipients of standard and expanded criteria donor grafts: toward increasing the pool of marginal donors. *Am J Transplant.* (2018) 18:1151–7. doi: 10.1111/ajt.14651
9. Tittelbach-Helmrich D, Thurow C, Arwinski S, Schleicher C, Hopt UT, Bausch D, et al. Poor organ quality and donor-recipient age mismatch rather than poor donation rates account for the decrease in deceased kidney transplantation rates in a Germany Transplant Center. *Transpl Int.* (2015) 28:191–8. doi: 10.1111/tri.12478
10. Watson CJ, Johnson RJ, Birch R, Collett D, Bradley JA. A simplified donor risk index for predicting outcome after deceased donor kidney transplantation. *Transplantation.* (2012) 93:314–8. doi: 10.1097/TP.0b013e31823f14d4
11. Rao PS, Schaubel DE, Guidinger MK, Andreoni KA, Wolfe RA, Merion RM, et al. A comprehensive risk quantification score for deceased donor kidneys: the kidney donor risk index. *Transplantation.* (2009) 88:231–6. doi: 10.1097/TP.0b013e3181ac620b
12. Lehner LJ, Kleinsteuber A, Halleck F, Khadzhynov D, Schrezenmeier E, Duerr M, et al. Assessment of the kidney donor profile index in a European cohort. *Nephrol Dial Transplant.* (2018) 33:1465–72. doi: 10.1093/ndt/gfy030
13. Peters-Sengers H, Heemskerk MBA, Geskus RB, Kers J, Homan van der Heide JJ, Berger SP, et al. Validation of the prognostic kidney donor risk index scoring system of deceased donors for renal transplantation in the Netherlands. *Transplantation.* (2018) 102:162–70. doi: 10.1097/TP.0000000000001889
14. Zens TJ, Danobeitia JS, Leverson G, Chlebeck PJ, Zitur LJ, Redfield RR, et al. The impact of kidney donor profile index on delayed graft function and transplant outcomes: a single-center analysis. *Clin Transplant.* (2018) 32:e13190. doi: 10.1111/ctr.13190
15. Noppakun K, Cosio FG, Dean PG, Taler SJ, Wauters R, Grande JP. Living donor age and kidney transplant outcomes. *Am J Transplant.* (2011) 11:1279–86. doi: 10.1111/j.1600-6143.2011.03552.x
16. Massie AB, Leanza J, Fahmy LM, Chow EK, Desai NM, Luo X, et al. A risk index for living donor kidney transplantation. *Am J Transplant.* (2016) 16:2077–84. doi: 10.1111/ajt.13709
17. Organ Procurement and Transplantation Network. *A Guide to Calculating and Interpreting the Kidney Donor Profle Index (KDPI) (PDF).* (2018). Available online at: https://optn.transplant.hrsa.gov/media/1512/guide_to_calculating_interpreting_kdpi.pdf (accessed August 21, 2018).
18. Organ Procurement and Transplantation Network. *KDRI to KDPI Mapping Table (PDF).* (2018). Available from: https://optn.transplant.hrsa.gov/media/2150/kdpi_mapping_table.pdf (accessed September 06, 2018).
19. Matas AJ, Smith JM, Skeans MA, Thompson B, Gustafson SK, Schnitzler MA, et al. OPTN/SRTR 2012 annual data report: kidney. *Am J Transplant.* (2014) 14:11–44. doi: 10.1111/ajt.12579
20. EBPG Expert Group on Renal Transplantation. European best practice guidelines for renal transplantation. Section IV: Long-term management of the transplant recipient. IV.13 Analysis of patient and graft survival. *Nephrol Dial Transplant.* (2002) 17(Suppl. 4):60–7. doi: 10.1093/ndt/17.suppl_4.55
21. Haas M, Loupy A, Lefaucheur C, Roufosse C, Glotz D, Seron D, et al. The Banff 2017 Kidney Meeting Report: Revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *Am J Transplant.* (2018) 18:293–307. doi: 10.1111/ajt.14625
22. Gwinner W, Hinzmann K, Erdbruegger U, Scheffner I, Broecker V, Vaske B, et al. Acute tubular injury in protocol biopsies of renal grafts: prevalence, associated factors and effect on long-term function. *Am J Transplant.* (2008) 8:1684–93. doi: 10.1111/j.1600-6143.2008.02293.x
23. Hall IE, Reese PP, Weng FL, Schroppel B, Doshi MD, Hasz RD, et al. Preimplant histologic acute tubular necrosis and allograft outcomes. *Clin J Am Soc Nephrol.* (2014) 9:573–82. doi: 10.2215/CJN.08270813
24. Harrell FE. *Regression Modeling Strategies: With Applications to Linear Models, Logistic and Ordinal Regression, and Survival Analysis.* New York, NY: Springer (2015).
25. Therneau T. *A Package for Survival Analysis in R.* R Package Version 3.2-13 (2021). Available online at: https://CRAN.R-project.org/package=survival (accessed March 3, 2020),
26. Therneau T, Grambsch P. *Modeling Survival Data: Extending the Cox Model.* New York, NY: Springer (2000).
27. Rehse G, Halleck F, Khadzhynov D, Lehner LJ, Kleinsteuber A, Staeck A, et al. Validation of the living kidney donor profile index in a european cohort and comparison of long-term outcomes with us results. *Nephrol Dial Transplant.* (2019) 34:1063–70. doi: 10.1093/ndt/gfy118
28. Dahmen M, Becker F, Pavenstädt H, Suwelack B, Schütte-Nütgen K, Reuter S. Validation of the Kidney Donor Profile Index (KDPI) to assess a deceased donor's kidneys' outcome in a European cohort. *Sci Rep.* (2019) 9:11234. doi: 10.1038/s41598-019-47772-7
29. Lee APK, Abramowicz D. Is the Kidney Donor Risk Index a step forward in the assessment of deceased donor kidney quality? *Nephrol Dial Transplant.* (2014) 30:1285–90. doi: 10.1093/ndt/gfu304

30. Bae S, Massie AB, Luo X, Anjum S, Desai NM, Segev DL. Changes in discard rate after the introduction of the Kidney Donor Profile Index (KDPI). *Am J Transplant.* (2016) 16:2202–7. doi: 10.1111/ajt.13769
31. Assfalg V, Misselwitz S, Renders L, Hüser N, Novotny A, Jäger C, et al. Kidney transplantation after rescue allocation-meticulous selection yields the chance for excellent outcome. *Nephrol Dial Transplant.* (2021) 36:551–60. doi: 10.1093/ndt/gfaa286
32. Rao PS, Merion RM, Ashby VB, Port FK, Wolfe RA, Kayler LK. Renal transplantation in elderly patients older than 70 years of age: results from the Scientific Registry of Transplant Recipients. *Transplantation.* (2007) 83:1069–74. doi: 10.1097/01.tp.0000259621.56861.31
33. Molnar MZ, Streja E, Kovesdy CP, Shah A, Huang E, Bunnapradist S, et al. Age and the associations of living donor and expanded criteria donor kidneys with kidney transplant outcomes. *Am J Kidney Dis.* (2012) 59:841–8. doi: 10.1053/j.ajkd.2011.12.014
34. Chen LX, Francalacci LC, Bang H, De Mattos A, Perez RV, Jen KY. Histopathologic findings on implantation renal allograft biopsies correlate with kidney donor profile index and 30-day serum creatinine. *Transplant Proc.* (2019) 51:639–46. doi: 10.1016/j.transproceed.2018.12.027
35. Del Moral Martín RMG, Retamero Díaz JA, Cava Molina M, Cobacho Tornel BM, Bravo Soto J, Osuna Ortega A, et al. Validation of KDRI/KDPI for the selection of expanded criteria kidney donors. *Nefrologia.* (2018) 38:297–303. doi: 10.1016/j.nefroe.2017.12.003
36. Wang CJ, Wetmore JB, Crary GS, Kasiske BL. The donor kidney biopsy and its implications in predicting graft outcomes: a systematic review. *Am J Transplant.* (2015) 15:1903–14. doi: 10.1111/ajt.13213
37. Azancot MA, Moreso F, Salcedo M, Cantarell C, Perello M, Torres IB, et al. The reproducibility and predictive value on outcome of renal biopsies from expanded criteria donors. *Kidney Int.* (2014) 85:1161–8. doi: 10.1038/ki.2013.461
38. Gandolfini I, Buzio C, Zanelli P, Palmisano A, Cremaschi E, Vaglio A, et al. The Kidney Donor Profile Index (KDPI) of marginal donors allocated by standardized pretransplant donor biopsy assessment: distribution and association with graft outcomes. *Am J Transplant.* (2014) 14:2515–25. doi: 10.1111/ajt.12928
39. Bahde R, Vowinkel T, Unser J, Anthoni C, Holzen JP, Suwelack B, et al. Prognostic factors for kidney allograft survival in the Eurotransplant Senior Program. *Ann Transplant.* (2014) 19:201–9. doi: 10.12659/AOT.890125
40. Centellas-Perez FJ, Tebar A, Llamas F, Lorenzo I, Sarduy P, Martinez-Diaz M, et al. Analysis of kidney donation and its relationship with graft failure of the recipient at 1 year. *Transplant Proc.* (2019) 51:314–20. doi: 10.1016/j.transproceed.2018.12.009
41. Aubert O, Kamar N, Vernerey D, Viglietti D, Martinez F, Duong-Van-Huyen JP, et al. Long term outcomes of transplantation using kidneys from expanded criteria donors: prospective, population based cohort study. *BMJ.* (2015) 351:h3557. doi: 10.1136/bmj.h3557
42. Hall IE, Reese PP, Doshi MD, Weng FL, Schroppel B, Asch WS, et al. Delayed graft function phenotypes and 12-month kidney transplant outcomes. *Transplantation.* (2017) 101:1913–23. doi: 10.1097/TP.0000000000001409
43. Gill J, Dong J, Rose C, Gill JS. The risk of allograft failure and the survival benefit of kidney transplantation are complicated by delayed graft function. *Kidney Int.* (2016) 89:1331–6. doi: 10.1016/j.kint.2016.01.028
44. Foroutan F, Friesen EL, Clark KE, Motaghi S, Zyla R, Lee Y, et al. Risk factors for 1-year graft loss after kidney transplantation: systematic review and meta-analysis. *Clin J Am Soc Nephrol.* (2019) 14:1642–50. doi: 10.2215/CJN.05560519
45. Tullius SG, Rabb H. Improving the supply and quality of deceased-donor organs for transplantation. *N Engl J Med.* (2018) 378:1920–9. doi: 10.1056/NEJMra1507080
46. DiRito JR, Hosgood SA, Tietjen GT, Nicholson ML. The future of marginal kidney repair in the context of normothermic machine perfusion. *Am J Transplant.* (2018) 18:2400–8. doi: 10.1111/ajt.14963
47. Kabagambe SK, Palma IP, Smolin Y, Boyer T, Palma I, Sageshima J, et al. Combined *ex vivo* hypothermic and normothermic perfusion for assessment of high-risk deceased donor human kidneys for transplantation. *Transplantation.* (2019) 103:392–400. doi: 10.1097/TP.0000000000002299
48. Ibrahim HN, Foley R, Tan L, Rogers T, Bailey RF, Guo H, et al. Long-term consequences of kidney donation. *N Engl J Med.* (2009) 360:459–69. doi: 10.1056/NEJMoa0804883
49. Assfalg V, Miller G, Stocker F, van Meel M, Groenevelt T, Tieken I, et al. Kidney transplantation after rescue allocation - the eurotransplant experience: a retrospective multicenter outcome analysis. *Transplantation.* (2021) doi: 10.1097/TP.0000000000003964. [Epub ahead of print].
50. McAdams-DeMarco MA, Law A, Salter ML, Boyarsky B, Gimenez L, Jaar BG, et al. Frailty as a novel predictor of mortality and hospitalization in individuals of all ages undergoing hemodialysis. *J Am Geriatr Soc.* (2013) 61:896–901. doi: 10.1111/jgs.12266

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.

frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 11 May 2022
doi: 10.3389/fmed.2022.885018

Dickkopf 3—A New Indicator for the Deterioration of Allograft Function After Kidney Transplantation

Antonia Schuster[1], Louisa Steines[1], Karolina Müller[2], Florian Zeman[2], Peter Findeisen[3], Bernhard Banas[1] and Tobias Bergler[1]*

[1] Department of Nephrology, University Hospital Regensburg, Regensburg, Germany, [2] Center for Clinical Studies, University Hospital Regensburg, Regensburg, Germany, [3] MVZ Laboratory Dr. Limbach, Heidelberg, Germany

Evidence of tubular atrophy and interstitial fibrosis is prognostically unfavorable and associated with a premature graft loss after kidney transplantation. Recently, Dickkopf 3 (DKK3), a profibrotic glycoprotein released by stressed tubular epithelial cells, has been identified to cause IF/TA by regulating the Wnt/β-catenin signaling and seems to engage a T-cell response. The aim of our study was to determine if a correlation between DKK3 and graft function exists and if DKK3 could be a new indicator to identify patients at risk for a deterioration in graft function. Patients, transplanted between 2016 and 2018, were analyzed with regard to DKK3 in the urine and graft function (creatinine, eGFR, albuminuria). Multivariable analyzes were used including known factors influencing graft function (PRA, donor age) to stress robustness of DKK3. The 3 and 12 month DKK3 values were significant predictors for subsequent graft function up to 36 months. An increase of DKK3 from month 3 to 12 of $\geq$ 25% showed a higher risk of an impaired graft function, with, e.g., a reduction in eGFR of about 9–10 ml/min in contrast to patients without intensified DKK3 increase. Induction therapy has an influence on DKK3 as patients induced with a T-cell depleting therapy showed a trend toward lower DKK3 values. In summary, our study is the first investigation of DKK3 in kidney transplant recipients and was able to show that DKK3 could forecast graft function. It is recommended to investigate the potential of DKK3 as a predictor of kidney function after transplantation in further studies.

Keywords: kidney transplantation, allograft survival, Dickkopf (DKK), albuminuria, glomerular filtration rate

OPEN ACCESS

Edited by:
Kathrin Eller,
Medical University of Graz, Austria

Reviewed by:
Brian Duncan Tait,
The University of Melbourne, Australia
Gaurav Gupta,
Virginia Commonwealth University, United States

****Correspondence:***
Antonia Schuster
antonia-margarete.schuster@ukr.de

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *27 February 2022*
Accepted: *19 April 2022*
Published: *11 May 2022*

Citation:
Schuster A, Steines L, Müller K, Zeman F, Findeisen P, Banas B and Bergler T (2022) Dickkopf 3—A New Indicator for the Deterioration of Allograft Function After Kidney Transplantation. Front. Med. 9:885018. doi: 10.3389/fmed.2022.885018

INTRODUCTION

Kidney transplantation remains the preferred treatment for patients with end stage kidney disease due to a better patient survival compared to dialysis. Despite good short-term results, ensuring a long-lasting graft function is an unsolved problem. To date, with serum creatinine, eGFR and albuminuria, only a few parameters are available in everyday clinical practice to monitor graft function. Even in the KDIGO guidelines, these parameters are named as the main monitoring tool (1). However, these parameters have not yet succeeded in ensuring graft survival. In literature, different biomarkers are being discussed in order to ensure a better risk assessment.

Park et al. showed that an eGFR decline of >−10% in a period of 3–12 months was associated with a greater risk of graft failure (2).

The main cause of late allograft loss is the development of interstitial fibrosis and tubular atrophy (IF/TA) (3). These histological changes describe the final stages of different processes (CNI- toxicity, etc.) and can be detected through biopsy (4).

The "iBox," a prediction score by the group of Loupy et al., aims to ensure a better graft monitoring and thus enable a patient-tailored diagnostic and therapy after transplantation (5). Trailin et al. were able to show that high levels of interleukin 2 in urine are associated with worsened eGFR (6). Kielar et al. showed that elevated neutrophil gelatinase-associated lipocalin (NGAL) levels in the urine were associated with an eGFR loss after transplantation (7). However, sufficient biomarkers to obtain robust and validated information about long-term allograft function and to identify patients at risk, are still lacking today.

Dickkopf 3 (DKK3) has been identified as a biomarker of kidney function in animal and clinical studies. These studies have been based on patients with acute kidney injury (AKI) or chronic kidney disease (CKD). In the context of AKI, DKK3 is currently seen as a prediction score for the development of a kidney failure (8).

DKK3, a profibrotic glycoprotein, belonging to the Dickkopf family consists of five proteins (DKK1-4, DKK like protein 1) that influence the Wnt signaling pathway through inhibition or activation. The Wnt signal pathway is an important signal transduction pathway in embryogenesis (9, 10). It is also relevant in tumor diseases (e.g., familial adenomatous polyposis) (11). In the context of kidney diseases, multiple functions are assigned, e.g., it is potentially associated with the development of ADPKD (10). DKK3 activates the canonical Wnt/b-catenin signaling pathway which induces gene expression (12).

Frederico et al. found that Dickkopf plays a role in embryonic development and was found in mesenchymal progenitor cells and mesenchymal cells. It is normally not detectable in adult cells (13). After kidney damage, DKK3 is expressed in the tubular epithelial cells and causes a profibrotic T-cell response (13). It can therefore be detected in the urine.

Studies on patients with CKD have shown that high Dickkopf values are associated with the increased incidence of tubulointerstitial fibrosis. Frederico et al. were able to show that DKK3 deficient mice showed less pronounced tubular atrophy and an improved kidney. This effect could also be demonstrated by an antibody-mediated blockade of DKK3 (13). Another study showed that higher Dickkopf values were associated with impaired kidney function and patients with high DKK3 values showed more tubulointerstitial fibrosis. The authors conclude that Dickkopf can be used as a biomarker for patients with a rapid eGFR loss over time, regardless of the underlying kidney disease (14).

Abbreviations: DKK3, Dickkopf 3; IF/TA, Interstitial fibrosis and tubular atrophy; eGFR, Estimated glomerular filtration rate; NGAL, Neutrophil gelatinase-associated lipocalin; AKI, Acute kidney injury; CKD, Chronic kidney disease; ADPKD, Autosomal dominant polycystic kidney disease; CDC-PRA, Complement dependent cytotoxicity- Panel reactive antibody; DSA, Donor specific antibody;rATG, Rabbit anti-thymocyte globulin; HRP, Horseradish peroxidase; TMB, Tetramethylbenzidine; IQR, Interquartile range; MLM, Mixed linear models; ECD, Extended donor criteria; Tregs, Regulatory T cells.

The role of Dickkopf 3 in the context of kidney transplantation is completely unclear. The aim of our study was to analyze DKK3 in the urine of transplanted patients, which represents the first investigation realized in such a cohort. For this purpose, a highly standardized cohort of kidney transplant recipients was investigated. In addition to kidney function, represented by creatinine, eGFR and albuminuria, both donor and recipient specific influencing factors (e.g., age, PRA level) were analyzed. The goal of our study was to determine if a correlation between the DKK3 values and graft function over the observation period of 3 years exists and if DKK3 could be further developed as a non-invasive marker to identify patients at high risk for a deterioration in transplant function.

MATERIALS AND METHODS

Patients' Baseline Characteristics

All patients being transplanted at our center between January 1, 2016 and December 31, 2018 were included ($n = 122$). All recipient-related data and transplantation-associated parameter were collected and archived as part of the "Regensburger Transplantationsnachsorge." This retrospective study was approved by the Ethical Committee of the University of Regensburg.

Baseline data of the recipients were recorded and the graft function represented by creatinine, eGFR (CKD-EPI) and urinary albumin/creatinine ratio up to 36 months after transplantation was analyzed. Each recipient was grouped according to its underlying immunological risk profile (CDC-PRA, DSA, etc.) before transplantation and thereafter treated by a pre-defined immunological algorithm (15). Induction therapy was done with a CD25 monoclonal antibody basiliximab (Novartis) in patients with low and medium risk and rATG (Sanofi) in high risk patients. Maintenance immunosuppression consisted of a calcineurin inhibitor (tacrolimus), a proliferation inhibitor (mycophenolic acid) and steroids (15). DKK3 was measured non-invasively in the urine 14 days, 3, 12, 18, 24, 30, and 36 months postTx. Since the majority of the patients were anuric at the time of transplantation, the determination of DKK3 on day 0 was dispensed.

DKK3 ELISA Analysis

Urinary midstream samples were collected from patients at the mentioned time points. The urine samples were stored at −80°C. DKK3 was measured with a commercially available ELISA according to the manufacturers' recommendations (DiaRen, Homburg, Germany). Urine samples were centrifuged at 370 g for 10 min. 100 μL of supernatant was mixed with 900 μL of sample buffer and 100 μL of the dilution was transferred to a microtiter plate coated with capture antibody and incubate for 30 min (23 ± 3°C). After repetitive washing (3x), the detection antibody was loaded with streptavidin-horseradish peroxidase (HRP) conjugate and rinsed again (3x). Substrate solution (100 μL of TMB/tetramethylbenzidine) was added and incubated for 30 min at room temperature. Finally, 100 μL of stop solution per well was added and the plate was immediately measured at 450 nm. For each microtiter plate, 6 standards were carried in

duplicate at DKK3 concentrations of 0, 30, 85, 245, 700, and 2,000 pg/mL. Concentration data in urine are not very meaningful because the results depend on the dilution state of the urine. Accordingly, urinary DKK3 levels were normalized to urinary creatinine concentrations to account for dilution of the urine (14). To exclude any bias, DKK3 in all samples was measured in a blinded manner.

Statistical Analysis

Descriptive analyses were done using absolute and percentual frequency (n, %), mean ± standard deviation, and median with corresponding interquartile range (IQR).

The course of DKK3, creatinine, eGFR, and albuminuria values from 14 days up to 3 years after transplantation were presented.

Mann- Whitney- *U*-tests were used to compare DKK3 crea ratio 3 months and 12 months postTx between patients treated with basiliximab or rATG. The time point 14d was excluded due to the presumably influence of reperfusion ischemia damage.

The associations between DKK3 crea ratio and graft function were assessed by three separate mixed linear models (MLM) including the measurement time points 12, 24, 30, and 36 months. It was examined whether the 3 or 12 month DKK3 value can predict graft function in the following course using six separate MLMs. The influence of changes in DKK3 values from month 3 to month 12 after transplantation on subsequent graft function 24, 30, and 36 month after transplantation was assessed by three separate MLMs. Changes in DKK3 were dichotomized in worse ($\geq$ 25% increase) and good ($<$ 25% increase). With these analyses, we investigated whether DKK3 represents an independent influencing factor on kidney function and if a change in DKK3 kinetics is relevant. 25% was chosen as cut-off, in accordance to the classification of an AKI, where a 25% deterioration in kidney function is classified as stage 1 (RIFLE criteria) (16). In the context of transplantation, a deterioration in creatinine of 0.3 mg/dl is considered relevant. This also corresponds to a loss of ∼25%.

The MLMs included factors that that are well-known to affect graft function, namely highest PRA level (17), cold and warm ischemia time (18, 19) and donor characteristics as age, hypertension, diabetes, and last creatinine (20, 21). MLM replaces missing values by using maximum likelihood estimates. All patients, even with missing graft function values at specific time points could be used for the analysis. Unstructured covariance type was used. As creatinine and albuminuria values were not normally distributed, values were logarithmised.

Statistical analyses were conducted with SPSS Statistics 26 (SPSS Inc, Chicago, Illinois). The level of significance was set at p two-sided $\leq$ 0.050. No adjustments for multiple testing were done.

RESULTS

Patients' Baseline Characteristics

A total of 122 patients were transplanted with 85 being men (70%) and 37 (30%) being women. Induction therapy with basiliximab was carried out in 82 patients (67%) and 39 patients received rATG (32%). One patient received no induction therapy (1%). The mean donor age was 56 years (IQR, 47–62). The cold ischemia time averaged 480 min (IQR, 166.3–679.3), the warm ischemia time was 42 min (IQR, 33–52). Donor-specific antibodies were detected in 11 patients prior to transplantation (9%). Out of the 122 transplants performed, 41 were from a living donation (33.6%) from which 17 were from blood relatives (41.5%) and 81 were from a cadaveric donation (66.4%). Forty-nine patients received an organ from a donor with extended donor criteria (ECD) (40.2%). Eight patients died during the follow-up. Further information are shown in **Table 1**.

TABLE 1 | Baseline characteristics of the study cohort.

	Study cohort (*n* = 122)
Donor- age (years)	53 ± 16
Donor- weight (kg)	78 ± 18.4
Donor- height (cm)	171.1 ± 13.2
Donor- sex (M:F)	57:65
Donor—hypertension (*n*/%)	42 (34.4)
Donor—diabetes (*n*/%)	10 (8.2)
Donor—last creatinine (median, IQR)	0.81 (0.68–1.08)
Recipient- weight (kg)	78.6 ± 14.1
Recipient- height (cm)	171.7 ± 9.2
Recipient- sex (M:F)	85:37
Re-Tx (*n*)	8 (7%)
Cause of end stage renal disease	
ADPKD	20 (16%)
IgA- Nephropathy	24 (20%)
Hypertensive nephropathy	23 (19%)
Diabetic nephropathy	11 (9%)
Other	44 (36%)
Mismatch	
HLA-A	0.81 ± 0.74
HLA-B	1.08 ± 0.75
HLA-DR	1.01 ± 0.7
PRA (%)- current	5.6 ± 21.2
PRA (%)- highest	13.5 ± 28.1
Ischemia time	
Cold ischemia time (min)	475.6 ± 297.6
Warm ischemia time (min)	44.6 ± 16.7
Rejection (*n*)	
TCMR	13 (11%)
AMR	5 (4%)
Borderline	4 (3%)
***De-novo* Donor specific antibodies**	
HLA class I (*n*/%)	6 (5%)
HLA class II (*n*/%)	10 (8%)
Graft loss (*n*/%)	6 (5%)
Death (*n*/%)	8 (7%)

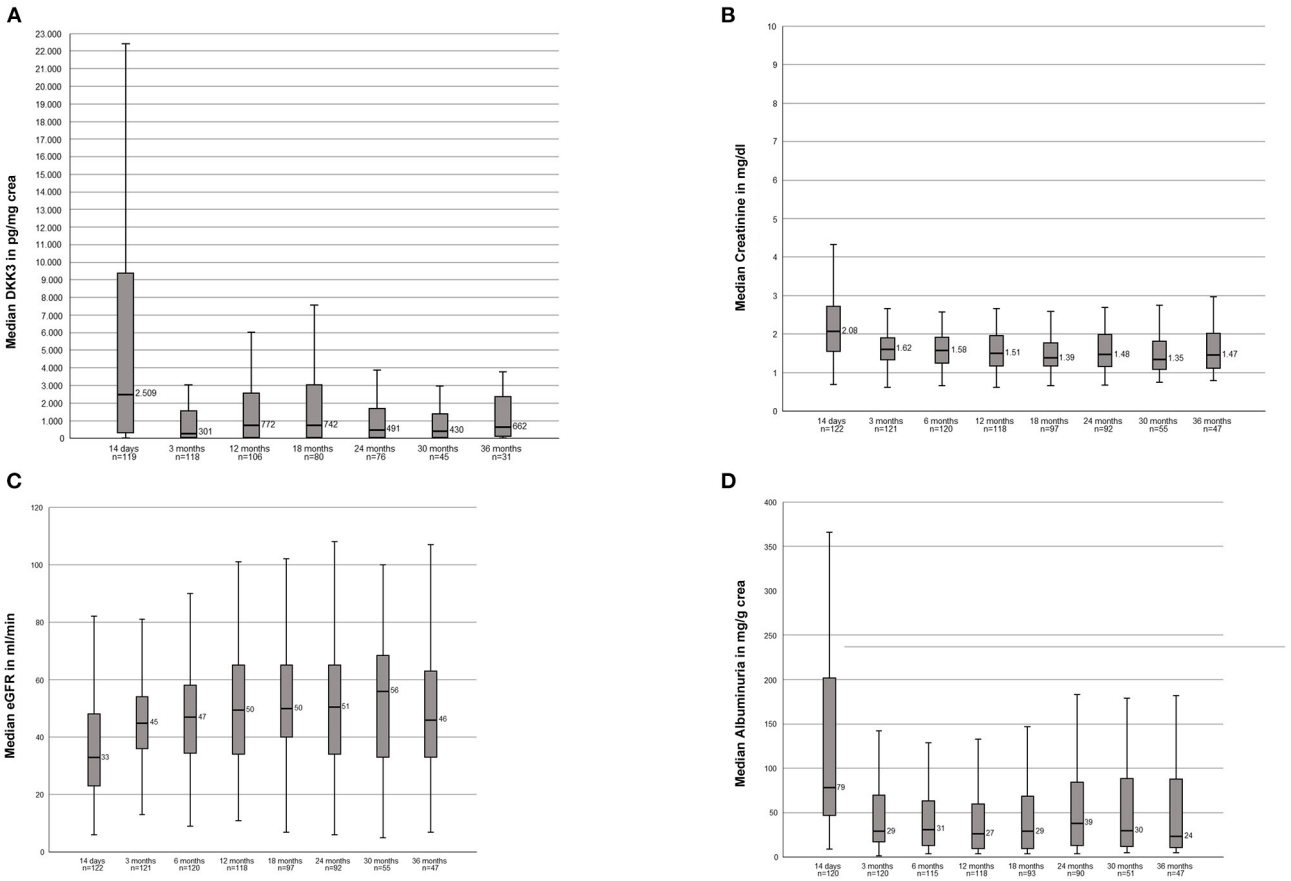


FIGURE 1 | (A) Course of the DKK3 values over the observation period of 3 years of the entire study population. **(B)** Course of the serum creatinine values over the observation period of 3 years of the entire study population. **(C)** Course of the eGFR over the observation period of 3 year of the entire study populations. **(D)** Course of the albuminuria over the observation period of 3 years of the entire study population.

Urinary DKK3 Crea Ratio and Resulting Allograft Function

DKK3 Crea Ratio

We analyzed the course of DKK3 over the observation period of all transplanted patients. The highest DKK3 value with a median of 2,509 pg/mg crea (IQR, 321-9636) were measured after 14 days. In the further course the following median values were measured: 3 months: 300.5 pg/mg crea (IQR, 33–1567); 12 months: 771.5 pg/mg crea (IQR, 45–2589); 18 months 742 pg/mg crea (IQR, 43–3059); 24 months: 491 pg/mg crea (IQR, 43–1693); 30 months: 430 pg/mg crea (IQR, 41–1521); and 36 months: 661 pg/mg crea (IQR, 83–2526) (**Figure 1A**).

Kidney Function Values of the Entire Study Population

Stable creatinine values over the entire 36 months were seen: 14d: 2.08 mg/dl (1.54–2.73); 3 months: 1.62 mg/dl; (1.34–1.92) 12 months: 1.51 mg/dl (1.18–1.96); 18 months: 1.39 mg/dl (1.17–1.82); 24 months: 1.48 mg/dl (1.16–2.01); 30 months: 1.35 mg/dl (1.05–1.84); 36 months: 1.47 mg/dl (1.11–2.03) (**Figure 1B**). In accordance resulting eGFR values were also stable within the observation period: 14d: 33 ml/min (22.8–48); 3 months: 45 ml/min (36–54.5); 12 months: 49.5 (34–63.5), 18 months: 50 ml/min (39.5–65); 24 months: 50.5 (34–65); 30 months: 56 ml/min (33–70); 36 months: 46 ml/min (32–64) (**Figure 1C**).

The following albuminuria values were measured: 14d: 78.50 mg/g crea (46.9–201.75); 3 months: 29.35 mg/g crea (16.95–70.40); 12 months: 26.75 mg/g crea (9.42–60.93); 18 months: 29.30 mg/g crea (9.63–69.85); 24 months: 38.50 mg/g crea (13–85.08); 30 months: 29.90 mg/g crea (11.2–90.60); 36 months: 23.80 mg/g crea (10.3–89.50). Two patients showed clear outliers at the time points 24 months (10,365 mg/g crea) and 30 months (11,079 mg/g crea). In both patients the albuminuria was associated with a subsequent graft loss (**Figure 1D**).

Impact of Chosen Induction Therapy on DKK3

Investigation whether the chosen induction therapy had an impact on the resulting DKK3 values showed no statistically significant differences. However, patients treated with basiliximab showed 3 and 12 month postTx continuously higher DKK3 values [3 months: median = 626 pg/mg crea (IQR,

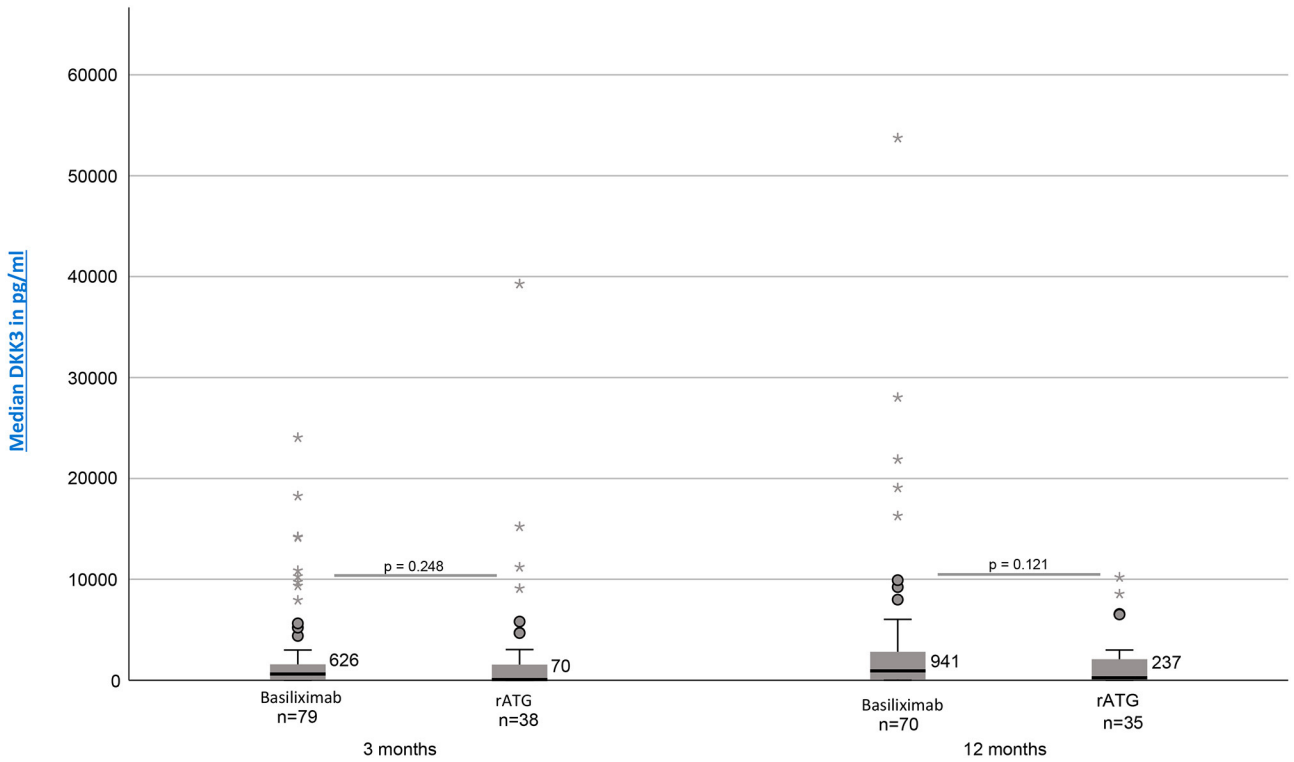


FIGURE 2 | DKK3 values at time point 3 and 12 months depending on induction therapy (Basiliximab vs. rATG).

38–1580, $n = 79$); 12 months: median = 941 pg/mg crea (IQR, 75–2837, $n = 70$)] than patients treated with rATG [3 months: median =70 pg/mg crea (IQR, 18–1761, $n = 38$); 12 months: median = 237 pg/mg crea (IQR, 32–2155, $n = 35$)] (3 months: $p = 0.248$; 12 months: $p = 0.121$) (**Figure 2**).

Impact of DKK3 on Simultaneously Measured Allograft Function

The mixed linear models showed that higher donor age ($p < 0.001$) and DKK3 expression level ($p = 0.011$) were significantly associated with impaired graft function. More precisely, if the donor age increased by 10 years, resulting creatinine increased by 0.11 mg/dl and if the DKK3 increased by 10.000, creatinine increased by 0.16 (95% CI 0.09–0.23). The analysis of the eGFR showed that donor age ($p < 0.001$) and last donor creatinine ($p = 0.03$) were the only influencing factor, whereas DKK3 did not reach the level of significance ($p = 0.13$). In the case of albuminuria, both donor age ($p < 0.001$), donor diabetes ($p = 0.03$) and the DKK3 value ($p < 0.001$) were statistically significant (Tables 1a–c of the **Supplement**).

Prediction of Subsequent Graft Function by DKK3

It was examined whether 3 or 12 month DKK3 values could predict subsequent allograft function. Higher DKK3 values 3 and 12 months after transplantation predicted higher subsequent creatinine values ($p < 0.050$) up to 36 months. Moreover, higher DKK3 values 3 and 12 months postTx predicted lower subsequent eGFR values ($p < 0.050$) in the same observation period. Higher DKK3 values 3 months after transplantation predicted higher albuminuria values 6 months ($p = 0.013$) and 12 months postTx ($p = 0.050$), but not on a later time point ($p > 0.050$). Higher DKK3 values 12 months after transplantation predicted higher subsequent albuminuria values up to month 36 ($p < 0.050$). Donor age was the only consistently significant parameter associated with graft function ($p < 0.050$), whereas the other analyzed parameters showed no consistent influence. More precisely, an increase in donor age by 10 years lead to a creatinine increase of 0.13 mg/dl, while an increase in DKK3 by 10.000 lead to a creatinine increase of 0.58 mg/dl (Tables 2a–f of the **Supplement**).

Impact of DKK3 Kinetics for Allograft Function

Comparing patients with a DKK3 increase $\geq$ 25% from time 3 to 12 months and patients with a decrease or an increase of <25% in the same period, patients with an increase of $\geq$ 25% in DKK3 values showed higher creatinine values ($p = 0.038$), a lower eGFR ($p = 0.018$) and higher albuminuria values ($p = 0.005$) for subsequent time points. These associations could be confirmed for graft function 30, and 36 months postTx ($p < 0.050$), except for albuminuria values 36 months after transplantation ($p = 0.092$) (**Tables 2A–C**). Roughly shown, less intense DKK3 increase between 3 and 12 months resulted in an

TABLE 2 | (A) Medium creatinine values depending on the DKK3 change of month 3–12 (≥ 25 vs. < 25%).

Time	DKK3 change	Mean value	Confidence interval 95%	
			Upper limit	Lower limit
(A)				
24 months	<25%	1.56	1.22	1.89
	≥ 25%	1.98	1.67	2.29
30 months	<25%	1.44	1.00	1.88
	≥ 25%	2.11	1.70	2.52
36 months	<25%	1.42	1.02	1.82
	≥ 25%	2.03	1.66	2.40
(B)				
24 months	<25%	54.51	48.86	60.16
	≥ 25%	47.49	42.25	52.74
30 months	<25%	55.13	49.38	60.88
	≥ 25%	47.01	41.74	52.27
36 months	<25%	57.13	51.16	63.10
	≥ 25%	48.22	42.70	53.75
(C)				
24 months	<25%	64.25	−308.44	436.94
	≥ 25%	393.57	47.44	739.71
30 months	<25%	46.35	−353.97	446.66
	≥ 25%	403.34	31.74	774.94
36 months	<25%	44.45	−92.21	181.11
	≥ 25%	213.80	86.92	340.68

(B) *Medium eGFR depending on the DKK3 change of month 3–12 (≥ 25 vs. < 25%).* ***(C)*** *Medium albuminuria depending on the DKK3 change of month 3–12 (≥ 25 vs. < 25%).*

eGFR differences of about 9–10 ml/min and in a 7–12 times lower albuminuria over the observation period (24 till 36 months) in contrast to patients with a DKK3 increase ≥ 25%.

DISCUSSION

In our study, we examined the influence of Dickkopf 3 on graft function in kidney transplant recipients. We were able to show that DKK3 correlates with resulting graft function, represented by creatinine, eGFR and albuminuria, over an observation period of 36 months. DKK3 can even predict kidney function as illustrated by the association of 3 and 12 months DKK3 values and subsequent allograft function. Furthermore, changes in DKK3 values from month 3 to 12 (≥25%) were associated with a significantly deteriorated graft function, being illustrated by tremendous differences in creatinine, eGFR, and albuminuria values. Our study is the first investigation of DKK3 referring to transplantation medicine.

Regarding the function of DKK3, studies have shown that DKK3 is secreted only by stressed tubular epithelial cells in the adult kidney. Using two animal models, an adenine-induced nephropathy and a model of an unilateral ureter obstruction, Gröne et al. showed by usage of a DKK3 knockout that DKK3 deficiency leads to a marked reduction in tubular damage and renal fibrosis. DKK3 deficiency triggered an antifibrotic T cell response and reduced activity of the WNT–β-catenin signaling pathway. These results could also be reproduced by an antibody-mediated blockade of DKK3. DKK3 therefore appears to be an important mediator of renal fibrosis and thus of deterioration in renal function (9).

Schunk et al. were able to show that patients after cardiac surgery and increased DKK3 scores (>471 pg/ml) had an increased risk for developing AKI (22). A comparable observation could be reproduced in our analysis. However, our data are more closely linked with chronic changes. Patients with a DKK3 dynamic of more than 25% showed a deteriorated graft function and also more albuminuria than patients with a smaller change in DKK3. Especially the changes in albuminuria, being 7–12 times higher in patients with intensified DKK3 increase, do not only link DKK3 expression levels with allograft function, but also with arising structural damage.

Zewinger et al. were able to show that high levels of DKK3 are associated with impaired function in patients with CKD. DKK3 could be seen as a predictor for an eGFR loss independent of the underlying disease. This study showed that high DKK3 values can function as a prognostic parameter regardless of the accompanying albuminuria (14). Similar results were showen by Sanchez-Alamo. By determining DKK3 in the urine, patients with a high risk of deterioration in kidney function could be identified, regardless of the underlying disease (23). A correlation between DKK3 and creatinine as well as the eGFR was also found in our work. In contrast to Zewinger, however, a significant influence of DKK3 could also be found for albuminuria.

We were able to see a trend toward lower DKK3 values after T-cell depleting induction therapy in comparison to an immunomodulatory therapy with basiliximab. Regarding the impact of immunosuppressives on the development of DKK3, no further data are available. But in literature, the influence of DKK3 on T- lymphocytes is discussed. As already mentioned, DKK3 seems to trigger a profibrotic T cell response. Federico et al. were also able to show that after an antibody-mediated blockade of DKK3, an increased presence of protective T cells (IFNγ-producing Th1 and Tregs) can be demonstrated (13). Taking this into account, the evidence of lower DKK3 values under a T-cell depleting therapy seems understandable. Further investigations on the influence of immunosuppressives would be useful to further evaluate specific "anti-DKK3 and therefore presumable anti-fibrotic immunosuppressive protocols."

In our cohort, we were able to recognize a total of 18 rejections over the entire observation period, both T cell-mediated and antibody-mediated rejections. Most of them occurred within the first 14 days. The analysis of the DKK3 values between patients with a rejection compared to patients without a rejection showed no statistically significant difference. However, it should be noted here that there is a relevant difference in the number of cases in the two groups as a possible confounding factor.

Our study is the first analyzing Dickkopf 3 after kidney transplantation. Nevertheless, it is a monocentric study with a limited case number. There are currently no special biomarkers postTx available to estimate the individual risk for a deterioration in graft function. DKK3 can be easily integrated into everyday clinical practice thanks to its detection in urine. Similar to

Zewinger in his study, we were also able to see a clear influence of DKK3 on graft function (14). The use of DKK3 as a predictor of graft function should therefore be considered and proofed in a multi-center clinical trial. Animal studies showed that an anti-DKK3 antibody could inhibit the development of fibrosis in mice. DKK3 thus also represents a possible therapeutic target. It should be noted critically that defined cut-off values for DKK3, from which a clinical consequence must result, are still missing. Looking at our study, the determination of DKK3 at time points 3 and 12 months after transplantation could be a helpful new screening parameter in the follow-up. Nevertheless, long-term analyzes and prospective multicenter studies would be necessary in order to address the still open questions and to deepen our findings made in a single-center study.

SIGNIFICANT STATEMENT

Whereas, in the context of chronic kidney disease, Dickkopf 3 has been recognized as a marker to identify patients at risk for a progressive loss of kidney function, to date, the impact of DKK3 after transplantation has not yet been analyzed.

In our study on kidney transplant recipients, DKK3 not only could precisely predict subsequent allograft function but an increase in DKK3 values within the first year after transplantation was associated with a deterioration in allograft function.

With the presented data, DKK3 can be considered as a new indicator of impaired graft function after transplantation. However, further prospective and interventional studies are needed to verify our findings.

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/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Committee of the University of Regensburg. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AS, BB, and TB: concept/design. AS, PF, and TB: data collection. AS, KM, FZ, and TB: statistics. AS, LS, and TB: data analysis/interpretation. AS, LS, BB, and TB: drafting article. AS, LS, KM, FZ, PF, BB, and TB: critical revision of article and approval of article. All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), project number 387509280, SFB 1350 Project B6 to TB and BB.

ACKNOWLEDGMENTS

We would like to thank Mrs. Stefanie Ellmann and Mrs. Alexandra Müller for their excellent technical assistance and Mrs. Lydia Walkowski for database management.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.885018/full#supplementary-material

REFERENCES

1. Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant.* (2009) 9(Suppl. 3):S1–155. doi: 10.1111/j.1600-6143.2009.02834.x
2. Park J-S, Oh IH, Lee CH, Kim G-H, Kang CM. The rate of decline of glomerular filtration rate is a predictor of long-term graft outcome after kidney transplantation. *Transplant Proc.* (2013) 45:1438–41. doi: 10.1016/j.transproceed.2012.10.052
3. Mannon RB, Matas AJ, Grande J, Leduc R, Connett J, Kasiske B, et al. Inflammation in areas of tubular atrophy in kidney allograft biopsies: a potent predictor of allograft failure. *Am J Transplant.* (2010) 10:2066–73. doi: 10.1111/j.1600-6143.2010.03240.x
4. Li X, Zhuang S. Recent advances in renal interstitial fibrosis and tubular atrophy after kidney transplantation. *Fibrogenesis Tissue Repair.* (2014) 7:15. doi: 10.1186/1755-1536-7-15
5. Loupy A, Aubert O, Orandi BJ, Naesens M, Bouatou Y, Raynaud M, et al. Prediction system for risk of allograft loss in patients receiving kidney transplants: international derivation and validation study. *BMJ.* (2019) 366:l4923. doi: 10.1136/bmj.l4923
6. Trailin AV, Pleten MV, Ostapenko TI, Iefimenko NF, Nykonenko OS. High urinary interleukin-2 in late post-transplant period portends a risk of decline in kidney allograft function: a preliminary study. *BMC Res Notes.* (2017) 10:605. doi: 10.1186/s13104-017-2936-7
7. Kielar M, Dumnicka P, Gala-Błądzińska A, Bedkowska-Prokop A, Ignacak E, Maziarz B, et al. Urinary NGAL measured after the first year post kidney transplantation predicts changes in glomerular filtration over one-year follow-up. *J Clin Med.* (2020) 10:43. doi: 10.3390/jcm10010043
8. Ostermann M, Zarbock A, Goldstein S, Kashani K, Macedo E, Murugan R, et al. Recommendations on acute kidney injury biomarkers from the acute disease quality initiative consensus conference: a consensus statement. *JAMA Netw Open.* (2020) 3:e2019209. doi: 10.1001/jamanetworkopen.2020.19209
9. Gröne EF, Federico G, Nelson PJ, Arnold B, Gröne H-J. The hormetic functions of Wnt pathways in tubular injury. *Pflugers Arch.* (2017) 469:899–906. doi: 10.1007/s00424-017-2018-7
10. Wang Y, Zhou CJ, Liu Y. Wnt signaling in kidney development and disease. *Prog Mol Biol Transl Sci.* (2018) 153:181–207. doi: 10.1016/bs.pmbts.2017.11.019
11. Caldwell GM, Jones CE, Ashley AM, Wei W, Hejmadi RK, Morton DG, et al. Wnt signalling in adenomas of familial adenomatous polyposis patients. *Br J Cancer.* (2010) 103:910–7. doi: 10.1038/sj.bjc.6605790

12. Schunk SJ, Speer T, Petrakis I, Fliser D. Dickkopf 3-a novel biomarker of the 'kidney injury continuum'. *Nephrol Dial Transplant*. (2020) 36:761–7. doi: 10.1093/ndt/gfaa003
13. Federico G, Meister M, Mathow D, Heine GH, Moldenhauer G, Popovic ZV, et al. Tubular Dickkopf-3 promotes the development of renal atrophy and fibrosis. *JCI Insight*. (2016) 1:e84916. doi: 10.1172/jci.insight.84916
14. Zewinger S, Rauen T, Rudnicki M, Federico G, Wagner M, Triem S, et al. Dickkopf-3 (DKK3) in urine identifies patients with short-term risk of eGFR loss. *J Am Soc Nephrol*. (2018) 29:2722–33. doi: 10.1681/ASN.2018040405
15. Schuster AM, Miesgang N, Steines L, Bach C, Banas B, Bergler T. B-cell activating factor BAFF as a novel alert marker for the immunological risk stratification after kidney transplantation. *Immunol Res*. (2021) 69:487–95. doi: 10.1007/s12026-021-09205-4
16. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the second international consensus conference of the Acute Dialysis Quality Initiative (ADQI) group. *Crit Care*. (2004) 8:R204-12. doi: 10.1186/cc2872
17. Meng HL, Jin XB, Li XT, Wang HW, Lü JJ. Impact of human leukocyte antigen matching and recipients' panel reactive antibodies on two-year outcome in presensitized renal allograft recipients. *Chin Med J*. (2009) 122:420–6. doi: 10.3760/cma.j.issn.0366-6999.2009.04.0012
18. Tennankore KK, Kim SJ, Alwayn IPJ, Kiberd BA. Prolonged warm ischemia time is associated with graft failure and mortality after kidney transplantation. *Kidney Int*. (2016) 89:648–58. doi: 10.1016/j.kint.2015.09.002
19. Quiroga I, McShane P, Koo DDH, Gray D, Friend PJ, Fuggle S, et al. Major effects of delayed graft function and cold ischaemia time on renal allograft survival. *Nephrol Dial Transplant*. (2006) 21:1689–96. doi: 10.1093/ndt/gfl042
20. Keith DS, Dematteos A, Golconda M, Prather J, Norman D. Effect of donor recipient age match on survival after first deceased donor renal transplantation. *J Am Soc Nephrol*. (2004) 15:1086–91. doi: 10.1097/01.ASN.0000119572.02053.F2
21. Oppenheimer F, Aljama P, Asensio Peinado C, Bustamante Bustamante J, Crespo Albiach JF, Guirado Perich L. The impact of donor age on the results of renal transplantation. *Nephrol Dial Transplant*. (2004) 19(Suppl. 3):iii11–5. doi: 10.1093/ndt/gfh1008
22. Schunk SJ, Zarbock A, Meersch M, Küllmar M, Kellum JA, Schmit D, et al. Association between urinary dickkopf-3, acute kidney injury, and subsequent loss of kidney function in patients undergoing cardiac surgery: an observational cohort study. *Lancet*. (2019) 394:488–96. doi: 10.1016/S0140-6736(19)30769-X
23. Sánchez-Álamo B, García-Iñigo FJ, Shabaka A, Acedo JM, Cases-Corona C, Domínguez-Torres P, et al. Urinary Dickkopf-3: a new biomarker for CKD progression and mortality. *Nephrol Dial Transplant*. (2021) 36:2199–207. doi: 10.1093/ndt/gfab198

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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 12 May 2022
doi: 10.3389/fmed.2022.889648

Poor Outcomes in Patients With Transplant Glomerulopathy Independent of Banff Categorization or Therapeutic Interventions

Kaiyin Wu[1], Danilo Schmidt[1], Covadonga López del Moral[1], Bilgin Osmanodja[1], Nils Lachmann[2], Fabian Halleck[1], Mira Choi[1], Friederike Bachmann[1], Simon Ronicke[1], Wiebke Duettmann[1], Marcel Naik[1], Eva Schrezenmeier[1], Birgit Rudolph[3] and Klemens Budde[1]*

[1] Department of Nephrology and Intensive Care, Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Berlin Institute of Health (BIH), Humboldt-Universität zu Berlin, Berlin, Germany, [2] HLA Laboratory, Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, BIH, Berlin, Germany, [3] Institute of Pathology, Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Berlin Institute of Health (BIH), Humboldt-Universitätzu Berlin, Berlin, Germany

OPEN ACCESS

Edited by:
Ondrej Viklicky, Institute for Clinical and Experimental Medicine (IKEM), Czechia

Reviewed by:
Anna Buxeda, Parc de Salut Mar, Spain
Marion Rabant, Hôpital Necker-Enfants Malades, France

***Correspondence:**
Kaiyin Wu
kaiyin.wu@charite.de

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *04 March 2022*
Accepted: *14 April 2022*
Published: *12 May 2022*

Citation:
Wu K, Schmidt D, López del Moral C, Osmanodja B, Lachmann N, Halleck F, Choi M, Bachmann F, Ronicke S, Duettmann W, Naik M, Schrezenmeier E, Rudolph B and Budde K (2022) Poor Outcomes in Patients With Transplant Glomerulopathy Independent of Banff Categorization or Therapeutic Interventions. Front. Med. 9:889648. doi: 10.3389/fmed.2022.889648

Background: Transplant glomerulopathy (TG) may indicate different disease entities including chronic AMR (antibody-mediated rejection). However, AMR criteria have been frequently changed, and long-term outcomes of allografts with AMR and TG according to Banff 2017 have rarely been investigated.

Methods: 282 kidney allograft recipients with biopsy-proven TG were retrospectively investigated and diagnosed according to Banff'17 criteria: chronic AMR (cAMR, $n = 72$), chronic active AMR (cAAMR, $n = 76$) and isolated TG (iTG, $n = 134$). Of which 25/72 (34.7%) patients of cAMR group and 46/76 (60.5%) of cAAMR group were treated with antihumoral therapy (AHT).

Results: Up to 5 years after indication biopsy, no statistically significant differences were detected among iTG, cAMR and cAAMR groups in annual eGFR decline (−3.0 vs. −2.0 vs. −2.8 ml/min/1.73 m^2 per year), 5-year median eGFR (21.5 vs. 16.0 vs. 20.0 ml/min/1.73 m^2), 5-year graft survival rates (34.1 vs. 40.6 vs. 31.8%) as well as urinary protein excretion during follow-up. In addition, cAMR and cAAMR patients treated with AHT had similar graft and patient survival rates in comparison with those free of AHT, and similar comparing with iTG group. The TG scores were not associated with 5-year postbiopsy graft failure; whereas the patients with higher scores of chronic allograft scarring (by mm-, ci- and ct-lesions) had significantly lower graft survival rates than those with mild scores. The logistic-regression analysis demonstrated that Banff mm-, ah-, t-, ci-, ct-lesions and the eGFR level at biopsy were associated with 5-year graft failure.

Conclusions: The occurrence of TG is closely associated with graft failure independent of disease categories and TG score, and the long-term clinical outcomes were not influenced by AHT. The Banff lesions indicating progressive scarring might be better suited to predict an unfavorable outcome.

Keywords: kidney transplantation, transplant glomerulopathy, chronic antibody-mediated rejection, antihumoral therapy, graft survival

INTRODUCTION

In the last decades it has been recognized, that antibody-mediated rejection (AMR) is an important cause for late allograft failure >1 year after transplantation (1). In our single center AMR was responsible for approximately 1/3 of death-censored allograft losses (2); in a multicenter cohort study AMR caused late allograft dysfunction in up to 60% of renal transplant recipients (3). In clinical reality AMR is frequently a chronic progressive disease process, which starts with the formation of donor specific anti-HLA antibodies (DSA) (4). Next, DSA lead to active AMR in presence of C4d deposition or at least moderate microcirculation inflammation (MVI) (5); over time, TG (defined as Banff cg-lesion) characterized with duplication of the glomerular basement membrane becomes more and more evident, and eventually, results in increasing proteinuria, progressive dysfunction and late allograft loss (6–8). The Banff 2005 report (9) defined chronic active AMR (cAAMR) with three salient features: (i) histological evidence of chronic graft injury (in most cases presence of TG), (ii) the immunohistological evidence of antibody-endothelial interactions by capillary C4d deposition; and (iii) the serological evidence of DSA. Later, a C4d-negative cAAMR was recognized in Banff 2013 report (10), and peritubular capillary C4d deposits could be replaced by at least moderate microcirculation inflammation (MVI). However, it is not uncommon for the three diagnostic features of cAAMR to appear as an incomplete combination, and different features of disease activity in the biopsy may be more reflective of the variable phenotypes of AMR (11). As a consequence, the Banff 2017 report (12) permits the diagnosis of chronic AMR (cAMR) with TG and current or recent DSA in absence of the capillary C4d deposits or at least moderate MVI.

Until recently, some clinical studies have reported that active AMR can be reversed to some degree by a combination of different antihumoral therapies (AHT) including antibody-depletion with plasmapheresis (PPh) or immunoadsorption (IA), immunomodulation with intravenous immunoglobulins (IVIG) with the aid of T- or B-cell-depleting agents (13, 14). The development of strategies to reverse or at least to halt cAAMR remains an unmet medical need, there is no accepted treatment for cAAMR (15, 16). Although TG is the diagnostic hallmark of cAAMR in late stage of transplantation (17), the data on its prognosis and treatment are still limited (18). Moreover, some researches suggest that the presence of TG is relevant to a reduced response to alloantibody removal therapy leading to inevitable late graft failure (19, 20).

According to the most recent Banff criteria (12), cases with TG can be classified into three categories: iTG, cAMR and cAAMR. However, there is no convincing data about the relative impact of these three TG categories on long-term allograft outcomes, partly due to frequent changes in the Banff criteria for AMR since 2001 (21). Thus, more data on cAMR and cAAMR according to the most recent Banff 2017 classification are needed and whether the grading of cg-lesion has any prognostic relevance, which would ease the design of adequate clinical trials to develop effective therapies for the late onset AMR. In addition, only limited data on isolated TG (without the presence for other diseases in the absence of DSA) exist. Therefore, we conducted a single-center retrospective study to investigate the clinical outcomes of allografts with TG with or without AMR according to Banff 2017 criteria and evaluated the prognostic relevance of TG categories and the utility of AHT.

MATERIALS AND METHODS

Study Population and Data Collection

We reviewed all adult patients (≥18 years) who received a single kidney transplantation at the transplant centre of Charité Campus Mitte and Charité Virchow Klinikum. Between Jan, 2000 and Dec, 2019, TG according to Banff 2017 (12) was found in 665 out of 7146 indication biopsies from 494 kidney allograft recipients. 146 patients were excluded because of missing HLA examinations at time of biopsy, 44 patients had incomplete data or were lost to follow-up shortly after biopsy, 21 patients had recurrent or de novo glomerulonephritis; finally, 282 patients with biopsy-proven TG were identified and included into this retrospective study (**Figure 1**).

All enrolled patients with TG visited routinely the transplantation clinic for follow-up care. The demographic, transplantation characteristics, immunosuppression, and treatment were registered at each outpatient clinic visit in the database (22) and the measurements of eGFR and proteinuria were taken 6 months before and at studied biopsy as well as every 3 months after diagnosis. Database was almost complete with <10% missing values in different data fields. In case of missing values at a certain time point, the next available value was entered. If there were several measurements in one time interval, the measurements at- or nearest to the planned follow-up were entered for analysis. In addition, measurements taken during hospitalization were omitted from analysis to minimize bias due to intercurrent illness and treatment, for example, infection and the admission of intravenous fluids etc.

In order to observe the effects of AHT on the graft outcomes, taking into consideration that in the present study all patients displayed TG, a minimum sample size of 25 patients per group was necessary to detect an eGFR decline difference of 10 ± 10 mL/min/1.73 m^2 per year between the AHT and free of AHT group (23).

All clinical and laboratory data were selected in the transplant database system (22) and assessed for completeness by a single investigator (S.D). The clinical information was collected from the patients' charts in accordance with the institutional review boards.

Biopsy and Histopathology

A indication biopsy was performed when the serum creatinine (Scr) rose above 25% from the baseline and/or proteinuria (PU) increased significantly. The biopsy specimens were processed with standard techniques in the institute of pathology, Charité Campus Mitte. All histological slides of recruited biopsies were selected from the archive, reevaluated by two nephropathologists (B.R and K.W) based on the updated Banff classification 2017 (12). TG was distinguished from

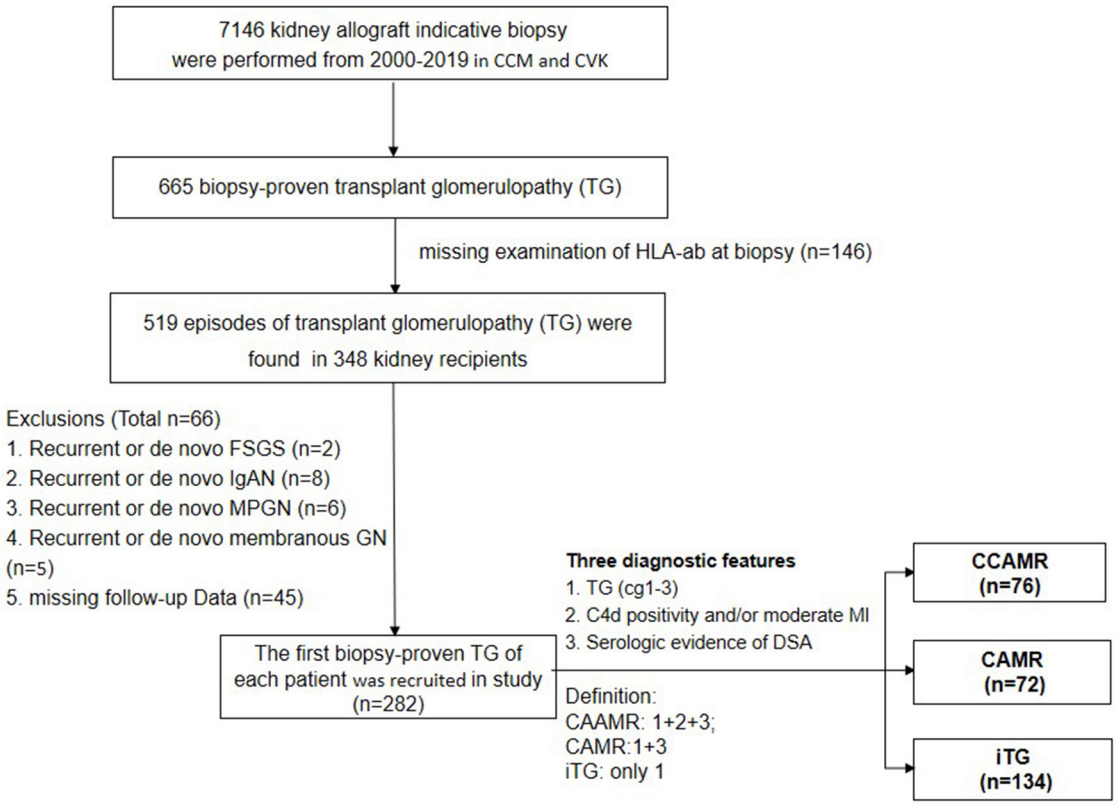


FIGURE 1 | Flow chart of patients enrolled in this study. CCM, Charite Campus Mitte; CVK, Charite Virchow Klinikum. FSGS, focal segmental glomerulosclerosis; IgAN, IgA nephropathy; PGN, membranous proliferative glomerulonephritis; membranos GN, membranous glomerulopathy; iTG, solated transplant glomerulopathy; cAMR, chronic antibody-mediated rejection; cAAMR, chronic active antibody-mediated rejection.

recurrent or de novo immune complex glomerulopathy by the immunofluorescent and electron microscopy, in particular from membranous/membranoproliferative glomerulonephritis; hepatitis C associated glomerulonephritis and lupus nephritis (24). In addition, TG was separated from thrombotic microangiopathy (TMA) by histological evaluation and review of clinical data (25). TMA was diagnosed based on the presence of typical clinical signs such as coombs negative haemolytic anemia together with thrombocytopenia and one or more of the following histologic conditions (26): fibrin thrombi in glomeruli and/or small arteries and arterioles; endothelial swelling with luminal compromise of the glomerular capillaries; mucoid concentric subintimal thickening of small arteries/arterioles with fragmented and/or hemolyzed erythrocytes; intracapillary or arteriolar thrombosis; vascular fibrinoid necrosis. C4d deposition is detected by indirect immunofluorescence on paraffin sections of formalin-fixed tissue (polyclonal anti-C4d antibody, Dianovo, Germany); more than 1% peritubular capillaries with linear deposition of C4d are considered as positive reaction. The categorization of TG is decided according to Banff report 2017 (12): cAAMR is diagnosed by coexistence of TG, DSA, C4d deposits and /or at least moderate (g+ptc $\geq$2) MVI; cAMR is considered when both TG and DSA are presented without clues of C4d deposits or at least moderate (g+ptc $\geq$2) MVI; the cases with TG but in absence of DSA and C4d positivity or with maximal mild MVI (g+ptc $<$2) are defined as isolated TG. In addition, the isometric vacuolization of proximal tubular epithelium with hyaline vasculopathy, striped pattern interstitial fibrosis and proportional tubular atrophy are considered as calcineurin-inhibitor (CNI) Nephrotoxicity. All Banff lesions are graded on a scale of 0-3 according to the proportion of cortical area affected, with higher scores indicating more severe abnormalities.

HLA-Antibody Screening

All patients were transplanted with a compliment dependent cytotoxicity (CDC)-negative cross-match. The serum samples at the time of biopsy were evaluated and tested for the presence of donor-specific antibodies against HLA (DSA). If DSA were found to be present, it was determined whether they constituted de novo DSA.

Patient serum samples were collected post biopsy and qualitatively screened for the presence of donor-specific antibodies against HLA (DSA) by two ELISA based screening systems (PRA-STAT and LAT) from 2000 to 2006 and the Luminex assay (27) from 2007 on (Immunocor Transplant

Diagnostics Inc., Stamford, CT, USA). Samples that were considered positive for HLA-ab specificities were further analyzed with a Luminex Single Antigen assay (One Lambda, Canoga Park, CA, USA). As an indicator for the antibody level, the maximal fluorescent intensity (MFI) of the immunodominant donor-specific antibody was used. HLA-Ab were considered positive when exceeding a plausible MFI value >500 (28). The values of MFI of immunodominant donor-specific HLA antibodies against class I (panel A) or class II (panel B) antigens were examined at biopsy and during the first year after studied biopsy. All tests were performed according to the manufacturer's guidelines and the DSA level was monitored in regular intervals as previously described (29).

Immunosuppression and Therapeutic Strategies

The maintennance immunosupression is shown in **Table 1**. The doses of cyclosporine A (CyA) and tacrolimus (Tac) were adjusted according to whole blood trough levels. 15/72 (20.8%) patients in cAMR group and 16/76 (21.1%) patients in cAAMR group were treated with six sessions PPh (30) followed by intravenous immunoglobulins (IVIG) at 1.5–2.0 g/kg. 6/72 (8.3%) patients in cAMR group and 11/76 (14.5%) patients in cAAMR group received a single dose of rituximab (375 mg/m^2 body surface area) 1 week after the last IVIG infusion; 4/72 (5.6%) patients in cAMR group and 7/76 (9.2%) patients in cAAMR received bortezomib at 1.3 mg/m^2 administered intravenously twice weekly on days 1, 4, 8 and 11 after the first IVIG infusion (31). In addition, 6/76 (7.9%) patients in cAAMR group were given 500 mg cyclophosphamide intravenously for 3 rounds after the last IVIG infusion (32) and for 2/76 (2.6%) patients with refractory cAAMR, eculizumab was used as a salvage treatment, a 900-mg dose was repeated weekly until the DSA MFI decreased to 5000. The cases showing concomitant TCMR were given 500 mg methylprednisolone for 3 days and thereafter tapered to maintenance dose at 4 mg/d. After intervention the patients received trimethoprim-sulfamethoxazole as prophylaxis for pneumocystis jirovecii for 6 months. When severe CNI nephrotoxicity (scores of ah, ci and ct-lesion ≥2) was observed, a change in immunosuppression was performed with minimization the doses of CyA/Tac or switch from CNI to a CNI-free immunosuppressive regimen with mTor Inhibitors or belatacept (33).

In addition, patients with hypertension received at least one antihypertensive drug and patients with daily urinary protein excretion (e.g., >1 g/L) were treated with the maximum tolerable dose of an angiotensin converting enzyme inhibitors (ACEi) and/or angiotensin receptor blockers (ARB) with the aid of AHT in patients of cAMR and cAAMR group.

Clinical Outcomes

All patients were followed up until the end of our study on 31.12.2020 or irreversible return to the chronic dialysis or retransplantation. Change in renal allograft function in time was evaluated by estimated glomerular filtration rate (eGFR ml/min/1.73 m^2) and urinary protein excretion. The eGFR value was calculated using formula of the Modification of Diet in Renal Disease (MDRD) (34). The influence of TG categories and AHT on eGFR slope was evaluated using linear mixed models with eGFR levels from 0, 12, 24, 36, 48, and 60 months postbiopsy as dependent variables, the interaction of TG categories or AHT and time as fixed effects. The covariance structure was specified as an autoregressive model of the first order. In model A patients experiencing graft loss or after death, the value of eGFR was not imputed. For an additional sensitivity analyses (model B), eGFR after graft loss or death was set to 5 ml/min/1.73 m^2. The effect of TG categories and AHT on long-term outcome was analyzed for patient and graft survival over a 5-year period after indication biopsy.

Statistical Analysis

Continuous data were expressed as median (IQR) and categorical variables were expressed as N and percentage of total. Mann-Whitney U test was used for comparison of continuous variables and chi-square for categorical data. The calculation of patient- and graft survival was analyzed by Kaplan-Meier curves and log-rank test. For univariate analysis of the histological factors influencing the 5-year death-censored graft failure we performed a Kaplan-Meier analysis for each histological Banff lesion comparing mild (score 0-1) and severe (score 2-3) lesion scores. The Log Rank test was used for statistical comparison between cases with mild and severe grade of each Banff lesion, and the Banff lesions with p-values < 0.05 were selected for further multivariable analysis. For multivariable modeling, a binary-logistic regression analysis was employed to examine the effects of three selected clinical factors (receiving antihumoral therapy, eGFR and proteinuria at biopsy) on overall graft survival, patient survival and death-censored graft survival. Adjusted estimates from multivariable models are presented as odds ratios (OR) with 95% confidence intervals (CI). All statistics were performed by using SPSS16.0 (SPSS Inc., Chicago, IL), P-value < 0.05 was considered as significant.

RESULTS

Clinical Characteristics at Studied Biopsy

In total, 282 patients with first episode of biopsy-proven TG and complete follow-up were enrolled in this study and were reclassified into cAMR ($n = 72$), cAAMR ($n = 76$) and iTG ($n = 134$) groups. Moreover, 25/72 (34.7%) patients in cAMR group and 46/76 (60.5%) patients in cAAMR group were treated with AHT primarily consisting of high-dose IVIG and PPh (**Table 1**). The basic demographics (including age, sex, body mass index) as well as transplant characteristics (including the presence of DGF, HLA-mismatches, PRA max before and at transplantation, type of donation) are summarized in **Table 1**. Baseline characteristics did not differ significantly among three groups with exception of significantly more male recipients and living donors in cAAMR group in comparison with iTG and cAMR group as well as the evidently higher fraction of living donation in cAAMR group vs. iTG group.

TABLE 1 | Demographics and clinical characteristics.

	iTG ($n = 134$)	cAMR ($n = 72$)	cAAMR ($n = 76$)	Overall ($n = 282$)	*P*-value
Demographics					
Recipient age (years, median IQR)	40.1 (18–68)	40.5 (18–70)	41.5 (18–78)	40.5 (18–78)	0.91
Recipient gender (m/f)	74/60	35/37	52/24*,#	161/121	**0.04**
Recipients BMI (kg/m^2 median, IQR)	25.7 (17.9–36.7)	24.8 (18.3–35.5)	22.8 (19.7–34.4)	24.3 (17.9-36.7)	0.40
First kidney transplant N (%)	113 (84.4%)	57 (79.2%)	64 (84.2%)	234 (83.0%)	0.53
PRA at Tx >10% N (%)	16 (11.9%)	18 (18.1%)	8 (10.5%)	42 (14.9%)	0.34
PRA max before Tx >30% N (%)	23 (17.0%)	18 (18.1%)	10 (13.2%)	52 (18.1%)	0.15
Board HLA-mismatches (N, median IQR)	3.0 (0–6)	2.9 (0–6)	3.2 (0–6)	3.1 (0–6)	0.11
CIT (hours median IQR)	12.1 (0.5–30.5)	5.8 (0.5–28.0)	6.6 (1.0–22.0)	10.0 (0.5–30.5)	0.27
Presence of DGF N (%)	41 (41.4%)	28 (44.4%)	23 (33.3%)	92 (39.8%)	0.39
Donor age (years, median, IQR)	45.3 (3.0-83.0)	49.0 (2.0-94)	48.0 (4.0-80)	48.0 (2.0-94.0)	0.46
Donor gender (m/f)	77/57	34/38	32/44	143/139	0.06
Living donation N (%)	24 (18.2%)	18 (25.0%)	29 (38.2%)**	71 (25.4%)	**0.006**
Clinical characteristics					
Follow-up after Bx (years, median IQR)	18.3 (1.1–36.3)	15.0 (2.6–29.0)	13.4 (5.0–27.8)	15.9 (1.1–36.3)	0.18
Time of Bx after Bx (years, median IQR)	7.3 (0.3–25.6)	7.1 (0.3–18.7)	6.1 (0.5–20.1)	6.9 (0.3–25.6)	0.13
Follow-up after Bx (years, median IQR)	10.3 (0.6–21.0)	7.6 (0.6-18.5)	6.6 (0.2–14.7)	7.8 (0.2–21.0)	0.54
Time from Bx to detectable DSA (years, median IQR)	–	5.7 (0.0–16.2)	5.0 (0.0–20.1)	5.4 (0.0–20.1)	–
HLA-antibody class type I N(%)	0/134 (0.0%)	12 (16.6%)**	12 (15.7%)**	24 (8.5%)	**<0.001**
HLA-antibody class type II N(%)	0/134 (0.0%)	52 (72.2%)**	36 (47.4%)**,##	88 (31.2%)	**<0.001**
HLA-antibody class type I+II N(%)	0/134 (0.0%)	8 (11.1%)*	28 (36.8%)**,##	36 (12.8%)	**<0.001**
Maintenance immunosuppression regimens at Bx N (%)					
Tac+MMF/MPA+PDN	50 (37.0 %)	42 (58.3 %)	39 (53.4 %)	131 (46.5%)	0.58
CyA+MMF/MPA+PDN	38 (28.1 %)	18 (25.0 %)	17 (23.3 %)	73 (25.9%)	0.49
Rap+MMF/MPA+PDN	4 (3.0 %)	1 (1.4 %)	4 (5.5 %)	9 (3.2%)	0.70
Tac+MMF/MPA	4 (3.0 %)	2 (2.8 %)	5 (6.8 %)	11 (3.9%)	0.45
CyA+MMF/MPA	10 (7.4 %)	3 (4.2 %)	4 (5.5 %)	17 (6.0%)	0.66
CyA+Azathioprine+PDN	21 (15.6 %)	4 (5.6 %)	1 (1.4 %)	26 (7.3%)	0.08
Tac+PDN	3 (2.2 %)	1 (1.4 %)	1 (1.4 %)	5 (1.8%)	0.81
CyA+PDN	3 (2.2 %)	0 (0.0 %)	1 (1.4 %)	4 (1.4%)	0.78
MMF/MPA+PDN	2 (1.5 %)	1 (1.4 %)	1 (1.4 %)	4 (46.5%)	0.93
(D) Antihumoral treatment (AHT) N (%)					
PPh+IVIG	0/134 (0.0%)	15 (20.8 %)**	16 (21.1 %)	31(11.0%)	**<0.001**
PPh+IVIG+rituximab$^+$	0/134 (0.0%)	6 (8.3 %)	11 (14.5 %)	17 (6.0%)	**<0.001**
PPh+IVIG+bortezomib$^+$	0/134 (0.0%)	4 (5.6 %)*	7 (9.2 %)*,#	11 (3.9%)	**0.01**
PPh+IVIG+cyclophosphamide$^+$	0/134 (0.0%)	0 (0.0 %)	6 (7.9 %)*,#	6 (2.1%)	**0.03**
PPh+IVIG+eculizumab$^+$	0/134 (0.0%)	0 (0.0 %)	2 (2.6 %)	2 (0.7%)	0.08
Patients receiving AHT$^+$	0/134 (0.0%)	25 (34.7 %)**	42 (55,3 %)**,##	67 (23.8 %)	**<0.001**
Steroid bolus	13 (9.6 %)	9 (12.5 %)	30 (40.0 %)**,##	52 (18.4%)	**<0.001**
(E) Presence of adverse events in the 12 months post Bx					
Urinary tract infection N (median IQR)	0.2 (0–5)	0.3 (0–5)	0.4 (0–5)	0.3 (0–5)	0.12
Respiratory tract infection N (median IQR)	0.3 (0–1)	0.2 (0–1)	0.3 (0–1)	0.3 (0–1)	0.18
CMV infectious colitis N (median IQR)	0.1 (0–1)	0.1 (0–2)	0.1 (0–1)	0.1 (0–2)	0.33
Polyoma virus nephropathy N (median IQR)	0.2 (0–2)	0.1 (0–1)	0.1 (0–1)	0.1 (0–2)	0.19
(F) The level of HbA1c and blood pressure at Bx					
HbA1c level (%median IQR)	5.3 (4.7–7.2)	5.4 (4.6–7.7)	5.2 (4.3–7.5)	5.3 (4.6–7.7)	0.75
SBP level (mmHg median IQR)	140 (100–221)	140 (110–204)	139 (72–180)	140 (72–221)	0.83
DBP level (mmHg median IQR)	84 (55–119)	80 (60–110)	80 (60–101)	82 (55–119)	0.92

(Continued)

TABLE 1 | Continued

	iTG ($n = 134$)	cAMR ($n = 72$)	cAAMR ($n = 76$)	Overall ($n = 282$)	*P*-value
(G) Antihypertensive therapy after Bx					
ACEi N (%)	34 (25.2 %)	20 (27.8 %)	23 (30.3 %)	77 (27.3%)	0.43
ARB N (%)	29 (21.3 %)	12 (16.7 %)	25 (32.9%)	66 (23.4%)	0.56
CCB N (%)	39 (28.9 %)	18 (13.3 %)	32 (42.1 %)	89 (31.6%)	0.58
Beta-blocker N (%)	8 (5.9 %)	4 (5.6 %)	9 (11.8 %)	21 (7.4%)	0.52

IQR, interquartile range; BMI, body mass index; ESRD, end stage renal disease; CIT, cold ischemic time.
PRA, panel reactive antibody; HLA, human leukocyte antigen; DSA, donor-specific anti-HLA antibodies.
Bx, the studied biopsies; MMF, mycophenolate mofetil; MPA, mycophenolic acid; Tac, Tacrolimus; CyA, Cyclosporin A; Rap, rapamycin; PDN, Predinisolon; ACEi, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blocker; CCB, calcium canal antagonist; MFI, mean fluorescent intensity; HbA1c, hemoglobin A1c; SBP, systolic blood pressure; DBP, diastolic blood pressure.
$^{**}p < 0.01$, comparing with iTG; $^{*}p < 0.05$, comparing with iTG.
$^{\#\#}p < 0.01$, comparing with cAMR; $^{\#}p < 0.05$, comparing with cAMR.
The bold values indicates all p-values less than 0.05.

TABLE 2 | Morphologic results of studied biopsies and of 60-month follow-up.

	iTG ($n = 134$)	cAMR ($n = 72$)	cAAMR ($n = 76$)	Overall ($n = 282$)	*P*-value
Total detected glomeruli N (IQR)	11 (7–53)	12 (7–50)	13 (7–51)	12 (7–53)	0.05
Global glomerulosclerosis % (IQR)	13 (0–80)	14 (0–75)	16 (0–65)	13 (0–80)	0.89
Total interlobular arteries N (IQR)	1.5 (1–7)	1.5 (1–4)	1.5 (1–8)	1.5 (1–8)	0.79
Histological scores of Banff-lesions at Bx (scores median IQR)					
g (0–3)	0.1 (0–1)	0.1 (0–3)	1.8 (0–3)$^{**,\#\#}$	0.4 (0–3)	**<0.001**
ptc (0–3)	0.1 (0–3)	0.1 (0–3)	1.6 (0–3)$^{**,\#\#}$	0.3 (0–3)	**<0.001**
cg (0–3)	2.0 (1–3)	2.2 (1–3)	2.6 (1–3)$^{*,\#}$	1.0 (1–3)	**<0.001**
C4d (0–3)	0.0 (0–0)	0.0 (0–0)	0.6 (0–3)$^{**,\#\#}$	0.2 (0–3)	**<0.001**
v (0–3)	0.0 (0–2)	0.1 (0–2)	0.2 (0–3)	0.1 (0–3)	**0.03**
ci (0–3)	1.0 (0–3)	0.7 (0–3)	0.9 (0–3)	0.9 (0-3)	0.13
ct (0–3)	1.0 (0–3)	0.7 (0–3)	0.9 (0–3)	0.9 (0-3)	0.15
i (0–3)	0.8 (0–3)	0.7 (0–3)	1.0 (0–3)	0.8 (0–3)	0.26
mm (0–3)	1.1 (0–3)	0.9 (0–3)	0.9 (0–3)	1.0 (0–3)	0.28
ah (0–3)	2.4 (0–3)	2.4 (0–3)	2.5 (0–3)	2.4 (0–3)	0.61
t (0–3)	0.3 (0–3)	0.4 (0–3)	0.4 (0–3)	0.4 (0–3)	0.79
cv (0–3)	1.8 (0–3)	1.9 (0–3)	1.9 (0–3)	1.8 (0–3)	0.86
At least moderate MVI N (%)	0 (0.0%)	0 (0.0%)	69 (90.8%)$^{**,\#\#}$	69 (24.5%)	**<0.001**
Advanced IFTA (ci3+ct3) N (%)	7 (5.3%)	4 (5.6%)	10 (13.2%)	21 (7.5%)	0.15
Concomitant TCMR N (%)	13 (9.6 %)	9 (12.5 %)	30 (40.0 %)$^{**,\#\#}$	52 (18.4%)	**<0.001**
Histological diagnosis of indication biopsies during 60-month postbiopsy follow-up					
≥1 for-cause Bx after studied Bx N (%)	54/134 (40.3%)	29/72 (40.3%)	44/76 (57.9%)$^{*,\#}$	127/282 (45.0%)	**0.02**
≥1 episode of iTG, N (%)	35/54 (64.8%)	0/29 (0.0 %)**	0/44 (0.0 %)**	35/127 (27.6%)	**<0.001**
≥1 episode of cAMR, N (%)	9/54 (16.7 %)	21/29 (72.4 %)**	12/44 (27.3 %)$^{\#\#}$	42/127 (33.1%)	**<0.001**
≥1 episode of cAAMR, N (%)	1/54 (0.7 %)	10/29 (34.5 %)	33/44 (75.0 %)$^{**,\#\#}$	44/127 (34.6%)	**<0.001**
≥1 episode of advanced IFTA (ci3+ct3), N (%)	13/54 (24.1%)	3/29 (10.3%)	1/44 (2.3%)	17/127 (13.4%)	**0.04**

Banff scored lesions: glomerulitis (g); peritubular capillaritis (ptc); transplant glomerulopathy (cg); intimal arteritis (v); interstitial inflammation (i); tubulitis (t); mesangial matrix increase (mm); vascular intimal thickening (cv); arteriolar hyaline thickening (ah); interstitial fibrosis (ci) and tubular atrophy (ct); at least moderate MVI: g+ptc ≥2; advanced IFTA: ci3+ct3; Bx, the studied biopsies; concomnitant TCMR, co-existed borderline rejection and Banff TCMR typies.
$^{**}p<0.01$, comparing with iTG; $^{*}p<0.05$, comparing with iTG.
$^{\#\#}p<0.01$, comparing with cAMR; $^{\#}p<0.05$, comparing with cAMR.
The bold values indicates all p-values less than 0.05.

Transplant Characteristics at Transplantation and Studied Biopsy

TG was first diagnosed at a median of 6.9 (0.3–25.6) years without notable differences among iTG, cAMR and cAAMR groups and similar follow-up (**Table 1**). DSA were detected at a median time of 5,7 years post transplantation in cAMR group and 5.0 years in cAAMR group ($P = 0.89$).

In the cAMR group, 12/72 (16.7%) patients had only class I HLA-antibodies vs. 12/76 (15.8%) patients of cAAMR group ($P = 0.91$). In 52/72 (72.2%) patients of cAMR group and 35/76

TABLE 3A | Variation of DSA, estimated glomerular filtration rate (eGFR), and daily proteinuria pre-, at-, and post-studied biopsies in relation to TG categories.

	iTG ($n = 134$)	cAMR ($n = 72$)	cAAMR ($n = 76$)	Overall ($n = 282$)	*p*–value
DSA–MFI intensicy at and after Bx (median IQR)					
MFI_max at Bx (median IQR)	–	5071 (380–23137)	9758 (327–22438)	8701 (327–23137)	0.15
MFI_max at 6 months post Bx (median IQR)	–	5109 (528–25113)	10688 (330–23320)	9310 (330–25113)	0.08
MFI_max at 1 year post Bx (median IQR)	–	5018 (343–23302)	13012 (397–26436)	9039 (343–26436)	0.05
Model A: The eGFR values before and after Bx (ml/min/1.73 m^2 median IQR)					
eGFR 6 months before Bx	28.2 (15.3–66.1)	33.3 (10.5–88.6)	41.5 (11.9–83.6)**	35.9 (7.7–88.6)	**0.03**
eGFR at Bx	24.7 (4.0–70.0)	28.0 (5.4–77.8)	29.8 (7.5–57.8)	26.0 (4.0–77.8)	0.26
eGFR 6 months after Bx	23.8 (6.2–69.0)	23.0 (9.8–88.7)	21.0 (9.8–68.0)	22.4 (6.2–88.7)	0.91
eGFR 1 year after Bx	26.4 (6.7–70.6)	25.6 (7.6–86.1)	26.0 (6.6–69.6)	26.2 (4.0–139.0)	0.96
eGFR3 years after Bx	25.0 (8.0–46.0)	24.4 (5.0–67.0)	17.8 (9.9–70.0)	22.5 (5.0–70.0)	0.99
eGFR 5 years after Bx	21.5 (6.0–57.0)	16.0 (5.0–51.0)	20.0 (10.0–70.9)	20.0 (5.0–70.9)	0.43
Model A: The decline of eGFR at and after the studied Bx (ml/min/1.73 m^2 median IQR)					
Δ eGFR 6 months before Bx	−3.1 (−19.8–13.5)	−5.1 (−57.3–6.0)*	−11.0 (−53.8–32.5)**	−6.5 (−57.3–32.5)	**0.02**
Δ eGFR Bx to 6 months after Bx	−2.9 (−32.2–19.9)	−1.4 (−17.4–10.9)	−5.2 (−32.0–27.3)	−3.4 (−32.2–27.3)	0.58
Δ eGFR Bx to 1 year after Bx	−4.3 (−32.2–15.6)	−5.8 (−18.7–17.7)	−4.8 (−28.4–21.4)	−5.0 (−32.2–21.4)	0.92
Δ eGFR Bx to 3 years after Bx	−6.4 (−23.5–12.1)	−9.8 (−22.4–15.0)	−9.0 (−48.1–30.7)	−8.7 (−48.1–30.7)	0.45
Δ eGFR Bx to 5 years after Bx	−6.3 (−52.3–16.0)	−9.5 (−43.0–31.6)	−5.7 (−27.2–30.2)	−6.3 (−52.3–31.6)	0.60
Model B: The eGFR values before and after Bx (ml/min/1.73 m^2 median IQR)					
eGFR 6 months before Bx	28.2 (15.3–66.1)	33.3 (10.5–88.6)	41.5(11.9–83.6)**	35.9 (7.7–88.6)	**0.03**
eGFR at Bx	24.7 (4.0–70.0)	28.1 (5.4–77.8)	29.8 (7.5–57.8)	25.8 (4.0–77.8)	0.16
eGFR 6 months after Bx	16.9 (5.0–69.0)	17.5 (5.0–86.1)	22.7 (4.0–139.0)	16.2 (5.0–88.7)	0.26
eGFR 1 year after Bx	13.8 (5.0–70.6)	14.8 (5.0–76.3)	19.8 (5.0–68.0)	17.0 (4.0–139.0)	0.05
eGFR3 years after Bx	5.8 (5.0–46.0)	11.1 (5.0–67.0)	8.3 (5.0–70.0)	8.9 (5.0–70.0)	0.10
eGFR 5 years after Bx	5.4 (5.0–57.0)	7.1 (5.0–51.0)	6.3 (5.0–70.9)	6.5 (5.0–70.9)	0.12
Model B: The decline of eGFR at and after Bx (ml/min/1.73m^2 median IQR)					
Δ eGFR 6 months before Bx	−3.1 (−19.8–13.5)	−5.1 (−57.3–6.0)*	−11.0 (−53.8–32.5)**	−6.5 (−57.3–32.5)	**0.02**
Δ eGFR Bx to 6 months after Bx	−3.0 (−32.2–19.9)	−2.7 (−17.4–10.9)	−4.9 (−32.0–27.3)	−3.9 (−32.2–27.3)	0.85
Δ eGFR Bx to 1 year after Bx	−5.5 (−34.0–15.6)	−6.1 (−23.9–17.7)	−5.0 (−28.4–21.4)	−5.6 (−34.0–21.4)	0.61
Δ eGFR Bx to 3 years after Bx	−12.0 (−70.0–11.7)	−11.9 (−37.6–15.0)	−15.0 (−50.2–29.3)	−12.1 (−70.0–29.3)	0.48
Δ eGFR Bx to 5 years after Bx	−12.1 (−65.0–16.0)	−11.4 (−43.0–41.4)	−19.7 (−50.2–30.2)	−13.0 (−65.0–41.4)	0.49
The proteinuria excretion before and after Bx (mg/day median IQR)					
PU 6 months before Bx	896 (39–6758)	709(40–5312)	866 (67–12181)	835 (39–12181)	0.54
PU at Bx	1474 (54–6962)	1271 (87–8366)	955 (90–6540)	1081(54–8366)	0.30
PU 6 months after Bx	1040 (93–5807)	1019(48–11597)	1062 (65–9886)	1040 (48–11597)	0.73
PU 1 year after Bx	810 (82–5373)	871 (82–5074)	934 (66–6605)	869 (82–6605)	0.74
PU 3 years after Bx	645 (171–1681)	998 (67–5204)	667 (176–3186)	725 (67–5204)	0.32
PU 5 years after Bx	496 (94–7688)	949 (94–2459)	761 (73–4078)	629 (73–7688)	0.87
The variation of proteinuria at and after Bx (mg/day median IQR)					
Δ PU 6 months before Bx	163 (−3454–5744)	−42 (−4465–3187)	116 (−3722–5050)	121 (−4465–5744)	0.09
Δ PU Bx to 6 months after Bx	−125 (−5578–8732)	44 (−950–8201)	123 (−1920–6079)	−5 (−5578–8734)	0.07
Δ PU Bx to 1 year after Bx	−143 (−5781–3099)	40 (−3619–1577)	77 (−2184–7430)	−16 (−5781–7430)	0.06
Δ PU Bx to 3 years after Bx	132 (−5308–2434)	480 (−3000–3420)	48 (−1713–3211)	106 (−5308–3420)	0.21
Δ PU Bx to 5 years after Bx	146 (−2606–2429)	885 (−1566–1232)	272 (−653–1891)	147 (−2606–2429)	0.85

The values were expressed as median and IQR.
MFI, mean fluorescent intensity; eGFR, estimated glomerular filtration rate; ΔeGFR, difference of eGFR value.
PU, daily urine protein excretion; ΔPU, difference of PU value.
Model A, the eGFR values after graft loss or death were not imputed.
Model B, the eGFR values after graft loss or death was imputed as 5 ml/min/1.73 m^2.
***$p < 0.01$, comparing with iTG; *$p < 0.05$, comparing with iTG.*
The bold values indicates all p-values less than 0.05.

(46.1%) patients of cAAMR group, only class II HLA-antibodies were detected ($P = 0.002$), and in 8/72 (11.1%) patients of cAMR group and 28/76 (36.8%) patients of cAAMR group, both class I and II HLA-antibodies ($P < 0.001$). The vast majority of DSA were found to be de novo DSA (93.1% of cAMR group vs. 97.4% of cAAMR group, $P = 0.79$) and patients with cAMR

had a predominance of only class II DSA, while cAAMR had more frequently class I and II DSAs. The median value of the immunodominant DSA (MFI_max) tended to be higher in cAAMR group than in cAMR group without reaching the significantly different level.

No significant differences of the distribution of the maintenance immunosuppression regimens and ACE inhibitors or ARBs were found among iTG, cAMR and cAAMR groups. The AHT regimens were given with the comparable fraction to the patients in cAMR and cAAMR groups (**Table 1**).

Histological Evaluation of the Studied Biopsies

The detailed biopsy diagnoses and kidney pathology lesion scores are shown in **Table 2**. A median of 12 glomeruli (1, 7–49) was available per biopsy, a median of 13% (0–80%) glomeruli presented with global sclerosis; no significant differences were found for the number of detectable glomeruli ($P = 0.05$) and the percentage of glomerulosclerosis ($P = 0.79$) among iTG, cAMR and cAAMR groups. The median g-, ptc- and cg-lesion scores in cAAMR group were significantly higher than those in iTG and cAMR group (each comparison: $P < 0.001$). A concomitant TCMR including borderline rejection was found in 13/134 (9.6%) patients of iTG group and 9/72 (12.5%) patients of cAMR group vs. 30/76 (40.0%) patients of cAAMR group ($P < 0.001$). Furthermore, among three TG groups no significant differences were found with respect to the chronic interstitial fibrosis/ tubular atrophy (by ci- and ct-lesion) or chronic vascular change (by ah- and cv-lesion).

During follow-up, a total of 190 indication biopsies in 127 patients (54, 29 and 44 patients in iTG, cAMR and cAAMR groups, respectively) were performed. Most biopsies confirmed the previous diagnosis and only a few patients changed categories (**Table 2**). Moreover, the advanced IFTA characterized with highest score of ci and ct (ci3+ct3) was found in 13/54 (24.1%) patient of iTG group, 3/29 (10.3%) patients of cAMR group and 1/44 (2.3%) patient of cAAMR group ($P = 0.04$).

Effect of TG Categories on the Kidney Allograft Function

The median eGFR (**Table 3A**) 6 months before biopsy was 28.2 ml/min/1.73 m^2 in iTG group and 33.3 ml/min/1.73 m^2 in cAMR group, which were significantly lower than 41.5 ml/min/1.73 m^2 in cAAMR group ($P = 0.03$). After biopsy, most patients had a progressive decline in renal function and median eGFR during follow up was similar among groups (each comparison among three groups at time post biopsy $P > 0.05$) without imputation (model A **Figure 2A**) and with imputation of graft loss (model B **Figure 2B**).

The evolution of eGFR is analyzed by linear mixed model and illustrated in **Supplementary Table 1** with and without imputation for graft loss or death, which did not reveal any significant difference of TG categories in association with eGFR decline ($F = 1.3$, $P = 0.28$), and there was no statistical difference in eGFR decline among three groups. The mean annual eGFR decline of iTG, cAMR and cAAMR group were −3.2 (95%CI, −5.2 to −1.2), −2.5 (95%CI, −4.5 to 0.5) and −2.9 (95%CI, −4.9 to 0.9) ml/min/1.73m^2/year, respectively (each comparison: $P > 0.05$). The difference of annual eGFR decline (ml/min/1.73 m^2 per year) was not significant when the comparison was performed between each two TG categories. In addition, there was no significant difference in proteinuria pre-, at- and post-diagnosis among iTG, cAMR and cAAMR groups (comparison in each time yields $P > 0.05$).

Effect of AHT on Renal Allograft Function

In order to analyze the effect of AHT on DSA intensity and allograft function, cAMR and cAAMR groups were further divided into subgroups based on AHT. As shown in **Table 3B**, no significant differences were found with regard to DSA intensity, allograft function and proteinuria, and no statistical significances were found in comparison to untreated iTG group. The association of AHT with eGFR slope was analyzed by linear mixed model and is shown in **Supplementary Table 2**. The difference of annual eGFR decline (ml/min/1.73 m^2/year) was not significant when the comparison was performed between different groups with and without treatment, irrespective of imputation. Similarly, proteinuria was similar in all groups during follow-up ($P > 0.05$).

Patient and Graft Outcomes

Importantly, the rates of graft survival (GS), death censored graft survival (DCGS) and patient survival (PS) at 1-, 3- and 5-year post transplantation were comparable between groups (**Figures 3**, **4**). 5-year Kaplan-Meier estimate for DCGS after diagnosis of iTG, cAMR and cAAMR were 35.9, 44.8, and 33.9%, respectively ($P = 0.75$) and rates of GS, DCGS and PS were comparable among iTG, cAMR and cAAMR groups at each time during follow-up (**Figure 3**). Finally, 5-year Kaplan-Meier estimate for overall graft survival (including patient death) of iTG, cAMR and cAAMR were 34.1, 40.6, and 31.8%, respectively ($P = 0.84$).

The role of AHT on the long-term graft outcome is shown in **Figure 4**. Up to 5-year post studied biopsies, there were no significant differences of the GS, DCGS and PS rates upon comparison between the patients with or without AHT in cAMR and cAAMR group and similar in comparison with iTG group ($P > 0.05$).

During the 12 months after diagnosis, the episodes of urinary tract and respiratory tract infections that required hospitalization occurred with comparable frequency among iTG, cAMR and cAAMR groups.

Correlation of Histological and Clinical Features With 5-Year Outcome

Each Banff lesion was divided into mild grade (score 0-1) and severe grade (score 2-3). After exclusion of thirteen patients, who died with a functioning graft, we found significant differences in 5-year death-censored graft survival when comparing mild and severe grade of Banff mm-, ah-, cv-, t-, ci- and ct-lesion in univariate Kaplan-Meier analysis (**Supplementary Table 3**),

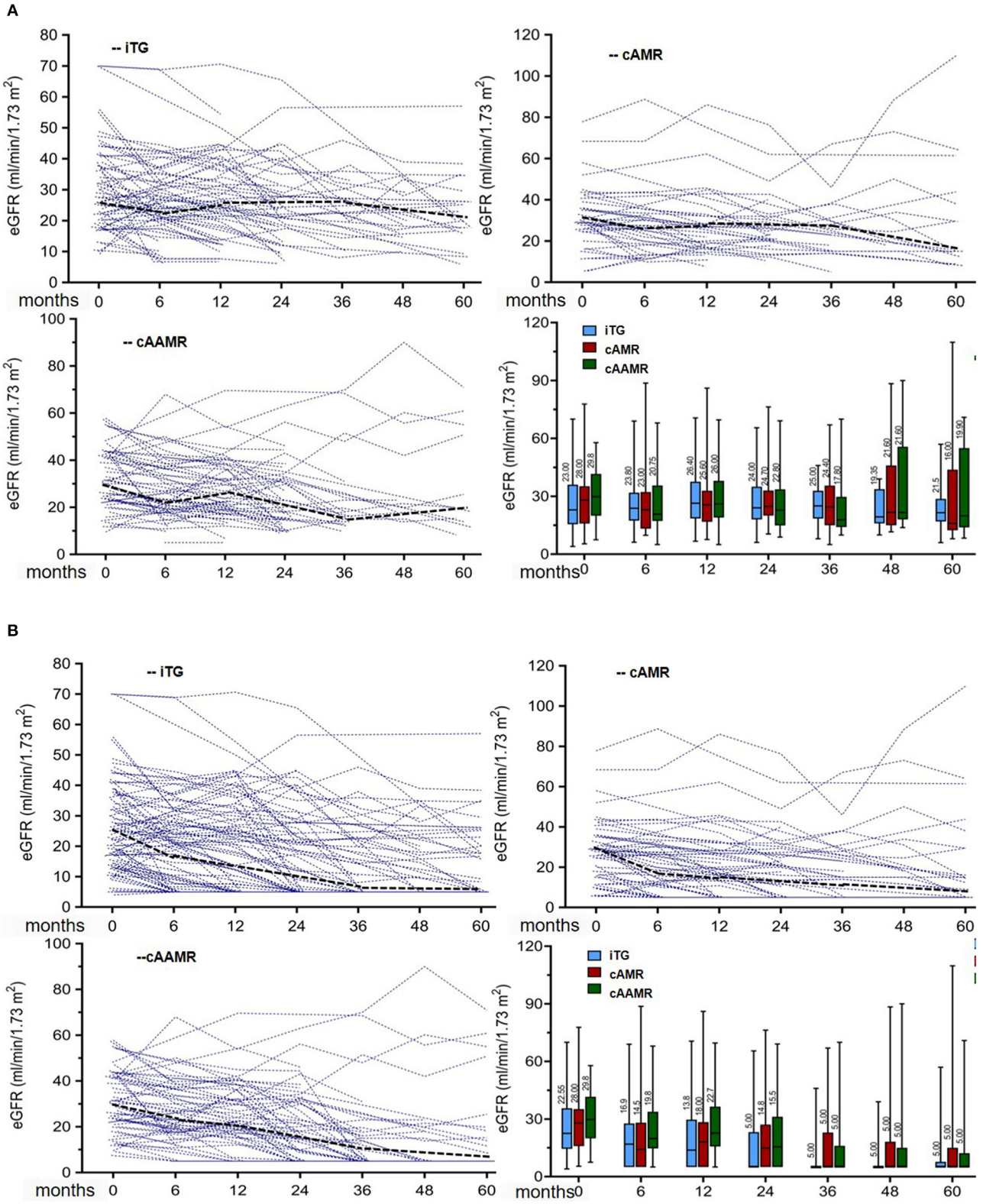


FIGURE 2 | (A) Effects of TG categories on the evolution of eGFR, for patient death or return to dialysis, no data of eGFR were imputed. **(B)** Effects of TG categories on the evolution of eGFR, for patient return to dialysis, eGFR were imputed as 5 ml/min/1.73 $m^{2.}$ Individual eGFR course (thin dashed lines) and median eGFR (fat dashed lines) in relation to TG categories. Analyses are on the basis of serial eGFR measurements at 0, 6, 12, 18, 24, 36, 48, and 60 months. Box plots indicate the median, the interquartile range, the minimum, and the maximum of the measures.

TABLE 3B | Variation of DSA, estimated glomerular filtration rate (eGFR), and proteinuria in relation to antihumoral therapy.

	iTG (n = 134)	cAMR free of AHT (n = 47)	cAMR with AHT (n = 25)	cAAMR free of AHT (n = 30)	cAAMR with AHT (n = 46)	p-value
DSA-MFI intensity at and after Bx (median IQR)						
MFI_max at Bx	–	8046 (380–23137)	4273 (648–25113)	11782 (386–22277)	8941 (327–22438)	0.12
MFI_max at 6 months after Bx	–	8798 (320–21105)	3984 (405–22516)	11153 (1863–18265)	10001 (330–23320)	0.91
MFI_max at 1 year after Bx	–	6913 (420–23076)	3606 (343–23302)	9321 (1505–22460)	14784 (397–26436)	0.05
Model A: The eGFR values before and after Bx (ml/min/1.73 m^2 median IQR)						
eGFR 6 months before Bx	28.2 (15.3–66.1)	29.0 (18.9–81.8)	41.5 (10.5–88.6)	36.0 (15.0–72.2)	42.0 (11.9–83.6)**	0.06
eGFR at Bx	24.7 (4.0–70.0)	28.3 (5.4–77.8)	27.7 (5.4–52.0)	26.3 (9.1–57.8)	30.5 (7.5–57.4)	0.54
eGFR 6 months after Bx	23.8 (6.2–69.0)	26.2 (11.6–88.7)	14.9 (9.8–43.8)	22.5 (10.8–48.9)	19.8 (9.8–68.0)	0.52
eGFR 1 year after Bx	26.4 (6.7–70.6)	26.6 (13.2–86.1)	19.7(7.6–62.2)	26.0 (12.1–69.6)	26.8(6.6–54.4)	0.84
eGFR3 years after Bx	25.0 (8.0–46.0)	20.5 (5.0–46.0)	25.9 (14.0–67.0)	16.0 (15.0–68.6)	18.0 (9.9–70.0)	0.78
eGFR 5 years after Bx	21.5 (6.0–57.0)	15.5 (8.0–109.8)	29.5 (8.8–64.0)	12.8 (8.3–60.9)	25.5 (15.3–70.9)	0.63
Model A: The decline of eGFR at and after Bx (ml/min/1.73 m^2 median IQR)						
Δ eGFR 6 months before Bx	−3.1 (−19.8–13.5)	−2.4 (−13.5–6.0)	−12.5 (−57.3– −0.5)*	−15.2 (−24.5– −0.9)*, #	−11.1 (−53.8–32.5)**	0.005
Δ eGFR 6 months after Bx	−3.4 (−22–16.7)	−1.8 (−18.8–25.0)	−4.3 (−18.8–7.0)	−2.3 (−26.7–22.3)	−2.4 (−14.6–38.4)	0.58
Δ eGFR 1 year after Bx	−4.1 (−24.1–15.6)	−4.7 (−17.0–21.1)	−7.5 (−24.8–15.0)	−6.5 (−37.2–13.3)	−3.7 (−27.0–21.4)	0.13
Δ eGFR 3 years after Bx	−6.4 (−23.5–12.1)	−13.0 (−22.4–9.4)	−3.5 (−20.0–15.0)	−9.7 (−28.3–20.4)	−8.1 (−48.1–30.7)	0.28
Δ eGFR 5 years after Bx	−6.3 (−52.3–16.0)	−15.9 (−43.0–31.6)	−3.5 (−17.0–8.0)	−8.3 (−27.2–12.7)	−5.6 (−26.2–30.2)	0.62
Model B: The eGFR values before and after the studied Bx (ml/min/1.73 m^2 median IQR)						
eGFR 6 months before Bx	28.2 (15.3–66.1)	29.0 (18.9–81.8)	41.5 (10.5–88.6)	36.0 (15.0–72.2)	42.0 (11.9–83.6)**	0.06
eGFR at Bx	24.7 (4.0–70.0)	28.3 (5.4–77.8)	27.7 (5.4–52.0)	26.3 (9.1–57.8)	30.5 (7.5–57.4)	0.39
eGFR 6 months after Bx	16.9 (5.0–69.0)	19.1 (5.0-88.7)	12.2 (5.0–43.8)	20.8 (5.0–48.9)	19.7 (5.0–68.0)	0.43
eGFR 1 year after Bx	13.8(5.0–70.6)	19.0 (5.0–86.1)	16.8 (5.0–62.2)	21.1 (5.0–69.6)	23.2 (5.0–54.4)	0.19
eGFR3 years after Bx	5.8 (5.0–46.0)	12.4 (5.0–46.0)	9.9 (5.0–67.0)	7.5 (5.0–68.6)	9.4 (5.0–70.0)	0.25
eGFR 5 years after Bx	5.4 (5.0–57.0)	8.0 (5.0–109.8)	6.6 (5.0–64.0)	6.3 (5.0–60.9)	8.8 (5.0–70.9)	0.22
Model B: The decline of eGFR at and after Bx (ml/min/1.73 m^2 median IQR)						
Δ eGFR 6 months before Bx	−3.1(−19.8–13.5)	−2.4 (−13.5–6.0)	−12.5 (−57.3–−0.5)*	−15.2 (−24.5– −0.9)*,Δ	−11.1 (-53.8–32.5)**	0.005
Δ eGFR 6 months after Bx	−3.0 (−32.2–19.9)	−1.7 (−16.3–10.9)	−4.5(−17.4–7.6)	−4.7 (−18.0–13.0)	−4.9 (−32.0–27.3)	0.98
Δ eGFR 1 year after Bx	−5.4 (−34.0–15.6)	−6.7 (−17.0–17.7)	−5.7 (−23.9–15.0)	−3.9 (−18.4–21.4)	−5.4 (−28.4–18.3)	0.78
Δ eGFR 3 years after Bx	−12.0 (−70.0–11.7)	−13.0 (−24.7–8.6)	−9.0 (−37.6–15.0)	−17.9 (−50.2–20.4)	−13.0 (−49.6–29.3)	0.75
Δ eGFR 5 years after Bx	−12.1 (−65.0–16.0)	−13.2 (−43.0–41.4)	−7.7 (−37.6–12.0)	−22.6 (−50.2–12.7)	−18.9 (−42.1–30.2)	0.66
The proteinuria values at and after Bx (mg/day median IQR)						
PU 6 months before Bx	896 (39–6758)	991(59–5155)	653 (45–2613)	866 (67–12181)	955 (90–6540)	0.54
PU at Bx	1474 (54–6962)	918 (48–11579)	969 (143–5812)	852 (78–4563)	1061.5 (65–9886)	0.48
PU 6 months after Bx	1040 (9–5807)	665(89–6989)	1114 (208–3732)	1058(59–6605)	998 (41–12355)	0.19
PU 1 year after Bx	684 (84–3812)	1037 (137–3325)	462 (125–3732)	165 (60–2637)	800 (41–12355)	0.58
PU 3 years after Bx	766 (75–4661)	1656 (75–3420)	841 (445–2600)	613 (184–1042)	540 (203–3172)	0.43
PU 5 years after Bx	539 (50–3581)	1365 (50–2206)	909 (199–1641)	622 (107–1818)	629 (158–2,404)	0.88
The variation of proteinuria at and after Bx (mg/day median IQR)						
Δ PU 6 months before Bx	163 (−3,454–5,744)	−21 (−4,465–1,598)	−65 (−950–3,187)	132 (−3732–5,050)	84 (−2,066–4,057)	0.11
Δ PU 6 months after Bx	−125 (−5,578–8,732)	−24 (−833–7,465)	79 (−950–8,201)	73 (−506–2,066)	143 (−1,920–6,079)	0.23
Δ PU 1 year after Bx	−143 (−5,781–3,099)	40 (−1,127–753)	117 (−3,619–1,577)	−115 (−405–2,005)	96 (−2,184–7,430)	0.12
Δ PU 3 years after Bx	132 (−5,308–2,434)	862 (−288–3,420)	−420 (−3,000–2,191)	220 (−184–525)	6 (−1,713–3,211)	0.22
Δ PU 5 years after Bx	146 (−2,606–2,429)	885 (−47–1,129)	637 (−1,566–1,232)	116 (−1,087–2,343)	427 (−653–1,891)	0.97

The values were expressed as median and IQR.

AHT, antihumoral therapy; MFI, mean fluorescent intensity; eGFR, estimated glomerular filtration rate; ΔeGFR, difference of eGFR value; PU, daily urine protein excretion; ΔPU, difference of PU value.

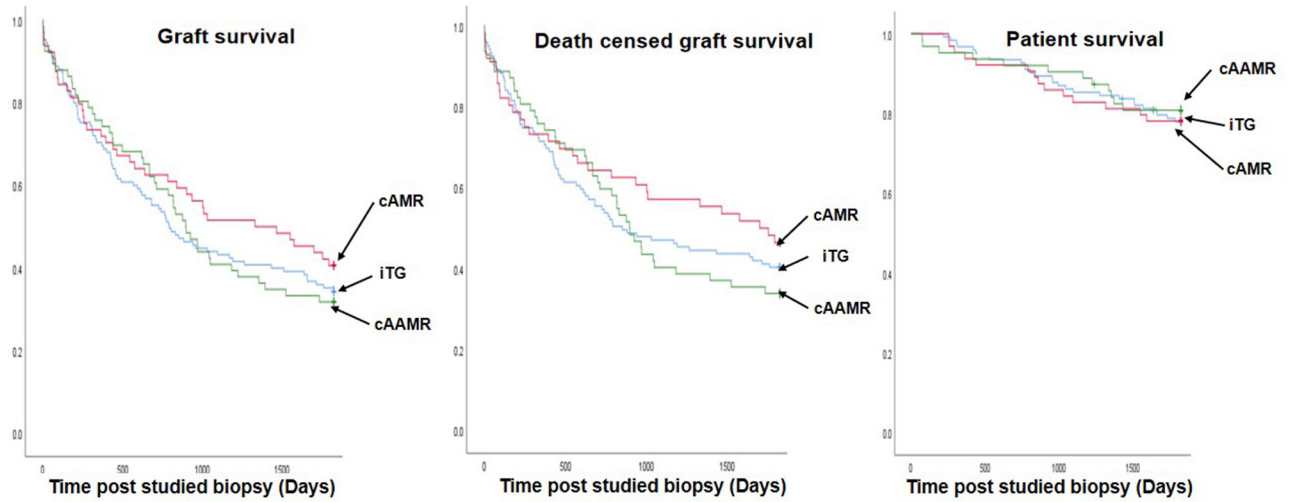
Model A, the eGFR values after graft loss or death were not imputed; Model B, the eGFR values after graft loss or death were imputed as 5 ml/min/1.73 m^2.

***$p < 0.01$, comparing with iTG; *$p < 0.05$, comparing with iTG.*

#$p < 0.05$, comparing with cAMR.

Δ$p < 0.05$, comparing with cAMR free of AHT.

The bold values indicates all p-values less than 0.05.



	Rate of graft survival					Rate of death-censored graft survival					Rate of patient survival				
	1 year post Bx	2 year post Bx	3 year post Bx	4 year post Bx	5 year post Bx	1 year post Bx	2 year post Bx	3 year post Bx	4 year post Bx	5 year post Bx	1 year post Bx	2 year post Bx	3 year post Bx	4 year post Bx	5 year post Bx
iTG	72.4 %	57.5 %	45.9 %	40.3 %	34.1 %	72.9 %	57.6 %	46.7 %	42.0 %	35.9 %	97.0 %	94.0 %	86.7 %	84.7 %	78.5 %
cAMR	75.0 %	66.7 %	55.1 %	52.9 %	40.6 %	78.3 %	70.6 %	60.3 %	58.1 %	44.8 %	94.4 %	93.1 %	84.1 %	82.1 %	78.1 %
cAAMR	78.9 %	64.0 %	46.6 %	39.4 %	31.8 %	80.0 %	65.8 %	47.9 %	41.8 %	33.9 %	96.1 %	93.3 %	91.7 %	82.4 %	81.0 %
overall	74.8%	61.6%	48.0%	43.3%	35.2%	76.2%	63.0%	50.4%	46.0%	37.6%	96.1%	93.6%	87.3%	83.4%	79.0%
log rank p-value	0.31	0.27	0.45	0.57	0.84	0.24	0.16	0.42	0.49	0.75	0.62	0.80	0.41	0.67	0.78

FIGURE 3 | The comparison of 5-year graft survival **(left)**, death censored graft survival **(middle)** and 5-year patient survival **(right)** among iTG, cAMR and cAAMR groups. Bx, the studied biopsies.

TABLE 4 | Binary logistic-regression analysis of clinical and histologicl factors associated with 5-year outcome after diagnosis of transplant glomerulopathy.

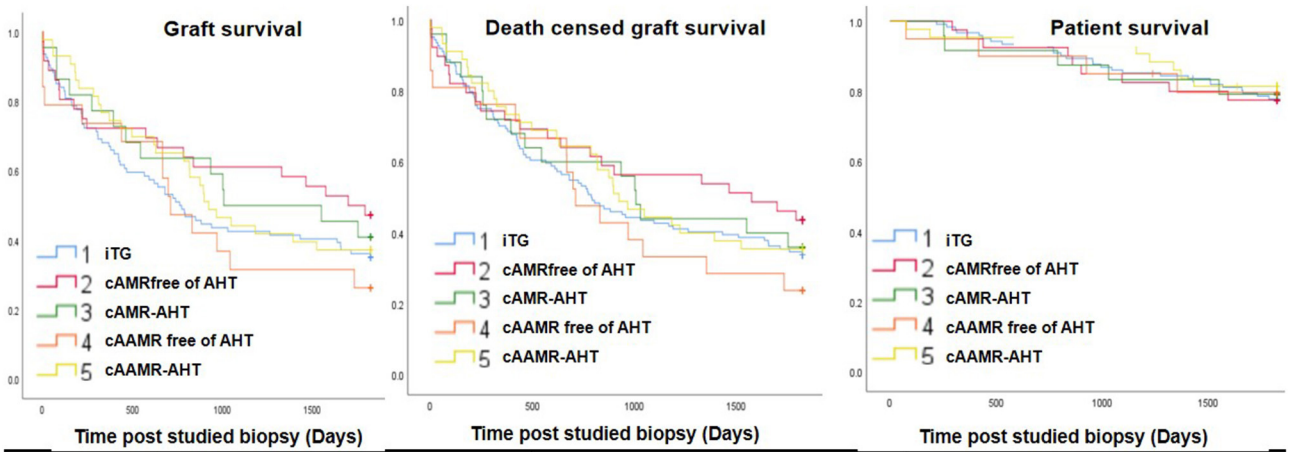
	Graft loss				Patient death				Death-censored graft loss			
	OR	95% CI		*p*-value	OR	95% CI		*p*-value	OR	95% CI		*p*-value
Clinical factors												
eGFR value at Bx	0.97	0.95	0.99	**0.02**	0.96	0.92	0.99	0.05	0.97	0.95	0.99	**0.02**
PU value at Bx	1.00	1.00	1.01	0.10	1.00	1.00	100	0.43	1.00	1.00	1.01	0.06
Receiving antihumoral therapy	0.82	0.38	1.79	0.62	0.75	0.26	2.13	0.58	0.73	0.33	1.62	0.44
Histological factors												
mm >1	3.19	1.60	6.35	**0.001**	1.83	0.91	3.67	0.09	3.33	1.66	6.72	**0.001**
ci >1	2.39	1.26	4.55	**0.008**	1.49	0.77	2.89	0.24	2.56	1.33	4.91	**0.005**
ct >1	2.32	1.22	4.42	**0.01**	1.53	0.79	3.00	0.21	2.48	1.29	4.76	**0.006**
ah >1	2.79	1.24	6.27	**0.01**	1.34	0.46	3.87	0.59	2.77	1.20	6.40	**0.02**
t >1	2.98	1.05	8.48	**0.04**	1.13	0.39	3.25	0.83	3.02	1.04	8.76	**0.04**
cv >1	1.70	0.90	3.21	0.11	1.46	0.66	3.24	0.36	1.44	0.75	2.77	0.28

eGFR, estimated glomerular filtration rate; PU, daily urine protein excretion.
OR, odds ratio; CI, conference intervals for odds ratio.
The bold values indicates all p-values less than 0.05.

these six Banff lesions were consequently selected for further multivariable analysis. Based on clinical experience, we performed a binary-logistic regression to assess the association of three selected clinical variables (eGFR and proteinuria at biopsy, and receiving AHT) with 5-year postbiopsy graft survival, patient survival and death-censored graft survival. The Banff mm-, ah-, t-, ci- and ct-lesions as well as eGFR level at biopsy were closely associated with 5-year graft failure (**Table 4**).

DISCUSSION

Late graft failure is a common problem after kidney transplantation presenting a seriously debilitating and life-threatening condition (35); AMR is considered as the major cause of late allograft loss outside of death with functioning graft and TG is recognized as a key histological change of chronic antibody-mediated injury during late allograft dysfunction (8). There is a need for robust surrogate endpoints in transplantation,



	Rate of graft survival					Rate of DCGS (death-censored graft survival)					Rate of Patient Survival				
	1 year post Bx	2 year post Bx	3 year post Bx	4 year post Bx	5 year post Bx	1 year post Bx	2 year post Bx	3 year post Bx	4 year post Bx	5 year post Bx	1 year post Bx	2 year post Bx	3 year post Bx	4 year post Bx	5 year post Bx
1. iTG	72.4 %	57.5 %	45.9 %	40.3 %	34.1 %	72.9 %	57.6 %	46.7 %	42.0 %	35.9 %	97.0 %	94.0 %	86.7 %	84.7 %	78.5 %
2. cAMR free of AHT	75.6 %	68.9 %	59.5 %	57.1 %	43.6 %	77.3 %	72.1 %	64.1 %	61.5 %	47.2 %	95.6 %	93.3 %	83.3 %	81.0 %	77.5 %
3.cAMR with AHT	74.1 %	63.0 %	48.1 %	46.2 %	36.0 %	80.0 %	68.0 %	54.2 %	52.2 %	40.9 %	92.6 %	92.6 %	85.2 %	84.0 %	79.2 %
4.cAAMR free of AHT	79.2 %	52.2 %	39.1 %	31.8 %	23.8 %	79.2 %	54.5 %	40.9 %	35.0 %	26.3 %	95.8 %	91.3 %	87.0 %	81.8 %	80.0 %
5. cAAMR with AHT	78.8 %	69.2 %	50.0 %	42.9 %	35.6 %	80.4 %	70.6 %	51.0 %	44.7 %	37.2 %	96.2 %	94.2 %	93.9 %	82.6 %	81.4 %
log rank p-value	0.32	0.28	0.54	0.65	0.90	0.23	0.24	0.50	0.67	0.79	0.64	0.87	0.28	0.87	0.65

FIGURE 4 | The effect of AHT on the 5-year post Bx graft survival rates **(left)**, DCGS rates **(middle)** and patient survival rates **(right)**. AHT, antihumoral therapy; Bx, the studied biopsies.

that adequately predict long-term graft outcome and facilitates the performance of clinical trials (36). So far, several biomarkers have been considered as proposed endpoints for kidney allograft dysfunction (37) but there is rather limited experience of these surrogate endpoints on graft outcome. Only a few studies have sufficient numbers, long-term follow-up and are fulfilling the most recent diagnostic criteria for AMR (1, 38, 39). In the Banff 2017 report (12) the diagnosis of cAMR is well-defined and differentiated from the cAAMR. However, there is still significant ambiguity and knowledge gaps for the different histopathologic forms of TG (40). In this respective analysis, 282 patients developing TG after transplantation were investigated. using the strict, most recent Banff criteria and individual features (12), patients with TG were devided into iTG, cAMR and cAAMR categories, and the evolution of allograft function and long-term graft outcomes analyzed. Our analysis showed no significant differences in eGFR decline, proteinuria, DSA intensity and morphologic features among iTG, cAMR and cAAMR groups; moreover, no obvious benefit of AHT was found in treating patients of cAMR or cAAMR groups because on average more than 60% patients lost the allograft function within 5-year postbiopsy follow-up.

The development of TG is viewed as a structural 'end-product' of the antibody-mediated pathophysiological process (41), however, the quality and quantity (titer) of circulating DSAs may impact the clinical manifestation of the AMR (42, 43), and discrepancies between histological and serological findings are commonly exist (44). In this study, the patients with cAMR had lower DSA intensity, less C4d positivity and less frequent combined class I and II DSAs compared to cAAMR group. Previous studies showed that patients with exclusively weak or no complement-activating DSAs tended to experience less disease activity and eventually had better outcomes (45). Our data provide further evidence for a fluctuating activity and/or patchy distribution of AMR activity in the kidney, supporting the hypothesis that cAMR and cAAMR are a spectrum of the same disease due to a shared underlying pathophysiology.

Nearly all therapeutic approaches for treating AMR aim to remove circulating DSAs and to decrease DSA production (46) in order to reduce of DSA intensity and AMR-activity. However, irrespective of AHT, the cAMR and cAAMR patients had some longitudinal variation of DSA-MFI values without significant intergroup differences. Although IVIG/PPh is regarded as the "standard care of AMR" (47, 48), the DSA-producing plasma cells are not affected (49). In an attempt to prevent further antibody production, some patients received additional rituximab or bortezomib therapy. A prospective, randomized study (23) reported that treatment of late AMR with rituximab in combination with steroids IVIG and PPh did not improve any outcome parameter compared to placebo (23). Similarly a randomized trial did not show any therapeutic efficacy for bortezomib (50). The current evidence is in line with our data and supports that there is no proven treatment for cAMR and cAAMR (19).

This is one of the first studies to report a large cohort of iTG according to Banff 2017 criteria (12). TG is a frequent histological finding and could be a sign of AMR, but there is evidence that many TG cases do not have detectable DSA nor evidence for antibody interaction with graft vascular endothelium (51). A retrospective analysis of TG in 954 kidney transplant recipients (3,744 biopsies including protocol biopsies) observed TG in 10% of patients independent of HLA mismatches, and >75% of TG cases had no HLA-DSA. They concluded that iTG represents a different phenotype that had lower levels of concomitant inflammation and graft loss compared with HLA-DSA+ TG (52). In our study iTG was observed in 47.5% of indication biopsies without signs for AMR, and we could not detect significant differences in outcomes among iTG, cAMR and cAAMR during a 5-year follow-up. HLA-DSA negative TG may also be caused by antibodies against non-HLA targets including non-HLA antibodies (e.g., against minor histocompatibility antigens) or other targets such as endothelial antigens or vimentin (53) and the failure to demonstrate DSA in iTG cases does not rule out the contribution of other antibodies in the pathophysiology of TG (54). Alternatively, the absorption of low antibody levels by the allograft may result in a lack of circulating DSA (55). Until we have fully deciphered the pathophysiology we should consider iTG as a rather frequent separate disease category in the long-term course after transplantation, indicating structural damage of the glomerular basement as evidenced by proteinuria, and resulting in suboptimal outcomes.

Although TG is a heterogeneous condition, the underlying disease processes often share a final common clinical pathway of declining kidney graft function and increasing proteinuria (56). Several publications advocate the use of eGFR slope as a surrogate for clinical outcome in kidney disease trials (57, 58), although annualized GFR loss does not meet all criteria for a valid surrogate endpoint (59). In our study the three TG groups had a comparable annual eGFR decline and similar long-term outcomes without an effect of AHT. Also proteinuria is considered a potential useful biomarker which is associated with structural injury of glomerular basement membrane and a decline in kidney function (60). In our study, the urinary protein excretion was comparable among iTG, cAMR, and cAAMR groups but failed to reach statistical significance in the multivariable models for long-term outcomes.

Late graft failure often coincides with cumulative chronic histologic injury (61), which has previously been identified as strongly associated with allograft loss, irrespective of diagnosis (62). The biopsies performed in late period of transplantation are particularly dominated by non-specific chronic lesions and IFTA (63). Our biopsies with TG displayed moderate to severe transplant vasculopathy (by ah- and cv-lesions), which might further contribute to late graft loss (64). Although the median scores of ci- and ct-lesions in our patients with TG were not advanced, the presence of IFTA in combination with transplant vasculopathy might also indicate some potential CNI-nephrotoxicity. The long-term exposure to CNI has been proven as one of the major risk factors leading to arterial intimal fibroproliferation and neointimal thickening, eventually resulting in graft ischemia and striped IFTA (65) and predicting rather poor graft survival (66). In addition, the AHT regimen with enhanced immunosuppression led to a higher number of over immunosuppression and conferred a substantial risk of drug-toxicities, which was closely associated with the deterioration of the tubulointerstitial fibrosis and inferior late graft survival (67). Several studies highlight the importance of progressive fibrosis as a key pathway to graft failure and a target for intervention independent of the role of AMR in late graft failure (11, 62). Therefore, the ideal therapeutic guidelines for TG remain to be determined, and the choice of appropriate medication dosage, paired with careful patient monitoring and adjustment of baseline immunosuppression, needs to be investigated.

AMR is often initially detected with concomitant TCMR, and the treatment of concomitant TCMR is recommended in all cases of AMR (19, 68). We found significantly more concomitant TCMR in the cAAMR group than in the iTG and cAMR groups, in parallel with an evidently rapid decline in eGFR before studied biopsy. An additional steroid bolus was given to treat the mixed TCMR, and afterward the median eGFR decline at each time post studied biopsy between the cAMR and cAAMR groups, which might be explained by an adequate response of concomitant TCMR to steroids while the clincal course of AMR was not affected.

It is important to point toward the limitations of our study. First, the retrospective design of our study has inherent limitations and although all data were captured since 2000 in an electronic database, different biases are always present in retrospective data collections. Second, our results are obtained from indication biopsies and indication for biopsies may have changed over time. Our center does not perform protocol biopsies, which might have identified early subclinical lesions, which theoretically could better correlate with outcome than advanced lesions detected in indication biopsies. Third, TG is not per se a diagnosis, but a histologic lesion, which can be seen as a uniform response pattern of the glomerular basement membrane to different injuries, including AMR (18). Therefore it is difficult to completely exclude TMA of other causes or de novo/recurrent glomerulonephritis, which may have been misdiagnosed as TG in the absence of immune complexes. However, we relied on the most recent consensus from the Banff 2017 classification and it seems unlikely that such misdiagnoses have introduced a significant bias in this study.

In summary, our observational study demonstrates that the occurrence of TG is associated with poor long-term graft outcomes independent of the TG categories and scores. Therefore our data point toward the limitations of TG grading as a suitable potential surrogate endpoint for clinical trials. Given that late graft failure (excluding death) is often multifactorial (3), and TG may arise as a uniform "response to injury pattern" from different underlying diseases the isolated histopathological finding of TG as single surrogate endpoint may not fully reflect the complexity of graft loss in kidney transplantation and cg grading was not associated with outcome. Contrary, Banff scores associated with chronic scarring might be better suited to predict

an unfavorable outcome in patients with TG. Importantly, AHT in patients with AMR had no relevant effect on the fluctuating course of DSA, eGFR decline and long-term allograft outcome. Our findings clearly support the need for prospective, randomized trials in this area. Meanwhile, when approaching the use of existing AHT agents for treating cAMR or cAAMR, less may be more.

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/s.

AUTHOR CONTRIBUTIONS

KW and BR participated in evaluation of pathologic slides, research design, and writing. DS participated in data administration. CL and NL participated in detection of DSA. BO, FH, MN, MC, FB, SR, WD, and ES participated in the designation and performance of the research. KB participated in the research design and paper writing. All authors have read and agreed to the published version of the manuscript.

FUNDING

MN, ES, and WD are participants in the BIH Charité Digital Clinician Scientist Program funded by the Charité-Universitätsmedizin Berlin, the Berlin Institute of Health and the German Research Foundation (DFG).

ACKNOWLEDGMENTS

The authors thank the technicians of the Charité-Universitätsmedizin Berlin Department of Nephrology and Pathology for their contributions to this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.889648/full#supplementary-material

REFERENCES

1. Loupy A, Lefaucheur C. Antibody-mediated rejection of solid-organ allografts. *N Eng J Med.* (2018) 379:1150–60. doi: 10.1056/NEJMra1802677
2. Mayrdorfer M, Liefeldt L, Wu K, Rudolph B, Zhang Q, Friedersdorff F, et al. Exploring the complexity of death-censored kidney allograft failure. *J Am Soc Nephrol.* (2021) 32:1513–26. doi: 10.1681/ASN.2020081215
3. Gaston RS, Cecka JM, Kasiske BL, Fieberg AM, Leduc R, Cosio FC, et al. Evidence for antibodymediated injury as a major determinant of late kidney allograft failure. *Transplantation.* (2010) 90:68–74. doi: 10.1097/TP.0b013e3181e065de
4. Montgomery RA, Cozzi E, West LJ, Warren DS. Humoral immunity and antibody-mediated rejection in solid organ transplantation. *Semin Immunol.* (2011) 23:224–34. doi: 10.1016/j.smim.2011.08.021
5. Gupta A, Broin PO, Bao Y, Pullman J, Kamal L, Ajaimy M, et al. Clinical and molecular significance of microvascular inflammation in transplant kidney biopsies. *Kidney Int.* (2016) 89:217–25. doi: 10.1038/ki.2015.276
6. Einecke G, Sis B, Reeve J, Mengel M, Campbell PM, Hidalgo LG, et al. Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *Am J Transplant.* (2009) 9:2520–31. doi: 10.1111/j.1600-6143.2009.02799.x
7. Banfi G, Villa M, Cresseri D, Cresseri D, Ponticelli C. The clinical impact of chronic tranplant glomerulopathy in cyclosporine era. *Transplantation.* (2005) 80:1392–97. doi: 10.1097/01.tp.0000181167.88133.d2
8. Remport A, Ivanyi B, Mathe Z, Tinckam K, Mucsi I, Molnar MZ. Better understanding of transplant glomerulopathy secondary to chronic antibody-mediated rejection. *Nephrol Dial Transplant.* (2015) 30:1825–33. doi: 10.1093/ndt/gfu371
9. Solez K, Colvin RB, Racusen LC, Sis B, Halloran PF, Birk PE, et al. Banff ′05 meeting report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). *Am J Transplant.* (2007) 7:518–26. doi: 10.1111/j.1600-6143.2006.01688.x
10. Haas M, Sis B, Racusen LC, Solez K, Glotz D, Colvin RB, et al. Banff 2013 meeting report: Inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. *Am J Transplant.* (2014) 14:272–83. doi: 10.1111/ajt.12590
11. Wu K, Schmidt D, Lopez Del Moral C, Osmanodja B, Lachmann N, Zhang Q, et al. Poor log-term renal allograft survival in patients with chronic antibody-mediated rejection, irrespective of treanment-A single center retrospective study. *J Clin Med.* (2021) 11:199. doi: 10.3390/jcm11010199
12. Haas M, Loupy A, Lefaucheur C, Roufosse C, Glotz D, Seron D, et al. The Banff 2017 kidney meeting report: Revised diagnostic criteria for chronic active T-cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *Am J Transplant.* (2018) 18:293–07. doi: 10.1111/ajt.14625
13. Böhmig GA, Eskandary F, Doberer K, Halloran PF. The therapeutic challenge of late antibody-mediated kidney allograft rejection. *Transpl Int.* (2019) 32:775–88. doi: 10.1111/tri.13436
14. Archdeacon P, Chan M, Neuland C, Velidedeoglu E, Meyer J, Tracy L, et al. Summary of FDA antibody-mediated rejection workshop. *Am J Transplant.* (2011) 11:896–906. doi: 10.1097/TP.0000000000002141
15. Meier-Kriesche HU, Schold JD, Srinivas TR, Kaplan B. Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. *Am J Transplant.* (2004) 4:378–83. doi: 10.1111/j.1600-6143.2004.00332.x
16. Coemans M, Süsal C, Döhler B, Anglicheau D, Giral M, Bestard O, et al. Analyses of the short- and long-term graft survival after kidney transplantation in Europe between 1986 and 2015. *Kidney Int.* (2018) 94:964–73. doi: 10.1016/j.kint.2018.05.018
17. Cosio FG, Grande JP, Wadei H, Larson TS, Griffin MD, Stegall MD. Predicting subsequent decline in kidney allograft function from early surveillance biopsies. *Am J Transplant.* (2005) 5:2464–72. doi: 10.1111/j.1600-6143.2005.01050.x
18. Baid-Agrawal S, Farris AB III, Pascual M, Mauiyyedi S, Farrell ML, Tolkoff-Rubin N, et al. Overlapping pathways to transplant glomerulopathy: chronic humoral rejection, hepatitis C infection, and thrombotic microangiopathy. *Kidney Int.* (2011) 80:879–85. doi: 10.1038/ki.2011.194
19. Schinstock CA, Mannon RB, Budde K, Chong AS, Haas M, Knechtle S, et al. Recommended treatment for antibody-mediated rejection after kidney transplantation: the 2019 expert consensus from the transplantion society working group. *Transplantation.* (2020) 104:911–22. doi: 10.1097/TP.0000000000003095
20. Sis B, Campbell PM, Mueller T, Hunter C, Cockfield SM, Cruz J, et al. Transplant glomerulopathy, late antibody-mediated rejection and the ABCD

tetrad in kidney allograft biopsies for cause. *Am J Transplant.* (2007) 7:1743–52. doi: 10.1111/j.1600-6143.2007.01836.x
21. Stegall MD, Morris RE, Alloway RR, Mannon RB. Developing new immunosuppression for the next generation of transplant recipients: the path forward. *Am J Transplant.* (2016) 16:1094–101. doi: 10.1111/ajt.13582
22. Schmidt D, Osmanodja B, Pfefferkorn M, Graf V, Raschke D, Duettmann W, et al. TBase—an integrated electronic health record and research database for kidney transplant recipients. *J Vis Exp.* (2021) (170):e61971. doi: 10.3791/61971
23. Moreso F, Crespo M, Ruiz JC, Torres A, Gutierrez-Dalmau A, Osuna A, et al. Treatmet of chronic antibody mediated rejection with intravenous imunoglobulins and rituximab: a multicenter, prespective, randomized, double-blind clinical trial. *Am J Transplant.* (2018) 18:927–35. doi: 10.1111/ajt.14520
24. Gloor JM, Sethi S, Stegall MD, Park WD, Moore SB, DeGoey S, et al. Transplant glomerulopathy: subclinical incidence and association with alloantibody. *Am J Transplant.* (2007) 7:2124–32. doi: 10.1111/j.1600-6143.2007.01895.x
25. Schwimmer J, Nadasdy TA, Spitalnik PF, Kaplan KL, Zand MS. De novo thrombotic microangiopathy in renal transplant recipients: a comparison of hemolytic uremic syndrome with localized renal thrombotic microangiopathy. *Am J Kidney Dis.* (2003) 41:471–9. doi: 10.1053/ajkd.2003.50058
26. Broecker V, Bardsley V, Torpey N, Perera R, Montero R, Dorling A, et al. Clinical-pathological correlations in post-transplant thrombotic microangiopathy. *Histopathology.* (2019) 75:88–103. doi: 10.1111/his.13855
27. Tait DB, Süsal C, Gebel MH, Nickerson PW, Zachary AA, Claas FHJ, et al. Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. *Transplantation.* (2013) 95:19–47. doi: 10.1097/TP.0b013e31827a19cc
28. Ziemann M, Suwelack B, Banas B, Budde K, Einecke G, Hauser I, et al. Determinaation of unacceptale HLA antien mismatches in kidney transplant recipients. *HLA*. (2022) 86:1–15. doi: 10.1111/tan.14521
29. Huber L, Lachmann N, Dürr M, Matz M, Liefeldt L, Neumayer HH, et al. Identification and therapeutic management of highly sensitized patients undergoing renal transplantation. *Drugs.* (2012) 72:1335–54. doi: 10.2165/11631110-000000000-00000
30. Waiser J, Duerr M, Schönemann C, Rudolph B, Wu K, Halleck F, et al. Rituximab in combination with bortezomib, plasmapheresis, and high-dose IVIG to treat antibody-mediated renal allograft rejection. *Transplant Direct.* (2016) 2:e91–9. doi: 10.1097/TXD.0000000000000604
31. Waiser J, Budde K, Schütz M, Liefeldt L, Rudolph B, Schönemann C, et al. Comparison between bortezomib and rituximab in the treatment of antibody-mediated renal allograft rejection. *Nephrol Dial Transplant.* (2012) 27:1246–51. doi: 10.1093/ndt/gfr465
32. Waiser J, Duerr M, Budde K, Rudolph B, Wu K, Bachmann F, et al. Treatment of acute antibody-mediated renal allograft rejection with Cyclophosphaide. *Transplantation.* (2017) 101:2545–52. doi: 10.1097/TP.0000000000001617
33. Choi M, Bachmann F, Wu K, Lachmann N, Schmidt D, Brakemeier S, et al. Microvasular inflammation is a risk factor in kidney transplant recipients with very late conversion from calcineurin inhibitor-based regimens to belatacept. *BMC Nephrol.* (2020) 21:354–64. doi: 10.1186/s12882-020-01992-6
34. Levey AS, Coresh J, Greene T, Stevens LA, Zhang ZPL, Hendriksen S, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med.* (2006) 145:247–54. doi: 10.1373/clinchem.2006.077180
35. Perl J. Kidney transplant failure: failing kidneys, failing care? *Clin J Am Soc Nephrol.* (2014) 9:1153–55. doi: 10.2215/CJN.04670514
36. Laaksonen MA, Webster AC, McCaughan GW, Grulich AE, Vajdic CM. Longitudinal immunosuppresion mize isclassification bias in solid organ transplantation cohorts*Clin Transplant*. (2019) 32:e13470. doi: 10.1111/ctr.13470
37. Stemer G, Lemmens-Gruber R. Clinical pharmacy services and solid organ transplantation: a literature review. *Pharm World Sci.* (2010) 32:7–18. doi: 10.1007/s11096-009-9351-7
38. Loupy A, Suberbielle-Boissel C, Hill GS, Lefaucheur C, Anglicheau D, Zuber J, et al. Outcome of subclinical antibody-mediated rejection in kidney transplant recipients with preformed donor-specific antibodies. *Am J Transplant.* (2009) 9:2561–70. doi: 10.1111/j.1600-6143.2009.02813.x
39. Senev A, Coemans M, Lerut E, Van Sandt V, Daniëls L, Kuypers D, et al. Histological picture of antibody-mediated rejection without donor-specific anti-HLA antibodies: cinical presentation and implications for outcome. *Am J Transplant.* (2019) 19:763–80. doi: 10.1111/ajt.15074
40. Callemeyn J, Ameye H, Lerut E, Senev A, Coemans M, von Loon E, et al. Revisiting the changes in the Banff classification for antibody-mediated rejection after kidney transplantation. *Am J Transplant.* (2021) 21:2413–23. doi: 10.1111/ajt.16474
41. Haas M. The relationship between pathologic lesions of active and chronic antibody-mediated rejection in renal allografts. *Am J Transplant.* (2018) 18:2849–56. doi: 10.1111/ajt.15088
42. Heilman RL, Nijim A, Desmarteau YM, Khamash H, Pando MJ, Chakera HA, et al. De Novo donor-specific human leukocyte antigen antibodies early after kidney transplantation. *Transplantation.* (2014) 98:1310–15. doi: 10.1097/TP.0000000000000216
43. Lefaucheur C, Loupy A, Hill GS, Andrade J, Nochy D, Antoine C, et al. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. *J Am Soc Nephrol.* (2010) 21:1398–406. doi: 10.1681/ASN.2009101065
44. Loupy A, Jordan SC. Transplantation: Donor-specific HLA antibodies and renal allograft failure. *Nat Rev Nephrol.* (2013) 9:130–1. doi: 10.1038/nrneph.2013.18
45. Hönger G, Hopfer H, Arnold ML, Spriewald BM, Schaub S, Amico P. Pretransplant IgG subclasses of donor-specific human leukocyte antigen antibodies and development of antibodymediated rejection. *Transplantation.* (2011) 92:41–7. doi: 10.1097/TP.0b013e31821cdf0d
46. Takemoto SK, Zeevi A, Feng S, Colvin RB, Jordan S, Kobashigawa J, et al. National conference to assess antibody-mediated rejection in solid organ transplantation. *Am J Transplant.* (2004) 4:1033–41. doi: 10.1111/j.1600-6143.2004.00500.x
47. Stegall MD, Gloor J, Winters JL, Moore SB, Degoey SA. Comparison of plasmapheresis vs. high–dose IVIG desensitization in renal allograft recipients with high levels of donor specific alloantibody. *Am J Transplant.* (2006) 6:346–51. doi: 10.1111/j.1600-6143.2005.01178.x
48. Walsh RC, Brailey P, Girnita A, Alloway RR, Shields AR, Wall GE, et al. Early and late acute antibody-mediated rejection differ immunologically and in response to proteasome inhibition. *Transplantation.* (2011) 91:1218–26. doi: 10.1097/TP.0b013e318218e901
49. Gupta G, Abu Jawdeh BG, Bhasin B, Arend L, Trollinger B, Kraus E, et al. Late antibody-mediated rejection in renal allografts: Outcome after conventional and novel therapies. *Transplantation.* (2014) 97:1240–6. doi: 10.1097/01.TP.0000442503.85766.91
50. Touzot M, Couvrat-Desvergnes G, Castagnet S, Cesbron A, Renaudin K, Cantarovich D, et al. Differential modulation of donor-specific antibodies after B-cell depleting therapies to cure chronic antibody mediated rejection. *Transplantation.* (2015) 99:63–8. doi: 10.1097/TP.0000000000000285
51. Lesage J, Noel R, Lapointe I, Côté I, Wagner E, Désy O, et al. Donor-specific antibodies, C4d and their relationship with the prognosis of transplant glomerulopathy. *Transplantation.* (2015) 99:69–76. doi: 10.1097/TP.0000000000000310
52. Senev A, Van Loon E, Lerut E, Callemeyn J, Coemans M, Van Sandt V, et al. Risk factors, histopathological features, and graft outcome of transplant glomerulopathy in the absence of donor-specific HLA antibodies. *Kidney Int.* (2021) 100:401–14. doi: 10.1016/j.kint.2021.01.029
53. Kardol-Hoefnagel T, Otten HG. A comprehensive overview of the clinical relevance and treatment options for antibody-mediated rejection associated with non-HLA Antibodies. *Transplantation.* (2021) 105:1459–70. doi: 10.1097/TP.0000000000003551
54. Joosten SA, Sijpkens YW, van Ham V, Trouw LA, van der Vlag J, van den Heuvel B, et al. Antibody response against the glomerular basement membrane protein agrin in patients with transplant glomerulopathy. *Am J Transplant*. (2005) 5:383–93. doi: 10.1111/j.1600-6143.2005.00690.x
55. Martin L, Guignier F, Mousson C, Rageot D, Justrabo E, Rifle G. Detection of donor-specific anti-HLA antibodies with flow cytometry in eluates and sera from renal transplant recipient with chronic allograft nephropathy.

Transplantation. (2003) 76:395–400. doi: 10.1097/01.TP.0000078895.24606.45
56. Fotheringham J, Angel CA, Mckane W. Transplant glomerulopathy: morphology, associations and mechanism. *Nephron Clin Pract.* (2009) 113:c1–7. doi: 10.1159/000228069
57. Levey AS, Gansevoort RT, Coresh J, Inker LA, Heerspink HL, Grams ME, et al. Change in albuminuria and GFR as end points for clnical trials in early stages of CKD: a scientific workshop sponsored by the national kidney foundationin in collaboration with the US food and drug administration and European medicines agency. *Am J Kidney Dis.* (2020) 75:84–104. doi: 10.1053/j.ajkd.2019.06.009
58. Grams ME, Sang Y, Ballew SH. Matsushita Kunihiro, Astor BC, Carrero JJ, et al. Evaluating glomerular filtration rate slope as a surrogate end point for ESKD in clinical trials: an individual participant meta-analysis of observational data. *J Am Soc Nephrol.* (2019) 30:1746–55. doi: 10.1681/ASN.2019010008
59. McNeill GB, Martin JH. How reliable is eGFR when calculating drug dosage in acute medical admissions. *Intern Med J.* (2011) 41:327–31. doi: 10.1111/j.1445-5994.2010.02307.x
60. Cravedi P, Remuzzi G. Pathophysiology of proteinuria and its value as an outcome measure in chronic kidney disease. *Br J Clin Pharmacol.* (2013) 76:516–23. doi: 10.1111/bcp.12104
61. Wekerle T. T cell subsets predicting belatacept-resistant rejection: finding the root where the trouble starts. *Am J Transplant.* (2017) 17:2235–7. doi: 10.1111/ajt.14390
62. Naesens M, Kuypers DR, De Vusser K, Evenepoel P, Claes K, Bammens B, et al. The histology of kidney transplant failure: A long-term follow-up study. *Transplantation.* (2014) 98:427–35. doi: 10.1097/TP.0000000000000183
63. Chand S, Atkinson D, Collins C, Briggs D, Ball S, Sharif A, et al. The spectrum of renal allograft failure. *PLoS ONE.* (2016) 11:e0168878. doi: 10.1371/journal.pone.0162278
64. Lefaucheur C, Loupy A, Vernerey D, Duog-Van-Huyen JP, Suberbielle C, Anglicheau D, et al. Antibody-mediated vascular rejection of kidney allografts: a population-based study. *Lancet.* (2013) 381:313–9. doi: 10.1016/S0140-6736(12)61265-3
65. Hillebrands JL, Rozing J. Chronic transplant dysfunction and transplant arteriosclerosis: New insights into underlying mechanisms. *Expert Rev Mol Med.* (2003) 5:1–23. doi: 10.1017/S1462399403005057X
66. Patri P, Seshan SV, Matignon M, Matignon M, Desvaux D, Lee JR, et al. Development and validation of a prognostic index for allograft outcome in kidney recipients with transplant glomerulopathy. *Kidney Int.* (2016) 89:450–8. doi: 10.1038/ki.2015.288
67. Dunn TB, Noreen H, Gillingham K, Maurer D, Ozturk OG, Pruett TL, et al. Revisiting traditional risk factors for rejection and graft loss after kidney transplantation. *Am J Transplant.* (2011) 11: 2132–43. doi: 10.1111/j.1600-6143.2011.03640.x
68. Randhawa P. T-cell-mediated rejection of the kidney in the era of donor-specific antibodies: Diagnostic challenges and clinical significance. *Curr Opin Organ Transplant.* (2015) 20:325–32. doi: 10.1097/MOT.0000000000000189

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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

CASE REPORT
published: 24 May 2022
doi: 10.3389/fmed.2022.906925

Case Report: Let Us Not Forget the Treatment That Some Patients Have Received—The Brief 50-Year History of a Kidney Transplant Survivor

Espen Nordheim[1,2]*, *Melinda Raki*[3] *and Karsten Midtvedt*[1]

[1] Department of Transplantation Medicine, Section of Nephrology, Oslo University Hospital, Rikshospitalet, Oslo, Norway, [2] Department of Nephrology, Oslo University Hospital, Oslo, Norway, [3] Department of Pathology, Oslo University Hospital, Rikshospitalet, Oslo, Norway

OPEN ACCESS

Edited by:
Kathrin Eller,
Medical University of Graz, Austria

Reviewed by:
Noble Johan,
Centre Hospitalier Universitaire de Grenoble, France
Georg Böhmig,
Medical University of Vienna, Austria

***Correspondence:**
Espen Nordheim
b23697@ous-hf.no

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *29 March 2022*
Accepted: *03 May 2022*
Published: *24 May 2022*

Citation:
Nordheim E, Raki M and Midtvedt K (2022) Case Report: Let Us Not Forget the Treatment That Some Patients Have Received—The Brief 50-Year History of a Kidney Transplant Survivor. Front. Med. 9:906925. doi: 10.3389/fmed.2022.906925

Background: There has been a considerable improvement in post-transplant care since the early 1960s. Some patients we meet in the clinic have personally experienced this progress and have histories to tell that one must not forget. This is the brief history of a long-time "transplant survivor."

Case Presentation: In 1970, a young woman developed acute oedema, proteinuria, hypertension and oliguria during pregnancy. Labor was induced, but neither the child nor the kidney function could be saved. Our patient started dialysis, and 4 years later received a kidney transplant donated by her father (then 55 years of age). Maintenance immunosuppression consisted of prednisolone and azathioprine until 2011, when azathioprine was switched to everolimus due to skin cancer. Before this, our patient was highly satisfied with prednisolone/azathioprine, despite discussions regarding newer immunosuppressive drugs, and always reminded the treating physician that one should "never change a winning team." Retrospectively, the avoidance of calcineurin inhibitors might have been beneficial for this patient who still has preserved an excellent renal function with s-creatinine levels around 100 μmol/L and just had sparse fibrosis detected in a recently performed transplant biopsy. The transplanted kidney is now 101 years old and is still working 24/7.

Conclusions: Our patient received a kidney transplant for 46 years ago and still has a remarkably stable transplant function with s-creatinine levels around 100 μmol/L. This case report illustrates the potential endurance of the kidneys and is a reminder to keep taking individualized treatment decisions even though new treatment alternatives promise superiority.

Keywords: kidney, transplantation - kidney, biopsy, immunosuppressants, history

BACKGROUND

Recently, a 72-year-old Caucasian woman who has been followed at our unit for 50 years came for a regular out- patient visit. She developed renal failure in 1970 and received a kidney transplant in 1974. Her kidney transplant has been well- functioning ever since, despite 46 years' treatment with immunosuppressive medication. **In April 2020 when the kidney transplant had passed 101 years of age, a biopsy was taken (Figure 1),** demonstrating only sparse fibrosis.

This is the brief history of a long-time transplant survivor.

CASE PRESENTATION

Our patients' medical history started in 1970 when she was pregnant (para 1). At the end of the last trimester, she developed oedema and proteinuria without signs of hypertension earlier during the pregnancy. Six days before the estimated time of delivery, she developed severe vaginal bleedings, hypertension (150/130 mmHg), proteinuria (2 g/24 h), oedema and eventually oliguria. Placental bleeding was suspected leading to an emergency induced labor, which resulted in stillbirth. Post-delivery blood pressure stabilized without antihypertensive treatment, but oliguria persisted and eventually our patient became anuric, thus peritoneal dialysis was started.

As the clinical presentation was considered atypical for pregnancy- related kidney disease, it was decided to perform a kidney biopsy. After the first attempt with a blindly sampled percutaneous procedure not obtaining any representative material, an open biopsy procedure was chosen for the second attempt. The pathologists described generalized cortical necrosis in the kidney biopsies, thought to be caused by severe pre-eclampsia. Urine production gradually increased and dialysis could be halted after about 5 weeks. After cessation of dialysis, renal function was stable with creatinine clearance levels around 15–16 ml/min and proteinuria 1.1 g/24 h. Blood pressure levels remained elevated at 160–180/100–110 mmHg, but no antihypertensive treatment was started. At a routine consultation in October 1973, the treating physician described her as "*wellbeing*" even though hemoglobin level of 4.7 g/dl and s-creatinine at 1122 μmol/L (12.7 mg/dl) was remarked. Our patient was informed to start oral iron supplementation and that ….*there was an indication for kidney transplantation!* Subsequently, pre- transplant work- up was initiated what included evaluation of family members as potential donors. The father of our patient (then aged 55) was accepted as donor and the transplantation was scheduled for January 1974. Human Leucocyte Antigen (HLA) - typing for HLA-A and HLA-B was performed in both donor and recipient and two HLA-mismatches were found, which was categorized as a D-match. Our patient needed to restart dialysis 2 months before the scheduled transplantation; at this point haemodialysis *via* an arterial- venous shunt (1) (**Figure 2**) was chosen.

Abbreviations: HLA, human leukocyte antigen; mTOR, inhibitor of the mammalian target of rapamycin; 6-TGN, 6-thioguanine nucleotides.

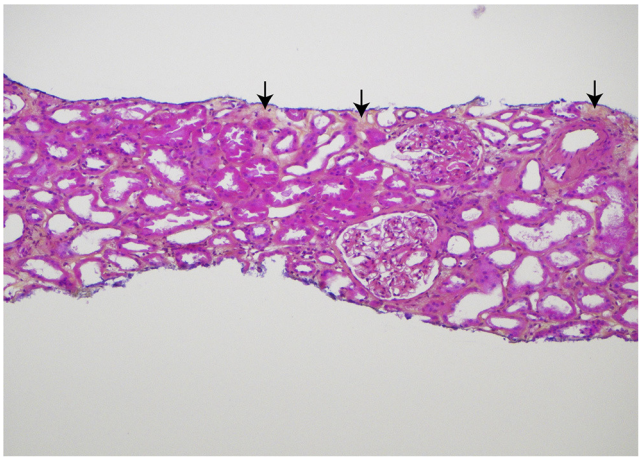


FIGURE 1 | Histologic findings in the core needle biopsy of the 101-year old kidney transplant, sampled April 2020. Hematoxylin, eosin, and saffron (HES) stained section demonstrating only sparse, focal interstitial fibrosis (yellow areas with arrows). There is no interstitial inflammation and only a slight, segmental increase of the mesangial matrix in some glomeruli. Original magnification ×100. Published in agreement with the patient.

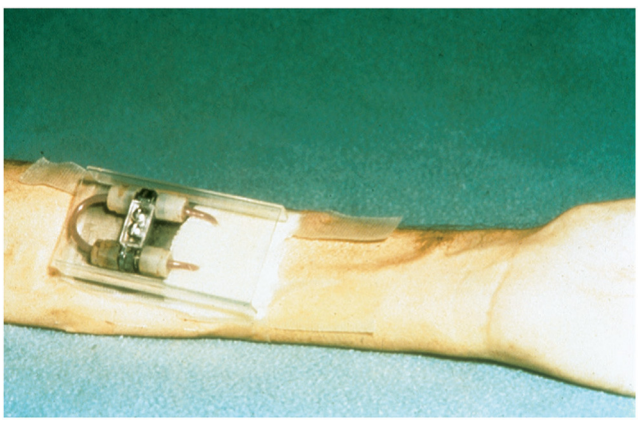


FIGURE 2 | "Schribner shunt" in place, at the left arm of the patient after 4 weeks attached to a stainless steel arm plate protected by a plastic cover placed over the shunt. Reproduced from Quinton et al. (1) with permission of Wolters Kluwer Health, Inc.

The kidney transplantation performed January 1974 included simultaneous bilateral nephrectomy common at the time (2). An accidental bleeding during the transplant procedure led to a per-operative splenectomy. Total cold ischemia time of 44 min was registered for the kidney transplant and 3,000 ml infusion fluids were given to the transplant recipient together with 300 mg hydrocortisone and 175 mg azathioprine as initial immunosuppression.

A clinical rejection was suspected on post- transplant day 6 due to an increase in s-creatinine- from 106 μmol/L (1.2 mg/dl) to 150 μmol/L (1.7 mg/dl). Anti- rejection treatment consisting of 5 gram intravenous methylprednisolone and radiation therapy [150 Roentgen × 3 (equivalent to 1.5 Gy × 3)] was started

without histological verification of the rejection diagnosis. Renal function stabilized [creatinine 115 μmol/L (1.3 mg/dl)] after the rejection episode and the patient was discharged at day 12 with the following daily medication: prednisolone 50 mg q.d, azathioprine 175 m g q.d., furosemide 40 mg t.d.s. and no anti-hypertensive treatment.

At the clinical visit at 1 year after transplantation she reported to be very well. The clinician noted cushingoid characteristics, 124/60 mmHg blood pressure and creatinine clearance 93 ml/min. Our patient was informed to continue following medication: prednisolone 175 mg q.d., azathioprine 225 mg q.d and furosemide 40 mg q.d, in addition to iron supplements and antacids. Eighteen months after transplantation the prednisolone dose was tapered to 10 mg q.d. and azathioprine dose to 100 mg q.d.

The following years went without any specific concerns. Renal function remained stable with serum creatinine values around 105 μmol/L (1.3 mg/dl).

From 15 years on after transplantation a broad specter of skin manifestations was diagnosed and treated: solar keratosis, fibroepithelial polyps, seborrheic keratosis, nodular basal cell carcinoma and squamous cell carcinoma. The different skin lesions slowly improved after azathioprine was switched to everolimus, an inhibitor of the mammalian target of rapamycin (mTORi) in 2011 (trough 4–8 μg). After the drug switch, serum cholesterol levels increased, followed by intensified lipid-lowering therapy. In 2017, she developed symptoms of angina pectoris. Coronary angiography revealed left coronary artery stenosis and a drug eluting stent was successfully implanted. Bone density has been measured regularly. The first signs of osteopenia were registered in 1998 and regional osteoporosis was diagnosed in 2019.

In April 2020, her blood pressure was 124/60 mmHg and serum creatinine value was 113 μmol/L. Current medication consisted of prednisolone 5 mg × 1, Everolimus 1 mg × 2, acetylsalicylic acid 75 mg × 1, rosovastatin 10 mg, ezetimib 10 mg, in addition to a combination of calcium and vitamin D at 1,000 mg/800 units.

DISCUSSION

This patient is a living witness of modern nephrology history. Hemoglobin levels below 5 g/dl due to renal anemia was treated with blood-transfusions and iron supplements in the 1960–70s; recombinant erythropoietin arrived on the marked in the late 1980s (3). Blood access for receiving haemodialysis prior to transplantation was achieved through an indwelling shunt placed externally on the forehand (**Figure 2**). The first kidney transplantation in Norway was performed in 1956, but the official transplant program was only 6 years old when our patient was transplanted in 1974.

Short and long-term outcome following kidney transplantation in the 70s was poor. One-year rejection rates were 70%−80% while the 1- and 5-year patient survival was 60 and 45%, respectively (4). In 1974, the pre- transplant immunological testing was restricted to HLA-A and HLA-B phenotyping in addition to cross- matching, and mismatches

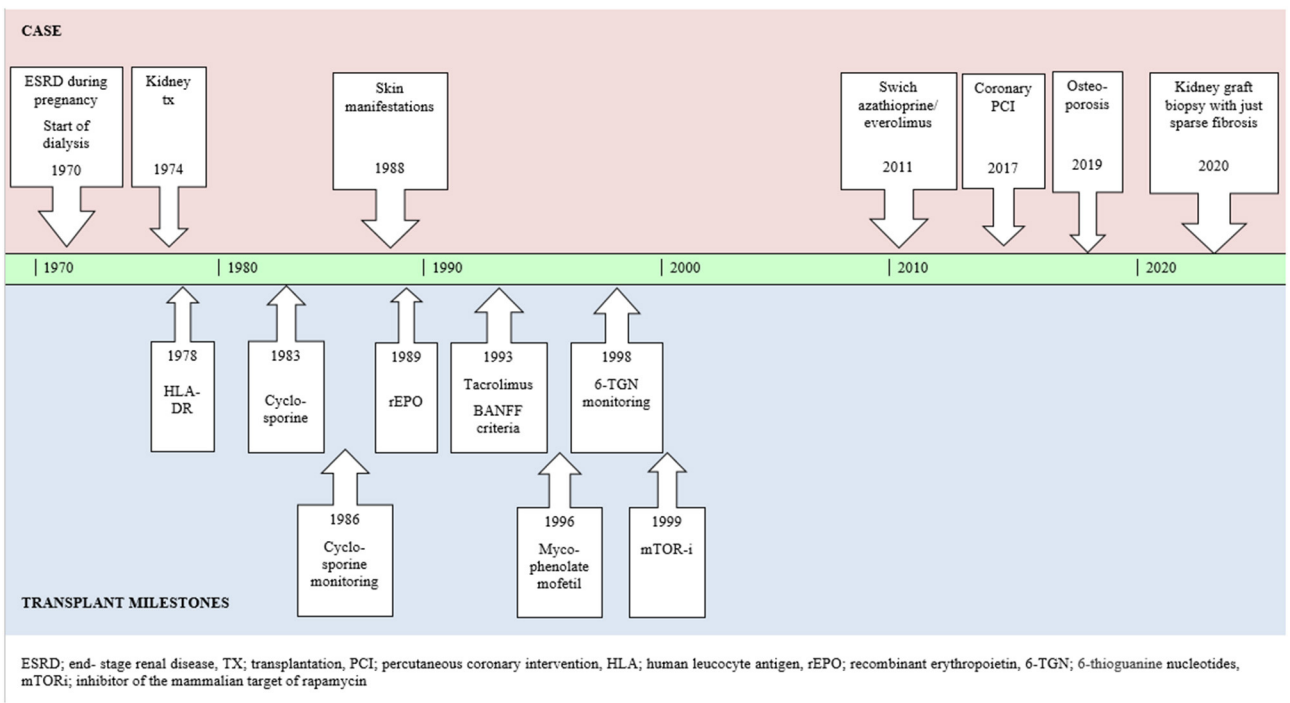


FIGURE 3 | Case report summarized in timeline including relevant milestones of the kidney transplant history.

were graded from A to G in most Scandinavian centers (5). Four years later, after the introduction of HLA-DR typing; 1-year graft survival was 55% for HLA-DR incompatible kidney transplants and 87% for HLA-DR compatible transplants in our center (5). Prednisolone and azathioprine were the only two immunosuppressive drugs available in transplantation at the time. One dose fitted all and individual azathioprine treatment, based on 6-thioguanine nucleotides (6-TGN) - monitoring, were still two decades away (6). Radiation therapy and 5 g of methylprednisolone was used for treatment of rejection suspected from clinical markers alone; more standardized rejection criteria based on histology findings was not introduced until 1993 (7). Radiation treatment has later been abandoned in kidney transplantation (8). Even though methylprednisolone is still in use, the recommended doses are much lower and usually only utilized in the case of biopsy-proven rejection.

In this early transplant era, 15% of the patients died of infections during the first year in our center. Pneumocystis jiroveci prophylaxis was not routinely applied in kidney transplant recipients until late 1990's.

Switches to "new and better" immunosuppressive treatment was repeatedly discussed with the patient as cyclosporine (1983), tacrolimus (1993) and mycophenolate mofetil (1996) became available (**Figure 3**). However, our patient felt confident with her treatment and did not want to "take the risk" of changing a medication she experienced as safe and was familiar with. Retrospectively, avoidence of the nephrotoxic calcineurin-inhibitors might have been beneficial for our patient to preserve excellent renal function.

The introduction of the calsinurin inhibitors (CNI) cyclosporine/tacrolimus was of significant importance improved graft and patient survival following kidney transplantation (9–12). Shortly after the introduction of cyclosporin Myers et al. (13) demonstated how "long- term" use of cyclosporin was associated with an irreversible deterioration of renal function due to tubulo- intestinal injury and glomerulosclerosis. These findings have been confirmed by others both for cyclosporin and tacrolimus (14–17). One must remember that in this early phase of CNI use the dosing was much higher and often in mg/kg and not according to measured concentration (trough values) The concept of CNI- toxicity is multifactorial with both demografic and pharmacogenetic flexibilty and is still beeing discussed (18).

Calcineurin inhibitors are still the cornerstones in maintenance immunosuppression after kidney transplantation; and tacrolimus has largely become the first choice due to better tolerability, rejection prevention and graft survival. Low-dose tacrolimus protocols have been implemented in several centers after it was found safe and advantageous for renal function when combined with mycophenolate mofetil and corticosteroids after renal transplantation (19, 20). A tacrolimus-based immunosuppressive regime was given to over 90% of new adult kidney transplant recipients in the United States in 2020 (21). CNI- free protocols after renal transplantation are available, which includes mTOR- inhibitors (22) or belatacept (23) but often lead to more rejections.

Our patient did, however, switch from azathioprine to everolimus in 2011 after being treated for severeal skin cancers, as the mTORs then had demonstrated a possible reduced risk for skin cancer (24).

After this swich, a severe worsening of her blood lipid profile was registered, a well-known side- effect of everolimus (25). Fluvastatin was initiated in order to reduce the cardiovascular risk (26) and later replaced with rosuvastatin (27). Despite these preventive efforts, our patient developed symptomatic angina, which was efficiently treated with percutaneous coronary intervention in 2017.

This kidney transplant has been through 101 rough years, but still there is only sparse fibrosis in the recent transplant biopsy which by our pathology unit was evaluated as a normal kidney transplant biopsy according to the Banff classification: The s-creatinine remains at levels around 100 μmol/L (1.1 g/dl) and just sparse proteinuria has been registered. It is out of the range for this report to answer how old a transplanted kidney can get, but we do think this case illustrates which endurance the kidneys might have but also that the expression "never change a winning team" might be relevant in the navigation of different immunosuppressive regimens in the follow-up of kidney transplant recipients.

The story doesn't end here but goes on and just like in the fairytales … *the patient and her transplanted kidney lived happily ever after……*

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

EN, MR, and KM created the idea and reviewed and finished the manuscript. EN drafted the manuscript. All authors have read and approved the manuscript.

ACKNOWLEDGMENTS

We would like to thank our patient who has given her written consent for sharing her medical history for this case report and for allowing us to demonstrate the picture of the kidney transplant biopsy in this publication.

REFERENCES

1. Quinton W, Dillard D, Scribner BH. Cannulation of blood vessels for prolonged hemodialysis. *Trans Am Soc Artif Intern Organs.* (1960) 6:104–13.
2. Midtvedt K, Hartmann A, Bentdal O, Brekke IB, Fauchald P. Bilateral nephrectomy simultaneously with renal allografting does not alleviate hypertension 3 months following living-donor transplantation. *Nephrol Dial Transplant.* (1996) 11:2045–9. doi: 10.1093/oxfordjournals.ndt.a027094
3. Eschbach JW, Abdulhadi MH, Browne JK, Delano BG, Downing MR, Egrie JC, et al. Recombinant human erythropoietin in anemic patients with end-stage renal disease. Results of a phase III multicenter clinical trial. *Ann Intern Med.* (1989) 111:992–1000. doi: 10.7326/0003-4819-111-12-992
4. Albrechtsen D, Pfeffer PF. *En Gave For Livet*. Vol 1. Bergen: Vigmostad & Bjørke AS (2011), p. 58–60.
5. Albrechtsen D, Flatmark A, Jervell J, Solheim B, Thorsby E. HLA-DR antigen matching in cadaver renal transplantation. *Lancet.* (1978) 1:825. doi: 10.1016/S0140-6736(78)93026-X
6. Bergan S, Rugstad HE, Bentdal O, Sødal G, Hartmann A, Leivestad T, et al. Monitored high-dose azathioprine treatment reduces acute rejection episodes after renal transplantation. *Transplantation.* (1998) 66:334–9. doi: 10.1097/00007890-199808150-00010
7. Solez K, Axelsen RA, Benediktsson H, Burdick JF, Cohen AH, Colvin RB, et al. International standardization of criteria for the histologic diagnosis of renal allograft rejection: the Banff working classification of kidney transplant pathology. *Kidney Int.* (1993) 44:411–22. doi: 10.1038/ki.1993.259
8. Godfrey AM, Salaman JR. Radiotherapy in treatment of acute rejection of human renal allografts. *Lancet.* (1976) 1:938–9. doi: 10.1016/S0140-6736(76)92715-X
9. Calne RY, White DJ, Thiru S, Evans DB, McMaster P, Dunn DC, et al. Cyclosporin A in patients receiving renal allografts from cadaver donors. *Lancet.* (1978) 2:1323–7. doi: 10.1016/S0140-6736(78)91970-0
10. Calne RY, Rolles K, White DJ, Thiru S, Evans DB, McMaster P, et al. Cyclosporin A initially as the only immunosuppressant in 34 recipients of cadaveric organs: 32 kidneys, 2 pancreases, and 2 livers. *Lancet.* (1979) 2:1033–6. doi: 10.1016/S0140-6736(79)92440-1
11. Starzl TE, Todo S, Fung J, Demetris AJ, Venkataramman R, Jain A, et al. 506 for liver, kidney, and pancreas transplantation. *Lancet.* (1989) 2:1000–4. doi: 10.1016/S0140-6736(89)91014-3
12. Fung JJ, Abu-Elmagd K, Todo S, Shapiro R, Tzakis A, Jordan M, et al. Overview of FK506 in transplantation. *Clin Transpl.* (1990) 115–21.
13. Myers BD, Ross J, Newton L, Luetscher J, Perlroth M. Cyclosporine-associated chronic nephropathy. *N Engl J Med.* (1984) 311:699–705. doi: 10.1056/NEJM198409133111103
14. Palestine AG, Austin HA 3rd, Balow JE, Antonovych TT, Sabnis SG, Preuss HG, et al. Renal histopathologic alterations in patients treated with cyclosporine for uveitis. *N Engl J Med.* (1986) 314:1293–8. doi: 10.1056/NEJM198605153142005
15. Klintmalm G, Bohman SO, Sundelin B, Wilczek H. Interstitial fibrosis in renal allografts after 12 to 46 months of cyclosporin treatment: beneficial effect of low doses in early post-transplantation period. *Lancet.* (1984) 2:950–4. doi: 10.1016/S0140-6736(84)91166-8
16. Starzl TE, Fung J, Jordan M, Shapiro R, Tzakis A, McCauley J, et al. Kidney transplantation under FK 506. *JAMA.* (1990) 264:63–7. doi: 10.1001/jama.264.1.63
17. Randhawa PS, Shapiro R, Jordan ML, Starzl TE, Demetris AJ. The histopathological changes associated with allograft rejection and drug toxicity in renal transplant recipients maintained on FK506. Clinical significance and comparison with cyclosporine. *Am J Surg Pathol.* (1993) 17:60–8. doi: 10.1097/00000478-199301000-00007
18. Xia T, Zhu S, Wen Y, Gao S, Li M, Tao X, et al. Risk factors for calcineurin inhibitor nephrotoxicity after renal transplantation: a systematic review and meta-analysis. *Drug Des Devel Ther.* (2018) 12:417–28. doi: 10.2147/DDDT.S149340
19. Ekberg H, Tedesco-Silva H, Demirbas A, Vítko S, Nashan B, Gürkan A, et al. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med.* (2007) 357:2562–75. doi: 10.1056/NEJMoa067411
20. Størset E, Åsberg A, Hartmann A, Reisaeter AV, Holdaas H, Skauby M, et al. Low-target tacrolimus in de novo standard risk renal transplant recipients: a single-centre experience. *Nephrology.* (2016) 21:821–7. doi: 10.1111/nep.12738
21. Lentine KL, Smith JM, Hart A, Miller J, Skeans MA, Larkin L, et al. OPTN/SRTR 2020 annual data report: kidney. *Am J Transplant.* (2022) 22(Suppl 2):21–136. doi: 10.1111/ajt.16982
22. Holdaas H, Rostaing L, Serón D, Cole E, Chapman J, Fellstrøm B, et al. Conversion of long-term kidney transplant recipients from calcineurin inhibitor therapy to everolimus: a randomized, multicenter, 24-month study. *Transplantation.* (2011) 92:410–8. doi: 10.1097/TP.0b013e318224c12d
23. Vincenti F, Charpentier B, Vanrenterghem Y, Rostaing L, Bresnahan B, Darji P, et al. A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study). *Am J Transplant*. 2010;10:535-4611 doi: 10.1111/j.1600-6143.2009.03005.x
24. Campistol JM, Eris J, Oberbauer R, Friend P, Hutchison B, Morales JM, et al. Sirolimus therapy after early cyclosporine withdrawal reduces the risk for cancer in adult renal transplantation. *J Am Soc Nephrol.* (2006) 17:581–9. doi: 10.1681/ASN.2005090993
25. Budde K, Becker T, Arns W, Sommerer C, Reinke P, Eisenberger U, et al. Everolimus-based, calcineurin-inhibitor-free regimen in recipients of de-novo kidney transplants: an open-label, randomised, controlled trial. *Lancet.* (2011) 377:837–47. doi: 10.1016/S0140-6736(10)62318-5
26. Holdaas H, Fellström B, Jardine AG, Holme I, Nyberg G, Fauchald P, et al. Effect of fluvastatin on cardiac outcomes in renal transplant recipients: a multicentre, randomised, placebo-controlled trial. *Lancet.* (2003) 361:2024–31. doi: 10.1016/S0140-6736(03)13638-0
27. Robertsen I, Asberg A, Granseth T, Vethe NT, Akhlaghi F, Ghareeb M, et al. More potent lipid-lowering effect by rosuvastatin compared with fluvastatin in everolimus-treated renal transplant recipients. *Transplantation.* (2014) 97:1266–71. doi: 10.1097/01.TP.0000443225.66960.7e

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.

frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 10 June 2022
doi: 10.3389/fmed.2022.904795

Disqualification of Donor and Recipient Candidates From the Living Kidney Donation Program: Experience of a Single-Center in Germany

Melissa Grigorescu[1,2], Stephan Kemmner[3], Ulf Schönermarck[1], Isidora Sajin[3], Wolfgang Guenther[3], Tiago Lemos Cerqueira[2,4], Ben Illigens[2], Timo Siepmann[5], Bruno Meiser[3], Markus Guba[3,6], Michael Fischereder[1,3†] and Manfred Johannes Stangl[3,6†]*

[1] Division of Nephrology, Department of Internal Medicine IV, University Hospital Munich, Ludwig-Maximilians University (LMU), Munich, Germany, [2] German Sites Development Principles and Practice of Clinical Research Harvard T.H., Chan School of Public Health, Dresden International University, Dresden, Germany, [3] Transplant Center, University Hospital of Munich, Ludwig-Maximilians University (LMU), Munich, Germany, [4] Department of Kidney Transplant, Hospital Evangelico de Minas Gerais, Belo Horizonte, Brazil, [5] Department of Neurology, University Hospital Carl Gustav Carus, Technische Universitaet Dresden, Dresden, Germany, [6] Department of General, Visceral, and Transplant Surgery, University Hospital of Munich, Ludwig-Maximilians University (LMU), Munich, Germany

Background: Kidney transplantation is the best treatment option for patients with end-stage kidney disease (ESKD) with a superiority of graft survival after living kidney donation (LKD) compared to deceased donation. However, a large part of potential donors and recipients are ineligible for LKD. Here, we analyze the leading causes for disqualification of potential living donor-recipient pairs from the LKD program and the health-related consequences for ESKD patients excluded from the LKD program in a German transplant center.

Methods: In this single-center retrospective cohort study we evaluated all candidates (potential donors and recipients) presenting for assessment of LKD from 2012 to 2020 at our transplant center. Thereby we focused on candidates excluded from the LKD program. Main reasons for disqualification were categorized as medical (donor-related), psychosocial, immunological, recipient-related, and unknown.

Results: Overall, 601 donor-recipient pairs were referred to our transplant center for LKD assessment during the observation time. Out of those, 326 (54.2%) discontinued the program with 52 (8.7%) dropouts and 274 (45.6%) donor-recipient pairs being ineligible for LKD. Donor-related medical contraindications were the main reason for disqualification [139 out of 274 (50.7%) potential donors] followed by recipient-related contraindications [60 out of 274 (21.9%) of potential donor-recipient pairs]. Only 77 out of 257 (29.9%) potential recipients excluded from the LKD program received a kidney transplant afterward with a median waiting time of 2 (IQR: 1.0–4.0) years. Overall, 18 (7.0%) ESKD patients initially declined for LKD died in this period.

OPEN ACCESS

Edited by:
Miriam Banas,
University Hospital Regensburg, Germany

Reviewed by:
Christian Morath,
Heidelberg University, Germany
Tobias Bergler,
University Hospital Regensburg, Germany
Christian Hugo,
University Hospital Carl Gustav Carus, Germany

***Correspondence:**
Melissa Grigorescu
melissa.grigorescu_vlas@med.uni-muenchen.de

[†]These authors share senior authorship

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: 25 March 2022
Accepted: 23 May 2022
Published: 10 June 2022

Citation:
Grigorescu M, Kemmner S, Schönermarck U, Sajin I, Guenther W, Cerqueira TL, Illigens B, Siepmann T, Meiser B, Guba M, Fischereder M and Stangl MJ (2022) Disqualification of Donor and Recipient Candidates From the Living Kidney Donation Program: Experience of a Single-Center in Germany. Front. Med. 9:904795. doi: 10.3389/fmed.2022.904795

Conclusion: A large percentage of donor-recipient pairs are disqualified from the German LKD program, mostly due to medical reasons related to the donor and with partly severe consequences for the potential recipients. For these, alternative solutions that promptly enable kidney transplantation are essential for improving patient quality of life and survival.

Keywords: living kidney donation, living donor candidates, disqualification living kidney donors, end-stage kidney disease, kidney transplantation

INTRODUCTION

Although kidney transplantation (KTx) confers the best survival benefit for patients with end-stage kidney disease (ESKD), the number of patients on the waiting list for KTx significantly exceeds the available donor kidneys worldwide (1). Living kidney donation (LKD) is one way to close this shortage with improved long-term graft and patient survival compared to KTx after deceased donation (2). Reports on global LKD rates vary widely, with countries such as Japan reporting a 90% LKD rate whereas northern-European countries attain roughly 15–30% (1, 3, 4). In Germany, LKD represents 25–30% of all donations from 2012 to 2020 with a slight decrease in the past years (1). The benefits of LKD over deceased KTx are mainly given by the overall better organ quality and the feasibility of pre-emptive transplantation as well as ABO- and human leucocyte antigen (HLA)-incompatible transplantation (5, 6). However, these recipient-related benefits should be carefully weighed against the perioperative morbidity, mortality and long-term risks for cardiovascular morbidity that potential healthy donors are exposed (6). Current guidelines for LKD evaluation providing recommendations for the transplant community show some differences in acceptable thresholds for living donors, which, among other factors, explain the variability of donor acceptance in transplant programs worldwide (7–11). These differences are evidenced by several studies reporting on the proportion and the reasons for exclusion of prospective living donors (12–14). However, data on why potential donors are disqualified for LKD in Germany are lacking. This explorative analysis evaluates the exclusion rates and the reasons for disqualification of potential donors and recipients for LKD in a transplant center in Germany. We further report the health-related consequences for ESKD patients excluded from the LKD program.

MATERIALS AND METHODS

This is a single center, retrospective cohort study concerning all potential kidney donors and respective recipients that presented for initial assessment at the LKD program of the transplant center of the LMU University Hospital in Munich from January 2012 to December 2020. The follow-up period for patients with ESKD was until December 2021. The study protocol was approved by the local ethics committee of the LMU Munich (Project number 21-0563).

The Living Kidney Donation Evaluation Program at the Ludwig-Maximilians University Munich Hospital

Potential donors and recipients were evaluated according to the LKD program protocol of our institution. **Figure 1** illustrates a flow chart of the LKD evaluation program. First, ESKD patients and potential donors are referred by a primary care nephrologist for the initial assessment. A team consisting of a transplant coordinator, a transplant surgeon and a nephrologist conduct the first consult. Blood samples from potential donors and recipients are obtained for immunological analysis. The immunology department reports on blood group, HLA typing, antibody detection, and crossmatch. The potential recipient is evaluated independently from the donor and, if no contraindications are yielded, the patient can be listed at the deceased donor waiting-list of the Eurotransplant kidney allocation system (ETKAS) or Eurotransplant senior program (ESP) of Eurotransplant (ET). The donor medical work-up progresses simultaneously according to recommendations of the KDIGO Guidelines. If the donor does not present contraindications, both recipient and donor undergo psychological evaluation, where the individuals and the relationship between them are examined by a psychologist. Upon completion, both donor and recipient must present for final assessment at our transplant center. Here, a nephrologist, a transplant surgeon and a general practitioner re-evaluate the findings of both candidates. Finally, assessment by an independent ethics committee of the state's medical association is necessary. After acceptance by all the above, surgery is planned as best estimated by the medical staff, the donor and the recipient. Candidates (potential donors and recipients) withdrawing the LKD program for personal reasons or voluntarily changing the transplant center before assessment completion are categorized as drop-outs (**Figure 1**, highlighted in gray). All other candidates (potential donors and recipients) that yield any contraindications are highlighted in red (**Figure 1**).

Disqualification Criteria and Study Population

The study population included all potential donors and recipients that presented for the first assessment of the LKD

Abbreviations: CKD, chronic kidney disease; DSO, Deutsche Stiftung Organtransplantation; eGFR, estimated glomerular filtration rate; ESKD, End-stage kidney disease; ESP, Eurotransplant senior program; ET, Eurotransplant; ETKAS, Eurotransplant kidney allocation system; HLA, human leucocyte antigen; IQR, Interquartile range; KDIGO, Kidney disease: Improving Global Outcomes; KTx, Kidney transplantation; LKD, Living kidney donation; LKDPI, Living Kidney Donor Profile Index; mGFR, measured glomerular filtration rate; SOLKID, Safety of the Living Kidney Donor.

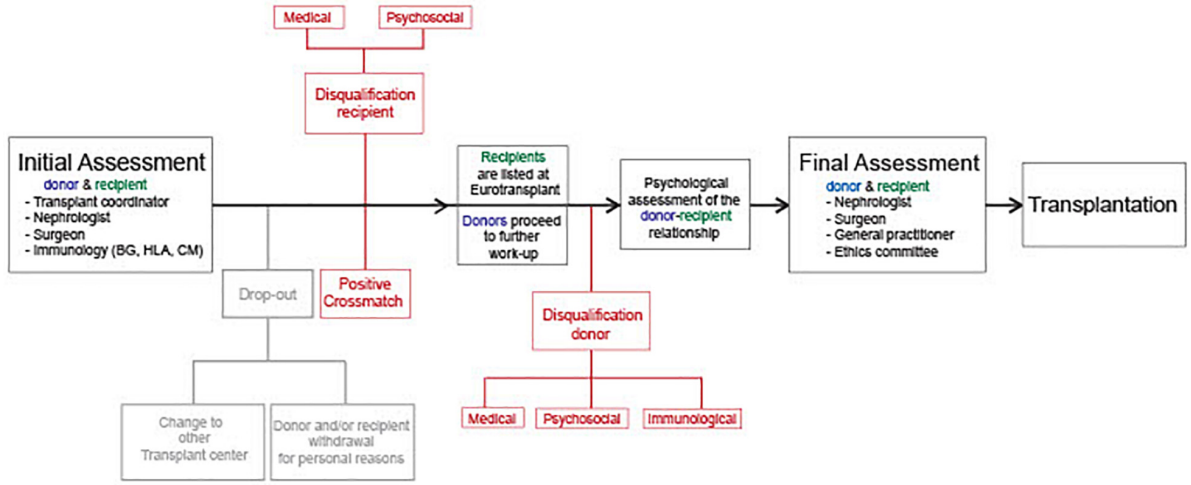


FIGURE 1 | Flow chart of the LKD program: Flow chart depicting the assessment of potential living kidney donors and recipients at the LMU University Hospital Munich. The black arrow represents a timeline with the stages of the living kidney donor (LKD) program leading to successful transplantation. Donors and/or recipients withdrawing the LKD program due to relocation to another transplant center or undetermined personal reasons are highlighted in gray. Recipients and/or potential donors disqualified from the LKD program are represented in red. BG, blood group; HLA, human leucocyte antigen; CM, crossmatch.

program at our transplant center. For the present analysis, donors and recipients were analyzed as couples in order of presentation (donor-recipient pairs). However, potential recipients were allowed to present with two or more donors, representing an independent donor-recipient pair. The criteria for disqualification of the potential donor-recipient pairs at the LKD program were categorized as medical (donor-related), immunological, psychosocial, recipient-related and unknown. The latter includes all donor-recipient pairs excluded from LKD where reasons for disqualification were not documented. Absolute and relative contraindications for potential donors assessed for LKD are listed in **Table 1**. It is worth mentioning that potential donors with an initially estimated glomerular filtration rate (eGFR) and a calculated creatinine clearance by 24-h collection urine around the threshold of acceptance were subsequently referred to renal nuclear scan (specifically Technetium-99m-diethylene-triamine-pentaacetate (Tc-99m-DTPA) scan) for further evaluation. Therefore, disqualified donors due to impaired kidney function were finally excluded based on measured GFR in Tc-99m-DTPA scans (see **Table 1**). Potential donors with relative contraindications were analyzed in a case-dependent manner depending on the individual risk (**Table 1**). Absolute and relative contraindications were based on KDIGO Guidelines and adjusted to the current version of the manual for evaluation of kidney transplant candidates by the working group of kidney transplant centers in North Rhine-Westphalia (15). Of note, ABO- and HLA-incompatibility were not considered absolute contraindications, contrary to previous published data (**Table 1**) (16). This is due to meanwhile established treatment methods that enable ABO- and HLA-incompatible transplantations (17). ABO-incompatible transplantations were analyzed case dependently. No IgG/IgM isoagglutinin-titer threshold was defined as exclusion criteria; however, preoperative desensitization was mandatory. Also, HLA-incompatible transplantations were analyzed in a case dependent manner. Recipients with a high titer of donor-specific antibodies (DSA) (i.e., mean fluorescent intensity (MFI) > 10,000 as well as a positive B- and T-cell cross-match were excluded. Patients with either Luminex-detected DSA with an MFI > 3,000 and a negative cross-match or, a positive CDC-B-cell and/or Luminex cross-match and MFI < 3,000 were accepted after individual case discussion. Pre-operative desensitization was mandatory if accepted for LKD.

Recipient-related contraindications included any relevant medical or psychological conditions attaining a higher risk for the recipient. **Table 2** shows the most relevant absolute and relative medical and psychological conditions that exclude potential recipients from the LKD program based on KDIGO Guidelines (18). Patients with multiple comorbidities were recipients with at least three advanced medical conditions, among them at least one or the combination of them implying a significant reduction of the patients' estimated survival according to the standards in Germany (**Table 2**). Under relative contraindications we include conditions which can be changed or resolved over time, therefore only delaying LKD assessment, and/or conditions that should be assessed individually. Here, a too long dialysis vintage (i.e., over 8 years) and thus a period of time resembling the average waiting time for ESKD patients on the deceased kidney transplant list in Germany with a reasonable chance of receiving a deceased kidney in a short period of time, and a stable kidney function, defined by an eGFR of at least 15 ml/min and a low likelihood for progression of ESKD in need for renal replacement therapy for the next 6 months, were included. In many cases, potential donors and recipients presented with more than one contraindication for LKD. Donor-recipient pairs presenting with more than one relative

TABLE 1 | Absolute and relative contraindications of potential donors for LKD.

Absolute
Medical
Age < 18 years old
Impaired kidney function[#]
mGFR < 70 ml/min 1.73 m^2
Nephrological
Manifest kidney disease (e.g., Alport syndrome)
Glomerular microhematuria (with signs of kidney disease)
Proteinuria and/or Albuminuria (>300 mg/d)
Cardiovascular
Hypertension [poorly controlled (>140/90 mmHg) with more than two medications]
Diabetes (any type) or pathological oGTT
Active smoking
Arteriosclerosis (as assessed by Doppler ultrasound or CT scan)[§]
BMI > 35 kg/m^2 (without weight loss)
Urological
Incidental abnormal kidney cysts, vessels or ureter
Unclear incidental macrohematuria
Nephrolithiasis or high risk for nephrolithiasis
Malignancy
Active (excluding treatable *in situ* carcinoma such as prostate cancer Gleason < 6
Non-melanoma skin cancer, *in situ* bladder-carcinoma, *in situ* cervical cancer)
In recent past medical history
Active infectious disease (Hepatitis B/C, HIV, TBC)
Genetic disorders associated with kidney disease (e.g., polycystic kidney disease)
Psychiatric disease
Immunological*
Positive crossmatch
Psychosocial
No meaningful relationship between donor and recipient*
Signs of coercion*
Uncertainty for transplantation
Active substance abuse (alcohol, illicit drugs)
Relative
Medical
Age (18–35 years old)
Case-dependent
Immunological*
HLA Antibodies
Blood group incompatibility
Psychosocial
Case-dependent

*LKD, living kidney donation. *Donor- and recipient related contraindication. [#]As assessed by renal nuclear scan. [§]Risk assessment by the radiologist and transplant surgeon. mGFR, measured glomerular filtration rate; BMI, body mass index; oGTT, oral glucose tolerance test; HIV, human immunodeficiency virus; HLA, human leucocyte antigen; TBC, tuberculosis.*

TABLE 2 | Absolute and relative contraindications for potential recipients for LKD.

Absolute
Medical
Cardiovascular*
Severe cardiac disease with uncorrectable symptoms (NYHA III/IV),
ventricular dysfunction (ejection fraction < 30%), severe valvular disease)
Pulmonary*
Severe irreversible obstructive or restrictive disease
Gastroenterological*
Acute decompensated liver cirrhosis**
Malignancy*
Active (except *in situ*/low grade carcinoma: e.g., prostate cancer with Gleason score < 6 or
incidental detected renal tumors < 1 cm max diameter)
In recent past medical history (only low-grade tumor at least 2 years low grade tumor without recurrence)
Multiple comorbidities[§]
Neurological*
Progressive central neurodegenerative disease
Unstable psychiatric disorder*
Psychosocial
No meaningful relationship between donor and recipient
Coercion
Non-adherence
Uncertainty for transplantation
Relative
Medical
BMI > 35 kg/m^2 (without weight loss)
Cardiovascular*
Active, symptomatic cardiac disease (unassessed)
Active, symptomatic peripheral arterial disease
Neurological*
Recent stroke or transient ischemic attack
Gastroenterological*
Active disease (e.g., peptic ulcers, acute pancreatitis, infections, uncontrolled inflammatory bowel disease, acute hepatitis)
Endocrinological*
Severe hyperparathyroidism (PTH > 800 pg/ml under conservative therapy and unsuitable for surgery)
Infectious disease (urinary tract infection, Anti-HCV positive)
Long dialysis vintage (over 8 years)
Stable kidney function (eGFR > 15 ml/min without worsening to RRT in 6 months)

*LKD, living kidney donation. *In all categories, the statement of an expert in the field (e.g., cardiologist, pulmonologist, oncologist) was included in the evaluation process. **Consider simultaneous liver-kidney transplantation. [§]Patients with at least three medical conditions in which at least one of them or de combination leads to a significant reduction of the patients' survival as of Germany's current standards. NYHA, New York, Heart Association (classification of symptomatic heart failure); BMI, body mass index; HIV, human immunodeficiency virus; PTH, parathormone; HCV, hepatitis C; eGFR, estimated glomerular filtration rate.*

contraindication were evaluated in a multidisciplinary team as mentioned above.

Data Acquisition, Statistical Analysis and Endpoints

All data was collected between August and December 2021 from patient files and the hospital information system (KAS and LAMP, SAP) in the transplant center or from the donor and recipient data in the Eurotransplant Network Information System (ENIS). Statistical analyses were performed using Microsoft Excel version Microsoft Office 365 (Microsoft Corporation, Redmond, Washington, U.S.), and GraphPad Prism version 7.05 (GraphPad Software, LLC, San Diego,

California, United States). Continuous variables were assessed for normality using histograms and Shapiro-Wilk test. Measures of central tendency and dispersion were expressed as mean and standard deviation for normally distributed data, and median and interquartile range for non-normally distributed data. Categorical variables are expressed as number of cases and percentage of total (%). For comparing continuous variables student's *t*-test and Mann-Whitney-*U*-test were used for normally and non-normally distributed data, respectively. Categorical variables were compared using Fisher's exact test or Pearson's Chi-square test. A p-value < 0.05 was considered as statistically significant. Missing data from the LKD program assessment were assumed as missing completely at random (MCAR). Missing data from recipient follow-up were assumed as missing not at random (MNAR). The primary outcome includes the rate and the summary of reasons for disqualification of potential living kidney donors and recipients. The secondary outcome is the impact on the potential recipients in respect to transplantation and mortality.

RESULTS

Between 2012 and 2020, 601 potential living kidney donor-recipient pairs presented for initial assessment at the transplant center of our institution. 275 (45.8%) proceeded for living kidney donation after successfully completing the LKD program. In total, 326 (54.2%) potential donor-recipient pairs did not complete the LKD program. Out of these, 52 (8.7%) accounted for drop-outs with 25 (4.2%) prospective donor-recipient pairs relocating to another transplant center and other 27 (4.5%) (22 potential donors and 5 potential recipients) withdrawing from the program for personal reasons. Overall, 274 (45.6%) potential donor-recipient pairs were disqualified for LKD. The study flow diagram is depicted in **Figure 2**. Among all evaluated candidates (accepted and declined donor-recipient pairs), the proportion of men as potential recipients (independent of the evaluation outcome) was higher than of women (340 vs. 192, respectively) (**Supplementary Table 2**). Accordingly, women presented more frequently as potential donors (independent of the evaluation outcome) than men (314 vs. 235, respectively, $p \leq 0.0001$) (**Supplementary Table 2**).

The proportion of potential donors-recipient pairs excluded for LKD between 2012 and 2020 per year at our transplant center is depicted in **Figure 3**. The graphic shows the highest disqualification rates in the years 2014–2016 with over 60% of potential donor-recipient pairs being ineligible for LKD. During that period, the absolute number of potential donor-recipient pairs evaluated for LKD was also higher and, compared to other years, potential recipients presented more frequently with two or more donors for the initial LKD evaluation. From 2017 until 2020, a marked reduction in disqualification rates and absolute number of evaluated donor-recipient pairs was observed. However, the overall number of donor-recipient pairs accepted for LKD per year remained similar during the evaluation period (**Figure 3**).

General Characteristics of Potential Donors and Relationship to Respective Recipients

General characteristics of potential donors declined for LKD and donors accepted for LKD are shown in **Table 3**. Median age at presentation was 55.5 (IQR: 48.0–63.0) and 56.0 (IQR: 49.0–61.0) years in disqualified and accepted donors, respectively, without a statistical difference between groups ($p = 0.82$). There was overall a higher proportion of women presenting as potential donors (56.6 and 57.8% in disqualified donors and accepted donors, respectively, $p = 0.79$). Conversely, the donor-recipient relationship differed significantly between the groups with parents (45.1%) showing the highest rate among accepted donors, and spouses (37.8%) the highest rate among disqualified potential donors ($p = 0.0017$). No acquaintances were accepted as donors for LKD (see **Table 3**).

Reasons for Disqualification of Potential Living Kidney Donor-Recipient Pairs

In the 9-year period, 274 (45.6%) potential donor-recipient pairs were ineligible for living kidney transplantation. The reasons for disqualification of the donor-recipient pairs are depicted in **Figure 4**. Half of the potential donor-recipient pairs [139 (50.7%) out of 274] were ineligible due to medical reasons related to the donor. Recipient-related issues were the second highest cause for exclusion with 60 (21.9%) cases, followed by immunological and psychosocial issues related to the donor [52 (18.9%) and 41 (14.9%) out of 274 cases, respectively]. In 16 (5.8%) cases, no specific reason for exclusion was documented (**Figure 4**, denoted as unknown). Only in 3 cases potential donors were excluded due to the presence of an alternative, more suitable candidate. It is worth mentioning that some of the disqualified donor-recipient pairs exhibited two or more reasons for disqualification. In one case, a potential donor was diagnosed with an esophageal submucosal mass, delaying the work-up due to its clarification. Meanwhile, profound non-adherence of the potential recipient was documented. Consequently, this donor-recipient pair was disqualified from the LKD program upon interdisciplinary decision. Another notable example shows a potential recipient with a low titer of donor specific HLA antibodies, considered a relative contraindication. However, the potential recipient yielded psychological issues in the following work-up, excluding the donor-recipient pair from the program.

The leading cause for exclusion due to medical reasons among donors (139 of potential donors) was reduced kidney function in 42 (30.2%) cases, followed by cardiovascular risk factors including a body mass index (BMI) over 35 kg/m^2 in 23 (16.5%) cases without weight loss in the follow-up examination and poorly controlled hypertension in 17 (12.9%) cases (**Table 4**). Remarkably, 15.1% (21 out of 139 potential donors with medical contraindications) were diagnosed with a malignant disease during work-up, with prostate cancer representing one third of the newly diagnosed malignancies (7 out of 21 cases), followed by renal cell carcinoma (4 out of 21 cases) (**Supplementary Table 1**). All patients with incidental prostate cancer had a Gleason score of at least 7. Patients

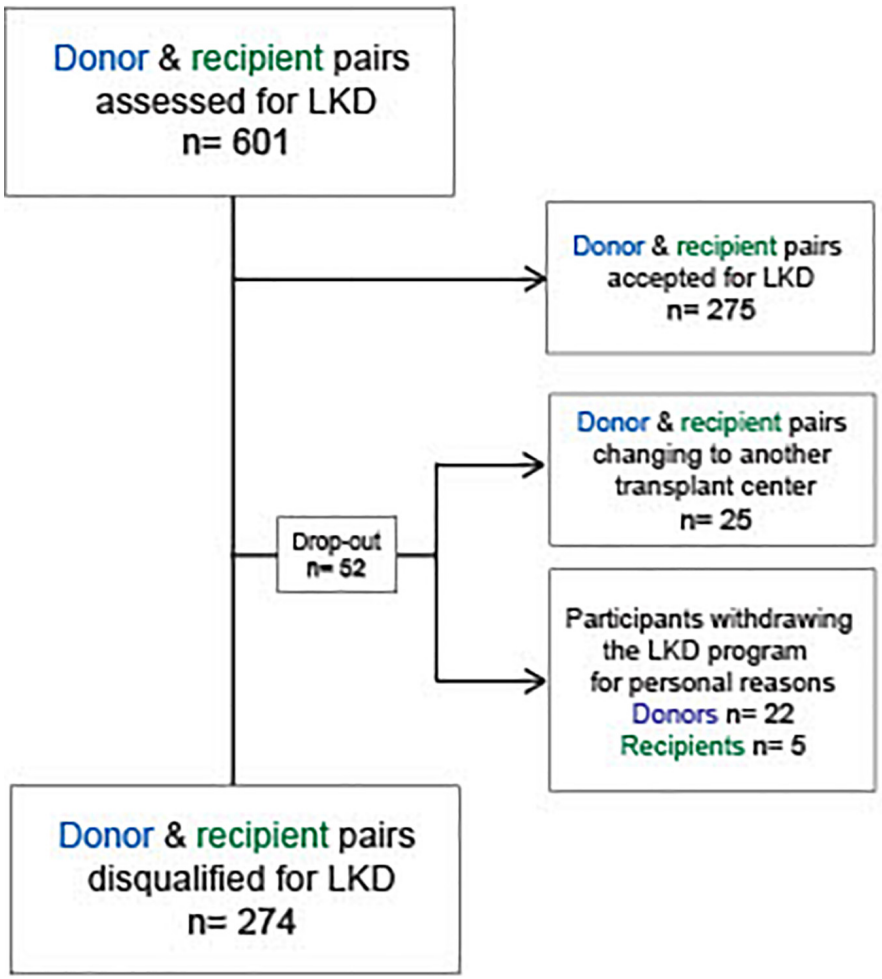


FIGURE 2 | Flow diagram: Study design. LKD, living kidney donation; n, number.

with adequate treatment and at least a 2-year recurrence-free period were reconsidered for LKD. Further incidental malignant diseases are listed in **Supplementary Table 1**. Overall, more men were newly diagnosed with a malignant disease [13 (61.9%) out of 21 potential donors]. The mean age of potential donors with incidental malignant disease was 63.8 ± 9.3 years and 65.1 ± 12.1 years in donors with incidental prostate cancer (**Supplementary Table 1**). Other relevant medical exclusion criteria involved nephrological issues [14 (10.1%) out of 139 potential donors with medical contraindications] with incidental diagnosis of proteinuria or manifest kidney disease (**Table 4**). For example, one potential donor was diagnosed with Alport syndrome and another with hypertensive nephropathy. Furthermore, three blood-related donors were excluded due to genetical abnormalities that increased the risk for kidney disease of the donor. Two potential donors presented genetical variants leading to focal segmental glomerulosclerosis and one potential donor had a genetical variant that increased the risk for developing atypical hemolytic uremic syndrome. It is worth mentioning that also among donors excluded for medical reasons, 44 yielded two or more absolute and/or relative exclusion criteria.

Overall, 52 (19.0%) out of the 274 potential donor-recipient pairs assessed were declined due to immunological reasons. 21 (40.4%) out of 52 cases had a positive crossmatch. In the remaining 31 (59.6%) out of 52 cases, donor specific HLA antibodies were detected and yielded an increased immunological risk, accounting for a relative contraindication. Immunological contraindications were more frequent in female recipients than in men [29 (55.8%) vs. 23 (44.2%) of potential recipients, respectively]. No donor-recipient pairs were excluded due to ABO-incompatibility with some of the participants undergoing ABO-incompatible transplantation upon desensitization of the recipient. However, in some cases an alternative ABO-compatible candidate was considered as more suitable for LKD.

Relevant psychosocial reasons for exclusion of the donor represented 14.9% (41 out of 274 declined potential donors). Ten (23.8%) out of 41 potential donors were declined due to psychological assessment, mostly due to insufficient bond between the potential donor and recipient (**Table 4**). Uncertainty

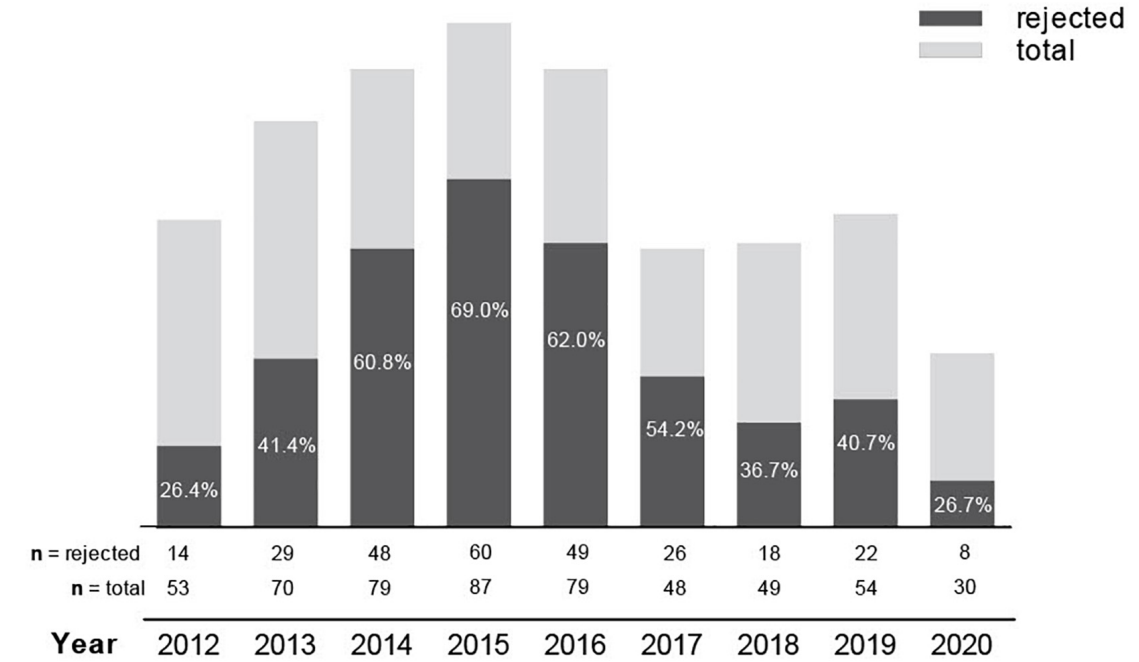


FIGURE 3 | Percentage of potential donors disqualified for LKD per year from 2012 to 2020:%, percentage; n, number.

for transplantation was also a frequent cause for exclusion with 12.2%. Other social aspects (15 out of 42 cases) leading to exclusion of the donor included complex social circumstances such as being a single parent of small children or conflicts between the potential donor and recipient. Less common reasons in our cohort were signs of coercion, financial problems, signs of non-adherence and religion-related reasons.

TABLE 3 | General characteristics of disqualified donors and donors completing the LKD program.

Characteristics	Disqualified donors *n* = 274	Accepted donors *n* = 275	*p*-value
Age in years in median (IQR)	55.5 (48.0–63.0)	56.0 (49.0–61.0)	0.82
Range	25–87	29–80	
Gender, *n* (%)			
Male	119 (43.4)	116 (42.2)	0.79
Female	155 (56.6)	159 (57.8)	
Relationship to recipient, *n* (%)			
Parents	80 (29.2)	124 (45.1)	0.0017
Spouse or partner	105 (38.3)	91 (33.1)	
Sibling	39 (14.2)	35 (12.7)	
Second degree relative	17 (6.2)	8 (2.9)	
Friend	18 (6.6)	9 (3.3)	
Other relatives*	10 (3.6)	8 (2.9)	
Acquaintance	4 (1.5)	0 (0.0)	

*Comparison of groups by Fisher's exact test or Pearson's Chi-square test for categorical data and Mann-Whitney U-test for continuous non-parametric data. *Includes stepfather, father-in-law, mother-in-law, brother-in-law, sister-in-law. n, absolute number; IQR, interquartile range; %, percentage.*

General Characteristics of Recipients of Disqualified Donor-Recipient Pairs and Recipients Who Underwent Living Kidney Transplantation

The following section focuses on all potential recipients declined for LKD, independent of the reason (donor- or recipient-related). Out of the 326 potential donor-recipient pairs who did not conclude the LKD program, 32 recipients presented with two or more potential donors, leading to a total of 257 potential recipients disqualified from the program in this time period (after excluding donor-recipient pairs relocating to another transplant center and recipient drop-outs). **Table 5** shows the general characteristics of recipients disqualified for LKD and recipients accepted for LKD. Patients who underwent living donation were significantly younger than recipients disqualified from the LKD program [44 (29.0–55.0) years and 49 (36.5–58.0) years, respectively, $p = 0.0007$]. The proportion of men as potential recipients for LKD was higher in both groups (185 (67.3%) successfully transplanted recipients and 155 (60.3%) recipients of disqualified donor-recipient pairs) with no significant difference between accepted and declined recipients ($p = 0.104$). The rate of pre-emptive evaluated recipients with a successful LKD and recipients disqualified for LKD was not different (33.1 and 28.0%, respectively, $p = 0.22$). Also, no significant difference was found in respect to the proportion of patients with a previous kidney transplant between successfully transplanted recipients and recipients from disqualified donor-recipient pairs [39 (14.2%) vs. 46 (17.9%), respectively, $p = 0.29$] (**Table 5**). Finally, the median dialysis vintage of ESKD patients accepted for LKD was 0.75

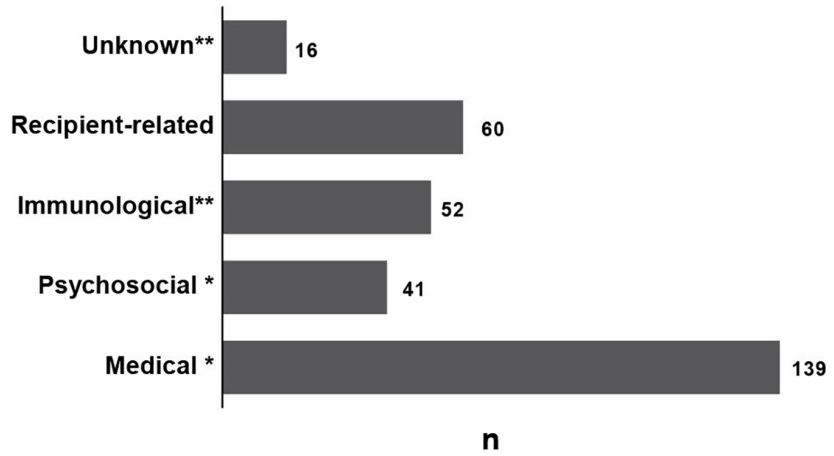


FIGURE 4 | Reasons for disqualification of the potential donor-recipient pairs from the LKD program: n: number. *Donor-related contraindications. **Donor- and/or recipient-related contraindications.

(IQR: 0.75–1.75) years until successfully performed kidney transplantation.

Recipient-Related Reasons for Disqualification and Outcomes of Recipients Disqualified From the Living Kidney Donation Program

We report that 60 (21.9%) out of 274 potential donor-recipient pairs were ineligible for LKD due to recipient-related issues. Median age in this group was 50 (41.8–63.8) years. There was no significant difference in terms of gender within this group [35 (58.3%) men vs. 25 (41.7%) women declined, $p = 0.76$]. **Table 6** displays the medical and psychosocial reasons for exclusion of potential recipients from the LKD program. In most of the cases, recipients were declined due to medical reasons. Multiple comorbidities and acceptable or improved kidney function of the potential recipients were among the leading causes for exclusion [9 (15.0%) and 11 (18.3%), respectively]. Also in this group, incident malignant disease represented an important exclusion criterion with 11 (18.3%) out of 60 cases disqualified (**Table 6**). More men were diagnosed with incidental malignant disease among potential recipients (6 (75%) out of 8 potential recipients) (**Supplementary Table 1**). Three of them were diagnosed with prostate cancer. Cardiovascular complications as well as long dialysis vintage were found in 5 (8.1%) cases, respectively. Four (6.3%) patients received a deceased kidney during the work-up or changed to the ESP program, while other four (6.3%) were listed for kidney-pancreas transplantation, due to better outcomes. Three patients (4.8%) died during the work up.

Overall, 78.9% (203 out of 257) of potential recipients initially declined from the LKD program remained in contact with our transplant center. Following disqualification, 77 (29.9%) ESKD patients received a kidney transplant and almost half of those (48.1%) received a kidney from an alternative living donor. The median time to KTx was overall 2 (IQR: 1.0–4.0) years. The latter was significantly shorter for recipients of living kidney donors than for recipients of deceased donors (1 (0–2) year vs. 4 (1.5–5.0) years, respectively, $p = 0.0001$). 18 (7.0%) out of 257 potential recipients initially declined at the LKD program died within the follow-up period, with only three of them receiving a deceased kidney transplant after exclusion from the LKD program. Unfortunately, we have no information regarding transplantation or death rate of 54 (21.1%) out of all potential recipients initially declined at our LKD program.

DISCUSSION

In Germany, only 20–30% of kidney transplants are from living donors in spite of its clear benefit for ESKD patients compared to deceased KTx (2). High disqualification rates of potential donors upon evaluation account for this problem. Nevertheless, thorough screening and clinical assessment of potential healthy living donors remains indispensable to avoid any potential harm upon transplantation. Early published data show LKD is safe for living kidney donors. However, recent reports do highlight a low but significant increase in cardiovascular and ESKD risk for patients after donor nephrectomy (15, 17). This prompts healthcare professionals to be more restrictive toward acceptance of potential donors, leading to high rates of exclusion (14, 18). Additionally, differences in guidelines for the assessment of LKD have led to variations in the acceptance of potential donors among transplant centers worldwide (19). Thus, the aim of this study was to analyze the rates of exclusion of potential donor-recipient pairs in a transplant center in Germany with a thorough description of the causes and possible consequences for waitlisted patients with ESKD.

We found that 45.5% of donor-recipient pairs at our transplant center were ineligible for LKD and further 8.6% dropped-out from the program. Interestingly, the rate of potential donor-recipient pairs disqualified for LKD per year peaked between 2014 and 2016, with more recipients presenting with two or more potential donors to the initial assessment.

TABLE 4 | Donor-related reasons for disqualification from the LKD program.

Medical *n* (%)	***n* = 139**
mGFR < 70 mL/min/1.73 $m^{2\#}$	42 (30.2)
Nephrological*	14 (10.1)
Urological**	12 (8.6)
Cardiovascular risk factors	
Hypertension (poorly controlled with more than two medications)	17 (12.9)
Diabetes or pathological oGTT	14 (10.1)
Smoking	9 (6.5)
Arteriosclerosis	6 (4.3)
BMI > 35 kg/m^2 (without weight loss in the work-up)	23 (16.5)
Age (too young)	7 (5.0)
Malignancy	28 (20.1)
In recent past medical history	7 (5.0)
Diagnosed during work-up	21 (15.1)
Psychiatric	7 (5.0)
Lung disease	4 (2.9)
Genetical predisposition for kidney disease	3 (2.2)
Active infectious disease (Hepatitis B/C, TBC, or HIV)	4 (2.9)
Other***	5 (3.6)
Psychosocial *n* (%)	***n* = 41**
Psychological assessment	10 (24.3)
Insufficient bond between donor and recipient	5 (12.2)
Other social aspects****	15 (36.5)
Uncertainty for transplantation	5 (12.2)
Signs of coercion	2 (4.9)
Financial problems	6 (14.6)
Non-adherence	4 (9.8)
Religion	2 (4.9)
Immunological[§] *n* (%)	***n* = 52**
Positive crossmatch	21 (40.4)
Donor specific HLA Antibodies	31 (59.6)

[#]*As assessed by renal nuclear scan. *Includes incidental unclear microhematuria and/or proteinuria, newly diagnosed kidney disease (e.g., Alport syndrome). **Includes incidental abnormal kidney cysts, abnormal kidney vessels or ureter, unclear incidental macrohematuria and/or nephrolithiasis or high risk for nephrolithiasis. ***Includes neurological abnormalities (newly diagnosed multiple sclerosis), one case of Merkelsson-Rosenthal Syndrome, and gastrointestinal abnormalities. ****Includes difficult social circumstances such as single parents of small children, planning child conception. Some candidates qualified for more than one category. n, number; mGFR, measured glomerular filtration rate; oGTT, oral glucose tolerance test; HIV, Human immunodeficiency virus; HLA, human leucocyte antigen; TBC, tuberculosis.*

[§]*Donor-related contraindications depending on the potential recipient.*

TABLE 5 | Baseline characteristics of recipients from disqualified donors and recipients who underwent LKD.

General characteristics	**Recipients disqualified for LKD* *n* = 257**	**Recipients who underwent LKD *n* = 275**	***p*-value**
Age in years (median, IQR)	49 (36.5–58.0)	44 (29.0–55.0)	0.0007
Range	2–80	1–77	
Gender, *n* (%)			
Male	155 (60.3)	185 (67.3)	0.104
Female	102 (39.7)	90 (32.8)	
Preemptive transplantation, *n* (%)	72 (28.0)	91 (33.1)	0.22
Previous kidney transplant, *n* (%)	46 (17.9)	39 (14.2)	0.29

*Comparison of groups by Fisher's exact test or Pearson's Chi-square test for categorical data and Mann-Whitney U-test for continuous non-parametric data. n, number; %, percent; IQR, interquartile range. *Independent on the reason for disqualification (donor- or recipient-related).*

TABLE 6 | Recipient-related reasons for disqualification from LKD program.

Recipient-related contraindications	***n* = 60**
Medical *n* (%)	
Multiple comorbidities	9 (15.0)
BMI > 35 kg/m^2 (without weight loss during evaluation)	3 (5.0)
Malignancy	11 (18.3)
Prostate cancer	3 (5.0)
Other malignancies*	8 (13.3)
Cardiovascular complications	5 (8.3)
Death during LKD evaluation	3 (5.0)
Long dialysis vintage	5 (8.3)
Stable kidney function	11 (18.3)
Received deceased kidney or changed to ESP program	4 (6.7)
Listed for simultaneous kidney-pancreas transplantation	4 (6.7)
Other**	9 (15.0)
Psychosocial n (%)	
Psychological assessment	4 (6.7)
Non-adherence	1 (1.7)
Other***	2 (3.3)

**Includes Non-Hodgkin lymphoma, leukemia, melanoma. **Includes uncontrolled hyperparathyroidism, multiple abscesses, chronic pancreatitis. ***Includes insecurity and anxiety of the recipient toward LKD. Some patients qualified for more than one category. n, number; %, percent.*

Especially percentages of potential donors being declined for immunological and medical reasons were higher during those years. As both cross-match examinations and medical screening can also be performed by referring nephrologists prior to donor evaluation at our center, we feel the discrepancy reflects donor selection by referring nephrologists prior to presentation to our center. Additionally, data from the "Deutsche Stiftung Organtransplantation" (DSO) has revealed a marked variability in the rate of LKD, deceased kidney transplantations, and waitlisted ESKD patients in Germany over the past 20 years (19, 20). It is possible that due to the short period of time used for our analysis (9 years), such inherent variations were only insufficiently detected. Moreover, the substantial reduction in 2020 might have been a consequence of the surging global coronavirus disease 2019 pandemic.

Similar to other studies, we report that almost half of the donor-recipient pairs evaluated for LKD at our transplant center are disqualified (21–23). In 50.7% of the cases donor-related medical contraindications were the reason for exclusion with reduced mGFR and cardiovascular risk factors (obesity, hypertension and diabetes) as leading causes. Villafuerte-Ledesma et al. and Lapasia et al. report similar results (22, 23). By contrast, a study from Ireland reported different results with reduced eGFR and diabetes not playing a significant role in disqualification rates (12). This was also observed by Perlis et al., where urological pathologies prevailed as

cause for disqualification of potential donors (24). However, these results should be interpreted with caution, as the structure of the LKD evaluation programs of each center differs considerably and, in the latter, patients with absolute contraindications (such as reduced eGFR) had already been excluded in a preliminary screening process. We believe that such differences among transplant centers worldwide are partly responsible for the varied disqualification rates and should be considered by clinicians when evaluating donor and recipient candidates for LKD.

One interesting aspect of our study is the high incidence of malignant disease among potential living donors with one third of the cases presenting incidental prostate cancer. These observations are probably related to the age of this group of potential donors (mean age: 65.1 ± 12.1 years), which resembles the worldwide mean age of diagnosis of prostate cancer at 66 years (25). No other studies report these findings. Unlike our results, several studies report an overall younger population presenting as potential donors with a mean age ranging between 40 and 45 years (12, 22, 26, 27). Only Gregorini et al. and Villafuerte-Ledesma et al. reported a comparable mean donor age between 53 and 55 years old (21, 23). Furthermore, our data show no difference in respect to the age of accepted and declined donors, suggesting that at our transplant center older age *per se* is not linked to donor-disqualification. On the contrary, in Spain and in Ireland older donors were more likely to be excluded from LKD (12, 23).

Corresponding to other transplant centers worldwide, we observed substantial gender differences among potential recipients and donors for LKD. Women presented overall more frequently as potential donors, independent of the evaluation outcome, which is analogous to previous published data (22, 28). Altruism and a more paternalistic approach of women toward their relatives have been associated with this finding (29). The higher proportion of men in need of a kidney transplant has been documented in other studies as well (12, 23), which has been associated to a higher risk of progression of chronic kidney disease (CKD) and ESKD among men (30). In addition, women waitlisted for a kidney transplant (especially deceased KTx) have often increased levels of preformed antibodies, reducing the likelihood of a successful transplantation (31). Nevertheless, additional factors such as socioeconomical and cultural issues should be addressed in future studies as alternative explanations for the gender disparity and potentially reduce the gap (30).

In this study, potential recipients accepted for LKD were substantially younger than potential recipients disqualified for LKD. Similar data has been reported by the DSO, where the percentage of ESKD patients between 16 and 55 years receiving a living donation was higher than in patients of the same age group receiving a deceased kidney donation (20). Reasons for this discrepancy might be related to the cause for ESKD, comorbidities in the ESKD older population and timing of patient referral by the primary care nephrologist. This trend highlights that kidney transplantation in the increasingly older ESKD population in Germany is mostly dependent on deceased kidney donation, reducing their probability for receiving a kidney transplant due to the longer waiting times. Therefore, timely evaluation of recipient candidates should be pursued by treating physicians in order to make kidney transplantation an available treatment for this population.

The second most common cause for disqualification of donor-recipient pairs for LKD were recipient-related contraindications accounting for 21.9%, similar to the numbers presented by German registries in 2020 where a third of the waitlisted ESKD patients were reported unsuitable for kidney transplantation (20). Overall, medical contraindications were the most common cause for recipient disqualification from LKD. However, stable kidney function was also seen in 18.3% of the cases, reflecting the timely presentation of potential recipients and potential donors for assessment at out transplant center and the improvement of therapies for patients with CKD. One seldomly reported cause for disqualification in other centers was a too long dialysis vintage. At our transplant center, this was weighed in patients with a dialysis vintage that resembled the average waiting times for receiving a deceased kidney transplant in Germany, whereby the benefit of LKD compared to deceased kidney donation is mostly lost.

Immunological contraindications (including mostly a positive cross-match and/or presence of donor specific HLA-antibodies) accounted for disqualification of 18.9% of donor-recipient pairs assessed. ABO-incompatibility was considered a relative contraindication and no patients were excluded for this reason in our study. This is different from previous published data, where potential donors were automatically excluded upon ABO-incompatibility and this alone represented a relevant cause for disqualification ranging from 12 to 20% (16, 22, 23, 32). New therapeutic strategies have allowed for prior desensitization of recipient candidates, enabling LKD under these conditions and thus reducing disqualification rates significantly (17). However, not all patients qualify for this therapeutic approach. Careful weighing of risk and benefits and assessment by an interdisciplinary team of experts remains indispensable. Otherwise, presence of donor specific antibodies was a relevant relative contraindication present in about half of patients with immunological contraindications, implying that HLA-incompatibility still signifies a higher risk for clinicians. Nevertheless, ABO-incompatible transplantations and HLA-desensitization have shown promising results with comparable graft and patient survival and should be available for all candidates assessed for LKD (17).

We report that almost 15% of potential donors presented psychosocial contraindications for LKD, a rather higher proportion compared to other studies (14, 33). We show there is a wide range of reasons in this regard, including an insufficient bond between donor and recipient, uncertainty for transplantation, non-adherence and legal issues as signs of coercion. Psychosocial factors should not be underestimated regarding KTx and LKD, especially in Germany where the number of LKD has shown a progressive decline, in part due to more stringent criteria regarding psychosocial factors for donor selection (34). One group from the United Kingdom proved that socioeconomical, geographical and demographic factors are strongly associated with the likelihood of receiving a LKD compared to clinical factors (35). Disqualification due to uncertainty for transplantation remains a relevant issue and highlights the need for a better education of potential

donors and recipients regarding perioperative risks and long-term consequences after donor-nephrectomy.

The long waiting times for deceased kidney transplantation in Germany (mean waiting time 8 years as of 2022) remain an important issue and are substantially longer compared to other countries (1, 36). In our study, only one third of recipient candidates initially disqualified from the LKD program obtained a kidney transplant in the following period, with about half of them receiving a deceased KTx. In addition, the median waiting time for potential recipients with deceased KTx was considerably longer, which is linked to poorer graft survival and patient prognosis. Up to 7.0% of patients died within the observation period, highlighting the severe health-related consequences waitlisted patients are subject to, in part due to the long waiting times in Germany. Only a few European countries, among them Germany, use the opt-in or informed consent system for acquiring deceased organ donors, which markedly reduces the number of available donors. Our observations clearly emphasize the need for implementing further strategies to increase the number of donor candidates, including living kidney donors.

Taken together, our study underlines the importance of a thorough clinical evaluation of potential donors and recipients for LKD, validating previous data from around the world. Further strategies, such as risk-stratification scores [e.g., living kidney donor profile index (LKDPI)], among others, should support clinicians in the decision-making process in order to provide patients with the best treatment modality (37). Furthermore, German society should evaluate the possibility of expanding the living donor pool by allowing paired exchanges or cross-over LKD and pooled donation. LKD has proven to be not only better for patient survival but also to be more cost-effective than other ESKD treatment modalities (38).

This study has some limitations. The observational, retrospective design limits the completeness of data. Additionally, data was analyzed in a period of time where changes in guidelines and clinical practice might have influenced disqualification rates. This is a single-center study and differences to other transplant centers in Germany might be considerable, therefore limiting generalizability. Nevertheless, our study is the first analysis from a German center providing information on disqualification of living kidney donor-recipient pairs. The recently introduced German living donor registry (Safety of the Living Kidney Donor (SOLKID) has encouraged the development of risk stratification scores to identify the population with increased medical and psychosocial risk upon donor nephrectomy (39). Nevertheless, additional studies from other German transplant centers are necessary in order to increase the available data and therefore create better strategies for living donor assessment and management of candidates for LKD.

In conclusion, half of potential donor-recipient pairs assessed at our LKD program are not eligible for transplantation with only a third of declined potential recipients receiving an alternative organ in the following years. Further efforts are still necessary to increase the living donor pool and reduce the gap between transplanted and wait-listed patients, always protecting the living donor from any harm.

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 Local Ethics Committee of the LMU University Munich. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MGr, SK, MF, and MS designed the concept of the retrospective study. MGr and TC wrote the manuscript. SK, MF, MS, US, and TS edited and revised the manuscript. MGr, IS, WG, and TC prepared clinical data for analysis. MGu, MS, SK, MF, US, BI, BM, and TS oversaw the study and critically discussed the manuscript. All authors substantially contributed to the manuscript.

ACKNOWLEDGMENTS

This work is part of a Master's thesis of the Master's Program in Clinical Research, Dresden International University, Dresden, Germany. We would like to thank all patients and their families for their contributions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.904795/full#supplementary-material

REFERENCES

1. Deutsche Stiftung Organtransplantation. *Statistiken zur Organtransplantation im Überblick [Internet]. Nierentransplantation in Deutschland, Jahresbericht*. Frankfurt am Main: Deutsche Stiftung Organtransplantation (2022).
2. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med*. (2000) 342:605–12.
3. Yagisawa T, Mieno M, Ichimaru N, Morita K, Nakamura M, Hotta K, et al. Trends of kidney transplantation in Japan in 2018: data from the kidney transplant registry. *Ren Replace Ther*. (2019) 5:3.
4. Scandiatransplant. *Annual Scandiatransplantdata Report 2020*. (2020). Available online at: http://www.scandiatransplant.org/resources/AnnualScandiatransplantdatareport_2020.pdf (accessed Jan 31, 2022).
5. Meier-Kriesche HU, Kaplan B. Waiting time on dialysis as the strongest modifiable risk factor for renal transplant outcomes.

Transplantation. (2002) 74:1377–81. doi: 10.1097/00007890-200211270-00005
6. Baid-Agrawal S, Frei UA. Living donor renal transplantation: recent developments and perspectives. *Nat Clin Pract Nephrol.* (2007) 3:31–41. doi: 10.1038/ncpneph0383
7. Claisse G, Gaillard F, Mariat C. Living kidney donor evaluation. *Transplantation.* (2020) 104:2487–96.
8. Richardson R, Connelly M, Dipchand C, Garg AX, Ghanekar A, Houde I, et al. Kidney paired donation protocol for participating donors 2014. *Transplantation.* (2015) 99(Suppl. 10):S1–88. doi: 10.1097/TP.0000000000000918
9. Lentine KL, Kasiske BL, Levey AS, Adams PL, Alberú J, Bakr MA, et al. KDIGO clinical practice guideline on the evaluation and care of living kidney donors. *Transplantation.* (2017) 101:S1–109.
10. Andrews PA, Burnapp L. British transplantation society / renal association UK guidelines for living donor kidney transplantation 2018. *Transplantation.* (2018) 102:e307. doi: 10.1097/TP.0000000000002253
11. The European Renal Best Practice (ERBP) Transplantation Guideline Development Group, Abramowicz D, Cochat P, Claas F, Dudley C, Harden P, et al. Guideline. *Nephrol Dial Transplant.* (2013) 28:ii1–71.
12. Connaughton DM, Harmon G, Cooney A, Williams Y, O'Regan J, O'Neill D, et al. The Irish living kidney donor program – why potential donors do not proceed to live kidney donation? *Clin Transplant.* (2016) 30:17–25.
13. Fehrman-Ekholm I, Gabel H, Magnusson G. Reasons for not accepting living kidney donors1. *Transplantation.* (1996) 61:1264–5.
14. Gozdowska J, Jankowski K, Bieniasz M, Wszoła M, Domagała P, Kieszek R, et al. Characteristics of potential living kidney donors and recipients: donor disqualification reasons—experience of a polish center. *Transplant Proc.* (2013) 45:1347–50. doi: 10.1016/j.transproceed.2013.01.030
15. Konsensus der Arbeitsgemeinschaft der Nierentransplantationszentren. *Nordrhein-Westfalens (Aachen BBDEKLKMM Sowie H (Saale), HSKLMMMM (Rechts der I. MANUAL zur Vereinheitlichung der Evaluation vor Nierentransplantation und Nierenlebendspende, der Wartelistenführung vor Nierentransplantation und zur Nachsorge nach Nierentransplantation und Nierenlebendspende*. (2018). Available online at: https://www.d-t-g-online.de/images/Downloads/Manual_Nierentransplantation_10-2018.pdf
16. Calder FR, Chang RW. Panning for gold: screening for potential live kidney donors. *Nephrol Dial Transpl.* (2004) 19:1276–80. doi: 10.1093/ndt/gfh045
17. Kosoku A, Uchida J, Nishide S, Kabei K, Shimada H, Iwai T, et al. ABO-incompatible kidney transplantation as a renal replacement therapy—a single low-volume center experience in Japan. *PLoS One.* (2018) 13:e0208638. doi: 10.1371/journal.pone.0208638
18. Chadban SJ, Ahn C, Axelrod DA, Foster BJ, Kasiske BL, Kher V, et al. KDIGO clinical practice guideline on the evaluation and management of candidates for kidney transplantation. *Transplantation.* (2020) 104:S11–103.
19. Deutsche Stiftung Organtransplantation. *Grafiken zum Tätigkeitsbericht 2016*. Frankfurt am Main: Deutsche Stiftung Organtransplantation (2016). https://dso.de/BerichteTransplantationszentren/Grafiken%20D%202016%20Niere.pdf
20. Deutsche Stiftung Organtransplantation. *Grafiken zum Tätigkeitsbericht 2020*. Frankfurt am Main: Deutsche Stiftung Organtransplantation (2020). https://dso.de/BerichteTransplantationszentren/Grafiken%20D%202020%20Niere.pdf
21. Gregorini M, Martinelli V, Ticozzelli E, Canevari M, Fasoli G, Pattonieri EF, et al. Living kidney donation is recipient age sensitive and has a high rate of donor organ disqualifications. *Transpl Proc.* (2019) 51:120–3. doi: 10.1016/j.transproceed.2018.03.136
22. Lapasia JB, Kong SY, Busque S, Scandling JD, Chertow GM, Tan JC. Living donor evaluation and exclusion: the Stanford experience. *Clin Transpl.* (2011) 25:697–704. doi: 10.1111/j.1399-0012.2010.01336.x
23. Villafuerte-Ledesma HM, de Sousa-Amorim E, Peri L, Musquera M, Palou E, Lozano M, et al. Impact of discards for living donor kidney transplantation in a transplant program. *Transpl Proc.* (2019) 51:3222–6.
24. Perlis N, Connelly M, D'A Honey JR, Pace KT, Stewart R. Evaluating potential live-renal donors: causes for rejection, deferral and planned procedure type, a single-centre experience. *Can Urol Assoc J.* (2013) 7:41. doi: 10.5489/cuaj.216
25. Rawla P. Epidemiology of prostate cancer. *World J Oncol.* (2019) 10:63–89.
26. Norman SP, Song PXK, Hu Y, Ojo AO. Transition from donor candidates to live kidney donors: the impact of race and undiagnosed medical disease states. *Clin Transpl.* (2011) 25:136–45. doi: 10.1111/j.1399-0012.2009.01188.x
27. Bailey PK, Tomson CRV, MacNeill S, Marsden A, Cook D, Cooke R, et al. A multicenter cohort study of potential living kidney donors provides predictors of living kidney donation and non-donation. *Kidney Int.* (2017) 92:1249–60. doi: 10.1016/j.kint.2017.04.020
28. Ojo A, Port FK. Influence of race and gender on related donor renal transplantation rates. *Am J Kidney Dis.* (1993) 22:835–41. doi: 10.1016/s0272-6386(12)70343-8
29. Rota-Musoll L, Brigidi S, Molina-Robles E, Oriol-Vila E, Perez-Oller L, Subirana-Casacuberta M. An intersectional gender analysis in kidney transplantation: women who donate a kidney. *BMC Nephrol.* (2021) 22:59. doi: 10.1186/s12882-021-02262-9
30. Carrero JJ, Hecking M, Chesnaye NC, Jager KJ. Sex and gender disparities in the epidemiology and outcomes of chronic kidney disease. *Nat Rev Nephrol.* (2018) 14:151–64. doi: 10.1038/nrneph.2017.181
31. Wolfe RA, Ashby VB, Milford EL, Bloembergen WE, Agodoa LYC, Held PJ, et al. Differences in access to cadaveric renal transplantation in the United States. *Am J Kidney Dis.* (2000) 36:1025–33. doi: 10.1053/ajkd.2000.19106
32. Beekman GM, van Dorp WT, van Es LA, van Bockel JH, van Saase JL, van der Woude FJ, et al. Analysis of donor selection procedure in 139 living-related kidney donors and follow-up results for donors and recipients. *Nephrol Dial Transplant.* (1994) 9:163–8.
33. Wafa EW, Donia AF, Ali-el-Dein B, el Agroudy AE, Rifaie A, Moustafa A, et al. Evaluation and selection of potential live kidney donors. *J Urol.* (2004) 171:1424–7. doi: 10.1097/01.ju.0000116431.65651.58
34. Deutsches Ärzteblatt. *Bundesgerichtshof Stellt Hohe Anforderungen an Aufklärung vor Lebendorganspende*. (2019). Available online at: https://www.aerzteblatt.de/nachrichten/100738/Bundesgerichtshof-stellthohe Anforderungen-an-Aufklaerung-vor-Lebendorganspende (accessed April 20, 2022).
35. Wu DA, Robb ML, Watson CJE, Forsythe JLR, Tomson CRV, Cairns J, et al. Barriers to living donor kidney transplantation in the United Kingdom: a national observational study. *Nephrol Dial Transpl.* (2017) 32:890–900. doi: 10.1093/ndt/gfx036
36. National Kidney Foundation. *Organ Donation and Transplantation Statistics*. (2014). Available online at: https://www.kidney.org/news/newsroom/factsheets/Organ-Donation-and-Transplantation-Stats (accessed April 20, 2022).
37. Rehse G, Halleck F, Khadzhynov D, Lehner LJ, Kleinsteuber A, Staeck A, et al. Validation of the living kidney donor profile index in a European cohort and comparison of long-term outcomes with US results. *Nephrol Dial Transpl.* (2019) 34:1063–70. doi: 10.1093/ndt/gfy118
38. Ehlert J. *"Wie Kann die Lebendspende Gefördert Werden?" [Internet]. Deutsche Transplant Gesellschaft (DTG) 2020*. (2020). Available online at: https://www.transplant-campus.de/nierentransplantation-lebertransplantation-herztransplantation-highlights/allgemein/kongresse/dtg-2020-wie-kann-die-lebendspende-gefoerdert-werden/ (accessed Jan 31, 2022).
39. Suwelack B, Dugas M, Koch M, Sommerer C, Urban M, Gerß J, et al. Die sicherheit des lebendnierenspenders – das deutsche lebendspende register SOLKID-GNR – entstehung und struktur eines nationalen registers in der versorgungsforschung. *Das Gesundheitswesen.* (2021) 83:S33–8.

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.

frontiers | Frontiers in Medicine

TYPE Correction
PUBLISHED 17 November 2022
DOI 10.3389/fmed.2022.1004413

OPEN ACCESS

APPROVED BY
Frontiers Editorial Office,
Frontiers Media SA, Switzerland

*CORRESPONDENCE
Melissa Grigorescu
melissa.grigorescu_vlas
@med.uni-muenchen.de

†These authors share senior authorship

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 27 July 2022
ACCEPTED 01 November 2022
PUBLISHED 17 November 2022

CITATION
Grigorescu M, Kemmner S, Schönermarck U, Sajin I, Guenther W, Cerqueira TL, Illigens B, Siepmann T, Meiser B, Guba M, Fischereder M and Stangl MJ (2022) Corrigendum: Disqualification of donor and recipient candidates from the living kidney donation program: Experience of a single-center in Germany. *Front. Med.* 9:1004413. doi: 10.3389/fmed.2022.1004413

Corrigendum: Disqualification of donor and recipient candidates from the living kidney donation program: Experience of a single-center in Germany

Melissa Grigorescu[1,2*], Stephan Kemmner[3], Ulf Schönermarck[1], Isidora Sajin[3], Wolfgang Guenther[3], Tiago Lemos Cerqueira[2,4], Ben Illigens[2], Timo Siepmann[5], Bruno Meiser[3], Markus Guba[3,6], Michael Fischereder[1,3†] and Manfred Johannes Stangl[3,6†]

[1]Division of Nephrology, Department of Internal Medicine IV, University Hospital Munich, Ludwig-Maximilians University (LMU), Munich, Germany, [2]German Sites Development Principles and Practice of Clinical Research Harvard T.H., Chan School of Public Health, Dresden International University, Dresden, Germany, [3]Transplant Center, University Hospital of Munich, Ludwig-Maximilians University (LMU), Munich, Germany, [4]Department of Kidney Transplant, Hospital Evangelico de Minas Gerais, Belo Horizonte, Brazil, [5]Department of Neurology, University Hospital Carl Gustav Carus, Technische Universitaet Dresden, Dresden, Germany, [6]Department of General, Visceral, and Transplant Surgery, University Hospital of Munich, Ludwig-Maximilians University (LMU), Munich, Germany

KEYWORDS

living kidney donation, living donor candidates, disqualification living kidney donors, end-stage kidney disease, kidney transplantation

A corrigendum on
Disqualification of donor and recipient candidates from the living kidney donation program: Experience of a single-center in Germany

by Grigorescu, M., Kemmner, S., Schönermarck, U., Sajin, I., Guenther, W., Cerqueira, T. L., Illigens, B., Siepmann, T., Meiser, B., Guba, M., Fischereder, M., and Stangl, M. J. (2022). *Front. Med.* 9:904795. doi: 10.3389/fmed.2022.904795

In the published article, there was an error regarding the affiliation for "Timo Siepmann." Instead of having affiliation 2: *German Sites Development Principles and Practice of Clinical Research Harvard T.H., Chan School of Public Health, Dresden International University, Dresden, Germany* he should have the following affiliation: *5: Department of Neurology, University Hospital Carl Gustav Carus, Technische Universitaet Dresden, Dresden, Germany*.

In the published article, an author name was incorrectly written as "Manfred Johannes Stang." The correct spelling is "Manfred Johannes Stangl."

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

Publisher's note

frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 04 July 2022
doi: 10.3389/fmed.2022.910987

Anti-SARS-CoV-2 Revaccination Success in Kidney Transplant Recipients With No Initial Humoral Response Is Linked to Primary Vaccine Type

Julian Stumpf[1,2], Jörg Schwöbel[3], Claudia Karger[4], Holger Schirutschke[5], René Mauer[6], Anna Klimova[7], Torsten Tonn[8,9] and Christian Hugo[1,2]*

[1] Medizinische Klinik und Poliklinik III, Universitätsklinikum Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany, [2] Kuratorium für Heimdialyse (KfH)-Nierenzentrum Dresden, Dresden, Germany, [3] Dialysezentrum Chemnitz, Chemnitz, Germany, [4] Kuratorium für Heimdialyse (KfH)-Nierenzentrum am Klinikum St. Georg, Leipzig, Germany, [5] Patienten-Heimversorgung Gemeinnützige Stiftung (PHV) Dialysezentrum Dresden Friedrichstadt, Dresden, Germany, [6] Faculty of Medicine Carl Gustav Carus, Institute for Medical Informatics and Biometry, Technische Universität Dresden, Dresden, Germany, [7] National Center for Tumor Diseases Dresden, Dresden, Germany, [8] Institute for Transfusion Medicine, German Red Cross Blood Donation Service North-East, Dresden, Germany, [9] Faculty of Medicine Carl Gustav Carus, Transfusion Medicine, Technische Universität Dresden, Dresden, Germany

OPEN ACCESS

Edited by:
Miriam Banas,
University Hospital Regensburg, Germany

Reviewed by:
Alexander R. Rosenkranz,
Medical University of Graz, Austria
Renata Maria Grifantini,
National Institute of Molecular Genetics (INGM), Italy

***Correspondence:**
Julian Stumpf
julian.stumpf@uniklinikum-dresden.de

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: 01 April 2022
Accepted: 07 June 2022
Published: 04 July 2022

Citation:
Stumpf J, Schwöbel J, Karger C, Schirutschke H, Mauer R, Klimova A, Tonn T and Hugo C (2022) Anti-SARS-CoV-2 Revaccination Success in Kidney Transplant Recipients With No Initial Humoral Response Is Linked to Primary Vaccine Type. Front. Med. 9:910987. doi: 10.3389/fmed.2022.910987

Background: While anti-SARS-CoV-2 vaccination success in kidney transplant recipients (KTR) after two doses and 1273-mRNA was associated with higher seroconversion rates compared to BNT162b2-mRNA in our "DIA-Vacc Study" (NCT04799808), it remains unclear whether this may also be the case in non-responding KTR after a third vaccination dose.

Materials and Methods: Non-responding KTR (after two mRNA vaccinations) were investigated 4.5–6 months after study enrollment at first vaccination. One hundred sixty-six of 193 received a third vaccination between 3.5 and 5 months after the initial study enrollment and were always investigated 4 weeks later, exploring humoral immune response (ELISA) and specific cellular responses (interferon-γ release assay). Sixty-seven of 193 measurements in KTR were done immediately before the third vaccination or in KTR without further vaccination at 4.5–6 months.

Results: Of 193 KTR with no initial immune response 4 weeks after the second vaccination, 106/87 were immunized twice with 1273-mRNA/BNT162b2-mRNA, respectively. Additional mRNA booster vaccination led to positive seroconversion rates of 30–50%, while 16% of the initial non-responders demonstrated a delayed seroconversion without any booster vaccination. Using logistic regression analysis, a positive IgG response after the third vaccination was 23% more likely if the primary vaccine type was 1273-mRNA compared to BNT162b2-mRNA (OR = 4.420, 95% CI [1.208–16.173], $p = 0.025$). Primary vaccine type, a weak anti-SpikeS1 IgG response 4 weeks after second vaccination (3.2–35.2 BAU/ml, $p < 0.001$) and a lack of MMF/MPA

as part of the immunosuppressive treatment (trend, $p = 0.06$) but no other variables studied correlated with seroconversion success.

Conclusion: This observational study adds important evidence toward using 1273-mRNA as the primary mRNA vaccine type for immunosuppressed KTR.

Keywords: revaccination, kidney transplant recipient (KTR), SARS-CoV-2, humoral response, 1273-mRNA, BNT162b2-mRNA, clinical decision making, guidelines

INTRODUCTION

SARS-CoV-2 (Severe Acute Respiratory Syndrome Corona Virus-2) causes COVID-19 disease. More than 2 years have passed since its initial discovery in Wuhan, China in December 2019, and SARS-CoV-2 infection has rapidly evolved into an international pandemic with devastating consequences (1, 2). Seroconversion rates for the general population after two doses of mRNA vaccination (3, 4) and the usefulness of a third vaccine booster dose, particularly for protection against new viral variants (5) have been reported. Others and we demonstrated that kidney transplant recipients (KTR) have a markedly decreased seroconversion rate after two doses of mRNA vaccination (6, 7) resulting in reduced protection against COVID-19. On the other hand, due to higher mortality in KTR, successful vaccination to protect against COVID-19 disease is crucial for this population. It is worth noting that vector vaccines (such as CoronaVac) have even lower seroconversion rates (8). Short-term seroconversion rates in 2x mRNA vaccinated but non-responding KTR receiving the third vaccination with mRNA vaccine varies between a third (9) and a half (10, 11) but side-by-side comparisons of 1273-mRNA and BNT162b2-mRNA are lacking. While 1273-mRNA was associated with higher seroconversion rates after two vaccinations compared to BNT162b2-mRNA in our observational, multicenter cohort DIA-Vacc study (6), it remains unclear whether this may also be the case in non-responding KTR after a third vaccination. We also asked the question, of whether vaccine type for the initial two vaccinations or the third "booster" vaccination is more relevant for seroconversion success. Within the DIAVacc study cohort, we now report seroconversion rates after an approximately half-year in non-responsive KTR who either did not receive another booster vaccination or were exposed to a third vaccination using either 1273-mRNA, BNT162b2-mRNA, or vector vaccines in various combinations.

MATERIALS AND METHODS

Contextual Information

Background information of the underlying DIA-Vacc study (NCT number: 04799808), which investigated the time point 8 weeks after the first vaccination, has already been published elsewhere (6). However, as some parts of it are indispensable for the understanding and interpretation of the present work, excerpts from it are included here together with new notes. In all DIA-Vacc study vaccination participants (eligibility if >18 years old and signed informed consent) SARS-CoV-2 antibody formation was analyzed. Previous or current COVID-19 disease, specific IgG- or IgA-antibodies against the Spike protein S1 (*de novo* development as the primary study aim) and IgG-antibodies against the nucleocapsid protein subunit (NCP, to exclude previous and current infection), as well as antibodies against the receptor-binding domain (RBD), were assessed. In a representative subcohort, interferon-γ release assays (IGRA) were done to analyze the development of a T-cellular immune response after vaccination/disease. The study time points were before (T0), 8 weeks (T2), and 6 months (T3) after the start of vaccination (6). In the observational DIA-Vacc study, medical personnel, dialysis patients, and KTR were vaccinated against SARS-CoV-2 using either BNT162b2- or 1273-mRNA. The first vaccination dose was administered between 15 January and 24 February, followed by a second dose 3 or 4 weeks later, depending on the vaccine type. Only the first 26 of 36 nephrology centers, providing 3,101 participants, were accepted for the study due to funding restrictions. By vaccine availability during January (BNT162b2-mRNA) and February (1273-mRNA) 2021, only the first four dialysis centers assigned to the vaccination campaign, received BNT162b2-mRNA, while all the other following dialysis centers received 1273-mRNA vaccine for both (first and second dose) vaccinations. Neither any dialysis center nor any participant nor the study center (Dresden) had a choice or influence regarding the type of vaccine, which was assigned in the order of contacting the central vaccination institute in Saxony. The central vaccination institute distributed information about the start of the vaccination campaign *via* email at the same time to all dialysis centers.

Current Information

In the study presented here, we analyzed KTR who did not show a *de novo* positive humoral response at T2 (10) as defined by either IgG- or IgA- anti-SpikeS1 antibodies to the first and second mRNA vaccination. An optional third vaccination was offered between 3.5 and 5 months after T0 and always investigated 4 weeks later, targeting the highest humoral response (**Figure 1**). Since at that time no recommendation for a third vaccination was given by the German national authorities, the decision for an additional booster vaccination and choice of vaccine-type was in the hands of the dialysis centers. In addition to the mRNA vaccines BNT162b2-mRNA and 1273-mRNA, a vector vaccine was used in eight cases as a third dose after two vaccinations with mRNA vaccines (5x AZD1222 and 3x Ad26.COV2.S). COVID-19 diseased patients (symptomatically and asymptomatically, the latter being assessed by NCP seroconversion), during and after

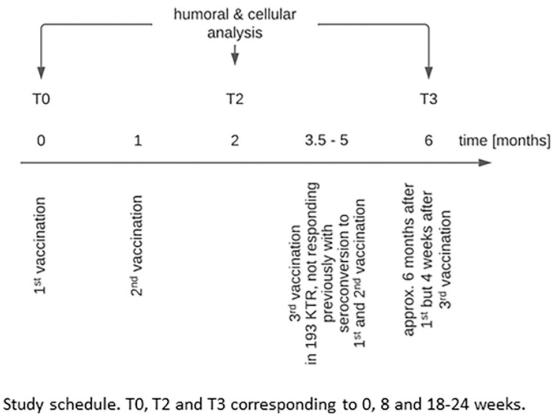


FIGURE 1 | Study schedule. T0, T2, and T3 correspond to 0, 8, and 18–24 weeks.

vaccination (up to T3) were excluded to evaluate the vaccination-related immune response. Patients were tested for SARS-CoV-2 infection by RT-PCR in the dialysis centers, if they presented one of the classic symptoms (fever, cough, shortness of breath, myalgias, diarrhea, or other symptoms consistent with such an infection) or if they were in contact with a person with RT-PCR-confirmed disease. Routine PCR screening without a cause was not part of the good medical practice of the dialysis centers.

For all antibody measurements, Euroimmun ELISAs on Euroimmun analyzers were used (12–16).

Endpoints

The primary endpoint of the study was the positive humoral immune response 4 weeks after a third vaccination dose as defined by *de novo* positivity of either IgG- or IgA- anti-SpikeS1 antibodies (**Table 1** and **Figure 2**) without the development of virus-specific NCP antibodies. Secondary endpoints were the development of vaccination-induced serological or T-cellular response parameters and titers.

Trends of antibody and IGRA titers (**Table 2**) are described in more detail at the end of the corresponding **Table 3**, **Figure 3A** (anti-SpikeS1 IgG), and **Figure 4A** (anti-RBD IgG). Likewise explained is the interval categorization (referred to as "levels" in the data analysis) to analyze the effect of the vaccines on the exact anti-SpikeS1 and –RBD IgG titer levels. In addition, change in levels between T2 and T3, varying from 0 to 5, was calculated for each patient (**Figure 3B** for anti-SpikeS1 and **Figure 4B** for anti-RBD IgG) and the dependence on the type of vaccine (**Figure 3C** for anti-SpikeS1 and **Figure 4C** for anti-RBD IgG) and the different vaccine combinations (**Figure 3D** for anti-SpikeS1 and **Figure 4D** for anti-RBD IgG) was investigated.

Statistical Analysis

In the descriptive analysis of the main study endpoints, categorical variables were summarized as absolute frequencies or percentages, and continuous variables were summarized using the mean and standard deviation or median and interquartile range (IQR). Time trends in anti-SpikeS1 IgG and –RBD IgG responses, as well as between-group differences, were analyzed either by the Wilcoxon signed-rank test, Mann–Whitney U test, or the chi-squared test, as appropriate.

As was observed in a number of studies (17, 18), a substantial difference in seroconversion response may occur after administering different vaccines. The analysis of IgG seroconversion predictors was carried out using multiple logistic regression analysis (**Table 4**). Hereby, we included the vaccine

TABLE 1 | Humoral and T-cellular response rates 6 months (T3) after the 1st vaccination.

Variable	Category	3x BNT162b2	2x BNT162b2, 1x 1273	2x 1273, 1x BNT162b2	3x 1273	2x mRNA, 1x vector	*p*_Value	2x mRNA
Patient number	N	57	22	16	63	8		67
Humoral response								
IgG-Ab or IgA-Ab SpikeS1 positive	n of total n (%)	17/57 (29.8%)	8/22 (36.4%)	8/16 (50%)	31/63 (49.2%)	1/8 (12.5%)	0.092	11/67 (16.4%)
IgG-Ab Spike S1 positive	n of total n (%)	12/57 (21.1%)	6/22 (27.3%)	7/16 (43.8%)	29/63 (46%)	1/8 (12.5%)	0.025	10/67 (14.9%)
RBD positive	n of total n (%)	11/57 (19.3%)	5/22 (22.7%)	3/16 (18.8%)	20/63 (31.7%)	1/8 (12.5%)	0.464	6/67 (9%)
IgA-Ab SpikeS1 positive	n of total n (%)	12/57 (21.1%)	6/22 (27.3%)	4/16 (25%)	21/63 (33.3%)	1/8 (12.5%)	0.524	7/67 (10.4%)
Interferon-γ release assay (IGRA)—T-cellular response								
IGRA positive	n of total n (%)	1/16 (6.2%)	0/7 (0%)	0/0 (0%)	6/16 (37.5%)	1/7 (14.3%)	0.06	5/25 (20%)

Humoral vaccination responses were assessed as positive when de novo production of the anti-SpikeS1 IgG and (anti-SpikeS1 IgA or IgG endpoint of the original DIA-Vacc study)/or IgA or IgG protein or anti-RBD IgG subunit was above positivity level. A positive T-cellular response to vaccination as assessed by interferon-γ release assay (IGRA) turned from a negative result on T0 to positive on T3, respectively (≥100 mIU/ml, as being recommended by the manufacturers). For this evaluation, all participants with asymptomatic or documented symptomatic** COVID-19 disease before and during vaccination up to T3 (6 months) were excluded.*

BNT162b2 = BNT162b2-mRNA or tozinameran or brand name Comirnaty; 1273 = 1273-mRNA or brand name Spikevax.

**Asymptomatic COVID-19 disease definition–neither knowledge nor symptoms of COVID-19 disease, but IgG-antibody reaction to nucleocapsid (T0, T2, or T3) or the Spike protein subunit S1 (only T0) of the SARS-CoV-2 virus is positive.*

***Symptomatic COVID-19 disease definition–SARS-CoV-2 PCR positive patients with clinical symptoms.*

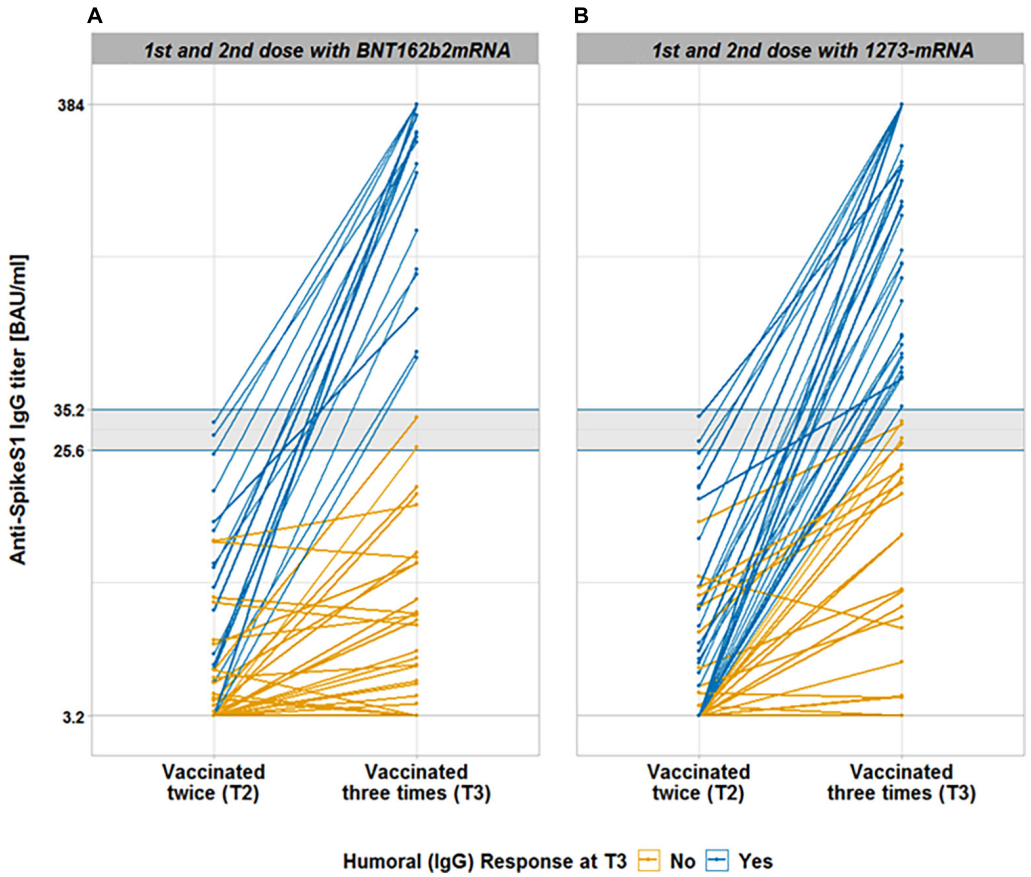


FIGURE 2 | Anti-SpikeS1 IgG antibody titer after 3rd vaccination in 193 kidney transplant recipients, not responding previously with seroconversion to 1st and 2nd vaccination. This figure shows anti-SpikeS1 IgG antibody titers after the 2nd and 3rd vaccination, depending on the vaccine type of the 1st and 2nd vaccine type being BNT162b2-mRNA **(A)** and 1273-mRNA **(B)**. The gray lines indicate the upper and lower threshold of the test (384 and 3.2 BAU/ml), respectively. Colored horizontal lines enclose and delineate the gray area for anti-SpikeS1 IgG antibody titer positivity (25.6 and <35.2 BAU/ml). The gray area still indicates a lack of seroconversion. A "weak" reaction (>3.2 and <35.2 BAU/ml) was distinguished from a negative reaction, the latter indicating 3.2 BAU/ml, in our statistical approach, while all values below 25.6 BAU/ml represent a negative antibody reaction according to the manufacture leaf. Blue progress lines indicate the antibody titer of kidney transplant recipients who seroconverted after a 3rd dose, whereas yellow progress lines indicate a lack of seroconversion.

type from the first/second as well as the third vaccination as potential predictors. The relative change in antibody titers after a third dose vs. the number of weeks passed between the second and third dose, as well as its dependence on primary and booster vaccine types, are shown in **Figures 5A,B** (anti-SpikeS1) and **Figures 6A,B** (anti-RBD IgG). Anti-SpikeS1 IgG (**Figures 5C,D**) and anti-RBD IgG (**Figures 6C,D**) titers after the second dose of vaccinations (despite being below positivity level) were plotted against titers after the third dose of vaccination to examine titer dependencies as predictors of an immune response. As other aspects could also influence seroconversion rates, we also investigated gender, age, time after transplantation, and hepatitis B vaccination failure on dialysis, as well as immunosuppressive therapy with MMF/MPA and the comorbidity diabetes mellitus.

For hypothesis testing, a significance level of 5% (two-sided) was chosen. Data analysis was implemented in the R Environment for Statistical Computing (19), version 4.0.4.

RESULTS

Follow-up data were available in 193 KTR (58 ± 13.6 years, 66% men, **Tables 5**, **6**) not responding with seroconversion as defined by an insufficient humoral immune response at T2 (<1.1 ratios for anti-SpikeS1 IgA ab and <35.2 BAU/ml for IgG ab), of which 106/87 were immunized twice with 1273-mRNA or BNT162b2-mRNA, respectively. Of 193 KTR, 166 received an additional booster vaccination 3.5 to 5 months after the study started. Twenty-seven of 193 KTR did not receive any booster vaccination for up to 6 months, while 40/193 were additionally investigated as unboostered study participants around 5 months before receiving a booster vaccination and reevaluation at 6 months (**Figure 7**). The mean time on dialysis before transplantation is 6 years and the mean time after transplantation is 8.5 years. One in seven had been kidney transplanted before. Immunosuppressive therapy included a calcineurin inhibitor in 93%, MMF/MPA in 88%, and corticosteroids in only 47% of cases, whereas mTOR inhibitors

TABLE 2 | Humoral and T-cellular titers at study time points two (T2) and six (T3) months in different vaccine combinations.

Var	Type of vaccines	Category	T2	T3	*P*-value
IgG-Ab Spike S1	3x BNT162b2	Median (IQR)	3.2 (3.2–4.6)	3.8 (3.2–16.7)	<0.001
	2x BNT162b2, 1x 1273	Median (IQR)	3.2 (3.2–3.2)	3.5 (3.2–52.5)	0.003
	2x 1273, 1x BNT162b2	Median (IQR)	3.2 (3.2–6.2)	22.8 (3.2–52.6)	0.003
	3x 1273	Median (IQR)	3.2 (3.2–5)	28.1 (3.2–209.4)	<0.001
	2x mRNA, 1x vector vaccine	Median (IQR)	3.2 (3.2–3.2)	3.2 (3.2–6)	0.181
	2x mRNA	Median (IQR)	3.2 (3.2–6.4)	3.6 (3.2–21.9)	
RBD-IgG-Ab RBD	3x BNT162b2	Median (IQR)	3.8 (3.2–6.2)	10.2 (7.6–15.3)	0.088
	2x BNT162b2, 1x 1273	Median (IQR)	1.4 (1.1–4.8)	3.6 (1.6–21)	0.093
	2x 1273, 1x BNT162b2	Median (IQR)	0 (0–5.3)	4.2 (0–29.9)	0.5
	3x 1273	Median (IQR)	3.9 (1.9–7.7)	7.5 (0–58.2)	<0.001
	2x mRNA, 1x vector vaccine	Median (IQR)	2.7 (0–3.4)	0 (0–1.1)	0.529
	2x mRNA	Median (IQR)	4.5 (1.9–7.7)	3.9 (0–9.4)	
IgA-Ab Spike S1	3x BNT162b2	Median (IQR)	0.3 (0.2–0.5)	0.4 (0.2–0.7)	0.156
	2x BNT162b2, 1x 1273	Median (IQR)	0.4 (0.2–0.7)	0.5 (0.3–1.3)	0.001
	2x 1273, 1x BNT162b2	Median (IQR)	0.3 (0.2–0.3)	0.3 (0.2–0.7)	0.289
	3x 1273	Median (IQR)	0.3 (0.2–0.5)	0.5 (0.3–1.4)	<0.001
	2x mRNA, 1x vector vaccine	Median (IQR)	0.3 (0.1–0.4)	0.3 (0.2–0.4)	0.313
	2x mRNA	Median (IQR)	0.4 (0.2–0.6)	0.3 (0.2–0.6)	
Interferon-γ release assays (IGRA)	3x BNT162b2	Median (IQR)	13.3 (0.9–38.9)	8.3 (1.3–60.3)	0.266
	2x BNT162b2, 1x 1273	Median (IQR)	0 (0–0.1)	17.2 (5.5–43.4)	0.5
	2x 1273, 1x BNT162b2	Median (IQR)	29.3 (29.3–152.8)		
	3x 1273	Median (IQR)	23.6 (3.7–151.4)	28.6 (6.1–200.4)	0.952
	2x mRNA,1x vector vaccine	Median (IQR)	7.1 (0–183.6)	11.9 (7.1–31.3)	0.062
	2x mRNA	Median (IQR)	26.1 (11.8–444.3)	23.7 (5.8–107)	

This table compares the average titer levels (median/interquartile range = IQR) on T3 with T2 (different columns) for the different anti-SpikeS1 IgA, IgG, RBD-IgG antibodies as well as for cellular immunity via Interferon-γ release assay = IGRA measurements in patients who received different combinations of mRNA vaccine (different rows). For this evaluation, all participants with asymptomatic or documented symptomatic** COVID-19 disease before and during vaccination up to T3 (6 months) were excluded. BNT162b2 = BNT162b2-mRNA or tozinameran or brand name Comirnaty; 1273 = 1273-mRNA or brand name Spikevax.*

**Asymptomatic COVID-19 disease definition–neither knowledge nor symptoms of COVID-19 disease, but IgG-antibody reaction to nucleocapsid (T0, T2, or T3) or the Spike protein subunit S1 (only T0) of the SARS-CoV-2 virus is positive.*

***Symptomatic COVID-19 disease definition–SARS-CoV-2 PCR positive patients with clinical symptoms.*

were used in 11% and Belatacept in only 7%. Further baseline characteristics can be found in **Table 5** and a schedule in **Figure 1**.

Study End Points

Humoral and Cellular Response Rates 6 Months After the First Vaccination (T3) in Kidney Transplant Recipients Not Responding Previously With Seroconversion 8 Weeks After the First Vaccination

Seroconversion results of initially non-responsive KTR based on different third vaccine types (no vaccine vs. BNT162b2-mRNA vs. 1273-mRNA vs. vector vaccine) varied between 13 and 50% (**Table 1**).

Four weeks after a third vaccination, humoral response in terms of seroconversion of anti-SpikeS1 IgG or IgA was observed in 29.8 and 36.4% of KTR vaccinated three times with BNT162b2-mRNA or twice with BNT162b2-mRNA plus once using 1273-mRNA. In contrast, the seroconversion rate of those vaccinated with 1273-mRNA was 50 and 49.2%, depending on the vaccine combination used (2x 1273-mRNA plus 1x BNT162b2-mRNA and 3x 1273-mRNA, respectively). Comparing all 3-fold vaccine combinations, however, statistical significance was missed, but a strong trend emerged ($p = 0.092$, **Table 1**). Regarding the seroconversion of anti-SpikeS1 IgG, response rates of 21.1 (3x BNT162b2-mRNA) and 27.3% (2x BNT162b2-mRNA plus 1x 1273-mRNA) were observed. In contrast, again higher seroconversion rates of 43.8 (2x 1273-mRNA plus 1x BNT162b2-mRNA) and 46% (3x 1273-mRNA) were achieved with the 1273-mRNA-based vaccine combinations. The difference comparing all 3-fold vaccine combinations was statistically significant ($p = 0.025$, **Table 1**). Looking at anti-RBD IgG when comparing all 3-fold vaccine combinations performed, there was no significant difference (**Table 1**). BNT162b2-mRNA based combinations showed rates of 19.3 (3x BNT162b2-mRNA) and 22.7% (2x BNT162b2-mRNA plus 1x 1273-mRNA) and 1273-mRNA based combinations of 18.8 and 31.7% (2x 1273-mRNA plus 1x BNT162b2-mRNA and 3x 1273-mRNA, respectively). Considering anti-SpikeS1 IgA conversion rates, again there was no significant difference in response rates, considering all 3-fold vaccine combinations ($p = 0.524$, **Table 1**) of 21.1 (3x BNT162b2-mRNA), 27.3 (2x BNT162b2-mRNA plus 1x 1273-mRNA), 25 (2x 1273-mRNA plus 1x BNT162b2-mRNA) and 33.3% (3x 1273-mRNA). The T-cellular immune response could only be examined in part in the KTR and borderline non-significant differences were found ($p = 0.06$, again in regard to a comparison of all 3-fold vaccine combinations).

TABLE 3 | Interval categorization into "level" of anti-SpikeS1-IgG and anti-RBD ranges of all participants.

Level	Interval [unit]	Participants at T2	Participants at T3
IgG level	**Interval [BAU/ml]**	***N***	***N***
–1	IgG < 25.6	154	98
0	25.6 ≤ IgG < 35.2	4	6
1	35.2 ≤ IgG < 100	0	15
2	100 ≤ IgG < 200	0	9
3	200 ≤ IgG < 300	0	10
4	IgG ≥ 300	0	20
RBD level	**Interval [% inhibition]**	***N***	***N***
–1	RBD < 20	93	65
0	20 ≤ RBD < 35	0	5
1	35 ≤ RBD < 50	0	2
2	50 ≤ RBD < 65	0	2
3	65 ≤ RBD < 80	0	3
4	RBD ≥ 80	0	16

The detectable ranges of anti-SpikeS1 and –RBD IgG antibody values are categorized into six intervals, labeled from –1 to 4 (referred to as "levels" in the data analysis). The limit of the "–1" level is defined by the manufacturer's test limit on negativity. The limit of the next higher level "0" follows directly upwards and includes the gray area of the corresponding test (below the positivity threshold). The limits of the other levels are chosen arbitrarily ("1," "2," "3," and "4") and represent the remaining linear test range in approximately equal intervals.

Trend differences, analogous to humoral seroconversion rates, could thereby be confirmed in distinct vaccine combinations. A tendency toward a better response rate for triple vaccinations with 1273-mRNA is 37.5%, compared to 6.2% for triple vaccinations with BNT162b2-mRNA (**Table 1**). The number of measured KTR who were vaccinated heterologically using 2x mRNA plus 1x vector vaccine is small. Response rates resemble spontaneous delayed seroconversion rates of the unboostered KTR (**Table 1**).

The unboostered KTR showed combined delayed seroconversion rates of anti-SpikeS1 IgA or IgG of 16.4%, 6 months after the first mRNA vaccination. Hereby, the rates for anti-SpikeS1 IgG and anti-RBD IgG alone were 14.9 and 9%, respectively. Humoral immunity as anti-SpikeS1 IgA was 10.4% in this group and T-cellular immune reaction measured by interferon-γ release assays showed a spontaneous delayed response rate of 20% (**Table 1**).

Next, we explored whether IgG seroconversion success after booster vaccination depends on the initial or booster vaccine type, immunosuppressive agents, comorbidities, etc. as evaluated before for seroconversion success after two vaccinations (6). Those were further examined *via* a logistic regression approach.

Predictors for Seroconversion After a Third mRNA Vaccination in Kidney Transplant Recipients, Not Responding Previously With Seroconversion 8 Weeks After the First Vaccination

Using logistic regression analysis, we found that a humoral response after revaccination was 23% more likely if the primary vaccine was 2x 1273-mRNA than 2x BNT162b2-mRNA (OR = 4.420, 95% CI [1.208–16.173], p = 0.025, **Table 3** and **Figure 2**). The median antibody titers when 1273-mRNA was used as the exclusive vaccine type were 28.1 BAU/ml for anti-SpikeS1 IgG compared with the titers of 3.8 BAU/ml when BNT162b2-mRNA was used (**Table 2**). The effect of the third vaccine type was non-significant [$\chi^2_{(2)}$ = 0.41, p = 0.639, **Table 4**]. Neither differed time between the second and third vaccination, gender, age, time after transplantation, hepatitis B vaccination failure on dialysis, and diabetes mellitus as comorbidity significantly in the multiple logistic regression analysis. Seroconversion success was observed in 53 vs. 20% of patients with a weak (3.2–35.2 BAU/ml) vs. negative (<3.2 BAU/ml) anti-SpikeS1 IgG response at T2, respectively (OR 1.360, 95% CI [1.167–1.584], p = 0.001, **Table 4**). The anti-SpikeS1 IgG threshold distributions were similar for primary vaccine subgroups [$\chi^2_{(1)}$ = 0.23, p = 0.630], but differed significantly after revaccination [$\chi^2_{(2)}$ = 10.76, p = 0.005]. KTR with primary 1273-mRNA were consistently more likely to respond with seroconversion than those with primary BNT162b2-mRNA: 66.7 vs. 41.2% (p = 0.074) in the weak anti-SpikeS1 IgG group and 32.1 vs. 6.3% success rate (p = 0.003) in the negative anti-SpikeS1 IgG group. This overall advantage for primary 1273-mRNA compared to BNT162b2-mRNA vaccination is also appreciated in **Figure 5C**, where the orange line (1273-mRNA) is above the green line (BNT162b2-mRNA) for most patients' values except the very few in the highest range of the weak anti-SpikeS1 IgG group. Looking at different mRNA-based vaccine combinations, all vaccine combinations show a booster success correlation to a weak compared to no anti-SpikeS1 IgG reaction after two vaccinations, since all lines showed an incline (**Figure 5D**). As indicated by **Figure 5D**, those ostensibly relying on 3x 1273-mRNA (orange line) or heterologous 2x 1273-1xBNT162b2-mRNA (blue line) vaccination lead not only to higher seroconversion rates but also to markedly higher IgG levels independent on no or a weak response after two vaccinations.

A similar picture indicating advantages for 1273-mRNA emerges when looking at anti-RBD IgG seroconversion rates considering the primary vaccine types (**Figure 6C**–1273-mRNA in orange and BNT162b2-mRNA in green). With regard to RBD-level changes after different vaccine combinations, 3xBNT162b2-mRNA appears to be the least effective and without correlation to some weak RBD-level induction after two vaccinations (**Figure 6D**–green line without incline). RBD-stimulation after three vaccinations appeared positively related to all other vaccine combinations (**Figure 6D**–orange, black, and blue inclining lines). For RBD-level changes, the most effective vaccine combination appears to be the heterologous 2x 1273-1xBNT162b2-mRNA (blue line), while 3x 1273-mRNA and heterologous 2xBNT162b2-/1x1273-mRNA were intermediate (orange and black lines in **Figure 6D**).

Logistic regression revealed that MMF/MPA in the context of immunosuppressive therapy was borderline significant as another predictor of seroconversion after a third mRNA vaccination in KTR. Thereby, the use of MPA/MMF is associated with a worse seroconversion rate (OR = 7.086, 95% CI [0.917–54.730], p = 0.060, **Table 4**).

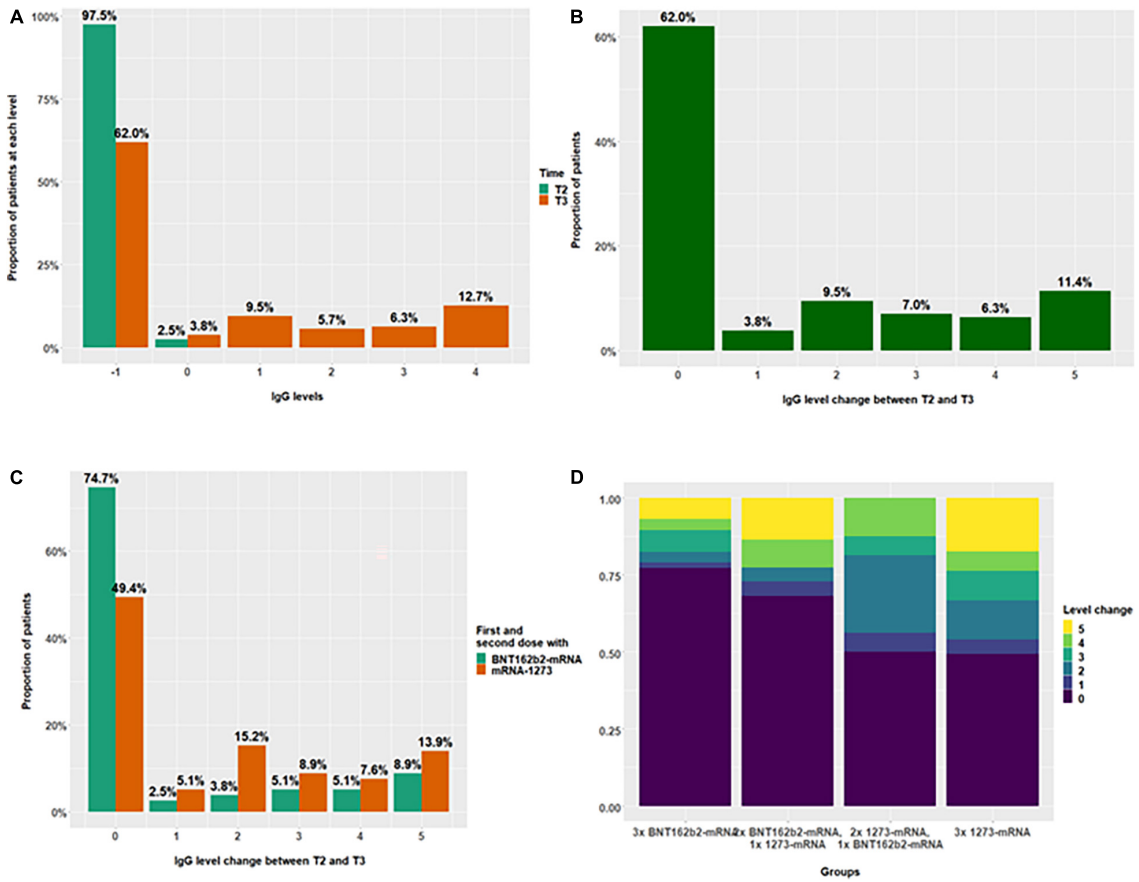


FIGURE 3 | Distribution of anti-SpikeS1 IgG level change after the 3rd vaccination in 193 kidney transplant recipients, not responding previously with seroconversion to the 1st and 2nd vaccination. The distributions of IgG levels according to interval categorizations at T2 and T3 **(A)**, as well as their change after the third vaccination are summarized in panels **(B–D)**. Level "–1" is assigned to negative test values (anti-SpikeS1 IgG < 25.6 BAU/ml). Values below the corresponding positivity threshold (35.2 BAU/ml) but above the threshold for negativity were assigned to level "0". The remaining test values were divided into four intervals of approximately equal length (level "1" <100 and ≥35.2; level "2" <200 and ≥100; level "3" <300 and ≥200; level "4" ≥300). These intervals can be used to quantify the change of IgG levels between T2 and T3, where, for example, a positive change corresponds to an increase in IgG, with a change of five being the maximum increase, which occurred in 11.4% **(B)**. Level changes (proportions of patients) between T2 and T3 depending on the vaccine used for 1st and 2nd vaccination are shown in panel **(C)**, whereas, the proportions with regard to the different vaccine combinations are shown in panel **(D)**.

Anti-Spike S1 IgG seroconversion rates as a function of the vaccine type used for the first and second vaccination, 4 weeks after the latter (T2, all without positive seroconversion) and then again 4 weeks after the third vaccination (T3) are depicted in **Figure 2**. We demonstrate higher response rates (blue lines vs. non-responders in yellow lines) in KTR primarily immunized with 1273-mRNA compared to BNT162b2-mRNA.

Analysis of Humoral and T-Cellular Titer Levels 6 Months After the First Vaccination (T3) in Kidney Transplant Recipients Not Responding Previously With Seroconversion 8 Weeks After the First Vaccination

Titer levels, two (T2) and six (T3) months after initial vaccination, concerning the humoral and T-cellular vaccination response are shown with median and IQR, for all vaccine combinations investigated, in **Table 2**.

Comparing all triple mRNA vaccine combinations, median anti-SpikeS1 IgG levels between T3 and T2 evaluations differed significantly. In BNT162b2-mRNA based vaccination regimes, there was an increase from 3.2 to 3.8 (3x BNT162b2-mRNA) or from 3.2 to 3.5 BAU/ml (2x BNT162b2-mRNA plus 1x 1273-mRNA). In 1273-mRNA-based vaccination regimes, IgG titers increased from 3.2 to 28.1 (3x 1273-mRNA) and from 3.2 to 22.8 BAU/ml (2x 1273-mRNA plus 1x BNT162b2-mRNA). Heterologous combination with vector vaccine showed no median increase in titer. Interestingly, after 2x mRNA vaccination, unboostered KTR revealed some delayed (the latter because without booster or indication of SARS-CoV-2 exposure) increase from 3.2 to 3.6 BAU/ml.

Regarding anti-RBD IgG (indicating neutralizing activity against the SARS-CoV-2 virus variants alpha to delta), statistically significant median titer increases were only seen in the 3-fold 1273-mRNA vaccinated KTR group (3.9–7.5% inhibition, $p < 0.001$), while all other triple mRNA or vector vaccinated

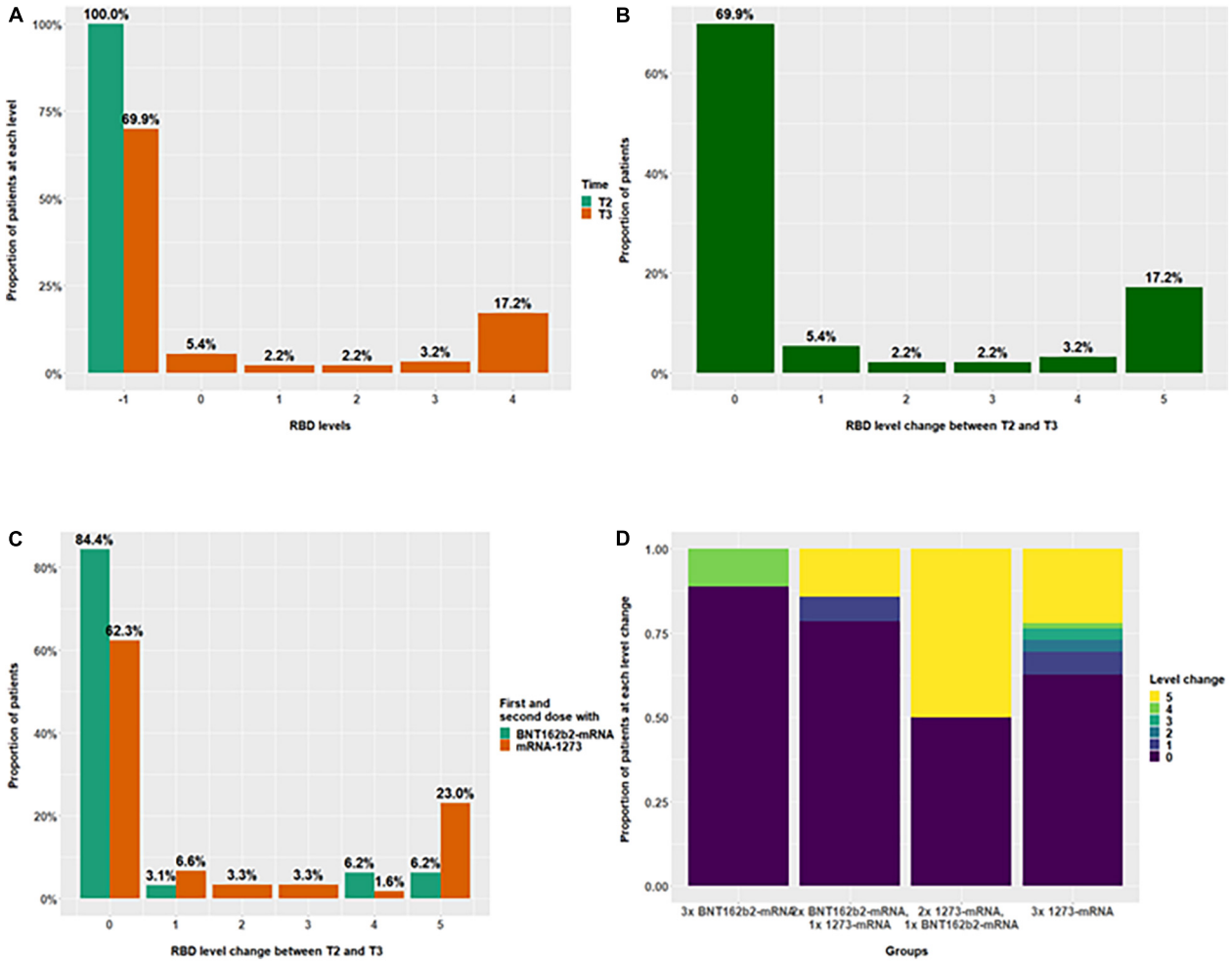


FIGURE 4 | Distribution of anti-RBD IgG level change after the 3rd vaccination in 193 kidney transplant recipients, not responding previously with seroconversion to the 1st and 2nd vaccination. The distributions of RBD levels according to interval categorizations at T2 and T3 **(A)**, as well as their change after the third vaccination are summarized in **Figures 5B–D**. Level "–1" is assigned to negative test values (anti-RBD IgG < 20% inhibition [IH]). Values below the corresponding positivity threshold (35% IH) but above the threshold for negativity were assigned to level "0". The remaining test values were divided into four intervals of approximately equal length (level "1" <50 and ≥35; level "2" <65 and ≥50; level "3" <80 and ≥65; level "4" ≥80). These intervals can be used to quantify the change of RBD levels between T2 and T3, where, for example, a positive change corresponds to an increase in RBD, with a change of five being the maximum increase, which occurred in 17.2% **(B)**. Level changes (proportions of patients) between T2 and T3 depending on the vaccine used for 1st and 2nd vaccination are shown in panel **(C)**, whereas, the proportions with regard to the different vaccine combinations are shown in panel **(D)**.

TABLE 4 | Achieving humoral IgG response after booster vaccination (logistic regression).

Risk factor	OR	95% CI	*P-value*
Anti-SpikeS1 IgG after two doses	1.360	[1.167,1.584]	<0.001
Time between 2nd and 3rd doses	0.972	[0.855,1.106]	0.668
1st and 2nd dose with 1273-mRNA (ref = BNT162b2-mRNA)	4.420	[1.208,16.173]	0.025
3rd dose with 1273-mRNA (ref = BNT162b2-mRNA)	1.080	[0.537,2.171]	0.830
Sex (ref = female)	1.339	[0.547,3.278]	0.523
Age	0.975	[0.946,1.005]	0.107
Time after transplantation (years)	1.028	[0.954,1.106]	0.471
HepB vaccination failure	1.502	[0.231,9.748]	0.670
MMF/MPA (ref = yes)	7.086	[0.917,54.730]	0.060
Diabetes mellitus (ref = yes)	0.610	[0.182,2.044]	0.423

Logistic regression on achieving anti-SpikeS1 IgG response 4 weeks after 3rd SARS-CoV-2 vaccination. MMF-MPA, mycophenolate mofetil or mycophenolic acid.

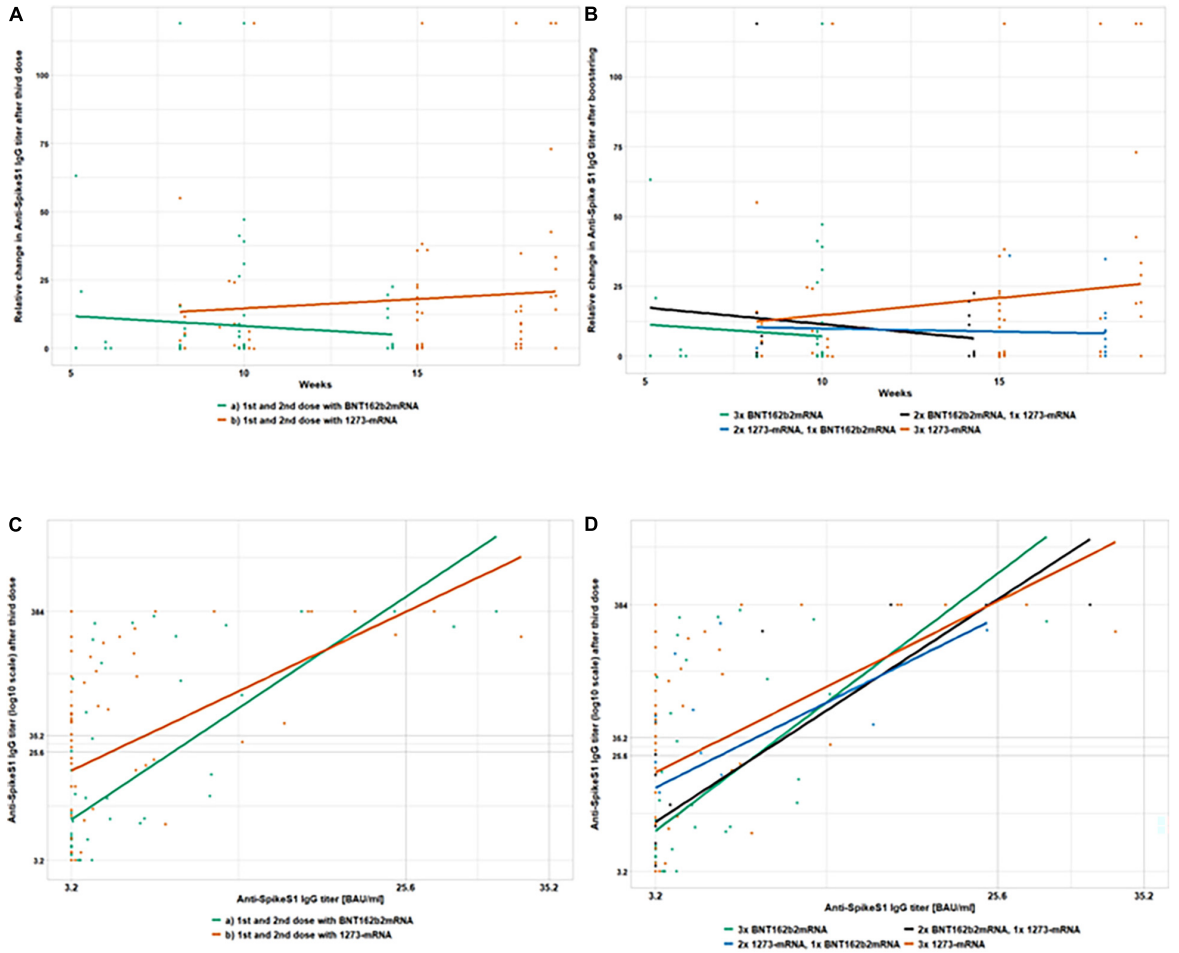


FIGURE 5 | Absolute and relative changes in anti-SpikeS1 IgG antibody titer after the 3rd vaccination in 193 kidney transplant recipients, not responding previously with seroconversion to the 1st and 2nd vaccination. This figure shows absolute and relative changes in anti-SpikeS1 IgG antibody titers between the 2nd and 3rd vaccination. At T2, 4 weeks after the second vaccination dose and T3, 3.5–5 months after the first but always 4 weeks after the third vaccination dose in 193 kidney transplant recipients, anti-SpikeS1 IgG titers are depicted in four different sub-panels. The relative change in antibody titers after a third dose vs. the number of weeks passed between the second and third dose, as well as its dependence on primary **(A)** and booster **(B)** vaccine types, are shown. In panel **(A)** the horizontal progression of the orange (1273-mRNA) and green (BNT162b2) lines indicate the lack of dependence on the timing of booster vaccination. The height indicates the titer levels 4 weeks after the third vaccination. The same applies to the horizontal courses of the green (3x BNTb2 mRNA), black (2x BNT162b2 mRNA, 1x 1273 mRNA), blue (2x 1273 mRNA, 1x BNT162b2 mRNA), and orange (3x 1273 mRNA) lines in panel **(B)** which also show a lack of dependence on the time of booster vaccination. Again, the heights represent the titer heights 4 weeks after the third vaccination. In panels **(C,D)** anti-SpikeS1 IgG titers after the second dose of vaccinations (despite being below positivity level) were plotted against titers after the third dose of vaccination to examine titer dependencies as predictors of an immune response. Gray lines enclose the upper and lower threshold of the test (384 and 3.2 BAU/ml, respectively), as well as the gray area of the test (25.6 and 35.2 BAU/ml). In panel **(C)** the incline of the orange (1273-mRNA) and green (BNT162b2) lines indicate a booster success correlation to a weak compared to no anti-SpikeS1 IgG reaction after two vaccination. The height indicates the titer levels and thus the strength of seroconversion 4 weeks after the third vaccination. The same applies to the incline of the green (3x BNTb2 mRNA), black (2x BNT162b2 mRNA, 1x 1273 mRNA), blue (2x 1273 mRNA, 1x BNT162b2 mRNA), and orange (3x 1273 mRNA) lines in panel **(D)** which also show a booster success correlation to a weak compared to no anti-SpikeS1 IgG reaction after two vaccination. Heights again indicate the titer levels and thus the strength of seroconversion 4 weeks after the third vaccination.

or unboostered KTR did not show a significant increase in median RBD titers.

Triple mRNA vaccinated KTR demonstrated increasing median titers from T2 to T3 with respect to anti-SpikeS1 IgA. The greatest increase in titer was seen again in the 3-fold 1273-mRNA vaccinated KTR, from 0.3 (IQR.2–0.5) to 0.5 ratio (IQR.3-1.4, $p < 0.001$). The group of heterologous vector vaccinated KTR showed no increase in median titers similar to those vaccinated only two times.

T-cellular immunity results were not available for all triple-vaccinated KTR at both time points. Interestingly, in 3x BNT162b2-mRNA vaccinated KTR, median titer decreased from 13.3 to 8.3 mIU/ml but showed an increase in the range of values (IQR from 0.9–38.9 to 1.3–60.3). In contrast, after 2x BNT162b2-mRNA plus 1x 1273-mRNA, there was a median titer increase from 0 to 17.2 mIU/ml. No IGRA measurements were available for 3x 1273-mRNA plus 1x BNT162b2-mRNA vaccinated KTR at T3. The only group in which the 75% percentile of levels

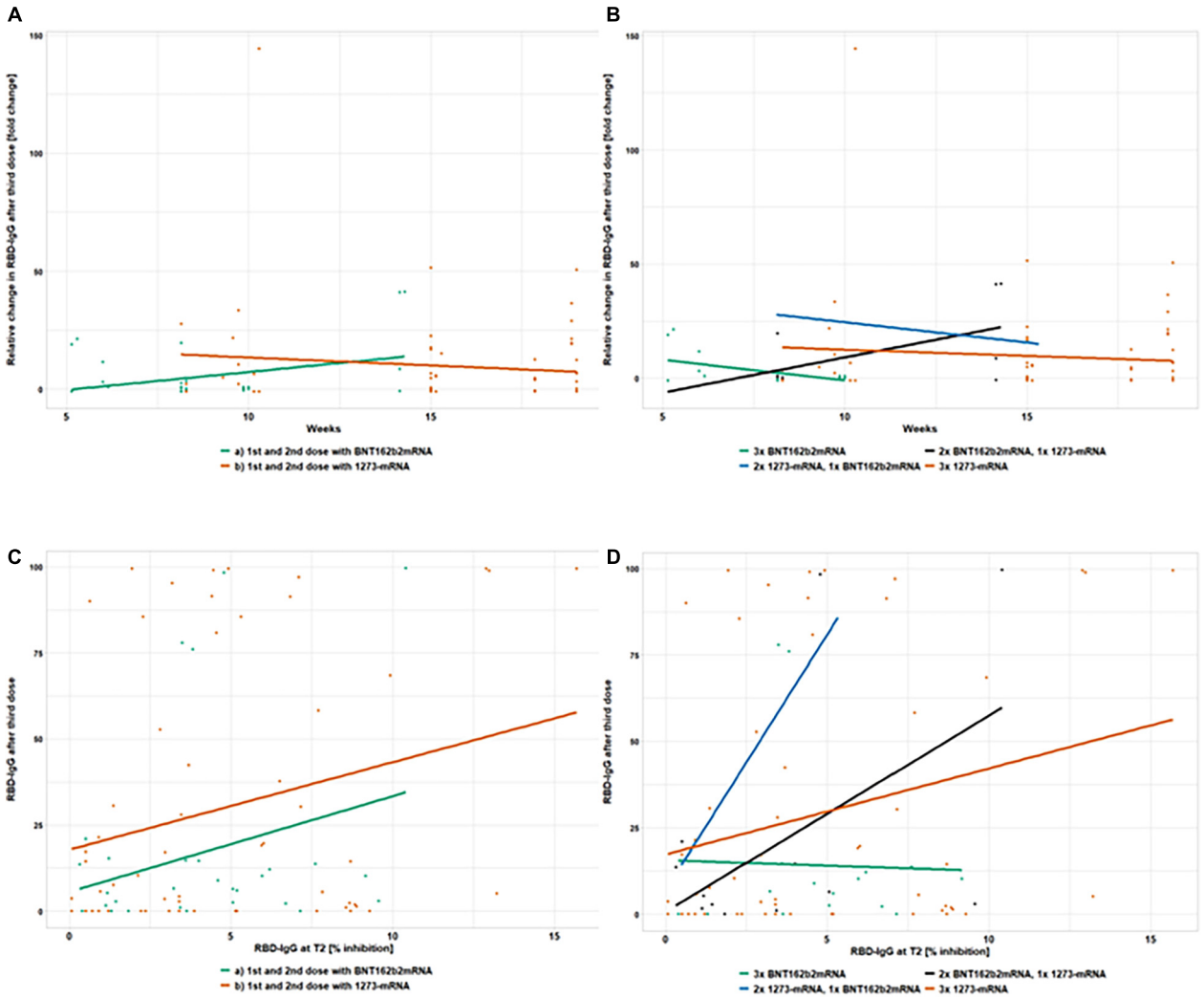


FIGURE 6 | Absolute and relative changes in anti-RBD IgG antibody titer after the 3rd vaccination in 193 kidney transplant recipients, not responding previously with seroconversion to the 1st and 2nd vaccination. This figure shows absolute and relative changes in anti-RBD IgG antibody titers between the 2nd and 3rd vaccination. At T2, 4 weeks after the second vaccination dose and T3, 3.5–5 months after the first but always 4 weeks after the third vaccination dose in 193 kidney transplant recipients, anti-RBD IgG titers are depicted in four different sub-panels. The relative change in antibody titers after a third dose vs. the number of weeks passed between the second and third dose, as well as its dependence on primary **(A)** and booster **(B)** vaccine types, are shown. In panel **(A)** the horizontal progression of the orange (1273-mRNA) and green (BNT162b2) lines indicate the lack of dependence on the timing of booster vaccination. The height indicates the titer levels 4 weeks after the third vaccination. The same applies to the horizontal courses of the green (3x BNTb2 mRNA), black (2x BNT162b2 mRNA, 1x 1273 mRNA), blue (2x 1273 mRNA, 1x BNT162b2 mRNA), and orange (3x 1273 mRNA) lines in panel **(B)** which also show a lack of dependence on the time of booster vaccination. Again, the heights represent the titer heights 4 weeks after the third vaccination. In panels **(C,D)** anti-RBD IgG titers after the second dose of vaccinations (despite being below positivity level) were plotted against titers after the third dose of vaccination to examine titer dependencies as predictors of an immune response. Gray lines enclose the upper and lower threshold of the test (100 and 0% inhibition, respectively). In panel **(C)** the incline of the orange (1273-mRNA) and green (BNT162b2) lines indicate a booster success correlation to a weak compared to no anti-RBD IgG reaction after two vaccination. The height indicates the titer levels and thus the strength of seroconversion 4 weeks after the third vaccination. The same applies to the incline of the green (3x BNTb2 mRNA), black (2x BNT162b2 mRNA, 1x 1273 mRNA), blue (2x 1273 mRNA, 1x BNT162b2 mRNA), and orange (3x 1273 mRNA) lines in panel **(D)** which also show a booster success correlation to a weak compared to no anti-RBD IgG reaction after two vaccination. Heights again indicate the titer levels and thus the strength of seroconversion 4 weeks after the third vaccination.

(upper limit of the IQR) was above the positive test threshold, and thus another 25% of the values above it, were 3x 1273-mRNA vaccinated KTR (titer increased from 23.6 to 28.6 mIU/ml while IQR spread from 3.7–151.4 to 6.1–200.4). Even though heterologous vector vaccinated KTR formally show an increase in median titers (7.1 to 11.9 mIU/ml), the IQR on the other hand reduced from 0–183.6 to 7.1–31.3 at the respective time points. In unboostered KTR with 2x mRNA vaccination, both the median titers decreased from 26.1 to 23.7 mIU/ml and the IQR decreased from 11.8–444.3 to 5.8–107.

For better illustration, we categorized the anti-SpikeS1 IgG (**Table 3** and **Figure 3A**) and anti-RBD IgG titer levels (**Table 3**

TABLE 5 | Baseline characteristics of SARS-CoV-2 unexposed entire cohort corresponding to T2 non-seroconverted kidney transplant recipients and the boostered cohort corresponding to three vaccine dose kidney transplant recipients.

Variable	Category	Entire cohort	Boostered cohort
Number	Evaluable	193	166
Age (years)	Mean ± SD	58 ± 13.6	58.4 ± 13.5
Male sex	n/%	128/66.3	117/70.5
BMI (kg/m^2)	mean ± SD	25.9 ± 5	26 ± 4.9
Cause of end stage renal disease	n/%	116/60.1	99/59.6
Diabetes-Hypertension-Vascular disease	n/%	35/18.1	32/19.3
Glomerulonephritis-Interstitial nephritis	n/%	49/25.4	41/24.7
Vasculitis	n/%	7/3.6	6/3.6
Polycystic kidney disease	n/%	25/13	20/12
Unknown	n/%	77/39.9	67/40.4
Drug treated comorbidities	n/%	176/91.2	149/89.8
Diabetes mellitus	n/%	35/18.1	32/19.3
Cardiovascular disease	n/%	170/88.1	143/86.1
Lung disease	n/%	15/7.8	11/6.6
Liver cirrhosis	n/%	3/1.6	3/1.8
Cancer	n/%	5/2.6	3/1.8
None	n/%	17/8.8	17/10.2
Time on dialysis (years)	Mean ± SD	6.1 ± 6.2	6.3 ± 6.3
Time on transplantation (years)	Mean ± SD	8.5 ± 6.3	8.2 ± 6.1
Previous transplantation	n/%	28/14.5	23/13.9
Hepatitis B vaccination failure	n/%	10/5.2	9/5.4
Flu vaccination winter 2020/2021	n/%	106/54.9	90/54.2
On immunosuppressive therapy	n/%	193/100	166/100
Corticosteroids	n/%	91/47.2	78/47
Calcineurin-inhibitor	n/%	179/92.7	154/92.8
MMF/MPA	n/%	170/88.1	146/88
mTOR-Inhibitor	n/%	22/11.4	18/10.8
Belatacept	n/%	14/7.3	12/7.2
T-cell depleting ab	n/%	0/0	0/0
B-cell depleting ab n	n/%	1/0.5	1/0.6
Other	n/%	2/1	2/1.2
Type of vaccine			
BNT162b2-mRNA	n/%	87/45.1	83/50
1273-mRNA	n/%	106/54.9	83/50

The entire cohort consists of non-responding KTR after two mRNA vaccinations. Boostered cohort consists of non-responding KTR after two mRNA vaccinations who received the third vaccination.
For this evaluation, all patients with asymptomatic or documented symptomatic** COVID-19 disease before and during vaccination up to T3 (6 months) were excluded. Hepatitis B vaccination failure definition–patients with unsuccessful vaccination after at least four attempts; MMF-MPA, mycophenolate mofetil or mycophenolic acid.*
**Asymptomatic COVID-19 disease definition–neither knowledge nor symptoms of COVID-19 disease, but IgG-antibody reaction to nucleocapsid (T0, T1, T2, or T3) or the Spike protein subunit S1 (only T0) of the SARS-CoV-2 virus is positive.*
***Symptomatic COVID-19 disease definition–SARS-CoV-2 PCR positive patients with clinical symptoms.*

and **Figure 4A**) into six intervals each and plotted them accordingly at times T2 and T3. The exact limits of the intervals (referred to as levels) can be found in the corresponding tables mentioned above. Regarding anti-SpikeS1 IgG levels, **Figure 3A** shows the changed distribution of the different IgG titer levels 4 weeks after the third vaccination at T3 (brown, indicating positive seroconversion of 34.2%) compared to before at T2 (green) in primary non-responders. In addition to this, we counted numbers of positive level changes graduating the extent of a positive response. To achieve a seroconversion from an initial negative test result, a maximum increase of 2 level changes is needed (minimally, if previously in the gray area, only 1 level change in increase is needed). These level changes are displayed in **Figure 3B** for anti-SpikeS1 IgG, subdivided into the primary vaccines in **Figure 3C** and subdivided into the different 3-fold mRNA vaccine combinations in **Figure 3D** of the corresponding plot. The level changes of anti-SpikeS1 IgG measurements of $\geq$2 add up to 34.2% (**Figure 3B**). When analyzed by the corresponding primary vaccination regimen, there is a proportional advantage in the use of 1273-mRNA as the initial vaccine for KTR (**Figure 3C**), as there was for the overall response for each level change. This is further emphasized in the graphical representation of the level changes subdivided into the different 3-fold mRNA vaccine combinations (**Figure 3D**).

TABLE 6 | Baseline characteristics of different vaccine combinations.

Variable		3x BNT162b2	2x BNT162b2, 1x 1273	2x 1273, 1x BNT162b2	3x 1273	2x mRNA, 1x vector	2x mRNA
Number	Evaluable	57	22	16	63	8	67
Age (years)	Mean ± SD	55.6 ± 15.5	60 ± 13.4	58.9 ± 13.3	59.9 ± 12.3	60.8 ± 7.4	57.1 ± 12.9
Male Sex	n/%	39/68.4	19/86.4	9/56.2	45/71.4	5/62.5	40/59.7
BMI (kg/m2)	Mean ± SD	25.6 ± 4.9	26.1 ± 3.7	26.3 ± 4.3	25.8 ± 4.6	28.8 ± 9.9	26.2 ± 5
Cause of end stage renal disease	n/%	36/63.2	16/72.7	12/75	29/46	6/75	44/65.7
Diabetes-hypertension-vascular disease	n/%	13/22.8	2/9.1	4/25	11/17.5	2/25	10/14.9
Glomerulonephritis-interstitial nephritis	n/%	13/22.8	9/40.9	7/43.8	9/14.3	3/37.5	21/31.3
Vasculitis	n/%	3/5.3	1/4.5	1/6.2	0/0	1/12.5	2/3
Polycystic kidney disease	n/%	7/12.3	4/18.2	0/0	9/14.3	0/0	11/16.4
Unknown	n/%	21/36.8	6/27.3	4/25	34/54	2/25	23/34.3
Drug treated comorbidities	n/%	51/89.5	21/95.5	14/87.5	56/88.9	7/87.5	60/89.6
Diabetes mellitus	n/%	14/24.6	3/13.6	2/12.5	10/15.9	3/37.5	7/10.4
Cardiovascular disease	n/%	46/80.7	21/95.5	14/87.5	56/88.9	6/75	60/89.6
Lung disease	n/%	5/8.8	0/0	1/6.2	4/6.3	1/12.5	6/9
Liver cirrhosis	n/%	2/3.5	1/4.5	0/0	0/0	0/0	0/0
Cancer	n/%	2/3.5	1/4.5	0/0	0/0	0/0	2/3
None	n/%	6/10.5	1/4.5	2/12.5	7/11.1	1/12.5	7/10.4
Time on dialysis (years)	Mean ± SD	6.3 ± 6.9	5.8 ± 3.5	8.9 ± 9.2	5.3 ± 4.5	7.7 ± 4.2	6 ± 6.8
Time on transplantation (years)	Mean ± SD	6.9 ± 6	8.4 ± 5.7	8.8 ± 7.6	9.3 ± 5.8	6.4 ± 7.3	10 ± 6.9
Previous transplantation	n/%	9/15.8	5/22.7	3/18.8	5/7.9	1/12.5	8/11.9
Hepatitis B vaccination failure	n/%	4/7	1/4.5	0/0	3/4.8	1/12.5	5/7.5
Flu vaccination winter 2020/2021	n/%	26/45.6	16/72.7	10/62.5	33/52.4	5/62.5	46/68.7
On immunosuppressive therapy	n/%	57/100	22/100	16/100	63/100	8/100	67/100
Corticosteroids	n/%	28/49.1	10/45.5	13/81.2	24/38.1	3/37.5	35/52.2
Calcineurin-inhibitor	n/%	51/89.5	19/86.4	16/100	60/95.2	8/100	63/94
MMF/MPA	n/%	49/86	19/86.4	14/87.5	57/90.5	7/87.5	62/92.5
mTOR-inhibitor	n/%	9/15.8	5/22.7	1/6.2	3/4.8	0/0	5/7.5
Belatacept	n/%	6/10.5	1/4.5	0/0	5/7.9	0/0	4/6
T-cell depleting ab	n/%	0/0	0/0	0/0	0/0	0/0	0/0
B-cell depleting ab n	n/%	0/0	0/0	0/0	1/1.6	0/0	0/0
Other	n/%	0/0	0/0	1/6.2	1/1.6	0/0	1/1.5

BNT162b2 = BNT162b2-mRNA or tozinameran or brand name Comirnaty; 1273 = 1273-mRNA or brand name Spikevax; vector = vector vaccine consisting of AZD1222 in 5 cases and Ad26.COV2.S in 3 cases.

For this evaluation, all patients with asymptomatic or documented symptomatic** COVID-19 disease before and during vaccination up to T3 (6 months) were excluded. Hepatitis B vaccination failure definition–patients with unsuccessful vaccination after at least four attempts; MMF-MPA = mycophenolate mofetil or mycophenolic acid.*

**Asymptomatic COVID-19 disease definition–neither knowledge nor symptoms of COVID-19 disease, but IgG-antibody reaction to nucleocapsid (T0, T1, T2, or T3) or the Spike protein subunit S1 (only T0) of the SARS-CoV-2 virus is positive.*

***Symptomatic COVID-19 disease definition–SARS-CoV-2 PCR positive patients with clinical symptoms.*

Hereby, the individual level changes show broader corresponding bands for combinations containing 1273-mRNA, especially those with 1273-mRNA as the primary (2x) vaccine regimen.

The serological response of anti-RBD IgG at T3 compared to T2 (level $\geq$ 2) indicating neutralizing capacity is less than one quarter (24.8%, **Figure 4A**) and corresponds exactly to the level changes (**Figure 4B**). Taking into account the level changes subdivided into the primary vaccine types, it is striking that 31.2% of the primary 1273-mRNA vaccinated KTR make a level change of $\geq$2 levels, whereas this is only the case in 12.4% of the corresponding primary BNT162b2-mRNA vaccinated KTR (p = 0.08). Again, the subdivision according to 3-fold mRNA vaccine combinations shows that the bands of the higher-level changes are broader in the vaccine combinations containing

1273-mRNA, especially those with 1273-mRNA as the primary (2x) vaccine regimen.

DISCUSSION

Our study in 193 non-responding KTR after 2x mRNA vaccination showed that successful seroconversion after the third vaccination is dependent on the choice of vaccine type, the level of weak (below positivity threshold) IgG titer stimulation after 2x mRNA vaccination, and the use/lack of MMF/MPA as an immunosuppressive drug.

The dependence of seroconversion success on the vaccine type was particularly evident when comparing the homologous vaccine combinations of 3-fold 1273-mRNA and 3-fold BNT162b2-mRNA (**Table 1**). This result in non-responding KTR is predominantly driven *via* increased rates of antiSpikeS1 IgG antibody induction. It is consistent with former studies by us and others examining organ transplant recipients after two mRNA vaccinations (6, 7, 9), in which markedly higher seroconversion rates had been shown in 1273-mRNA vaccinated kidney transplant recipients (6) (49 vs. 26% in BNT162b2-mRNA). In our current study, we unexpectedly found that 1273-mRNA compared to BNT162b2-mRNA, as the third vaccine, is not critical for this improved seroconversion rate but rather the original use of 1273-mRNA as the primary vaccine type for the first two vaccinations. When BNT162b2-mRNA is being used as the third vaccine after primary 1273-mRNA immunizations, equivalent IgG seroconversion rates up to almost 50% compared to 3x 1273-mRNA can be achieved. Independent of the choice of the third mRNA vaccine type, seroconversion rates with BNT162b2-mRNA as the primary vaccine regimen did not even reach seroconversion rates of 25%. Apart from seroconversion rates, these results showing dependence on the primary vaccine type were also mimicked considering the extent of antiSpikeS1 IgG titer level changes as quantified by intervals in our study. While high RBD-IgG level changes after the third vaccination seemed to be more frequent when 1273-mRNA compared to BNT162b2-mRNA has been used as the primary vaccine type supporting these data, RBD-IgG or IgA antibody seroconversion rates were not similarly influenced by mRNA vaccine type. T-cell immune response measurements support the success of 3x 1273-mRNA vaccinations but were not frequently done enough to compare the different vaccine combinations statistically.

The simplest explanation for the higher immunogenicity of the 1273-mRNA vaccine in KTR was and still is the 3-fold higher mRNA dose, better thermostability, and easier handling of the 1273-mRNA compared to the BNT162b2-mRNA vaccine. Nevertheless, differences in antigenic motifs or mRNA modifications and different formulations may also play a role. It remains unclear, why, this effect of 1273-mRNA is especially important in the primary immunization process and can be less attributed to the third vaccine dose in our study. A prolonged vaccination interval as a potential differential influence on seroconversion has already been demonstrated for the BNT162b2-mRNA vaccine in the United Kingdom when the interval was extended from 3–4 to 6–14 weeks (20, 21) also had to be excluded. In contrast to the United Kingdom study, our study did not show any difference in the levels of anti-SpikeS1 IgG vaccination response between early and late third vaccination time points (period 8–12 weeks after the second vaccination, see horizontal lines in **Figures 5A**, 6A than lines ascending with increasing time interval [over the x-axis to the right]). The afore-mentioned concept of T-cell exhaustion (21) cannot be replicated in the present data set, but the time intervals chosen in our study were also less heterogeneous than in the United Kingdom study. For interpretation of our and the United Kingdom data, our finding of a spontaneous seroconversion rate in up to 1/6 of the KTR, immunized with only two vaccine doses, also suggests a severely delayed immunological responsiveness under immunosuppressive therapy. The small group of KTR immunized with heterologous 2x mRNA and 1x vector vaccine showed similar immune response rates in any test system examined as unboostered KTR with only 2x mRNA. While the interpretation of these data needs to be done with caution, this limited effect as a boostering vaccine has already been described in the literature for vector vaccines and is confirmed in the present population (22).

In line with previous studies for other humoral test systems (11), weak (below the positivity level of the test but above the detectability threshold), compared to negative responders were more likely to show seroconversion after the third vaccination. Taking into account all limitations of the test systems with gray range, detection thresholds, and linearity of the measurement ranges, we believe that anti-SpikeS1 IgG titers >3.2 and <35.2 BAU/ml define a sub-cohort within the immunosuppressed KTR that can be distinguished from an immunologically almost anergic group of non-responders (≤ 3.2 BAU/ml). Consistent with low humoral response rates 4 weeks after two mRNA vaccine doses, there is no evidence of increased rejection rates in COVID-19-diseased (23) or vaccinated (24) kidney transplant recipients. However, the present results show that serologic testing of the vaccination response in immunocompromised KTR makes sense defining cohorts with either successful seroconversion as well as non-responding patients with better or worse chances for a successful booster vaccination. In this sub-cohort of non-responding KTR with better chances for successful booster vaccination, no potentially risky reductions in immunosuppressive therapy, as already proposed by others (25), may be necessary to achieve high seroconversion rates as shown here. Hereby it also can be considered that successful seroconversion is accompanied by, albeit relative (depending on the variants of the virus), protection from severe or fatal infections.

Others and we demonstrated that besides the vaccine type used MMF/MPA, as part of the standard immunosuppressive therapy in KTR, predicts humoral response to mRNA COVID-19 vaccines (6, 26, 27). While this has been described as a predictor of humoral response after the second vaccine dose, we interpret MMF/MPA intake, despite borderline significance, as a predictor of humoral response also to a third vaccine dose. An unfavorable dose-dependent effect of MMF/MPA has been suggested (27). MMF/MPA as an anti-metabolite impairs not only B-cell proliferation and maturation into plasma blasts (28) but also

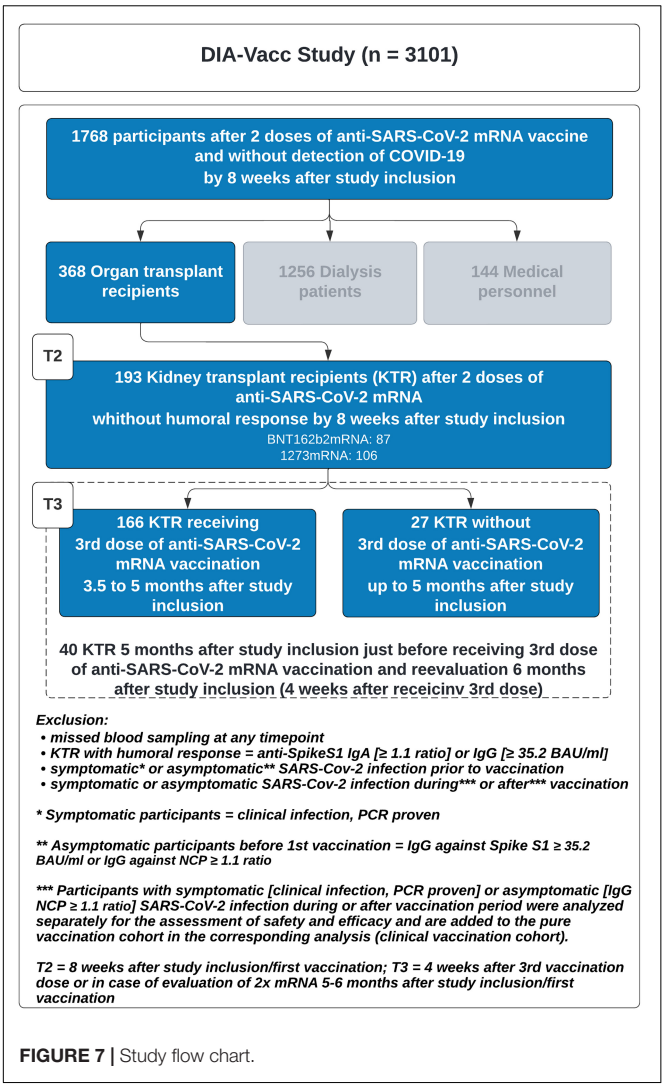


FIGURE 7 | Study flow chart.

expansion and activation of B cells (29, 30). Mechanistically, inhibition of the STAT3 pathway in particular is thought to be responsible for impaired differentiation of B-cells up to immunoglobulin secretion in the bone marrow (31, 32). The impact of MMF/MPA on B cells in an antigen-specific context up to the impairment of spike-specific CD27++CD38+plasma blast formation was recently shown by colleagues for the first time in a clinical setting (25). They also showed, that not only MMF/MPA dose modification could lead to an improved immune response but a temporary hold of MMF/MPA for 5 weeks is a feasible option to facilitate immunogenicity KTR (25). Although no increased rejection rates have been reported, this certainly remains the biggest concern of the approach proposed and should only be considered in the almost anergic non-responding group after 2x vaccinations ($\leq$3.2 BAU/ml).

Limitations of our approach include the non-randomized observational nature of our study and potential bias in patient selection, who participated only if interested in SARS-CoV-2 vaccination. For KTR with similar characteristics, these results should still be applicable but could also be confirmed in prospective controlled randomized trials. Another limitation is the lack of a detailed characterization of the T-cell mediated immune response as well as functional virus-related neutralization tests. Nevertheless, these tests are extremely work-intensive and not at all part of a standard diagnostic procedure for immune monitoring after vaccination and are not suited (especially not in an observational diagnostic study) to prove a causal link between vaccination-related immune response and disease incidence/mortality or even vaccination efficacy. Hereby it needs to be considered that even functional neutralization tests being performed *in vitro* never reflect real-life conditions, where in addition to the current immune status of the host, the route of viral transmission (inhalation vs. nasal mucosal contact), the viral load, virulence factors of the pathogen, and the type of viral variants (wild type, alpha, delta, omicron, etc.) play a role in the incidence and time course of the disease. With all these limitations in mind, Dolscheid-Pommerich and colleagues already described some correlations between the quantitative anti-SARS-CoV-2 IgG ELISA (as is also used here) and virus neutralization activity *in vitro* (33). This correlation may be true for our corresponding study period, while it is not applicable for the later appearing Omicron SARS-CoV-2 variant (VOC strain B.1.1.529).

In conclusion, this study provides important evidence for the use of 1273-mRNA as the primary mRNA vaccine type for immunocompromised KTR, which not only positively influence seroconversion after 2x vaccination but also improves the chance of seroconversion in non-responding KTR independent of the choice of the third mRNA vaccine. In addition, serologic testing should be performed subsequently in this vulnerable patient population to monitor and partly predict vaccination response. Weak (below positivity level) responders after two mRNA vaccinations have a good seroconversion chance after additional booster vaccinations despite current immunosuppressive therapy. Heterologous mRNA vaccine use as a third vaccination in non-responding KTR may be especially useful when BNT162b2-mRNA was used as the primary immunization. A temporary MMF/MPA withdrawal could be taken into consideration especially in KTR with no IgG response ($\leq$3.2 BAU/ml) after two mRNA vaccinations.

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 the Ethical Institutional Review Boards at Technische Universität Dresden (TU Dresden) responsible for the coordinating investigator (BO-EK-45012021), as well as at the University of Leipzig (046/21-lk) and Saxon

Medical Association (Sächsische Landesärztekammer—EK-BR-10/21-1) responsible for further participating trial sites. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JSt and CH contributed to the study design, data collection and interpretation, and drafting of the manuscript. JSc, CK, HS, RM, AK, and TT were involved in data acquisition and collection and study organization or contributed to data interpretation. AK and RM were involved in the statistical analysis or data management of the study. JSt, JSc, CK, HS, and CH were involved in patient recruitment and data collection. All authors have approved the final version for submission.

FUNDING

This study was funded by the Else Kröner Fresenius Stiftung, Bad Homburg v. d. H., grant number Fördervertrag EKFS 2021_EKSE.27 and the Sächsisches Staatsministerium für Wissenschaft, Kultur und Tourismus via Sächsische AufbauBank, grant number (SAB-Antragsnr.) 100592538.

ACKNOWLEDGMENTS

*Subgroup of the DIA-Vacc- Investigators

Members in alphabetical order within the corresponding centers:

Medizinische Klinik und Poliklinik III, Universitätsklinikum, Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany

Andrea Angermann, Patrick Arndt, Nicole Bunk, Susanne Dollfus, Petya Drinova, Annegret Fleischer, Chris Gebauer, Susann Graichen, Kerstin Haaser, Sabrina Heide, Gabriele Hirt, Friederike Kessel, Franziska Klemt, Hannah Kröger, Alexander Paliege, Jens Passauer, Miriam Poser, Margrit Reinhardt, Ronny Rettig, Maria Schuster, Jan Sradnick, Vladimir Todorov, Kerstin Zäumer

KfH-Nierenzentrum am Klinikum Chemnitz, Krankenhaus Küchwald, Chemnitz, Germany

Angela Grzegorek, Markus Hallmann, Saskia Härtwig, Christiane Hintzen-Kruse, Frauke Neidt und Söhne, Susann Reissland, Bianca Seidel, Torsten Siepmann, Anett Speer, Peggy Weiß, Christine Wiech

Division of Nephrology, University Hospital Leipzig, Leipzig, Germany

Anette Bachmann, Jonathan Christoph de Fallois, Jan Halbritter, Tom Lindner, Claudia Kreyßig, Christof Mayer, Benjamin Sandner

KfH-Nierenzentrum am Klinikum St. Georg, Leipzig, Germany

Joachim Beige, Kathrin Heipmann, Claudia Karger, Christian Schauff, Peyo Kolev Sivenov

Department of Nephrology and Rheumatology, Internal Medicine II, Martin-Luther-University Halle/Wittenberg, Halle, Germany

Joachim Beige

Dialysezentrum Chemnitz, Chemnitz, Germany

Markus Gläser, Ulrike Richardt, Anja Richter, Kathrin Scheibner, Jörg Schwöbel, Andreas Sohn

Dialysepraxis Leipzig, Leipzig, Germany

Leona Anders, Ingolf Bast, Daniel Grey

Nephrologisches Zentrum Freiberg, Freiberg, Germany

Robert Faulhaber-Walter, Anja Sawalha, Emad Sawalha

PHV Dialysezentrum Dresden Friedrichstadt, Dresden, Germany

Roland Behnisch, Peter Grossmann, Peter Heduschka. Holger Schirutschke

PHV Dialysezentrum Dresden-Johannstadt, Dresden, Germany

Steffen Bischoff, Petra Müller, Tatjana Seitz

KfH-Nierenzentrum Bautzen, Bautzen, Germany

Norgit Meyer, Sybille Stehr, Thomas Stehr, Patrice Steinert

Via medis Nierenzentrum Dresden MVZ GmbH, Dresden, Germany

Ivo Döhler, Annegret Pietzonka, Matthias Pietzonka

Institut für Transfusionsmedizin Plauen, DRK-Blutspendedienst Nord-Ost gemeinnützige GmbH, Plauen, Germany

Kerstin Frank, Nadine Hildebrandt, Andreas Karl

Coordinating Centre for Clinical Trials, Dresden, Germany

Stephan Friedrich, Xina Grählert

FURTHER SUPPORTERS

D. Michael Albrecht, Medizinischer Vorstand (Sprecher), Universitätsklinikum Carl Gustav Carus Dresden

Thomas Grünewald, Chemnitz, Vorsitzender Sächsische Impfkommission

Patricia Klein, Ärztliche Geschäftsführerin, Sächsische Landesärztekammer Körperschaft des öffentlichen Rechts

Marcus Strotkötter, Senior Operations Officer for Vaccination Center and Mobile Vaccination Teams at DRK Landesverband Sachsen e.V.

EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany provided antibody ELISAs and interferon-gamma release assays for this study.

REFERENCES

1. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3
2. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
3. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med.* (2020) 383:2603–15.

4. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med.* (2021) 384:403–16.
5. Gruell H, Vanshylla K, Tober-Lau P, Hillus D, Schommers P, Lehmann C, et al. mRNA booster immunization elicits potent neutralizing serum activity against the SARS-CoV-2 Omicron variant. *Nat Med.* (2022) 28:477–80. doi: 10.1038/s41591-021-01676-0
6. Stumpf J, Siepmann T, Lindner T, Karger C, Schwobel J, Anders L, et al. Humoral and cellular immunity to SARS-CoV-2 vaccination in renal transplant versus dialysis patients: a prospective, multicenter observational study using mRNA-1273 or BNT162b2 mRNA vaccine. *Lancet Reg Health Eur.* (2021) 9:100178.
7. Boyarsky BJ, Werbel WA, Avery RK, Tobian AAR, Massie AB, Segev DL, et al. Antibody response to 2-Dose SARS-CoV-2 mRNA vaccine series in solid organ transplant recipients. *JAMA.* (2021) 325:2204–6. doi: 10.1001/jama.2021.7489
8. Sadioğlu RE, Demir E, Evren E, Aktar M, Şafak S, Artan AS, et al. Antibody response to two doses of inactivated SARS-CoV-2 vaccine (CoronaVac) in kidney transplant recipients. *Transpl Infect Dis.* (2021) 23:e13740.
9. Stumpf J, Tonnus W, Paliege A, Rettig R, Steglich A, Gembardt F, et al. Cellular and humoral immune responses after 3 doses of BNT162b2 mRNA SARS-CoV-2 vaccine in kidney transplant. *Transplantation.* (2021) 105:e267–9.
10. Kamar N, Abravanel F, Marion O, Couat C, Izopet J, Del Bello A. Three doses of an mRNA covid-19 vaccine in solid-organ transplant recipients. *N Engl J Med.* (2021) 385:661–2.
11. Benotmane I, Gautier G, Perrin P, Olagne J, Cognard N, Fafi-Kremer S, et al. Antibody response after a third dose of the mRNA-1273 SARS-CoV-2 vaccine in kidney transplant recipients with minimal serologic response to 2 doses. *JAMA.* (2021) [Online ahead of print]. doi: 10.1001/jama.2021.12339
12. Padoan A, Sciacovelli L, Basso D, Negrini D, Zuin S, Cosma C, et al. IgA-Ab response to spike glycoprotein of SARS-CoV-2 in patients with COVID-19: a longitudinal study. *Clin Chim Acta.* (2020) 507:164–6. doi: 10.1016/j.cca.2020.04.026
13. Rubio-Acero R, Castelletti N, Fingerle V, Olbrich L, Bakuli A, Wölfel R, et al. In search for the SARS-CoV-2 protection correlate: a head-to-head comparison of two quantitative S1 assays in a group of pre-characterized oligo-/asymptomatic patients. *medRxiv[Preprint].* (2021). doi: 10.1101/2021.02.19.21252080
14. Müller L, Andrée M, Moskorz W, Drexler I, Walotka L, Grothmann R, et al. Age-dependent immune response to the Biontech/Pfizer BNT162b2 COVID-19 vaccination. *medRxiv.* (2021). doi: 10.1101/2021.03.03.21251066
15. Meyer B, Torriani G, Yerly S, Mazza L, Calame A, Arm-Vernez I, et al. Validation of a commercially available SARS-CoV-2 serological immunoassay. *Clin Microbiol Infect.* (2020) 26:1386–94.
16. Euroimmun Medizinische Labordiagnostika Ag. *SARS-CoV-2 NeutraLISA 2021*. (2021). Available online at: https://www.coronavirus-diagnostics.com/documents/Indications/Infections/Coronavirus/EI_2606_D_UK_F.pdf [accessed on March 31, 2021].
17. Shaw RH, Stuart A, Greenland M, Liu X, Nguyen Van-Tam JS, Snape MD, et al. Heterologous prime-boost COVID-19 vaccination: initial reactogenicity data. *Lancet.* (2021) 397:2043–6. doi: 10.1016/S0140-6736(21)01115-6
18. Munro APS, Janani L, Cornelius V, Aley PK, Babbage G, Baxter D, et al. Safety and immunogenicity of seven COVID-19 vaccines as a third dose (booster) following two doses of ChAdOx1 nCov-19 or BNT162b2 in the UK (COV-BOOST): a blinded, multicentre, randomised, controlled, phase 2 trial. *Lancet.* (2021) 398:2258–76.
19. R Core Team. *R: A Language and Environment for Statistical Computing. 4.0.* 1 ed. Vienna: R Foundation for Statistical Computing (2020).
20. Payne RP, Longet S, Austin JA, Skelly DT, Dejnirattisai W, Adele S, et al. Immunogenicity of standard and extended dosing intervals of BNT162b2 mRNA vaccine. *Cell.* (2021) 184:5699–714 e11.
21. Gutierrez-Bautista JF, Rodriguez-Nicolas A, Rosales-Castillo A, Jimenez P, Garrido F, Anderson P, et al. Negative clinical evolution in COVID-19 patients is frequently accompanied with an increased proportion of undifferentiated th cells and a strong underrepresentation of the th1 subset. *Front Immunol.* (2020) 11:596553. doi: 10.3389/fimmu.2020.596553
22. Bruminhent J, Setthaudom C, Kitpermkiat R, Kiertiburanakul S, Malathum K, Assanatham M, et al. Immunogenicity of ChAdOx1 nCoV-19 vaccine after a two-dose inactivated SARS-CoV-2 vaccination of dialysis patients and kidney transplant recipients. *Sci Rep.* (2022) 12:3587. doi: 10.1038/s41598-022-07574-w
23. Pascual J, Melilli E, Jimenez-Martin C, Gonzalez-Monte E, Zarraga S, Gutierrez-Dalmau A, et al. COVID-19-related mortality during the first 60 days after kidney transplantation. *Eur Urol.* (2020) 78:641–3.
24. Hall VG, Ferreira VH, Ku T, Ierullo M, Majchrzak-Kita B, Chaparro C, et al. Randomized trial of a third dose of mRNA-1273 vaccine in transplant recipients. *N Engl J Med.* (2021) 385:1244–6.
25. Schrezenmeier E, Rincon-Arevalo H, Jens A, Stefanski A-L, Hammett C, Osmanodja B, et al. Temporary hold of mycophenolate boosts SARS-CoV-2 vaccination-specific humoral and cellular immunity in kidney transplant recipients. *medRxiv[Preprint].* (2022). doi: 10.1101/2022.01.05.21268478
26. Debska-Slizien A, Slizien Z, Muchlado M, Kubanek A, Piotrowska M, Dabrowska M, et al. Predictors of humoral response to mRNA COVID19 vaccines in kidney transplant recipients: a longitudinal study-the COViNEPH project. *Vaccines* (2021) 9:1165. doi: 10.3390/vaccines9101165
27. Kantauskaite M, Muller L, Kolb T, Fischer S, Hillebrandt J, Ivens K, et al. Intensity of mycophenolate mofetil treatment is associated with an impaired immune response to SARS-CoV-2 vaccination in kidney transplant recipients. *Am J Transplant.* (2022) 22:634–9. doi: 10.1111/ajt.16851
28. Eickenberg S, Mickholz E, Jung E, Nofer JR, Pavenstadt HJ, Jacobi AM. Mycophenolic acid counteracts B cell proliferation and plasmablast formation in patients with systemic lupus erythematosus. *Arthritis Res Ther.* (2012) 14:R110. doi: 10.1186/ar3835
29. Matz M, Lehnert M, Lorkowski C, Fabritius K, Weber UA, Mashreghi MF, et al. Combined standard and novel immunosuppressive substances affect B-lymphocyte function. *Int Immunopharmacol.* (2013) 15:718–25. doi: 10.1016/j.intimp.2013.02.025
30. Karnell JL, Karnell FG III, Stephens GL, Rajan B, Morehouse C, Li Y, et al. Mycophenolic acid differentially impacts B cell function depending on the stage of differentiation. *J Immunol.* (2011) 187:3603–12. doi: 10.4049/jimmunol.1003319
31. Jego G, Bataille R, Pellat-Deceunynck C. Interleukin-6 is a growth factor for nonmalignant human plasmablasts. *Blood.* (2001) 97:1817–22. doi: 10.1182/blood.v97.6.1817
32. Cassese G, Arce S, Hauser AE, Lehnert K, Moewes B, Mostarac M, et al. Plasma cell survival is mediated by synergistic effects of cytokines and adhesion-dependent signals. *J Immunol.* (2003) 171:1684–90. doi: 10.4049/jimmunol.171.4.1684
33. Dolscheid-Pommerich R, Bartok E, Renn M, Kummerer BM, Schulte B, Schmithausen RM, et al. Correlation between a quantitative anti-SARS-CoV-2 IgG ELISA and neutralization activity. *J Med Virol.* (2022) 94:388–92.

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.

frontiers | Frontiers in Medicine

BRIEF RESEARCH REPORT
published: 07 July 2022
doi: 10.3389/fmed.2022.928542

MMF/MPA Is the Main Mediator of a Delayed Humoral Response With Reduced Antibody Decline in Kidney Transplant Recipients After SARS-CoV-2 mRNA Vaccination

*Julian Stumpf[1,2], Torsten Siepmann[3], Jörg Schwöbel[4], Grit Glombig[5], Alexander Paliege[1], Anne Steglich[1], Florian Gembardt[1], Friederike Kessel[1], Hannah Kröger[1], Patrick Arndt[1], Jan Sradnick[1], Kerstin Frank[6], Anna Klimova[7], René Mauer[8], Torsten Tonn[9,10] and Christian Hugo[1,2]**

[1] Medizinische Klinik und Poliklinik III, Universitätsklinikum, Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany, [2] KfH-Nierenzentrum Dresden, Dresden, Germany, [3] KfH-Nierenzentrum am Klinikum Chemnitz, Krankenhaus Küchwald, Chemnitz, Germany, [4] Dialysezentrum Chemnitz, Chemnitz, Germany, [5] KfH-Nierenzentrum am Klinikum St. Georg, Leipzig, Germany, [6] Institut für Transfusionsmedizin Plauen, DRK-Blutspendedienst Nord-Ost gemeinnützige GmbH, Plauen, Germany, [7] National Center for Tumor Diseases (NCT) Dresden, Dresden, Germany, [8] Faculty of Medicine Carl Gustav Carus, Institute for Medical Informatics and Biometry (IMB), Technische Universität Dresden, Dresden, Germany, [9] Institute for Transfusion Medicine, German Red Cross Blood Donation Service North-East, Dresden, Germany, [10] Faculty of Medicine Carl Gustav Carus, Transfusion Medicine, Technische Universität Dresden, Dresden, Germany

OPEN ACCESS

Edited by:
Georg Böhmig,
Medical University of Vienna, Austria

Reviewed by:
Roman Reindl-Schwaighofer,
Medical University of Vienna, Austria
Joanna Sobiak,
Poznan University of Medical Sciences, Poland

***Correspondence:**
Christian Hugo
christian.hugo@uniklinikum-dresden.de

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: 25 April 2022
Accepted: 07 June 2022
Published: 07 July 2022

Citation:
Stumpf J, Siepmann T, Schwöbel J, Glombig G, Paliege A, Steglich A, Gembardt F, Kessel F, Kröger H, Arndt P, Sradnick J, Frank K, Klimova A, Mauer R, Tonn T and Hugo C (2022) MMF/MPA Is the Main Mediator of a Delayed Humoral Response With Reduced Antibody Decline in Kidney Transplant Recipients After SARS-CoV-2 mRNA Vaccination. Front. Med. 9:928542. doi: 10.3389/fmed.2022.928542

Kidney transplant recipients (KTR) show significantly lower seroconversion rates after SARS-CoV-2 mRNA vaccination compared to dialysis patients (DP). Mycophenolate mofetil or mycophenolic acid (MMF/MPA) in particular has been identified as a risk factor for seroconversion failure. While the majority of all KTR worldwide receive MMF/MPA for immunosuppressive therapy, its impact on antibody decline in seroconverted KTR still remains unclear. In an observational study (NCT04799808), we investigated whether 132 seroconverted KTR (anti-spike S1 IgG or IgA positive after 2 vaccinations) show a more rapid antibody decline with MMF/MPA than those without this medication. A total of 2 months after mRNA vaccination, average anti-spike S1 IgG levels of KTR with MMF/MPA were lower than without ($p = 0.001$), while no differences between these two groups were observed after 6 months ($p = 0.366$). Similar results were obtained for anti-RBD IgG antibodies (T2 $p = 0.003$ and T3 $p = 0.135$). The probability of severe IgG decline with MMF/MPA was three times lower than without ($p = 0.003$, OR 0.236, 95% CI 0.091–0.609). In the multivariate analysis, neither immunosuppressants, such as calcineurin inhibitors, mTOR inhibitors (mTOR-I; mechanistic target of rapamycin), glucocorticoids, nor vaccine type, sex, or age showed a significant influence on IgG titer decline between 2 and 6 months. For the decision on additional booster vaccinations, we consider immunosurveillance to be needed as an integral part of renal transplant follow-up after SARS-CoV-2 mRNA vaccination. Not only the lack of seroconversion but also the peak and titer decline of the specific IgG and RBD IgG antibody formation after two mRNA vaccinations is significantly influenced by MMF/MPA.

Keywords: vaccination, kidney transplant recipients, SARS-CoV-2, humoral response, mycophenolic acid, clinical decision making, guidelines

INTRODUCTION

Immunosuppressive therapy in kidney transplant recipients (KTR) is the main determinant for highly impaired seroconversion rates compared to the normal population after SARS-CoV-2 mRNA vaccination (1–3). Hereby, studies including our Dia-Vacc study identified the anti-metabolite MMF/MPA (besides belatacept) as the critical immunosuppressive drug type being associated with seroconversion failure at 2 months after SARS-CoV-2 vaccination in KTR (1–3). During 6 months of follow-up investigations, seroconverted KTR [compared to medical personnel (MP)] were at risk for a strong decline in IgG and RBD-IgG antibodies but neither IgA antibodies nor cellular immunity (4). Hereby, antibody titers of KTR peaked at a lower level, and pronounced antibody decline was mixed with an increasing IgG or RBD-IgG response in at least 15% of patients. Despite MMF/MPA being given to the majority of all organ transplant recipients worldwide, its influence on antibody decline in seroconverted transplant recipients after SARS-CoV-2 mRNA vaccination is unclear. According to the pre-existing data on the impact of MMF/MPA on vaccination-related seroconversion and antibody formation, we hypothesized that MMF/MPA treatment may also lead to a pronounced antibody decline within additional 4 months of follow-up after seroconversion at the 8-week time point after mRNA vaccination starts in 132 KTR of the DIA-Vacc cohort.

METHODS

Study Design

In the 2- (T2) and 6-month (T3) evaluation (**Supplementary Figure 1**) of the prospective DIA-Vacc (1) observational study (NCT04799808), we analyzed the specific cellular (interferon-γ release assay) and humoral immune responses after 2x SARS-CoV-2 mRNA without a third vaccination in 112 out of 132 KTR (see **Supplementary Table 1**) with seroconversion (de novo IgA or IgG antibody positivity by ELISA (enzyme-linked immunosorbent assay), KTR_{112}). In addition, 26 out of 132 KTR with seroconversion represent a separate group of IgA de novo positive only, but IgG negative KTR_{IgA} at T2 (**Figure 1**, also referred to as "weakly seroconverted"). In 20 out of 26 cases of this KTR_{IgA} group by unanticipated, individual decision of the dialysis centers (procedure legally permitted in Germany), an additional third vaccination was done at 4.2 ± 1 months. At T0 (start of vaccination), T2, and T3, SARS-CoV-2-specific IgG or IgA antibody responses to Spike S1 protein and antibodies to the receptor binding domain (RBD-IgG) at T2 orT3 were assessed in all study participants (1). Titer levels and changes were classified differently depending on what was being looked at. For the assessment and comparison of subcohorts defined by a response of comparable expression (i.e., titer decrease to comparable absolute values), levels and corresponding limits were defined (see 2.4 Interval classification in levels). For the overall assessment and comparisons with regard to relative changes defined as increasing, equal, or decreasing titers, ranges were formed. For the latter, a range of 20% for the T3 compared to T2 change of antibody and interferon-γ release assay (IGRA) titers/values (increased or equal or decreased) was used and the percentage of patients within each range was calculated (**Table 1**). In addition, the antibody time course was analyzed on the interval scale. The detectable ranges of anti-spike S1-IgG and RBD-IgG antibody values were categorized into five intervals, labeled from 0 to 4 (referred to as "levels" in the data analysis), and the change in levels, varying from −4 to +4, was calculated for each patient. Level decreases from T2 to T3 by at least two units were defined as a strong decline (1).

Background Study Design

In the original investigator-driven, non-interventional, prospective, observational DIA-Vacc study (1), the first 26 out of all 36 regional nephrology centers were recruited. Further centers could not be considered due to funding restrictions. A total of 3,101 participants were enrolled to explore the time course of a specific cellular response or/and humoral seroconversion to disease and/or SARS-CoV-2 vaccination in MP, DP, and 368 KTR (see **Figure 1**). To report clean humoral seroconversion rates, as reported here, a "pure vaccination cohort" was created excluding retrospectively all symptomatically and asymptomatically COVID-19 infected or deceased participants before, during, and after vaccination (up to T2). For further description, see elsewhere (1). Another cohort called the "clinical vaccination cohort" consists of the "pure vaccination" cohort but includes all participants who experienced symptomatic or asymptomatic COVID-19 disease (or death) strictly during or after vaccination to assess the clinical outcome of vaccination. The study start (T0) was immediately before the first vaccination. Further monitoring of time points is described elsewhere (1). By vaccine availability, initially, only the first four dialysis centers were assigned to the vaccination campaign and received BNT162b2 mRNA, while all other following dialysis centers received the mRNA-1273 vaccine for both vaccinations. Neither any dialysis center nor any patient or MP or the study center (Dresden) had a choice or influence regarding the type of vaccine. All dialysis centers were informed *via* simultaneous email from the central vaccination institute, about the start of the vaccination campaign. In all study participants (eligibility if >18 years old and signed informed consent) at T0, T2, and T3, the above-mentioned antibody measurements were done, using Euroimmun ELISAs on Euroimmun analyzers (5–9). To explore the cellular SARS-CoV-2 immune response in subgroups, a SARS-CoV-2 specific interferon-γ release assay (Euroimmun-SARS-CoV-2-IGRA for research use only ET 2606-3003 & EQ 6841-96011,2) was applied (10). The sub-group for the IGRA was formed as follows: the analysis of T cells requires vivid cells. To reach high viability in IGRA samples, the procession should start at <24 h (established at <6 h) after collection. To ensure this high sample quality, four centers in the vicinity of the study coordination center were asked to participate in this sub-group analysis. The selection took into account that the centers treated a sufficient number of transplanted patients and that both vaccines were represented. The exact procedure and analysis are further described elsewhere (1).

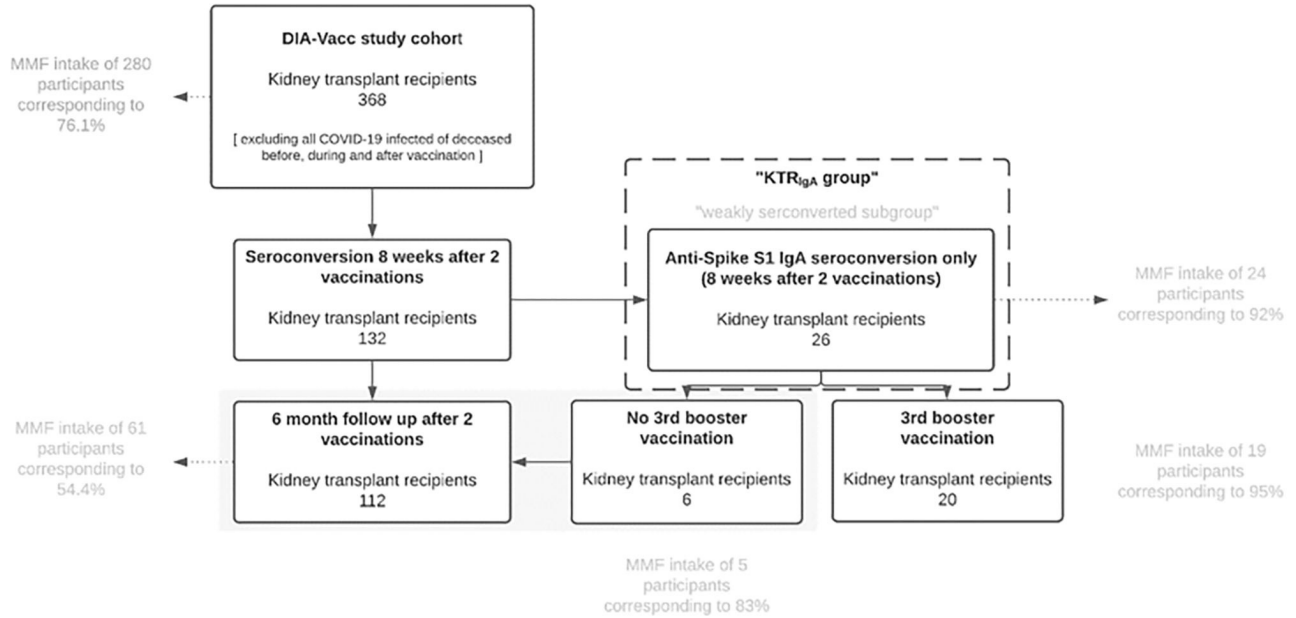


FIGURE 1 | KTR_{IgA} group. KTR, Kidney Transplant Recipients; MMF, mycophenolate mofetil or mycophenolic acid.

Statistical Analysis

In the descriptive analysis of the main study endpoints, categorical variables were summarized as absolute frequencies or percentages, and continuous variables were summarized using the mean and SD or median and interquartile range (IQR). Time trends in IgG and RBD-IgG responses and between-group differences were analyzed either by the Wilcoxon signed-rank test or the chi-squared test, as appropriate. The analysis of risk factors of patients with a strong antibody decline was carried out using multiple logistic regression. For hypothesis testing, a significance level of 5% (two-sided) was chosen. A Bonferroni correction was applied during *post hoc* testing of group effects. Data analysis was implemented in the R Environment for Statistical Computing (11), version 4.0.4.

Interval Classification in Levels

In the proposed interval classification, level 0 is assigned to IgG and RBD values below the corresponding positivity threshold [35.2 Binding Antibody Units/ml (BAU/ml) and 35%, respectively], and the remaining values are divided into four intervals of approximately equal length (**Supplementary Table 2**). These intervals can be used to quantify the change in IgG or RBD-IgG between T2 and T3, where, for example, a positive change corresponds to an increase in IgG or RBD-IgG, respectively, with a change of 4 being the maximum increase. Based on this definition, we referred to any decrease of more than one level (at least two) as a "strong declining response." The distributions of IgG and RBD-IgG levels at T2 and T3 and their change between T2 and T3 are summarized in **Supplementary Figures S2A–F** and separately dependent on the use of MMF/MPA.

Multivariate Analysis

Besides gender, age, and vaccine type, the association between different immunosuppressive drug types of drugs such as calcineurin inhibitors, corticosteroids, mTOR-inhibitors, and MMF/MPA, and strong declining IgG response was explored using a penalized logistic regression model estimated using the elastic net approach (12). **Supplementary Figure 3** illustrates a stepwise model selection procedure in which predictors (immunosuppressive 4 drug types) are added to a regression model one at a time, to maximize the goodness-of-fit, assessed from the deviance, given the current number of predictors. The slope of each path in **Supplementary Figure 3** changes as a new immunosuppressive drug enters the model. According to this plot, MMF/MPA has the strongest explanatory ability as a single predictor.

Definition of KTR_{IgA} Group

The KTR_{IgA} group ($n = 26$) is defined as a seroconversion group with de novo IgA positivity without a positive IgG response at T2 after 2x mRNA vaccination (< 35.2 BAU/ml according to manufacturer definition, see also **Figure 1**). In this group, 24 of 26 (92%) KTR were treated with MMF/MPA since most (20/26) of these had to be excluded due to an unanticipated third vaccination by the dialysis centers despite formal seroconversion. Nineteen of 24 of the MMF/MPA treated KTR_{IgA} group received an additional mRNA vaccine booster between T2 and T3. In contrast, 5 of 24 KTR_{IgA} patients with MMF/MPA were not vaccinated a third time.

TABLE 1 | Immune response rates 6 months after vaccination (T3) compared to T2 in the seroconverted Kidney transplant recipients $(KTR)_{112}$ cohort.

Variable	Category	KTR without MMF/MPA	KTR with MMF/MPA	*p*-value
Patient number	***n***	51	61	
Humoral responses				
IgG-Ab or IgA-Ab Spike S1 positive	*n* of total *n* (%)	45 / 51 (88.2 %)	53 / 61 (86.9 %)	1
IgA-Ab Spike S1 positive	*n* of total *n* (%)	29 / 51 (56.8 %)	37 / 61 (60.7 %)	0.831
IgA-Ab Spike S1 increasing	*n* of total *n* (%)	2 / 51 (3.9 %)	2 / 61 (3.3 %)	1
IgA-Ab Spike S1 equal	*n* of total *n* (%)	0 / 51 (0 %)	2 / 61 (3.3 %)	0.556
IgA-Ab Spike S1 decreasing	*n* of total *n* (%)	49 / 51 (96.1 %)	57 / 61 (93.4 %)	0.845
IgG-Ab Spike S1 positive	*n* of total *n* (%)	42 / 51 (82.4 %)	52 / 61 (85.3 %)	0.875
IgG-Ab Spike S1 increasing	*n* of total *n* (%)	2 / 51 (3.9 %)	15 / 61 (24.6 %)	0.006
IgG-Ab Spike S1 equal	*n* of total *n* (%)	14 / 51 (27.5 %)	17 / 61 (27.9 %)	1
IgG-Ab Spike S1 decreasing	*n* of total *n* (%)	35 / 51 (68.6 %)	29 / 61 (47.6 %)	0.04
RBD-IgG positive	*n* of total *n* (%)	35 / 51 (68.6 %)	31 / 61 (50.8 %)	0.086
RBD-IgG increasing	*n* of total *n* (%)	1 / 50 (2.0 %)	11 / 56 (19.6 %)	0.011
RBD-IgG equal	*n* of total *n* (%)	18 / 50 (36.0 %)	18 / 56 (32.1 %)	0.831
RBD-IgG decreasing	*n* of total *n* (%)	31 / 50 (62.0 %)	27 / 56 (48.2 %)	0.219
RBD-IgG de novo	*n* of total *n* (%)	1 / 50 (2.0 %)	5 / 56 (8.9 %)	0.263
Interferon-γ release assay (IGRA)– T-cellular response				
IGRA positive	*n* of total *n* (%)	8 / 20 (40.0 %)	7 / 22 (31.8 %)	0.818
IGRA increasing	*n* of total *n* (%)	6 / 18 (33.3 %)	6 / 17 (35.3 %)	1
IGRA equal	*n* of total *n* (%)	1 / 18 (5.6 %)	0 / 17 (0 %)	1
IGRA decreasing	*n* of total *n* (%)	11 / 18 (61.1 %)	11 / 17 (64.7 %)	1

MMF/MPA, mycophenolate mofetil or mycophenolic acid.

In Table 1 using 20% as a margin, the time course of antibody or IGRA titers at T3 compared to T2 time point were categorized into increased (>20%), equal (within 20% range), and decreased (<20%). De novo positivity on T3 means that despite overall seroconversion on T2 (for either IgA or IgG antibodies), the value for RBD-IgG was negative on T2 but positive on T3. Humoral vaccination responses were assessed as positive when de novo production of the antibody to the Spike S1 (IgA or IgG) protein or RBD (IgG) subunit was above the positivity level. A positive T-cellular response to vaccination as assessed by interferon-γ release assay (IGRA) turned from a negative result on T0 to positive on T3, respectively (≥100 mIU/ml, as being recommended by the manufacturers).

For this evaluation, all participants with asymptomatic or documented symptomatic** COVID-19 disease before and during vaccination up to T3 (6 months) were excluded.*

**Asymptomatic COVID-19 disease definition—neither knowledge nor symptoms of COVID-19 disease, but IgG-antibody reaction to nucleocapsid (T0, T2, or T3) or to the Spike protein subunit S1 (only T0) of the SARS-CoV-2 virus is positive.*

***Symptomatic COVID-19 disease definition—SARS-CoV-2 PCR positive patients with clinical symptoms.*

TABLE 2 | Multivariate analysis of IgG antibody decline between T2 and T3 in kidney transplant recipients after seroconversion [kidney transplant recipients $(KTR)_{112}$ cohort].

Risk factor	OR	95% CI	*P*-value
Age	1.034	[0.996, 1.075]	0.083
Sex (Ref. = female)	1.284	[0.504, 3.270]	0.600
Vaccine type (Ref. = mRNA-1273)	1.817	[0.655, 5.041]	0.251
Steroids (Ref. = none)	2.150	[0.845, 5.467]	0.108
CNI (Ref. = none)	1.338	[0.395, 4.533]	0.640
MMF/MPA (Ref. = none)	0.236	[0.091, 0.609]	0.003
mTOR-I (Ref. = none)	0.459	[0.139, 1.517]	0.202

mRNA-1273 represents Spikevax also called Moderna COVID-19 vaccine; the second vaccine (compared to) is BNT162b2-mRNA which stands for Comirnaty also known as Pfizer-BioNTech COVID-19 vaccine; CNI means calcineurin inhibitors; KTR, Kidney Transplant Recipient; MMF/MPA, mycophenolate mofetil or mycophenolic acid; mTOR-I means mTOR-inhibitors.

RESULTS

Kidney transplant recipients$_{112}$ group: separation of all 112 KTR in two groups with MMF/MPA ($n = 61$) and without MMF/MPA ($n = 51$) demonstrates that with the exception of immunosuppressive drug types both groups are well matched for patient characteristics (**Supplementary Table 1**). Multivariate analysis of the KTR_{112} group revealed that MMF/MPA but no

TABLE 3 | Antibody and interferon-γ release assay (IGRA) titers 2 (T2) and 6 months (T3) after vaccination in the seroconverted kidney transplant recipients $(KTR)_{112}$ cohort with and without mycophenolate mofetil or mycophenolic acid (MMF/MPA).

Variable	Group	Category	KTR without MMF/MPA	KTR with MMF/MPA	*p*-value
Humoral responses					
IgA-Ab spike S1	T2	Median (interquartile range)	4.3 (2.4–9)	5.2 (1.9–9)	0.827
IgA-Ab spike S1	T3	Median (interquartile range)	1.7 (0.6–3.9)	1.9 (0.8–4.2)	0.568
IgG-Ab spike S1	T2	Median (interquartile range)	384 (215.4–384)	167.8 (84.2–384)	0.001
IgG-Ab spike S1	T3	Median (interquartile range)	149.6 (51.2–375.3)	106.1 (61.1–263.4)	0.366
RBD-IgG-Ab spike S1	T2	Median (interquartile range)	84.8 (55.0–97.9)	59.1 (25.0–88.7)	0.003
RBD-IgG-Ab spike S1	T3	Median (interquartile range)	46.9 (30.7–81.5)	37.9 (17.8–69.3)	0.135
T-cellular response					
IGRA	T2	Median (interquartile range)	113.3 (13.5–289.6)	79.7 (14.3–454.3)	0.897
IGRA	T3	Median (interquartile range)	75.6 (14.4–176.1)	25.9 (16.1–169.6)	0.876

KTR, Kidney Transplant Recipient; MMF/MPA, mycophenolate mofetil or mycophenolic acid; Interferon-γ release assay = IGRA.

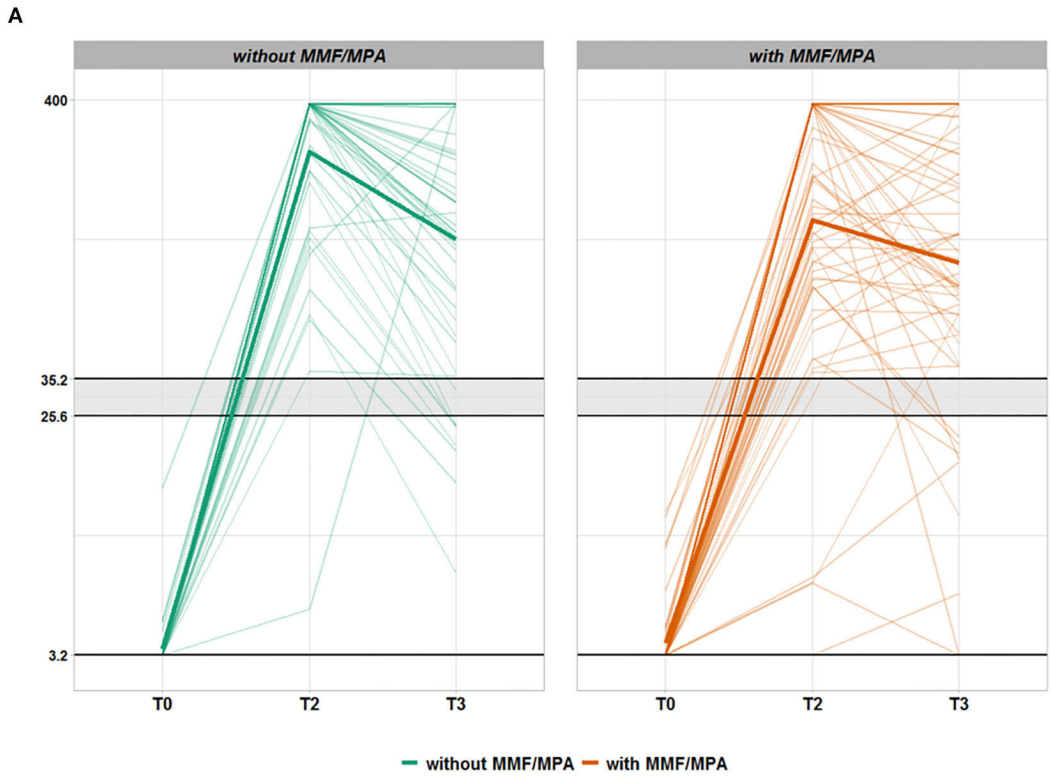


FIGURE 2A | Time course of anti-SARS-CoV-2 IgG antibodies in seroconverted kidney transplant recipients (KTR) without (green) or with (orange) mycophenolate mofetil or mycophenolic acid (MMF/MPA) treatment. Each thin line corresponds to the anti-spike S1 protein IgG antibody values (QuantiVac, Euroimmun) of a study participant from T0 (vaccination start) *via* T2 (8 weeks after vaccination start) to T3 (6 months after vaccination start). KTR being treated without MMF/MPA are represented in green and KTR exposed to MMF/MPA treatment are shown in orange. Only patients with successful de novo seroconversion at T2 (IgA or IgG antibody positivity against the SARS-CoV-2 S1 protein) after 2x mRNA vaccination and without SARS-CoV-2 nucleocapsid (NCP) antibodies were considered. The area shaded gray designates IgG borderline range below positivity level. The vertical axis is depicted on $\log_{10}$ scale with the corresponding unit BAU/ml.

other immunosuppressive drug such as calcineurin inhibitors, mTOR-inhibitors, or glucocorticoids significantly influenced vaccination-related IgG anti-spike S1 protein antibody titers and decline between 2 and 6 months (**Table 2**). While at 2 months, IgG levels of KTR_{112} with MMF/MPA were on average lower than those of KTR_{112} without MMF ($p = 0.001$), at 6 months no differences between these two groups were observed ($p = 0.366$) (**Table 3**, **Figures 2A/B**). As it can be observed in **Supplementary Figure 3**, there is a negative association between taking MMF/MPA and strong IgG decline, that is, patients taking MMF/MPA have a lower chance to experience a strong decline than patients taking other immunosuppressive. An

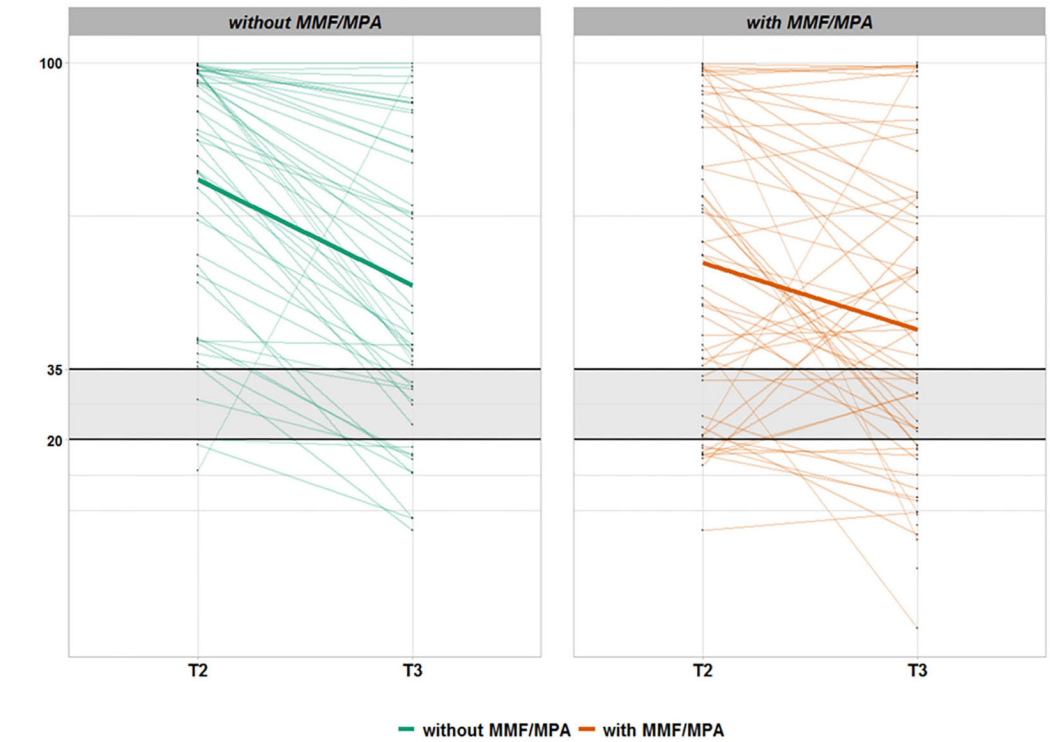


FIGURE 2B | Time course of anti-SARS-CoV-2 RBD-IgG antibodies in seroconverted kidney transplant recipients (KTR) without (green) or with (orange) mycophenolate mofetil or mycophenolic acid (MMF/MPA) treatment. Each thin line corresponds to the anti-spike S1 protein RBD-IgG antibody values (Euroimmun) of a study participant from T2 (8 weeks after vaccination start) to T3 (6 months after vaccination start). KTR being treated without MMF/MPA are represented in green and KTR exposed to MMF/MPA treatment are shown in orange. Only patients with successful de novo seroconversion at T2 (IgA or IgG antibody positivity against the SARS-CoV-2 S1 protein) after 2x mRNA vaccination and without SARS-CoV-2 nucleocapsid (NCP) antibodies were considered. The area shaded gray designates RBD-IgG borderline range below positivity level. The vertical axis is depicted on $\log_{10}$ scale with corresponding unit % inhibition.

overall decreasing trend occurred in both groups, but KTR_{112} with MMF/MPA were three times less likely to show a strong IgG decline than KTR_{112} without MMF/MPA ($p = 0.003$). A comparable difference for KTR_{112} with and without MMF/MPA was also observed for RBD-IgG: lower values for MMF at T2 ($p = 0.003$) and no significant difference at T3 ($p = 0.135$).

Using 20% as a margin, only 48% or 48% of patients with MMF/MPA but 69% or 62% of KTR without MMF showed decreased anti-spike S1 IgG or RBD-IgG antibody titers at T3, respectively (**Table 1**, **Figures 2A/B**). A total of 25% or 20% of KTR_{112} with MMF/MPA but only 4% or 2% of KTR without MMF/MPA showed IgG or RBD-IgG antibody increases up to T3. This delayed antibody response/increase in patients with MMF/MPA is also reflected by 9% of seroconverted KTR_{112}, who are characterized by *de novo* RBD-IgG positivity at T3.

In contrast, anti-spike S1 IgA protein antibody and cellular immunity rates were independent of MMF/MPA use (**Table 1**).

Only one KTR developed asymptomatic COVID-19 disease with anti-nucleocapsid antibody (NCP) seroconversion.

Kidney Transplant Recipients$_{IgA}$ Group

Most (20/26) of the "anti-spike S1 IgA antibody seroconverting only" KTR_{IgA} group (IgA but no IgG seroconversion) had to be excluded from the above evaluation due to an unanticipated third vaccination by the dialysis centers despite formal seroconversion. In this subgroup, 24 of 26 (92%) KTR were treated with MMF/MPA further supporting the general MMF/MPA-dependent IgG antibody results of our study. Nineteen of 24 of the MMF/MPA treated KTR_{IgA} patients received an additional mRNA vaccine booster at 4.2 ± 1 month demonstrating a marked IgG (**Supplementary Table 3**, **Supplementary Figure 4**) and RBD-IgG (**Supplementary Table 3**) increases in almost all patients between T2 and T3, respectively. In contrast, 5 of 24 KTR_{IgA} patients with MMF/MPA were not vaccinated a third time and remained at a much lower level of antibody titers T3. Nevertheless, two out of five of these "IgA only seroconverted" patients with MMF/MPA showed a delayed *de novo* positivity of IgG antibodies at T3 without any booster vaccination (**Supplementary Figure 4**).

DISCUSSION

The predominantly used immunosuppressive anti-metabolite MMF/MPA impairs both seroconversion rate and IgG and RBD-IgG titers in organ transplant recipients 2 months after SARS-CoV-2 mRNA vaccination (1–3). Our study data unexpectedly demonstrate that antibody decline in MMF/MPA treated, seroconverted patients, is reduced leading to equivalent seropositivity rates and titers after 6 months of follow-up compared to KTR without MMF/MPA. Our data suggest that MMF/MPA is responsible for a delayed humoral IgG immune response with a different time course specifically of IgG antibody development and decline compared to transplant recipients with immunosuppressive therapy without MMF/MPA, in which 35% were treated with mTOR-I. Almost all KTR with an increasing or *de novo* IgG or RBD-IgG antibody reaction between 2 and 6 months were found in the MMF/MPA group, where this occurred in about a quarter of patients. Interestingly, these MMF/MPA effects were not seen regarding a vaccination-related IgA- or T-cellular response. Whether these results represent an MMF/MPA-mediated problem of a delayed IgM/IgG but not IgA switch remains elusive. A similar delayed immune response in KTR was shown by others after COVID-19 disease (13). Here, an early anti-SARS-CoV-2 IgA and IgM response occurred in KTR, whereas the IgG response appeared delayed compared with immunocompetent individuals. While MMF/MPA similar to other anti-metabolites, such as azathioprine or mTOR-I, exerts a wide array of inhibitory effects on B-, T-, dendritic cells, macrophages, and endothelial cells (14), reduced IgG levels (15) and distinct effects on differential immunoglobulin classes (16), severe differences between MMF/MPA and mTOR-I have been demonstrated in KTR being exposed to either immunocyanin, pneumococcal polysaccharide (PPS), or tetanus toxoid (TT) (17). Hereby, only MMF/MPA severely reduced B-cell numbers and completely disturbed primary and secondary humoral responses, while treatment with the mTOR-I everolimus allowed primary immune responses and boosting of T-cell-dependent and -independent secondary humoral responses to the above vaccines. Nevertheless, vaccination-motivated stop or reduction of MMF/MPA dose and exposure or replacement by mTOR-I need to be balanced with rejection risk. While some transplant centers already consider a temporary stop of MMF/MPA treatment to achieve seroconversion in non-seroconverting KTR, our data demonstrate an MMF/MPA-mediated shift in the antibody time course associated with a decreased risk of decline suggests that this approach is not necessary for seroconverting KTR. In this context, it is interesting that the seroconverted KTR_{IgA} group with IgA but not IgG seroconversion was dominated by MMF/MPA treatment. Within this patient group, mRNA booster (third) vaccinations still led to marked IgG and RBD-IgG titer increases in almost all patients indicating the value of IgA antibody measurements. Nevertheless, despite no clinical consequence of this delayed immune response being visible in our study population, this situation may change dependent on regional pandemic conditions, where timely and strong protection may be required.

Considering the frequency and consequences of insufficient protection in the vulnerable population of transplant recipients, immune monitoring should be an integral part of patient care and used for the timing of additional booster vaccinations. Hereby, MMF/MPA seems to be the most critical drug changing not only the chance of seroconversion but also the peak level and time course of specific IgG and RBD-IgG antibody formation and decline after successful SARS-CoV-2 vaccination.

DATA AVAILABILITY STATEMENT

After publication of the primary objective, the data might be provided to interested scientists on request (e.g. for meta-analyses, health related registers or other scientific questions) in an anonymized way within five years, if the members of the DIA-Vacc group agree.

ETHICS STATEMENT

According to the professional code of conduct for doctors (§15) the clinical trial was submitted to the ethical institutional review boards at Technische Universität Dresden (TU Dresden) responsible for the coordinating investigator (BO-EK-45012021), as well as at the University of Leipzig (046/21-lk) and Saxon Medical Association (Sächsische Landesärztekammer - EK-BR-10/21-1) responsible for further participating trial sites.

AUTHOR CONTRIBUTIONS

Contributors JSt and CH contributed to the study design, data collection, data interpretation, and drafting of the manuscript. TS, JSc, GG, AP, AS, FG, FK, HK, PA, JSr, KF, and TT were involved in data acquisition and collection or study organization. AK and RM were involved in the statistical analysis or data management of the study. TS, JSc, GG, and AP were involved in patient recruitment and data collection. All authors contributed to the article and approved the submitted version.

FUNDING

This study was funded by the Else Kröner Fresenius Stiftung, Bad Homburg v. d. H., grant number Fördervertrag EKFS 2021_EKSE.27 and the "Sächsisches Staatsministerium für Wissenschaft, Kultur und Tourismus" *via* Sächsische AufbauBank, grant number (SAB-Antragsnr). (100592538).

ACKNOWLEDGMENTS

*DIA-Vacc- Investigators

Members in alphabetical order within the corresponding centers:

[1]*Medizinische Klinik und Poliklinik III, Universitätsklinikum, Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany*

Andrea Angermann, Nicole Bunk, Susanne Dollfus, Petya Drinova, Annegret Fleischer, Chris Gebauer, Susann Graichen, Kerstin Haaser, Sabrina Heide, Gabriele Hirt, Franziska Klemt, Markus Latk, Jens Passauer, Miriam Poser, Margrit Reinhardt, Ronny Rettig, Vladimir Todorov, Kerstin Zäumer

[3]*KfH-Nierenzentrum am Klinikum Chemnitz, Krankenhaus Küchwald, Chemnitz, Germany*

Angela Grzegorek, Markus Hallmann, Saskia Härtwig, Christiane Hintzen-Kruse, Frauke Neidt und Söhne, Susann Reissland, Bianca Seidel, Anett Speer, Peggy Weiß, Christine Wiech

[4]*Dialysezentrum Chemnitz, Chemnitz, Germany*

Markus Gläser, Ulrike Richardt, Anja Richter, Kathrin Scheibner, Andreas Sohn

[5]*KfH-Nierenzentrum am Klinikum St. Georg, Leipzig, Germany*

Joachim Beige, Kathrin Heipmann, Claudia Karger, Christian Schauff, Peyo Kolev Sivenov

[6]*PHV Dialysezentrum Dresden-Johannstadt, Dresden, Germany*

Steffen Bischoff, Petra Müller, Tatjana Seitz

[8]*Institut für Transfusionsmedizin Plauen, DRK-Blutspendedienst Nord-Ost gemeinnützige GmbH,* Plauen, Germany

Nadine Hildebrandt, Andreas Karl.

Further Supporters

D. Michael Albrecht, Medizinischer Vorstand (Sprecher), Universitätsklinikum Carl Gustav Carus Dresden

Thomas Grünewald, Chemnitz, Vorsitzender Sächsische Impfkommission

Patricia Klein, Ärztliche Geschäftsführerin, Sächsische Landesärztekammer Körperschaft des öffentlichen Rechts

Marcus Strotkötter, Senior Operations Officer for Vaccination Center and Mobile Vaccination Teams at DRK Landesverband Sachsen e.V.

EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany provided antibody ELISAs and interferon-gamma release assays for this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.928542/full#supplementary-material

REFERENCES

1. Stumpf J, Siepmann T, Lindner T, Karger C, Schwobel J, Anders L, et al. Humoral and cellular immunity to SARS-CoV-2 vaccination in renal transplant versus dialysis patients: a prospective, multicenter observational study using mRNA-1273 or BNT162b2 mRNA vaccine. *Lancet Reg Health Eur*. (2021) 9:100178. doi: 10.1016/j.lanepe.2021.100178
2. Grupper A, Rabinowich L, Schwartz D, Schwartz IF, Ben-Yehoyada M, Shashar M, et al. Reduced humoral response to mRNA SARS-CoV-2 BNT162b2 vaccine in kidney transplant recipients without prior exposure to the virus. *Am J Transplant*. (2021) 21:2719–26. doi: 10.1111/ajt.16615
3. Boyarsky BJ, Werbel WA, Avery RK, Tobian AAR, Massie AB, Segev DL, et al. Antibody response to 2-dose SARS-CoV-2 mRNA vaccine series in solid organ transplant recipients. *JAMA*. (2021) 325:2204–6. doi: 10.1001/jama.2021.7489
4. Stumpf J, Schwobel J, Lindner T, Anders L, Siepmann T, Karger C, et al. Risk of strong antibody decline in dialysis and transplant patients after SARS-CoV-2mRNA vaccination: six months data from the observational dia-vacc study. *Lancet Reg Health Eur*. (2022) 17:100371. doi: 10.1016/j.lanepe.2022.100371
5. Padoan A, Sciacovelli L, Basso D, Negrini D, Zuin S, Cosma C, et al. IgA-Ab response to spike glycoprotein of SARS-CoV-2 in patients with COVID-19: a longitudinal study. *Clin Chim Acta*. (2020) 507:164–6. doi: 10.1016/j.cca.2020.04.026
6. Rubio-Acero R, Castelletti N, Fingerle V, Olbrich L, Bakuli A, Wölfel R, et al. In search for the SARS-CoV-2 protection correlate: a head-to-head comparison of two quantitative S1 assays in a group of pre-characterized oligo-/asymptomatic patients. *Infect Dis Ther*. (2021) 10:1505–18. doi: 10.1007/s40121-021-00475-x
7. Müller L, Andrée M, Moskorz W, Drexler I, Walotka L, Grothmann R, et al. Age-dependent immune response to the Biontech/Pfizer BNT162b2 COVID-19 vaccination. *Clin Infect Dis*. 73:2065–72. doi: 10.1101/2021.03.03.21251066
8. Meyer B, Torriani G, Yerly S, Mazza L, Calame A, Arm-Vernez I, et al. Validation of a commercially available SARS-CoV-2 serological immunoassay. *Clin Microbiol Infect*. (2020) 26:1386–94. doi: 10.1016/j.cmi.2020.06.024
9. EUROIMMUN Medizinische Labordiagnostika AG aPc. *SARS-CoV-2 NeutraLISA*. (2021). Available online at: https://www.coronavirus-diagnostics.com/documents/Indications/Infections/Coronavirus/EI_2606_D_UK_F.pdf (accessed March 31, 2021).
10. Aiello A, Najafi Fard S, Petruccioli E, Petrone L, Vanini V, Farroni C, et al. Spike is the most recognized antigen in the whole-blood platform in both acute and convalescent COVID-19 patients. *Int J Infect Dis*. (2021) 106:338–47. doi: 10.1016/j.ijid.2021.04.034
11. R Core Team. *R: A Language and Environment for Statistical Computing*. 4.0.1 ed. Vienna: R Foundation for Statistical Computing (2020).
12. Friedman J, Hastie T, Tibshirani R. Regularization paths for generalized linear models *via* coordinate descent. *J Stat Softw*. (2010) 33:1–22. doi: 10.18637/jss.v033.i01
13. Cravedi P, Ahearn P, Wang L, Yalamarti T, Hartzell S, Azzi Y, et al. Delayed kinetics of IgG, but Not IgA, antispike antibodies in transplant recipients following SARS-CoV-2 infection. *J Am Soc Nephrol*. (2021) 32:3221–30. doi: 10.1681/ASN.2021040573
14. Ritter ML, Pirofski L. Mycophenolate mofetil: effects on cellular immune subsets, infectious complications, and antimicrobial activity. *Transpl Infect Dis*. (2009) 11:290–7. doi: 10.1111/j.1399-3062.2009.00407.x
15. Jonsson CA, Carlsten H. Mycophenolic acid inhibits inosine 5'-monophosphate dehydrogenase and suppresses immunoglobulin and cytokine production of B cells. *Int Immunopharmacol*. (2003) 3:31–7. doi: 10.1016/S1567-5769(02)00210-2
16. Zmonarski SC, Boratynska M, Madziarska K, Klinger M, Kusztel M, Patrzalek D, et al. Mycophenolate mofetil severely depresses antibody response to CMV infection in early posttransplant period. *Transplant Proc*. (2003) 35:2205–6. doi: 10.1016/S0041-1345(03)00764-4
17. Struijk GH, Minnee RC, Koch SD, Zwinderman AH, van Donselaar-van der Pant KA, Idu MM, et al. Maintenance immunosuppressive therapy with everolimus preserves humoral immune responses. *Kidney Int*. (2010) 78:934–40. doi: 10.1038/ki.2010.269

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.

frontiers | Frontiers in Medicine

SYSTEMATIC REVIEW
published: 14 July 2022
doi: 10.3389/fmed.2022.942665

Belatacept in Kidney Transplantation: What Are the True Benefits? A Systematic Review

Yannis Lombardi[1,2] *and Hélène François*[1,2,3]*

[1] Soins Intensifs Néphrologiques et Rein Aigu, Hôpital Tenon, Assistance Publique-Hôpitaux de Paris, 4 rue de la Chine, Paris, France, [2] Sorbonne Université, Paris, France, [3] INSERM UMR_S 1155, Paris, France

The current gold standard to prevent allograft rejection for maintenance immunosuppression in kidney transplantation currently consists in glucocorticoids, an antiproliferative agent and a calcineurin inhibitor (CNI), with better outcome for tacrolimus than cyclosporin. Although, CNI drastically improved early graft survival, so far, CNI have failed to significantly improve long-term survival mainly because of nephrotoxicity. In addition, CNI carry several other side effects such as an increased risk for cardiovascular events and for diabetes mellitus. Therefore, seeking alternatives to CNI remains of paramount importance in kidney transplantation. Belatacept is a fusion protein composed of the human IgG1 Fc fragment linked to the modified extracellular domain of cytotoxic T lymphocyte–associated antigen 4. In kidney transplant recipients, pivotal phase III randomized studies suggested clinical benefits of belatacept as an initial maintenance regimen, as compared with cyclosporine, mainly on kidney function. Recently, a randomized study also suggested a clinical benefit on renal function of a conversion from a CNI-based to a belatacept-based maintenance regimen in patients. However, conversion from CNIs to belatacept is probably associated with an increased risk of biopsy-proven acute rejection and should prompt close clinical surveillance. On the other hand, other studies suggest a decrease in *de novo* humoral transplant immunization. Belatacept is probably associated with an increase in both risk and severity of some infectious diseases, including EBV-linked post-transplantation lymphoproliferative disorders, and with a decreased response to vaccines. Most studies on belatacept are observational, retrospective, and non-comparative. Consequently, high-quality data about the safety and efficacy profile of belatacept, as compared with the current gold standard for maintenance regimens (tacrolimus-based), is uncertain. Our review will therefore focus on the most recent published data aiming at evaluating the evidence-based or the "true" benefits and risks of belatacept-based regimens in kidney transplantation.

Keywords: belatacept, kidney transplantation, immunosuppressive therapy, maintenance therapy, calcineurin avoidance, avoidance (withdrawal), CNI toxicity, costimulation blockade

OPEN ACCESS

Edited by:
Kathrin Eller,
Medical University of Graz, Austria

Reviewed by:
Wen Tian,
Stanford University, United States
Željka Večerić-Haler,
University Medical Centre Ljubljana, Slovenia

***Correspondence:**
Hélène François
helene.francois@aphp.fr

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *12 May 2022*
Accepted: *20 June 2022*
Published: *14 July 2022*

Citation:
Lombardi Y and François H (2022) Belatacept in Kidney Transplantation: What Are the True Benefits? A Systematic Review. Front. Med. 9:942665. doi: 10.3389/fmed.2022.942665

INTRODUCTION

The current gold standard to prevent allograft rejection in kidney transplantation currently consists in a maintenance treatment based on glucocorticoids, an antiproliferative agent and a calcineurin inhibitor (CNI) (1). Among calcineurin inhibitors, tacrolimus is the current gold standard due to better outcomes as compared to cyclosporin A (1). Indeed, CNI drastically improved early graft survival but, so far, have failed to improve significantly long-term survival mainly because of nephrotoxicity. In addition, CNI carry several other side effects such as an increased risk for cardiovascular events and for diabetes mellitus (2). Therefore, seeking alternatives to CNI remains of paramount importance in kidney transplantation.

Belatacept was designed as an alternative to calcineurin inhibitors-based regimens to prevent rejection–and consequently, graft loss–in recipients of kidney allografts. Belatacept is a recombinant immunoglobulin fusion protein, combining the modified extracellular B7-binding domain of Cytotoxic T-Lymphocyte-Associated protein 4 (CTLA4) with the constant fragment portion (Fc) of IgG1 (3). Due to a high affinity with CD80 (B7-1) and CD86 (B7-2), molecules expressed on Antigen Presenting Cells, belatacept acts as a highly potent costimulation inhibitor, preventing CD28-mediated T-cell activation (3).

Since belatacept appeared effective in preventing allograft rejection in non-human models of kidney transplantation without the burden of nephrotoxicity (3), subsequent clinical studies were led. Belatacept obtained US Food and Drug Administration's and European Medicines Agency's approval as an alternative for CNI in *de novo* kidney transplant recipients (KTRs) in 2011 (4), although initial trials were led against cyclosporin. Yet, in 2016, only 3.11% of *de novo* KTRs the United States received belatacept for initial maintenance therapy (5). Similarly, its use in France and in many countries in Europe has been limited because meta-analysis have failed to demonstrate significant benefits for long term graft survival compared to tacrolimus (6).

In addition, despite its lack of nephrotoxicity and a better renal graft function several questions remain that may hamper its use in clinical practice such as the risk of acute rejection, PTLD and infection.

In view of the recent published randomized trials that were led against tacrolimus (7, 8), we will hereafter review the benefits and risks of using belatacept in kidney transplantation, to provide an up to date and unbiased evaluation of belatacept use in kidney transplantation. To this end, we conducted a systematic review of the literature. Our focus will be on comparative original studies—and mainly Randomized Controlled Trials (RCTs)—studying the impact, on clinically pertinent outcomes, of using belatacept instead of CNI. We will also briefly review other studies.

SYSTEMATIC REVIEW OF THE LITTERATURE

We performed a systematic review of the current medical literature (**Figure 1**). We searched NCBI's PubMed database on 14/04/2022 using the query ["belatacept" AND (kidney OR renal)] and identified 475 citations. We assessed all corresponding abstracts.

We retrieved 404 articles on belatacept in kidney transplantation, among which 160/404 (39.6%) were not original studies (i.e., reviews, experts' opinions, comments, responses, etc..).

We retrieved 80 basic science studies and 164 clinical studies. Among clinical studies, 90/164 (54.9%) were non-comparative studies, meaning that no comparison was made between belatacept and other treatments.

RANDOMIZED CLINICAL TRIALS COMPARING CNI- AND BELATACEPT-BASED REGIMENS

We retrieved 38 published articles on RCTs comparing belatacept with at least one other treatment. Among these, 22/38 (57.9%) concerned Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial (BENEFIT) trials—either BENEFIT, BENEFIT-Extended Criteria Donors (BENEFIT-EXT), or both.

We will consider, for each RCT, the publication describing the longest follow-up for each RCT in the intention-to-treat population. In general, unless there is a significant contribution, we will not discuss results from publications describing short-term analyses, *post-hoc* analyses, subgroup analyses or meta-analyses of these RCTs.

Overall, 13 distinct RCTs were identified, 11 of which were trials directly comparing CNI- and belatacept-based regimens (**Table 1**). One study was not considered since it investigated the effect of belatacept to prevent humoral sensitization in patients with failed grafts. One study compared two belatacept regimens (every 4 weeks vs. every 8 weeks); its results are also reported in **Table 1**.

Among those 11 RCTs, 9/11 (81.8%) evaluated belatacept in *de novo* kidney transplant recipients (KTRs), and 2/11 evaluated it when started in stable kidney transplant recipients already receiving CNI. Studies in *de novo* KTRs included 2018 patients, 1208/2018 (59.8%) of whom were in BENEFIT and BENEFIT-EXT trials.

Control groups included 1001 patients who received CNI: in 3 trials, 478 patients received only cyclosporin A; in 6 trials, 211 patients received only tacrolimus; in 2 trials, 312 patients received either cyclosporin A or tacrolimus.

Standard regimen for *de novo* KTRs (called "less intensive"), used in all studies, consists in i.v. belatacept 10 mg/kg for 5 injections in 84 days (one every 2 weeks), then 5 mg/kg every month subsequently. This regimen is US Food and Drug Administration- and European Medicines Agency-approved. An

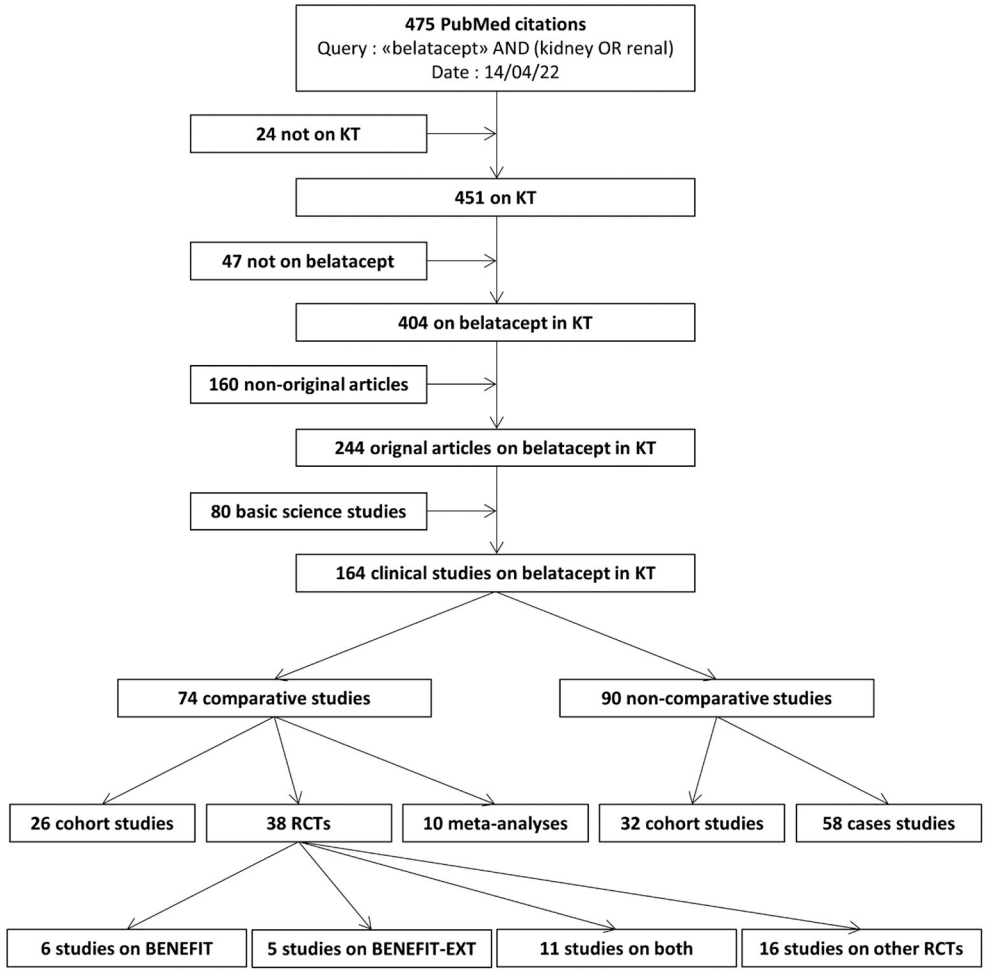


FIGURE 1 | Systematic review of the literature on belatacept in kidney transplantation. KT: kidney transplantation. RCT: randomized controlled trial.

alternative regimen (called "more intensive") consists in i.v. belatacept 10 mg/kg for 11 injections in 6 months, then 5 mg/kg every month subsequently, and was evaluated in three studies.

Death With a Functioning Graft

Two studies using cyclosporin A as a comparator (BENEFIT and BENEFIT-EXT), and none using tacrolimus, appeared to have sufficient power to perform statistical comparisons for this outcome. In these, no significant difference in the risk of death with a functioning graft were observed between patients receiving belatacept or cyclosporin A, regardless of belatacept dose.

Death-Censored Loss of Allograft Function

Here also, only BENEFIT trials appeared to have sufficient power for this outcome. In those, no significant difference in the risk of loss of allograft function were observed between patients receiving belatacept or cyclosporin A, regardless of belatacept dosage.

Graft Loss (Death or Loss of Allograft Function)

Three studies using cyclosporin A as a comparator, and none using tacrolimus, appeared to have sufficient power for this outcome [BENEFIT, BENEFIT-EXT, and the initial phase II study, whose final results were published by Vincenti et al. (9)].

In BENEFIT, belatacept at a standard dose ("less intensive") was associated with a significant decrease in the risk of graft loss, with a hazard ratio of 0.57 [95% CI: 0.35-0.94] during a 7-year follow-up, when compared with cyclosporin A, in patients receiving a kidney from a Standard Criteria Donor (SCD). All patients were treated by basiliximab at induction, and glucocorticoids and either mycophenolic acid or mycophenolate mofetil for maintenance.

In BENEFIT and the phase II trial, belatacept at a higher dose ("more intensive") was associated with a significant decrease in the risk of graft loss when compared with cyclosporin A.

In BENEFIT-EXT, in KTRs receiving a kidney from an Expanded Criteria Donora (ECD), regardless of the dose,

TABLE 1 | Summary of randomized controlled trials evaluating belatacept in kidney transplant recipients.

	Comparator	Intervention	Nb. of patients	Setting	Follow–up	Death	Graft failure	Death or graft failure	Rejections	CV events	Infections	Cancers
Belatacept vs. CNI, for *de novo* KT recipients												
Vincenti et al. (9)	CsA (C_0: 150–300 mg/l until M1, then 100–250)	First randomization: LI: 10 mg/kg, 6 inj./3 months MI: 10 mg/kg, 11 inj./6 months Second randomization: 4w: 5 mg/kg every 4w 8w: 5 mg/kg every 8w	(Total nb: 218) CsA: 73 LI: 71 MI: 74 CsA:71 4w: 62 8w: 60	Basiliximab for induction, steroids + MYC for maintenance	10 years	(Total nb: 15) CsA: 5/73 LI: 2/71 MI: 8/74	(Total nb: 8) CsA: 3/73 LI: 1/71 MI: 4/71	LI vs. CsA: HR 0.95 [0.38–2.36] **MI vs. CsA: HR 0.24 [0.24–0.91]** 4w vs. CsA: HR 0.55 [0.17–1.73] 8w vs. CsA: HR 0.52 [0.16–1.74]	LI vs. CsA: HR 1.61 [0.85–3.05] MI vs. CsA: HR 0.95 [0.47–1.92] 4w vs. CsA: HR 1.06 [0.35–3.17] 8w vs. CsA: HR 2.00 [0.75–5.35]	(Death from CV cause) CsA: 2/73 LI: 1/71 MI: 1/71	(Serious events) CsA: 15.0/100py LI: 6.7/100py MI: 10.4/100py CsA: 16.7/100py 4w: 6.0/100py 8w: 10.4/100py	CsA: 3.0/100py LI: 2.5/100py MI: 10.4/100py CsA: 3.3/100py 4w: 2.8/100py 8w: 3.3/100py
Vincenti et al. (10) BENEFIT	CsA (C_0: 150–300 mg/l until M1, then 100–250)	Bela LI then 4w Bela MI then 4w	(Total nb: 666) CsA: 221 LI: 226 MI: 219	SCD Basiliximab for induction, steroids + MYC for maintenance	7 years	(Total nb: 58) LI vs. CsA: HR 0.55 [0.30–1.04] MI vs. CsA: HR 0.62 [0.33–1.14]	(Total nb: 38) LI vs. CsA: 0.59 [0.28–1.25] MI vs. CsA: 0.56 [0.25–1.21]	**LI vs. CsA: HR 0.57 [0.35–0.94] MI vs. CsA: HR 0.57 [0.35–0.95]**	CsA: 11.4% LI: 18.3% MI: 24.4%	(Death from CV cause) CsA: 11/221 LI: 6/226 MI: 6/219 (Serious cardiac+vascular events) CsA: 2.0+1.8/100py LI: 1.4+1.5/100py MI: 2.2+2.9/100py	(Serious events) CsA: 13.3/100py LI: 10.7/100py MI: 10.6/100py	CsA: 2.6/100py LI: 1.8/100py MI: 2.1/100py
Durrbach et al. (11) BENEFIT–EXT	CsA (C_0: 150–300 mg/l until M1, then 100–250)	Bela LI then 4w Bela MI then 4w	(Total nb: 542) CsA: 184 LI: 175 MI: 183	ECD Basiliximab for induction, steroids + MYC for maintenance	7 years	(Total nb: 102) LI vs. CsA: HR 0.78 [0.45–1.35] MI vs. CsA: HR 0.70 [0.40–1.29]	(Total nb: 73) LI vs. CsA: 0.78 [0.45–1.35] MI vs. CsA: 0.70 [0.40–1.23]	LI vs. CsA: HR 0.93 [0.63–1.36] MI vs. CsA: HR 0.92 [0.63–1.34]	LI vs. CsA: HR 1.15 [0.70–1.90] MI vs. CsA: HR 1.22 [0.75–2.00]	(Death from CV cause) CsA: 8/184 LI: 12/175 MI: 12/183 (Serious events) CsA: 5.2/100py LI: 4.1/100py MI: 5.2/100py	(Serious events) CsA: 20.3/100py LI: 16.5/100py MI: 22.7/100py	CsA: 3.6/100py LI: 3.2/100py MI: 3.8/100py
Ferguson et al. (12)	Tac/MYC (C_0: 8–12 ng/ml until M1, then 5–10)	Bela/MYC Bela/Siro	(Total nb: 89) Tac/MYC: 30 Bela/MYC: 33 Bela/Siro: 26	rATG for induction No steroids for maintenance	1 year	(Total nb: 1) Tac/MYC: 0/30 Bela/MYC: 1/33 Bela/Siro: 0/26	(Total nb: 3) Tac/MYC: 0/30 Bela/MYC: 1/33 Bela/Siro: 2/26	Tac/MYC: 0/30 Bela/MYC: 2/33 Bela/Siro: 2/26	Tac/MYC: 1/30 Bela/MYC: 5/33 Bela/Siro: 1/26	–	Tac/MYC: 5/30 Bela/MYC: 7/33 Bela/Siro: 4/26	Tac/MYC: 1/30 Bela/MYC: 0/33 Bela/Siro: 1/26
de Graav et al. (13)	Tac (C_0: 10–15 ng/ml until S2, then 8–12 until M1, then 5–10)	Bela	(Total nb: 40) Tac: 20 Bela: 20	Basiliximab for induction, steroids + MYC for maintenance	1 year	(Total nb: 1) Tac: 1/20 Bela: 0/20	(Total nb: 3) Tac: 0/20 Bela: 3/20	Tac: 1/20 Bela: 3/20	Tac: 2/20 Bela: 11/20	Tac: 1.20/100py Bela: 0.95/100py	Tac: 1.90/100py Bela: 2.25/100py	Tac: 0/100py Bela: 0/100py

(Continued)

TABLE 1 | Continued

	Comparator	Intervention	Nb. of patients	Setting	Follow–up	Death	Graft failure	Death or graft failure	Rejections	CV events	Infections	Cancers
Newell et al. (14) CTOT−10	Alem/Tac (C_0: 8–12 ng/ml until M6, then 5–10)	Alem/Bela Bas/Tac/Bela: tacrolimus withdrawal in 3 months	(Total nb: 19) Alem/Tac: 6 Alem/Bela: 6 Bas/Tac/Bela: 7	No steroids for maintenance	1 year	(Total nb: 1) Alem/Tac: 1/6 Alem/Bela: 0/6 Bas/Tac/Bela: 0/7	(Total nb: 4) Alem/Tac: 1/6 Alem/Bela: 3/6 Bas/Tac/Bela: 0/7	Alem/Tac: 2/6 Alem/Bela: 3/6 Bas/Tac/Bela: 0/7	Alem/Tac: 3/6 Alem/Bela: 2/6 Bas/Tac/Bela: 5/7	–	–	–
Stock et al. (15) CTOT−15	MYC/Tac (C_0: 8–12 ng/ml until M6, then 5–8)	MYC/Tac/Bela: tacrolimus withdrawal, if possible, in 10 months	(Total nb: 43) MYC/Tac: 21 MYC/Tac/Bela: 22	Combined kidney and pancreas transplantation rATG for induction No steroids for maintenance	1 year	(Total nb: 1) MYC/Tac: 0/21 MYC/Tac/Bela: 1/22	(Total nb: 0)	MYC/Tac: 0/21 MYC/Tac/Bela: 1/22	(Treated episodes) MYC/Tac: 4/21 MYC/Tac/Bela: 4/22	–	MYC/Tac: 15/21 MYC/Tac/Bela: 19/22	–
Mannon et al. (16) CTOT−16	rATG/MYC/Tac (C_0: 8–12 ng/ml until M6, then 5–8)	rATG/MYC/Bela Bas/Tac/MYC/ Bela: tacrolimus withdrawal in 3 months	(Total nb: 68) rATG/MYC/Tac: 29 rATG/MYC/Bela: 29 Bas/Tac/MYC/ Bela: 10	No steroids for maintenance	1 year	(Total nb: 2) rATG/MYC/Tac: 2/29 rATG/MYC/Bela: 0/29 Bas/Tac/MYC/Bela: 0/11	(Total nb: 0)	rATG/MYC/Tac: 2/29 rATG/MYC/Bela: 0/29 Bas/Tac/MYC/ Bela: 0/11	(Treated episodes) rATG/MYC/Tac: 7/29 rATG/MYC/Bela: 14/29 Bas/Tac/MYC/Bela: 4/11	–	rATG/MYC/Tac: 14/29 rATG/MYC/Bela: 16/29 Bas/Tac/MYC/Bela: 3/11	–
Kaufman et al. (17) BEST	rATG/Tac (C_0: 8–12 ng/ml until M1, then 5–10)	rATG/Bela Alem/Bela	(Total nb: 333) rATG/Tac: 105 rATG/Bela: 104 Alem/Bela: 107	No steroids for maintenance	2 years	(Total nb: 7) rATG/Tac: 1/105 rATG/Bela: 4/104 Alem/Bela: 2/107	(Total nb: 2) rATG/Tac: 1/105 rATG/Bela: 1/104 Alem/Bela: 0/107	rATG/Tac: 2/105 rATG/Bela: 5/104 Alem/Bela: 2/107	rATG/Tac: 7/105 rATG/Bela: 26/104 Alem/Bela: 20/107	(Serious events) rATG/Tac: 3/105 rATG/Bela: 10/104 Alem/Bela: 1/107	(Serious events) rATG/Tac: 22/105 rATG/Bela: 24/104 Alem/Bela: 24/107	rATG/Tac: 7/105 rATG/Bela: 6/104 Alem/Bela: 7/107
Belatacept vs. CNI, for stable KT recipients already on CNI												
Grinyo et al. (7)	CNI (CsA or Tac)	Bela: 5 mg/kg 5 inj./2 months, then every 4w	(Total nb: 173) CNI: 89 Bela: 84	6–36 months after KT eGFR 35–75 ml/min	3 years	(Total nb: 2) CNI: 1/89 Bela: 1/84	(Total nb: 2) CNI: 1/89 Bela: 1/84	CNI: 2/89 Bela: 2/84	CNI: 3/89 Bela: 7/84	–	(Serious events) CNI: 10.2/100py Bela: 9.3/100py	CNI: 3.4/100py Bela: 3.0/100py
Budde et al. (8)	CNI (CsA or Tac)	Bela	(Total nb: 666) CNI: 223 Bela: 223	6–60 months after KT eGFR 30–75 ml/min	2 years	(Total nb: 8) CNI: 4/223 Bela: 4/223	(Total nb: 2) CNI: 2/223 Bela: 0/223	CNI: 6/223 Bela: 4/223	CNI: 9/223 Bela: 18/223	(Death from CV cause) CNI: 1/223 Bela: 3/223	(Serious events) CNI: 44/222 Bela: 37/221	CNI: 12/222 Bela: 18/221
Comparison of belatacept regimens, for stable KT recipients already on belatacept												
Badell et al. (18)	Bela 4w	Bela 8w	(Total nb: 163) 4w: 82 8w: 81	>12 months after KT eGFR >35 ml/min	1 year	(Total nb: 2) 4w: 2/82 8w: 0/81	(Total nb: 0) 4w: 0/82 8w: 0/81	4w: 2/82 8w: 0/81	4w: 2/82 8w: 5/81	–	(Any event) 4w: 24/82 8w: 23/81	4w: 1/82 8w: 4/81

When several articles were published on the same trial, only the one with the longest follow-up time was considered. Statistically significant differences are highlighted in bold. Bela: belatacept. LI, less intensive: MI, more intensive; CsA, cyclosporin A; Tac, tacrolimus; CNI, calcineurin inhibitors; KT, kidney transplant; MYC, mycophenolate mofetil or mycophenolic acid; rATG, rabbit antithymocyte globulin; Alem, alemtuzumab; Bas, basiliximab; HR, hazard ratio; Py, patient-year.

belatacept was not associated with a significant difference in the risk of graft loss in comparison with cyclosporin A.

Rejection

In 9 trials out of 11, belatacept was associated with a higher rate of rejection compared with CNI. Statistical comparisons were not systematically performed in these studies but, since this difference is consistently observed across trials in various settings, it most likely reflects a true difference.

Based on data from BENEFIT studies, we can estimate that, in *de novo* KTRs receiving an induction with basiliximab, the risk for biopsy-proven rejection within 7 years following KT using a kidney from an SCD is approximatively 15% higher with belatacept than with cyclosporin A; and 60% higher when using a kidney from an ECD.

Based on data from two RCTs (7, 8), we can estimate that in stable KTRs receiving CNI for more than 6 months, the risk for biopsy-proven rejection within 2–3 years following a switch from CNI to belatacept is increased by approximatively 100-150%, compared to remaining on CNI.

Overall, most rejection episodes occurred within a year following KT (in de novo KTRs) or switch (in stable KTRs).

Cardiovascular Events

No study had sufficient power to detect a significant difference in death from cardiovascular cause, and no study presented a survival analysis for this outcome.

In BENEFIT studies in general, observed rates of serious cardiovascular events were lower in patients treated with "less intensive" belatacept compared with CsA. In BENEFIT-EXT especially, a trial in which the absolute number of events is high (elderly patients with comorbidities), the rate of serious cardiovascular events was 5.2 per 100 patient-year with cyclosporin A and 4.1 per 100 patient-year with belatacept (relative risk reduction: 21%; absolute risk reduction:−1.1 per 100 patient-year; number needed to treat to avoid one serious cardiovascular event each year: 91 patients).

In the study by Kaufman et al. (17), in 209 patients that received rabbit antithymocyte globulin (rATG) in induction and a steroids-free regimen for maintenance that were followed for 2 years, the rate of serious cardiovascular events was 3.4 times higher in patients treated with belatacept than with tacrolimus (2.8% of patients on tacrolimus vs. 9.6% on belatacept). This difference was not observed when belatacept-treated patients received alemtuzumab instead of rATG.

Infectious Events

In the initial phase II study, then in BENEFIT and BENEFIT-EXT, a notable decrease in the risk of serious infectious events was noted in patients treated with belatacept, compared with cyclosporin A. For instance, in BENEFIT, the risk of serious infection was 13.3 per 100 patient-year on cyclosporin A and 10.7 per 100 patient-year on belatacept (relative risk reduction: 19.6%; absolute risk reduction:−2.6 per 100 patient-year; number needed to treat to avoid one serious infection each year: 38 patients).

Subsequent studies, that used tacrolimus as the main comparator, did not find such a high decrease in the risk of infection. For instance, in the study by Kaufman et al., the risk of serious infection during the 2-year follow-up was 22/105 (20.9%) on tacrolimus and 24/104 (23.1%) on belatacept. In the study by Budde et al., the risk of serious infection during the 2-year follow-up was 44/222 (19.8%) on CNI and 37/221 (16.7%) on belatacept.

No study was powered to detect significant differences in specific types of infection (e.g., opportunistic infection, CMV disease, BK virus nephropathy, EBV-induced post-transplantation lympoproliferative disorder (PTLD), etc..). However, in the initial phase II study, three patients randomized to receive belatacept developed EBV-induced PTLD, vs. none among cyclosporin A-treated controls. In two of them, the disease was the consequence of a primo infection. In BENEFIT, among EBV-seronegative patients, 5/369 developed EBV-induced PTLD on belatacept, vs. 0/184 on cyclosporin A. Consequently, due to an increase in risk for PTLD in case of EBV primo infection, belatacept is contraindicated for EBV-seronegative patients.

Cancers

The same limits about statistical power apply for cancers. In BENEFIT-EXT, a trial in which elderly patients were included and, consequently, in which the absolute risk for cancers was high, there was no obvious difference in the risk for cancer between belatacept- and cyclosporin A-treated patients (3.6/100 patient-year on cyclosporin A vs. 3.2 on belatacept, during a 7-year follow-up). In the study by Budde et al. (8), during a 2-year follow-up, 5.4% of patients in the CNI group (90% of whom received tacrolimus) developed a cancer, vs. 8.1% in the belatacept group.

No study had sufficient power to detect differences on the risk for specific cancers (e.g., non-skin cancers).

Estimated Glomerular Filtration Rate and Donor Specific Antibodies

In most studies, belatacept was associated with an increase in estimated glomerular filtration rate (eGFR) compared to CNI. This is likely a consequence of differential renal hemodynamic effects between both drugs. In BENEFIT, after a 7-year follow-up, mean eGFR increased from 66 to 72.1 ml/min/1.73 m^2 on belatacept, and decreased from 52.5 to 44.9 on cyclosporin A. In the study by Budde et al., mean eGFR increased by 5.2 ml/min/1.73 m^2 on belatacept and decreased by 1.9 on CNI.

Belatacept was also associated with a decrease in the risk to develop *de novo* donor specific antibodies (DSA). In BENEFIT, 4.6% of patients on belatacept developed *de novo* DSA during follow-up vs. 17.8% on cyclosporin A. In the study by Budde et al., 1% of patients switched to belatacept developed *de novo* DSA during follow-up vs. 7% on tacrolimus.

These results must be interpreted with caution as they do not necessarily mean that, on the long term, there would be differences on hard, clinically pertinent outcomes (such as graft loss or death). Indeed, eGFR slopes and *de novo* DSA are determined on the subgroup of patients alive, with a functioning graft and with available data, notably excluding patients that

lost their graft after a rejection (an event that is probably more frequent with belatacept), thus creating a potential differential bias. Furthermore, since rejections are more frequent with belatacept than with CNI, one cannot exclude that, on the long term, more patients would lose their grafts because of a rejection occurring on belatacept than because of a CNI-mediated nephrotoxicity.

Surrogate endpoints, developed to predict hard, clinically pertinent outcomes based on intermediate outcomes, have been validated in kidney transplantation (19). As they integrate various parameters (e.g., eGFR, donor specific antibodies, biopsy findings, proteinuria), observed differences between groups on these integrative criteria seem more reliable than differences in a sole parameter (e.g., eGFR) to predict long-term outcomes, and could help reduce follow-up in clinical trials with no loss in statistical power. More data are needed on this important matter.

Quality of Life

In a *post-hoc* study based on data from BENEFIT and BENEFIT-EXT, Dobbels et al. (20) found that belatacept, compared to cyclosporine, was associated with an increase in Physical Composite Scores at 3 years (49.2 vs. 47.1 in BENEFIT, 46.4 vs. 43.6 in BENEFIT-EXT, $p < 0.05$ for both comparisons) but not in Mental Composite Scores.

No study compared quality of life between patients receiving belatacept and tacrolimus.

NON-RANDOMIZED STUDIES

Hereafter, we will review non-randomized clinical studies involving belatacept in KT. We will only consider studies that add significant contribution to data from RCTs, either because they strongly comfort their findings or because they make new ones. We will distinguish between comparative (i.e., where there is a control group of CNI-treated patients) and non-comparative studies. Among comparative studies, we will distinguish between those providing an adjusted analysis (i.e., with statistical methods to consider selection bias between groups) and those providing none (i.e., crude comparison between groups).

Comparative Studies, Adjusted Analyses

In a registry study of 50 244 *de novo* KTRs in the US, 458 of whom received belatacept, Wen et al. (21) found that belatacept was associated with a 2.36 times increase in adjusted hazard of rejection during a 1-year follow-up, compared with tacrolimus. There was a decrease in risk for new onset diabetes on belatacept (3.8% vs. 2.2%). There were no significant differences in risk for death, loss of allograft function, PTLD or cancer.

In a propensity-matched registry study on 657 *de novo* KTRs treated with belatacept in the US, and on 3 210 controls on tacrolimus, Cohen et al. (22) found that belatacept was associated with a 3.12 times higher odds of rejection during the first year following KT. During a maximal follow-up of 8 years, there were no differences in risk for death or loss of allograft function between belatacept and tacrolimus.

In a propensity-matched cohort study of 181 KTRs switched to belatacept in Paris, France, and on 181 controls on CNI, Chavarot et al. (23) found that belatacept was associated with a 6.3 times increase in risk of CMV-disease during follow-up (17.7% vs. 2.8%). Most CMV diseases on belatacept were atypical, late onset, had gastrointestinal involvement, and 10% (4/40) were life-threatening.

In a cohort study of 609 KTRs in the US, 24 of which were receiving belatacept, Ou et al. (24) found, in a weighted analysis, that belatacept was associated with a 16.7-fold lower odds of responding to anti-SARS-CoV2 mRNA-based vaccination, compared to comparable patients not receiving belatacept. Overall, after two doses, 5% of patients to belatacept responded to vaccination, compared to 50% in comparable patients not receiving belatacept.

In a cohort study of 563 KTRs in Berlin, Germany, 45 of which were receiving belatacept, Liefeld et al. (25) found, in a multivariate analysis, that belatacept was associated with an absence of response to anti-SARS-CoV2 mRNA-based vaccine. Specifically, none of the patients receiving belatacept showed a seroconversion after two doses of vaccine, vs. 24% of patients on tacrolimus.

Comparative Studies, Unadjusted Analyses

In a cohort study of 11 453 *de novo* KTRs in São Paulo, Brazil, 34 of whom received belatacept, Viana et al. (26) found that belatacept-treated patients had the highest risk of developing tuberculosis during follow-up (14.7% vs. 1.6% among patients receiving CNI; unadjusted HR 13.14 [95%CI: 5.3-32.8]).

In a cohort study of 168 *de novo* KTRs in Atlanta, 104 of whom were treated by belatacept, Karadkhele et al. (27) found that the risk for CMV viremia was higher on belatacept than on tacrolimus during a 2-year follow-up (50% vs. 34.4%, $p = 0.047$). Of note, all patients were CMV-seronegative patients receiving kidneys from CMV-seropositive patients, and all received valganciclovir in primary prophylaxis for 6 months following KT. Among patients that developed CMV viremia, the rate of resistance to ganciclovir was higher on belatacept than on tacrolimus (21.1% vs. 1.6%, $p < 0.001$).

In a cohort study on 49 *de novo* KTRs treated with belatacept in France, and on 74 controls treated with tacrolimus, Leibler et al. (28) found that the risk of acute T-cell mediated rejection was higher on belatacept during a 1-year follow-up (25.4% vs. 5.6%, $p = 0.003$). There was no difference in the risk for acute antibody mediated rejection. Of note, all patients had pre-formed donor specific antibodies (median fluorescence intensity 500 to 3000), received thymoglobulin as an induction therapy and had protocol biopsies at 3 months and 12 months.

In a cohort study of 60 *de novo* KTRs treated with belatacept in Atlanta, USA, and on 44 controls treated with tacrolimus, Parsons et al. (29) found that belatacept was associated with a significant reduction in cPRA as compared with tacrolimus. All patients had calculated panel reactive antibody (cPRA) higher than 97% and no DSA. Of note, this reduction was predominantly due to a decrease on the strength of anti-HLA class I antibodies.

Non-comparative Studies

In a cohort study of 453 KTRs switched from CNI to belatacept in France, Bertrand et al. (30) found that opportunistic infections

TABLE 2 | Current evidence, based on data from comparative studies, on the benefits to use belatacept instead of tacrolimus for kidney transplant recipients.

	De novo KTRs	Switch from tacrolimus
Hard, clinically pertinent outcomes		
Death with a functioning graft	No proven benefit vs. tacrolimus	
Loss of graft function	No proven benefit vs. tacrolimus	
Rejections	Higher risk with belatacept than with tacrolimus (Mostly T-cell mediated, mostly within a year after initiation)	
Cardiovascular events	No proven benefit vs. tacrolimus	
Infectious events	No proven benefit vs. tacrolimus	Higher risk for CMV disease with belatacept
Cancers	No proven benefit vs. tacrolimus	
Surrogate endpoints		
Estimated GFR	Higher estimated GFR with belatacept than with tacrolimus	
Donor specific antibodies	Less *de novo* DSA with belatacept than with tacrolimus	
Glycemic control	Better glycemic control with belatacept than with tacrolimus	

GFR, glomerular filtration rate; DSA, donor specific antibodies; KTR, kidney transplant recipient.

developed in 43 patients (9.3%) post-conversion, during a mean follow-up of 20.1 months. The risk for opportunistic infections was of 6.5 per 100 person-year (among which CMV disease: 2.8 per 100 person-year; Pneumocystis pneumonia: 1.6 per 100 person-year). Two patients developed PTLD, two patients developed JC virus infection with neurological symptoms, no patients developed BK virus nephropathy. At 1-year post-conversion, 22/453 (4.8%) patients died with a functioning graft, 42/453 (9.3%) were alive with a non-functioning graft, 24/453 (5.3%) had experienced a rejection.

In a cohort study of 103 KTRs switched from CNI to belatacept in Grenoble, France, Terrec et al. (31) found that glycated hemoglobin A1c (HbA1c) levels decreased from 6.2% pre-switch to 5.8% after 6 months of treatment ($p < 0.001$). Overall, beneficial effects on glycemic control were found whether patients had preexisting diabetes at the time of conversion or not.

Belatacept for Non-kidney Solid Organ Transplant Recipients

In an international randomized controlled study of 260 liver transplant recipients, 153 of whom received belatacept, Klintmalm et al. (32) found that belatacept was associated with an increase in risk for a composite outcome (rejection, graft loss or death) within 6 months following transplantation. In an extended follow-up, two patients on belatacept developed PTLD, and an increase in mortality was noted in the subgroup of patients receiving belatacept at high dose. The study was then stopped.

In a randomized controlled study of 27 lung transplant recipients in the US, 13 of whom received belatacept, Huang et al. (33) found that belatacept was associated with an increase in risk of death following transplantation, as compared with CNI (3/13 death in the belatacept group, vs. 0/14 in the CNI group). The study was prematurely stopped.

In a randomized controlled study of 43 kidney-pancreas transplant recipients in the US, 22 of whom received belatacept, Stock et al. (15) found that belatacept was associated with an increase in risk of pancreas rejection (5/22 patients on belatacept vs. 1/21 patients on CNI). Among patients on belatacept, 1/22 patients died (vs. none on CNI) and 2/22 patients had a partial or total loss of pancreatic function (vs. none on CNI). The study was prematurely stopped.

In a multicenter retrospective non-comparative cohort study of 40 heart transplant recipients in France switched from CNI to belatacept, mainly due to impaired renal function on CNI, Launay et al. (34) found that belatacept was associated with a high rate discontinuation and adverse effects. At the end of follow-up, 4/40 (10%) of patients had died (2 of fatal rejection, 1 of invasive infection, 1 of non-compliance). Discontinuation rate was of 16/40 (40%). Most patients had an increase in eGFR after conversion, but one patient started renal replacement therapy despite CNI withdrawal.

Based on these studies, the increase in risk for rejection with belatacept seems more problematic in non-kidney solid organ transplantations than in kidney transplantation, with less clear benefits on renal function. So far, belatacept use in daily practice is restricted to few patients with very specific indications (35).

SYNHTESIS

A synthesis of the current state of the medical literature on belatacept in kidney transplant recipients is provided in **Table 2**.

CONCLUSION

Belatacept is a non-nephrotoxic non-diabetogenic immunosuppressive drug developed to increase graft survival, as compared to the current gold standard, tacrolimus—which is a nephrotoxic and diabetogenic drug. Despite proven beneficial effects of belatacept on glomerular filtration rate, glycemic control, and the appearance of de novo donor specific antibodies, there are, currently, no truly evidence-based benefits on renal graft survival, as compared with tacrolimus.

This can be the consequence of insufficiently powered studies to detect a better graft survival. On the other end, a lack of effect of belatacept is also possible since its benefits (absence of nephrotoxicity, less donor specific antibodies) may

be undermined by adverse effects (higher rate of rejection and of CMV disease) which impair graft and patient survival.

For *de novo* kidney transplant recipients, the current state of the medical literature does not support the use of belatacept instead of tacrolimus. This is a direct consequence of the lack of RCTs with enough statistical power to compare belatacept against tacrolimus, in this setting, on hard, clinically pertinent outcomes. Such trials are urgently needed.

For stable kidney transplant recipients already receiving tacrolimus, additional RCTs and long-term follow-up of previous trials are needed to determine whether the observed differences in surrogate endpoints favoring belatacept (better eGFR, less *de novo* DSA, better glycemic control) will result in differences on clinically pertinent outcomes (death, loss of allograft function).

Belatacept is associated with an increase in risk for rejection, especially within the first year after treatment initiation. There is evidence that belatacept is associated with an increase in risk for CMV-disease. Due to this increased risk, patients on belatacept should be closely monitored, especially within the first year after initiation. Belatacept is associated with a reduced response rate to anti-SARS-CoV2 mRNA-based vaccination.

Most patients included in control groups in RCTs on belatacept received cyclosporin A, which is not the current gold standard for KTRs. Consequently, belatacept has not been routinely used in KTRs since its approval. Therefore, since few KTRs received belatacept since its approval, data from observational post-approval studies are of poor quality, with small sample sizes and are mostly non comparative, adding very few significant information.

As of 2022, most questions on belatacept (and all the important ones) are unanswered from an evidence-based medicine perspective. RCTs using tacrolimus as a comparator with long term follow up are essential to definitively establish the true benefits of belatacept in kidney 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.

AUTHOR CONTRIBUTIONS

YL: methodology, conceptualization, data curation, visualization, investigation, and writing–original draft. HF: project administration, supervision, methodology, conceptualization, investigation, and writing–review and editing. Both authors contributed to the article and approved the submitted version.

REFERENCES

1. Kasiske BL, Zeier MG, Chapman JR, Craig JC, Ekberg H, Garvey CA, et al. KDIGO clinical practice guideline for the care of kidney transplant recipients: a summary. *Kidney Int*. (2010) 77:299–311. doi: 10.1038/ki.2009.377
2. Naesens M, Kuypers DRJ, Sarwal M. Calcineurin inhibitor nephrotoxicity. *Clin J Am Soc Nephrol*. (2009) 4:481–508. doi: 10.2215/CJN.04800908
3. Larsen CP, Pearson TC, Adams AB, Tso P, Shirasugi N, Strobert E, et al. Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties. *Am J Transplant*. (2005) 5:443–53. doi: 10.1111/j.1600-6143.2005.00749.x
4. Archdeacon P, Dixon C, Belen O, Albrecht R, Meyer J. Summary of the US FDA approval of belatacept. *Am J Transplant*. (2012) 12:554–62. doi: 10.1111/j.1600-6143.2011.03976.x
5. Karadkhele G, Duneton C, Garro R, Badell IR, Pearson TC, Larsen CP, et al. Temporal trends and current use of *de novo* belatacept in kidney transplant recipients in the United States. *Clin Transplant*. (2022) 36:e14531. doi: 10.1111/ctr.14531
6. NULOJIX (bélatacept), immunosuppresseur sélectif. Haute Autorité de Santé. Disponible sur: https://www.has-sante.fr/jcms/c_2723467/fr/nulojix-belatacept-immunosuppresseur-selectif
7. Grinyó JM, Del Carmen Rial M, Alberu J, Steinberg SM, Manfro RC, Nainan G, et al. Safety and efficacy outcomes 3 years after switching to belatacept from a calcineurin inhibitor in kidney transplant recipients: results from a phase 2 randomized trial. *Am J Kidney Dis Off J Natl Kidney Found*. (2017) 69:587–94. doi: 10.1053/j.ajkd.2016.09.021
8. Budde K, Prashar R, Haller H, Rial M, Kamar N, Agarwal A, et al. Conversion from Calcineurin Inhibitor to Belatacept-based Maintenance Immunosuppression in Renal Transplant Recipients: a Randomized Phase 3b Trial. *J Am Soc Nephrol*. (2021) 32:3252–64. doi: 10.1681/ASN.2021050628
9. Vincenti F, Blancho G, Durrbach A, Grannas G, Grinyó J, Meier-Kriesche HU, et al. Ten-year outcomes in a randomized phase II study of kidney transplant recipients administered belatacept 4-weekly or 8-weekly. *Am J Transplant*. (2017) 17:3219–27. doi: 10.1111/ajt.14452
10. Vincenti F, Rostaing L, Grinyo J, Rice K, Steinberg S, Gaite L, et al. Belatacept and long-term outcomes in kidney transplantation. *N Engl J Med*. (2016) 374:333–43. doi: 10.1056/NEJMoa1506027
11. Durrbach A, Pestana JM, Florman S, Del Carmen Rial M, Rostaing L, Kuypers D, et al. Long-Term outcomes in belatacept- versus cyclosporine-treated recipients of extended criteria donor kidneys: final results from BENEFIT-EXT, a phase III randomized study. *Am J Transplant*. (2016) 16:3192–201. doi: 10.1111/ajt.13830
12. Ferguson R, Grinyó J, Vincenti F, Kaufman DB, Woodle ES, Marder BA, et al. Immunosuppression with belatacept-based, corticosteroid-avoiding regimens in *de novo* kidney transplant recipients. *Am J Transplant*. (2011) 11:66–76. doi: 10.1111/j.1600-6143.2010.03338.x
13. de Graav GN, Baan CC, Clahsen-van Groningen MC, Kraaijeveld R, Dieterich M, Verschoor W, et al. A randomized controlled clinical trial comparing belatacept with tacrolimus after *de novo* kidney transplantation. *Transplantation*. (2017) 101:2571–81. doi: 10.1097/TP.0000000000001755
14. Newell KA, Mehta AK, Larsen CP, Stock PG, Farris AB, Mehta SG, et al. Lessons learned: early termination of a randomized trial of calcineurin inhibitor and corticosteroid avoidance using belatacept. *Am J Transplant*. (2017) 17:2712–9. doi: 10.1111/ajt.14377
15. Stock PG, Mannon RB, Armstrong B, Watson N, Ikle D, Robien MA, et al. Challenges of calcineurin inhibitor withdrawal following combined pancreas and kidney transplantation: results of a prospective, randomized clinical trial. *Am J Transplant*. (2020) 20:1668–78. doi: 10.1111/ajt.15817
16. Mannon RB, Armstrong B, Stock PG, Mehta AK, Farris AB, Watson N, et al. Avoidance of CNI and steroids using belatacept-results of the clinical trials in organ transplantation 16 trial. *Am J Transplant*. (2020) 20:3599–608. doi: 10.1111/ajt.16152
17. Kaufman DB, Woodle ES, Shields AR, Leone J, Matas A, Wiseman A, et al. Belatacept for simultaneous calcineurin inhibitor and chronic corticosteroid immunosuppression avoidance: two-year results of a prospective, randomized multicenter trial. *Clin J Am Soc Nephrol*. (2021) 16:1387–97. doi: 10.2215/CJN.13100820
18. Badell IR, Parsons RF, Karadkhele G, Cristea O, Mead S, Thomas S, et al. Every 2-month belatacept maintenance therapy in kidney transplant recipients

greater than 1-year posttransplant: A randomized, noninferiority trial. *Am J Transplant*. (2021) 21:3066–76. doi: 10.1111/ajt.16538
19. Aubert O, Divard G, Pascual J, Oppenheimer F, Sommerer C, Citterio F, et al. Application of the iBox prognostication system as a surrogate endpoint in the TRANSFORM randomised controlled trial: proof-of-concept study. *BMJ Open*. (2021) 11:e052138. doi: 10.1136/bmjopen-2021-052138
20. Dobbels F, Wong S, Min Y, Sam J, Kalsekar A. Beneficial effect of belatacept on health-related quality of life and perceived side effects: results from the BENEFIT and BENEFIT-EXT trials. *Transplantation*. (2014) 98:960–8. doi: 10.1097/TP.0000000000000159
21. Wen X, Casey MJ, Santos AH, Hartzema A, Womer KL. Comparison of utilization and clinical outcomes for belatacept- and tacrolimus-based immunosuppression in renal transplant recipients. *Am J Transplant*. (2016) 16:3202–11. doi: 10.1111/ajt.13853
22. Cohen JB, Eddinger KC, Forde KA, Abt PL, Sawinski D. Belatacept compared with tacrolimus for kidney transplantation: a propensity score matched cohort study. *Transplantation*. (2017) 101:2582–9. doi: 10.1097/TP.0000000000001589
23. Chavarot N, Divard G, Scemla A, Amrouche L, Aubert O, Leruez-Ville M, et al. Increased incidence and unusual presentations of CMV disease in kidney transplant recipients after conversion to belatacept. *Am J Transplant*. (2021) 21:2448–58. doi: 10.1111/ajt.16430
24. Ou MT, Boyarsky BJ, Chiang TPY, Bae S, Werbel WA, Avery RK, et al. Immunogenicity and Reactogenicity After SARS-CoV-2 mRNA vaccination in kidney transplant recipients taking belatacept. *Transplantation*. (2021) 105:2119–23. doi: 10.1097/TP.0000000000003824
25. Liefeldt L, Glander P, Klotsche J, Straub-Hohenbleicher H, Budde K, Eberspächer B, et al. Predictors of serological response to SARS-CoV-2 vaccination in kidney transplant patients: baseline characteristics, immunosuppression, and the role of IMPDH Monitoring. *J Clin Med*. (2022) 11:1697. doi: 10.3390/jcm11061697
26. Viana LA, Cristelli MP, Santos DW, Tavares MG, Dantas MTC, Felipe CR, et al. Influence of epidemiology, immunosuppressive regimens, clinical presentation, and treatment on kidney transplant outcomes of patients diagnosed with tuberculosis: a retrospective cohort analysis. *Am J Transplant*. (2019) 19:1421–31. doi: 10.1111/ajt.15220
27. Karadkhele G, Hogan J, Magua W, Zhang W, Badell IR, Mehta A, et al. CMV high-risk status and posttransplant outcomes in kidney transplant recipients treated with belatacept. *Am J Transplant*. (2021) 21:208–21. doi: 10.1111/ajt.16132
28. Leibler C, Matignon M, Pilon C, Montespan F, Bigot J, Lang P, et al. Kidney transplant recipients treated with belatacept exhibit increased naïve and transitional B cells. *Am J Transplant*. (2014) 14:1173–82. doi: 10.1111/ajt.12721
29. Parsons RF, Zahid A, Bumb S, Decker H, Sullivan HC, Eun-Hyung Lee F, et al. The impact of belatacept on third-party HLA alloantibodies in highly sensitized kidney transplant recipients. *Am J Transplant*. (2020) 20:573–81. doi: 10.1111/ajt.15585
30. Bertrand D, Terrec F, Etienne I, Chavarot N, Sberro R, Gatault P, et al. Opportunistic infections and efficacy following conversion to belatacept-based therapy after kidney transplantation: a french multicenter cohort. *J Clin Med*. (2020) 9:3479. doi: 10.3390/jcm9113479
31. Terrec F, Jouve T, Malvezzi P, Janbon B, Naciri Bennani H, Rostaing L, et al. Belatacept use after kidney transplantation and its effects on risk of infection and COVID-19 vaccine response. *J Clin Med*. (2021) 10:5159. doi: 10.3390/jcm10215159
32. Klintmalm GB, Feng S, Lake JR, Vargas HE, Wekerle T, Agnes S, et al. Belatacept-based immunosuppression in *de novo* liver transplant recipients: 1-year experience from a phase II randomized study. *Am J Transplant*. (2014) 14:1817–27. doi: 10.1111/ajt.12810
33. Huang HJ, Schechtman K, Askar M, Bernadt C, Mittler B, Dore P, et al. A pilot randomized controlled trial of *de novo* belatacept-based immunosuppression following anti-thymocyte globulin induction in lung transplantation. *Am J Transplant*. (2022). doi: 10.1111/ajt.17028
34. Launay M, Guitard J, Dorent R, Prevot Y, Prion F, Beaumont L, et al. Belatacept-based immunosuppression: a calcineurin inhibitor-sparing regimen in heart transplant recipients. *Am J Transplant*. (2020) 20:553–63. doi: 10.1111/ajt.15584
35. Mujtaba MA, Sharfuddin AA, Taber T, Chen J, Phillips CL, Goble M, et al. Conversion from tacrolimus to belatacept to prevent the progression of chronic kidney disease in pancreas transplantation: case report of two patients. *Am J Transplant*. (2014) 14:2657–61. doi: 10.1111/ajt.12863

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.

frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 15 July 2022
doi: 10.3389/fmed.2022.928516

OPEN ACCESS

Edited by:
Miriam Banas,
University Hospital Regensburg, Germany

Reviewed by:
Brian Duncan Tait,
The University of Melbourne, Australia
Swati Jain,
University of Colorado Anschutz Medical Campus, United States

***Correspondence:**
Emil Grothgar
Emil.grothgar@charite.de
Nina Goerlich
nina.goerlich@charite.de

†ORCID:
Emil Grothgar
orcid.org/0000-0002-7742-8916
Nina Goerlich
orcid.org/0000-0002-8029-2300
Philipp Enghard
orcid.org/0000-0002-7254-3931

‡*These authors have contributed equally to this work*

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *25 April 2022*
Accepted: *23 June 2022*
Published: *15 July 2022*

Citation:
Grothgar E, Goerlich N, Samans B, Skopnik CM, Metzke D, Klocke J, Prskalo L, Freund P, Wagner L, Duerr M, Matz M, Olek S, Budde K, Paliege A and Enghard P (2022) Urinary CD8+HLA-DR+ T Cell Abundance Non-invasively Predicts Kidney Transplant Rejection. Front. Med. 9:928516. doi: 10.3389/fmed.2022.928516

Urinary CD8+HLA-DR+ T Cell Abundance Non-invasively Predicts Kidney Transplant Rejection

***Emil Grothgar*[1,2*†‡], *Nina Goerlich*[1,2,3*†‡], *Bjoern Samans*[4‡], *Christopher M. Skopnik*[1,2], *Diana Metzke*[1,2], *Jan Klocke*[1,2], *Luka Prskalo*[1,2], *Paul Freund*[1,2], *Leonie Wagner*[1,2], *Michael Duerr*[1], *Mareen Matz*[3], *Sven Olek*[4], *Klemens Budde*[1], *Alexander Paliege*[5‡] *and Philipp Enghard*[1,2†‡]**

[1] *Department of Nephrology and Intensive Care, Charité–Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany,* [2] *German Rheumatism Research Center Berlin (DRFZ), Berlin, Germany,* [3] *Berlin Institute of Health (BIH) at Charité–Universitätsmedizin Berlin, Berlin, Germany,* [4] *Ivana Türbachova Laboratory for Epigenetics, Precision for Medicine GmbH, Berlin, Germany,* [5] *Universitätsklinkum Carl Gustav Carus, Dresden, Germany*

Early detection of kidney transplant (KT) rejection remains a challenge in patient care. Non-invasive biomarkers hold high potential to detect rejection, adjust immunosuppression, and monitor KT patients. So far, no approach has fully satisfied requirements to innovate routine monitoring of KT patients. In this two-center study we analyzed a total of 380 urine samples. T cells and tubular epithelial cells were quantified in KT patients with graft deterioration using flow cytometry. Epigenetic urine cell quantification was used to confirm flow cytometric results. Moreover, a cohort of KT patients was followed up during the first year after transplantation, tracking cell subsets over time. Abundance of urinary cell counts differed in patients with and without rejection. Most strikingly, various T cell subsets were enriched in patients with T cell-mediated rejection (TCMR) compared to patients without TCMR. Among T cell subsets, CD8+HLA-DR+ T cells were most distinctive (AUC = 0.91, Spec.: 95.9%, Sens.: 76.5%). Epigenetic analysis confirmed T cell and tubular epithelial cell quantities as determined by flow cytometry. Urinary T cell abundance in new KT patients decreased during their first year after transplantation. In conclusion urinary T cells reflect intrarenal inflammation in TCMR. T cell subsets yield high potential to monitor KT patients and detect rejection. Hereby we present a promising biomarker to non-invasively diagnose TCMR.

Keywords: transplantation, kidney, urine, T cell, biomarker, CD8+HLA-DR+, allograft acute rejection, tubular epithelial cell

INTRODUCTION

With a global prevalence of 9–15%, and rising, chronic kidney disease is a major contributor to morbidity and mortality worldwide (1, 2). Kidney transplantation is the therapy of choice in end stage kidney disease (3). However, allograft rejection (AR) leading to reduced allograft function or even graft loss remains a major challenge affecting more than 10 % of patients within the first year after transplantation (4). Established parameters like serum creatinine and proteinuria do not provide definite information about graft pathology and only increase once allograft function is already impaired (5). Transplant biopsy, the diagnostic gold standard to detect rejection, is limited by its invasive nature.

Previous studies discovered that non-invasive biomarkers hold high potential to detect rejection, adjust immunosuppression and monitor kidney transplant (KT) patients (6, 7). Various omics-based urinary biomarkers correlated with kidney inflammation and rejection (8–10). Apart from soluble factors, urine samples serve as non-invasive source for cellular components derived from the allograft. Such urinary cells hold potential as AR biomarkers since they may reflect detrimental processes in the transplant. Our group previously demonstrated that urinary cells can be used to monitor kidney damage and kidney inflammation precisely (11, 12). Other groups linked urine-derived cells to AR (13–15). More specifically, urinary HLA-DR+ cells and CD8+ T cells analyzed by flow cytometry (FC) have been suggested as promising biomarkers to detect rejection (13, 15–18). Previous trials also reported tubular epithelial cells (TEC) to represent damage in AR (19–21). Our group recently developed a biomarker combination involving urinary T cells and TEC detected by FC to identify patients with kidney transplant rejection (22).

However, many of the proposed biomarkers showed insufficient sensitivity and specificity, and were often only analyzed in small and single-centered explorative trials. Accordingly, diagnostic yield of promising biomarkers could not be proven in confirmatory trials if they had been done at all.

The current study extends previous research by (a) validating our previous findings in a multi-center setting, (b) adding an additional method (epigenetic qPCR analysis) proving the concept of urinary cells as non-invasive biomarker of rejection, (c) performing deeper phenotyping of urinary T cells and (d) describing urinary cell population trajectories during the first year after kidney transplantation to determine biomarker applicability.

Abbreviations: ABMR, antibody mediated rejection; AR, allograft rejection; AUC, area under the curve; BR, borderline rejection; BSA, bovine serum albumin; DMSO, dimethylsulfoxide; EDTA, Ethylenediaminetetraacetic acid; FC, flow cytometry; FCS, fetal calve serum; FSC, forward scatter; IU, imidazolidinyl urea; KT, kidney transplant; MOPS, 3-(N-morpholino)propanesulfonic acid; noRX, no rejection; PBE, bovine serum albumin and 2 mM Ethylenediaminetetraacetic acid; PBS, phosphate-buffered saline; ROC, receiver operating characteristic; SSC, side scatter; TCM, central memory T cell; TCMR, T cell-mediated rejection; TEC, tubular epithelial cell; TEM, effector memory T cell; TEMRA, effector memory T cell re-expressing CD45RA; THFA, tetrahydrofurfuryl alcohol; TNV, naïve T cell.

TABLE 1 | Patient characteristics.

Characteristic	Cohort 1	Cohort 2	Cohort 3
Mean age in years ± SD	55 (± 14)	51 (± 16)	54 (± 13)
Male/Female	54/36	100/41	19/17
Mean years post KT ± SD	6 (± 7)	5 (± 6)	First year follow-up
KT donor			
Living related	20	21	6
Living unrelated	13	28	5
Cadaveric	57	92	25

Demographic details of patients included in statistical analysis. Patients who failed quality control for epigenetic analysis are not show.

This unique design allowed us to comprehensively investigate urinary cells as biomarkers in KT monitoring. To find the putatively best biomarker among T cell subsets, we investigated CD4+, CD8+, effector memory, central memory, effector memory T cells re-expressing CD45RA (termed TEMRA), and HLA-DR+ T cells. Additionally, as a surrogate for intrarenal tissue damage urinary proximal and distal TEC were quantified.

METHODS

Patients

380 urine samples of KT patients were analyzed in three different cohorts. Detailed patient characteristics are shown in **Table 1**, schematic illustration of cohorts is presented in **Figure 1**.

For cohort 1, we collected 90 urine samples between 2019 and 2021 for flow cytometric analysis from patients with graft deterioration and diagnostic biopsy of the Department of Nephrology, Charité University Hospital, Berlin and from Carl Gustav Carus University Hospital, Dresden, Germany.

For cohort 2, between 2010 and 2018, 218 urine samples were collected from patients at the Department of Nephrology, Charité University Hospital, Berlin and were subject to epigenetic analysis. Among these samples, 164 were collected from patients with graft deterioration and, as control group, 54 from patients with stable graft function, defined as no fluctuation of more than +/– 0.3 mg/dl creatinine compared to the prior visit. Professional diagnoses by board certified nephropathologists from renal biopsies served to uniquely group graft deterioration into borderline rejection (BR), T cell mediated rejection (TCMR), and antibody mediated rejection (ABMR), other specific pathohistological diagnosis (other), or no rejection (noRX). Children, patients on menstruation, patients with overt causes for transplant deterioration other than rejection, such as urinary tract infections or postrenal causes of acute kidney injury, and patients with already commenced rejection therapy were excluded from the study.

For cohort 3, 72 samples from newly transplanted patients were collected as follow-up during the first year after transplantation. Differences in urinary cell trajectories during that period may prospectively identify patients developing rejection. Planned urine sample acquisitions at one, 3 and 12 months after transplantation were subject to variation in

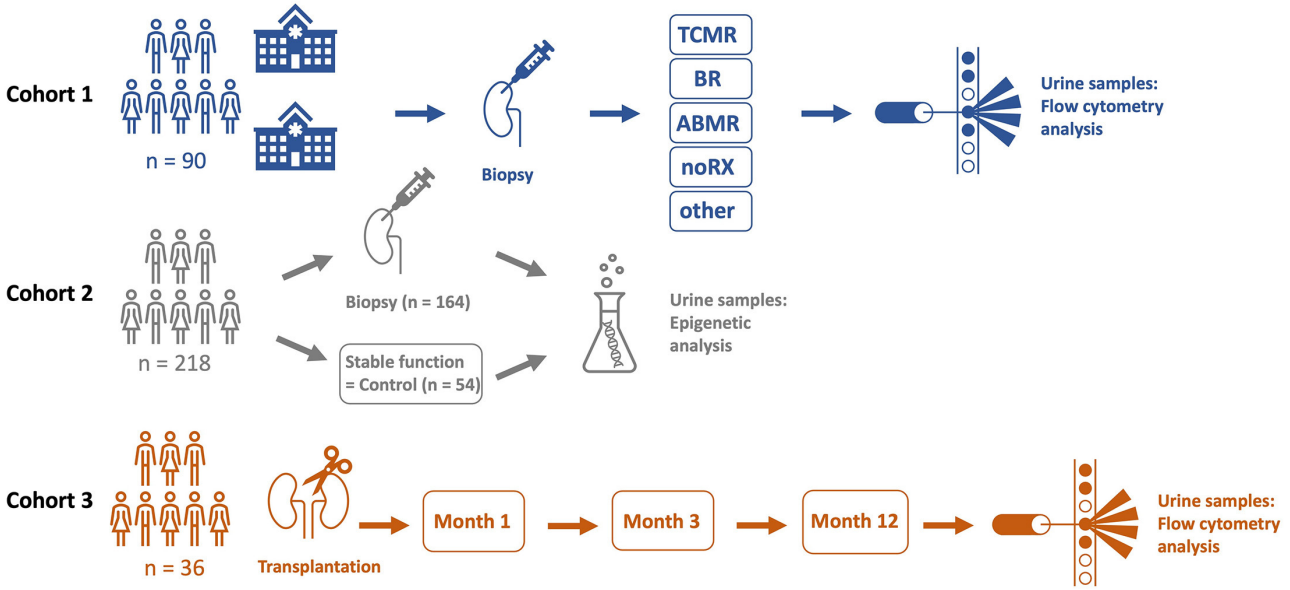


FIGURE 1 | A total of three different cohorts were analyzed in this trial. Cohort 1 included 90 kidney transplant (KT) patients from two hospitals (Charité University Hospital, Berlin, Germany and Carl Gustav Carus University Hospital, Dresden, Germany) who underwent kidney biopsy due to graft deterioration. Patients were categorized by histopathological diagnosis and urine samples were analyzed by flow cytometry. In cohort 2, urine samples of 218 KT patients were subject to epigenetic qPCR analysis. 164 patients of cohort 2 underwent kidney biopsy because of graft deterioration, 54 stable KT patients served as a control group. Cohort 3 included 36 KT patients. Urine samples were analyzed on three scheduled visits by flow cytometry in a follow-up setting during the first year after transplantation.

schedule due to the COVID-19 pandemic. Sample collection was done at the Department of Nephrology, Charité University Hospital, Berlin.

Sample Preparation

For cohort 1 and 2, we collected urine samples up to 72 h prior to transplant biopsy. Samples from prospective cohort (cohort 3) were collected on scheduled follow-up visits. We used spontaneously voided urine. We developed a urine-cup-based fixation system with imidazolidinyl urea (IU, Sigma-Aldrich) and 3-(N-morpholino)propanesulfonic acid (MOPS, Carl Roth GmbH + Co. KG) to preserve urine samples (23). Specimen were stored at 4°C for up to 7 days, centrifuged (600 g, 6 min) and frozen in 90% fetal calve serum (FCS) and 10% dimethylsulfoxide (DMSO) (cohort 1 and 3). Preparing samples for epigenetic qPCR analysis (cohort 2), urine specimen was centrifuged immediately (1,500 g, 10 min) and frozen at −80°C. All samples were stored at −80°C for a median of 3 years.

To conduct flow cytometry analysis, we defrosted samples in phosphate-buffered saline (PBS), pH 7.2 with 0.2 % bovine serum albumin (BSA) and 2 mM Ethylenediaminetetraacetic acid (EDTA) (PBE) and strained through a 30 µm cell strainer (Miltenyi Biotech). *PermWash 10X Solution* (BD) was used to permeabilize cells for intracellular staining of TEC. Fc receptors were blocked with *FcR Blocking Reagent (human)* (Miltenyi Biotech) to reduce unspecific binding and labeled for 15 min on ice with fluorochrome-conjugated monoclonal antibodies in the dark. The following antibodies were used: for T cells anti-CD3-APCeF780 (eBioscience, SK7, mo IgG1k), -CD4-PEVio770 (Miltenyi Biotec, REA623, REA) -CD8-APC (Biolegend, SK1, mo IgG1k) -CD45RO-PE (Biolegend, UCHL1, mo IgG1k2), -CD45-BUV805 (BD, 3D12, rat IgG1ak), -CCR7-BV421 (Biolegend, G043H7, mo IgG2ak), -HLA-DR-BUV395 (BD, G46-6, mo IgG2ak), -CD28-FITC (Biolegend, CD28.2, mo IgG1k) and for tubular epithelial cells anti-Cytokeratin-FITC (Miltenyi Biotec, CK3-6H5, mo IgG1k), -Vimentin-APC (Miltenyi Biotec, REA409, REA), -CD10-PeVio770 (Miltenyi Biotec, REA877, REA), -CD13-APCVio770 (Miltenyi Biotec, REA263, REA), -CD227-PE (Miltenyi Biotec, REA448, REA), -CD326-BV711 (Biolegend, 9C4, mo IgG2b). Samples were analyzed on a BD FACSymphony™ A5 Cell Analyzer. Gating strategies are depicted in **Figures 2A,B**. Acquired cell numbers were normalized to a volume of 100 mL urine. FC data was analyzed with *FlowJo 10.7* (BD Biosciences).

For epigenetic analysis, DNA from urine was obtained, processed, and analyzed using the method published by *Pradhan et al.* with some modifications (24). Workflow for epigenetic analysis of urine samples is depicted in **Figure 2D**. In short, urine sediment (∼75 µl) was lysed by adding 67 µl lysis buffer [54.25 µl ATL buffer (Qiagen), 9 µl Proteinase K (30 mg/ml, CAS 39450-01-6)], and 3.75 µl spiking plasmid essential for absolute quantification (400.000 copies/µl, Genscript) to urine sediment followed by an incubation step (56°C for 1.5 h, 900 rpm) to make genomic DNA of urinary nucleated cells accessible for bisulfite-treatment. Bisulfite-conversion was performed by adding 270

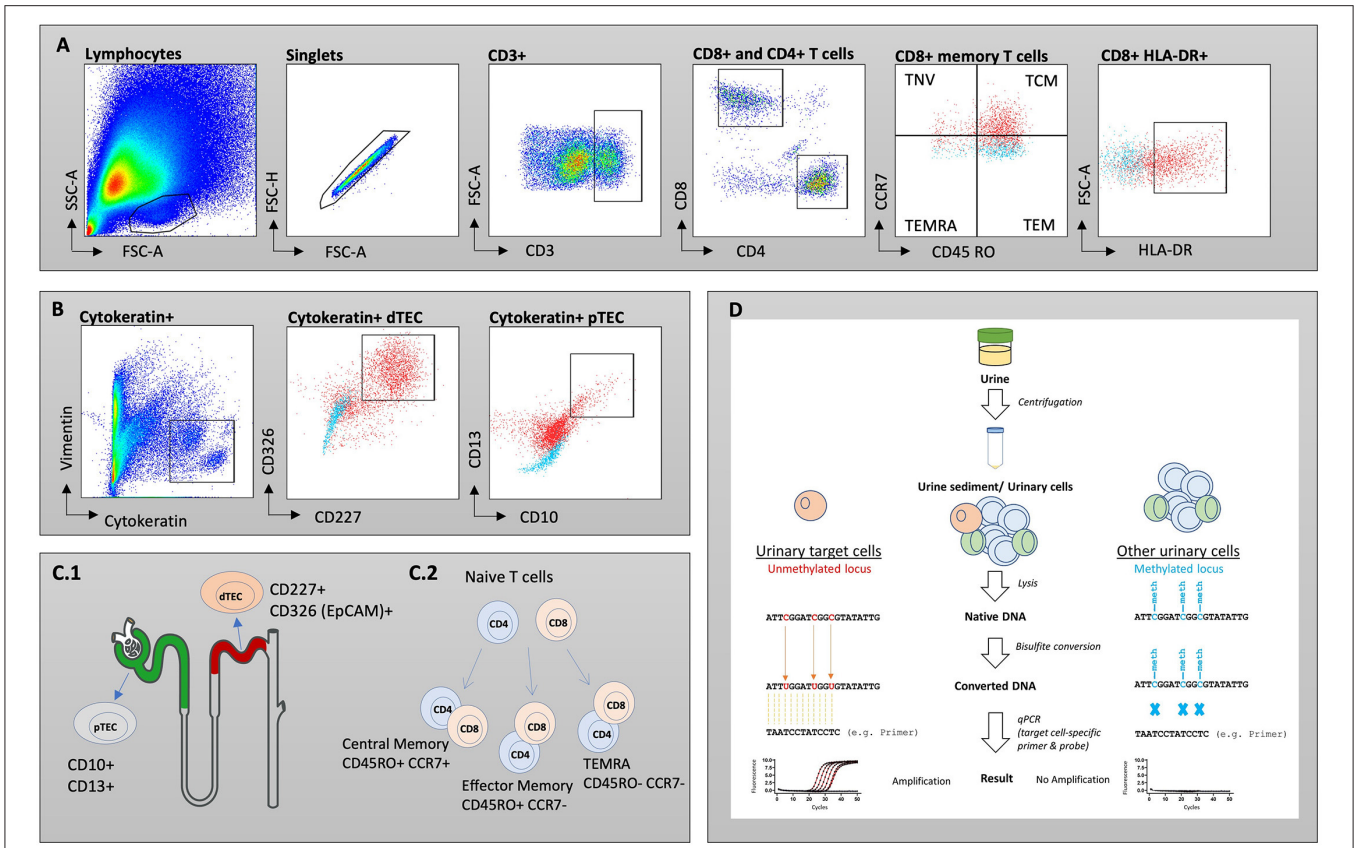


FIGURE 2 | Gating strategies for T cell subsets **(A)** and tubular epithelial cells (TEC) **(B)**. Isotype controls are displayed as blue, while full stains are represented in red. **(C.1)** Schematic overview of investigated subsets. Proximal TECs were defined CD10+ and CD13+, while distal TECs were characterized being CD227+ and CD326(EpCAM)+. **(C.2)** Maturation of naïve T cells into memory T cells. **(D)** Workflow for epigenetic analysis of urine samples. SSC, side scatter; FSC, forward scatter; TNV, naïve T cells; TEM, T effector memory cells; TCM, T central memory cells; TEMRA, T effector memory cells re-expressing CD45RA.

µl ammonium bisulfite [65–75% (w/w), CAS-No.: 10192-30-0] and 90 µL of tetrahydrofurfuryl alcohol (THFA, purity ≥ 98%, CAS No.: 97-99-4). After bead-based purification (Dynabeads My Silane Genomic DNA Kit, Invitrogen), a qPCR-based approach (demethyl-specific primers and probes) was used to determine CD3+ and CD3+CD8+ T cells and proximal TEC based on cell type-specific demethylated genomic regions. Cell type-specific epigenetic markers were identified by bisulfite-sequencing and cell counts were calculated according to Baron et al. (25) (**Supplementary Figure 1**). Oligonucleotides for bisulfite-sequencing and for demethyl-specific qPCR are listed in **Supplementary Table 1**.

Statistical Analysis

Mann-Whitney test was used to test for significantly different cell counts between groups with $p < 0.05$ being considered as significant. Friedman and Wilcoxon test were used to detect differences in the longitudinal cohort. Bonferroni correction was used to correct for multiple testing. Medians, means, Mann-Whitney, Friedman, and Wilcoxon tests, Bonferroni correction and receiver operating characteristic (ROC) curves were calculated using R version 4.1.0. (26).

RESULTS

Urinary T Cell Abundance Is Enriched in TCMR

To study populations of T cells and TEC derived from urine in patients with kidney graft deterioration, we grouped participants based on the results of their KT biopsy. In cohort 1, 17 patients were diagnosed with TCMR, 24 patients with BR, 6 patients showed ABMR, 21 patients were grouped as noRX and 22 patients presented with other specific pathologies on their biopsy results. All 90 urine samples of this cohort were analyzed by FC. Patients with inconclusive biopsy results were excluded from statistical analysis. Stack plots shown in **Figure 3A** give an overview of cell counts per population in each group. Patients with TCMR presented with the most urinary cells in total (26,061 cells/100 ml urine on average). Together with ABMR patients, they also had the highest fraction of urinary immune cells (combined CD4+ and CD8+ fraction: 40–46%, **Figure 3B**). In contrast, patients with BR, noRX or other graft pathologies presented predominantly with distal TEC (Fraction: 80–88%, **Figure 3B**). The fewest urinary cells were found in patients with noRX (2,743 cells/100 ml urine on average). Patients with TCMR presented with significantly increased urinary CD8+ T

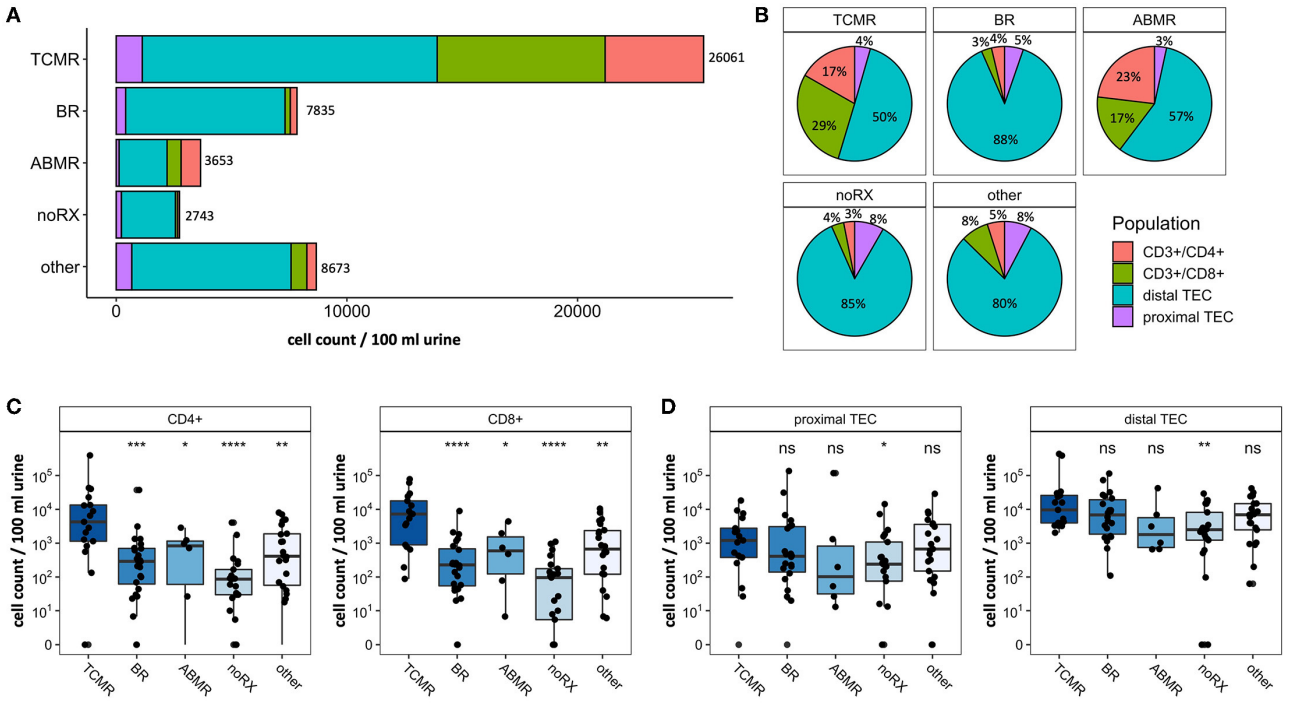


FIGURE 3 | Absolute cell counts recorded by flow cytometry in patients undergoing renal biopsy due to graft deterioration. Patients are subdivided into five groups based on histopathological results from biopsy. **(A)** Stack plot for population proportions. Each stack illustrates the mean absolute cell count per population in each group. **(B)** Pie charts representing composition of urinary cells (selected populations) per group. **(C)** CD4+ and CD8+ T cell counts per 100 ml urine shown for different biopsy groups. **(D)** Proximal and distal TEC counts per 100 ml urine shown for different biopsy groups. Significance levels indicate comparison with TCMR; ns, no significance; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. TCMR, T cell-mediated rejection; BR, Borderline rejection; ABMR, antibody-mediated rejection; noRX, no rejection; other, other pathologies; TEC, tubular epithelial cell.

cell counts per 100 ml urine compared to patients with other biopsy results (TCMR vs. BR: $p < 0.0001$; TCMR vs. ABMR: $p < 0.05$; TCMR vs. noRX: $p < 0.0001$, TCMR vs. other: $p < 0.01$). CD4+ T cells showed a likewise tendency (TCMR vs. BR: $p < 0.0001$; TCMR vs. ABMR: $p < 0.05$; TCMR vs. noRX: $p < 0.0001$, TCMR vs. other: $p < 0.001$; **Figure 3C**).

In addition to T cells, we quantified subsets of urinary TEC (**Figure 3D**). Schematic overview of analyzed TEC populations is depicted in **Figure 2C.1**. Proximal TEC, defined as Cytokeratin+, CD10+ and CD13+, did not differ significantly between patient groups. In contrast, cell counts of distal TEC (Cytokeratin+, CD227+, CD326+) were higher in patients with TCMR than in patients with noRX ($p < 0.05$). The ratio of T cells and TEC did not improve discrimination between groups.

Epigenetic Analyses Qualitatively Confirm T Cell and TEC Quantities as Determined by Flow Cytometry

For validation purposes, we assessed urinary cells by epigenetic qPCR analysis. In 218 urine samples from kidney transplant patients, we quantified T cells and TEC. The cohort consisted of 164 KT patients with graft deterioration and suspected rejection undergoing transplant biopsy and 54 KT patients with stable kidney function without biopsy as control group. Patients undergoing biopsy were grouped based on histological results. One hundred forty-one samples passed quality control for epigenetic qPCR analysis. They were included in statistical analysis and are depicted in **Figure 4**. Patients with TCMR showed significantly more CD3+ T cells and CD8+ T cells than patients with noRX or than the control group. Quantity of CD3+ or CD8+ T cells did not discriminate between patients with TCMR and patients with BR or other diagnoses. Epigenetic quantification of proximal TEC showed no difference between disease groups. Therefore, epigenetic qPCR analyses confirmed FC findings showing significantly different amounts of urinary T cells in TCMR, with however imperfect delineation from other patients.

Subsets of Urinary CD8+ T Cells Enable Improved Discrimination of TCMR

Since CD8+ T cell populations derived from urine showed significant differences in patients with TCMR and patients with other causes of graft deterioration, we further investigated their subsets and activation to optimize their potential as biomarkers to detect rejection. Subsets were quantified for naïve, TEMRA effector memory and center memory T cells. Schematic overview of T cell subsets is depicted in **Figure 2C.2**. Moreover, HLA-DR+ and CD28+ expression as activation marker was analyzed

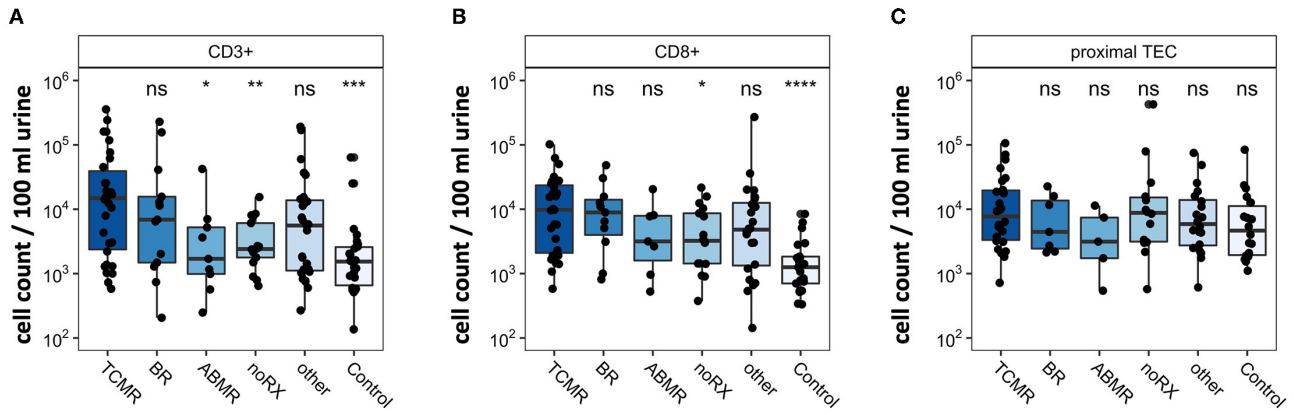


FIGURE 4 | Epigenetic quantification of cell populations in patients with renal biopsy due to graft deterioration. Patients are subdivided into five groups based on histopathological results from biopsy. The sixth group, Control, includes transplant patients with stable graft function. Counts per 100 ml urine were analyzed in **(A)** CD3+ T cells, **(B)** CD8+T cells, and **(C)** proximal TEC. Significance levels indicate comparison with TCMR; ns, no significance; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$. TCMR, T cell-mediated rejection; BR, Borderline rejection; ABMR, antibody-mediated rejection; noRX, no rejection; other, other pathologies; TEC, tubular epithelial cell.

(**Supplementary Figure 2**). Most strikingly among CD8+ T cells were CD8+HLA-DR+ and CD8+CD45RO+CCR7- (T effector memory cell, TEM) (**Figure 5A,** representative gating strategy including isotype controls: **Figures 5D,E**). Next, we assessed if our analyzed CD8+ subsets were able to distinguish patients with TCMR from all patients without TCMR and found a significant separation between these two groups (noTCMR = BR + ABMR + noRX + others; $n = 73$, TCMR vs. no TCMR: $p < 0.0001$; **Figure 5B**). To assess the diagnostic ability of CD8+HLA-DR+ and CD8+CD45RO+CCR7-, we calculated ROC curves (displayed in **Figure 5C**). The area under the curve (AUC) to diagnose TCMR using CD8+TEM cells was 0.89. CD8+HLA-DR+ T cells yielded an even better AUC value of 0.91, resulting in the most promising biomarker to distinguish patients with TCMR from all other patients. Setting a cut-off of 262.5 CD8+HLA-DR+ T cells/100 ml urine shows a sensitivity of 76.47 % and a specificity of 95.89 % to diagnose TCMR.

Urinary T Cell and TEC Abundance Remain Low Over Time in the First Year After Kidney Transplantation

The first year after kidney transplantation is characterized by a particular high risk for rejection. The intrarenal reorganizing and adaptation processes in that time period after KT may however affect the applicability of biomarkers to detect rejection. In order to assess the applicability of our biomarkers in that time period, we analyzed urine samples of 36 newly transplanted patients. Our goal was to analyze three samples per patient, obtained one, 3 and 12 months after transplantation. Due to COVID19 regulations, clinic visits were canceled or changed to telemedicine visits, resulting in 9 patients each donating only one sample, while 18 other patients only provided two samples during the first year after transplantation. Nine patients fulfilled the initially planned regime of three visits including sample collections (cell trajectories for each individual patient are depicted in **Supplementary Figure 3**). Only two biopsy proven rejections occurred, diagnosed 3 and 4 months after the last visit and urine analysis in this trial. Therefore, no meaningful comparison of urinary cell counts and rejection was possible.

All included patients showed sufficient graft function 12 months after transplantation (creatinine mean 1.77 mg/dl, range 0.9–4.05 mg/dl). **Figures 6A–D** shows the trajectory of cell counts for CD4+ T cells, CD8+ T cells, proximal TEC, and distal TEC within the first year post transplantation. T cell counts in stable KT patients were low after first month post transplantation (median CD4+: 277 cells/100 ml urine; median CD8+: 506 cells/100 ml urine) and even showed a tendency to decrease over the first year after KT. The trajectories provide insights into regular development of urinary cell counts in patients without complications (defined as biopsy proven rejection, surgical complications or transplant associated hospitalization). **Figure 6E** shows progression of urinary CD8+ HLA-DR+ T cell populations. Applying our prior calculated cut-off for diagnosing TCMR (line), median cell counts were below cut-off level already 1 month after transplantation. These results suggest that our urine FC biomarker can feasibly be used within the first year after transplantation.

DISCUSSION

In this first multicenter study on FC urine analysis in KT patients, we reveal CD8+ HLA-DR+ T cells as a potential TCMR biomarker with high precision. Urine FC findings were validated *via* epigenetic analysis and longitudinal analysis of urinary cell abundance over the first year after KT suggest that the biomarker can be applied even in this early, AR-prone phase.

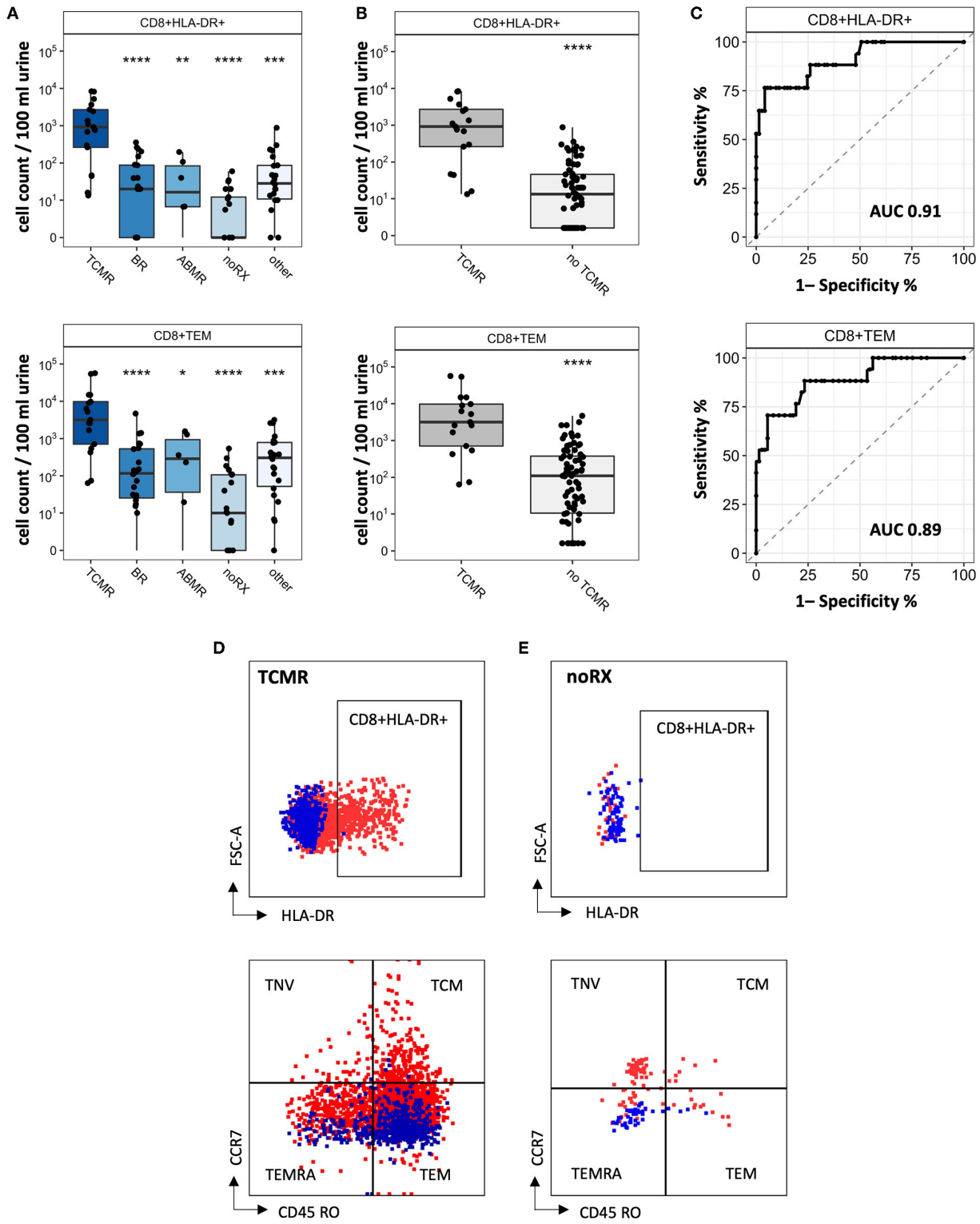


FIGURE 5 | CD8+ T cell subsets as biomarker for detection of KT rejection. **(A)** Cell counts for CD8+HLA-DR+ and CD8+TEM per biopsy group. **(B)** Cell counts from patients with TCMR compared to all other patients (= no TCMR). **(C)** ROC curves to distinguish TCMR from no TCMR. Representative FC gating for CD8+HLA-DR+ and CD8+TEM in **(D)** TCMR patients and **(E)** noRX patients. Isotype controls are displayed as blue, while full stains are represented in red. Significance levels indicate comparison with TCMR; ns, no significance; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. TCMR, T cell-mediated rejection; BR, Borderline rejection; ABMR, antibody-mediated rejection; noRX, no rejection; other, other pathologies; no TCMR, no T cell-mediated rejection; AUC, area under the curve; TNV, naïve T cells; TEM, T effector memory cells; TCM, T central memory cells; TEMRA, T effector memory cells re-expressing CD45RA.

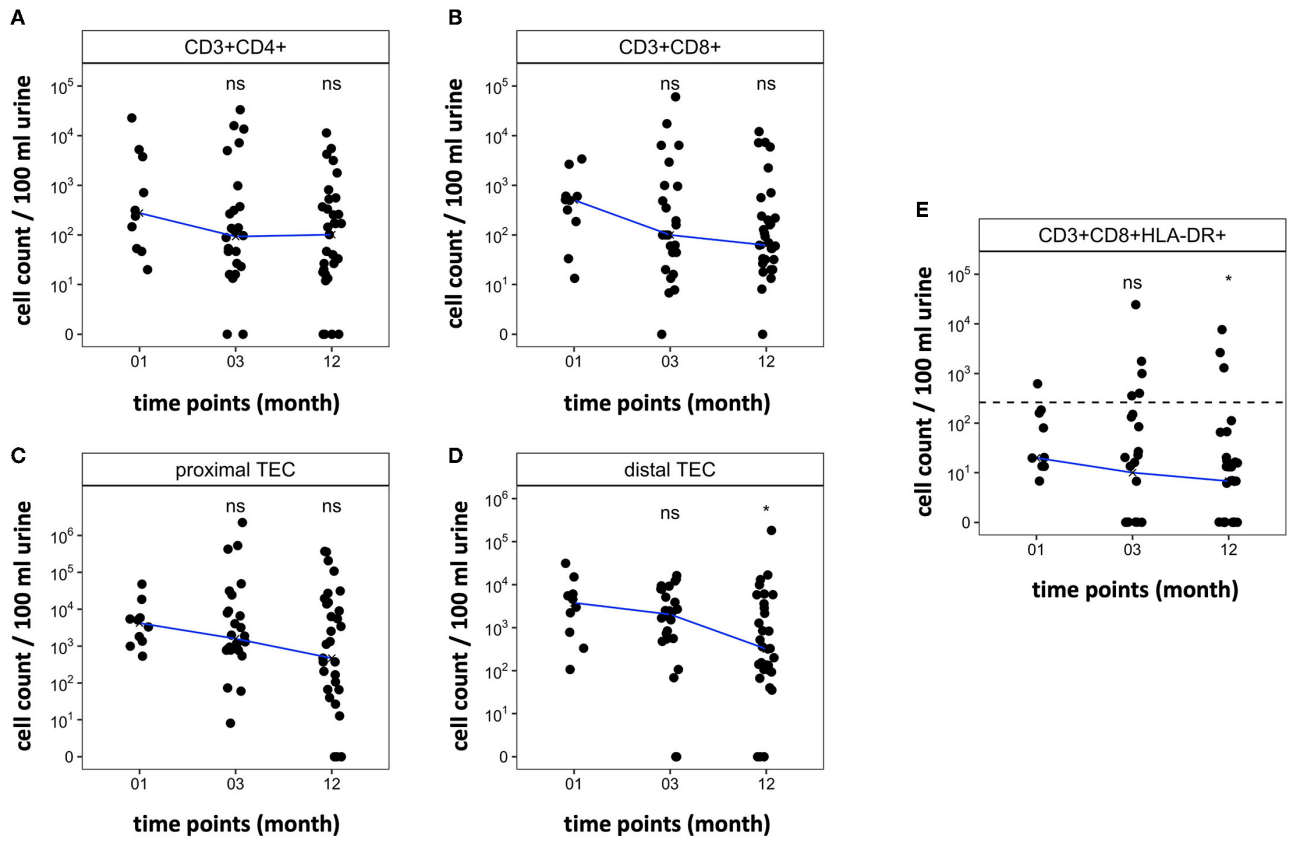


FIGURE 6 | Trajectory of cell counts in patients within the first year after kidney transplantation without rejection. Samples were collected 1, 3, and 12 months post-surgery. Median cell count per time point displayed as line. Cell abundance at different time points was compared. **(A,B)** CD4+ and CD8+ T cell counts decrease within the first year after transplantation. **(C,D)** Cell counts of TEC decrease during the first year after transplantation. **(E)** CD8+ HLA-DR+ T cell populations decrease during the first year after transplantation. Dashed line marks CD8+HLA-DR+ T cell cut-off at 262.5 CD8+HLA-DR+/100 ml urine that showed a sensitivity of 76.47% and specificity of 95.89% to diagnose TCMR in cohort 1. *$P < 0.05$, ns, no significance; TEC, tubular epithelial cell.

The Amount of Urinary T Cells Differs Significantly in Patients With and Without TCMR

Urinary T cell counts are significantly increased in TCMR. Our findings regarding CD8+ T cells distinguishing TCMR from other groups are consistent with results of other prior studies (16–18, 22). Abundance of T cells derived from urine even correlated with histopathological findings like tubulitis and interstitial inflammation. This underlines their ability to mirror graft pathology (22). In line with previous research, our findings emphasize the crucial role of CD8+ T cells in rejection. However, while the vast majority of past studies analyzed very small samples sizes, we propose our findings to be more robust due to a larger patient group with rejection and a multicenter setting.

Urinary TEC are abundant in all patient groups with graft deterioration. Contrary to our initial beliefs, we could not show differences in patients with rejection and without rejection, except for significantly more distal TEC in TCMR compared to noRX. The reason for that might be TEC reflecting unspecific kidney damage irrespective of the cause. Additionally, urinary TEC may also reflect increased turnover of the renal epithelium.

Epigenetic qPCR Analyses Qualitatively Confirmed T Cell and TEC Quantities as Determined by Flow Cytometry

As predicted and assessed by FC, we found higher T cell populations in patients with TCMR using epigenetic qPCR. These findings are in line with abundant previous research stressing T cells' potential as diagnostic tool (13, 16, 17). Epigenetic analysis has been utilized in KT biomarker development in regard to donor-derived cell-free DNA analysis before (27). However, to our knowledge it has not been adapted to analyze urinary cell populations in AR, making this the first trial to apply epigenetic qPCR analysis of urinary cells in patients with graft deterioration. The epigenetic qPCR is an established method for quantifying immune cells in blood or tissues and was used in different studies before (28, 29). Here, this method was applied in addition to FC to validate our findings with a complementary method. Epigenetic qPCR enabled us to analyze samples frozen without any additives stabilizing the cellular integrity as a prerequisite for FC. Using epigenetic qPCR we were able to confirm significantly higher median T cell counts in the TCMR group compared to noRX or Control group in an

independent cohort. Due to its methodical robustness, epigenetic qPCR could be an alternative to FC in samples stored without a dedicated protocol for flow cytometric analysis of intact cells.

Subsets of Urinary CD8+ T Cells Enable Improved Discrimination of TCMR

We found activated CD8+ TEM and CD8+ HLA-DR+ T cell subsets to separate patients with TCMR best from all other examined groups. Pathophysiologically, this makes a lot of sense, since these subsets are suspected to drive tubulitis and interstitial inflammation in AR. Our findings are also in line with previous research, describing HLA-DR positive cells in urine samples with AR (13, 15, 16). With CD8+ HLA-DR+ T cell counts as TCMR biomarker, we surpassed the diagnostic ability of our previously proposed FC TCMR biomarker (22). CD8+ HLA-DR+ cells also show a better performance than transcriptomics and sophisticated urinary protein analyses (9). We think, an implementation of specific urinary cell populations, such as CD8+ HLA-DR+ T cells, to other combined biomarker types, such as *Q Score/Qsant*, could provide powerful precision to diagnose AR (10). However, detection of patients with ABMR *via* FC remains challenging.

Long-Term Follow-Up of KT Patients Shows Low Amounts of Urinary T Cells and TEC in the First Year in Patients Without Rejection

When examining trajectories of urinary cells within the first year after transplantation, we discovered, as predicted, only moderate urinary cell counts which showed a tendency to decrease over time in patients without rejection episodes. Existing trials assessing prediction of rejection episodes by urine analysis in follow-up settings focus on gross proteinuria (30, 31) or on specific immune cell associated metabolites (32, 33). Our study therefore extends previous findings, shifting its focus on cell populations and their trajectories, which have not been described in a longitudinal setting before. Plus, our results show that cut-off levels for CD8+ HLA-DR+ T cells to diagnose rejection can be applied within the first months after transplantation.

Practical Implications

Although further studies are needed to draw definitive conclusions, results of our trial present evidence that detailed phenotyping of urinary immune cells with FC provides a promising approach to monitor KT patients and detect rejection. With CD8+ HLA-DR+ T cells revealing the best performance in diagnosing TCMR and the broad availability of FC in routine laboratories, an implementation into clinical care could be realized using existing infrastructure. As suggested by 1 year-trajectories, our biomarker could also be applied within the first year after transplantation and add value in monitoring KT patients.

Limitations

First, although we conducted a multicentric approach to assess diagnostic performance of urine FC, sample sizes are still confined and rejection incidence (fortunately) is relatively low, making a final evaluation of the diagnostic quality challenging. However, we were able to include patients from two different centers and achieve promising distinction of patients with TCMR from others using FC. Future experimental studies are needed to fully uncover the diagnostic ability of T cell subsets. Second, predictive utility of our non-invasive biomarker candidates remains inconclusive due to low rejection prevalence within the first year in our cohort. Nevertheless, we were able to describe cell population trajectories and share insights into processes within the first year after transplantation. We propose a multicentric longitudinal prospective trial including KT patients to analyze urine samples by FC at regular clinic visits for a longer time span. Lastly, urine FC comes along with certain challenges, such as autofluorescence and issues in investigating rare cell subsets. Therefore, an even deeper phenotyping of immune cells with FC seems effortful. To gain deeper insights, other methods such as mass cytometry or single cell sequencing could provide a solution. More studies are needed to achieve a more fine-grained understanding of "urine prints" among KT patients with graft deterioration. These disease-specific cell patterns might mirror intrarenal pathologies and provide innovative diagnostic tools.

CONCLUSION

The current study is a unique investigation phenotyping urinary immune cells by FC as a biomarker to detect KT rejection. We extend previous research by examining urinary cell populations in a multicenter setting and by validating findings conducting epigenetic qPCR analysis. Moreover, this trial includes a longitudinal design to determine biomarker applicability during the most prone timespan for rejection—the first year after transplantation. Our data shows that urinary CD8+ HLA-DR+ T cell have the highest potential to diagnose TCMR, with a cut-off that can be implemented during the first year after transplantation. This study lays the foundation and might catalyze future research exploring urinary immune cell signatures to non-invasively diagnose rejection and monitor KT patients.

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 Charité EA1/284/19. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EG and NG conducted flow cytometry experiments and created the manuscript. BS and SO established protocols for epigenetic

analysis of urinary cells. BS conducted all epigenetic analyses. CS, PE, and JK established the staining protocol and reviewed the article. DM, LW, PF, and LP provided material and expertise in method development and reviewed the manuscript. MD and KB significantly supported patient recruitment and trial management. MM collected, stored, and processed all samples for epigenetic analysis. PE and AP conceptualized and designed this trial. PE provided intellectual content of critical importance to the work described and gave final approval of the version to be published. All authors supported manuscript writing and gave final approval of the manuscript.

FUNDING

This work was supported by grants from the Berlin Institute of Health (BIH) and SPARK Berlin. JK was supported by a research scholarship of the Deutsche Gesellschaft für Nephrologie (DGfN). NG was participant in the BIH Charité Junior Clinician Scientist Program funded by the Charité–Universitätsmedizin Berlin and the Berlin Institute of Health at Charité (BIH).

ACKNOWLEDGMENTS

We thank the Flow Cytometry and Cell Sorting Facility and lab managers at DRFZ for technical support and helpful insights. We thank all patients for participating.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.928516/full#supplementary-material

REFERENCES

1. Lv JC, Zhang LX. Prevalence and disease burden of chronic kidney disease. *Adv Exp Med Biol.* (2019) 1165:3–15. doi: 10.1007/978-981-13-8871-2_1
2. Thurlow JS, Joshi M, Yan G, Norris KC, Agodoa LY, Yuan CM, et al. Global epidemiology of end-stage kidney disease and disparities in kidney replacement therapy. *Am J Nephrol.* (2021) 52:98–107. doi: 10.1159/000514550
3. Tonelli M, Wiebe N, Knoll G, Bello A, Browne S, Jadhav D, et al. Systematic review: kidney transplantation compared with dialysis in clinically relevant outcomes. *Am J Transplant.* (2011) 11:2093–109. doi: 10.1111/j.1600-6143.2011.03686.x
4. Hart A, Smith JM, Skeans MA, Gustafson SK, Wilk AR, Castro S, et al. OPTN/SRTR 2018 annual data report: kidney. *Am J Transplant.* (2020) 20:20–130. doi: 10.1111/ajt.15672
5. COPE. *Official Journal of the international Society of Nephrology KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease.* Available online at: www.publicationethics.org (accessed April 23, 2022).
6. Eikmans M, Gielis EM, Ledeganck KJ, Yang J, Abramowicz D, Claas FFJ. Non-invasive biomarkers of acute rejection in kidney transplantation: novel targets and strategies. *Front Med.* (2019) 5:358. doi: 10.3389/fmed.2018.00358
7. Van Loon E, Naesens M. Blood transcriptomics as non-invasive marker for kidney transplant rejection. *Nephrol Ther.* (2021) 17S:S78–82. doi: 10.1016/j.nephro.2020.02.012
8. Guzzi F, Cirillo L, Buti E, Becherucci F, Errichiello C, Roperto RM, et al. Urinary biomarkers for diagnosis and prediction of acute kidney allograft rejection: a systematic review. *Int J Mol Sci.* (2020) 21:1–21. doi: 10.3390/ijms21186889
9. Jin PH, Sarwal RD, Sarwal MM. Urinary biomarkers for kidney allograft injury. *Transplantation.* (2022) 106:1330–8. doi: 10.1097/TP.0000000000004017
10. Yang JYC, Sarwal RD, Sigdel TK, Damm I, Rosenbaum B, Liberto JM, et al. A urine score for non-invasive accurate diagnosis and prediction of kidney transplant rejection. *Sci Transl Med.* (2020) 12:eaba2501. doi: 10.1126/scitranslmed.aba2501
11. Enghard P, Rieder C, Kopetschke K, Klocke JR, Undeutsch R, Biesen R, et al. Urinary CD4 T cells identify SLE patients with proliferative lupus nephritis and can be used to monitor treatment response. *Ann Rheum Dis.* (2014) 73:277–83. doi: 10.1136/annrheumdis-2012-202784
12. Bertolo M, Baumgart S, Durek P, Peddinghaus A, Mei H, Rose T, et al. Deep phenotyping of urinary leukocytes by mass cytometry reveals a leukocyte signature for early and non-invasive prediction of response to treatment in active lupus nephritis. *Front Immunol.* (2020) 11:256. doi: 10.3389/fimmu.2020.00256
13. Roberti I, Panico M, Reisman L. Urine flow cytometry as a tool to differentiate acute allograft rejection from other causes of acute renal graft dysfunction. *Transplantation.* (1997) 64:731–4. doi: 10.1097/00007890-199709150-00012
14. Yu D-S, Sun G-H, Lee S-S, Wu C-J, Ma C-P, Chang S-Y. Flow-Cytometric measurement of cellular changes in urine: a simple and rapid method for perioperatively monitoring patients after kidney transplantation. *Urol Int.* (1999) 62:143–6. doi: 10.1159/000030378
15. Roberti I, Reisman L. Serial evaluation of cell surface markers for immune activation after acute renal allograft rejection by urine flow cytometry. *Transplantation.* (2001) 71:1317–20. doi: 10.1097/00007890-200105150-00024
16. Nanni-Costa A, Iannelli S, Vangelista A, Buscaroli A, Liviano G, Raimondi C, et al. Flow cytometry evaluation of urinary sediment in renal transplantation. *Transpl Int.* (1992) 5:S8–12. doi: 10.1111/tri.1992.5.s1.8
17. Galante NZ, Câmara NOS, Kallas EG, Salomão R, Pacheco-Silva A, Medina-Pestana JO. Noninvasive immune monitoring assessed by flow cytometry and real time RT-PCR in urine of renal transplantation recipients. *Transpl Immunol.* (2006) 16:73–80. doi: 10.1016/j.trim.2006.03.014
18. van Doesum WB, Abdulahad WH, van Dijk MCRF, Dolff S, van Son WJ, Stegeman CA, et al. Characterization of urinary CD4+ and CD8+ T cells in kidney transplantation patients with polyomavirus BK infection and allograft rejection. *Transpl Infect Dis.* (2014) 16:733–43. doi: 10.1111/tid.12273
19. Nguan CYC, Du C. Renal tubular epithelial cells as immunoregulatory cells in renal allograft rejection. *Transplant Rev.* (2009) 23:129–38. doi: 10.1016/j.trre.2009.02.003
20. Ting YT, Coates PT, Walker RJ, McLellan AD. Urinary tubular biomarkers as potential early predictors of renal allograft rejection. *Nephrology.* (2012) 17:11–6. doi: 10.1111/j.1440-1797.2011.01536.x
21. Havasi A, Dong Z. Autophagy and tubular cell death in the kidney. *Semin Nephrol.* (2016) 36:174. doi: 10.1016/j.semnephrol.2016.03.005
22. Goerlich N, Brand HA, Langhans V, Tesch S, Schachtner T, Koch B, et al. Kidney transplant monitoring by urinary flow cytometry: biomarker combination of T cells, renal tubular epithelial cells, and podocalyxin-positive cells detects rejection. *Sci Rep.* (2020) 10:796. doi: 10.1038/s41598-020-57524-7
23. Freund P, Skopnik CM, Metzke D, Goerlich N, Klocke J, Grothgar E, et al. Addition of formaldehyde releaser imidazolidinyl urea and MOPS buffer to urine samples enables delayed processing for flow cytometric analysis of urinary cells. *medRxiv [Preprint].* (2022). doi: 10.1101/2022.04.07.22273579
24. Pradhan SK, Guzman J, Dargitz C, Switalski S, Landon M, Olek S, et al. Determination of immune cell identity and purity using epigenetic-based quantitative PCR. *J Vis Exp.* (2020) 2020:e60465. doi: 10.3791/60465
25. Baron U, Werner J, Schildknecht K, Schulze JJ, Mulu A, Liebert UG, et al. Epigenetic immune cell counting in human

blood samples for immunodiagnostics. *Sci Transl Med.* (2018) 10:eaan3508. doi: 10.1126/scitranslmed.aan3508
26. R Core Team. *R: The R Project for Statistical Computing*. Available online at: https://www.r-project.org/ (accessed March 9, 2022).
27. Paul RS, Almokayad I, Collins A, Raj D, Jagadeesan M. Donor-derived cell-free DNA: advancing a novel assay to new heights in renal transplantation. *Transplant Direct.* (2021) 7:3–438. doi: 10.1097/TXD.0000000000001098
28. Burska AN, Thu A, Parmar R, Bzoma I, Samans B, Raschke E, et al. Quantifying circulating Th17 cells by qPCR: potential as diagnostic biomarker for rheumatoid arthritis. *Rheumatology.* (2019) 58:2015–24. doi: 10.1093/rheumatology/kez162
29. Le Cornet C, Schildknecht K, Chornet AR, Fortner RT, Maldonado SG, Katzke VA, et al. Circulating immune cell composition and cancer risk: a prospective study using epigenetic cell count measures. *Cancer Res.* (2020) 80:1885–92. doi: 10.1158/0008-5472.CAN-19-3178
30. Belmar Vega L, Rodrigo Calabia E, Gómez Román JJ, Ruiz San Millán JC, Martín Penagos L, Arias Rodríguez M. Relationship between albuminuria during the first year and antibody-mediated rejection in protocol biopsies in kidney transplant recipients. *Transplant Proc.* (2016) 48:2950–2. doi: 10.1016/j.transproceed.2016.09.012
31. Oblak M, Mlinšek G, Kojc N, Frelih M, Buturović-Ponikvar J, Arnol M. Spot urine protein excretion in the first year following kidney transplantation associates with allograft rejection phenotype at 1-year surveillance biopsies: an observational national-cohort study. *Front Med.* (2021) 8:781195. doi: 10.3389/fmed.2021.781195
32. Hricik DE, Nickerson P, Formica RN, Poggio ED, Rush D, Newell KA, et al. Multicenter validation of urinary CXCL9 as a risk-stratifying biomarker for kidney transplant injury. *Am J Transplant.* (2013) 13:2634. doi: 10.1111/ajt.12426
33. Hricik DE, Augustine J, Nickerson P, Formica RN, Poggio ED, Rush D, et al. Interferon gamma ELISPOT testing as a risk-stratifying biomarker for kidney transplant injury: results from the CTOT-01 multicenter study. *Am J Transplant.* (2015) 15:3166. doi: 10.1111/ajt.13401

Conflict of Interest: BS and SO are employed by Precision for Medicine GmbH.

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

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

BRIEF RESEARCH REPORT
published: 22 July 2022
doi: 10.3389/fmed.2022.936126

Three-Month Follow-Up of Heterologous vs. Homologous Third SARS-CoV-2 Vaccination in Kidney Transplant Recipients: Secondary Analysis of a Randomized Controlled Trial

Andreas Heinzel[1†], Eva Schrezenmeier[2†], Florina Regele[1], Karin Hu[1], Lukas Raab[1], Michael Eder[1], Christof Aigner[1], Rhea Jabbour[1], Constantin Aschauer[1], Ana-Luisa Stefanski[3], Thomas Dörner[3], Klemens Budde[2], Roman Reindl-Schwaighofer[1] and Rainer Oberbauer[1]*

[1] Division of Nephrology and Dialysis, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria, [2] Department of Nephrology and Medical Intensive Care, Charité-Universitätsmedizin Berlin, Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany, [3] Department of Rheumatology and Clinical Immunology, Charité-Universitätsmedizin Berlin, Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany

Response to SARS-CoV-2-vaccines in kidney-transplant recipients (KTR) is severely reduced. Heterologous3rd vaccination combining mRNA and vector vaccines did not increase seroconversion at 4 weeks after vaccination, but evolution of antibody levels beyond the first month remains unknown. We have recently completed a randomized-controlled trial on heterologous (Ad26COVS1) vs. homologous (BNT162b2 or mRNA-1273) 3rd vaccination in 201 KTR not developing SARS-CoV-2-spike-protein antibodies following two doses of mRNA vaccine (EurdraCT: 2021-002927-39). Here, we report seroconversion at the second follow-up at 3 months after the 3rd vaccination (prespecified secondary endpoint). In addition, higher cut-off levels associated with neutralizing capacity and protective immunity were applied (i.e., > 15, > 100, > 141, and > 264 BAU/ml). A total of 169 patients were available for the 3-month follow-up. Overall, seroconversion at 3 months was similar between both groups (45 vs. 50% for mRNA and the vector group, respectively; $p = 0.539$). However, when applying higher cut-off levels, a significantly larger number of individuals in the vector group reached antibody levels > 141 and > 264 BAU/ml at the 3-month follow-up (141 BAU/ml: 4 vs. 15%, $p = 0.009$ and 264 BAU/ml: 1 vs. 10%, $p = 0.018$ for mRNA vs. the vector vaccine group, respectively). In line, antibody levels in seroconverted patients further increased from month 1 to month 3 in the vector group while remaining unchanged in the mRNA group (median increase: mRNA = 1.35 U/ml and vector = 27.6 U/ml, $p = 0.004$). Despite a similar overall seroconversion rate at 3 months following 3rd vaccination in KTR, a heterologous 3rd booster vaccination with Ad26COVS1 resulted in significantly higher antibody levels in responders.

Keywords: COVID-19, kidney transplantation, COVID-19 vaccination, heterologous vaccination, third vaccination

OPEN ACCESS

Edited by:
Ondrej Viklicky, Institute for Clinical and Experimental Medicine (IKEM), Czechia

Reviewed by:
Ivan Zahradka, Institute for Clinical and Experimental Medicine (IKEM), Czechia
Sira Nanthapisal, Thammasat University, Thailand
Sascha Kahlfuss, Universitätsklinikum Magdeburg, Germany

***Correspondence:**
Roman Reindl-Schwaighofer roman.reindl-schwaighofer@meduniwien.ac.at

†These authors share first authorship

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *04 May 2022*
Accepted: *13 June 2022*
Published: *22 July 2022*

Citation:
Heinzel A, Schrezenmeier E, Regele F, Hu K, Raab L, Eder M, Aigner C, Jabbour R, Aschauer C, Stefanski A-L, Dörner T, Budde K, Reindl-Schwaighofer R and Oberbauer R (2022) Three-Month Follow-Up of Heterologous vs. Homologous Third SARS-CoV-2 Vaccination in Kidney Transplant Recipients: Secondary Analysis of a Randomized Controlled Trial. Front. Med. 9:936126. doi: 10.3389/fmed.2022.936126

INTRODUCTION

Vaccine response in kidney transplant recipients (KTR) is severely reduced due to the mandatory immunosuppressive medication following transplantation. Subsequently, a significant number of KTR remains at risk for SARS-CoV-2 infection despite vaccination (1, 2). Strategies to improve vaccine response in this high-risk population for severe COVID-19 are urgently needed.

We have recently conducted a randomized, single-blinded, controlled trial in 201 patients, comparing a homologous vs. heterologous vaccination strategy in KTR who did not develop SARS-CoV-2 spike protein-specific antibodies after two doses of an mRNA vaccine: Overall, 39% of patients developed antibodies at 4 weeks after the 3rd dose, with no statistically significant difference between an additional dose of the same mRNA vaccine as used for the initial prime/boost vaccination (BNT162b2 or mRNA-1273, a 35% response rate) or a vector vaccine (Ad26COVS1, a 42% response rate) (3).

Other recent reports, however, have suggested a more pronounced induction of both, a SARS-CoV-2-specific CD4 T-cell response and antibodies, following heterologous vaccination that includes a vector-based vaccine in transplant recipients (4). In line, heterologous 3rd vaccination also increased overall T-cell response in patients treated with B-cell-depleting therapy (5).

Most analyses to date were limited to observation within the first 4 weeks after 3rd vaccination. Another recent observational study from France has reported changes in antibody levels in KTR from 1 month to 3 months after a 3rd mRNA vaccine, showing a significant reduction in antibody levels (6). However, data on trajectories of antibody levels beyond the first month following heterologous vaccination remain unknown. In the current analysis of our randomized controlled trial (RCT), including follow-up data on antibody levels until month 3, we aimed to assess changes in antibody over time (month 1 to month 3) following homologous vs. heterologous 3rdvaccination. We hypothesized that a heterologous 3rd vaccination using a vector vaccine would result in higher antibody levels at 3 months after vaccination compared to an additional homologous booster dose.

METHODS

Study Cohort and Trial Design

Study participants were followed up for antibody assessment at the outpatient's transplant clinic of the Medical University of Vienna for a second follow-up (FU) between 60 and 120 days after the 3rd vaccine dose (*3-month FU*, a pre-specified secondary endpoint). Details of randomization and treatment have been reported before (3). In short, 201 patients without detectable SARS-CoV-2 specific antibodies following two doses of a mRNA vaccine were randomized to a 3rd dose of the same mRNA vaccine (the mRNA group) or a dose of the vector vaccine Ad26COVS1. Clinical endpoints (death, COVID-19) were recorded for all study participants throughout the observation period until 31st of December 2021. The patients receiving a fourth vaccine dose or contracting COVID-19 before completion of the 3-month FU visit were excluded from analysis of vaccine efficacy.

Assessment of the Humoral Response

Antibody response was evaluated using the Roche Elecsys anti–SARS-CoV-2 S enzyme immunoassay (Roche, Switzerland), detecting antibodies against the receptor-binding domain of the SARS-CoV-2 spike protein (the cutoff at 0.8 U/ml according to the manufacturer's instructions). As additional endpoints, we applied higher cut-off levels that were also reported as secondary endpoints at the 1-month FU and that are associated with neutralizing capacity as well as reduced risk for COVID-19 infection: > 100 U/ml (7), > 141 BAU/ml (8), and > 264 BAU/ml (9). BAU/ml were converted to U/ml based on the conversion formula: U/ml = 0.972*BAU/ml.

Assessment of T-Cell Response

Besides the humoral response, we further analyzed SARS-CoV-2-specific CD4 and CD8 T-cell responses among humoral top responders at 4 weeks in both groups ($n = 18$ per group). The T-cell stimulation flow cytometric (FC) assessment of SARS-CoV-2-specific T-cells has been described before (10, 11). In brief, peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Paque density gradient centrifugation and cryopreserved until further analysis. For the identification of SARS-CoV-2-specific T-cells, 3–5 x 10^6 PBMCs were incubated for 18 h with overlapping 15-mer peptides, covering the complete SARS-CoV-2 spike protein wild-type variant (1 ug/ml per peptide; JPT, Germany) and subsequently subjected to FC analysis. SARS-CoV-2-specific CD4 T-cells were identified based on CD154 and CD137 co-expression, whereas co-expression of CD137 and IFN-γ was used for CD8 T-cells. The gating strategy is exemplified in **Supplementary Figure S1**. The patients were considered having SARS-CoV-2-specific T-cells when the number of identified cells in the stimulated sample exceeded the number of such cells in the unstimulated sample by at least 2-fold. To account for patient-specific background activation, frequencies of activated cells detected in control samples were subtracted from the stimulated samples prior to any subsequent analysis of fractions of SARS-CoV-2-specific T-cells.

Statistical Analysis

Patient demographics for continuous variables were reported as the median and interquartile range, except for patient age, which was reported as mean and standard deviation. Categorical variables were described by frequency and percentage. Differences between treatment groups for continuous and categorical variables were assessed by the Wilcoxon rank sum test and the Fisher's exact test, respectively. Occurrence of COVID-19 infections was visualized using a Kaplan–Meier graph. Wilcoxon rank sum tests were used for all comparison of absolute antibody concentrations as well as antibody level differences from 1-month to 3-month FU and detectable fractions of SARS-CoV-2-specific T-cells between groups. The number of seroconverted patients, number of patients with SARS-CoV-2-specific T-cells, and the number of patients exceeding defined antibody level cutoffs between groups were evaluated by means of the Fisher's exact test.

RESULTS

Study Population

From the initially enrolled $n = 201$ patients, blood samples from 169 patients were available for the 3-month FU analysis of vaccine efficacy: 85 and 84 patients in the mRNA and vector groups, respectively (CONSORT Flow Chart is provided in **Figure 1**). Patient characteristics are provided in **Table 1**. There was no statistically significant difference between the mRNA and vector vaccine groups. Overall, eight deaths and seven SARS-CoV-2 infections occurred in the study population within the observation period (death: four vs. four; COVID-19: three vs. four for mRNA vs. vector vaccine groups, respectively; **Figure 2**). All COVID-19 cases occurred in vaccine no-/low-responders (six individuals without antibody response and one individual < 15 U/ml); three patients had severe COVID-19, requiring ICU treatment (two patients in the vector group died as well as one patient from the mRNA group, who was on extra-corporal membrane oxygenation).

Humoral Immune Response

The overall response rate to the 3rd vaccine dose at the 3-month FU was 47%, with no statistically significant difference in seroconversion between the mRNA and vector vaccine groups

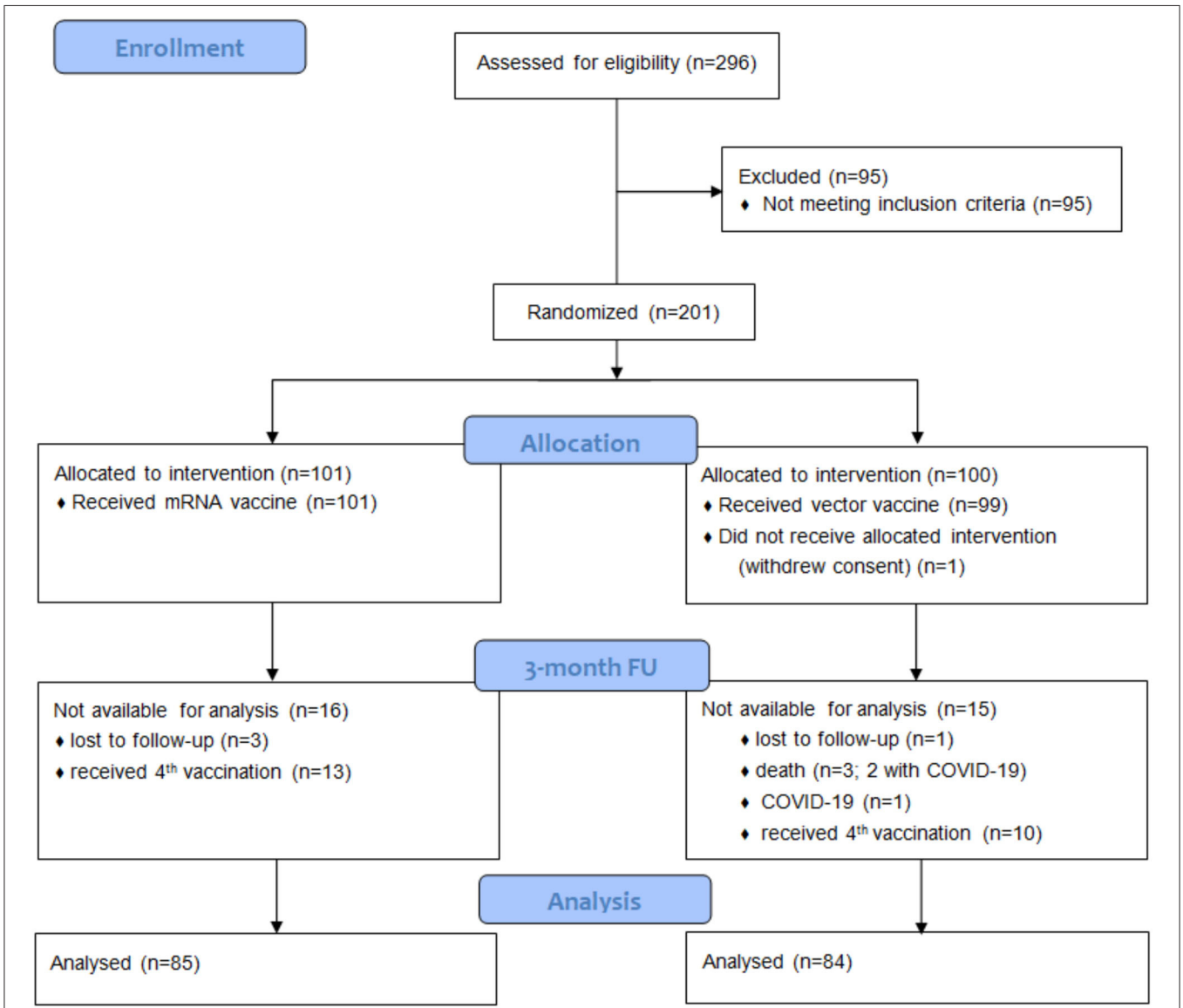


FIGURE 1 | CONSORT flow chart for the 3-month follow-up. Blood samples for evaluation of vaccine efficacy at the 3-month FU were available for 169 of the initially enrolled 201 patients: One patient had withdrawn consent before vaccination, and 23 patients were excluded after they had received a 4th vaccine dose before completing the 3-month FU visit; one patient died following myocardial infarction, two patients died due to COVID-19, one patient had mild COVID-19, and four patients had no blood draw within the observation period.

TABLE 1 | Demographics of the study population.

Variable	mRNA	Vector
N	85	84
Mean (SD) age, y	61 (13)	61 (12)
Sex		
Female	37 (44)	34 (40)
Male	48 (56)	50 (60)
Time since KTX, y	4.8 [2.4–8.6]	4.9 [1.6–7.4]
No. of KTX		
1	64 (75)	66 (79)
2	15 (18)	13 (15)
3	4 (5)	4 (5)
4	2 (2)	0 (0)
5	0 (0)	1 (1)
Donor type (living)	14 (16)	18 (21)
Initial vaccinations (mRNA-1273)	27 (32)	27 (32)
Maintanance immunosuppression		
Belatacept, MMF, steroids	6 (7)	6 (7)
Belatacept, azathioprine, steroids	0 (0)	1 (1)
Cyclosporin A, MMF, steroids	1 (1)	4 (5)
Cyclosporin A, MMF	3 (4)	1 (1)
Cyclosporin A, azathioprine, steroids	1 (1)	0 (0)
MMF, steroids	1 (1)	1 (1)
Tracolimus, MMF, steroids	66 (78)	62 (74)
Tracolimus, MMF	1 (1)	3 (4)
Tracrolimus, azathioprine, steroids	4 (5)	3 (4)
Tracrolimus, steroids	2 (2)	2 (2)
Tracrolimus, leflunomide, steroids	0 (0)	1 (1)
ATG in past year	1 (1)	2 (2)
Nontriple immunosuppression	7 (8)	7 (8)
Time between second and third vaccination, d	78 [55–87]	80.5 [57–90.25]
Time between third vaccination and one-month follow-up visit, d	31 [28–32]	30 [28–33]
Time between third vaccination and three-month follow-up visit, d	81 [74–88]	76 [69–89]

[mRNA: 45% and vector: 50% OR = 1.24, 95% CI = (0.65, 2.37), $p = 0.539$]. Absolute antibody titers between the two groups were also not significantly different (median mRNA: 0.2 U/ml and vector: 0.81 U/ml, $p = 0.104$). However, when examining higher antibody cut-off levels that were also included in our primary analysis at the 1-month FU, we observed that a significantly higher number of patients in the vector group reached antibody levels above 141 and 264 BAU/ml [141 BAU/ml: 4 vs. 15% OR = 4.96, 95% CI = (1.29, 28.21), $p = 0.009$ and 264 BAU/ml: 1 vs. 10% OR = 8.75, 95% CI = (1.13, 396.17), $p = 0.018$, for mRNA vs. vector vaccine groups, respectively, **Table 2**]. In contrast, no difference between the groups was observed for any of the antibody level cut-offs at the 1-month FU (**Table 2**).

Change in Serostatus Between Month 1 vs. Month 3

In both groups, a comparable number of patients who had not seroconverted at the one-month FU became seropositive in the subsequent months [8 and 8% OR = 1.01, 95% CI = (0.29, 3.56), $p = 1$ for mRNA and vector, respectively]. With the exception of a single patient in the vector group, all the patients who showed seroconversion at the 1-month FU had antibody levels above the 0.8 U/ml cutoff at the 3-month FU. **Figure 3A** visualizes changes in serostatus, including increase above 141 BAU/ml as surrogate for protective immunity.

Evolution of Antibody Levels Beyond the 1st Month

Of particular note, evolution of antibody levels in patients with seroconversion at the 1-month FU differed significantly between the two groups. Antibody levels in the vector group further increased after the 1-month FU while remaining approximately unchanged in the mRNA group (median of differences mRNA: 1.35 U/ml and vector: 27.6 U/ml, $p = 0.004$, **Figure 3B**). Consequently, absolute antibody levels were significantly different between the two treatment groups at the 3-month FU (median mRNA: 25.8 U/ml and vector: 77.7 U/ml, $p = 0.038$), even though they were not significantly different at the 1-month FU (mRNA: 19.7 U/ml and vector: 22.1 U/ml, $p = 0.753$).

T-Cell Response

We also analyzed the T-cell response at the 1 month_FU in 18 patients among the top responders to the 3rd vaccine from both groups to see if the subsequent increase in antibody levels in the vector group was preceded by a higher SARS-CoV-2-specific T-cell response. After the 3rd vaccination, 83 and 36% of the patients had SARS-CoV-2-specific CD4 and CD8 cells, respectively. The number of patients with SARS-CoV-2-specific CD4 and CD8 T-cells was comparable between the treatment groups [CD4 mRNA: 89% and vector: 78% OR = 0.45, 95% CI = (0.04, 3.68), $p = 0.658$; CD8 mRNA: 33% and vector: 39% OR = 1.26, 95% CI = (0.27–6.19), $p = 1$, **Figure 3C**]. In the patients with SARS-CoV-2-specific CD4 and CD8 T-cells, a median of 0.033 and 0.003% overall CD4 and CD8 cells was SARS-CoV-2-specific. Interestingly, these numbers were also comparable between the two treatment groups (CD4 mRNA: 0.038% and vector: 0.024% $p = 0.547$; CD8 mRNA: 0.006% and vector: 0.003% $p = 0.295$, **Figure 3D**).

DISCUSSION

In this 3-month FU analysis of our RCT on homologous vs. heterologous 3rd vaccination in KTR, we observed an increase in antibody levels from month 1 to month 3 in individuals receiving a heterologous 3rd vaccination dose, with the vector vaccine Ad26COVS1. In contrast, antibody levels in individuals receiving a homologous 3rd vaccination with an additional dose of mRNA remained unchanged from the 1-month FU to the 3-month FU, resulting in overall lower antibody levels in the homologous vaccination group. Consequently,

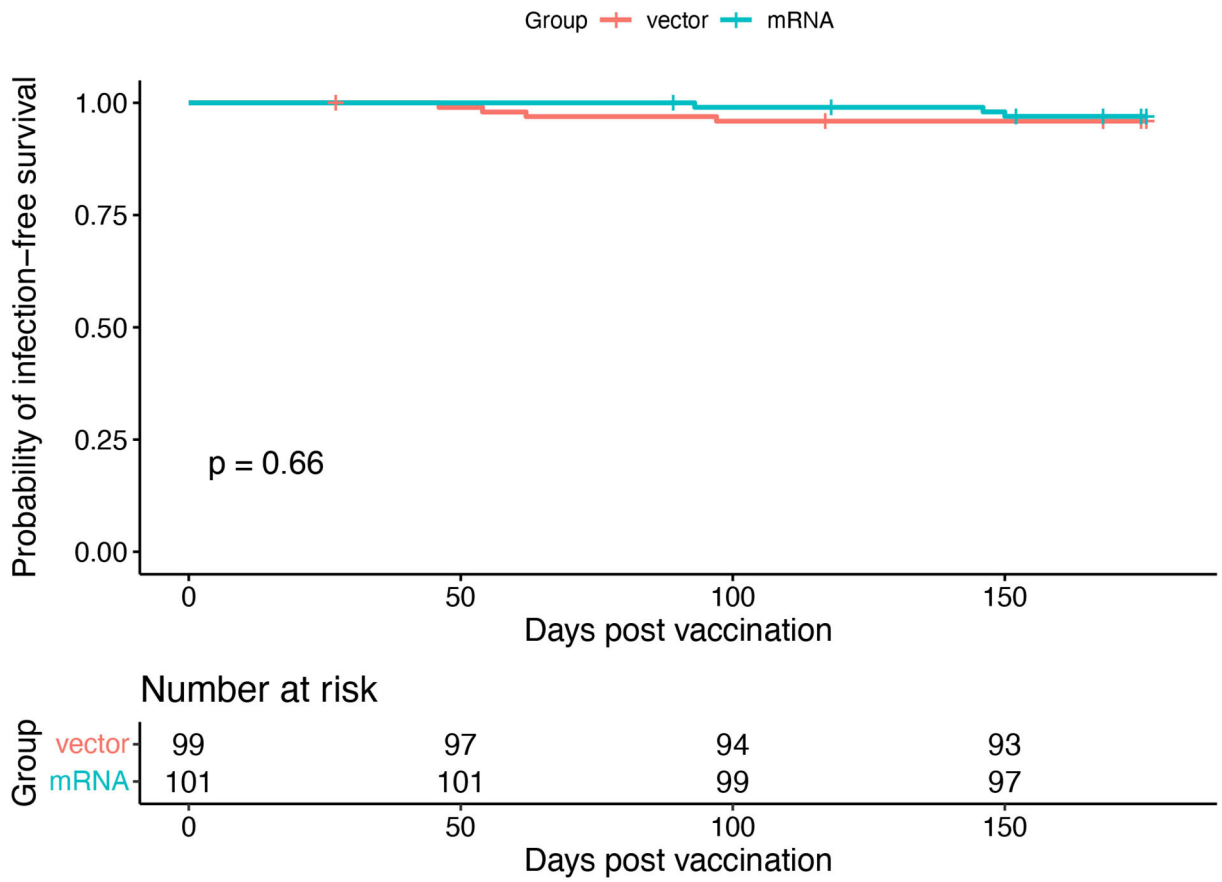


FIGURE 2 | Occurrence of COVID-19 infections. A Kaplan–Meier graph of COVID-19 infection-free duration after homologous and heterologous third SARS-CoV-2 vaccination throughout the observation period. In case of non-COVID-19-related death, the follow-up period was censored at the date of death.

TABLE 2 | The response rate to 3rd SARS-CoV-2 vaccination at different pre-specified cut-off levels for the 1- and 3-month follow-up.

	One-month FU				Three-month FU			
Cutoff	**mRNA %**	**Vector %**	**P**	**OR 95%CI**	**mRNA %**	**Vector %**	**p**	**OR 95%CI**
0.8 U/mL	36	43	0.434	1.3 [0.67, 2.54]	45	50	0.539	1.24 [0.65, 2.37]
15 U/mL	22	26	0.594	1.23 [0.57, 2.66]	24	31	0.304	1.45 [0.7, 3.06]
100 U/mL	7	12	0.307	1.77 [0.55, 6.25]	8	17	0.108	2.22 [0.78, 6.89]
141 BAU/mL	5	8	0.37	1.83 [0.45, 8.89]	4	15	0.009	4.96 [1.29, 28.21]
264 BAU/mL	4	4	1	1.01 [0.13, 7.78]	1	10	0.018	8.75 [1.13, 396.17]

there were a significantly higher number of individuals with antibody levels above antibody thresholds reported in the literature to be associated with neutralizing capacity despite a comparable overall seroconversion rate. Especially in the face of new variants that evade immune response (i.e., *Omicron* BA.1 and BA.2), higher antibody levels are needed for infection prevention, but cut-off levels conveying protective immunity remain undefined (12).

Interestingly, in both groups, 8% of KTR developed antibodies between completion of the primary endpoint at 4 weeks and the follow-up at 3 months. All the participants (excluding seven patients who tested positive for SARS-CoV-2 infection) had negative nucleocapsid antibody results at the 1- and 3-month follow-up, supporting delayed seroconversion rather than subclinical infections.

The difference in vaccine response > 141 BAU/ml and > 264 BAU/ml between both groups was only partly driven by an increase of responders in the heterologous vaccination group, but also a decline of antibody levels in individuals above these thresholds, following homologs vaccination. However, median antibody levels in the homologous vaccination group remained overall stable while increasing the vector group.

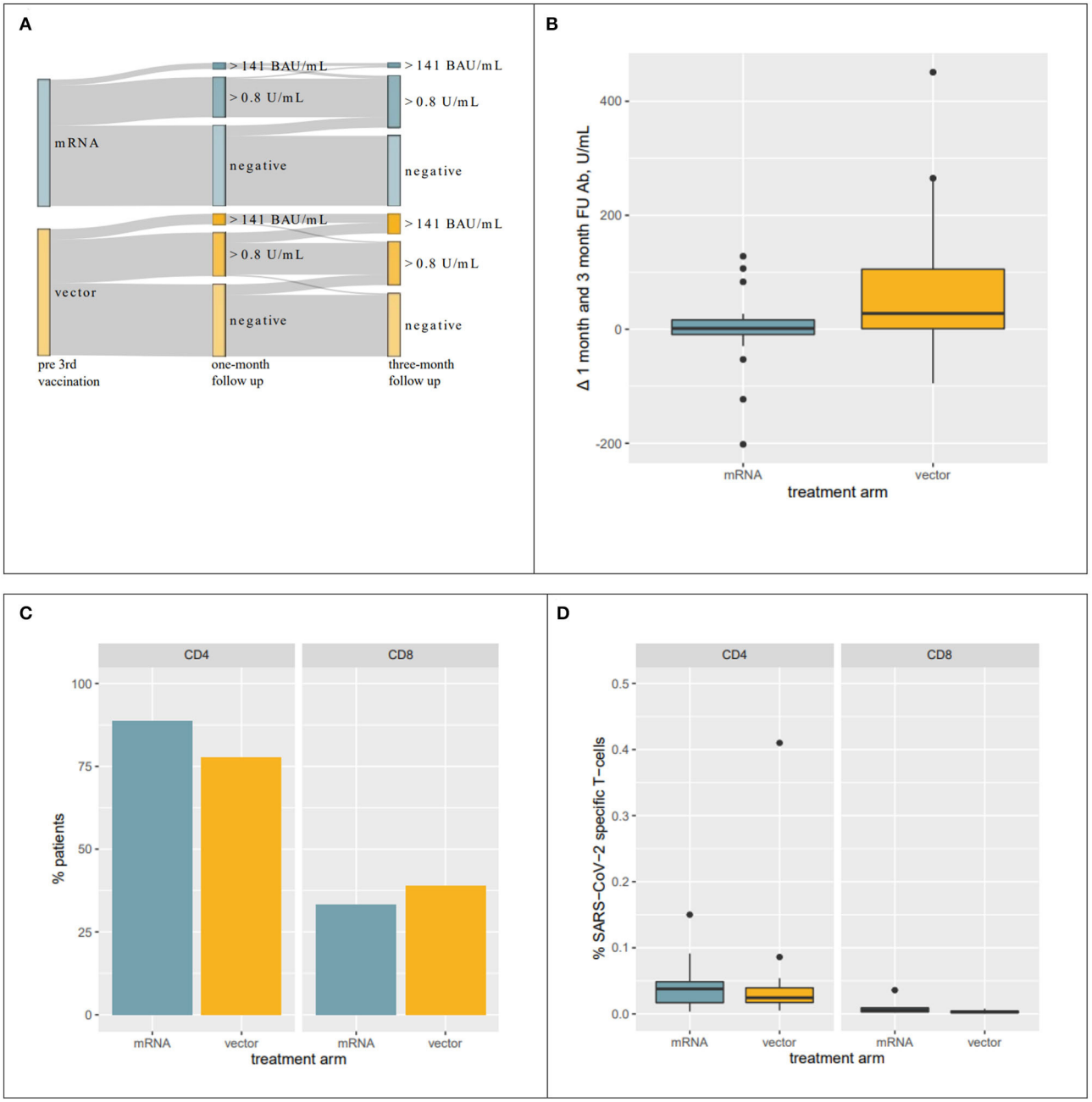


FIGURE 3 | Response to vaccination. **(A)** Sankey Diagram visualizing changes in the response rate to 3rd vaccination. A significantly larger proportion of individuals developed antibody levels > 141 BAU/ml. **(B)** Boxplots visualizing changes in antibody levels from 1- to 3-month FU in patients who seroconverted within 1 month after receiving their 3rd vaccination. Antibody levels in individuals receiving a heterologous 3rd vaccination further increased while remaining unaltered in patients receiving mRNA vaccines. **(C)** Percentage of patients with SARS-CoV-2-specific CD4 and CD8 T-cells among the top humoral responders at the 1-month FU. **(D)** Percentages of SARS-CoV-2-specific T-cells in patients with SARS-CoV-2-specific CD4 and CD8 T-cells.

Overall, four percent of study participants contracted COVID-19 in the observation period. Clinical endpoints were similar between both intervention groups, and COVID-19 infections only occurred in no/low responders (<0.4 or < 15 U/ml, respectively). Three KTRs had severe COVID-19, requiring intensive-care treatment, and two of these patients subsequently died. One fatality was in a vaccine low responder (5.9U/ml), suggesting that low-level antibody responses do not provide protection from severe disease. This is in line with reports that antibody levels > 141 or 264 BAU/ml are required for effective protection from symptomatic infections with the SARS-CoV-2 *alpha* variant (8, 9). We have previously compared antibody

levels (BAU/ml) and neutralizing capacity in serum samples, following third vaccination: all samples with BAU > 141 BAU/ml also had neutralizing capacity (3).

Interestingly, there was no difference in the SARS-CoV-2-specific CD4 or CD8 T-cell response at 4 weeks after vaccination, comparing homologous or heterologous vaccination strategies. This contrasts with other reports in immunized individuals that suggest higher levels of T-cell response, following heterologous vaccination (5, 13), although clear thresholds or correlates of T-cell protection remain to be delineated. In animal models, adenovirus-based vector vaccines also induced a stronger T-cell response (14, 15). Data from the general populations show higher antibody and T-cell responses, following heterologous vaccination compared to homologous mRNA and vector vaccination strategies (16–18). However, most studies used the vector vaccine ChAdOx1 as opposed to Ad26COVS1. Overall, impact of heterologous vaccination on antibody levels in immunized patients was inconclusive, with some suggesting higher antibody levels in the heterologous group (KTR), while another showed a lower seroconversion rate in the heterologous vaccination group (patients treated with rituximab) (5, 13).

A limitation of this study is the incomplete follow-up as 23 patients had received a fourth vaccine dose before completing the 3-month FU and were, therefore, excluded from analysis of vaccine efficacy. However, the overall follow-up rate was still at 85% at 3-month FU. To identify a potential imbalance, we reanalyzed the primary endpoint at 1-month FU only including KTR who completed the 3-month FU and found in line with our previous report of the entire cohort no statistically significant differences between the treatment groups (**Table 2**). Applicability of previously identified antibody cut-off levels for infection prevention (i.e., > 141 BAU/ml or > 264 BAU/ml) to new immune-evasion variants (e.g., Omicron) remains unclear, and much higher levels are most likely required for protective immunity. Until now, no such cut-off levels have been reported in the literature. The primary objective of the present trial, however, was the comparison of the immune response, following homologous and heterologous vaccination: Increased immunogenicity of the heterologous vaccination approach may, therefore, also play an important role in the response to future variant-specific vaccines. In addition, different antibody detection platforms are used across the literature that shows different sensitivity or specificity to detect SARS-CoV-2 antibodies. All the samples were tested using the same platform, and we used the WHO standardized units reported as BAU/ml for the reported cut-off levels derived from the literature to allow for comparability across different platforms (19). The cut-off BAU < 264 BAU/ml has been suggested as a cut-off to select individuals requiring additional immunization (20).

The strength of the study is the randomized controlled trial design and the pre-specified secondary endpoint at 3-month FU. To date, it has remained the only published RCT on heterologous third boost vaccination using Ad26COVS1 as a vector vaccine.

CONCLUSION

Despite similar overall seroconversion rates and comparable antibody levels at 4 weeks, heterologous 3rd boost vaccination using Ad26COVS1 results in significantly higher antibody levels in KTR over a 3-month follow-up period compared to additional homologous vaccination. More individuals in the heterologous vaccination group reached antibody levels associated with protective immunity against the SARS-CoV-2 *alpha* variant at 3 months.

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 the Medical University of Vienna. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AH, ES, RO, and RR-S conceptualized the study and wrote the manuscript. FR, KH, LR, ME, CAi, RJ, CAs, A-LS, TD, and KB contributed to data acquisition, data analysis, and writing the of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the Medical-Scientific Fund of the Mayor of the Federal Capital of Vienna (Project-Nr. 21182) and the Christine Vranitzky-Stiftung Research Grant 2020.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.936126/full#supplementary-material

REFERENCES

1. Grupper A, Rabinowich L, Schwartz D, Schwartz IF, Ben-Yehoyada M, Shashar M, et al. Reduced humoral response to mRNA SARS-CoV-2 BNT162b2 vaccine in kidney transplant recipients without prior exposure to the virus *Am J Transplant.* (2021) 21:2719–26. doi: 10.1111/ajt.16615
2. Boyarsky BJ, Werbel WA, Avery RK, Tobian AAR, Massie AB, Segev DL, et al. Antibody response to 2-dose SARS-CoV-2 mRNA vaccine series in solid organ transplant recipients. *JAMA.* (2021) 325:2204–6. doi: 10.1001/jama.2021.7489
3. Reindl-Schwaighofer R, Heinzel A, Mayrdorfer M, Jabbour R, Hofbauer TM, Merrelaar A, et al. Comparison of SARS-CoV-2 antibody response 4 weeks after homologous vs heterologous third vaccine dose in kidney transplant recipients: a randomized clinical

trial. *JAMA Intern Med.* (2022) 182:165–71. doi: 10.1001/jamainternmed.2021.7372
4. Schmidt T, Klemis V, Schub D, Schneitler S, Reichert MC, Wilkens H, et al. Cellular immunity predominates over humoral immunity after homologous and heterologous mRNA and vector-based COVID-19 vaccine regimens in solid organ transplant recipients. *Am J Transplant.* (2021) 21:3990–4002. doi: 10.1111/ajt.16818
5. Bonelli M, Mrak D, Tobudic S, Sieghart D, Koblischke M, Mandl P, et al. Additional heterologous versus homologous booster vaccination in immunosuppressed patients without SARS-CoV-2 antibody seroconversion after primary mRNA vaccination: a randomised controlled trial. *Ann Rheum Dis.* (2022) 81:687–94. doi: 10.1101/2021.09.05.21263125
6. Kamar N, Abravanel F, Marion O, Esposito L, Hebral AL, Medrano C, et al. Anti-SARS-CoV-2 spike protein and neutralizing antibodies at 1 and 3 months after three doses of SARS-CoV-2 vaccine in a large cohort of solid organ transplant patients. *Am J Transplant.* (2022) 22:1467–74. doi: 10.1111/ajt.16950
7. Hall VG, Ferreira VH, Ku T, Ierullo M, Majchrzak-Kita B, Chaparro C, et al. Randomized trial of a third dose of mRNA-1273 vaccine in transplant recipients. *N Engl J Med.* (2021) 385:1244–6. doi: 10.1056/NEJMc2111462
8. Dimeglio C, Herin F, Martin-Blondel G, Miedouge M, Izopet J. Antibody titers and protection against a SARS-CoV-2 infection. *J Infect.* (2022) 84:248–88. doi: 10.1016/j.jinf.2021.09.013
9. Feng S, Phillips DJ, White T, Sayal H, Aley PK, Bibi S, et al. Oxford, correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *Nat Med.* (2021) 27:2032–40. doi: 10.1101/2021.06.21.21258528
10. Sattler A, Angermair S, Stockmann H, Heim KM, Khadzhynov D, Treskatsch S, et al. SARS-CoV-2-specific T cell responses and correlations with COVID-19 patient predisposition. *J Clin Invest.* (2020) 130:6477–89. doi: 10.1172/JCI140965
11. Stefanski AL, Rincon-Arevalo H, Schrezenmeier E, Karberg K, Szelinski F, Ritter J, et al. B cell numbers predict humoral and cellular response upon SARS-CoV-2 vaccination among patients treated with rituximab. *Arthritis Rheumatol.* (2021) 74:934–47. doi: 10.1101/2021.07.19.21260803
12. Hoffmann M, Kruger N, Schulz S, Cossmann A, Rocha C, Kempf A, et al. The Omicron variant is highly resistant against antibody-mediated neutralization: Implications for control of the COVID-19 pandemic. *Cell.* (2022) 185:447–56.e11. doi: 10.1016/j.cell.2021.12.032
13. Schmidt T, Klemis V, Schub D, Mihm J, Hielscher F, Marx S, et al. Immunogenicity and reactogenicity of heterologous ChAdOx1 nCoV-19/mRNA vaccination. *Nat Med.* (2021) 27:1530–5. doi: 10.1038/s41591-021-01464-w
14. Spencer AJ, McKay PF, Belij-Rammerstorfer S, Ulaszewska M, Bissett CD, Hu K, et al. Heterologous vaccination regimens with self-amplifying RNA and adenoviral COVID vaccines induce robust immune responses in mice. *Nat Commun.* (2021) 12:2893. doi: 10.1038/s41467-021-23173-1
15. He Q, Mao Q, An C, Zhang J, Gao F, Bian L, et al. Heterologous prime-boost: breaking the protective immune response bottleneck of COVID-19 vaccine candidates. *Emerg Microbes Infect.* (2021) 10:629–37. doi: 10.1080/22221751.2021.1902245
16. Shaw RH, Stuart A, Greenland M, Liu X, Van-Tam JSN, Snape MD, et al. Heterologous prime-boost COVID-19 vaccination: initial reactogenicity data. *Lancet.* (2021) 397:2043–6. doi: 10.1016/S0140-6736(21)01115-6
17. Hillus D, Schwarz T, Tober-Lau P, Vanshylla K, Hastor H, Thibeault C, et al. Safety, reactogenicity, and immunogenicity of homologous and heterologous prime-boost immunisation with ChAdOx1 nCoV-19 and BNT162b2: a prospective cohort study. *Lancet Respir Med.* (2021) 9:1255–65. doi: 10.1016/S2213-2600(21)00357-X
18. Tenbusch M, Schumacher S, Vogel E, Priller A, Held J, Steininger P, et al. Heterologous prime-boost vaccination with ChAdOx1 nCoV-19 and BNT162b2. *Lancet Infect Dis.* (2021) 21:1212–3. doi: 10.1101/2021.07.03.21258887
19. Infantino M, Pieri M, Nuccetelli M, Grossi V, Lari B, Tomassetti F, et al. The WHO International Standard for COVID-19 serological tests: towards harmonization of anti-spike assays. *Int Immunopharmacol.* (2021) 100:108095. doi: 10.1016/j.intimp.2021.108095
20. Caillard S, Thaunat O. COVID-19 vaccination in kidney transplant recipients. *Nat Rev Nephrol.* (2021) 17:785–7. doi: 10.1038/s41581-021-00491-7

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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

TYPE Correction
PUBLISHED 10 October 2022
DOI 10.3389/fmed.2022.1048395

OPEN ACCESS

APPROVED BY
Frontiers Editorial Office,
Frontiers Media SA, Switzerland

*CORRESPONDENCE
Roman Reindl-Schwaighofer
roman.reindl-schwaighofer@meduniwien.ac.at

†These authors share first authorship

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 19 September 2022
ACCEPTED 20 September 2022
PUBLISHED 10 October 2022

CITATION
Heinzel A, Schrezenmeier E, Regele F, Hu K, Raab L, Eder M, Aigner C, Jabbour R, Aschauer C, Stefanski A-L, Dörner T, Budde K, Reindl-Schwaighofer R and Oberbauer R (2022) Corrigendum: Three-Month follow-up of heterologous vs. homologous third SARS-CoV-2 vaccination in kidney transplant recipients: Secondary analysis of a randomized controlled trial. *Front. Med.* 9:1048395. doi: 10.3389/fmed.2022.1048395

Corrigendum: Three-Month follow-up of heterologous vs. homologous third SARS-CoV-2 vaccination in kidney transplant recipients: Secondary analysis of a randomized controlled trial

Andreas Heinzel[1†], Eva Schrezenmeier[2†], Florina Regele[1], Karin Hu[1], Lukas Raab[1], Michael Eder[1], Christof Aigner[1], Rhea Jabbour[1], Constantin Aschauer[1], Ana-Luisa Stefanski[3], Thomas Dörner[3], Klemens Budde[2], Roman Reindl-Schwaighofer[1*] and Rainer Oberbauer[1]

[1]Division of Nephrology and Dialysis, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria, [2]Department of Nephrology and Medical Intensive Care, Charité-Universitätsmedizin Berlin, Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany, [3]Department of Rheumatology and Clinical Immunology, Charité-Universitätsmedizin Berlin, Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany

KEYWORDS

COVID-19, kidney transplantation, COVID-19 vaccination, heterologous vaccination, third vaccination

A corrigendum on

Three-Month follow-up of heterologous vs. homologous third SARS-CoV-2 vaccination in kidney transplant recipients: Secondary analysis of a randomized controlled trial

by Heinzel, A., Schrezenmeier, E., Regele, F., Hu, K., Raab, L., Eder, M., Aigner, C., Jabbour, R., Aschauer, C., Stefanski, A.-L., Dörner, T., Budde, K., Reindl-Schwaighofer, R., and Oberbauer, R. (2022). *Front. Med.* 9:936126. doi: 10.3389/fmed.2022.936126

In the published article, an author name was incorrectly written as "Schretzenmeier." The correct spelling is "Schrezenmeier."

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 29 July 2022
DOI 10.3389/fmed.2022.923524

OPEN ACCESS

EDITED BY
Georg Böhmig,
Medical University of Vienna, Austria

REVIEWED BY
Nirmala Parajuli,
University of Arkansas for Medical Sciences, United States
Sahra Pajenda,
Medical University of Vienna, Austria

*CORRESPONDENCE
Eva Kieslichova
evki@ikem.cz

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 19 April 2022
ACCEPTED 08 July 2022
PUBLISHED 29 July 2022

CITATION
Protus M, Uchytilova E, Indrova V, Lelito J, Viklicky O, Hruba P and Kieslichova E (2022) Sepsis affects kidney graft function and one-year mortality of the recipients in contrast with systemic inflammatory response. *Front. Med.* 9:923524. doi: 10.3389/fmed.2022.923524

Sepsis affects kidney graft function and one-year mortality of the recipients in contrast with systemic inflammatory response

Marek Protus[1,2], Eva Uchytilova[1,2], Veronika Indrova[1], Jan Lelito[1], Ondrej Viklicky[2,3], Petra Hruba[4] and Eva Kieslichova[1,2]*

[1]Department of Anesthesiology, Resuscitation and Intensive Care, Institute for Clinical and Experimental Medicine, Prague, Czechia, [2]First Faculty of Medicine, Charles University, Prague, Czechia, [3]Department of Nephrology, Transplant Centre, Institute for Clinical and Experimental Medicine, Prague, Czechia, [4]Transplantation Laboratory, Experimental Medicine Centre, Institute for Clinical and Experimental Medicine, Prague, Czechia

Background: Infections remain a major cause of morbidity and mortality after kidney transplantation. The aim of our study was to determine the effect of sepsis on kidney graft function and recipient mortality.

Methods: A prospective, observational, single-center study was performed. Selected clinical and biochemical parameters were recorded and compared between an experimental group (with sepsis, $n = 34$) and a control group (with systemic inflammatory response syndrome, $n = 31$) comprising kidney allograft recipients.

Results: Sepsis worsened both patient (HR = 14.77, $p = 0.007$) and graft survival (HR = 15.07, $p = 0.007$). Overall one-year mortality was associated with age (HR = 1.08, $p = 0.048$), APACHE II score (HR = 1.13, $p = 0.035$), and combination immunosuppression therapy (HR = 0.1, $p = 0.006$), while graft survival was associated with APACHE II (HR = 1.25, $p = 0.004$) and immunosuppression. In sepsis patients, mortality correlated with the maximal dose of noradrenalin (HR = 100.96, $p = 0.008$), fungal infection (HR = 5.64, $p = 0.024$), SAPS II score (HR = 1.06, $p = 0.033$), and mechanical ventilation (HR = 5.97, $p = 0.033$), while graft survival was influenced by renal replacement therapy (HR = 21.16, $p = 0.005$), APACHE II (HR = 1.19, $p = 0.035$), and duration of mechanical ventilation (HR = 1.01, $p = 0.015$).

Conclusion: In contrast with systemic inflammatory response syndrome, septic kidney allograft injury is associated with early graft loss and may represent a significant risk of mortality.

KEYWORDS

kidney transplantation, sepsis, systemic inflammatory response syndrome, mortality, graft loss

Introduction

The number of kidney transplantations is steadily increasing (1). Although allograft survival has improved (2), the potential for surgical complications combined with the impact of immunosuppression predisposes recipients to infectious complications (3, 4). In particular, bloodstream infections (BSI) remain a major cause of morbidity, graft dysfunction and mortality after transplantation. When accompanied by septic shock, mortality can reach up to 50% (5).

Predisposing factors include those present in the recipient or donor before transplantation as well as those secondary to intraoperative and post-transplant events (6). Knowledge of the previous and current immunosuppression burden as well as the time course of infectious episodes after transplantation can guide clinicians toward devising the most appropriate treatment. In the first 6 months, infections are usually related to postoperative complications, manipulation of the urinary tract or viral reactivation. Urinary tract infections (UTI) are also the main source of BSI, followed by catheter-related and wound infections (5).

Perioperative antibiotic prophylaxis should be carefully considered based on the epidemiological situation at the transplant center concerned, the possible colonization of the recipient, and other risk factors. Despite a decrease in the incidence of infectious complications due to routine perioperative and long-term prophylaxis, recipients remain at significant risk of developing infections from multidrug-resistant (MDR) pathogens. MDR bacteremia results in significantly poorer clinical outcomes and higher overall case-fatality rates compared with other etiologies (7).

Septic acute kidney injury (AKI) is defined as the acute impairment of function and organ damage linked with long-term adverse outcomes depending on the extent of acute injury superimposed on the underlying organ reserve (8). Early and appropriate antimicrobial therapy along with septic source control is a cornerstone in its prevention (9). Since septic AKI is not characterized by renal hypoperfusion, restricting resuscitation fluid volumes is feasible (10). While the level of renal protection provided by the most commonly used vasopressors is comparable (11), maintaing the target mean arterial pressure is likely more important (12). Ultimately, a proportion of sepsis patients will undergo renal replacement therapy (RRT). Although commencing RRT at an early phase of both sepsis and AKI can prevent fluid overload and organ injury by removing inflammatory mediators, it can also expose patients to a number of adverse effects, including inadequate antibiotic dosing (13).

Immunosuppressants are used in many different combinations after kidney transplantation, depending on the risk of rejection in the individual patient, time course following transplantation, previous adverse effects and local protocols. The risk of rejection, potentiated by a reduction or discontinuation of immunosuppressive therapy during sepsis, should always be balanced against life-threatening septic complications (14).

The primary aim of our study was to determine the influence of sepsis on kidney allograft function and to identify possible risk factors that contribute to the development of septic acute kidney allograft injury. The secondary aim was to evaluate the mortality of sepsis patients in comparison with kidney allograft recipients with systemic inflammatory response syndrome (SIRS).

Materials and methods

Study design and patient population

This prospective observational study was performed between 2018 and 2020 at the intensive care unit (ICU) of the Transplant Centre at the Institute for Clinical and Experimental Medicine (IKEM), Prague. Consecutive kidney transplant patients admitted to ICU for management of a first episode of sepsis were prospectively included. Inclusion criteria were as follows: kidney transplant patients with a first episode of sepsis; age $\geq$18 years; ICU stay $\geq$24 h. Exclusion criteria were: age $<$18 years; objection to participating in the study; severe underlying disease with poor prognosis and/or a life expectancy of less than 24 h; clinical history involving loss of a previous organ transplant graft; recent discontinuation of immunosuppression therapy. The absence of any antibiotic treatment within at least 1 month prior to enrollment was conditional. In all experimental patients, the first dose of antibiotics was given after enrollment as part of sepsis therapy. The control group included kidney transplant patients diagnosed with SIRS without infection within the first 30 days after transplantation. Exclusion criteria for the control group were as follows: clinical signs of systemic or local infection within 30 days after transplantation; age $<$ 18 years; objection to participating in the study. In cases involving repeat admissions of a patient (in either group) to ICU, only the first admission was considered.

Data collection and interventions

Selected clinical and biochemical data were recorded for both groups. Clinical data were derived from the medical records, clinical examinations, and anamneses of patients. Biochemical examinations of serum biochemistry, blood counts, blood coagulation parameters, and laboratory markers of sepsis/SIRS were performed at an accredited laboratory (ISO 15189) at IKEM's Department of Laboratory Methods. Pathogen detection (fungal and bacterial) was performed using standard microbiological examination procedures, and detection of

microbial nucleic acids by polymerase chain reaction (PCR). Acute graft rejection was diagnosed according to specific (oliguria, serum biochemistry abnormalities) or non-specific (generalized malaise, fever, and anorexia) symptoms and confirmed by immunological testing and histology of the graft biopsy. A graft biopsy was performed in all cases of suspected acute graft rejection, with histology evaluated by experienced pathologists from IKEM's Department of Clinical and Transplant Pathology.

Selected clinical parameters and laboratory markers were recorded in order to estimate possible organ dysfunction and severity using the Acute Physiology and Chronic Health Evaluation II (APACHE II) score, the Simplified Acute Physiology Score (SAPS II) and, in patients with sepsis, the Sepsis-related Organ Failure Assessment score (SOFA). The APACHE severity score was calculated from the worst parameters obtained within the first 24 h after admission to ICU, the SAPS II score was collected within the first 24 h of the ICU stay, and the SOFA score was calculated daily during the ICU stay. The following clinical and laboratory markers were recorded: demographic data, comorbid conditions, prophylaxis, type of immunosuppression, sites and type of infection (community- or hospital-acquired), septic shock development in the sepsis group. Vital signs such as mental status, temperature, hemodynamic and ventilation parameters, urine output and fluid balance were also recorded.

Interventions such as antibiotic use, vasopressor administration (including epinephrine, norepinephrine, and dobutamine), mechanical ventilation (MV), renal replacement therapy (RRT), and nutritional therapy were recorded. Any infectious episodes, acute tubular necrosis or acute rejection of the allograft occurring within 1 year of inclusion were monitored. In the sepsis group, the time from transplantation to inclusion (days), reduction or withdrawal of immunosuppression, and the number of days without immunosuppression were recorded. Sepsis was diagnosed in accordance with the Surviving Sepsis Campaign (SSC) consensus guidelines based on clinical examination, imaging methods, and laboratory testing, including microbiological identification of the infectious agent by microbiological, immunological and molecular-biological techniques. Sepsis treatment was carried out according to standards based on antimicrobial therapy, source control, early goal-directed therapy (EGDT), and supportive treatment (15, 16).

Immunosuppression

Our standard immunosuppressive protocol consisted of induction agents and a combination of extended-release tacrolimus (Advagraf, Astellas), mammalian target of rapamycin inhibitors (mTORi, Rapamune, Pfizer), mycophenolic acid (Myfortic, Novartis) and prednisone. The standard protocol was adjusted according to individual immunological risk. Episodes of rejection were treated with intravenous steroids or lymphocyte-depleting agents.

Reduction or withdrawal of immunosuppression in sepsis patients was performed according to standard procedures used at our transplant center. Nevertheless, corticosteroid administration was not discontinued in order to allow for septic shock-associated adrenocortical insufficiency. The suitability of sepsis patients to resume immunosuppressive therapy was assessed daily.

Outcomes

Clinical outcomes of patients, represented by in-hospital mortality, one-year mortality and kidney allograft function, were assessed one year after inclusion in the study. Allograft function was classified as impaired in cases where serum creatinine (stable before septic episode) increased above 150 μmol/l and subsequently failed to return to the preceding value within the defined time period. Allograft function was defined as lost in cases where chronic hemodialysis treatment was reinitiated within 1 year of enrollment. Selected parameters were compared between the group of kidney transplant patients with sepsis and the group of kidney transplant patients with SIRS, including the number and type of consecutive infectious episodes. Hospital-acquired infections were defined as healthcare-associated infections in cases where the first symptoms occurred more than 48 h after admission to hospital. Risk factors for one-year mortality, impaired kidney function and loss of kidney graft function within one year after inclusion were also identified.

Statistical analysis

Continuous variables were reported as medians and interquartiles with range determination (minimum, maximum). Categorical variables were expressed as *n* and a percentage of the total. Continuous variables were compared using the two-sample Wilcoxon rank-sum test and categorical variabes using Fisher's exact test. Survival analysis was performed using the Kaplan–Meier method, with differences between groups compared using the log-rank test. Univariable Cox proportional-hazards models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) of potential risk factors for patient and graft survival. Binary logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) of potential risk factors for kidney graft dysfunction one year after study enrollment. A p-value < 0.05 was considered statistically significant throughout the study. Statistical analysis was performed using R-studio software, version 4.1.3 (2022-03-10) (Development for R. RStudio, Inc.,

Boston, MA, United States) and JMP 15.2.0, 2019 (SAS Institute, Inc).

Results

The experimental group consisted of 34 kidney transplant recipients readmitted to hospital because of sepsis; the control group comprised 31 kidney transplant recipients with SIRS only and without clinical or laboratory signs of BSI admitted to ICU immediately after transplantation.

Demographic and clinical characteristics

In principle, baseline demographic and clinical characteristics did not significantly differ between groups. The median age was 60 years for sepsis patients and 48 years for controls. Males accounted for 47% of patients in the sepsis group and 71% in the control group. The median body mass index was 26.4 for the sepsis group and 25.8 for controls. The APACHE II severity score was significantly lower in controls with a median of 12, while the sepsis group had a median of 19.5 ($p = 0.001$) (**Table 1**). The median time from transplantation to enrollment was 254 days in the sepsis group (**Table 2**).

Patients from both groups had similar comorbidities, with 70% of sepsis patients and 100% of control patients suffering from hypertension, which was the most common comorbidity in both groups followed by ischemic heart disease. There was a slight diference in some other comorbidities between the two groups. Signs of chronic heart failure were identified in 6% of control patients. The second most frequent disease was type two diabetes, found in 29% of sepsis patients and 23% of controls. Type one diabetes was less common, diagnosed in 18% of sepsis patients and 13% of controls. Cancer was identified in 12% of sepsis patients, with the same percentage of patients from this group displaying chronic obstructive pulmonary disease (COPD). While no patient in the control group had cancer, 3% of controls exhibited COPD. Two control patients had a history of liver disease.

In the majority of cases (71% of sepsis patients, 94% of controls), chronic immunosuppressive therapy administered to kidney recipients was based on tacrolimus, with cyclosporine used in 12% of sepsis patients and 6% of controls. As part of combination immunosuppression treatment, mycophenolate mofetil was given to 62% of sepsis patients, a significantly lower percentage than controls (94%) ($p = 0.003$). Only 3% of sepsis patients and 6% of controls had mTORi (**Table 1**).

With respect to the type of kidney transplantation, no differences were found between the two groups. Most patients received a first kidney graft, represented by 76% of sepsis patients and 80% of controls. In sepsis patients, only 6% underwent a second kidney transplantation, while 3% underwent a third kidney transplantation. Similarly, in control patients, only 10% underwent a second transplantation, but no patients underwent a third transplantation. The remaining 15% of sepsis patients and 10% of control patients underwent a first simultaneous pancreas and kidney transplantation (**Table 1**).

Clinical outcomes

The main aims of our study were to evaluate kidney graft function one year after a sepsis event and then to compare outcomes with graft function in SIRS (control) patients. Graft function remained stable in only 13 sepsis patients (38%) in comparison with 23 control patients (74%) ($p = 0.006$). In 20% of sepsis patients and 26% of controls, graft function was classified as impaired one year after enrollment. An episode of acute tubular necrosis (ATN) occurred within the year in 35% of sepsis patients and 29% of controls, although these differences were not statistically significant. Seven kidney recipients (20%) from the sepsis group lost graft function completely within 1 year after the sepsis event before returning to hemodialysis, whereas in the control group all grafts remained functional to such a degree that no patient required dialysis within the defined time period ($p = 0.011$). A biopsy was performed in suspected cases of allograft rejection within 1 month of the sepsis episode, representing a total of 14 patients from the sepsis group (41%). Acute tubular necrosis was identified in 7 patients and tubular atrophy in 2 patients. Rejection changes were not observed in any of the biopsies.

The median hospital stay in patients with sepsis was 20.5 days, significantly longer than the median hospital stay in control patients (13 days) ($p = 0.001$). In-hospital mortality did not differ significantly between the two groups. However, one-year mortality was higher in the sepsis group ($p = 0.012$) (**Table 2**) with significantly impaired 1-year survival (logrank $p = 0.0087$) (**Figure 1**).

Infectious complications

Sepsis patients also proved more susceptible to infectious complications. Six (18%) developed one complication, while 20 (59%) developed more than one infectious complication within the defined time period. These cases occurred significantly more frequently than controls ($p = 0.012$). Unsurprisingly, the urinary tract was the most common site of infection (65% of sepsis patients and 52% of controls) followed by abdominal and respiratory infections. Three patients from the sepsis group developed a biliary tract infection. In 12 out of 34 patients from the sepsis group, BSI confirmed by positive hemoculture occurred repeatedly. As anticipated, more hospital-acquired than community-acquired infections were recorded within the

TABLE 1 Baseline demographic and clinical characteristics of the study population with a comparison of variables between both groups.

Variable	SIRS group	SIRS group % or range	Sepsis group	Sepsis group % or range	*P*-value
Age (years)	48 (44, 63)	19–78	60 (49.5, 68)	22–82	0.165
Sex (male)	22/31	71%	16/34	47%	0.077
APACHE II	12 (10, 14)	8–26	19.5 (15.8, 25)	7–33	**0.001***
BMI	25.8 (23.5, 30.4)	17.9–39.1	26.4 (23.4, 29.0)	20–39	0.564
Comorbidities					
Type I diabetes	4/31	13%	6/34	18%	0.736
Type II diabetes	7/31	23%	10/34	29%	0.582
COPD	1/31	3%	4/34	12%	0.358
Cancer	0/31	0%	4/34	12%	0.115
IHD	8/31	26%	18/34	53%	**0.042***
Hypertension	31/31	100%	24/34	70%	**0.005***
CHF	2/31	6%	0/34	0	0.602
Liver disease	2/31	6%	0/34	0	0.223
Chronic immunosuppression					
Tacrolimus	29/31	94%	24/34	71%	0.086
Cyclosporine	2/31	6%	4/34	12%	0.674
MMF	29/31	94%	21/34	62%	**0.003***
mTORi	2/31	6%	1/34	3%	1.0
Type of transplantation					
1st, 2nd, 3rd kidney	25/3/0	80%/10%/0%	26/2/1	76%/6%/3%	-
Pancreas and kidney	3/31	10%	5/34	15%	-

Data are presented as n (%) or medians and interquartile ranges. SIRS: systemic inflammatory response syndrome, APACHE II, Acute Physiology and Chronic Health Evaluation II; BMI, body mass index; COPD, chronic obstructive pulmonary disease; IHD, ischemic heart disease; CHF, chronic heart failure; MMF, mycophenolate mofetil; mTROi, mammalian target of rapamycin inhibitor. Boldface indicates statistical significance where $p < 0.05$ (*).

TABLE 2 Kidney graft function one year after enrollment, time from transplantation to enrollment in days, length of stay, in-hospital mortality, and one-year mortality.

Variable	SIRS group	SIRS group % or range	Sepsis group	Sepsis group % or range	*P*-value
Stable	23/31	74%	13/34	38%	**0.006***
Impaired function	8/31	26%	7/34	20%	0.770
Loss of graft function	0/31	0	7/34	20%	**0.011***
Hemodialysis	0/31	0	7/34	20%	**0.011***
Episode of ATN after enrollment (1 year)	9/31	29%	12/34	35%	0.608
Days from transplantation to enrollment	0	–	254 (50.8, 3333.5)	5–6882	–
Length of stay (days)	13 (10, 14)	7–28	20.5 (12.8, 34)	1–104	**0.001***
In-hospital mortality	0/31	0	2/34	6%	0.493
1-year mortality	0/31	0	7/34	20%	**0.012***

Data are presented as *n* (%) or medians and interquartile ranges. SIRS: systemic inflammatory response syndrome, impaired function indicates serum creatinine >150 μmol/l, ATN: acute tubular necrosis. Boldface indicates statistical significance where $p < 0.05$ (*).

year in both groups of patients. Overall, the number of infectious episodes of both types was higher in the sepsis group, as mentioned above (Table 3).

Risk factors associated with mortality

Taking kidney graft recipients with sepsis separately, we analyzed possible risk factors associated with increased 1-year mortality. According to univariable Cox regression, the following were significant mortality factors: SAPS II score (HR = 1.06, $p = 0.033$), the presence of fungal infection (HR = 5.64, $p = 0.024$), the need for mechanical ventilation (HR = 5.97, $p = 0.033$), but not duration, and the maximum dose of norepinephrine (HR = 100.96, $p = 0.008$). However, none of the demographic characteristics and comorbidities, APACHE and SOFA scores, the duration of immunosuppression withdrawal due to sepsis, or the site

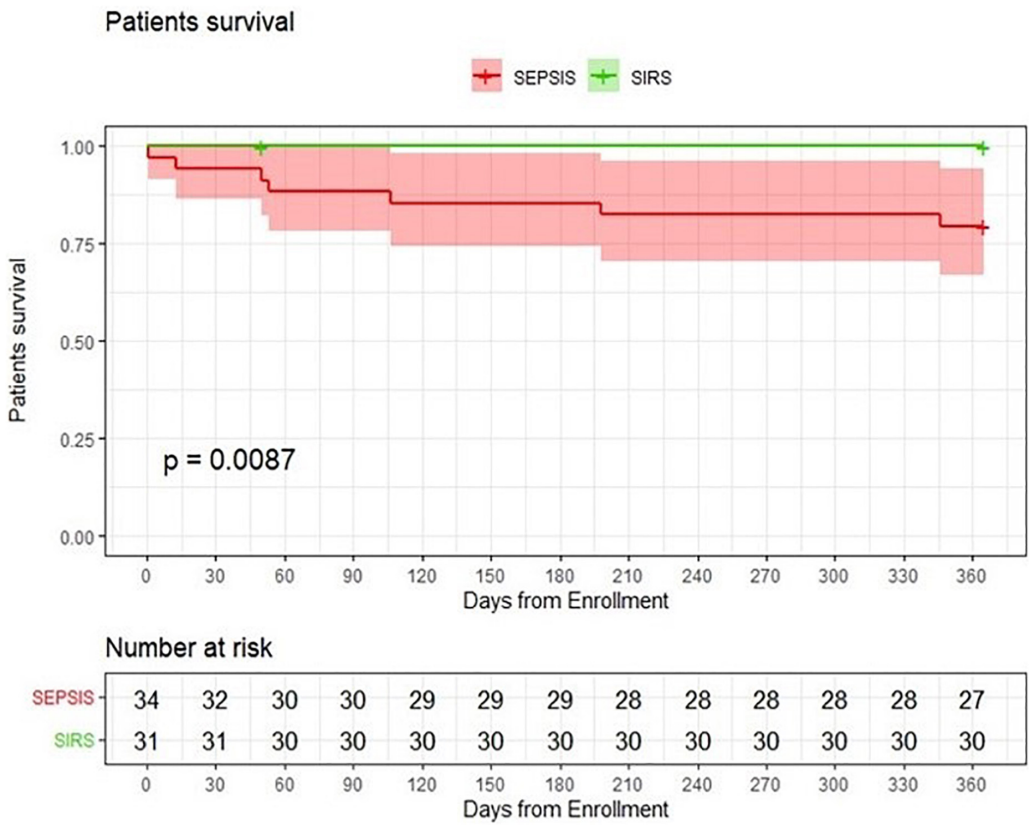


FIGURE 1
Probability of patients survival. Comparison of kidney transplant patients with sepsis with a group of kidney transplant patients with SIRS.

TABLE 3 Infectious complications within the first year after enrollment.

Variable	SIRS group n/N	SIRS group % or range	Sepsis group n/N	Sepsis group % or range	*P*-value
One sepsis event	8/31	26%	6/34	18%	0.559
More than one sepsis event	8/31	26%	20/34	59%	**0.012***
Site of infection					
Urinary	16/31	52%	22/34	65%	0.322
Lung	2/31	6%	3/34	9%	1.0
Biliary tract	0	0	3/34	9%	0.240
Abdomen	2/31	6%	7/34	20%	0.153
Positive hemoculture	0		12/34	35%	-
Type of infection					
Community-acquired	9/31	29%	13/34	38%	0.600
Hospital-acquired	12/31	39%	20/34	59%	0.138

SIRS, systemic inflammatory response syndrome. Boldface indicates statistical significance where $p < 0.05$ (*).

and source of infection played a significant role. Unexpectedly, we found that neither the development of septic shock nor any clinical sign of organ dysfunction due to sepsis, such as acute lung injury (ALI), acute kidney injury requiring renal replacement therapy, lactic acidosis, low platelet count, or elevated serum bilirubin level, significantly affected the one-year mortality of patients (Table 4A).

Analyzing the data on all kidney graft recipients from both groups together, we found that patients with sepsis had a 14.8-times-worse one-year survival (HR = 14.77, $p = 0.007$). Immunosuppression protocol without MMF (HR = 0.1, $p = 0.006$), APACHE II score (HR = 1.13, $p = 0.035$), and age (HR = 1.08, $p = 0.048$) was associated with 1-year mortality based on univariable Cox regression. On the other hand,

TABLE 4A Univariable Cox regression analysis of possible risk factors associated with one-year mortality in patients with sepsis ($n = 34$).

Variable	HR (95% CI)	*P*-value
BMI	1.02 (0.89–1.18)	0.750
DM I + II	0.47 (0.09–2.41)	0.363
APACHE II score	1.06 (0.94–1.2)	0.340
SOFA score	1.03 (0.99–1.71)	0.060
SAPS II score	1.06 (1.01–1.12)	**0.033***
Days without immunosuppression	1.00 (1.00–1.01)	0.518
Community-acquired	0.31 (0.04–2.56)	0.275
Hospital-acquired	3.25 (0.39–27.04)	0.275
Fungal infection	5.64 (1.25–25.37)	**0.024***
MDR bacteria	6.35 (0.76–52.85)	0.087
Viral infection	1.55 (0.19–12.87)	0.686
G- infection	0.65 (0.14–2.89)	0.567
G+ infection	1.13 (0.14–9.39)	0.911
G- and G+ infection	1.58 (0.31–8.18)	0.584
Acute lung injury	2.21 (0.49–9.87)	0.300
Lactic acidosis	5.0 (0.6–41.62)	0.137
Acute kidney injury	0.41 (0.08–2.14)	0.291
Serum bilirubin 20 μmol/l	2.11 (0.47–9.42)	0.329
Thrombocytopoenia	0.39 (0.05–3.23)	0.382
Septic shock	5.00 (0.6–41.62)	0.137
Mechanical ventilation	5.97 (1.15–30.93)	**0.033***
Duration of mechanical ventilation	1.01 (1–1.02)	0.209
RRT	1.07 (0.21–5.53)	0.934
Noradrenaline maximum dose	100.96 (3.41–2985.66)	**0.008***

APACHE II, Acute Physiology and Chronic Health Evaluation II; BMI, body mass index; DM I + II, diabetes mellitus type I and type II; SOFA, Sequential Organ Failure Assessment score; SAPS II, Simplified Acute Physiology Score II; RRT, renal replacement therapy; HR, hazard ratio; CI, confidence interval; MDR, multidrug-resistant; G-, gram-negative bacteria; G+, gram-positive bacteria. Boldface indicates statistical significance where $p < 0.05$ (*).

comorbidities such as hypertension, both types of diabetes, sex, and BMI did not play a significant role. We found no association between immunosuppression protocol (tacrolimus, cyclosporine, exclusively corticosteroid immunosupression) and one-year mortality (Table 4B).

Risk factors associated with graft dysfunction

Binary logistic regression was used to identify risk factors associated with impairment of graft function in kidney transplant recipients from both groups. Surprisingly, the only significant risk factor linked to impairment of kidney graft function within one year was BMI ($p = 0.042$), whereas age, APACHE II score, the source of infection and recurrent infections within the defined period did not seem to play an important role (Supplementary Table 1).

TABLE 4B Univariable Cox regression analysis of all-cause one-year mortality for the whole patient cohort ($n = 65$).

Variable	HR (95% CI)	*P*-value
Immunosuppression without MMF	0.10 (0.02–0.52)	**0.006***
APACHE II	1.13 (1.08–1.26)	**0.035***
Age	1.08 (1.00–1.16)	**0.048***
Hypertension	0.31 (0.06–1.58)	0.156
Prednisone only	4.35 (0.52–36.23)	0.174
Sex (male)	0.51 (0.12–2.30)	0.384
DM I + II	0.57 (0.11–2.95)	0.503
IHD	0.58 (0.11–2.97)	0.51
Cyclosporine	1.72 (0.21–14.32)	0.615
BMI	1.01 (0.87–1.17)	0.896
Tacrolimus	1.09 (0.13–9.03)	0.938
Sepsis patients	14.77 (1.80–1917.57)	**0.007***

APACHE II, Acute Physiology and Chronic Health Evaluation II; BMI, body mass index; DM I + II, diabetes mellitus type I and type II; HR, hazard ratio; CI, confidence interval; SIRS, systemic inflammatory response syndrome; MMF, mycophenolate mofetil; IHD, ischemic heart disease. Boldface indicates statistical significance where $p < 0.05$ (*).

To identify the risk factors associated with complete loss of graft function in the sepsis group, univariable Cox regression analysis was performed. Based on our results, APACHE II score (HR = 1.19, $p = 0.035$), duration of mechanical ventilation (HR = 1.01, $p = 0.015$), and the need for renal replacement therapy during sepsis (HR = 21.16, $p = 0.005$) were crucial factors. Conversely, other demographic parameters and comorbidities (BMI, age, sex, and diabetes), immunosuppression-free duration, source of infection, type of microorganism, SOFA, SAPS II score, or presence of septic shock requiring vasopressor circulatory support accompanied by ALI, as well as lactic acidosis, elevated serum bilirubin or low platelet count, did not play a significant role (Table 5A).

Finally, univariate Cox regression was used to analyze the risk factors associated with loss of kidney graft function in both patient groups together. Sepsis *per se* increased the risk of graft loss 15-fold (HR = 15.07, $p = 0.007$). Interestingly, immunosuppression without MMF (HR = 0.20, $p = 0.038$), APACHE II (HR = 1.25, $p = 0.004$), and immunosuppression based on tacrolimus (HR = 0.21, $p = 0.041$) proved significant. Graft survival was not significantly affected by any demographic factor (age, sex, and BMI), comorbidity (hypertension, diabetes, and ischemic heart disease) or immunosuppression protocol based either on cyclosporine or prednisone only (Table 5B and Figure 2).

Discussion

In general, sepsis is a leading cause of ICU admission, and is associated with a high mortality rate (17, 18). To determine

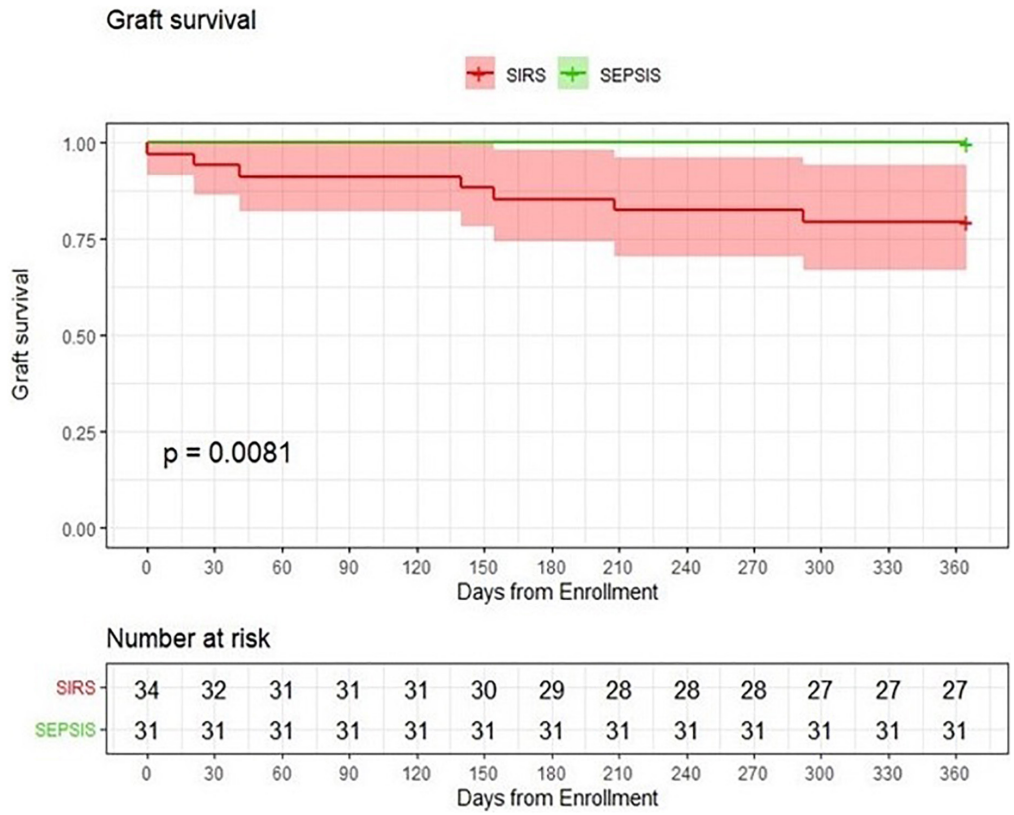


FIGURE 2
Probability of graft survival. Comparison of kidney transplant patients with sepsis with a group of kidney transplant patients with SIRS.

the negative impact of sepsis on kidney graft function from a long-term perspective, we chose kidney allograft recipients with SIRS after transplantation (i.e., with similar clinical signs to sepsis, but without the presence of infection) as controls. These patients could be used as suitable controls, because any surgery (including kidney transplantation) may lead to SIRS by itself and may affect the allograft function. These patients were followed for one year after transplantation and all their grafts remained functional within this defined time period. Baseline characteristics such as demographic parameters, presence of comorbidities and immunosuppressive therapy were similar in both groups. The APACHE II score was statistically significantly higher in the group of patients with sepsis, reflecting the severity of the condition upon ICU admission.

According to our findings, sepsis significantly affected kidney graft function: 20% of patients lost graft function within one year of the septic episode and returned to dialysis. Sepsis patients also suffered from more subsequent infections and had higher one-year mortality: fungal infections, median SAPS II score, respiratory failure, and hemodynamic instability were all identified as significant one-year mortality factors. The development of septic acute kidney graft injury requiring RRT seems to be a crucial risk factor for complete loss of graft function, unlike the number of subsequent infections or duration of immunosuppression withdrawal. Notably, all recipients who required RRT due to sepsis and lost graft function died within a year of the septic event. The tacrolimus-based immunosuppression protocol was associated with loss of kidney graft functin as well as immunosuppression without MMF (**Tables 5A**,**B**). The longer time period between transplantation and enrollment in sepsis patients or selection bias may explain why these patients were placed on tacrolimus and significantly fewer MMF (as presented in **Table 1**).

Renal circulation plays an important role in the pathogenesis of AKI. Therefore, in instances of systemic vasodilatation, the use of vasoactive drugs is necessary in order to maintain the perfusion pressure of the kidney graft and preserve kidney function (19). However, it is also necessary to ensure sufficient intravascular volume first before titrating the appropriate dose in order to prevent further medullary hypoxia (12). This is probably why, in our study, the maximum (and not the cumulative) dose of norepinephrine proved a significant mortality factor in kidney transplant recipients with sepsis. The maximum norepinephrine dose indicates the degree of hemodynamic instability, which determines the severity of a patient's clinical condition. On the other hand, neither the use

TABLE 5A Univariable Cox regression of possible risk factors associated with kidney graft failure in the sepsis group one year after enrollment ($n = 34$).

Variable	HR (95%CI)	*P*-value
BMI	1.02 (0.88–1.18)	0.789
Age (years)	1.03 (0.97–1.08)	0.392
Sex (male)	7.78 (0.94–64.74)	0.058
DM I + II	0.98 (0.22–4.39)	0.980
APACHE II score	1.19 (1.01–1.41)	**0.035***
SOFA score	1.1 (0.88–1.38)	0.391
SAPS II score	1.02 (0.97–1.07)	0.437
Days without immunosuppression	0.99 (0.96–1.03)	0.631
Community-acquired	0.33 (0.04–2.72)	0.302
Hospital-acquired	3.05 (0.37–25.35)	0.302
Fungal infection	0.97 (0.12–8.03)	0.974
MDR bacteria	0.32 (0.06–1.65)	0.173
G- infection	1.24 (0.24–6.4)	0.797
G+ infection	1.18 (0.14–9.84)	0.877
G- and G+ infection	0.62 (0.08–5.15)	0.658
Acute lung injury	2.13 (0.48–9.55)	0.323
Lactic acidosis	0.91 (0.2–4.05)	0.898
Serum bilirubin 20 μmol/l	0.46 (0.06–3.79)	0.467
Thrombocytopoenia	3.51 (0.79–15.72)	0.1
Septic shock	0.91 (0.2–4.05)	0.898
Mechanical ventilation	3.02 (0.67–13.52)	0.149
Duration of mechanical ventilation	1.01 (1.00–1.02)	**0.015***
RRT	21.16 (2.53–177.11)	**0.005***
Noradrenaline maximum dose	0.46 (0.02–10.99)	0.633

APACHE II, Acute Physiology and Chronic Health Evaluation II; BMI, body mass index; DM I + II, diabetes mellitus type I and type II; SOFA, Sequential Organ Failure Assessment score; SAPS II, Simplified Acute Physiology Score II; RRT, renal replacement therapy; HR, hazard ratio; CI, confidence interval; MDR, multidrug-resistant; G-, gram-negative bacteria; G+, gram-positive bacteria. Boldface indicates statistical significance where $p < 0.05$ (*).

TABLE 5B Univariable Cox regression of risk factors associated with kidney graft failure one year after enrollment ($n = 65$).

Variable	HR (95% CI)	*P*-value
Immunosuppression without MMF	0.20 (0.05–0.91)	**0.038***
APACHE II	1.25 (1.08–1.46)	**0.004***
Age	1.04 (0.98–1.10)	0.192
Hypertension	0.87 (0.11–7.24)	0.898
Prednisone only	4.68 (0.56–38.94)	0.154
Sex (male)	4.46 (0.54–37.08)	0.166
DM I + II	1.15 (0.26–5.16)	0.852
IHD	4.13 (0.8–21.28)	0.09
Cyclosporine	4.30 (0.83–22.19)	0.081
BMI	1.01 (0.87–1.17)	0.952
Tacrolimus	0.21 (0.05–0.94)	**0.041***
Sepsis patients	15.07 (1.84–1955.60)	**0.007***

APACHE II, Acute Physiology and Chronic Health Evaluation II; BMI, body mass index; DM I + II, diabetes mellitus type I and type II; HR, hazard ratio; CI, confidence interval; SIRS, systemic inflammatory response syndrome; MMF, mycophenolate mofetil; IHD, ischemic heart disease. Boldface indicates statistical significance where $p < 0.05$ (*).

of norepinephrine *per se* nor its maximum dose was associated with impaired or lost kidney graft function in kidney graft recipients with sepsis. In this context, it can be assumed that the fluid management and dosage of vasopressor circulatory support ensured the appropriate conditions for the preservation of kidney graft function.

In a study by the RESITRA group, crude one-year BSI-associated mortality in transplant recipients was 7.8% (5). However, in our study, 20% of sepsis patients died within one year, a difference possibly explained by variations in study design and cohort size. While the RESITRA study was multicenter in design, containing data on recipients of different solid organs as well as hematopoietic stem cells, our work was performed in a single transplant center and focused on kidney transplant recipients only.

Given that transplant patients are more vulnerable in a critical condition due to chronic immunosuppression and comorbidities, a tailored treatment approach is required. A recent retrospective multicenter study documented better results in the treatment of sepsis in immunosuppressed patients in hospitals that had a higher number of these specific patients (20). Even though the cohort of patients in this study was largely heterogeneous and transplanted patients formed only part of the cohort, it can be concluded that treatment of sepsis in transplant patients should be performed under the supervision of an experienced specialist.

Sepsis and its consequences have been the focus of many studies, but little is known about the consequences of sepsis in transplanted patients requiring long-term immunosuppression to prevent rejection (21). A retrospective multicenter cohort study, which examined in-hospital mortality of various organ transplant patients with sepsis (22), found that, contrary to expectations, in-hospital mortality was lower in transplanted than in non-transplanted patients. In this study, in-hospital mortality of transplant recipients with severe sepsis was 5.5%, whereas in non-transplanted patients it was 8.7%. In our cohort, in-hospital mortality rate was 6%. However, the comparison is not relevant given that we applied the current definition of sepsis (only sepsis, not severe sepsis according to the SSC definition). Furthermore, our cohort consisted of kidney transplant recipients only. Lower 28-day and 90-day mortality rates were also reported by another case-control study (23) in recipients of various organs (only 12.2% kidney) with bacteremic sepsis compared to non-transplanted patients. The overall 28-day mortality and 90-day mortality reported in this study was 8.1 and 14.6%, respectively. These findings may be attributed to a greater level of specialized care, a focus on detecting sepsis in these patients at an earlier stage, and the likely benefit of immunosuppression in the modulation of the inflammatory response.

Although reduction and/or withdrawal of immunosuppression is a generally accepted part of sepsis therapy in transplant recipients, data concering its impact on overall clinical outcomes and allograft function are scarce. Specifically, there is no consensus on the management of immunosuppressive drugs in critically ill patients with sepsis, nor is it fully clear whether short-term withdrawal for a necessary period of time in a sepsis setting leads to a significantly higher incidence of allograft rejection (21, 24, 25). Based on the biopsy findings in our cohort, it can be concluded that a transient reduction in immunosuppression during sepsis did not lead to the development of rejection.

In our study, we failed to demonstrate an association between temporary discontinuation of immunosuppression during sepsis and loss of kidney graft function or mortality within 1 year after sepsis.

Sepsis survivors have an increased risk of sepsis recurrence, which can be related to a compromised immune system, impaired organ function, or reduced functional reserve of the organism in response to an insult (16). Within one year after sepsis, we observed a higher incidence of hospital and community-acquired infections in kidney transplant patients with sepsis than in the control group with SIRS, even though the difference was not statistically significant. In both groups of patients, we observed a higher incidence of hospital-acquired infections than of community-acquired infections, a difference that trended toward statistical significance.

In agreement with previous findings (4, 5, 26), in our patients, the urinary tract proved the most common site as well as source of BSI, of which MDR microorganisms played a significant role.

The main strength of our study is the high homogenity of the cohort enrolled, which contained only kidney allograft recipients from a single center. Also, patients were treated by a uniform team and according to the same protocols. Sepsis was diagnosed and treated in line with recent guidelines (SSC), as was the reduction or possible withdrawal of immunosuppressive therapy. Another advantage of our study is its prospective design; most studies on sepsis in transplant patients are retrospective (22, 23, 27). The majority of previous studies have compared selected parameters in transplanted and non-transplanted patients with sepsis. In this context, our study is unusual in that it compares kidney transplant patients with sepsis and with SIRS. Our comparison of patients with sepsis and SIRS demonstrates the negative impact of infection and organ dysfunction on the prognosis of kidney recipients.

Nonetheless, our study has some limitations. Firstly, its observational design by itself. The main weakness relates to the to the small number of patients in the cohort. The study was restricted in its focus on carefully selecting patients meeting strict exclusion criteria. A major limitation in terms of recruiting patients was the condition of no antibiotic treatment within one month before enrollment, and requirement to be enrolled before the first dose of antibiotics was administered. As a result, some sepsis transplant patients meeting the other criteria were not enrolled and some degree of selection bias could arise. The control group included kidney transplant patients who were diagnosed with SIRS without infection during 30 days after transplantation.

In conclusion, our prospective single-center study confirms that sepsis in kidney transplant patients is associated with increased mortality and places them at high risk of losing kidney allograft function. However, it seems that SIRS without infection does not have negative consequences for one-year mortality and allograft function in kidney transplant patients. The requirement for renal replacement therapy in sepsis patients appears to have a particularly negative impact on the long-term function of the transplanted kidney.

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 Thomayer's Hospital and Institute for Clinical and Experimental Medicine, Prague, Czech Republic (Docket No.: G-16-06-17). The patients/participants provided their written informed consent to participate in this study.

Author contributions

MP: patient recruitment, data acquisition, analysis and interpretation, and manuscript preparation and critical revision. EU: data analysis and interpretation and manuscript preparation and critical revision. VI and JL: patient recruitment and data collection. OV: critical revision of the manuscript. PH: data analysis and interpretation. EK: article creation, concept and design, data analysis and interpretation, and manuscript preparation and critical revision. All authors contributed to this article and approved the submitted version.

Funding

This study was supported by Ministry of Health, Czechia, conceptual development of research organisations (Institute for Clinical and Experimental Medicine - IKEM, IN 00023001).

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.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.923524/full#supplementary-material

References

1. Koordinační středisko transplantací. *Coordination Center for Transplantation Czech Republic*. Available online at: https://kst.cz/#co_ctime (accessed June 15, 2022) (2019).

2. Viklicky O, Fronek J, Trunecka P, Pirk J, Lischke R. Organ transplantation in the Czech Republic. *Transplantation.* (2017) 101:2259–61. doi: 10.1097/TP.0000000000001871

3. Dharnidharka VR, Stablein DM, Harmon WE. Post-transplant infections now exceed acute rejection as cause for hospitalization: a report of the NAPRTCS. *Am J Transplant.* (2004) 4:384–9. doi: 10.1111/j.1600-6143.2004.00350.x

4. Karuthu S, Blumberg EA. Common infections in kidney transplant recipients. *Clin J Am Soc Nephrol.* (2012) 7:2058–70. doi: 10.2215/CJN.04410512

5. Moreno A, Cervera C, Gavalda J, Rovira M, de la Camara R, Jarque I, et al. Bloodstream infections among transplant recipients: results of a nationwide surveillance in Spain. *Am J Transplant.* (2007) 7:2579–86. doi: 10.1111/j.1600-6143.2007.01964.x

6. Kalil AC, Sandkovsky U, Florescu DF. Severe infections in critically ill solid organ transplant recipients. *Clin Microbiol Infect.* (2018) 24:1257–63. doi: 10.1016/j.cmi.2018.04.022

7. Bodro M, Sabe N, Tubau F, Llado L, Baliellas C, Roca J, et al. Risk factors and outcomes of bacteremia caused by drug-resistant ESKAPE pathogens in solid organ transplant recipients. *Transplantation.* (2013) 96:843–9. doi: 10.1097/TP.0b013e3182a049fd

8. Bellomo R, Kellum JA, Ronco C, Wald R, Martensson J, Maiden M, et al. Acute kidney injury in sepsis. *Intensive Care Med.* (2017) 43:826–8. doi: 10.1007/s00134-017-4755-7

9. Bagshaw SM, Lapinsky S, Dial S, Arabi Y, Dodek P, Wood G, et al. Cooperative Antimicrobial Therapy of Septic Shock (CATSS) Database Research Group. Acute kidney injury in septic shock: clinical outcomes and impact of duration of hypotension prior to initiation of antimicrobial therapy. *Intensive Care Med.* (2009) 35:871–81. doi: 10.1007/s00134-008-1367-2

10. Hjortrup PB, Haase N, Bundgaard H, Thomsen SL, Winding R, Pettila V, et al. Restricting volumes of resuscitation fluid in adults with septic shock after initial management: the CLASSIC randomised, parallel-group, multicentter feasibility trial. *Intensive Care Med.* (2016) 42:1695–705. doi: 10.1007/s00134-016-4500-7

11. De Backer D, Aldecoa C, Njimi H, Vincent J-L. Dopamine versus norepinephrine in the treatment of septic shock: a meta-analysis. *Crit Care Med.* (2012) 40:725–30. doi: 10.1097/CCM.0b013e31823778ee

12. Asfar P, Meziani F, Hamel J-F, Grelon F, Megarbane B, Anguel N, et al. SEPSISPAM Investigators. High versus low blood pressure target in patiens with septic shock. *N Engl J Med.* (2014) 370:1583–93. doi: 10.1056/NEJMoa1312173

13. Zarbock A, Kellum JA, Schmidt C, Van Aken H, Wempe C, Payenstadt H, et al. Effect of early vs delayed initiation of renal replacement therapy on mortality in critically ill patiens with acute kidney injury: the ELAN randomised clinical trial. *JAMA.* (2016) 315:2190–9. doi: 10.1001/jama.2016.5828

14. Tu GW, Ju MJ, Zheng YJ, Zhu DM, Xu M, Rong RM, et al. An interdisciplinary approach for renal transplant recipients with severe pneumonia: a single ICU experience. *Intensive Care Med.* (2014) 40:914–5. doi: 10.1007/s00134-014-3296-6

15. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. *Intensive Care Med.* (2017) 43:304–77. doi: 10.1007/s00134-017-4683-6

16. Evans L, Rhodes A, Alhazzani W, Antonelli M, Coopersmith CM, French C, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. *Crit Care Med.* (2021) 49:e1063–143.

17. Schlapbach LJ, Kissoo N, Alhawsawi A, Aljuaid MH, Daniel R, Gorordo-Delso LA, et al. World Sepsis Day: a global agenda to target a leading cause of morbidity and mortality. *Am J Physiol Lung Cell Mol Physiol.* (2020) 319:L518–22. doi: 10.1152/ajplung.00369.2020

18. Wang M, Jiang L, Zhu B, Li W, Du B, Kang Y, et al. The prevalence, risk factors, and outcome of sepsis in critically ill patients in China: a multicenter prospective cohort study. *Front Med.* (2020) 7:593808 . doi: 10.3389/fmed.2020.593808

19. Langenberg C, Bellomo R, May C, Wan L, Egi M, Morgera S. Renal blood flow in sepsis. *Crit Care.* (2005) 9:363–74. doi: 10.1186/cc3540

20. Greenberg JA, Hohmann SF, James BD, Shah RC, Hall JB, Kress JP, et al. Hospital volume of immunosuppressed patients with sepsis and sepsis mortality. *Ann Am Thorac Soc.* (2018) 15:962–9. doi: 10.1513/AnnalsATS.201710-819OC

21. Bafi AT, Tomotani DYV. Rezende de Freitas FG. Sepsis in solid-organ transplant patients. *Shock.* (2017) 47(Suppl 1):12–6. doi: 10.1097/SHK.0000000000000700

22. Donnelly JP, Locke JE, MacLennan PA, McGwin G Jr., Mannon RB, Safford MM, et al. Inpatient mortality among solid organ transplant recipients hospitalized for sepsis and severe sepsis. *Clin Infect Dis.* (2016) 63:186–94. doi: 10.1093/cid/ciw295

23. Kalil AC, Syed A, Rupp ME, Chambers H, Vargas L, Maskin A, et al. Is bacteremic sepsis associated with higher mortality in transplant recipients than in nontransplant patients? A matched case-control propensity-adjusted study. *Clin Infect Dis.* (2015) 60:1590. doi: 10.1093/cid/ciu789

24. Chou NK, Ko WJ, Chi NH, Chen YS, Yu HY, Hsu RB, et al. Sparing immunosuppression in heart transplant recipients with severe sepsis. *Transplant Proc.* (2006) 38:2145–6. doi: 10.1016/j.transproceed.2006.06.009

25. Bige N, Zafrani L, Lambert J, Peraldi MN, Snanoudj R, Reuter D, et al. Severe infections requiring intensive care unit admission in kidney transplant recipients: impact on graft outcome. *Transpl Infect Dis.* (2014) 16:588–96. doi: 10.1111/tid.12249

26. Kotagiri P, Chembolli D, Ryan J, Hughes PD, Toussaint ND. Urinary tract infections in the first year post-kidney transplantation: potential benefits of treating asymptomatic bacteriuria. *Transplant Proc.* (2017) 49:2070–5. doi: 10.1016/j.transproceed.2017.07.008

27. Schachtner T, Stein M, Reinke P. Sepsis after renal transplantation: clinical, immunological, and microbiological risk factors. *Transpl Infect Dis.* (2017) 19:e12695. doi: 10.1111/tid.12695

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 12 August 2022
DOI 10.3389/fmed.2022.863962

OPEN ACCESS

EDITED BY
Kathrin Eller,
Medical University of Graz, Austria

REVIEWED BY
Judith Kahn,
Medical University of Graz, Austria
Ajay Kumar Baranwal,
Armed Forces Medical College Pune, India

*CORRESPONDENCE
Wei Gao
gaowei2906@xjtufh.edu.cn
Junkai Du
68631784@qq.com

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 27 January 2022
ACCEPTED 28 July 2022
PUBLISHED 12 August 2022

CITATION
Li Y, Wang B, Wang L, Shi K, Zhao W, Gao S, Chen J, Ding C, Du J and Gao W (2022) Postoperative day 1 serum cystatin C level predicts postoperative delayed graft function after kidney transplantation. *Front. Med.* 9:863962. doi: 10.3389/fmed.2022.863962

Postoperative day 1 serum cystatin C level predicts postoperative delayed graft function after kidney transplantation

Yajuan Li [1,2], Bo Wang[1], Le Wang[1], Kewei Shi[1], Wangcheng Zhao[1], Sai Gao[1], Jiayu Chen[1], Chenguang Ding[3], Junkai Du[4]* and Wei Gao[1]*

[1]Department of Anesthesiology and Center for Brain Science and Center for Translational Medicine, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China, [2]Department of Anesthesiology, 521 Hospital of Norinco Group, Xi'an, China, [3]Department of Renal Transplantation, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China, [4]Department of Emergency, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

Background: Delayed graft function (DGF) commonly occurs after kidney transplantation, but no clinical predictors for guiding post-transplant management are available.

Materials and methods: Data including demographics, surgery, anesthesia, postoperative day 1 serum cystatin C (S-CysC) level, kidney functions, and postoperative complications in 603 kidney transplant recipients who met the enrollment criteria from January 2017 to December 2018 were collected and analyzed to form the Intention-To-Treat (ITT) set. All perioperative data were screened using the least absolute shrinkage and selection operator. The discrimination, calibration, and clinical effectiveness of the predictor were verified with area under curve (AUC), calibration plot, clinical decision curve, and impact curve. The predictor was trained in Per-Protocol set, validated in the ITT set, and its stability was further tested in the bootstrap resample data.

Result: Patients with DGF had significantly higher postoperative day 1 S-CysC level (4.2 ± 1.2 vs. 2.8 ± 0.9 mg/L; $P < 0.001$), serum creatinine level (821.1 ± 301.7 vs. 554.3 ± 223.2 μmol/L; $P < 0.001$) and dialysis postoperative (74 [82.2%] vs. 25 [5.9%]; $P < 0.001$) compared with patients without DGF. Among 41 potential predictors, S-CysC was the most effective in the parsimonious model, and its diagnostic cut-off value was 3.80 mg/L with the risk score (OR, 13.45; 95% CI, 8.02–22.57; $P < 0.001$). Its specificity

and sensitivity indicated by AUC was 0.832 (95% CI, 0.779–0.884; $P < 0.001$) with well fit calibration. S-CysC yielded up to 50% of clinical benefit rate with 1:4 of cost/benefit ratio.

Conclusion: The postoperative day 1 S-CysC level predicts DGF and may be used as a predictor of DGF but warrants further study.

KEYWORDS

kidney transplantation, serum cystatin C, delayed graft function, least absolute shrinkage and selection operator, area under curve, clinical decision curve

Introduction

Delayed graft function (DGF) is common after kidney transplantation. Once DGF occurs, 3.2 years graft survival decreases by 40%, 3 years death increases by 53%, and 3.5 years acute rejection increases by 38% (1–3). Current laboratory measurements, such as serum creatine (Scr), is inaccurate, and kidney graft biopsies are extremely invasive (4, 5). An early and non-invasive predictor of DGF is urgently needed to optimize timely postoperative clinical management. Pretransplant parameters have been analyzed with multivariate regressions for the formulation of predictive models that identify high-risk patients with DGF (6). The Irish model has an accuracy of 70% and has 16 clinical parameters of recipient- and donor-related factors, including cold ischemic time, donor terminal creatinine, donor body mass index, donation after cardiac death, and donor age (7). However, this model was built from the data of the United States Renal Data System in 2003 and does not meet the requirements of current clinical practice as marginal donor kidney grafts are widely used nowadays (8).

Scr, as the most used renal function biomarker, is used in the diagnosis of DGF, but it has low sensitivity when predicting DGF (9). Indeed, Scr may not rise before 50% loss of renal function and can be influenced by diet and muscle metabolism (10) and is the balance between creatinine production and excretion rather than a product of renal tubular injury (11). DGF in transplantation had been proved it was a specific manifestation of acute tubular necrosis (12). The products of renal tubular injury, such as kidney injury molecule-1 (KIM-1), interleukin-18 (IL-18), β-trace protein, and neutrophil gelatinase-associated lipocalin (NGAL), have promising diagnostic value for acute kidney injury or ischemic injury, but they have not been widely used clinically yet (13).

Previous studies reported Serum cystatin C (S-CysC) increased 24 h earlier than Scr after unilateral nephrectomy in kidney organ donors (14). S-CysC showed larger area under the curve than Scr in the prediction of postoperative renal dysfunction (0.73 vs 0.65; $P = 0.01$) (15). S-CysC is a 13.4 kDa cysteine protease inhibitor produced by nucleated cells at a constant rate and taken up by renal tubular epithelial cells without tubular secretion but is not re-absorbed into the circulation after being freely filtered by the glomeruli (16). Given that a significant increase in S-CysC level in the blood indicates tubular dysfunction, it has been used as a biomarker of glomerular filtration in chronic kidney disease (17–19). In prior study, the ROC curves showed that S-CysC had the largest AUC and the highest sensitivity and the highest diagnostic efficiency on postoperative day 1 after kidney transplantation (20). The first postoperative day S-CysC may be a potential predictor of DGF, but its clinical value has not been established and validated because of small sample size (18, 21). Therefore, we conducted this large-sample-size case control study to investigate the value of the first postoperative day S-CysC for predicting DGF.

Materials and methods

Study design

This study was approved by the ethics committee of the First Affiliated Hospital of Xi'an Jiaotong University (XJTUIAF2019LSL–008). This study was in accordance with the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis. Written informed consent was waived because de-identified retrospective data were used. All the medical procedures adhered to the principles of the Declaration of Helsinki and the Istanbul Declaration, and all renal grafts were voluntarily donated. All organs (except kinship donor kidneys) were obtained by the Organ Procurement Organization of the First Affiliated Hospital of Xi'an Jiaotong

Abbreviations: AUC, area under curve; DGF, delayed graft function; DCD, donation after cardiac death; DBD, donation after brain death; DCA, decision curve analysis; ITT set, Intention-To-Treat set; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; LASSO, least absolute shrinkage and selection operator; NGAL, neutrophil gelatinase-associated lipocalin; PP set, Per-Protocol set; ROC, receiver-operating characteristic curve; S-CysC, serum cystatin C; Scr, serum creatinine; β-TP, β-trace protein.

University, supervised by the Red Cross Society of Shaanxi Province, and were allocated by China Organ Transplant Response System. Receptors were included in this cohort when they met the following criteria: (1) older than 18, (2) underwent kidney transplantation for end-stage kidney disease under general anesthesia, and (3) admitted to the First Affiliated Hospital of Xi'an Jiaotong University from January 1, 2017 to December 31, 2018. Donors who met any of the following criteria were excluded from the study: (1) combined kidney and other organ transplantation, (2) re-transplantation, and (3) missing clinical records of the S-CysC (24 h) or creatinine (72 h). All perioperative data (clinical symptoms, perioperative characteristics, postoperative kidney function examination, and postoperative complications) in kidney transplant patients from January 1, 2017 to December 31, 2018 were collected, cross-checked, and de-identified by a team of experienced clinicians. DGF was defined as post-transplant graft kidney dysfunction with no spontaneous 10% decline in serum creatinine in 72 h, and dialysis is required 72 h after transplantation (22). The data of included patients formed the Intention-To-Treat (ITT) set, which served as the validation set, and patients without missing values, formed the Per-Protocol set (PP), which served as the training set.

Perioperative transplant procedures

The data of preoperative donors and recipients were obtained from the registry system of organ donation database and then evaluated and recorded in electronic medical record system by surgeons and anesthesiologists. Anesthesia management, surgery, and perioperative care followed standard institutional protocols. A triple immunosuppressive regimen with calcineurin inhibitors (CNIs), entericcoated mycophenolate sodium (EC-MPS; Myfortic, Novartis Pharma, Basel, Switzerland) and prednisone were treated all recipients. Cyclosporine A (CsA; Sandimmun Optoral, Novartis Pharma, Nuremberg, Germany) and tacrolimus (TAC; Prograf, Astellas Pharma, Deerfield, IL, United States) composed the CNIs. The initial dosages of CsA, TAC, EC-MPS and prednisone were 4.0–4.5 and 0.06–0.08 mg/kg/day, 1,080–1,440 and 10–20 mg/day, respectively. Rabbit anti-thymocyte globulin (rATG; thymoglobulin, Genzyme Ireland, Waterford, Ireland) at a dosage of 1.25–1.50 mg/kg/day as induction therapy during the surgery were given to all recipients in a total of 4–6 days after kidney transplantation.

Donor and recipient characteristics

The collective data of all the recipients and donors were obtained and presented. For each patient, the baseline characters were screened: age, gender, body mass index, nationality, smoke, dialysis (hemodialysis, peritoneal dialysis, and hemodialysis vs. peritoneal dialysis), dialysis duration, comorbidities (hypertension, diabetes, coronary heart disease, cerebral infarction, phthisis, and hepatitis), pathogenesis of end-stage kidney disease (chronic glomerulonephritis, IgA nephropathy, and other kidney disease). The donor characteristics were as follows: donor (donation after cardiac death, donation after brain death, and kinsfolk), right or left kidney, and duration of ischemia (warm ischemia and cold ischemia). Operation factors, such as American Society of Anesthesiologist classification, iliac fossa, operation location, vascular anastomosis (internal iliac artery and arteria iliac externa), and time of operation. Intraoperative medication (propofol, dexmedetomidine, sevoflurane, sufentanil, remifentanil, and cisatracuramide) and intake and output volumes (crystal, colloid, intraoperative blood transfusion, intraoperative blood plasma, bleeding, and urine volume) were collected. Postoperative kidney function indexes, including S-CysC, Scr, glomerular filtration rate (GFR), urea nitrogen (BUN), and uric acid (UA) on the first day after surgery were compared.

Statistical analyses

Between the DGF and non-DGF groups in the ITT set, the normally distributed continuous variables were presented as means ± standard deviations (SDs); otherwise, they were presented as medians (interquartile ranges). The categorical variables were reported as numbers (percentages). They were analyzed with independent-sample student's *t*-tests, Mann–Whitney *U* test, Chi-square test, and Fisher's exact test. All perioperative variables in the PP set were entered in the Least Absolute Shrinkage and Selection Operator (LASSO) selection process for the generation of a single predictive model of DGF. Missing predictor values in the ITT set ($N = 517$) were imputed through multiple imputation with chained equations. We used L1-penalized LASSO for multivariable analyses, augmented with 10-fold cross validation for internal validation. This logistic regression model penalizes the absolute size of the coefficients of a regression model to minimize the potential collinearity of variables measured from the same patient and model overfitting. The optimal diagnostic model and the most parsimonious model of LASSO regression were identified with minimum criteria and one standard error of the minimum criteria (the 1-SE criterion) in the 5 times multiple interpolation ITT sets. To compare the predictive effect of optimal model and the most parsimonious model in ITT set without multiple interpolation, the same items among the 5 optimal diagnostic models were collected by univariate analysis with $P < 0.1$, calculated their relative risk by multivariate analysis, and compared with the most parsimonious model by using the area under the receiver operating characteristic curve (AUC) and the DeLong method.

The internal validation of the single predictor was tested in the PP and ITT sets: (1) The predictive accuracy estimates and mean absolute error were calculated by 200 bootstrap resamples, (2) The calibration curves of the predictor on DGF were plotted and tested by Hosmer–Lemeshow test. (3) The optimal cut-off value of the single predictor was calculated by Youden's index, and the relative risk was calculated by univariate logistic regression. (4) The clinical value of the predictor for DGF diagnosis was finalized through decision curve analysis (DCA). (5) A clinical impact plot was used in depicting the estimated number of high-risk patients and the true positive cases. All data were analyzed using R software (version 4.0.2) and Empower (X&Y Solutions, Inc., Boston, MA, United States). Packages in R that were used in this study were "rms," "rmda" and "glmnet." The reported statistical significance levels were two-side, with a $P < 0.05$ considered to be statistically significant.

Results

Development cohort

A total 603 kidney transplant patients received kidney transplants between January 1, 2017 and December 31, 2018. The ITT set had 517 patients, and 310 of these patients had missing data and formed the PP set (Figure 1). No significant difference in DGF incidence was found between the PP (19.35%) and ITT (17.41%). No significant differences in the demographic and clinical characteristics of the recipients were found between the DGF (n = 90) and non-DGF (n = 427) groups except for pneumonia (12 [2.8%] vs. 7 [7.8%]; P = 0.023; Table 1). The incidence rate of DGF in the different types of donors were 18.3%, 16.7% and 14.3%, respectively, corresponding to circulatory death, brain-death and living donation. The DGF group had a longer operation time, larger doses of propofol and remifentanil, and lower urine volume ($P < 0.05$; Table 2). The DGF group showed worse kidney function values (S-CysC, Scr, GFR, UA, and BUN) on the postoperative 1st day and longer length of hospital stay and progressed higher incidence of hospitalized complications, such as postoperative severe cardiovascular events (cardiac failure, arrhythmia, and acute coronary attack), pulmonary infection, gastrointestinal hemorrhage, acute rejection, renal artery stenosis, renal venous thrombosis, perirenal hemorrhage, and postoperative dialysis (Table 3).

Predictor selection

In the PP set, 41 variables measured at the hospital admission (Tables 1, 2 and the kidney function variables on the first day after surgery of Table 3) were included in the LASSO regression. After the cross-validated error plot and the most parsimonious model of the LASSO regression, the S-CysC on the postoperative 1st day was identified as the single DGF predictor (Figure 2). S-CysC was the independent risk factor (β, 3.61; 95% CI, 2.53–5.15; $P < 0.001$), and the diagnostic cutoff value of the model was 3.80 mg/L (OR 10.96; 95% CI, 5.78–20.77; $P < 0.001$; Table 4). The sensitivity of PP set is 0.62 (0.48, 0.74) and the specificity is 0.87 (0.82, 0.91). The predictive effect of S-CysC on DGF preliminarily showed good discrimination with 0.797 (95% CI, 0.725–0.870; $P <$ 0.001) of AUC and well-fit calibration curves, yielding approximately 50% of clinical benefit rate and predicting positives cases with 1:4 cost/benefit ratio in the PP set on the basis of 19.35% DGF incidence (Supplementary Figures 1, 2). The five times multiple imputation data of LASSO regression agreed with the S-CysC as the single DGF predictor as the most parsimonious model (Supplementary Figure 3). The postoperative 1st day S-CysC and Scr, preoperative pneumonia, and the interoperative dose of propofol between the DGF and No-DGF group showed the P value less than 0.1 (Supplementary Table 1). The postoperative 1st day S-CysC and preoperative pneumonia were the risk factors of DGF (β: 3.52, 95% CI: 2.43–5.10; OR: 3.45, 95%CI: 1.03–11.61, respectively; Supplementary Table 2).

Predictor validation

The AUC of S-CysC on DGF in ITT set was 0.832 (95% CI, 0.779–0.884; $P <$ 0.001), and the 200 repetitions of bootstrapping validation further confirmed this value. The calibration curve of S-CysC for the probability of DGF indicated the consistency between prediction and observation in the ITT dataset (Figure 3). The Hosmer–Lemeshow test between the apparent red line (S-CysC predictive model) and the ideal dotted line had no significant difference (P = 0.142), suggesting that the predictive model fitted well with the ideal model The decision curve analysis demonstrated that using this model to predict the diagnosis of DGF would have more benefits than those in all dialysis or non-dialysis patient when the threshold probability of a patient was 3–78% (Figure 4A).The incidence of DGF was 17.41% in the ITT set, and the net benefit was 50% when the model was used to make the clinical decision, compared with the -20% of net benefit in all dialysis patients and 0% of net benefit in non-dialysis patients. The clinical impact curve of the S-CysC based on the risk model showed the predicted positives cases included all the actual positives cases with 1:4 cost/benefit ratio based on the incidence of DGF (Figure 4B). Both the optimal diagnostic model and the most parsimonious model showed well predictive AUC, accuracy, sensitivity, and specificity, and had no significantly different in AUC (0.835, 95%CI 0.784–0.886 vs. 0.832, 95%CI 0.779–0.884; P = 0.584; Supplementary Table 3).

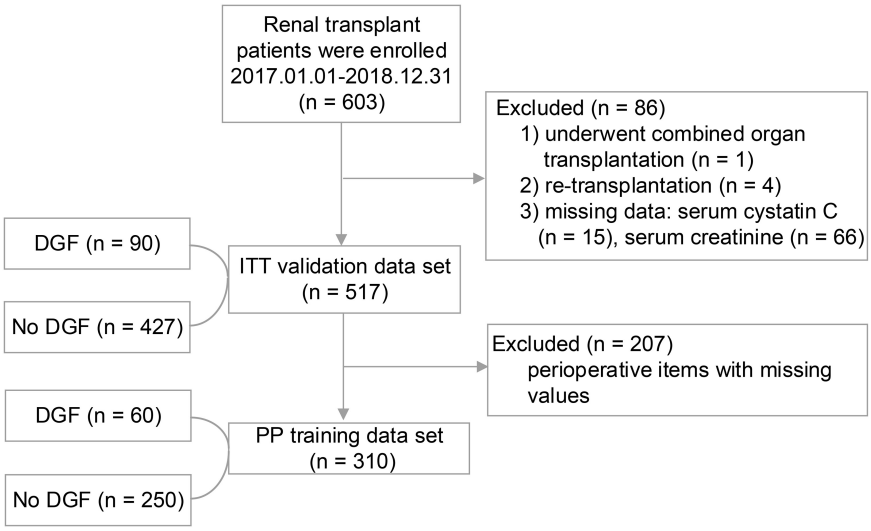


FIGURE 1
The study flowchart. During the study period, 603 patients underwent renal transplant surgery. A total of 86 (14.26%) patients were excluded because they did not satisfy the inclusion criteria. In the end, a total of 517 (Intention-To-Treat set) first time renal transplant recipients were included in the study, of whom 207 were excluded from the Per-Protocol analysis.

TABLE 1 Demographics and clinical characteristics among patients in the development cohort who did or did not develop DGF.

Characteristic	Total	Non-DGF	DGF	*P*-value
Male, *n* (%)	372 (72.0%)	303 (71.0%)	69 (76.7%)	0.176
Age, mean (SD), y	35.9 (9.2)	35.6 (9.1)	37.7 (9.4)	0.050
BMI, mean (SD)	21.0 (3.0)	21.0 (3.0)	21.3 (3.2)	0.457
Smoke, *n* (%)	162 (31.3%)	131 (30.7%)	31 (34.4%)	0.484
Nationality, *n* (%)				0.805
Han Nationality	481 (93.0%)	396 (92.7%)	85 (94.4%)	
Hui Nationality	19 (3.7%)	16 (3.7%)	3 (3.3%)	
Other Nationality	17 (3.3%)	15 (3.5%)	2 (2.2%)	
Dialysis, *n* (%)				0.770
Hemodialysis	431 (83.5%)	358 (84.0%)	73 (81.1%)	
Peritoneal dialysis	57 (11.0%)	46 (10.8%)	11 (12.2%)	
Hemodialysis vs. peritoneal dialysis	28 (5.4%)	22 (5.2%)	6 (6.7%)	
Dialysis duration, median (IQR), m	15.0 (8.0–28.0)	15.0 (8.0–27.8)	14.0 (7.6–27.8)	0.644
Comorbidities, *n* (%)				
Hypertension	403 (78.0%)	328 (76.8%)	75 (83.3%)	0.175
Diabetes	15 (2.9%)	11 (2.6%)	4 (4.4%)	0.337
Coronary heart disease	21 (4.1%)	15 (3.5%)	6 (6.7%)	0.168
Cerebral infarction	19 (3.7%)	14 (3.3%)	5 (5.6%)	0.297
Pneumonia	19 (3.7%)	12 (2.8%)	7 (7.8%)	**0.023**
Hepatitis	36 (7.0%)	27 (6.3%)	9 (10.0%)	0.213
Causes of ESRD, *n* (%)				
Chronic glomerulonephritis	394 (76.2%)	324 (75.9%)	70 (77.8%)	0.701
IgA nephropathy	79 (15.3%)	65 (15.2%)	14 (15.6%)	0.936
Other kidney disease	57 (11.0%)	51 (11.9%)	6 (6.7%)	0.335

BMI, body mass index; ESRD, end stage renal disease.

TABLE 2 Surgical information is presented as mean (SD), median (IQR) or number (%).

Characteristic	Total	Non-DGF	DGF	*P*-value
Donor, *n* (%)				
DCD	180 (41.9%)	144 (40.3%)	36 (49.3%)	0.525
DBD	180 (41.9%)	152 (42.6%)	28 (38.4%)	0.884
Kinsfolk	70 (16.3%)	61 (17.1%)	9 (12.3%)	0.512
Kidney side, *n* (%)				0.912
Left	273 (52.8%)	226 (52.9%)	47 (52.2%)	
Right	244 (47.2%)	201 (47.1%)	43 (47.8%)	
Duration of ischemia				
Warm ischemia (min)	8.5 (4.8)	8.5 (4.8)	8.5 (4.5)	0.958
Cold ischemia (h)	5.9 (3.6)	5.9 (3.7)	5.7 (2.9)	0.663
Notch location, *n* (%)				0.620
Left iliac fossa	149 (28.8%)	125 (29.3%)	24 (26.7%)	
Right iliac fossa	368 (71.2%)	302 (70.7%)	66 (73.3%)	
Vascular anastomosis, *n* (%)				0.050
Internal iliac artery	166 (32.1%)	145 (34.0%)	21 (23.3%)	
Arteria iliac externa	351 (67.9%)	282 (66.0%)	69 (76.7%)	
ASA, *n* (%)				0.965
II	57 (11.0%)	47 (11.0)	10 (11.0%)	
III	293 (56.7%)	241 (56.4%)	52 (57.8)	
IV	167 (32.3%)	139 (32.6%)	28 (31.1%)	
Intraoperative medication, mean (SD) or median (IQR)				
Propofol, mg	1,412.3 (624.2)	1,381.4 (600.6)	1,558.6 (711.5)	0.014
Sufentanil, μg	30.6 (5.8)	30.6 (5.9)	30.4 (5.3)	0.765
Remifentanil, mg	2,203.1 (1,669.4–2,921.6)	2,161.2 (1,656.0–2,837.3)	2,468.3 (1,761.0–3,334.5)	**0.035**
Cisatracuramide, mg	23.8 (8.5)	23.5 (8.4)	25.3 (9.1)	0.067
Dexmedetomidine, μg	100.1 (70.0–149.7)	95.3 (70.0–146.6)	116.9 (72.0–155.8)	0.133
Sevoflurane, ml	4.9 (1.4)	4.9 (1.6)	5.0 (1.6)2	0.511
Operative Time, h	3.3 (0.7)	3.3 (0.7)	3.5 (0.8)	**0.029**
Intraoperative volume infusion and loss, mean (SD) or median (IQR)				
Crystal, ml	1,904.9 (546.3)	1,899.8 (563.5)	1,929.4 (457.8)	0.640
Colloid, ml	894.6 (340.4)	883.6 (330.5)	946.7 (381.7)	0.110
Red blood cells, *n*%	118 (22.9%)	100 (23.5%)	18 (20.0%)	0.476
Plasma, *n*%	49 (9.5%)	41 (9.6%)	8 (8.9%)	0.829
Bleeding, ml	150.0 (100.0–200.0)	150.0 (100.0–200.0)	150.0 (100.0–300.0)	0.678
Urine volume, ml	300.0 (150.0–500.0)	300.0 (200.0–500.0)	200.0 (100.0–300.0)	<**0.001**

DBD, donation after brain death; DCD, donation after cardiocirculatory death; ASA, American Standards Association. The *P* values in hold is $P < 0.05$.

Discussion

Our current retrospective study investigated the postoperative first day clinical routine renal function biomarker of S-CysC as the single predictor of DGF by the most parsimonious model of LASSO regression. The predictive cutoff value of S-CysC showed 3.80 mg/L, whose accuracy, sensitivity and specificity, respectively, were 83.6, 67.8, and 86.9%, and whose AUC had no significantly different compared with the AUC of optimal model (**Supplementary Tables 1–3**). The PP set, bootstrapping ITT set, and multiple imputation ITT set corresponded to the S-CysC AUCs of 0.797, 0.828, and 0.832, respectively. S-CysC showed a well-fitted calibration curve, yielding approximately 50% of clinical benefit rate and predicting positives cases with 1:4 cost/benefit ratio. The predictive effect was repeatedly validated in the ITT set with multiple interpolation data and in the data of bootstrap resamples. Our single center and retrospective study design suggested that the first postoperative S-CysC level may predict DGF.

The donor, recipient, and perioperative-related risk factors contributed to DGF incidence in 10–30% patients after kidney engraftment (23, 24). Previous predictive models focused on preoperative transplant decision, and the widely used

TABLE 3 Renal function on the first day after surgery, postoperative complications while in hospital and length of stay.

Characteristic	Total	Non-DGF	DGF	*P*-value
Kidney function				
Serum cystatin C, mg/L	3.0 (1.1)	2.8 (0.9)	4.2 (1.2)	<**0.001**
Serum UA, μmol/L	368.9 (94.0)	361.7 (91.9)	402.9 (97.3)	<**0.001**
Serum BUN, mmol/L	18.1 (6.1)	17.4 (5.7)	21.6 (6.6)	<**0.001**
Serum eGFR, ml/min/1.73m^2	9.3 (6.7–13.3)	10.2 (7.3–14.8)	6.2 (4.8–9.0)	<**0.001**
Serum SCR, μmol/L	600.8 (259.0)	554.3 (223.2)	821.1(301.7)	<**0.001**
Postoperative complications in hospital, *n*%				
Cardiovascular events	24 (4.6%)	13 (3.0%)	11 (12.2%)	<**0.001**
Pulmonary infection	54 (10.4%)	34 (8.0%)	20 (22.2%)	<**0.001**
Gastrointestinal hemorrhage	5 (1.0%)	2 (0.5%)	3 (3.3%)	**0.012**
CRAD	2 (0.4%)	1 (0.2%)	1 (1.1%)	0.223
Renal infarction	2 (0.4%)	1 (0.2%)	1 (1.1%)	0.223
Acute rejection	9 (1.7%)	4 (0.9%)	5 (5.6%)	**0.002**
RAS	7 (1.4%)	1 (0.2%)	6 (6.7%)	<**0.001**
RVT	15 (2.9%)	4 (0.9%)	11 (12.2%)	<**0.001**
Perirenal infection	12 (2.3%)	10 (2.3%)	2 (2.2%)	0.945
Perirenal hemorrhage	6 (1.2%)	3 (0.7%)	3 (3.3%)	**0.034**
Urinary fistule	29 (5.6%)	23 (5.4%)	6 (6.7%)	0.631
Postoperative dialysis	41 (7.930%)	25 (5.855%)	16 (17.778%)	<**0.001**
Length of stay, day	21.5 (9.3)	20.0 (7.3)	27.5 (11.8)	<**0.001**

Data are presented as mean (SD), median (IQR) or number (%). Cardiovascular events are defined as postoperative cardiac failure, arrhythmia and acute coronary attack. GFR, glomerular filtration rate; SCR, serum creatinine; BUA, urea nitrogen; UA, uric acid; CRAD, chronic renal allograft dysfunction; RAS, renal artery stenosis; RVT, renal venous thrombosis. Postoperative dialysis: As an adverse event, during hospitalization after kidney transplantation, Incidence of dialysis 72 h after surgery. The *P* values in hold is $P < 0.05$.

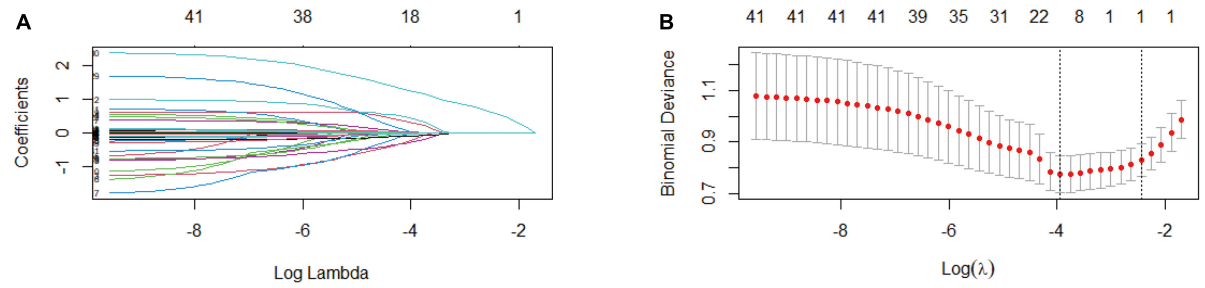


FIGURE 2
Exclude all missing values feature selection (Per-Protocol set) using the least absolute shrinkage and selection operator (LASSO) binary logistic regression model (n = 310). **(A)** LASSO coefficient profiles of the 41 baseline features, where the minimum lambda resulted in the single candidates of serum cystatin C (S-CysC) with non-zero coefficients. **(B)** Dotted vertical lines in the LASSO regression showed the optimal diagnostic model (left vertical line) and the most parsimonious model (right vertical line). The LASSO regression identifies S-CysC as the single predictor from the most parsimonious model.

TABLE 4 The logistic regression serum cystatin C and its Youden's index cut-off point.

Exposure	PP set (N = 310) β/OR 95% CI	ITT set (N = 517) β/OR 95% CI
Serum cystatin C	3.61 (2.53, 5.15) < **0.001**	3.83 (2.89, 5.08) < **0.001**
Serum cystatin C		
<3.80 mg/L	1	1
≥3.80 mg/L	10.96 (5.78, 20.77) < **0.001**	13.45 (8.02, 22.57) < **0.001**

The *P* values in hold is $P < 0.05$.

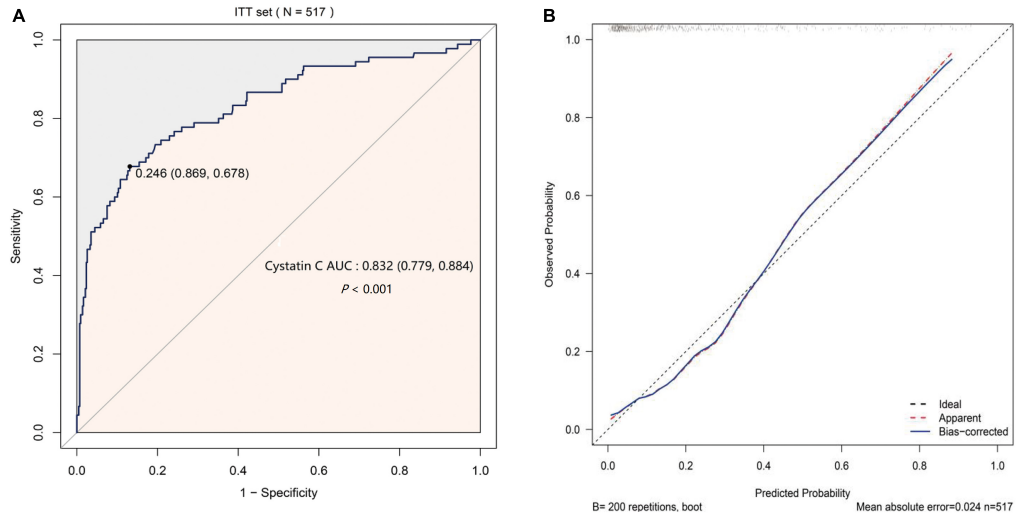


FIGURE 3
The predicted validation of serum cystatin C (S-CysC) in the Intention-To-Treat set with all values feature selection (n = 517). **(A)** The receiver operating characteristic curve of single S-CysC. **(B)** The calibration curve of single S-CysC on the delayed graft function (DGF) prediction. The ideal line showed the ideal estimated probabilities correspond to the actual observation; the apparent red line showed the predictive capability of the model; the bias-corrected blue line showed the predictive stability of the bootstrap corrected model. The apparent red line and the ideal dotted line had no significant different by Hosmer–Lemeshow test (P = 0.142), suggesting a well fit between the model and the ideal data. The apparent red line well coincided with bias-corrected blue line illustrated the stability of the prediction of S-CysC on DGF.

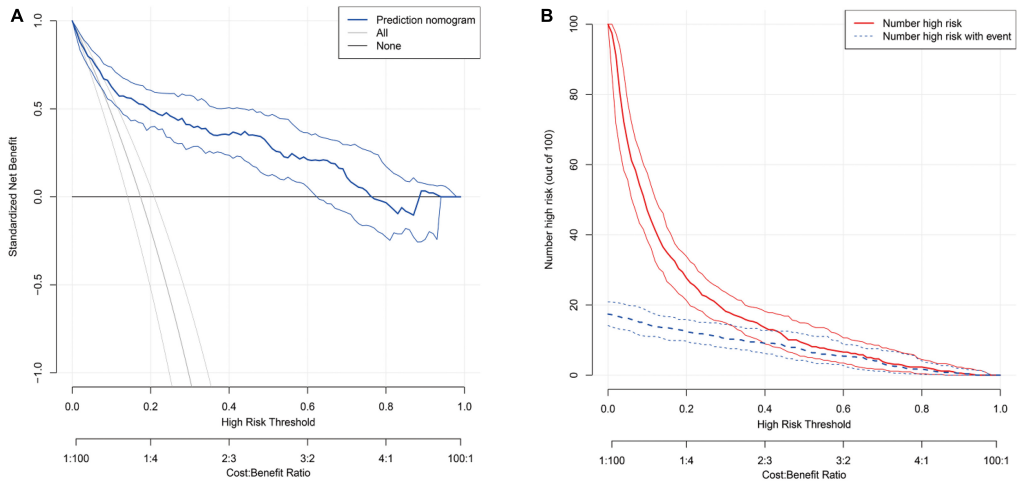


FIGURE 4
(A) The decision curve for the predicting delayed graft function (DGF) in the Intention-To-Treat set (n = 517). The thick blue line represents the model; the light gray line represents the assumption that all patients have DGF; the thick gray line represents the assumption that all patients have non-DGF. The threshold probability in the Per-Protocol set and Intention-To-Treat data set both are about 20%, using serum cystatin C (S-CysC) to diagnose DGF could yield a clinical benefit rate of 50%. **(B)** The clinical impact curve of the S-CysC based risk model showed the predicted positives cases included all the actual positives cases with 1:4 cost/benefit ratio. Of 100 patients, the heavy red solid line showed the total number who would be deemed high risk for each risk threshold. The dotted blue line shows how many of those would be true positives (cases).

marginal kidneys for limited donor kidney compelled clinicians to optimize postoperative clinical management decisions (25). Previous models consisted of various pretransplant items tested with simple multivariate regression and only AUC and related P value (7, 26). A randomized controlled trial with 78 patients reported that S-CysC combined with recipient's and donor's age, cold ischemia time, and urine output can predict DGF with 0.89 of AUC (18). A prospective cohort study with 40 patients reported that a formula with Scr, malondialdehyde, and S-CysC predicts DGF with 0.96 of AUC (21). However, neither of these

studies proved the predictive effect of single S-CysC on DGF. Our study demonstrated the S-CysC is a single predictor of DGF with different predictor selections and verification. In addition, S-CysC as the single predictor of DGF was trained with LASSO and logistic regression from all preoperative and interoperative variables in the PP set (**Figure 2**). The AUC of S-CysC was 0.797 (95% CI, 0.725–0.870; $P < 0.001$). S-CysC had 50% of the net benefit of the 1:4 cost/benefit ratio based on 19.35% of the DGF incidence (**Supplementary Figures 1**, **2**). Further, S-CysC was verified in the ITT set, in which the AUC was 0.832 (95% CI, 0.779–0.884; $P < 0.001$), and the 200 repetitions of bootstrapping validation further confirmed that the AUC was 0.828. The calibration plot diagram showed a good consistency between the actual and predicted diagnoses. The Hosmer–Lemeshow test further illustrated the predicted diagnoses, and the ideal dotted line had no significant difference (**Figure 3**). The kidney transplantation patients obtained a net benefit of 50% from the clinical decision of the model treatment. The net benefit had a 1:4 cost/benefit ratio based on 17.41% incidence of the DGF groups. All dialysis patients had -20% net benefits, and non-dialysis patients had 0 net benefit (**Figure 4**).

In this study, the Scr levels of the DGF group increased by 266.8 μmol/L relative to those of the non-DGF group ($P < 0.001$), but Scr level was not selected as the single predictor by the LASSO regression. The cohort study with 91 patients reported that Scr on postoperative first day is not predictive for AUC 0.53 (95% CI, 0.35–0.71) (27). Scr is unfit as predictor because it is derived from the balance between creatinine production and excretion, delaying the diagnosis of acute kidney injury for 48–72 h (28). Several renal tubular injury biomarkers, such as KIM-1, IL-18, and NGAL showed AUC values of 0.50 (95% CI, 0.36–0.64), 0.82 (95% CI, 0.72–0.92), and 0.82 (95% CI, 0.72–0.92) but still not available for routine use (27). S-CysC, as one of the routine renal function items, normally is reabsorbed by renal tubular epithelial cells with a low blood concentration, but it is significantly increased once the tubular is injured (29). The retrospective analysis with 47 patients showed that S-CysC, serum NGAL, and urine NGAL reflected renal function sensitively, and S-CysC reached to 4.77 mg/L with a sensitivity of 0.818 and specificity of 0.889 (30).

The strength of our study were the logical and strict predictor selection, verification, and manifestation in a large sample size of 517 patients. S-CysC was screened from all perioperative data by the most parsimonious diagnostic LASSO regression of DGF. Meanwhile, AUC, the clinical utility of the model, DCA, and clinical impact curve analysis were all implemented. Finally, the predictive effect was validated with the ITT set, bootstrap resample data, and multiple interpolation data. The postoperative first clinical routine S-CysC as a single predictor of DGF facilitates the postoperative individual patient management and hospital resources allocation in the high-risk patients with DGF. The DGF high-risk patients will be performed ultrasound examination to exclude surgery-related complications; adjusted the immunosuppressors; provided critical care or dialysis whenever need. In the future, however, the limitation of our study is a single center and retrospective study and hence its value to predict DGF warrants further prospective study. Meanwhile, it would have been more appropriate to combine S-CysC with the biomarkers of renal tubular injury, such as KIM-1, IL-18, and NGAL. Multi-biomarkers study would have helped to characterize better the complexity of DGF.

In conclusion, the postoperative first day S-CysC level may be a single predictor of DGF with good discrimination, calibration, and clinical benefit and may be used in routine clinical use, although validation studies are still needed.

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 authors.

Ethics statement

The studies involving human participants were reviewed and approved by the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

YL and WG: study conception. YL, BW, LW, KS, WZ, SG, JC, CD, and JD: acquisition or interpretation of data. WG and YL: statistical analyses. YL, BW, and WG: drafted the manuscript. YL, BW, WG, CD, and JD: critically revised the manuscript for important intelectual content. WG: obtained funding. WG, YL, BW, LW, KS, WZ, SG, CD, and JD: provided administrative, technical, or material support. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the National Nature Science Foundation of China (Nos. 81771485 and 81971290), the Key Research and Development Program of Shaanxi Province (No. 2020SF-136), the Fundamental Research Funds for the Central Universities (No. xjj2018262), and Young Talent Support Plan of Shaanxi Province; The Clinical Research Award of the First Affiliated Hospital of Xi'an Jiaotong University, China (No. XJTU1AF2021CRF-012).

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.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.863962/full#supplementary-material

References

1. Yarlagadda SG, Coca SG, Formica RN Jr., Poggio ED, Parikh CR. Association between delayed graft function and allograft and patient survival: a systematic review and meta-analysis. *Nephrol Dial Transplant.* (2009) 24:1039–47. doi: 10.1093/ndt/gfn667

2. Tapiawala SN, Tinckam KJ, Cardella CJ, Schiff J, Cattran DC, Cole EH, et al. Delayed graft function and the risk for death with a functioning graft. *J Am Soc Nephrol.* (2010) 21:153–61. doi: 10.1681/ASN.2009040412

3. Perico N, Cattaneo D, Sayegh MH, Remuzzi G. Delayed graft function in kidney transplantation. *Lancet.* (2004) 364:1814–27. doi: 10.1016/s0140-6736(04)17406-0

4. Musial K, Augustynowicz M, Miskiewicz-Migon I, Kalwak K, Ussowicz M, Zwolinska D. Clusterin as a new marker of kidney injury in children undergoing allogeneic hematopoietic stem cell transplantation-a pilot study. *J Clin Med.* (2020) 9:2599. doi: 10.3390/jcm9082599

5. Preda A, Van Dijk LC, Van Oostaijen JA, Pattynama PM. Complication rate and diagnostic yield of 515 consecutive ultrasound-guided biopsies of renal allografts and native kidneys using a 14-gauge Biopty gun. *Eur Radiol.* (2003) 13:527–30. doi: 10.1007/s00330-002-1482-3

6. Kawakita S, Beaumont JL, Jucaud V, Everly MJ. Personalized prediction of delayed graft function for recipients of deceased donor kidney transplants with machine learning. *Sci Rep.* (2020) 10:18409. doi: 10.1038/s41598-020-75473-z

7. Irish WD, McCollum DA, Tesi RJ, Owen AB, Brennan DC, Bailly JE, et al. Nomogram for predicting the likelihood of delayed graft function in adult cadaveric renal transplant recipients. *J Am Soc Nephrol.* (2003) 14:2967–74. doi: 10.1097/01.asn.0000093254.31868.85

8. Grossberg JA, Reinert SE, Monaco AP, Gohh R, Morrissey PE. Utility of a mathematical nomogram to predict delayed graft function: a single-center experience. *Transplantation.* (2006) 81:155–9. doi: 10.1097/01.tp.0000188621.54448.c8

9. Yuan SM. Acute kidney injury after pediatric cardiac surgery. *Pediatr Neonatol.* (2019) 60:3–11. doi: 10.1016/j.pedneo.2018.03.007

10. Thameem F, Voruganti VS, Blangero J, Comuzzie AG, Abboud HE. Evaluation of neurotrophic tyrosine receptor kinase 2 (NTRK2) as a positional candidate gene for variation in estimated glomerular filtration rate (eGFR) in Mexican American participants of San Antonio Family Heart study. *J Biomed Sci.* (2015) 22:23. doi: 10.1186/s12929-015-0123-5

11. Yildirim D, Donmez T, Sunamak O, Mirapoglu S, Hut A, Erdogan NR, et al. The effects of prolonged CO2 insufflation on kidney function in a rat pneumoperitoneum model. *Wideochir Inne Tech Maloinwazyjne.* (2017) 12:125–34. doi: 10.5114/wiitm.2017.67210

12. Chen R, Wang H, Song L, Hou J, Peng J, Dai H, et al. Predictors and one-year outcomes of patients with delayed graft function after deceased donor kidney transplantation. *BMC Nephrol.* (2020) 21:526. doi: 10.1186/s12882-020-02181-1

13. Han F, Wan S, Sun Q, Chen N, Li H, Zheng L, et al. Donor plasma mitochondrial dna is correlated with posttransplant renal allograft function. *Transplantation.* (2019) 103:2347–58. doi: 10.1097/TP.0000000000002598

14. Breidthardt T, Sabti Z, Ziller R, Rassouli F, Twerenbold R, Kozhuharov N, et al. Diagnostic and prognostic value of cystatin C in acute heart failure. *Clin Biochem.* (2017) 50:1007–13. doi: 10.1016/j.clinbiochem.2017.07.016

15. Lee SH, Youn YN, Choo HC, Lee S, Yoo KJ. Cystatin C as a predictive marker of renal dysfunction and mid-term outcomes following off-pump coronary artery bypass grafting. *Heart.* (2015) 101:1562–8. doi: 10.1136/heartjnl-2015-307986

16. Filler G, Bhayana V, Schott C. Diaz-Gonzalez de Ferris ME. How should we assess renal function in neonates and infants? *Acta Paediatr.* (2021) 110:773–80. doi: 10.1111/apa.15557

17. Levey AS, James MT. Acute kidney injury. *Ann Intern Med.* (2017) 167:ITC66–80. doi: 10.7326/AITC201711070

18. Hall IE, Doshi MD, Poggio ED, Parikh CR. A comparison of alternative serum biomarkers with creatinine for predicting allograft function after kidney transplantation. *Transplantation.* (2011) 91:48–56. doi: 10.1097/TP.0b013e3181fc4b3a

19. Ismail OZ, Bhayana V, Kadour M, Lepage N, Gowrishankar M, Filler G. Improving the translation of novel biomarkers to clinical practice: the story of cystatin C implementation in Canada: a professional practice column. *Clin Biochem.* (2017) 50:380–4. doi: 10.1016/j.clinbiochem.2017.01.005

20. Zhou C, Chen Y, He X, Xue D. The value of cystatin C in predicting perioperative and long-term prognosis of renal transplantation. *Scand J Clin Lab Invest.* (2022) 82:1–5. doi: 10.1080/00365513.2021.1989714

21. Fonseca I, Reguengo H, Oliveira JC, Martins LS, Malheiro J, Almeida M, et al. A triple-biomarker approach for the detection of delayed graft function after kidney transplantation using serum creatinine, cystatin C, and malondialdehyde. *Clin Biochem.* (2015) 48:1033–8. doi: 10.1016/j.clinbiochem.2015.07.007

22. Mogulla MR, Bhattacharjya S, Clayton PA. Risk factors for and outcomes of delayed graft function in live donor kidney transplantation – a retrospective study. *Transpl Int.* (2019) 32:1151–60. doi: 10.1111/tri.13472

23. Bahl D, Haddad Z, Datoo A, Qazi YA. Delayed graft function in kidney transplantation. *Curr Opin Organ Transplant.* (2019) 24:82–6. doi: 10.1097/mot.0000000000000604

24. Mezzolla V, Pontrelli P, Fiorentino M, Stasi A, Pesce F, Franzin R, et al. Emerging biomarkers of delayed graft function in kidney transplantation. *Transplant Rev (Orlando).* (2021) 35:100629. doi: 10.1016/j.trre.2021.100629

25. Khalid U, Newbury LJ, Simpson K, Jenkins RH, Bowen T, Bates L, et al. A urinary microRNA panel that is an early predictive biomarker of delayed graft function following kidney transplantation. *Sci Rep.* (2019) 9:3584. doi: 10.1038/s41598-019-38642-3

26. Irish WD, Ilsley JN, Schnitzler MA, Feng S, Brennan DC. A risk prediction model for delayed graft function in the current era of deceased donor renal transplantation. *Am J Transplant.* (2010) 10:2279–86. doi: 10.1111/j.1600-6143.2010.03179.x

27. Hall IE, Yarlagadda SG, Coca SG, Wang Z, Doshi M, Devarajan P, et al. IL-18 and urinary NGAL predict dialysis and graft recovery after kidney transplantation. *J Am Soc Nephrol.* (2010) 21:189–97. doi: 10.1681/ASN.2009030264

28. Sterling KA, Tehrani T, Rudnick MR. Clinical significance and preventive strategies for contrast-induced nephropathy. *Curr Opin Nephrol Hypertens.* (2008) 17:616–23. doi: 10.1097/MNH.0b013e32830f45a3

29. Bang JY, Kim SG, Oh J, Kim SO, Go YJ, Hwang GS, et al. Impact of remote ischemic preconditioning conducted in living kidney donors on renal function in donors and recipients following living donor kidney transplantation: a randomized clinical trial. *J Clin Med.* (2019) 8:713. doi: 10.3390/jcm8050713

30. Li F, Hu L, Zhao X, Ge W, Pan H, Zhang W, et al. The value of cystatin C and urinary and serum neutrophil gelatinase-associated lipocalin during the perioperative period of renal transplantation. *Transl Androl Urol.* (2019) 8:432–41. doi: 10.21037/tau.2019.08.12

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 25 August 2022
DOI 10.3389/fmed.2022.928567

OPEN ACCESS

EDITED BY
Xiaopeng Hu,
Capital Medical University, China

REVIEWED BY
Yi Dong,
Fudan University, China
Cheng Yang,
Fudan University, China

*CORRESPONDENCE
Sarah Friedl
sarah.friedl@posteo.de

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 25 April 2022
ACCEPTED 29 July 2022
PUBLISHED 25 August 2022

CITATION
Friedl S, Jung EM, Bergler T, Tews HC, Banas MC, Banas B and Putz FJ (2022) Factors influencing the time-intensity curve analysis of contrast-enhanced ultrasound in kidney transplanted patients: Toward a standardized contrast-enhanced ultrasound examination.
Front. Med. 9:928567.
doi: 10.3389/fmed.2022.928567

Factors influencing the time-intensity curve analysis of contrast-enhanced ultrasound in kidney transplanted patients: Toward a standardized contrast-enhanced ultrasound examination

Sarah Friedl[1]*, Ernst Michael Jung[2], Tobias Bergler[1], Hauke C. Tews[3], Miriam C. Banas[1], Bernhard Banas[1] and Franz Josef Putz[1]

[1]Department of Nephrology, University of Regensburg, Regensburg, Germany, [2]Department of Radiology, Interdisciplinary Ultrasound, University of Regensburg, Regensburg, Germany, [3]Department of Internal Medicine I, Gastroenterology, Hepatology, Endocrinology, Rheumatology and Infectious Diseases, University Hospital, Regensburg, Germany

Background: Time-intensity curve analysis (TIC analysis) based on contrast-enhanced ultrasound (CEUS) provides quantifiable information about the microcirculation of different tissues. TIC analysis of kidney transplantations is still a field of research, and standardized study protocols are missing though being mandatory for the interpretation of TIC parameters in the clinical context. The aim of this study was to evaluate the impact of different sizes and forms of regions of interest (ROIs) on the variance of different TIC parameters and the level of interoperator variance between the different ROI methods in kidney transplantations.

Methods: In 25 renal transplanted patients, 33 CEUS of the transplanted kidney were performed, and TIC analysis with ROIs sized 5 mm^2 (ROI_5), 10 mm^2 (ROI_{10}), and ROIs circumscribing the outlines of anatomical regions (ROI_{Anat}) were analyzed based on CEUS examination. The TIC analysis was repeated by a second independent operator for ROI_5 and ROI_{Anat}.

Results: Statistical analysis revealed significant differences between TIC parameters of different ROI methods, and overall, the interoperator variance was low. But a greater ROI surface (ROI_{10}) led to higher values of the intensity parameters A and AUC compared with ROI_5 ($p < 0.05$). The difference in the ROI form led to high variation of certain TIC parameters between ROI_5 and ROI_{Anat} in the myelon [intraclass correlation coefficient (A, ICC = 0.578 (0.139–0.793); TIC parameter (TTP); and ICC = 0.679 (0.344–0.842) ($p < 0.05$)]. A mean variation of 1 cm of the depth of ROI_5 in the cortex did not show significant differences in the TIC parameters, though

there was an impact of depth of ROI_{Anat} on the values of TIC parameters. The interoperator variance in the cortex was low and equal for ROI_5 and ROI_{Anat}, but increased in the myelon, especially for ROI_{Anat}. Furthermore, the analysis revealed a strong correlation between the parameter AUC and the time interval applied for the TIC analysis in the cortex and myelon (r = 0.710, 0.674, $p < 0.000$).

Conclusion: Our findings suggest the application of multiple ROIs of 5 mm^2 in the cortex and medulla to perform TIC analysis of kidney transplants. For clinical interpretation of AUC, a standardized time interval for TIC analysis should be developed. After the standardization of the TIC analysis, the clinical predictive value could be investigated in further studies.

KEYWORDS

TIC-analysis, ROI, region of interest, CEUS, contrast-enhanced ultrasound, kidney transplantation, perfusion analysis

Introduction

Kidney transplantation is the treatment of choice for patients with end-stage renal disease besides various dialysis procedures (1). Compared with dialysis, patients after successful kidney transplantation benefit from a better quality of life, a higher functional level, and show longer survival (2, 3). With the eldering of society and advanced medical care, the mismatch between organ demand and availability is increasing. In this context, it is important to maintain the function of the allograft as long as possible. The main reason for long-term allograft loss is a combination of immunological and different non-immunological factors (4). In the context of immune responses, inflammation and degenerative changes occur and lead to changes in microcirculation and limitation of allograft function (5). Chronic allograft nephropathy often starts developing within the first year post-transplantation, (6) and until recently, the invasive biopsy is the gold standard for diagnostics. However, the utility of protocol biopsies is useful to determine the degree of chronic damage but is discussed controversially because of their invasive nature and is not performed in every transplant center (7). Recently, more and more progress was made in non-invasive methods to assess transplant function. In this study, especially, biomarkers in serum (8) and urine (9) have been developed. In the field of apparative diagnostics, there is a focus on modern MRI techniques (10) and CT perfusion imaging (11).

Contrast-enhanced ultrasound (CEUS) allows the description of the microcirculation of organs and is more and more used in the examination of kidneys and kidney transplants. Time-intensity curve analysis (TIC analysis) in kidney transplantation is a novel technique of perfusion analysis, and there are promising data that TIC analysis could provide useful information to determine the prognosis of allograft early and non-invasively (12, 13). TIC analysis allows the objective measurement of the contrast kinetics within a defined region of interests (ROIs) and therefore describes the microcirculation. Based on CEUS examination, perfusion parameters are calculated using integrated or external software that applies a perfusion model in a selected ROI in the kidney. The advantages of CEUS are its availability, low cost, and safe application without nephrotoxic effects, so this technique can be applied to a broad mass of patients, especially as chronic kidney disease is no contraindication in comparison to other perfusion imaging modalities, such as contrast-enhanced CT scans (14). Currently, results of TIC analysis are only comparable to a limited extent, and TIC analysis in kidney transplants is still considered a field of research (7). Numerous factors such as instrument settings during CEUS examination, application of contrast medium, patient-related data (i.e., blood pressure and body mass index), and different analysis software have been shown to influence perfusion parameters (15–17). Although TIC analysis is an emerging field of research, there is neither clarification about the impact of size, form, and localization of the different ROIs nor do we know much about the interoperator variance of TIC analysis.

In this study, we evaluated different methods of TIC analysis in renal transplantations and compared different factors influencing the quality of the measurement parameters (e.g., depth of the kidney and length of the cine loop). By repeating the measurements by another investigator, we checked the interreader variance. The aim of this study was to develop a standardized TIC analysis protocol with low intraoperator and interoperator variance and high feasibility.

Materials and methods

Patients and contrast-enhanced ultrasound examination

Between May 2017 and January 2019, 25 patients aged from 22 to 79 years with kidney transplants (mean organ age since KTx 5.18 years) received 33 CEUS at the University Hospital Regensburg by an experienced sonographer. Kidney-transplanted patients (>18 years) with a stable graft function and a CEUS examination suiting the study protocol were included in the study. Patients with pathologies of the transplanted kidneys (e.g., infarction, renal artery stenosis, infection, and ureteral obstruction) and patients with unstable hemodynamics were excluded from the study. In addition, CEUS studies that did not meet the quality requirements for the subsequent TIC analysis (e.g., stable image and length) were also excluded. There were no significant differences in the hemodynamics (e.g., blood pressure and cardiac function) of the patients.

Before CEUS, a complete status of the transplanted kidney was obtained including a B-mode scan and color-coded Doppler sonography. CEUS was performed in the "low-MI technique" (MI, mechanical index) with MI values < 0.09 (12). The setting of depth, gain, and focus was adjusted to the optimal display, with focus at the deepest point of the transplant. After giving written informed consent, patients received a 1.5 ml bolus of ultrasound contrast agent (sulfur hexafluoride microbubbles, SonoVue®, Bracco, Italy) followed by a 10 ml saline flush *via* intravenous administration in the cubital vein. After the injection of the contrast agent was completed, a timer was started. All examinations, including TIC analysis, were stored digitally (DICOM format). CEUS examination and data collection were permitted by the Ethical Committee of the University of Regensburg (17-662-101_P1, 17-662-101_P2, and 17-662-101_P3).

Time-intensity curve analysis

TIC analysis was performed based on 33 CEUS examinations by two operators separately. Both operators were blinded to the clinical parameters and the transplant outcomes. To check the robustness of the investigation and the ease of application, the investigations were carried out by two operators with different levels of experience. Operator 1 was an advanced medical student, and Operator 2 was a nephrologist experienced in the field of CEUS. The analysis was carried out using the integrated software of Logiq E9 (GE Healthcare, United States). A mathematical model for typical "Wash-in" kinetics was used for curve fitting. The starting point of TIC analysis was set at the arrival of the contrast agent in the central artery of the kidney (18), and the end of TIC analysis was set after 60 s on average or TIC analysis was determined earlier by the end of the video clip. We applied three different methods of ROI to perform the TIC analysis.

ROI_5 and ROI_{10} 3–5 regions were placed in the renal cortex and the myelon, respectively. The shape is circular and has a fixed size of 5 mm^2 in ROI_5 and 10 mm^2 in ROI_{10}. TIC parameters of ROI_5 and ROI_{10} were calculated as averages of the multiple ROIs (**Figure 1A**).

ROI_{Anat} describes the anatomical region (i.e., the total kidney, the whole cortex, the upper and the lower cortex, and one representative myelon). The regions were identified in the B-Mode scan, and the anatomical outline was circumscribed. Therefore, the size of the regions varies from patient to patient but may reflect the size and quality of the transplanted organ (**Figure 1B**).

The internal device software calculated the intensity-related TIC parameters including A, AUC, Grad [in arbitrary units (a.u.)], and the time-related TIC parameter (TTP) [in seconds (s)] (**Figure 1C**). A second operator, an experienced CEUS examiner, repeated the 33 TIC analysis with ROI_5 and ROI_{Anat} methods in the cortex and myelon. We investigated the differences and correlations between TIC parameters derived from different ROI methods. Furthermore, we analyzed the impact of ROI depth and the time interval of TIC analysis on TIC parameters and compared the interoperator variance of ROI_5 and ROI_{Anat} between the two operators.

Statistics

Results were expressed as mean ± *SD* if not indicated otherwise. The differences between groups were compared using the Wilcoxon rank test and the Friedman test (paired samples). The intraclass correlation coefficient was calculated using a two-way mixed model and absolute agreement, and then classification by Koo and Li was applied (19). Pearson correlation analysis determined the relation between TIC parameters and the time interval of TIC analysis. A *p*-value of < 0.05 was considered significant. All data were analyzed using IBM SPSS Statistics version 25.0 (IBM, Armonk, NY, United States).

Results

Baseline characteristics

TIC analysis was performed based on 33 CEUS examinations of 25 renal transplants of different patients in the Department of Nephrology at the University Hospital Regensburg. Since the examination of the transplanted kidney was often carried out as part of ultrasound follow-up examinations (e.g., when checking for complicated kidney

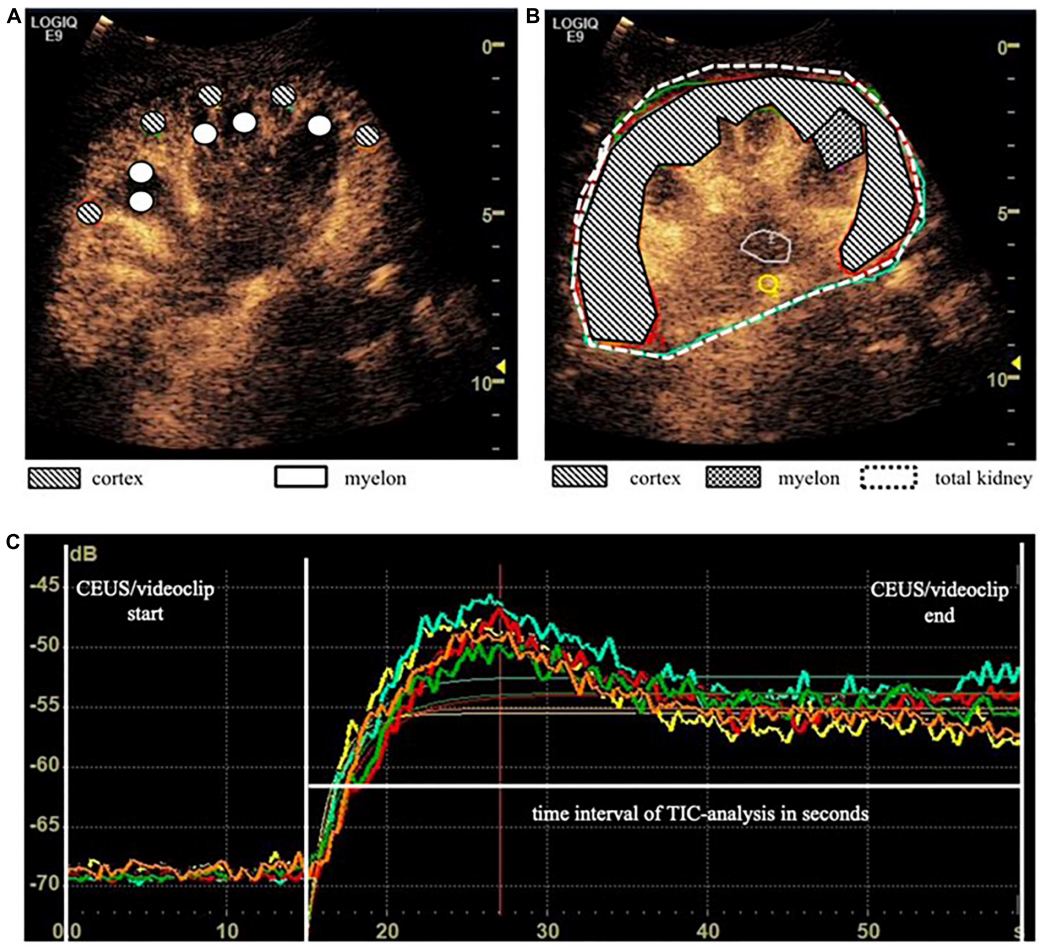


FIGURE 1
Time-intensity curve analysis (TIC analysis) was calculated based on different region of interest (ROI) methods. **(A)** ROI_5 and ROI_{10} consisted of 3 to 5 × 5 mm^2 and 10 mm^2 placed in the cortex and myelon. TIC parameters of $ROI_{5/10}$ were calculated as averages of the multiple ROIs. **(B)** ROI_{Anat} was an anatomical outline of the total kidney, the whole cortex, the upper and the lower cortex, and one representative myelon. **(C)** TIC curves based on ROI_5 in the cortex.

cysts), it occurred that seven patients received a second CEUS, and one patient received a third CEUS. The average patient age was 54.73 ± 13.66 years (22–79 years), and the majority were men (64%, 16 cases), and the average age of kidney transplant at CEUS was 5.18 ± 4.86 years (0.0–249 months). In 28 cases, laboratory data were available at the time point of the CEUS with a mean creatinine level of 2.53 ± 1.59 mg/dl and a mean eGFR (CKD-EPI) of 40.04 ± 25.28 ml/min/1.73 m^2. For the CEUS examination, we included patients of all CKD stages (Table 1 and Figure 2).

TABLE 1 Patient baseline characteristics.

CEUS—*n*	33
Male—n (%)	16 (64%)
Patient age—years	54.73 ± 13.66
Kidney transplant age—years	5.18 ± 4.86
eGFR at CEUS—ml/min/1,73 m^2	37,0 ± 23,0
Serum creatinine level—mg/dl	2.53 ± 1.59

Influence of size and form of region of interest

First, we compared the TIC parameters of all methods, and most frequently, differences showed up between ROI_{Anat} and ROI_{10}. In the myelon the differences between ROI_5 and ROI_{10} were significant but in view of the measured values, the difference was rather low with a deviation of the mean < 10% ($\Delta A = -1.24 \pm 0.55$ a.u.; $\Delta AUC = 29.98 \pm 15.44$ a.u.; $p < 0.05$), and the ICC remained high in cortex and myelon. Then, we compared the ROIs with fixed surface area (ROI_5 and ROI_{10}) to the ROI_{Anat} method and found variations for TIC parameter

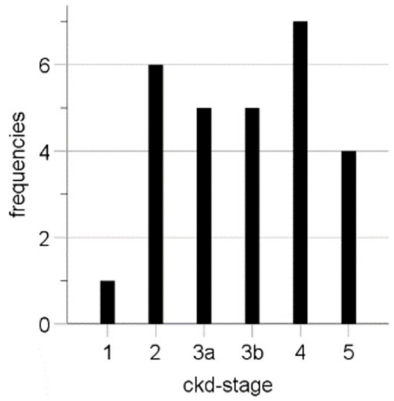


FIGURE 2
CKD stages at CEUS of examined kidney transplants.

Grad and AUC in the cortex and myelon. The ICC between ROI_5 and ROI_{Anat} decreased in the myelon for parameters A, TTP, and Grad, and if one considered not solely the IC coefficient but also the 95% confidence interval of ICC, the agreement between the two methods must be interpreted as bad ($p < 0.05$). Notably, ROI_5 was the only method that measured differences between the cortex and myelon for all TIC parameters (Tables 2–4).

Influence of the localization and depth of the regions of interest

We investigated the influence of depth of ROI on the TIC parameters. The standardized 5 mm^2 ROIs no. 1–5 were placed in different regions of the cortex and ROI no. 5 was on average 1 cm deeper than ROI no. 1 (3.5 ± 1.3 cm vs. 4.5 ± 1.7 cm). Nevertheless, the TIC parameters derived by ROI no. 1–5 did not show significant differences (Table 5). Using the ROI_{Anat} method, we investigated differences between ROI "upper/lower/total cortex." The intensity parameters A and AUC were higher, and the TTP was prolonged in "lower" and "total cortex" vs. "upper cortex." There were no differences for TIC parameter Grad (Figure 3 and Table 6).

TABLE 2 Differences in time-intensity curve (TIC) parameter between ROI_5, ROI_{10}, and ROI_{Anat}.

	ROI_{Anat}	ROI_5	ROI_{10}	*P*-value		
				1	2	3
Cortex						
A	20.94 ± 6.11	20.35 ± 5.87	20.99 ± 6.86	0.396	0.432	0.574
TTP	15.12 ± 6.11	14.55 ± 5.19	15.45 ± 7.11	0.177	0.550	0.526
AUC	620.60 ± 294.99	589.95 ± 278.88	564.19 ± 312.39	0.189	0.098	0.026*
Grad	1.44 ± 0.66	1.61 ± 0.66	1.54 ± 0.7	0.001*	0.191	0.145
Myelon						
A	18.90 ± 7.63	19.07 ± 6.04	20.31 ± 6.59	0.755	0.025*	0.145
TTP	20.55 ± 7.67	20.55 ± 7.67	19.56 ± 7.78	0.728	0.280	0.782
AUC	502.37 ± 284.65	532.27 ± 292.62	562.18 ± 308.06	0.339	0.014*	0.008*
Grad	0.97 ± 0.44	1.09 ± 0.50	1.20 ± 0.65	0.118	0.095	0.019*

p-value group: 1 = ROI_5 vs. ROI_{Anat}, 2 = ROI_5 vs. ROI_{10}, and 3 = ROI_{10} vs. ROI_{Anat} (n = 33). A, AUC, Grad in a.u.; TTP in seconds.
*$p < 0.05$.

TABLE 3 Intraclass correlation of ROI_5 and ROI_{Anat} and ROI_5 and ROI_{10}.

	ROI_5 vs. ROI_{Anat}		ROI_5 vs. ROI_{10}	
	ICC (95%–CI)	*P*-value	ICC (95%–CI)	*P*-value
Cortex				
A	0.873 (0.745–0.937)	0.000	0.887 (0.772–0.944)	0.000
TTP	0.939 (0.876–0.970)	0.000	0.878 (0.754–0.939)	0.000
AUC	0.958 (0.916–0.979)	0.000	0.972 (0.943–0.986)	0.000
Grad	0.951 (0.831–0.981)	0.000	0.931 (0.862–0.966)	0.000
Myelon				
A	0.679 (0.344–0.842)	0.001	0.928 (0.844–0.965)	0.000
TTP	0.578 (0.139–0.793)	0.009	0.859 (0.716–0.930)	0.000
AUC	0.941 (0.882–0.971)	0.000	0.983 (0.962–0.992)	0.000
Grad	0.757 (0.513–0.879)	0.000	0.881 (0.758–0.941)	0.000

Intraclass correlation coefficient (ICC) classification: bad < 0.5, moderate 0.5–0.75, good 0.75–0.9, and excellent correlation > 0.9. TIC analysis was performed by operator 1 (n = 33). A, AUC, Grad in a.u.; TTP in seconds.

TABLE 4 Differences of TIC parameters between cortex and myelon.

	Cortex	Myelon	*P*-value
ROI_5			
A	20.35	19.07	0.007
TTP	14.55	20.55	0.000
AUC	589.95	532.27	0.001
Grad	1.61	1.09	0.000
ROI_{10}			
A	20.99	20.31	0.480#
TTP	15.45	19.57	0.000
AUC	564.89	562.18	0.600#
Grad	1.54	1.20	0.000
ROI_{Anat}			
A	20.94	18.90	0.098#
TTP	15.21	19.89	0.000
AUC	610.61	502.37	0.000
Grad	1.44	0.97	0.000

#p > 0.05 (n = 33). A, AUC, Grad in a.u.; TTP in seconds.

Interoperator variance

We investigated the interoperator variance of TIC analysis between two operators using ROI_5 and ROI_{Anat} methods. Apart from the TIC parameter Grad, which showed a slight

TABLE 5 In ROI_5, variation of ROIs in depth does not affect TIC parameter values.

	nr. 1	nr. 5	*P*-value
Depth in cm	**3.5 ± 1.3**	**4.5 ± 1.7**	**0.014***
A	20.86 ± 6.54	20.28 ± 6.31	0.875
TTP	14.08 ± 5.94	14.49 ± 4.82	0.652
AUC	598.95 ± 291.21	611.00 ± 273.59	0.597
Grad	1.65 ± 0.67	1.6 ± 0.70	0.984

Five regions of interest (ROIs) sized 5 mm^2 were placed in the cortex at different distances from the ultrasound probe. The value "depths in cm" describes the distance between ROI in the parenchyma and the ultrasound probe measured in cm. The TIC parameters derived by ROI no. 1 did not differ significantly from TIC parameters derived by ROI no. 5 (Friedman test, $p > 0.05$), though ROI no. 5 was localized on average 1.05 cm deeper in the cortex than ROI no. 1 ($p < 0.05$). N = 31 (in two TIC analyses, just four ROIs could be placed sufficiently). A, AUC, Grad in a.u.; TTP in seconds.
*$p < 0.05$.

bias of 0.14 between operators 1 and 2 in the myelon, there were no significant differences between the two operators (Table 7). Yet, in the myelon, the deviation between the two operators increased compared with the cortex and was generally higher with ROI_{Anat} than with ROI_5 (Table 8). The higher interoperator variance for method ROI_{Anat} is especially reflected in a greater level of agreement (LoA) in the myelon for parameters A and TTP (Table 9 and Figure 4).

Influence of the time interval of the cine-loop

As TIC analysis was carried out retrospectively, the duration of CEUS video clips available for TIC analysis differed in some cases and resulted in a variation in time. This is due to the fact of slightly different circulation times between the patients. The mean time interval used for TIC analysis was 47.31 ± 15.18 s, and Table 10 shows a strong correlation between the time interval of TIC analysis and the TIC parameter AUC in the cortex and myelon ($r = 0.710$ and 0.674, $p < 0.000$). Compared with the correlation between AUC and the time interval, the correlation between TTP and time interval was not significant ($r = 0.389$, $p > 0.05$), and TIC parameters A and Grad did not correlate with the time interval at all.

Discussion

TIC analysis of kidney transplants is a promising field of research to detect early signs of organ dysfunction through reduced microperfusion, especially in the cortical region of the kidney transplant. Unfortunately, to date, there is no standardized protocol to measure the different TIC parameters in organs with different compartments, e.g., transplanted kidneys. In this study, we tried to determine the factors, which influence the value of TIC parameter analysis in kidney transplants.

In general, the ROI should be large enough to also allow the detection of heterogeneous perfusion signs (20, 21). To date, there is no standardized protocol for the size or form of ROI for TIC analysis in kidney transplants resulting in an inhomogeneous use of ROI mainly sized 5 or 10 mm^2 (22–26) of an anatomical outline (27–32) or was clearly not indicated (33–36). Table 11 gives an overview of the localization, size, and form of ROIs of various studies with CEUS in kidneys. Leinonen et al.

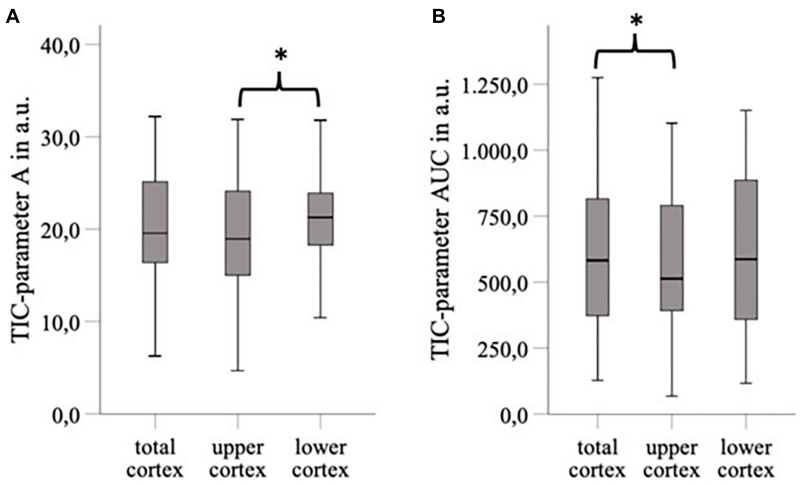


FIGURE 3
The area of ROI "total cortex" was approximately twice as big as the area of ROI "upper/lower cortex," and the ROI "lower cortex" and "total cortex" were placed on average 4.44 cm deeper than the "upper cortex" ($p < 0.05$). For TIC parameter, A was a significant difference between "upper cortex" vs. "lower cortex" (ΔA = 2,15 a.u., $p < 0.05$) **(A)** and for TIC parameter AUC between "upper cortex" and "total cortex" (ΔAUC = 39,21 a.u., $p < 0.05$) **(B)**. *$p < 0.05$.

TABLE 6 Impact of depths in ROI_{Anat} on TIC parameter values in the cortex.

	Cortex			*P*-value		
	Total	Upper	Lower	1	2	3
Depth in cm	8.33 + 1.79	3.48 + 0.96	7.50 + 1.30	0.000*	0.000*	0.003*
A	20.94 + 6.11	19.54 + 5.99	21.69 + 5.33	0.055	0.014*	0.313
TTP	15.21 + 6.11	14.47 + 4.44	17.57 + 10.09	0.147	0.140	0.161
AUC	610.61 + 294.99	571.40 + 272.72	613.82 + 302.25	0.024*	0.091	0.574
Grad	1.44 + 0.66	1.48 + 0.70	1.36 + 0.69	0.755	0.304	0.416

p-value group: 1 = upper vs. total. 2 = upper vs. lower 3 = total vs. lower; A, AUC, Grad in a.u.; TTP in seconds.
*p < 0.05.

TABLE 7 Differences of TIC parameters between operators 1 and 2.

	ROI_5			ROI_{Anat}		
	O1	O2	*P*-value	O1	O2	*P*-value
Cortex						
A	20.53 ± 5.8	20.46 ± 6.72	0.492	20.94 ± 6.11	23.09 ± 8.21	0.067
TTP	14.55 ± 5.19	15.83 ± 6.55	0.088	15.12 ± 6.11	25.52 ± 6.43	0.911
AUC	589.95 ± 277.87	573.54 ± 300.44	0.067	610.62 ± 294.99	655.14 ± 295.38	0.210
Grad	1.61 ± 0.66	1.50 ± 0.71	0.085	1.44 ± 0.66	1.45 ± 0.68	0.501
Myelon						
A	19.07 ± 6.04	19.92 ± 7.35	0.427	18.90 ± 7.63	20.50 ± 8.68	0.313
TTP	20.55 ± 7.67	21.63 ± 6.74	0.480	19.89 ± 6.50	21.66 ± 8.74	0.166
AUC	532.27 ± 292.62	535.76 ± 312.42	0.102	502.37 ± 284.65	538.71 ± 326.02	0.837
Grad	1.09 ± 0.50	0.95 ± 0.36	0.013*	0.97 ± 0.44	0.91 ± 0.36	0.503

Wilcoxon rank test, *p < 0.05, n = 33. O1 = operator 1, O2 = operator 2; A, AUC, Grad in a.u.; TTP in seconds.

TABLE 8 Intraclass correlation between TIC parameters of operators 1 and 2.

	ROI_5		ROI_{Anat}	
	ICC (95%–CI)	*P*-value	ICC (95%–CI)	*P*-value
Cortex				
A	0.915 (0.828–0.958)	0.000	0.579 (0.162–0.791)	0.007
TTP	0.834 (0.665–0.918)	0.000	0.903 (0.802–0.953)	0.000
AUC	0.922 (0.843–0.961)	0.000	0.929 (0.855–0.965)	0.000
Grad	0.917 (0.829–0.959)	0.000	0.952 (0.902–0.977)	0.000
Myleon				
A	0.738 (0.471–0.871)	0.000	0.717 (0.433–0.859)	0.000
TTP	0.824 (0.648–0.913)	0.000	0.543 (0.087–0.773)	0.014
AUC	0.879 (0.754–0.940)	0.000	0.880 (0.758–0.940)	0.000
Grad	0.752 (0.498–0.877)	0.000	0.701 (0.397–0.852)	0.001

Intraclass correlation coefficient classification: bad < 0.5, moderate 0.5–0.75, good 0.75–0.9, excellent correlation > 0.9; A, AUC, Grad in a.u.; TTP in second.

TABLE 9 Bland Altman statistics for ROI_5 and ROI_{Anat}.

	ROI_5		ROI_{Anat}	
	LoA (bias ± 1.96*SD)	*P*-value	LoA (bias ± 1.96*SD)	*P*-value
Cortex				
A	−0.11 ± 7.00	0,863	−2.38 ± 15.19	0.091
TTP	−1.28 ± 8.57	0.103	−0.21 ± 7.41	0.761
AUC	16.40 ± 307.92	0.552	−30.98 ± 294.05	0.253
Grad	0.12 ± 0.72	0.077	−0.02 ± 0.57	0.710
Myelon				
A	−0.85 ± 12.03	0.432	−1.60 ± 15.00	0.238
TTP	−1.08 ± 10.92	0.273	−1.76 ± 16.87	0.248
AUC	−3.49 ± 394.29	0.921	−36.35 ± 392.80	0.305
Grad	0.14 ± 0.70	0.044*	0.06 ± 0.75	0.378

p-value refers to bias (*p < 0.05); A, AUC, Grad in a.u.; TTP in seconds.

reported an inverse correlation between the size of the ROI and the intensity parameters (37). This goes along with our results, suggesting size impacts, especially TIC parameters representing the signal intensity like A and AUC. We recommend using a size of 5 mm^2 for various reasons. First, placement of up to five ROIs in cortex and myelon was in most cases possible with an ROI of 5 mm^2. In comparison, with 10 mm^2 in some cases, only three ROIs could be positioned, as the thin cortex did not allow the exact placement without including other structures, e.g., the medulla or vascular structures, and correct placement of ROI with 10 mm^2 size was more time-consuming than the positioning of 5 mm^2. Second, a greater surface of ROI makes it more likely to include vascular structures, e.g., AA. interlobares, AA. arcuatae, and AA. interlobularis in unnoticed manner, which should be avoided in the analysis, as this distorts the perfusion analysis of microcirculation (18, 37). The arteries show a faster and increased contrast enhancement, which then leads to significant changes in the TIC parameters in the ROIs. This could also be seen in our analysis. TIC parameters representing the signal intensity did not differ significantly in the cortex or the myelon by using ROI_{10} (area 10 mm^2) probably because other anatomical structures were included in the area of 10 mm^2.

The next question was whether it is necessary to include an entire anatomical region within the TIC analysis or only a representative, preformed area within this region. The rationale for using anatomic ROIs was that in standardized sections of the transplanted kidney, the size and configuration of the anatomic region could also provide an additional indication of the future renal function, which cannot be provided by single standardized sections of the anatomic region. When it comes to the form of ROI, a preformed size of 5 mm^2 offers more standardization than a freehand drawn outline of the anatomic

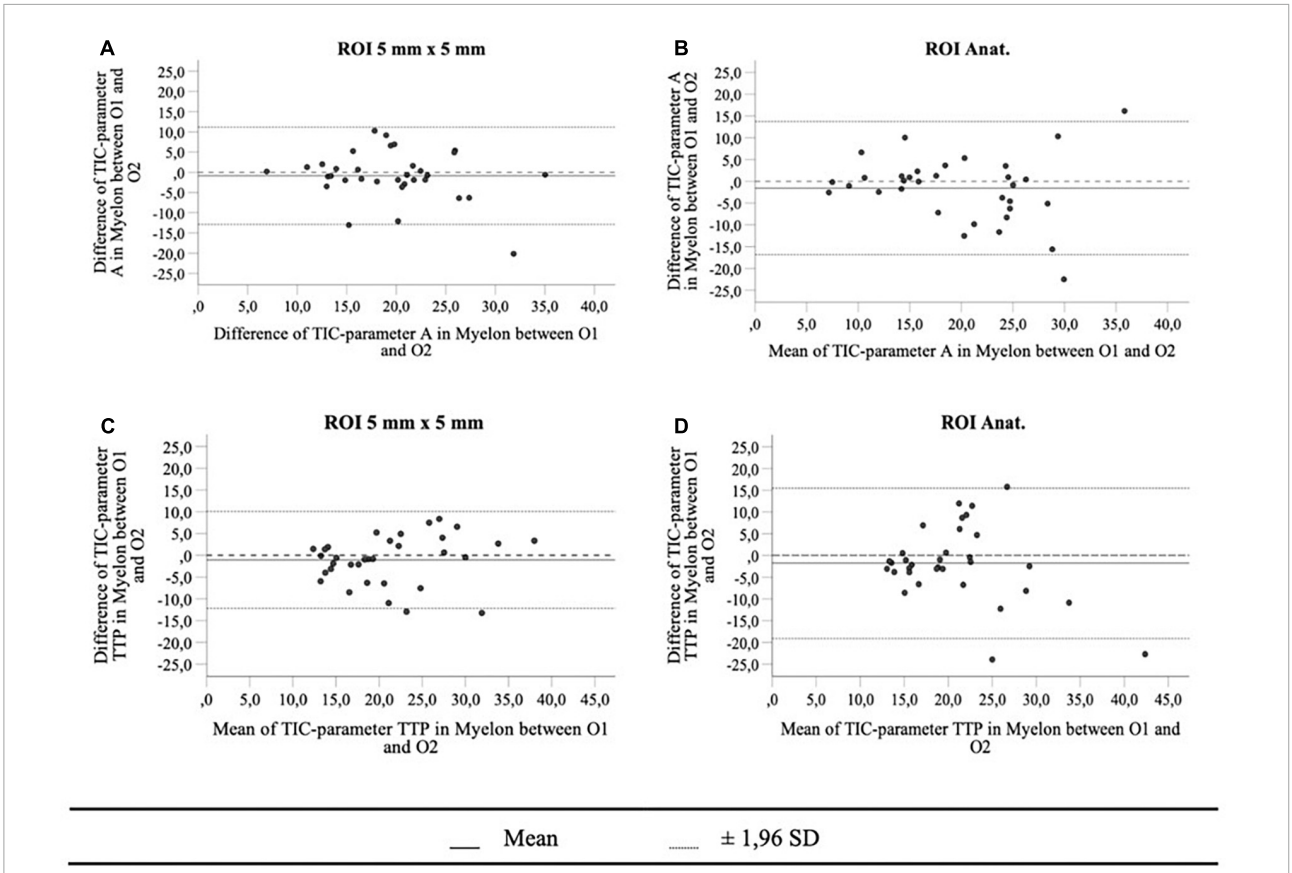


FIGURE 4
The Bland-Altman plots for TIC parameters A **(A,B)** and TTP in myelon **(C,D)** show a greater level of agreement (mean ± 1,96 *SD*) for method ROI_{Anat} than for ROI_5.

region (ROI_{Anat}). The high variation of TIC parameters between ROI_5 and ROI_{Anat} shows that both methods could not be used as equivalent. To decide on one of the two methods, we included the following aspects into consideration: first, the variation of TIC parameters especially within the myeloid structures between the ROI_5 and ROI_{Anat} could be explained by the non-myeloid structure being unintentionally included in the freehand drawn ROI. This is supported by the higher interoperator variance between operators 1 and 2 in the myelon for ROI_{Anat}. Second, the area of ROI_{Anat} varied, whereas the area of ROI_5 was constant. As Leinonen et al. reported, an equal area of ROI is a necessary criterion for constant TIC analysis (37). So far, there is no literature that distinguished these two methods before, but our results recommend an application of multiple ROIs sized 5 mm^2 for further TIC analysis.

Using ROI_5, the average difference in depth between the single ROI with 5 mm^2 was solely 1.0 cm and did not result in different TIC parameters. In contrast to that, with ROI_{Anat}, there was an average difference of 4.5 cm that led to differences in TIC parameters. The method ROI_{Anat} showed that not the size of ROI, but predominantly the depth of ROI influenced the values of intensity-related (A, AUC) and time-related TTP. It is up to the technique of ultrasound itself that signal attenuation correlates with distance to the ultrasound probe and may reflect in different values of TIC parameters (37). Nevertheless, with ROI_5, we recommended placing the ROIs in well-perfused and distinct regions that are representative of the anatomic region and handle depth as a secondary criterion for the location of ROIs.

TABLE 10 Pearson correlation coefficient between the time interval of TIC analysis and TIC parameters.

	Correlation coefficient, *r* (*P*-value)			
	A	**TTP**	**AUC**	**Grad**
Cortex	−0.257 (0.149)	−0.118 (0.513)	0.710 (0.000*)	0.287 (0.105)
Myelon	−0.225 (0.208)	0.389 (0.025*)	0.674 (0.000*)	−0.038 (0.833)

r > 0.5 is considered a strong correlation, *p < 0.05; A, AUC, Grad in a.u.; TTP in second.

The CEUS examination should be performed only by experienced investigators (12, 38) and yet the performance and subsequent assessment of the CEUS examination are highly examiner-dependent. The most important thing to mention in this study is that the examination is carried out without

TABLE 11 Comparison of different ROI-sizes and -forms used for the TIC-analysis in kidney transplants in different studies.

References	ROI form	ROI location	Number of ROIs per region	US-device	Software	Kinetics of CEUS	Aim of study	Study size
Wang et al. (22)	Square	Cortex; myelon;	1;1;	IU 22 (Philips)	QLAB (Philips)	Bolus	Evaluate perfusion parameters 1–6 months after transplantation	35
Yoon et al. (23)	Square	Cortex; myelon;	3;3;	IU 22 (Philips)	QLAB (Philips)	Bolus	Evaluate CEUS-parameters as predictors of outcome in acute kidney injury	48
Liang et al. (24)	Circular	Cortex; myelon; interlobar artery; segmental artery	1;1;1;1;	IU 22 (Philips)	Sonoliver (TomTec Imaging Systems)	Bolus	Evaluate CEUS in the assessment of renal allograft dysfunction	57
Cai et al. (25)	Circular	Cortex	2;	GE LOGIQ 9 (GE Healthcare)	Device internal software	Bolus	Compare TIC-parameters between normal graft and delayed graft function	44
Jin et al. (26)	Circular	Cortex	2;	GE LOGIQ 9 (GE Healthcare)	Device internal software	Bolus	Reliability of CEUS on the diagnosis of acute (AR) or chronic rejection (CR) after renal transplantation	79
Álvarez Rodríguez et al. (33)	Circular (no size)	Cortex; myelon, interlobar artery	1;	–	–	Bolus	Assess the effectiveness of CEUS in the early post-transplant period of kidneys	15
Benozzi et al. (34)	Circular (no size)	Cortex; corticomedullary axis;	2; 2;	–	–	Bolus	Compare CEUS to doppler-US in detection of early graft dysfunction	39
Fischer et al. (35)	Circular (no size)	Main artery; cortex; renal vein;	1;1;1;	Aplio (Toshiba)	Device internal software	Bolus	Evaluate kidney recipients in the early posttransplant phase by TIC-analysis	22
Fischer et al. (36)	Circular (no size)	Main artery; interlobar artery; cortex; renal vein;	1;1;1;1;	Aplio (Toshiba)	Device internal software	Bolus	Determine the value of CEUS in the assessment of early allograft dysfunction	45
Schwenger et al. (27)	Outline of the region	Cortex	1;	ATL HDI 5000 (Philips)	QLAB (Philips)	Flash replenishment	Feasibility of CEUS detecting CAN in comparison to color doppler US	26
Araújo and Suassuna (28)	Outline of the region	Cortex; myelon; segmental artery;	1;1;1;	Aplio 400 (Toshiba)	Device internal software	Bolus	Differences of TIC-analysis between early and late graft dysfunction	67
Brabrand et al. (29)	Outline of the region	Cortex; myelon;	1; 1;	Acuson Sequoia 512 (Siemens)	nordicICE; nordic imaging lab	Bolus	Evaluate changes in perfusion with CEUS due to global hypoxia in piglets	12
Jeong et al. (30)	Outline of the region	Cortex	1;	RS80A (Samsung Medison)	VueBox§ ; Bracco	Bolus	Evaluate clinical significance of CEUS in CKD	24
Stock et al. (31)	Outline of the region	Cortex; myelon; interlobar artery	3;2;1;	IU 22 (Philips)	VueBox§ , Bracco	Bolus	Evaluate renal perfusion with CEUS in cats with CKD	57
Kihm et al. (32)	Outline of the region	Cortex	1;	ATL TDI 5000 (Philips)	QLAB	Flash replenishment	Evaluate change in microperfusion due to ciclosporine A and tacrolimus by CEUS	32

movement and without pressure on the graft. Regardless of this, the TIC analysis allows objective quantification of perfusion separately from the CEUS examination. In this study, we analyzed the interoperator variance of TIC analysis between two investigators for ROI_5 and ROI_{Anat}. Overall, the agreement of TIC analysis between investigators 1 and 2 was high but in comparison to the cortex, the agreement decreased in the myelon. This is remarkable because although both investigators had different levels of experience, the results were consistent, despite the fact that renal tissue is very inhomogeneous, and different compartments were measured separately. Our results are supported by Nylund et al. who also found a

low interoperator variance of TIC analysis with inflammatory bowel disease (39). We preferred the standardized 5 mm^2 form ROI_5 instead of the anatomic form ROI_{Anat}. For ROI_{Anat}, the interoperator variance for the parameters A and TTP was so high that the clinical application is not reasonable and the method ROI_5 should be preferred.

TIC analyses were performed retrospectively after CEUS examination and consequently, the cine-loops lasted in some cases less than 60 s and led to a variation of the time interval for TIC analysis with a mean of 47.31 ± 15.18 s. This is due to the fact that in some patients, the arrival time in the kidney transplant was longer than in others, and the cine-loops were standardized to a length of 60 s after contrast-agent application. But this allowed us to determine the influence of the time interval of the cine-loop on the different TIC parameters. Our results showed a strong correlation between the time interval and the TIC parameter AUC. Many authors emphasize the use of AUC in the clinical context (40, 41), but if the TIC parameter is dependent on the time interval, its informative value is limited. Therefore, our results emphasize the need for a standardized start and endpoint of TIC analysis to generate a consistent time interval for TIC analysis. To date in many studies, there is no standardized length of the video clip, but this is crucial to define clear results and cutoff values of AUC and TTP in future studies.

In general, the time interval of the TIC analysis should include the contrast agent wash-in phase and representative parts of the wash-out phase. The entire wash-out phase of the contrast agent may take up to 10 min in the bolus model (18, 42), and integration of the entire wash-out phase into the TIC analysis would be too time-consuming, not practical, and inappropriate for the patient examination. With a view to a uniform time interval, the stop setting needs to be further evaluated in follow-up studies. An approach following Kay et al. would be conceivable. The authors normalized the time interval to 5 s after initiation of the contrast agent and described a correlation of AUC with eGFR 3 months after renal transplantation (43). Other experimental approaches would be a stop point 30 s after the arrival of the contrast agent to capture the cortical phase or after 60 s to capture portions of the medullary phase (12, 44).

The main limitation of this study is the limited number of subjects, and the results should be confirmed in a larger population. However, this study should generate hypotheses that should be tested in a larger cohort in a clinical context. In our study, we applied "Wash-in" kinetics as it best represents the perfusion. Eventually, patients with hyperdynamic circulation who show an early wash-out might lead to a bias in the TIC parameters. If extreme abnormalities in the visual evaluation of the perfusion kinetics were referred to as measuring errors, these CEUS examinations were excluded from the study. For the assessment of interoperator variance, the LoA has to be discussed in a clinical context (45). Consequently, till present, the assessment of interoperator variance is limited due to the lack of a generally applicable value range for TIC analysis with kidney transplants. Furthermore, no clinical parameters were included in this study, but this has to be the subject of further studies after the examination has been standardized.

Conclusion

Identifying kidney transplant recipients at increased risk for graft failure is one of the most important tasks in transplant medicine. TIC analysis could make a key contribution to improving long-term graft survival. But before TIC parameters can be used to define threshold values for good or limited future graft function, the procedure of TIC analysis should be standardized because TIC parameters are influenced by various factors. We recommended the use of an average of multiple ROIs of 5 mm^2 in the cortex and myelon. The method of ROI 5 mm^2 offers a standardized form and a sufficient, feasible size, which enables TIC analysis with low intraoperator and interoperator variance. The duration of the video clip should be set at 60 s after the contrast agent has reached the kidney transplant. With regard to further improvement of TIC analysis in kidney transplants, we emphasized concluding with one standardized method of ROI.

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 the Ethikkomitee Universitätsklinik Regensburg. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

SF collected, analyzed the data, wrote, reviewed, and edited the manuscript. FP conceptualized the study design, performed the CEUS examination, and edited and reviewed the manuscript. SF, EJ, TB, HT, MB, BB, and FP contributed to manuscript revision and read and approved the final

version. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the University Hospital of Regensburg (ReForM B under a grant to FP).

Acknowledgments

We thank Florian Zeman from the Centre for Clinical Studies of the University Hospital of Regensburg for his assistance with the statistical and biometric analysis of the data.

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.

Publisher's note

References

1. Suthanthiran M, Strom TB. Renal transplantation. *N Engl J Med.* (1994) 331:365–76. doi: 10.1056/NEJM199408113310606

2. Port FK, Wolfe RA, Mauger EA, Berling DP, Jiang K. Comparison of survival probabilities for dialysis patients vs cadaveric renal transplant recipients. *JAMA.* (1993) 270:1339–43. doi: 10.1001/jama.270.11.1339

3. Schnuelle P, Lorenz D, Trede M, van der Woude FJ. Impact of renal cadaveric transplantation on survival in end-stage renal failure: evidence for reduced mortality risk compared with hemodialysis during long-term follow-up. *J Am Soc Nephrol.* (1998) 9:2135–41. doi: 10.1681/ASN.V9112135

4. Gaston RS, Cecka JM, Kasiske BL, Fieberg AM, Leduc R, Cosio FC, et al. Evidence for antibody-mediated injury as a major determinant of late kidney allograft failure. *Transplantation.* (2010) 90:68–74. doi: 10.1097/TP.0b013e3181e065de

5. Einecke G, Sis B, Reeve J, Mengel M, Campbell PM, Hidalgo LG, et al. Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *Am J Transplant.* (2009) 9:2520–31. doi: 10.1111/j.1600-6143.2009.02799.x

6. Nankivell BJ, Borrows RJ, Fung CL-S, O'Connell PJ, Allen RDM, Chapman JR. The natural history of chronic allograft nephropathy. *N Engl J Med.* (2003) 349:2326–33. doi: 10.1056/NEJMoa020009

7. Ott U, Wolf G. Indikation zur nierenbiopsie nach nierentransplantation. *Nephrologe.* (2010) 5:466–72. doi: 10.1007/s11560-009-0376-7

8. Bassi R, Niewczas MA, Biancone L, Bussolino S, Merugumala S, Tezza S, et al. Metabolomic profiling in individuals with a failing kidney allograft. *PLoS One.* (2017) 12:e0169077. doi: 10.1371/journal.pone.0169077

9. Banas MC, Böhmig GA, Viklicky O, Rostaing LP, Jouve T, Guirado L, et al. A prospective multicenter trial to evaluate urinary metabolomics for non-invasive detection of renal allograft rejection (PARASOL): study protocol and patient recruitment. *Front Med.* (2021) 8:780585. doi: 10.3389/fmed.2021.780585

10. Raoul SP, Ipek IG, McLaughlin K, Yilmaz S, Mahallati H. Assessment of chronic renal allograft nephropathy using contrast-enhanced MRI: a pilot study. *AJR Am J Roentgenol.* (2010) 194:W407–13. doi: 10.2214/AJR.09.2912

11. Jeong S, Park SB, Chang IH, Shin J, Chi BH, Park HJ, et al. Estimation of renal function using kidney dynamic contrast material-enhanced CT perfusion: accuracy and feasibility. *Abdom Radiol.* (2021) 46:2045–51. doi: 10.1007/s00261-020-02826-7

12. Sidhu PS, Cantisani V, Dietrich CF, Gilja OH, Saftoiu A, Bartels E, et al. Die EFSUMB-Leitlinien und empfehlungen für den klinischen einsatz des kontrastverstärkten ultraschalls (CEUS) bei nicht-hepatischen anwendungen: update 2017 (Langversion). *Ultraschall Med.* (2018) 39:e2–44. doi: 10.1055/a-0586-1107

13. Yang C, Wu S, Yang P, Shang G, Qi R, Xu M, et al. Prediction of renal allograft chronic rejection using a model based on contrast-enhanced ultrasonography. *Microcirculation.* (2019) 26:e12544. doi: 10.1111/micc.12544

14. Rudnick MR, Leonberg-Yoo AK, Litt HI, Cohen RM, Hilton S, Reese PP. The controversy of contrast-induced nephropathy with intravenous contrast: what is the risk? *Am J Kidney Dis.* (2020) 75:105–13. doi: 10.1053/j.ajkd.2019.05.022

15. Tang M-X, Mulvana H, Gauthier T, Lim AKP, Cosgrove DO, Eckersley RJ, et al. Quantitative contrast-enhanced ultrasound imaging: a review of sources of variability. *Interface Focus.* (2011) 1:520–39. doi: 10.1098/rsfs.2011.0026

16. Gauthier TP, Averkiou MA, Leen ELS. Perfusion quantification using dynamic contrast-enhanced ultrasound: the impact of dynamic range and gain on time-intensity curves. *Ultrasonics.* (2011) 51:102–6. doi: 10.1016/j.ultras.2010.06.004

17. Pitre-Champagnat S, Coiffier B, Jourdain L, Benatsou B, Leguerney I, Lassau N. Toward a standardization of ultrasound scanners for dynamic contrast-enhanced ultrasonography: methodology and phantoms. *Ultrasound Med Biol.* (2017) 43:2670–7. doi: 10.1016/j.ultrasmedbio.2017.06.032

18. Greis C. Quantitative evaluation of microvascular blood flow by contrast-enhanced ultrasound (CEUS). *Clin Hemorheol Microcirc.* (2011) 49:137–49. doi: 10.3233/CH-2011-1464

19. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J Chiropr Med.* (2016) 15:155–63. doi: 10.1016/j.jcm.2016.02.012

20. Schneider AG, Goodwin MD, Schelleman A, Bailey M, Johnson L, Bellomo R. Contrast-enhanced ultrasonography to evaluate changes in renal cortical microcirculation induced by noradrenaline: a pilot study. *Crit Care.* (2014) 18:653. doi: 10.1186/s13054-014-0653-3

21. Wei K, Le E, Bin J-P, Coggins M, Thorpe J, Kaul S. Quantification of renal blood flow with contrast-enhanced ultrasound. *J Am Coll Cardiol.* (2001) 37:1135–40. doi: 10.1016/S0735-1097(00)01210-9

22. Wang X, Yu Z, Guo R, Yin H, Hu X. Assessment of postoperative perfusion with contrast-enhanced ultrasonography in kidney transplantation. *Int J Clin Exp Med.* (2015) 8:18399–405. doi: <doi>

23. Yoon HE, Kim DW, Kim D, Kim Y, Shin SJ, Shin YR. A pilot trial to evaluate the clinical usefulness of contrast-enhanced ultrasound in predicting renal outcomes in patients with acute kidney injury. *PLoS One.* (2020) 15:e0235130. doi: 10.1371/journal.pone.0235130

24. Liang WX, Cai MJ, Jiang L, Xie YQ, Yuan WL, Zhang H. Ultrasonic imaging characteristics of transplanted kidneys with delayed graft function. *Genet Mol Res.* (2014) 13:6878–84. doi: 10.4238/2014.August.29.9

25. Cai R, Tao L, Liang W, Lin M, Guo X, Huang X-E. Application of contrast-enhanced sonography on the diagnosis of acute and chronic rejection after renal transplantation. *Ultrasound Q.* (2020) 36:59–63. doi: 10.1097/RUQ.0000000000000449

26. Jin Y, Yang C, Wu S, Zhou S, Ji Z, Zhu T, et al. A novel simple noninvasive index to predict renal transplant acute rejection by contrast-enhanced ultrasonography. *Transplantation.* (2015) 99:636–41. doi: 10.1097/TP.0000000000000382

27. Schwenger V, Korosoglou G, Hinkel U-P, Morath C, Hansen A, Sommerer C, et al. Real-time contrast-enhanced sonography of renal transplant recipients predicts chronic allograft nephropathy. *Am J Transplant.* (2006) 6:609–15. doi: 10.1111/j.1600-6143.2005.01224.x

28. Araújo NC, Suassuna JHR. Time-intensity curve analysis of contrast-enhanced ultrasound is unable to differentiate renal dysfunction in the early post-transplant period - a prospective study. *BMC Nephrol.* (2018) 19:351. doi: 10.1186/s12882-018-1158-0

29. Brabrand K, Lange C, de Emblem KE, Reinholt FP, Saugstad OD, Stokke ES. Contrast-enhanced ultrasound identifies reduced overall and regional renal perfusion during global hypoxia in piglets. *Invest Radiol.* (2014) 49:540–6. doi: 10.1097/RLI.0000000000000053

30. Jeong S, Park SB, Kim S-H, Hwang JH, Shin J. Clinical significance of contrast-enhanced ultrasound in chronic kidney disease: a pilot study. *J Ultrasound.* (2019) 22:453–60. doi: 10.1007/s40477-019-00409-x

31. Stock E, Paepe D, Daminet S, Vandermeulen E, Duchateau L, Saunders JH, et al. Contrast-enhanced ultrasound examination for the assessment of renal perfusion in cats with chronic kidney disease. *J Vet Intern Med.* (2018) 32:260–6. doi: 10.1111/jvim.14869

32. Kihm LP, Blume C, Seckinger J, Hankel V, Stoffler D, Morath C, et al. Acute effects of calcineurin inhibitors on kidney allograft microperfusion visualized by contrast-enhanced sonography. *Transplantation.* (2012) 93:1125–9. doi: 10.1097/TP.0b013e31824f3dae

33. Álvarez Rodríguez S, Hevia Palacios V, Sanz Mayayo E, Gómez Dos Santos V, Díez Nicolás V, Sánchez Gallego MD, et al. The usefulness of contrast-enhanced ultrasound in the assessment of early kidney transplant function and complications. *Diagnostics.* (2017) 7:53. doi: 10.3390/diagnostics7030053

34. Benozzi L, Cappelli G, Granito M, Davoli D, Favali D, Montecchi MG, et al. Contrast-enhanced sonography in early kidney graft dysfunction. *Transplant Proc.* (2009) 41:1214–5. doi: 10.1016/j.transproceed.2009.03.029

35. Fischer T, Mühler M, Kröncke TJ, Lembcke A, Rudolph J, Diekmann F, et al. Early postoperative ultrasound of kidney transplants: evaluation of contrast medium dynamics using time-intensity curves. *Rofo.* (2004) 176:472–7. doi: 10.1055/s-2004-812992

36. Fischer T, Filimonow S, Dieckhöfer J, Slowinski T, Mühler M, Lembcke A, et al. Improved diagnosis of early kidney allograft dysfunction by ultrasound with echo enhancer–a new method for the diagnosis of renal perfusion. *Nephrol Dial Transplant.* (2006) 21:2921–9. doi: 10.1093/ndt/gfl313

37. Leinonen MR, Raekallio MR, Vainio OM, Ruohoniemi MO, O'Brien RT. The effect of the sample size and location on contrast ultrasound measurement of perfusion parameters. *Vet Radiol Ultrasound.* (2011) 52:82–7. doi: 10.1111/j.1740-8261.2010.01745.x

38. Bracco Imaging. *Fachinformation (Zusammenfassung der Merkmale des Arzneimittels).* Midrand: SonoVue (2022). doi: <doi>

39. Nylund K, Saevik F, Leh S, Pfeffer F, Hausken T, Gilja OH. Interobserver-analyse der CEUS abgeleiteten perfusion bei fibrotischem und entzündlichem morbus crohn. *Ultraschall Med.* (2019) 40:76–84. doi: 10.1055/s-0044-100492

40. Wang L, Wu J, Cheng J-F, Liu X-Y, Ma F, Guo L-H, et al. Diagnostic value of quantitative contrast-enhanced ultrasound (CEUS) for early detection of renal hyperperfusion in diabetic kidney disease. *J Nephrol.* (2015) 28:669–78. doi: 10.1007/s40620-015-0183-3

41. Zhang Q, Yu Z, Xu Y, Zeng S, Zhang Z, Xue W, et al. Use of contrast-enhanced ultrasonography to evaluate chronic allograft nephropathy in rats and correlations between time-intensity curve parameters and allograft fibrosis. *Ultrasound Med Biol.* (2016) 42:1574–83. doi: 10.1016/j.ultrasmedbio.2016.01.028

42. Tsuruoka K, Yasuda T, Koitabashi K, Yazawa M, Shimazaki M, Sakurada T, et al. Evaluation of renal microcirculation by contrast-enhanced ultrasound with Sonazoid as a contrast agent. *Int Heart J.* (2010) 51:176–82. doi: 10.1536/ihj.51.176

43. Kay DH, Mazonakis M, Geddes C, Baxter G. Ultrasonic microbubble contrast agents and the transplant kidney. *Clin Radiol.* (2009) 64:1081–7. doi: 10.1016/j.crad.2009.06.010

44. Bădulescu MR, Socaciu MA, Moisoiu T, Andries A, Iacob G, Badea R. Current status of imaging diagnosis in the transplanted kidney. a review of the literature with a special focus on contrast-enhanced ultrasonography. *Med Pharm Rep.* (2020) 93:133–44. doi: 10.15386/mpr-1536

45. Griffiths P, Murrells T. Reliability assessment and approaches to determining agreement between measurements: classic methods paper. *Int J Nurs Stud.* (2010) 47:937–8. doi: 10.1016/j.ijnurstu.2010.03.004

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 16 September 2022
DOI 10.3389/fmed.2022.943502

OPEN ACCESS

EDITED BY
Kathrin Eller,
Medical University of Graz, Austria

REVIEWED BY
Wen Tian,
Stanford University, United States
Brian Duncan Tait,
The University of Melbourne, Australia

*CORRESPONDENCE
Covadonga López del Moral
covadonga.lopezdelmoral@scsalud.es

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 13 May 2022
ACCEPTED 19 August 2022
PUBLISHED 16 September 2022

CITATION
López del Moral C, Wu K, Naik M, Osmanodja B, Akifova A, Lachmann N, Stauch D, Hergovits S, Choi M, Bachmann F, Halleck F, Schrezenmeier E, Schmidt D and Budde K (2022) The natural history of *de novo* donor-specific HLA antibodies after kidney transplantation. *Front. Med.* 9:943502. doi: 10.3389/fmed.2022.943502

The natural history of *de novo* donor-specific HLA antibodies after kidney transplantation

Covadonga López del Moral[1,2]*, Kaiyin Wu[3], Marcel Naik[1], Bilgin Osmanodja[1], Aylin Akifova[1], Nils Lachmann[4], Diana Stauch[4], Sabine Hergovits[4], Mira Choi[1], Friederike Bachmann[1], Fabian Halleck[1], Eva Schrezenmeier[1,5], Danilo Schmidt[1] and Klemens Budde[1]

[1]Department of Nephrology and Medical Intensive Care, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany, [2]Valdecilla Biomedical Research Institute (IDIVAL), Santander, Spain, [3]Department of Pathology, Charité – Universitätsmedizin Berlin, Berlin, Germany, [4]Institute for Transfusion Medicine, HLA-Laboratory, Charité – Universitätsmedizin Berlin, Berlin, Germany, [5]Berlin Institute of Health Charité – Universitätsmedizin Berlin, BIH Academy, Berlin, Germany

Background: *De novo* donor-specific HLA antibodies (dnDSA) are key factors in the diagnosis of antibody-mediated rejection (ABMR) and related to graft loss.

Methods: This retrospective study was designed to evaluate the natural course of dnDSA in graft function and kidney allograft survival and to assess the impact of mean fluorescence intensity (MFI) evolution as detected by annual Luminex® screening. All 400 kidney transplant recipients with 731 dnDSA against the last graft (01/03/2000-31/05/2021) were included.

Results: During 8.3 years of follow-up, ABMR occurred in 24.8% and graft loss in 33.3% of the cases, especially in patients with class I and II dnDSA, and those with multiple dnDSA. We observed frequent changes in MFI with 5-year allograft survivals post-dnDSA of 74.0% in patients with MFI reduction $\geq$ 50%, 62.4% with fluctuating MFI (MFI reduction $\geq$ 50% and doubling), and 52.7% with doubling MFI (log-rank $p < 0.001$). Interestingly, dnDSA in 168 (24.3%) cases became negative at some point during follow-up, and 38/400 (9.5%) patients became stable negative, which was associated with better graft survival. Multivariable analysis revealed the importance of MFI evolution and rejection, while class and number of dnDSA were not contributors in this model.

Conclusion: In summary, we provide an in-depth analysis of the natural course of dnDSA after kidney transplantation, first evidence for the

impact of MFI evolution on graft outcomes, and describe a relevant number of patients with a stable disappearance of dnDSA, related to better allograft survival.

KEYWORDS

donor-specific antibodies, mean fluorescence intensity, graft failure, antibody-mediated rejection, kidney transplantation

Introduction

Short-term graft survival has improved over the past decades in kidney transplantation, but no major changes in long-term survival have been achieved (1–4). Antibody-mediated rejection (ABMR) is an important cause of graft failure (5–11). Although non-HLA antibodies may also cause graft dysfunction (12–15), it is well-known that preformed or *de novo* HLA donor-specific antibodies (dnDSA) are strongly associated with rejection and graft failure (16–22). The development of *dn*DSA may occur at any time after transplantation, and different characteristics of DSA may determine the clinical phenotype of rejection (23–29). The presence of dnDSA has been reported in 13–27% of previously non-sensitized patients, but the indication and frequency of systematic DSA screening in stable patients are not currently established (30–32). High HLA mismatch is one of the risk factors for dnDSA development (33–36). Non-adherence to treatment, under-immunosuppression, and graft inflammation are other factors that are related to dnDSA formation (29). It has been reported that the presence of both class I and II dnDSAs is more strongly related to graft failure, but few studies have specifically analyzed the long-term effects of antibody class (27, 37–43), and the impact of the number of dnDSA per patient on graft survival is unknown.

The Luminex®-based single-antigen bead (SAB) assay is currently the most appropriate method for the detection of HLA antibodies, which allows for semiquantitative analysis of the level of anti-HLA antibodies by the mean fluorescence intensity (MFI) (44–46). It is assumed that antibodies with higher MFI values are more harmful and related to graft dysfunction, but the relationship between clinical outcomes and MFI level is not fully established. The correlation between MFI and the amount of bound HLA antibodies is not linear and can be affected by several factors, such as the inhibitory effect produced by complement (prozone effect) (45, 47, 48). Currently, there is no accepted MFI value that is clinically significant, and each laboratory has set its own MFI positivity threshold (32, 41, 46). The STAR 2017 Working Group (32) gave recommendations for HLA antibody testing, pointing out that differences of up to 25% or even 50% in MFI values should not be considered meaningful.

The purpose of the current study was to evaluate the natural history and clinical evolution of patients with dnDSA after kidney transplantation. We wanted to specifically address the relationship of dnDSA MFI values with graft failure. Changes in renal function were evaluated to assess the evolution of these analytical parameters after the occurrence of dnDSA.

Materials and methods

Patient population

For this retrospective analysis, we included all kidney transplant recipients with dnDSA from 01/03/2000 until 31/05/2021 (end of follow-up) at Charité-Universitätsmedizin Berlin (Germany). All patients with dnDSA against the last graft with complete HLA typing were included, excluding those patients with preformed DSA before transplantation. The primary outcome variable in our study was time to death-censored graft failure, defined as graft loss (i.e., the need for permanent dialysis, allograft nephrectomy, or re-transplantation). Patients who developed dnDSA after graft loss were excluded.

All data including estimated glomerular filtration rate (GFR, ml/min), proteinuria (mg/g creatinine), delayed graft function (DGF), defined as the need for dialysis within 7 days of transplant, and biopsy data were collected from the prospectively maintained database (TBase) (49). All rejections were categorized according to Banff 2017 classification (5, 50, 51). Calculated panel-reactive antibody (cPRA) was obtained through the Virtual PRA Calculator of the Eurotransplant Reference Laboratory.[1] No institutional review board approval was required for this retrospective analysis.

De novo donor-specific HLA antibodies

Regular annual monitoring of HLA antibodies was performed as described previously (26, 33) and in case of clinical signs of impaired allograft function. DnDSA were determined by Luminex® -based LABScreen® SAB assay (One Lambda, Canoga Park, CA). The general MFI positivity threshold in our laboratory was 1,000. Despite this, the first occurrence date in our study was defined as the date of the medical report by the immunology department in which dnDSA was first assigned, considering other factors such as plausibility

1 https://www.etrl.org/vPRA.aspx

(52) and evolution of HLA antibodies posttransplant, regardless of MFI value. The most probable two-field HLA typing of the donor (53) was considered to assign DSA and the respective MFI as appropriate as possible. For missing information on specific HLA loci (usually DQA and DPA), DRB1∼DQA1∼DQB1 and DPA1∼DPB1 haplotype frequencies were used to assign the most probable allele, according to extended haplotype frequencies previously described in the European population (54–56). The first appearance of each dnDSA and the date of the last negative sample were collected. Because each dnDSA had its own time of the first occurrence and its own MFI evolution, we also performed some analyses for different dnDSA as indicated.

De novo DSAs were categorized according to MFI on the date of the first occurrence (<500, 500–999, 1,000–2,999, 3,000–9,999, and ≥10,000), and they were also classified according to MFI evolution in the subsequent samples [MFI increase ≥ 50%, MFI reduction ≥ 50%, fluctuating MFI (increase and reduction ≥ 50%)]. In dnDSA with ≥ 50% MFI reduction. specific active treatment for ABMR was recorded (57), excluding changes in chronic baseline immunosuppression. The

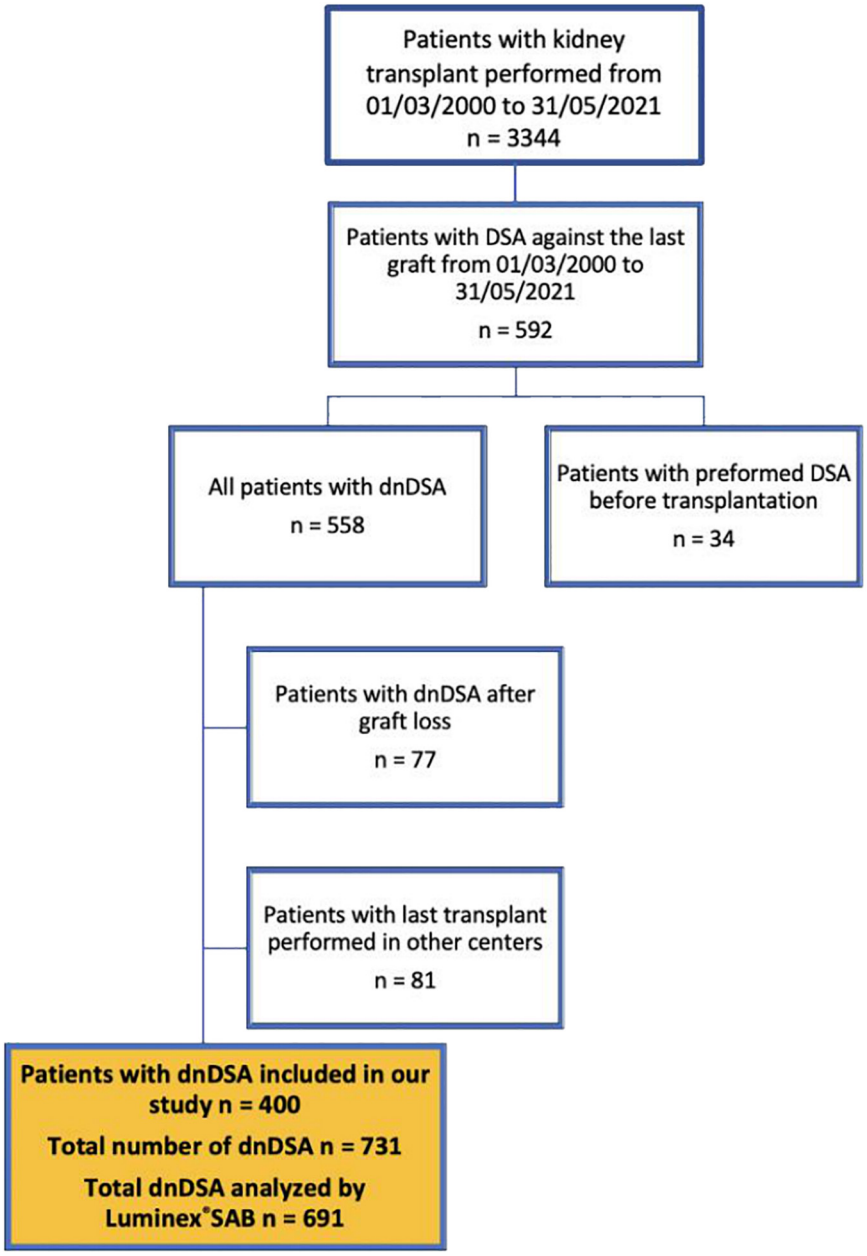


FIGURE 1
Flowchart of the patients in our study. DSA, donor-specific antibodies; dnDSA, *de novo* donor-specific antibodies; SAB, single antigen bead.

TABLE 1 Baseline characteristics of patients with dnDSA.

Variables	Patients with dnDSA (n = 400)
Recipient age at time of TX	46.1 (34.2–59.1)
Recipient sex (male, %)	62.5% (n = 250)
Follow-up (years) after TX	12.9 (9.6–16.3)
Follow-up (years) after dnDSA development*	8.3 (5.5–10.7)
Graft loss (%)	33.3% (n = 133)
• Time (years) from TX to graft loss	• 8.4 ± 4.9
• Time (years) from dnDSA to graft loss*	• 4.6 (1.7–8.1)
Death (%)	24.0% (n = 96)
• Time (years) from TX to death	• 8.9 ± 4.3
Patients alive with functioning graft (%)	53.0% (n = 212)
Donor age	50.0 (39.0–59.5)
Donor sex (male,%)	51.0% (n = 204)
Donor blood type	
• A	• 39.4% (n = 158)
• B	• 13.3% (n = 53)
• AB	• 5.8% (n = 23)
• 0	• 41.5% (n = 166)
Donor type	
• Deceased donor (100% DBD)	• 68.5% (n = 274)
• Living donor	• 31.5% (n = 126)
First kidney transplant (%)	88.7% (n = 355)
Combined transplant (%)	6.8% (n = 27)
	• 5.5% (n = 22): Pancreas-kidney transplant
	• 1.3% (n = 5): Liver-kidney transplant
Cold ischemia time (CIT, minutes)	420.0 (165.0–768.0)
Delayed graft function (DGF, %)	29.7% (n = 119)
•cPRA ≥ 5% at the time of TX (%) (Eurotransplant)	16.5% (n = 66)
•cPRA ≥ 85% at the time of TX (%) (Eurotransplant)	5.8% (n = 23)
cPRA ≥ 5% at the time of TX (%)	
•cPRA ≥ 5% class I (%)	• 16.8% (n = 67)
•cPRA ≥ 5% class II (%)	• 11.3% (n = 45)
cPRA ≥ 85% at the time of TX (%)	
•cPRA ≥ 85% class I (%)	• 3.8% (n = 15)
cPRA ≥ 85% class II (%)	• 2.5% (n = 10)
Initial IS	
•Triple standard therapy (calcineurin inhibitor, mycophenolate, and steroids)	• 24.5% (n = 98)
•Triple standard therapy + anti-IL2R	• 49.8% (n = 199)
•Triple standard therapy + ATG	• 5.8% (n = 23)
Others	• 19.9% (n = 80)
HLA mismatch A = 0 (%)	30.5% (n = 122)
HLA mismatch A = 1 (%)	51.9% (n = 208)
HLA mismatch A = 2 (%)	17.6% (n = 70)
HLA mismatch B = 0 (%)	12.2% (n = 49)
HLA mismatch B = 1 (%)	50.9% (n = 203)
HLA mismatch B = 2 (%)	36.9% (n = 148)
HLA mismatch DRB1 = 0 (%)	10.7% (n = 43)
HLA mismatch DRB1 = 1 (%)	60.3% (n = 241)
HLA mismatch DRB1 = 2 (%)	29.0% (n = 116)
HLA mismatch DQB1 = 0 (%)	11.0% (n = 44)
HLA mismatch DQB1 = 1 (%)	57.8% (n = 231)
HLA mismatch DQB1 = 2 (%)	31.2% (n = 125)
Graft nephrectomy (%) after dnDSA occurrence	10.3% (n = 41)
• Cause of graft nephrectomy	
○ Acute rejection	○ 14.6% (n = 6)
○ Chronic rejection	○ 56.1% (n = 23)
○ Surgical complications	○ 4.9% (n = 2)
○ Others	○ 24.4% (n = 10)
• Time (months) from TX to graft nephrectomy	○ 77.3 (30.7–138.1)

(Continued)

TABLE 1 (Continued)

Variables	Patients with dnDSA (n = 400)
Patients with allograft kidney biopsy (%) (all by clinical indication; independent of results)	72.0% (n = 288)
•Patients with allograft kidney biopsy after dnDSA occurrence*	• 63.9% (n = 184)
Number of allograft kidney biopsy per patient	1.0 (0.0–3.0)
Number of dnDSA per patient	1.0 (1.0–2.0)
Patients with ≥ 2 dnDSA (independent of class) (%)	43.5% (n = 174)
Patients with ≥ 4 dnDSA (independent of class) (%)	10.3% (n = 41)
Class dnDSA per patient	
• Patients with class I dnDSA only (%)	• 18.5% (n = 74)
• Patients with class II dnDSA only (%)	• 59.3% (n = 237)
• Patients with both class I and II dnDSA (%)	• 22.3% (n = 89)
Proteinuria (mg/g creatinine) at the time of first occurrence of dnDSA*	182.0 (100.2–502.0)
Patients with proteinuria ≥500 mg/g creatinine at the time of first occurrence of dnDSA (%)*	21.3% (n = 85)
eGFR (ml/min) at the time of first occurrence of dnDSA*	41.0 (29.0–54.2)
Creatinine (mg/dl) at the time of first occurrence of dnDSA*	1.6 (1.3–2.3)
TCMR before first occurrence of dnDSA (%)*	35.0% (n = 140)
TCMR (all episodes, independent of first occurrence of dnDSA) (Banff 2017 Classification)	45.8% (n = 183)
•Acute TCMR borderline	• 27.3% (n = 50)
• Acute TCMR IA	• 13.1% (n = 24)
• Acute TCMR IB	•8.2% (n = 15)
•Acute TCMR IIA	• 12.0% (n = 22)
•Acute TCMR IIB	• 2.2% (n = 4)
• Acute TCMR III	• 0.5% (n = 1)
•Episodes of different categories per patient	•36.7% (n = 67)
ABMR (all episodes, independent of first occurrence of dnDSA) (Banff 2017 Classification)**	24.8% (n = 99)
• Active ABMR	• 16.2% (n = 16)
• Chronic active ABMR	• 59.6% (n = 59)
• Chronic ABMR	• 10.1% (n = 10)
• Episodes of different categories per patient	• 14.1% (n = 14)

Variables with normal distribution: mean ± SD. Variables with non-normal distribution: median and IQR. *At the time of occurrence of the first dnDSA for patients with > 1 dnDSA. **All episodes of ABMR appeared at the time and/or after dnDSA the first occurrence. TX, transplant; dnDSA, *de novo* donor-specific antibody; DBD, donation after brain death; cPRA, calculated panel-reactive antibody; IS, immunosuppression; Anti-IL2R, anti-interleukin-2 receptor; ATG, antithymocyte globulin; HLA, human leukocyte antigen; eGFR, estimated glomerular filtration rate; TCMR, T-cell-mediated rejection; ABMR, antibody-mediated rejection.

frequency of negativity (MFI < 500) after the first occurrence of each dnDSA was analyzed, either temporary or stable negativity.

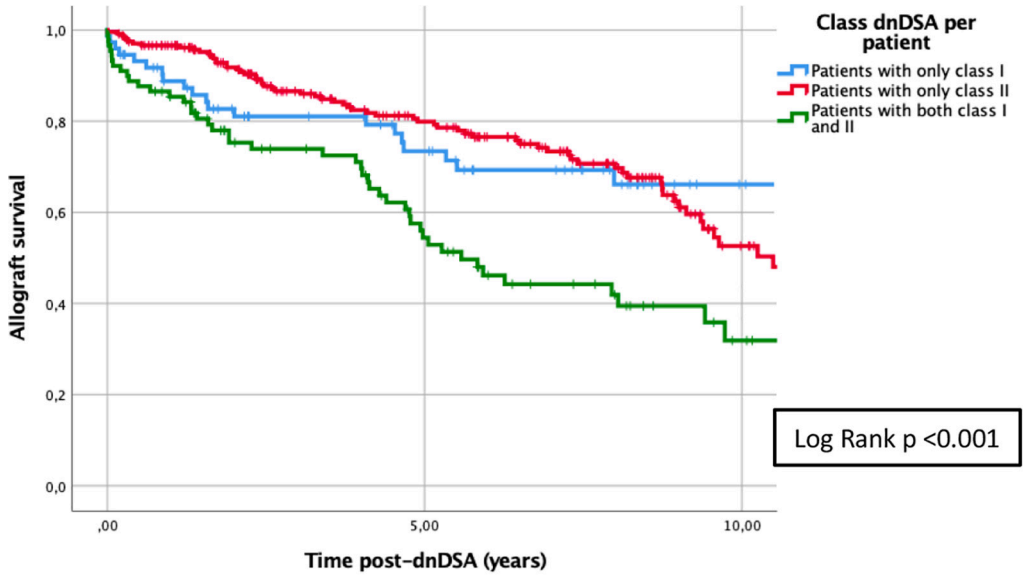
Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR) according to their distribution. Categorical variables were described as relative frequencies. A non-parametric test (Mann–Whitney U test) was used to compare variables with non-normal distribution. A chi-square test was used to compare the average values of categorical variables. Univariable and multivariable Cox regression analyses were performed to determine which clinical variables were associated with death-censored graft loss, and hazard ratios (HR) were reported with 95% confidence intervals. Missing laboratory values due to graft loss or lack of follow-up after dnDSA appearance were imputed using last observation carried forward (LOCF) analysis and automatic multiple imputation (MI) using five default imputations. Time-to-event outcome data were assessed by Kaplan–Meier plots and log-rank tests. P < 5% defined statistical significance. Statistical analysis was conducted using the SPSS statistical software package (IBM SPSS Statistics, Version 25.0. Armonk, NY: IBM Corp.).

Results

In total, we identified 400 patients with dnDSA (**Figure 1**), which accounts for 11.9% of the total population of 3,344 transplanted patients in the period from March 2000 until May 2021. The study cohort comprised mainly patients with a first single-kidney transplant from a deceased donor (**Table 1**) with a median follow-up of 8.3 years (IQR 5.5–10.7) after dnDSA appearance. By design of the study, none of the patients had DSA at the time of transplantation, and only a few were sensitized. Patients with dnDSA in our study had significantly lower long-term allograft survival compared to patients without dnDSA (Control group, n = 2,752), as shown in **Supplementary Figure 1**.

Regular annual DSA screening was performed for more than 18 years (26), with a median number of 1.6 (IQR 1.2–2.0) DSA determinations per patient/year. The median time from the last



Number at risk	At 1st occurrence	5 years	10 years
class I dnDSA	74	38	8
class II dnDSA	237	122	26
class I and II dnDSA	89	35	7

FIGURE 2
Kaplan–Meier survival analysis of death-censored graft failure for HLA class of dnDSA after the first occurrence of the first dnDSA. Five-year death-censored allograft survival post-dnDSA: 73.4% (±5.6%) for patients with class I dnDSA; 79.9% (±2.9%) for patients with class II dnDSA; and 54.4% (±5.9%) for patients with both class I and II dnDSA. Log-rank test $p < 0.001$. dnDSA, *de novo* donor-specific antibodies.

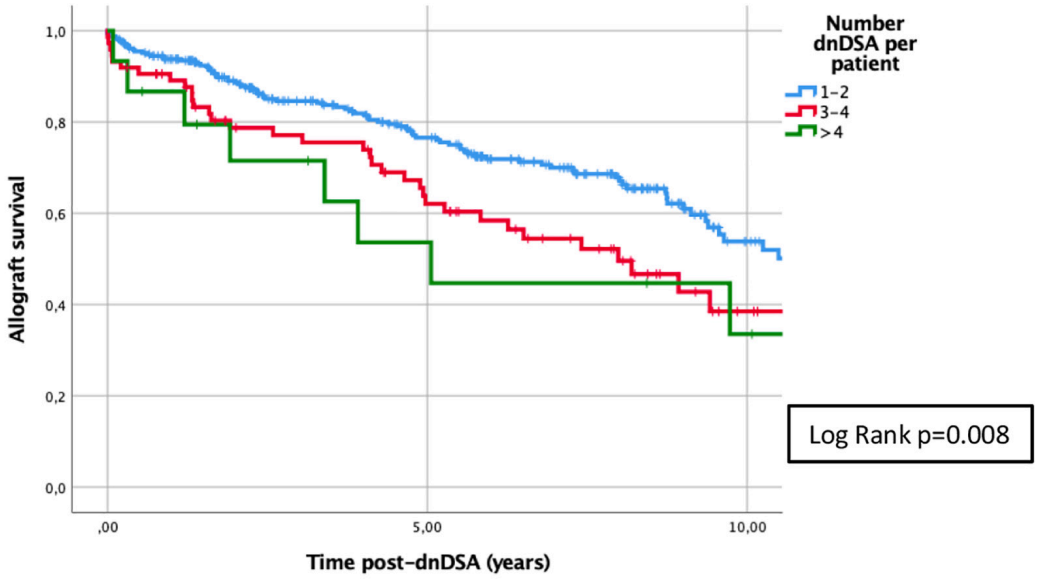
negative sample to the first positive dnDSA was 11.3 (IQR 4.7–20.3) months.

The median number of dnDSA per patient was 1.0, but 10.3% of patients had ≥4 dnDSA. In patients with >1 dnDSA (n = 174, 43.5%), 113 (64.9%) had all dnDSA with the same date of appearance. In the other patients (n = 61, 35.1%), the median time from the first occurrence to the next first appearance of other dnDSA was 14.4 months (IQR 4.7–43.7) (**Supplementary Figures 2–4**).

The biopsies of allograft kidneys were performed by clinical indication (rise in creatinine and/or proteinuria), and 72.0% of patients had at least one biopsy (**Table 1**). About 35.0% of patients had at least one episode of T-cell mediated rejection (TCMR) before the first appearance of dnDSA. All episodes of ABMR appeared at the time and/or after the first occurrence of dnDSA (**Supplementary Figure 5**). Only 26/400 (6.5%) patients had rejection at the time of the first appearance of dnDSA, which, however, accounted for 24.8% of all ABMR episodes. Patients with at least one rejection episode, either TCMR or ABMR, had significantly lower graft survival compared to those patients without rejection, as shown in **Supplementary Figures 6**, **7**. Analyzing the class of dnDSA, 18.5% of the patients presented only class I, 59.3% presented only class II, and 22.3% had both class I and II dnDSA. In patients with DQ-dnDSA (n = 260), 64.2% (n = 167) had only DQ-dnDSA, and 35.8% (n = 93) had DQ along with other dnDSA. In the latter group, most of the patients presented DQ at the time or before the appearance of other dnDSA (n = 79, 84.9%). These 79 patients had additional class I (50.6%), class II (27.8%), and both class I and II (21.5%) dnDSA. In patients with DQ-dnDSA which appeared before other dnDSA (n = 19), the median time from DQ to the occurrence of other dnDSA was 15.1 months (IQR 6.9–20.0). The class of dnDSA was associated with 5-year death-censored allograft survival (**Figure 2**). Similarly, death-censored allograft survival was related to the number of dnDSA (**Figure 3**).

Stratification by dnDSA (n = 731) (**Table 2**) revealed 231 (31.6%) class I and 500 (68.4%) class II dnDSA, including 363 class II-DQ dnDSA (72.6% of class II dnDSA). The median time from transplantation to the first occurrence of each dnDSA was 35.9 months, without significant differences between class I and II (p = 0.575) (**Supplementary Figure 8**).

Analyzing MFI at the time and after the first occurrence in Luminex-defined dnDSA (n = 691; **Table 3**), we had 6.0 (IQR



Number at risk	At 1st occurrence	5 years	10 years
1-2 dnDSA	311	153	32
3-4 dnDSA	74	36	6
>4 dnDSA	15	6	3

FIGURE 3
Kaplan–Meier survival analysis of death-censored graft failure for the number of dnDSA/patient after the first occurrence of the first dnDSA. Five-year death-censored allograft survival post-dnDSA: 76.6% (±2.7%) for patients with 1–2 dnDSA; 62.1% (±6.1%) for patients with 3–4 dnDSA; and 53.6% (±14.2%) for patients with > 4 dnDSA. Log-rank p = 0.008. dnDSA, *de novo* donor-specific antibodies.

4.0–9.0) samples/dnDSA with a median time between samples of 9.0 months (IQR 5.8–11.5). About 24.0% of dnDSA had doubling MFI during follow-up, in 36.9% we observed ≥50% MFI reduction, and 7.5% of dnDSA had fluctuating MFI. Analyzing these results per patient, 27.5% of patients had at least one dnDSA with doubling MFI, 42.5% with ≥50% MFI reduction, and 10.3% with fluctuating MFI. In dnDSA with ≥ 50% MFI reduction (n = 255), 25.5% (n = 65) had received some form of treatment (26), but 74.5% (n = 190) had a 'spontaneous' reduction. Interestingly, 168 (24.3%) dnDSA became negative at some point during follow-up and 100 (14.5%) dnDSA became stable negative. Altogether, 38/400 (9.5%) patients became stable negative.

The relationship between MFI evolution and graft loss is shown in Table 4. The number of dnDSA with doubling and fluctuating MFI was higher in the graft loss group (p < 0.001), and temporary and stable MFI negativity was significantly lower in the graft loss group (p = 0.034 and 0.004).

Specifically analyzing DQ-dnDSA (n = 363, 49.7% of total dnDSA), the proportion of DQ was significantly lower in the graft loss group (53.7 vs. 43.3%, p = 0.006). The number of DQ-dnDSA with MFI available at the first occurrence was 346 (Table 5). At first occurrence, most DQ dnDSA had MFI > 3,000 (74.9%). A ≥ 50% MFI reduction was observed in 31.2% (n = 108), and 7.2% (n = 25) became stable negative. In 84/108 (77.8%) cases, the MFI reduction occurred without treatment. The MFI evolution was associated with 5-year death-censored allograft survival (Figure 4).

Proteinuria and eGFR (observed values, LOCF, and MI) before and after dnDSA appearance are shown in Figures 5, 6 and Supplementary Tables 1, 2. The eGFR was already decreased at the time of the first appearance of dnDSA, with a negative slope after this date (-11.9 ml/min/10 years), clearly demonstrating the importance of imputation compared to observed values. Conversely, proteinuria increased at the time of the first occurrence, and we observed increasing proteinuria over time, especially when we used the multiple imputation method.

Different patient characteristics were associated with death-censored graft loss in univariable Cox regression analyses (Table 6). Interestingly, patients with class II dnDSA had significantly less graft loss (p = 0.007), and the presence

TABLE 2 Characteristics of dnDSA.

Variables	All dnDSA (n = 731)	dnDSA class I (n = 231)	dnDSA class II (n = 500)	p
HLA mismatch A:				**0.001**
HLA mismatch A = 0 (%)	28.8%	19.7%	33.0%	
HLA mismatch A = 1 (%)	52.4%	60.7%	48.6%	
HLA mismatch A = 2 (%)	18.8%	19.7%	18.4%	
HLA mismatch B:				**0.002**
HLA mismatch B = 0 (%)	10.1%	6.1%	11.9%	
HLA mismatch B = 1 (%)	50.1%	45.9%	52.0%	
HLA mismatch B = 2 (%)	39.8%	48.0%	36.0%	
HLA mismatch DRB1:				<**0.001**
HLA mismatch DR = 0 (%)	8.4%	15.7%	5.1%	
HLA mismatch DR = 1 (%)	60.4%	56.8%	62.1%	
HLA mismatch DR = 2 (%)	31.1%	27.5%	32.8%	
HLA mismatch DQB1:				**0.002**
HLA mismatch DQ = 0 (%)	9.2%	14.1%	6.9%	
HLA mismatch DQ = 1 (%)	56.9%	58.1%	56.3%	
HLA mismatch DQ = 2 (%)	33.9%	27.8%	36.8%	
Time (months) from TX to first occurrence of dnDSA	35.9 (14.2–84.7)	35.0 (12.8–85.2)	38.1 (14.2–84.7)	0.575
Time (months) from last negative sample to sample with positive dnDSA	11.3 (4.7–20.3)	9.2 (3.2–19.5)	11.5 (5.6–22.4)	0.120
ABMR (at the time or after each dnDSA) (Banff 2017 Classification)	29.1%	29.4%	29.0%	0.904
Categories:				
• Active ABMR	• 14.6%	• 16.2%	• 13.8%	<**0.001**
• Chronic active ABMR	• 48.4%	• 33.8%	• 55.2%	
• Chronic ABMR	• 6.1%	• 1.5%	• 8.3%	
• Episodes of different previous categories	• 30.9%	• 48.5%	• 22.7%	

Variables with non-normal distribution: median and IQR. HLA, human leukocyte antigen; TX, transplant; dnDSA, *de novo* donor-specific antibody; ABMR, antibody-mediated rejection.

of both class I and II dnDSAs was significantly associated with graft failure ($p < 0.001$). Patients with ≥ 4 dnDSA experienced significantly more frequent graft loss ($p < 0.001$). DGF was associated with graft loss in univariable analysis, and conversely, those patients with a combined transplant experienced significantly less graft failure. Patients with doubling and fluctuating MFI values of dnDSA had significantly more graft loss ($p < 0.001$ and 0.008, respectively), while patients with ≥50% MFI reduction ($p < 0.001$) and stable negative MFI ($p = 0.018$) of dnDSA were significantly associated with less graft failure. These results were confirmed by multivariable Cox regression analysis (**Table 7**). MFI ≥ 50% reduction of dnDSA was associated with a positive outcome in the multivariable model; however, patients with doubling and fluctuating MFI values of dnDSA were not associated with graft loss. DGF was associated with graft failure in this model, and having at least one episode of TCMR or ABMR was an independent risk factor for graft loss. Other than expected, the class and number of dnDSA were not significant in multivariable analysis.

Discussion

It is well-known that dnDSA may appear years after transplantation and are strongly related to ABMR and graft failure (23–29). Despite a huge body of literature, little is known about the natural history of dnDSA and the clinical consequences beyond graft loss. In our study, we performed regular annual screening for HLA antibodies in a large and well-described population with 8 years of follow-up after dnDSA development. dnDSA developed only in 12% of the total cohort transplanted in this 21-year time period. The median time from transplant to the first appearance of dnDSA is around 3 years with a broad range. Graft failure occurred in 33.3% of patients, which is less than expected and probably related to regular dnDSA screening (30–32, 58), enabling early detection of dnDSA. Renal function was already deteriorated at the first occurrence, and in 6.5% of patients, rejection was present at that time. In total, 24.8% developed rejection over the follow-up period, which is clearly associated with poor results. Here,

TABLE 3 MFI values at the first occurrence and MFI evolution of dnDSA analyzed by Luminex®.

Variables	All dnDSA (n = 691)	dnDSA class I (n = 221)	dnDSA class II (n = 470)	p
MFI at first occurrence of dnDSA				<**0.001**
• 1: MFI < 500	• 2.5%	• 5.0%	• 1.3%	
• 2: MFI 500–999	• 11.1%	• 21.7%	• 6.2%	
• 3: 1,000–2,999	• 30.5%	• 41.6%	• 25.3%	
• 4: 3,000–9,999	• 36.5%	• 28.1%	• 40.4%	
• 5: >10,000	• 19.4%	• 3.6%	• 26.8%	
MFI evolution of dnDSA after first occurrence^				0.080
• 1: MFI doubling	• 24.0%	• 23.1%	• 24.5%	
• 2: MFI reduction ≥50%	• 36.9%	• 41.2%	• 34.9%	
○ Specific active treatment for ABMR*	○ 25.5% (n = 65)	○ 26.4% (n = 24)	○ 25.0% (n = 41)	
• 3: MFI fluctuating (MFI doubling and reduction ≥50% at some point)	• 7.5%	• 9.5%	• 6.6%	
• 4: Other	• 24.0%	• 18.1%	• 26.8%	
• 5: No MFI evolution available	• 7.5%	• 8.1%	• 7.2%	
dnDSA becomes negative (MFI < 500) at some point during evolution	24.3%	37.1%	18.3%	<**0.001**
dnDSA becomes constant negative (MFI < 500) (Stable negative)**	14.5%	23.1%	10.4%	<**0.001**

^MFI evolution independent of biopsy-proven rejection and treatments. *p-value = 0.809. **Stable negative dnDSA defined as MFI < 500 in every sample after the first negative sample. MFI, mean fluorescence intensity; dnDSA, *de novo* donor-specific antibody; ABMR, antibody-mediated rejection.

we describe fluctuating or increasing MFI values in a substantial number of patients, which is associated with inferior outcomes. In our cohort, 27.5% of patients have doubling MFI of dnDSA during follow-up. However, for the first time, we also describe a relevant cohort of patients (9.5%) with a stable disappearance of dnDSA, associated with better outcomes. In summary, our study provides detailed and granular clinical data for the natural history of dnDSA, which provides a solid basis for further studies and risk stratification.

Due to the strong association between the development of anti-HLA antibodies after transplantation and graft failure, sequential monitoring of HLA antibodies posttransplant has been recommended in different studies (16–18). Although there are clear recommendations for the screening of dnDSA when there is impaired kidney function, the universal screening and its frequency in stable patients is not well established (30–32). In our patients the median time to dnDSA positivity after the last negative test result is 11.3 months, supporting regular annual screening even in low risk, pretransplant DSA-negative patients. Almost half of the patients (43.5%) developed > 1 dnDSA, which was detected in most patients at first occurrence and in the others after a median of 14.4 months. These results support the value of annual screening for HLA antibodies after kidney transplantation for early detection of dnDSA.

Different risk factors for the development of dnDSA are described, with high HLA mismatch being one of the most important factors (33–36). As expected, a greater HLA-A and -B mismatch is significantly related to class I dnDSA formation in our cohort, and conversely, higher HLA-DRB1 and HLA-DQB1 mismatches are associated with class II dnDSA development. Thus, our study provides additional evidence for good HLA matching, which might be the easiest way to prevent the development of dnDSA. Graft inflammation, such as TCMR, can increase immunogenicity and can also precipitate the formation of dnDSA (23, 28). We can confirm this strong association, as around one-third of our patients had experienced TCMR before the appearance of dnDSA. Despite this, our study was not designed to specifically evaluate potential risk factors for the development of dnDSA in detail, since this was not the objective of our analysis.

The important role of dnDSA in the development of ABMR and graft dysfunction is well defined (23–26). ABMR was already present in 6.5% of patients at first occurrence and increased to 24.8% after around 8 years of follow-up. It has been described that class I dnDSA are more related to active ABMR, and conversely, class II dnDSA are commonly associated with chronic changes (23, 27, 37, 38), which is confirmed in our large cohort. As expected, the development of ABMR is significantly associated with a 2.7-fold higher risk of graft loss in multivariable analysis. Surprisingly, TCMR was also strongly associated with graft loss (HR 2.5), which might be explained by the local inflammation produced by TCMR, and

TABLE 4 MFI values at the first occurrence and MFI evolution of dnDSA analyzed by Luminex® and relationship with graft loss.

Variables	All dnDSA *n* = 691	No graft loss (*n* = 430)	Graft loss (*n* = 261)	*p*
MFI at first occurrence of dnDSA				0.563
• 1: MFI < 500	• 2.5%	• 2.3%	• 2.7%	
• 2: MFI 500–999	• 11.1%	• 10.7%	• 11.9%	
• 3: 1,000–2,999	• 30.5%	• 32.8%	• 26.8%	
• 4: 3,000–9,999	• 36.5%	• 34.9%	• 39.1%	
• 5: > 10,000	• 19.4%	• 19.3%	• 19.5%	
MFI evolution of dnDSA after first occurrence^				<**0.001**
• 1: MFI doubling	• 24.0%	15.1%	38.1%	
• 2: MFI reduction ≥ 50%	• 36.9%	44.4%	24.5%	
• 3: MFI fluctuating (MFI doubling and reduction ≥ 50% at some point)	• 7.5%	4.9%	11.9%	
• 4: Other	• 24.0%	25.6%	21.5%	
• 5: No MFI evolution available	7.5%	10.0%	3.4%	
dnDSA becomes negative MFI < 500 at some point during evolution	24.3%	27.2%	19.8%	**0.034**
dnDSA becomes constant negative (MFI < 500) (Stable negative)*	14.5%	17.4%	9.6%	**0.004**

^MFI evolution independent of biopsy-proven rejection and treatments. *Stable negative dnDSA defined as MFI < 500 in every sample after the first negative sample. MFI, mean fluorescence intensity; dnDSA, *de novo* donor-specific antibody.

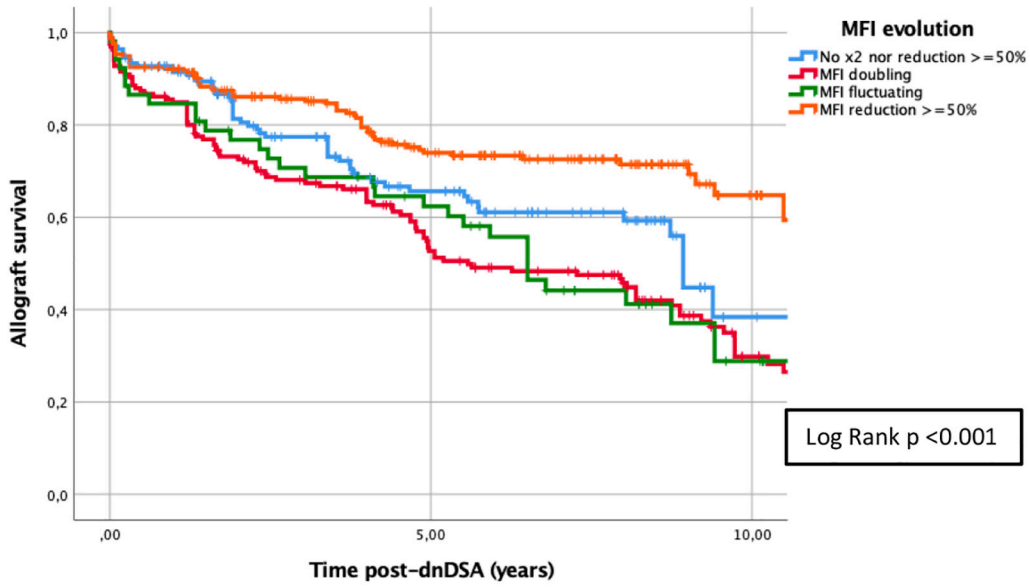
TABLE 5 MFI values at first occurrence and MFI evolution of DQ-dnDSA analyzed by Luminex®.

Variables	All DQ dnDSA (*n* = 346)
MFI at first occurrence of dnDSA	
• 1: MFI < 500	• 0.6%
• 2: MFI 500–999	• 2.9%
• 3: 1,000–2,999	• 21.7%
• 4: 3,000–9,999	• 42.8%
• 5: > 10,000	• 32.1%
MFI evolution of dnDSA after first occurrence^	
• 1: MFI doubling	• 26.6%
• 2: MFI reduction ≥ 50%	• 31.2%
• 3: MFI fluctuating (MFI doubling and reduction ≥ 50% at some point)	• 5.5%
• 4: Other	• 28.3%
• 5: No MFI evolution available	• 8.4%
dnDSA becomes negative MFI < 500 at some point during evolution	12.3%
dnDSA becomes constant negative (MFI < 500) (Stable negative)*	7.2%

^MFI evolution independent of biopsy-proven rejection and treatments. *Stable negative dnDSA defined as MFI < 500 in every sample after the first negative sample. MFI, mean fluorescence intensity; dnDSA, *de novo* donor-specific antibody.

tubulitis may result in subsequent irreversible nephron injury (59), which supports previous observations that TCMR is an independent and important risk factor for graft loss (5, 60, 61).

Previous literature suggested that class I dnDSA are less common and may appear sooner, while class II dnDSA, especially DQ, are frequently found and related to rejection and graft dysfunction (23, 27, 37–41, 62). In our study, there are no differences according to the class of dnDSA in the time of appearance after transplantation. We confirm that class II-DQ dnDSA are the most common dnDSA and potentially less harmful. In our analysis, the combination of class I and II dnDSA in particular has a negative impact on graft survival in



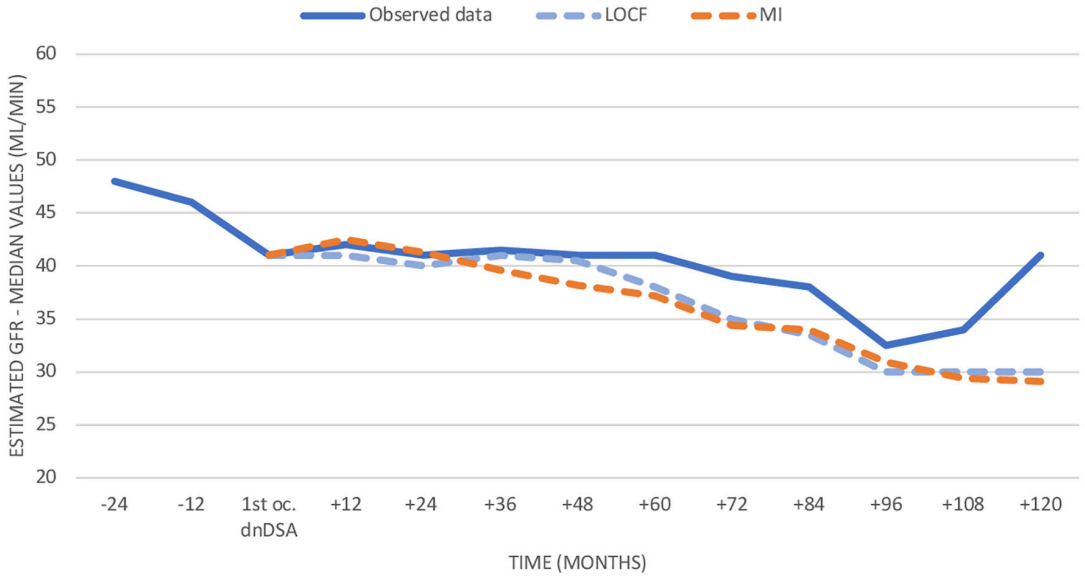
Number at risk	At 1st occurrence	5 years	10 years
MFI reduction≥50%	255	124	20
MFI stable	166	64	3
MFI fluctuating	52	29	6
MFI doubling	166	74	21

FIGURE 4
Kaplan–Meier survival analysis of death-censored graft failure for dnDSA-MFI evolution after the first occurrence of dnDSA. Five-year death-censored allograft survival post-dnDSA: 74.0% (±3.0%) when MFI reduction ≥ 50%; 65.6% (±4.2%) when no MFI reduction ≥50% nor MFI doubling; 62.4% (±6.9%) when MFI fluctuating; and 52.7% (±4.0%) when MFI doubling. Log-rank $p < 0.001$. dnDSA, *de novo* donor-specific antibodies; MFI, mean fluorescence intensity.

univariable analysis, which, however, was not supported in the multivariable model, being in line with previous studies (27, 43). The impact of the number of dnDSA per patient, independent of class, on graft survival is not known yet, since it has not been specifically analyzed in previous studies. In our cohort, 43.5% of patients have >1 dnDSA, and a higher number of dnDSA per patient is associated with inferior 5-year graft survival, although this is not supported by multivariable analysis.

Today, Luminex®-based SAB technology is standard, and provides semi-quantitative information on the antibody level through the MFI value (44–46). One of the main problems is the lack of consensus on MFI positivity thresholds (32, 44, 46). There is no clear association between the MFI level and clinical outcomes (44–48). In our center, the general MFI cut-off to determine positivity is 1,000, and most of the dnDSA have MFI ≥ 1,000 at first occurrence. However, 13.6% of dnDSA present MFI below the cut-off level. In this latter group, we defined dnDSA by plausibility, epitope sharing, and other factors beyond the simple MFI value (52). We observed higher MFI in patients with class II dnDSA at the time of the first appearance. Interestingly, we did not observe a clear relationship between MFI values at first occurrence and outcome. Therefore, our data do not support a fixed MFI threshold, as low plausible MFI values also may have detrimental effects. Instead, our data provide further evidence for the complexity and limitations of MFI values and their interpretation.

It has been described that changes in MFI values <25%, or in some cases <50%, are not considered clinically important (32), but until now no studies have analyzed the MFI evolution of dnDSA and its relationship with graft failure in greater detail. The evolution of MFI in our study is analyzed by classifying dnDSA into three categories, with MFI reduction ≥50% being the most frequently observed category (36.9%). In these cases, only one-quarter had received specific active treatment for ABMR. As around 75% of patients with dnDSA did not develop clinical ABMR, our data suggest that the indication for allograft biopsy or potentially harmful treatment should be specifically evaluated in each case, since the appearance of dnDSA is not always associated with ABMR and a spontaneous reduction of MFI, and even stable negativity, without active treatment is frequent. Analyzing the relationship



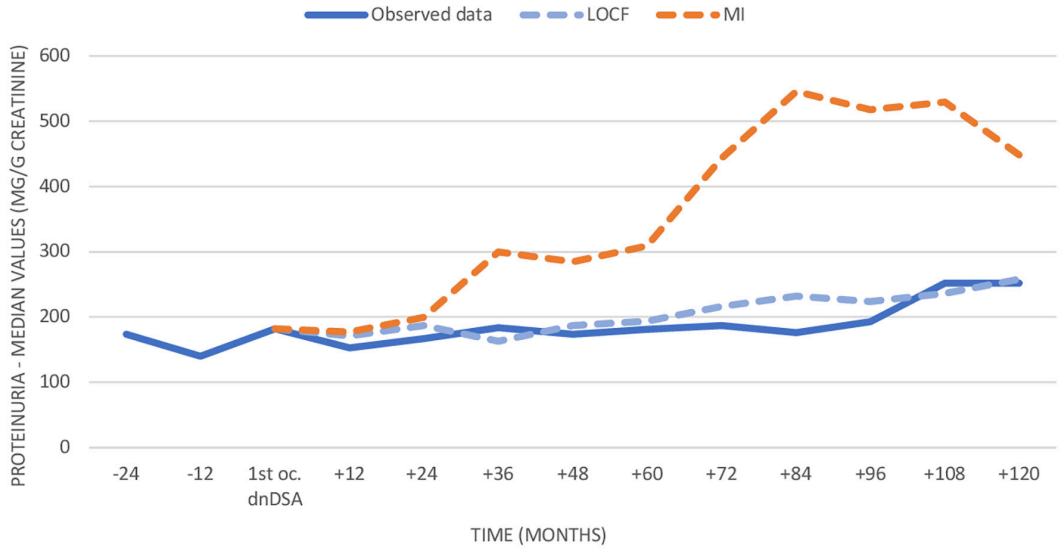
Time (months)	-24	-12	First dnDSA	+12	+24	+36	+48	+60	+72	+84	+96	+108	+120
Observed data (n)	n=147	n=174	n=306	n=235	n=175	n=234	n=211	n=192	n=156	n=121	n=102	n=65	n=37
	-24	-12	First dnDSA	+12	+24	+36	+48	+60	+72	+84	+96	+108	+120
LOCF (n)	n=147	n=174	n=306	n=324	n=336	n=267	n=276	n=281	n=281	n=282	n=282	n=284	n=284
	-24	-12	First dnDSA	+12	+24	+36	+48	+60	+72	+84	+96	+108	+120
MI (n)	n=147	n=174	n=306	n=330	n=330	n=330	n=330	n=330	n=330	n=330	n=330	n=330	n=330

FIGURE 5
Evolution of eGFR (ml/min) before and after the first occurrence of the first dnDSA. Missing values after dnDSA were imputed using last observation carried forward (LOCF) analysis and multiple imputation (MI). eGFR, estimated glomerular filtration rate; dnDSA, *de novo* donor-specific antibodies.

of MFI evolution with death-censored graft failure, MFI reduction ≥50% of dnDSA is a protective factor for graft loss, and this is supported by multivariable Cox regression analysis (p = 0.012). However, doubling and fluctuating MFI are related to graft failure in univariable analysis but are not contributors in the multivariable model (p = 0.054 and 0.419, respectively).

Specifically analyzing the MFI negativity, the proportion of dnDSA with stable negative MFI is 14.5%, with a greater MFI negativity in class I dnDSA (p < 0.001). Temporary and stable MFI negativity are significantly associated with better graft survival. This stable disappearance of dnDSA may have several causes, such as the development of anti-idiotypic antibodies that suppress DSA production (63, 64), which is not the objective of our analysis. Nevertheless, in our study, we show the relevance of stable negativity of dnDSA, this being related to better outcomes.

Evaluating DQ-dnDSA (39, 40, 62) MFI values and negativity, the MFI at the time of the first appearance is higher with MFI > 3,000 in 74.9%, and DQ-dnDSA are more persistent, being lower than the proportion of stable negative MFI (7.2%), although their presence is not significantly related to graft failure in our study. With our results, we can conclude that DQ-dnDSA are potentially less harmful to the graft or produce insidious and progressive chronic damage with late graft failure as described in some studies (23, 62); therefore, a longer follow-up is needed to evaluate long-term graft outcome. It has also been described that class II dnDSA, and therefore DQ, are usually non-complement binding IgG2 and IgG4 subclasses (23), suggesting a different, less studied, and complement-independent pathway of damage that could explain our findings. For the first time in our study, we provide important evidence about DQ evolution in our large cohort of patients, being the most frequent dnDSA after transplantation, presenting with higher MFI, and being more persistent. Accordingly, with our data, we support and highlight



Time (months)	-24	-12	First dnDSA	+12	+24	+36	+48	+60	+72	+84	+96	+108	+120
Observed data (n)	n=144	n=184	n=340	n=212	n=195	n=159	n=164	n=166	n=143	n=117	n=99	n=58	n=32
	-24	-12	First dnDSA	+12	+24	+36	+48	+60	+72	+84	+96	+108	+120
LOCF (n)	n=144	n=184	n=340	n=343	n=347	n=237	n=246	n=252	n=255	n=258	n=260	n=261	n=261
	-24	-12	First dnDSA	+12	+24	+36	+48	+60	+72	+84	+96	+108	+120
MI (n)	n=144	n=184	n=340	n=306	n=306	n=306	n=306	n=306	n=306	n=306	n=306	n=306	n=306

FIGURE 6
Evolution of proteinuria (mg/g creatinine) before and after first occurrence of first dnDSA. Missing values after dnDSA were imputed using last observation carried forward (LOCF) analysis and multiple imputation (MI). dnDSA, *de novo* donor-specific antibodies.

the need to expand knowledge about DQ-dnDSA and improve HLA-DQ matching strategies.

Changes in renal function were already registered in our study together with the first appearance of dnDSA. Although it has been described in some studies with sequential HLA antibody monitoring posttransplant that antibodies may appear before a rise in serum creatinine (17), in our cohort, renal function deteriorates at dnDSA first occurrence, and some patients already experience ABMR. Ten years after dnDSA, proteinuria and eGFR had worsened significantly, demonstrating the negative impact of dnDSA. Our data also highlight the importance of imputation methods, as results related to observed values are biased due to missing data in patients with graft loss.

The strength of our study is essentially to have a large and well-described cohort of patients with regular screening for DSA. In addition, our large and in-depth analysis of MFI by Luminex® with long follow-up enables us to specifically evaluate the characteristics of the patients and the MFI evolution of each dnDSA. Furthermore, having allograft biopsies and analytical data available already at first dnDSA appearance makes it possible to correlate early clinical features with long-term clinical outcomes.

Nevertheless, our study has several limitations. This is a retrospective analysis, and we did not evaluate in depth a control group and did not analyze the factors that may be associated with dnDSA formation in greater detail. For such a study a different methodology (e.g., matched pairs and propensity score matching) is needed in order to avoid survival bias. In our cohort, adherence to treatment and levels of immunosuppressive drugs are not evaluated at the time of appearance of dnDSA. The analysis of dnDSA by Luminex® with MFI data is currently the best tool available, although we must know the limitations of this assay. For patients with ≥50% reduction in MFI, we only registered specific active treatment for ABMR, but we were not able to analyze changes in chronic baseline immunosuppression. The evaluation of the class and level of dnDSA is key, but other characteristics, such as the complement binding capacity or IgG subclasses, also have an impact, which are not evaluated in our study as these tests are

TABLE 6 Univariable Cox regression for death-censored graft loss.

Univariable Cox regression for death-censored graft loss	HR	CI 95% INF	CI 95% SUP	*p*
Patients with only class I dnDSA	0.7	0.4	1.2	0.310
Patients with only class II dnDSA	0.6	0.4	0.8	**0.007**
Patients with both class I and II dnDSA	2.1	1.5	3.1	<**0.001**
Patients with ≥ 2 dnDSA (independent of class)	1.4	0.9	1.9	0.053
Patients with ≥ 4 dnDSA (independent of class)	2.4	1.5	3.7	<**0.001**
Number of dnDSA per patient	1.2	1.0	1.3	**0.001**
MFI evolution of dnDSA*				
• Patients with MFI doubling of dnDSA (%)	1.9	1.3	2.7	< **0.001**
• Patients with MFI reduction ≥ 50% of dnDSA (%)	0.4	0.3	0.7	< **0.001**
• Patients with MFI fluctuating of dnDSA (MFI doubling and reduction ≥ 50% at some point) (%)	1.8	1.1	2.8	**0.008**
• Patients with other MFI evolution of dnDSA (stable) (%)	1.3	0.9	1.9	0.114
Patients with stable negative MFI of all dnDSA**	0.3	0.1	0.8	**0.018**
Cold ischemia time (CIT, minutes)	1.0	1.0	1.0	0.106
Delayed graft function (DGF)	1.7	1.1	2.4	**0.004**
cPRA ≥ 5% at the time of TX (Eurotransplant)	1.1	0.7	1.8	0.433
cPRA ≥ 85% at the time of TX (Eurotransplant)	1.0	0.5	2.1	0.877
Donor type				
• Deceased donor	1.1	0.7	1.5	0.593
• Living donor	0.9	0.6	1.3	0.593
First kidney transplant	0.8	0.5	1.3	0.486
Combined transplant	0.3	0.1	0.9	**0.040**
TCMR (all episodes, independent of first occurrence of dnDSA) (Banff 2017 Classification)	3.4	2.3	5.0	<**0.001**
ABMR (all episodes, independent of first occurrence of dnDSA) (Banff 2017 Classification)***	4.1	2.9	5.7	<**0.001**

*MFI evolution of at least one dnDSA of the patient. **Patients with all dnDSA stable negative (stable negative MFI defined as MFI < 500 in every sample after the first negative sample of dnDSA). ***All episodes of ABMR appeared at the time and/or after dnDSA first occurrence. dnDSA, *de novo* donor-specific antibody; MFI, mean fluorescence intensity; cPRA, calculated panel-reactive antibody; TX, transplant; TCMR, T-cell-mediated rejection; ABMR, antibody-mediated rejection. HR, hazard ratio; CI, confidence interval; SUP, superior; INF, inferior.

TABLE 7 Multivariable Cox regression analysis for death-censored graft loss.

Multivariable Cox regression for death-censored graft loss	HR	CI 95% INF	CI 95% SUP	*p*
Patients with only class II dnDSA	0.7	0.4	1.2	0.271
Patients with both class I and II dnDSA	1.2	0.6	2.3	0.467
Number of dnDSA per patient	1.0	0.9	1.2	0.512
MFI evolution of dnDSA*				
• Patients with MFI doubling of dnDSA (%)	1.4	0.9	2.1	0.054
• Patients with MFI reduction ≥ 50% of dnDSA (%)	0.5	0.3	0.8	**0.012**
• Patients with MFI fluctuating of dnDSA (MFI doubling and reduction ≥ 50% at some point) (%)	1.2	0.7	1.9	0.419
Delayed graft function (DGF)	2.0	1.3	2.9	<**0.001**
Combined transplant	0.9	0.3	2.5	0.874
TCMR (Banff 2017 Classification)**	2.5	1.7	3.8	<**0.001**
ABMR (Banff 2017 Classification)**	2.7	1.8	4.1	<**0.001**

*MFI evolution of at least one dnDSA of the patient. **All episodes, independent of the first occurrence of dnDSA. dnDSA, *de novo* donor-specific antibody; MFI, mean fluorescence intensity; DGF, delayed graft function; TCMR, T-cell-mediated rejection; ABMR, antibody-mediated rejection. HR, hazard ratio; CI, confidence interval; SUP, superior; INF, inferior.

not performed routinely. Last classical antigen HLA mismatch is considered to describe dnDSA specificities in our cohort, without analyzing HLA epitope mismatch.

In summary, we are providing a large body of evidence for the natural course of dnDSA. We highlight the problem of the MFI positivity threshold, as even low, but plausible, MFI may

have a negative impact. We confirm the high frequency of DQ dnDSA, presenting with higher MFI at the time of appearance and being more persistent, but seem less harmful to the graft. For the first time, we describe that MFI evolution is associated with graft survival, demonstrating the positive effect of a ≥50% reduction in MFI values, and we observed that almost 10% of patients became stable negative, which is related to better outcomes. Our large observational study provides important evidence for a better understanding of the evolution of dnDSA in renal allograft recipients. Further studies are needed to distinguish those dnDSA which are harmful from those dnDSA with an uneventful clinical course. A better knowledge of relevant HLA epitopes or the use of novel biomarkers of graft dysfunction, such as cell-free DNA, may provide additional information to identify patients at risk.

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/s.

Ethics statement

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

Author contributions

CL, NL, and KB conceived and designed the study and wrote the article. MN and DSc provided technical support and acquired the data. CL analyzed and interpreted the data. NL, DSt, and SH performed HLA antibody testing. KW performed and interpreted histopathological examinations. MN, BO, AA, MC, FB, FH, and ES advised on the preparation of the article and provided conceptual advice. KB designed the study and supervised the research. All authors contributed to the article and approved the submitted version.

Funding

CL was supported by a research grant from the Valdecilla Biomedical Research Institute (IDIVAL).

Acknowledgments

We thank all the authors for their contributions.

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.943502/full#supplementary-material

References

1. Coemans M, Caner S, Döhler B, Anglicheau D, Giral M, Bestard O, et al. Analyses of the short- and long-term graft survival after kidney transplantation in Europe between 1986 and 2015. *Kidney Int.* (2018) 94:964–73. doi: 10.1016/j.kint.2018.05.018

2. Jardine AG, Hartmann A, Holdaas H. Long-term renal allograft survival: a quiet revolution. *Kidney Int.* (2018) 94:853–5. doi: 10.1016/j.kint.2018.08.005

3. Poggio ED, Augustine JJ, Arrigain S, Brennan DC, Schold JD. Long-term kidney transplant graft survival-Making progress when most needed. *Am J Transplant.* (2021) 21:2824–32. doi: 10.1111/ajt.16463

4. Hariharan S, Israni AK, Danovitch G. Long-Term survival after kidney transplantation. *N Engl J Med.* (2021) 385:729–43. doi: 10.1056/NEJMra2014530

5. Mayrdorfer M, Liefeldt L, Wu K, Rudolph B, Zhang Q, Friedersdorff F, et al. Exploring the complexity of death-censored kidney allograft failure. *J Am Soc Nephrol.* (2021) 32:1513–26. doi: 10.1681/ASN.2020081215

6. El-Zoghby ZM, Stegall MD, Lager DJ, Kremers WK, Amer H, Gloor JM, et al. Identifying specific causes of kidney allograft loss. *Am J Transplant.* (2009) 9:527–35. doi: 10.1111/j.1600-6143.2008.02519.x

7. Sellarés J, de Freitas DG, Mengel M, Reeve J, Einecke G, Sis B, et al. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant.* (2012) 12:388–99. doi: 10.1111/j.1600-6143.2011.03840.x

8. Naesens M, Kuypers DR, De Vusser K, Evenepoel P, Claes K, Bammens B, et al. The histology of kidney transplant failure: a long-term follow-up study. *Transplantation.* (2014) 98:427–35. doi: 10.1097/TP.0000000000000183

9. Chand S, Atkinson D, Collins C, Briggs D, Ball S, Sharif A, et al. The spectrum of renal allograft failure. *PLoS One.* (2016) 11:e0162278. doi: 10.1371/journal.pone.0162278

10. Van Loon E, Senev A, Lerut E, Coemans M, Callemeyn J, Van Keer JM, et al. Assessing the complex causes of kidney allograft loss. *Transplantation.* (2020) 104:2557–66. doi: 10.1097/TP.0000000000003192

11. Loupy A, Haas M, Roufosse C, Naesens M, Adam B, Afrouzian M, et al. The Banff 2019 kidney meeting report (I): updates on and clarification of criteria for T cell- and antibody-mediated rejection. *Am J Transplant.* (2020) 20:2318–31. doi: 10.1111/ajt.15898

12. Lefaucheur C, Louis K, Philippe A, Loupy A, Coates PT. The emerging field of non–human leukocyte antigen antibodies in transplant medicine and beyond. *Kidney Int.* (2021) 100:787–98. doi: 10.1016/j.kint.2021.04.044

13. Lefaucheur C, Viglietti D, Bouatou Y, Philippe A, Pievani D, Aubert O, et al. Non-HLA agonistic anti-angiotensin II type 1 receptor antibodies induce a distinctive phenotype of antibody-mediated rejection in kidney transplant recipients. *Kidney Int.* (2019) 96:189–201. doi: 10.1016/j.kint.2019.01.030

14. Cardinal H, Dieudé M, Hébert MJ. The emerging importance of Non-HLA autoantibodies in kidney transplant complications. *J Am Soc Nephrol.* (2017) 28:400–6. doi: 10.1681/ASN.2016070756

15. Clotet-Freixas S, Kotlyar M, McEvoy CM, Pastrello C, Rodríguez-Ramírez S, Farkona S, et al. Increased autoantibodies against Ro/SS-A, CENP-B, and La/SS-B in patients with kidney allograft antibody-mediated rejection. *Transplant Direct.* (2021) 7:e768. doi: 10.1097/TXD.0000000000001215

16. Lachmann, N, Terasaki PI, Schönemann C. Donor-specific HLA antibodies in chronic renal allograft rejection: a prospective trial with a four-year follow-up. *Clin Transpl.* (2006) 171–99.

17. Mao Q, Terasaki PI, Cai J, Briley K, Catrou P, Haisch C, et al. Extremely high association between appearance of HLA antibodies and failure of kidney grafts in a five-year longitudinal study. *Am J Transplant.* (2007) 4:864–71. doi: 10.1111/j.1600-6143.2006.01711.x

18. Zhu L, Lee P, Everly MJ, Terasaki PI. Detailed examination of HLA antibody development on renal allograft failure and function. *Clin Transpl.* (2008) 2008:171–87.

19. Haas M, Loupy A, Lefaucheur C, Roufosse C, Glotz D, Seron D, et al. The Banff 2017 kidney meeting report: revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *Am J Transplant.* (2018) 18:293–307. doi: 10.1111/ajt.14625

20. Callemeyn J, Ameye H, Lerut E, Senev A, Coemans M, Van Loon E, et al. Revisiting the changes in the Banff classification for antibody- mediated rejection after kidney transplantation. *Am J Transplant.* (2021) 21:2413–23. doi: 10.1111/ajt.16474

21. Aubert O, Loupy A, Hidalgo L, Duong van Huyen JP, Higgins S, et al. Antibody-Mediated rejection due to preexisting versus de novo donor-specific antibodies in kidney allograft recipients. *J Am Soc Nephrol.* (2017) 28:1912–23. doi: 10.1681/ASN.2016070797

22. Ziemann M, Altermann W, Angert K, Arns W, Bachmann A, Bakchoul T, et al. Preformed donor-specific HLA antibodies in living and deceased donor transplantation: a multicenter study. *Clin J Am Soc Nephrol.* (2019) 14:1056–66. doi: 10.2215/CJN.13401118

23. Zhang R. Donor-Specific antibodies in kidney transplant recipients. *Clin J Am Soc Nephrol.* (2018) 13:182–92. doi: 10.2215/CJN.00700117

24. Filippone EJ, Farber JL. Humoral immune response and allograft function in kidney transplantation. *Am J Kidney Dis.* (2015) 66:337–47. doi: 10.1053/j.ajkd.2015.03.033

25. Ginevri F, Nocera A, Comoli P, Innocente A, Cioni M, Parodi A, et al. Posttransplant de novo donor-specific hla antibodies identify pediatric kidney recipients at risk for late antibody-mediated rejection. *Am J Transplant.* (2012) 12:3355–62. doi: 10.1111/j.1600-6143.2012.04251.x

26. Lachmann N, Terasaki PI, Budde K, Liefeldt L, Kahl A, Reinke P, et al. Anti-human leukocyte antigen and donor-specific antibodies detected by luminex posttransplant serve as biomarkers for chronic rejection of renal allografts. *Transplantation.* (2009) 87:1505–13. doi: 10.1097/TP.0b013e3181a44206

27. Ntokou I-SA, Iniotaki AG, Kontou EN, Darema MN, Apostolaki MD, Kostakis AG, et al. Long-term follow up for anti-HLA donor specific antibodies postrenal transplantation: high immunogenicity of HLA class II graft molecules. *Transpl Int.* (2011) 24:1084–93. doi: 10.1111/j.1432-2277.2011.01312.x

28. Wiebe C, Gibson IW, Blydt-Hansen TD, Karpinski M, Ho J, Storsley LJ, et al. Evolution and clinical pathologic correlations of de novo donor specific HLA antibody post kidney transplant. *Am J Transplant.* (2012) 12:1157–67. doi: 10.1111/j.1600-6143.2012.04013.x

29. Everly MJ, Rebellato LM, Haisch CE, Ozawa M, Parker K, Briley KP, et al. Incidence and impact of de novo donor-specific alloantibody in primary renal allografts. *Transplantation.* (2013) 95:410–7. doi: 10.1097/TP.0b013e31827d62e3

30. Cun H, Hönger G, Kleiser M, Amico P, Wehmeier C, Steiger J, et al. Screening strategy for de novo donor-specific HLA antibodies beyond the first year after kidney transplantation: personalized or "one size fits all"? *Clin Transplant.* (2021) 35:e14170. doi: 10.1111/ctr.14170

31. Cooper JE, Gralla J, Cagle L, Goldberg R, Chan L, Wiseman AC. Inferior kidney allograft outcomes in patients with de novo donor-specific antibodies are due to acute rejection episodes. *Transplantation.* (2011) 91:1103–9. doi: 10.1097/TP.0b013e3182139da1

32. Tambur AR, Campbell P, Claas FH, Feng S, Gebel HM, Jackson AM, et al. Sensitization in transplantation: assessment of risk (STAR) 2017 working group meeting report. *Am J Transplant.* (2018) 18:1604–14. doi: 10.1111/ajt.14752

33. Lachmann N, Niemann M, Reinke P, Budde K, Schmidt D, Halleck F, et al. Donor-recipient matching based on predicted indirectly recognizable HLA epitopes independently predicts the incidence of de novo donor-specific HLA antibodies following renal transplantation. *Am J Transplant.* (2017) 17:3076–86. doi: 10.1111/ajt.14393

34. Unterrainer C, Döhler B, Niemann M, Lachmann N, Süsal C. Can PIRCHE-II matching outmatch traditional HLA matching? *Front Immunol.* (2021) 12:631246. doi: 10.3389/fimmu.2021.631246

35. Sakamoto S, Iwasaki K, Tomosugi T, Niemann M, Spierings E, Miwa Y, et al. Analysis of T and B cell epitopes to predict the risk of de novo donor-specific antibody (DSA) production after kidney transplantation: a two-center retrospective cohort study. *Front Immunol.* (2020) 11:2000. doi: 10.3389/fimmu.2020.02000

36. Bestard B, Meneghini M, Crespo E, Bemelman F, Koch M, Volk HD, et al. Preformed T cell alloimmunity and HLA eplet mismatch to guide immunosuppression minimization with tacrolimus monotherapy in kidney transplantation: results of the CELLIMIN trial. *Am J Transplant.* (2021) 21:2833–45. doi: 10.1111/ajt.16563

37. Lee PC, Zhu L, Terasaki PI, Everly MJ. HLA-specific antibodies developed in the first year posttransplant are predictive of chronic rejection and renal graft loss. *Transplantation.* (2009) 88:568–74. doi: 10.1097/TP.0b013e3181b11b72

38. Kumbala D, Zhang R. Essential concept of transplant immunology for clinical practice. *World J Transplant.* (2013) 3:113–8. doi: 10.5500/wjt.v3.i4.113

39. Cunha APL, Fabreti-Oliveira RA, Lasmar MF, Garcia JC, Vilela TP, Nascimento E. Clinical outcome of kidney transplant patients on the allograft function, loss, effects of HLA-DQB1-DSA +, and Graft Survival. *Transplant Proc.* (2021) 53:2188–96. doi: 10.1016/j.transproceed.2021.07.011

40. Senev A, Coemans M, Lerut E, Van Sandt V, Kerkhofs J, Daniëls L, et al. Eplet mismatch load and de novo occurrence of donor-specific Anti-HLA antibodies, rejection, and graft failure after kidney transplantation: an observational cohort study. *J Am Soc Nephrol.* (2020) 31:2193–204. doi: 10.1681/ASN.2020010019

41. Tambur AR, Campbell P, Chong AS, Feng S, Ford ML, Gebel H, et al. Sensitization in transplantation: assessment of risk (STAR) 2019 working group meeting report. *Am J Transplant.* (2020) 20:2652–68. doi: 10.1111/ajt.15937

42. Cooper JE, Gralla J, Chan L, Wiseman AC. Clinical significance of post kidney transplant de novo DSA in otherwise stable grafts. *Clin Transpl.* (2011) 2011:359–64.

43. Liu W, Zhao J, Kang ZY, Xiao YL, Yang L, Liu C, et al. De novo donor-specific HLA antibodies reduce graft survival rates and increase the risk of kidney transplant rejection: a single-center retrospective study. *Transpl Immunol.* (2021) 68:101430. doi: 10.1016/j.trim.2021.101430

44. Tambur AR, Herrera ND, Haarberg KM, Cusick MF, Gordon RA, Leventhal JR, et al. Assessing antibody strength: comparison of MFI, C1q, and titer information. *Am J Transplant.* (2015) 15:2421–30. doi: 10.1111/ajt.13295

45. Tambur AR, Wiebe C. HLA diagnostics: evaluating DSA strength by titration. *Transplantation.* (2018) 102:S23–30. doi: 10.1097/TP.0000000000001817

46. Süsal C, Wettstein D, Döhler B, Morath C, Ruhenstroth A, Scherer S, et al. Association of kidney graft loss with de novo produced donor-specific and non-donor-specific HLA antibodies detected by single antigen testing. *Transplantation.* (2015) 99:1976–80. doi: 10.1097/TP.0000000000000672

47. Reed EF, Rao P, Zhang Z, Gebel H, Bray RA, Guleria I, et al. Comprehensive assessment and standardization of solid phase multiplex-bead arrays for the detection of antibodies to HLA. *Am J Transplant.* (2013) 13:1859–70. doi: 10.1111/ajt.12287

48. Zachary AA, Lucas DP, Detrick B, Leffell MS. Naturally occurring interference in Luminex assays for HLA-specific antibodies: characteristics and resolution. *Hum Immunol.* (2009) 70:496–501. doi: 10.1016/j.humimm.2009.04.001

49. Schmidt D, Osmanodja B, Pfefferkorn M, Graf V, Raschke D, Duettmann W, et al. TBase - an integrated electronic health record and research database for kidney transplant recipients. *J Vis Exp.* (2021) 170:e61971. doi: 10.3791/61971

50. Zhang Q, Budde K, Schmidt D, Halleck F, Duerr M, Naik MG, et al. Clinicopathologic features and risk factors of proteinuria in transplant glomerulopathy. *Front Med.* (2021) 8:666319. doi: 10.3389/fmed.2021.666319

51. Wu K, Schmidt D, López Del Moral C, Osmanodja B, Lachmann N, Zhang Q, et al. Poor long-term renal allograft survival in patients with chronic antibody-mediated rejection, irrespective of treatment-a single center retrospective study. *J Clin Med.* (2021) 11:199. doi: 10.3390/jcm11010199

52. Ziemann M, Suwelack B, Banas B, Budde K, Einecke G, Hauser I, et al. Determination of unacceptable HLA antigen mismatches in kidney transplant recipients. *HLA.* (2021) 100:3–17. doi: 10.1111/tan.14521

53. Gragert L, Madbouly A, Freeman J, Maiers M. Six-locus high resolution HLA haplotype frequencies derived from mixed-resolution DNA typing for the entire US donor registry. *Hum Immunol.* (2013) 74:1313–20. doi: 10.1016/j.humimm.2013.06.025

54. Creary LE, Gangavarapu S, Mallempati KC, Montero-Martín G, Caillier SJ, Santaniello A, et al. Next-generation sequencing reveals new information about HLA allele and haplotype diversity in a large European American population - Table S13, S14. *Hum Immunol.* (2019) 80:807–22. doi: 10.1016/j.humimm.2019.07.275

55. Eberhard HP, Schmidt AH, Mytilineos J, Fleischhauer K, Müller CR. Common and well-documented HLA alleles of German stem cell donors by haplotype frequency estimation. *HLA.* (2018) 92:206–14. doi: 10.1111/tan.13378

56. HLA-Matchmaker (2020). Available online at: http://www.epitopes.net/downloads.html (DRDQDP Antibody Analysis Program V3.1). (accessed May 15, 2021).

57. Waiser J, Klotsche J, Lachmann N, Wu K, Rudolph B, Halleck F, et al. Predictors of graft survival at diagnosis of antibody-mediated renal allograft rejection: a retrospective single-center cohort study. *Transpl Int.* (2020) 33:149–60. doi: 10.1111/tri.13525

58. Kiberd BA, Miller A, Martin S, Tennankore KK. De novo donor-specific human leukocyte antigen antibody screening in kidney transplant recipients after the first year posttransplantation: a medical decision analysis. *Am J Transplant.* (2016) 16:3212–9. doi: 10.1111/ajt.13838

59. Halloran PF. T cell-mediated rejection of kidney transplants: a personal viewpoint. *Am J Transplant.* (2010) 10:1126–34. doi: 10.1111/j.1600-6143.2010.03053.x

60. Rampersad C, Balshaw R, Gibson IW, Ho J, Shaw J, Karpinski M, et al. The negative impact of T cell–mediated rejection on renal allograft survival in the modern era. *Am J Transplant.* (2021) 22:761–71. doi: 10.1111/ajt.16883

61. Wiebe C, Rush DN, Gibson IW, Pochinco D, Birk PE, Goldberg A, et al. Evidence for the alloimmune basis and prognostic significance of Borderline T cell–mediated rejection. *Am J Transplant.* (2020) 20:2499–508. doi: 10.1111/ajt.15860

62. Tambur AR, Kosmoliaptsis V, Claas FHJ, Mannon RB, Nickerson P, Naesens M. Significance of HLA-DQ in kidney transplantation: time to reevaluate human leukocyte antigen-matching priorities to improve transplant outcomes? An expert review and recommendations. *Kidney Int.* (2021) 100:1012–22. doi: 10.1016/j.kint.2021.06.026

63. Hardy MA, Suciu-Foca N, Reed E, Benvenisty AI, Smith C, Rose E, et al. Immunomodulation of kidney and heart transplants by anti-idiotypic antibodies. *Ann Surg.* (1991) 4:522–8. doi: 10.1097/00000658-199110000-00016

64. Rocha PN, Butterly DW, Greenberg A, Reddan DN, Tuttle-Newhall J, Collins BH, et al. Beneficial effect of plasmapheresis and intravenous immunoglobulin on renal allograft survival of patients with acute humoral rejection. *Transplantation.* (2003) 9:1490–5. doi: 10.1097/01.TP.0000060252.57111.AC

frontiers | Frontiers in Medicine

TYPE Systematic Review
PUBLISHED 21 September 2022
DOI 10.3389/fmed.2022.859318

OPEN ACCESS

EDITED BY
Kathrin Eller,
Medical University of Graz, Austria

REVIEWED BY
Werner Ribitsch,
Medical University of Graz, Austria
Andrea Angeletti,
Giannina Gaslini Institute (IRCCS), Italy

*CORRESPONDENCE
Zesong Li
Lzssc@email.szu.edu.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 21 January 2022
ACCEPTED 30 August 2022
PUBLISHED 21 September 2022

CITATION
Xu C, Lin S, Mao L and Li Z (2022) Neutrophil gelatinase-associated lipocalin as predictor of acute kidney injury requiring renal replacement therapy: A systematic review and meta-analysis.
Front. Med. 9:859318.
doi: 10.3389/fmed.2022.859318

Neutrophil gelatinase-associated lipocalin as predictor of acute kidney injury requiring renal replacement therapy: A systematic review and meta-analysis

Chunhua Xu[1,2,3†], Shan Lin[1,4†], Longyi Mao[1] and Zesong Li[1*]

[1]Guangdong Provincial Key Laboratory of Systems Biology and Synthetic Biology for Urogenital Tumors, Shenzhen Key Laboratory of Genitourinary Tumor, Department of Urology, The First Affiliated Hospital of Shenzhen University, Shenzhen Second People's Hospital (Shenzhen Institute of Translational Medicine), Shenzhen, Guangdong, China, [2]Guangdong Key Laboratory for Biomedical Measurements and Ultrasound Imaging, Shenzhen University Health Science Center, School of Biomedical Engineering, Shenzhen, Guangdong, China, [3]Shulan International Medical College, Zhejiang Shuren University, Hangzhou, China, [4]Department of Central Laboratory, Shenzhen Hospital, Beijing University of Chinese Medicine, Shenzhen, Guangdong, China

Background: Patients with severe acute kidney injury (AKI) may require renal replacement therapy (RRT), such as hemodialysis and peritoneal dialysis. Neutrophil gelatinase-associated lipocalin (NGAL) is a sensitive indicator for early diagnosis and recognition of AKI; however, its predictive value of AKI-associated need for RRT needs further evaluation.

Methods: Following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines, relevant articles were systematically searched and selected from seven databases. The random effects model was applied to evaluate the predictive performance of NGAL for AKI requiring RRT. The Newcastle–Ottawa Scale (NOS) was used to assess the quality of each included study.

Results: A total of 18 studies including 1,787 patients with AKI and having an average NOS score of 7.67 were included in the meta-analysis. For plasma/serum NGAL, the pooled sensitivity and specificity with corresponding 95% confidence interval (CI) were 0.75 (95% CI: 0.68–0.81) and 0.76 (95% CI: 0.70–0.81), respectively. The pooled positive likelihood ratio (PLR) was 2.9 (95% CI: 2.1–4.1), and the pooled negative likelihood ratio (NLR) was 0.34 (95% CI: 0.25–0.46). Subsequently, the pooled diagnostic odds ratio (DOR) was 9 (95% CI: 5–16) using a random effects model, and the area under the curve (AUC) of summary receiver operating characteristic to summarize predictive accuracy was 0.82 (95% CI: 0.79–0.85). For urine NGAL, the pooled sensitivity, specificity, PLR, NLR, DOR, and AUC values were 0.78 (95% CI: 0.61–0.90),

0.77 (95% CI: 0.65–0.85), 3.4 (95% CI: 2.4–4.8), 0.28 (95% CI: 0.15–0.52), 12 (95% CI: 6–24), and 0.84 (95% CI: 0.80–0.87), respectively.

Conclusion: Plasma/serum and urine NGAL levels performed comparably well in predicting AKI requiring RRT. Our findings suggested that NGAL is an effective predictive biomarker for the AKI-associated need for RRT. Nevertheless, more pieces of high-quality evidence and future trials with larger sample sizes are needed for further improvement of patient outcomes.

Systematic review registration: [https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022346595], identifier [CRD42022346595].

KEYWORDS

neutrophil gelatinase-associated lipocalin, acute kidney injury, renal replacement therapy, predictive value, systematic review and meta-analysis

Introduction

Acute kidney injury (AKI) has been emerged as a crucial public health issue, which affects millions of patients worldwide and is a common complication in patients hospitalized in the intensive care unit (ICU), and AKI is independently associated with significant morbidity and mortality (1–3). Non-AKI patients usually have better clinical outcomes than patients with AKI (4–6). The management of AKI usually involves several conservative interventions, such as avoidance of nephrotoxins and prompt resuscitation of circulation (4, 7). However, patients with AKI with severe metabolic disorders, such as acidosis, hyperkalemia, uremia, and fluid disorders, whose kidney function does not recover after certain interventions have to undergo renal replacement therapy (RRT) with a possibility of eventual kidney transplantation (5, 8). Despite considerable research on RRT, it is still unclear if and when RRT should be commenced to improve the outcome of patients with AKI (5, 9). Although early initiation of RRT may reduce the mortality of patients with AKI (10), it may cause a higher risk of treatment-related complications, such as bloodstream infections (11). More importantly, few studies have specifically evaluated the value of various biomarkers to predict AKI that may persist or worsen and progress to a certain stage, resulting in a necessary reception of RRT (12, 13). Therefore, a new biomarker that serves as an early predictor of AKI and an indicator of the need to undergo RRT may play a critical role in improving the prognosis of AKI.

Neutrophil gelatinase-associated lipocalin (NGAL), a secretory protein with a molecular weight of 25 kDa, belongs to the lipocalin superfamily and is released from injured tubular epithelial cells in response to various insults (5, 14). NGAL has already been acknowledged by nephrologists as one of the most promising biomarkers of upcoming AKI. Recently, plasma/serum and urine NGAL levels have been investigated as biomarkers for early prognostication of AKI (9). It is well known that AKI and chronic kidney disease (CKD) are interconnected syndromes as AKI may exacerbate CKD progression and CKD increases the risk of AKI. Serum and urinary NGAL levels were significantly higher in patients with CKD than in the normal population and were negatively correlated with the glomerular filtration rate (GFR). Although NGAL cutoff values and kinetics are significantly altered in patients with CKD, NGAL is considered not only a better indicator of a GFR decline than serum creatinine (sCr) but also a potent marker of the degree of kidney injury. According to the multivariate Cox proportional risk regression model, serum and urinary NGAL levels were independent predictors of the risk of CKD progression (15). Furthermore, several studies have reported that an increase in NGAL levels can be detected well before an increase in plasma creatinine (pCr) levels, which highlights the sensitivity of the former as a biomarker for diagnosing AKI (11, 16). For instance, pCr did not increase until 24–72 h postoperatively in patients undergoing elective cardiac surgery; however, increases in urine and plasma NGAL levels were identified as soon as 2 h postoperatively, with areas under receiver operating characteristic curve of 0.99 and 0.91, respectively (16). However, because of the lack of corresponding statistical data for early prediction of AKI requiring RRT (17–19), it remains controversial whether NGAL is a predictive biomarker of AKI requiring RRT. Therefore, the potential of NGAL for early prediction of AKI-associated RRT remains to be established.

To illuminate this issue, a systematic review and meta-analysis was conducted to evaluate the ability of the available physiological and molecular biomarkers for predicting the initiation of RRT in patients with AKI. This systematic review and meta-analysis was performed to explore the predictive evidence of AKI requiring RRT.

Methods

Data sources and searches

We performed a systematic search of the following databases: PubMed, Embase, the Web of Science, Cochrane Library (in English), Chinese National Knowledge Infrastructure,[1] and Wanfang Data (in Chinese).[2] The search duration was from inception to November 2021. The following search terms were used: ["Biomarkers" (MeSH) OR biomarker OR marker OR neutrophil gelatinase associated lipocalin OR NGAL OR neutrophil gelatinase-associated lipocalin] and (AKI OR acute kidney injury OR acute kidney failure OR acute renal failure), and (RRT OR renal replacement therapy). Abstracts with a complete section "Results" were included in this study. There were no language restrictions. This meta-analysis was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement issued in 2009 (Checklist file) (20, 21).

Study selection

All citations were reviewed, and the literature was retrieved by titles or abstracts, and subsequently, full texts were reviewed by two investigators (CX and SL) to determine the study eligibility. Any disagreements regarding study eligibility were resolved by consulting another investigator (ZL). Studies meeting the following inclusion criteria were included: (1) studies with participants aged ≥ 18 years; (2) studies using plasma/serum and/or urine NGAL for prediction of patients with AKI who might need RRT; (3) studies including AKI and non-AKI patients with sepsis who underwent RRT; (4) observational studies; and (5) studies with enough information to calculate true-positive, false-positive, false-negative, and true-negative values of NGAL as a predictor of AKI requiring RRT (contains AUC, sensitivity, and specificity values) or studies with these values provided. Studies were excluded if they met the following exclusion criteria: (1) studies with only animal or *in vitro* experiments; (2) studies lacking the information about predictive accuracy in control or experimental groups; (3) review articles, commentaries, poster presentations, letters, supplementary issues, and editorials; (4) studies with duplicate data or insufficient information; and (5) studies on individuals with prior kidney transplant, end-stage kidney disease, or prior RRT.

1 http://www.cnki.net/

2 http://www.wanfangdata.com.cn

Data extraction and quality assessment

A total of two investigators (CX and SL) independently extracted the data from each trial, and any disagreements between them were resolved by consulting a third investigator (ZL) and reaching a consensus. Data on the following variables from each article were documented and recalculated: first author, year of publication, study location, population type, gender, total sample size, AKI definition, number of patients with AKI, number of patients undergoing RRT, age, NGAL assay results, sample type, AUC (95% CI), and NGAL cutoff, sensitivity, and specificity. Absolute data of true-positive (TP), false-positive (FP), true-negative (TN), and false-negative (FN) rates or equivalent data were calculated or extracted.

The two investigators (CX and SL) independently assessed the methodological quality of the studies using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool (22). This tool is based on four key domains: index test, reference standard, patient selection, and flow and timing. Each domain is evaluated in the aspect of "risk of bias," and the first 3 domains are evaluated in the aspect of concern regarding applicability. The included studies collected response using "yes," "no," or "unclear" items. The responses of "yes" are considered positive responses for analysis herein.

The Newcastle–Ottawa Scale (NOS) was used to assess the quality of each included study (23). Based on several aspects of the study, such as comparability (maximum points, 2), outcomes (maximum points, 3), and selection (maximum points, 4), the quality of the study was judged using a "star" scoring system of NOS. The scores range from 0 (for worst) to 9 (for best). A study with a score no less than 7 was considered a high-quality study.

Data synthesis and analysis

We used STATA version 12.0 (Stata Corp, College Station, TX) to perform all statistical analyses, which included TP, TN, FP, and FN rates for each test in every study, to assess the sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) for each included study. Statistically significant heterogeneity was represented by $P < 0.05$ for Q statistic, and $I^2 > 50\%$ was considered to indicate substantial heterogeneity (24). The degree of heterogeneity between multiple studies was measured using the I^2 index, and I^2 values of <25, 25–50, and >50% indicated modest, moderate, and substantial heterogeneity, respectively. A random effects model was chosen if I^2 was greater than 50% (25). Any departure from the Hardy–Weinberg equilibrium (HWE) in the control group of each study was assessed using the χ^2 test, and significant deviations were represented by $P < 0.05$ (26).

Forest plots of accuracy indices were constructed, and a summary receiver operating characteristic (SROC) curve was

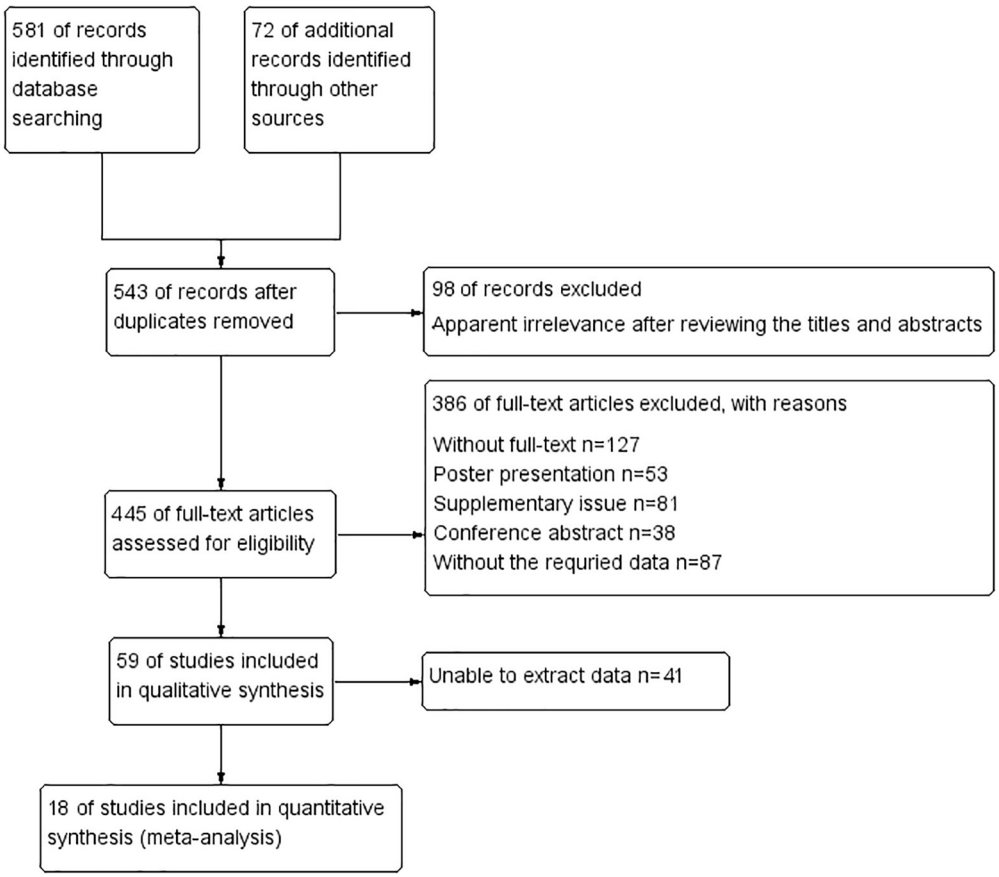


FIGURE 1
Selection process of the included studies in the meta-analysis.

constructed on the basis of TP and FP rates to describe the relationship between test sensitivity and specificity. NGAL has been defined as a useful risk predictor when the AUC $\geq$ 0.70, and the predictive performance for the prediction of AKI in RRT by NGAL was measured by calculating the AUC as an overall summary index (27).

Furthermore, subgroup analyses were performed according to the biological material, definition of AKI, geographic location, NGAL assay method, and AKI causes. Finally, Begg's and Egger's measures were assessed and calculated using Begg's funnel plots to detect publication biases (28, 29). A statistically significant difference was represented by $P < 0.05$ in the test results for the overall effect.

Results

Literature search

During the literature search, we initially identified 653 potentially relevant studies, and 543 studies remained after removing the duplicates found in electronic databases. Subsequently, 98 articles were identified as irrelevant after reviewing titles and abstracts and excluded. Then, 445 full-text articles were assessed for eligibility, and 386 of these articles were excluded as they did not meet the requirements of data extraction. After screening the full texts of the remaining articles, 41 studies were excluded as they did not meet our eligibility criteria. Finally, 18 articles that met the inclusion criteria were included in this meta-analysis. These studies encompassed a total of 5,441 participants who were included in the meta-analysis for prediction of AKI requiring RRT. The selection process of the included studies is shown in **Figure 1**.

Characteristics and quality of the included studies

All 18 selected articles were written in English. To analyze the quality of the included studies, the main characteristics were extracted, as presented in **Table 1**. The included studies were geographically diverse: 10 studies were conducted in Europe

TABLE 1 Main characteristics of the studies included in the meta-analysis.

References	Location	Sample size (male)	AKI definition	Cause	Source	AKI (*n*)	RRT (*n*)	NGAL assay	NOS
Albeladi and Algethamy (42)	Saudi Arabia	75 (38)	NR	ICU	Urinary	21	17	NR	8
Chen et al. (43)	China	110 (NR)	KDIGO	Sepsis	Serum/urinary	110	78	NR	7
Chun et al. (44)	Korea	76 (66)	NR	Burn	Plasma	32	20	NR	8
Constantin et al. (30)	France	88 (NR)	RIFLE	ICU	Plasma	36	7	NR	7
Cruz et al. (31)	Italy	301 (207)	RIFLE	ICU	Plasma	133	15	NR	8
Cruz et al. (32)	Italy	933 (NR)	NR	Critically ill	Plasma	284	40	ELISA	7
Gaipov (37)	Turkey	60 (42)	KDIGO	Heart surgery	Urinary	40	7	ELISA	8
Hanson et al. (41)	Australia	163 (136)	NR	Malaria	Urinary	84	43	ELISA	8
Hjortrup et al. (33)	Denmark	222 (126)	NR	Sepsis	Plasma/urinary	91	29	NR	8
Kaufmann et al. (35)	Germany	255 (184)	KDIGO	Sepsis	Plasma	33	33	ELISA	8
Linko et al. (34)	Finland	369 (243)	RIFLE	ICU	Plasma	47	47	NR	8
Lukasz et al. (38)	Germany	39 (28)	AKIN	Uremia	Serum	31	24	ELISA	7
Maisel et al. (39)	United States	927 (575)	KDIGO	Heart failure	Plasma	72	11	NR	8
Nisula et al. (14)	Finland	1042 (673)	KDIGO	Critically ill	Urinary	379	83	ELISA	8
Park et al. (45)	Korea	169 (96)	KDIGO	Sepsis	Serum	114	114	NR	7
Tiranathanagul et al. (46)	Thailand	47 (31)	AKIN	Critically ill	Plasma/urinary	47	18	ELISA	7
Tornblom et al. (36)	Finland	484 (310)	KDIGO	ICU	Urinary	217	46	ELISA	8
Wagener et al. (40)	United States	81 (53)	AKIN	ICU	Urinary	16	5	ELISA	8

10.3389/fmed.2022.859318

TABLE 2 Predictive value of NGAL on AKI requiring RRT in individual studies.

Study	AUC	95% CI	Cut-off value	Sensitivity	Specificity	Number of patients			
						TP	FP	FN	TN
Albeladi and Algethamy (42)	35.000	1.274–961.305	200 ng/mL	1.00	0.56	17	2	0	2
Chen et al. (43)	29.400	8.950–96.581	403 ng/mL	0.81	0.89	63	4	15	28
Chen et al. (43)	35.000	10.489–116.793	695 ng/mL	0.83	0.88	65	4	13	28
Chun et al. (44)	3.250	0.733–14.402	253 ng/mL	0.63	0.67	13	4	8	8
Constantin et al. (30)	15.750	1.630–152.179	303 ng/mL	0.90	0.72	6	8	1	21
Cruz et al. (31)	12.207	2.627–56.724	150 ng/mL	0.87	0.65	13	41	2	77
Cruz et al., (32)	3.107	1.485–6.501	NR	0.72	0.54	29	112	11	132
Gaipov (37)	20.172	1.064–382.451	6 ng/mL	0.94	0.58	7	14	0	19
Hanson et al. (41)	4.511	1.798–11.316	510 ng/mL	0.65	0.70	28	12	15	29
Hjortrup et al. (33)	4.040	1.573–10.376	641 ng/mL	0.69	0.64	20	22	9	40
Hjortrup et al. (33)	2.786	1.084–7.156	1832 ng/mL	0.46	0.77	13	14	16	48
Kaufmann et al. (35)	0.91	0.82–0.99	10.26 U/g	0.90	0.89	30	24	3	198
Linko et al. (34)	6.044	3.118–11.716	304 ng/mL	0.68	0.74	32	84	15	238
Lukasz et al. (38)	30.000	2.794–322.090	330 ng/mL	0.83	0.80	20	1	4	6
Maisel et al. (39)	18.375	3.504–96.363	125 ng/dL	0.80	0.80	9	12	2	49
Nisula et al. (14)	17.866	9.442–33.804	449 ng/mL	0.83	0.79	69	64	14	232
Park et al. (45)	2.405	1.209–4.783	576.5 ng/mL	0.61	0.58	70	23	44	32
Tiranathanagul et al. (46)	22.533	4.648–109.247	960 ng/mL	0.72	0.90	13	3	5	26
Tiranathanagul et al. (46)	10.833	2.383–49.242	2600 ng/mL	0.55	0.91	10	3	8	26
Tornblom et al. (36)	0.769	0.729–0.806	1000 ng/mL	0.53	0.92	24	14	22	157
Wagener et al. (40)	3.333	0.276–40.287	470 ng/mL	0.81	0.48	4	6	1	5

(14, 30–38), two in America (39, 40), one in Australia (41), and the remaining five studies in Asia (42–46). The 18 observational studies involved a total of 5,441 patients from 12 countries. All studies were single-center trials published between 2006 and 2021. Overall, 1,787 patients developed AKI and 637 patients received RRT. The use of plasma/serum NGAL and urine NGAL was almost equal among the studies. The definitions of AKI varied among individual studies. A total of 13 studies used the traditional method to define AKI, and the remaining five studies used a non-traditional method. Most studies evaluated the NGAL level in the plasma and urine, rather than in the serum. Commercial enzyme-linked immune sorbent assay (ELISA) was frequently used for NGAL measurements. Among the 18 studies, nine used ELISA and nine used other methods to measure the NGAL level. The performance of NGAL for predicting AKI requiring RRT is summarized in Table 2.

Assessment of methodological quality and publication bias

All studies were clearly defined with eligibility criteria and reasons for patient exclusion. The quality of each included study was assessed using the QUADAS tool, with all of them having high QUADAS scores (≥10). The overall quality of included trials was moderate. The results of the QUADAS-2 evaluation are shown in Figure 2.

Subsequently, publication bias assessment was conducted using a funnel plot. The results of the funnel plot are shown in Figure 3, which indicated no significant threshold effect and no significant asymmetry, suggesting that there was no evident publication bias in the present meta-analysis. Therefore, it is unlikely that unpublished studies would substantially alter our findings.

Neutrophil gelatinase-associated lipocalin for prediction of acute kidney injury requiring renal replacement therapy

Table 2 shows a total of 21 sets of data extracted from 18 eligible studies, including TP/FP/FN/TN value, sensitivity, specificity, positive predictive value, negative predictive value (NPV), AUC, various optimal cutoff values for different sample types of NGAL, the NGAL assay method, and the definition of AKI. In the 18 studies, we investigated the predictive value of plasma NGAL as a biomarker of AKI requiring RRT in 1787/5441 patients who developed AKI. The pooled results of these studies are summarized in Table 2. Taken together,

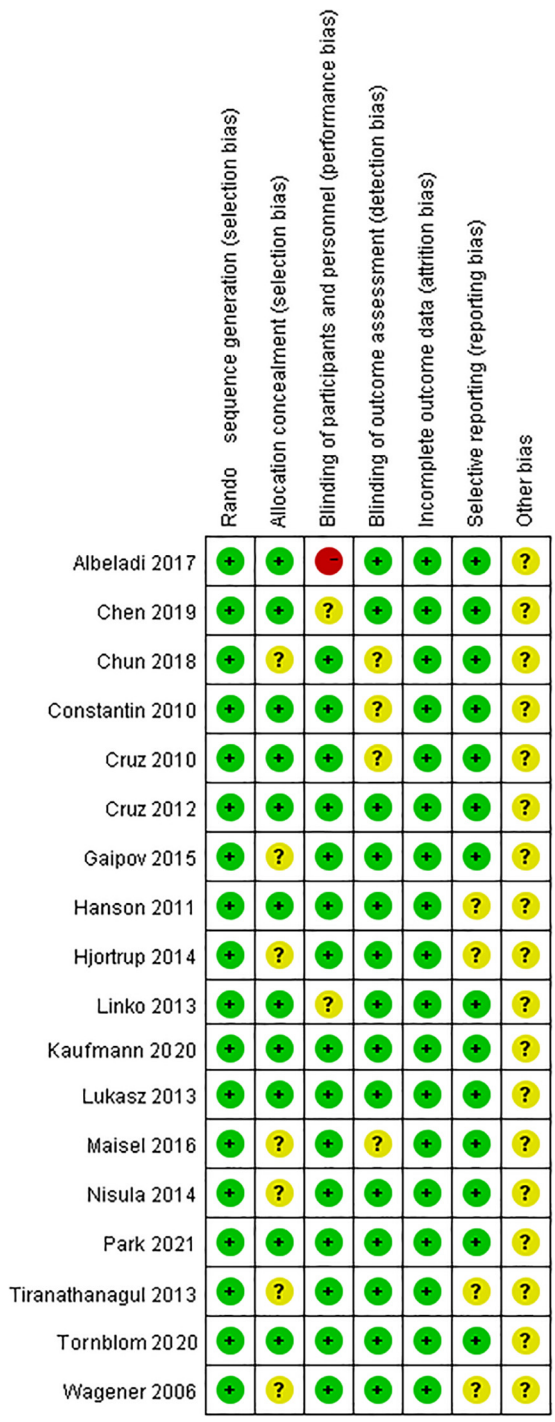


FIGURE 2
Quality assessment of included eligible studies using QUADAS-2.

the predictive value of NGAL for AKI requiring RRT from plasma/serum and urine samples is shown in Figure 4. For summary performance estimates, the pooled sensitivity and specificity with corresponding 95% CI were 0.75 (95% CI: 0.68–0.81) and 0.76 (95% CI: 0.70–0.81), respectively. The pooled PLR was 2.9 (95% CI: 2.4–4.1), and the pooled NLR was 0.34 (95% CI: 0.25–0.46). The pooled DOR was 9 (95% CI: 5–16) using a random effects model (Figure 4). Moreover, the AUC for SROC to summarize predictive accuracy was 0.82 (95% CI: 0.79–0.85; Figure 5). Even though the result of SROC for AKI requiring RRT was worse than that for AKI, its clinical application was still of great value.

Subgroup analysis

Subgroup analysis of this meta-analysis was performed, and the results are shown in Table 3. Based on the comparisons of DOR and AUC, the predictive value of NGAL for AKI requiring RRT showed some variability. For subgroup analysis of the biological material, the DOR and AUC of the urine NGAL level were significantly higher than those of the plasma/serum NGAL level in the prediction of AKI requiring RRT (DOR, 12; AUC, 0.84 vs. DOR, 9; AUC, 0.82). This showed that urine NGAL performed better for RRT prediction than plasma/serum NGAL. For subgroup analysis of geographic location, the value of the NGAL level to predict AKI requiring RRT in oriental was substantially higher than that in occidental (DOR, 14; AUC, 0.85 vs. DOR, 9; AUC, 0.81). Subsequently, for subgroup analysis of the NGAL assay method, we investigated the predictive accuracies of NGAL with and without ELISA. The findings revealed that the former had a higher predictive value for AKI requiring RRT (DOR, 14; AUC, 0.86 vs. DOR, 7; AUC, 0.79). For subgroup analysis of the definition of AKI, the traditional definition of AKI was associated with better DOR and AUC values (DOR, 13; AUC, 0.85) than the non-traditional definition of AKI (DOR, 4; AUC, 0.70). Moreover, subgroup analysis for different causes of AKI was conducted, and the results showed that AKI caused by sepsis/heart failure had the best predictive ability with optimal DOR and AUC (DOR, 10; AUC, 0.83), and AKI caused by ICU showed a similar predictive ability as AKI caused by critical illness (DOR, 9; AUC, 0.82 VS DOR, 9; AUC, 0.80). Based on the establishment of subgroup analyses of the 21 datasets from the 18 studies wherein multivariable analyses were provided, it would be reasonable to believe NGAL as an independent predictor of AKI requiring RRT.

Heterogeneity analysis

A total of 18 heterogeneous studies containing 21 datasets of plasma/serum NGAL and urine NGAL for the prediction of AKI requiring RRT were obtained. Overall, heterogeneity analyses were performed, and the SROC was constructed; the points in the plots did not show a "shoulder arm" pattern, which suggested no presence of the threshold effect. Subsequently, Begg's funnel plot and Egger's test were used to check for

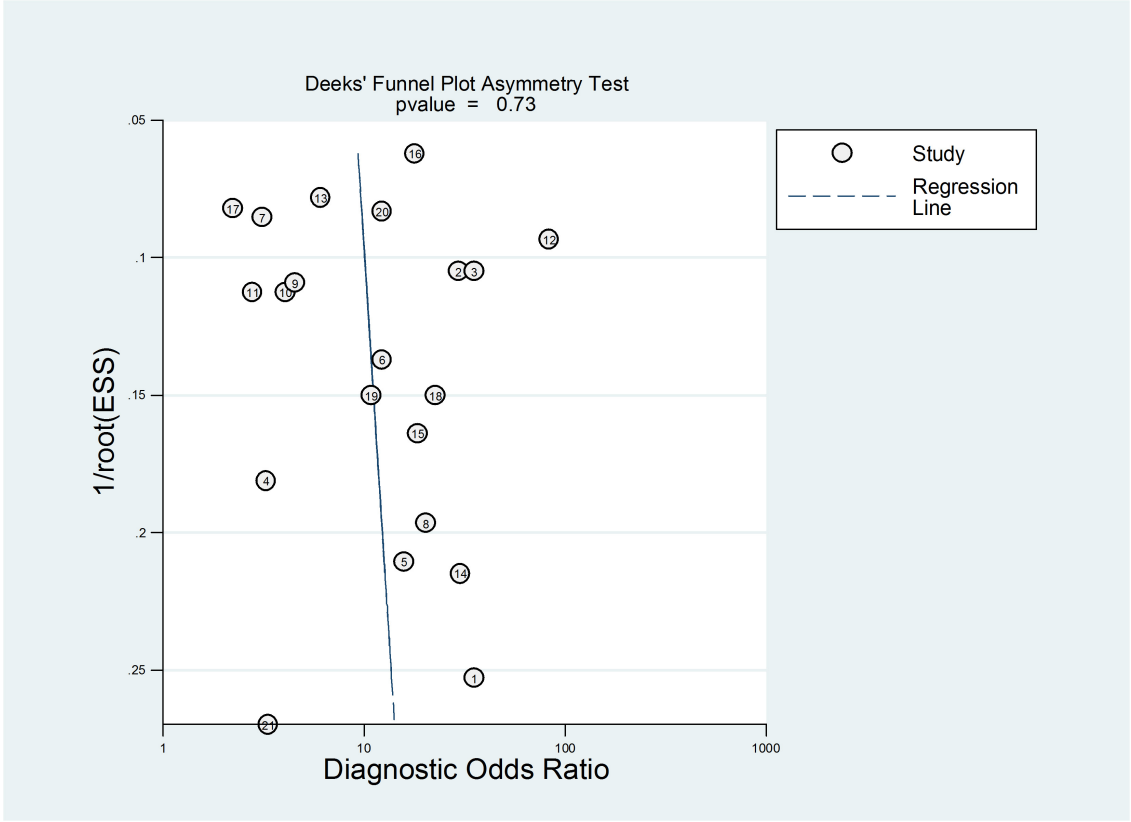


FIGURE 3
Deek's funnel plot assessment of potential publication bias.

publication bias, and the funnel plots are shown in **Figure 6**. The results indicated a low probability of publication bias.

To explore other possible reasons for heterogeneity, meta-regression and subgroup analyses were performed. The main sources of heterogeneity could be the geographic location (occidental or oriental) and definition of AKI (traditional or non-traditional). However, the specimen type (urine or plasma/serum) and the NGAL assay (ELISA or others) may not be the sources of heterogeneity for NGAL.

Discussion

In the present systematic review and meta-analysis, we assessed the predictive accuracy of NGAL in 1,787 patients with AKI requiring RRT. The results of this meta-analysis revealed NGAL as a valuable renal biomarker to predict the need for RRT with high sensitivity, specificity, and DOR in patients scheduled to undergo AKI. Furthermore, subgroup analyses indicated that plasma/serum and urine NGAL had comparable predictive values for AKI requiring RRT. Moreover, the definition of AKI and/or the geographic location of the patients affected the efficiency of the NGAL level for predicting AKI requiring RRT. Of note, although the predictive value of NGAL has been shown in AKI requiring RRT, it is hard to suggest NGAL as a promising biomarker that may guide clinical decision regarding the timing of initiation of RRT among patients with AKI due to the lack of established cutoff values of NGAL for the initiation of RRT. In addition, whether mild AKI or severe AKI requiring RRT will be developed is difficult to be predicted only by a certain increase in NGAL levels. As a matter of fact, clinical manifestations and variables measured on clinical laboratory platforms are the common factors that should be taken into consideration in clinical practice to decide the initiation of RRT, such as severely decreased renal function indicated by sharply increased serum creatinine or dramatically decreased GFR, acidosis, severe edema, hyperkalemia, and so on. Furthermore, NGAL levels not only reflects kidney injury but may also be influenced by systemic conditions such as sepsis or originating from non-kidney tissues. A increase in NGAL indicates the possibility of AKI and provides appropriate preparations for subsequent possible therapies.

Neutrophil gelatinase-associated lipocalin has been implicated in a variety of processes, including cell

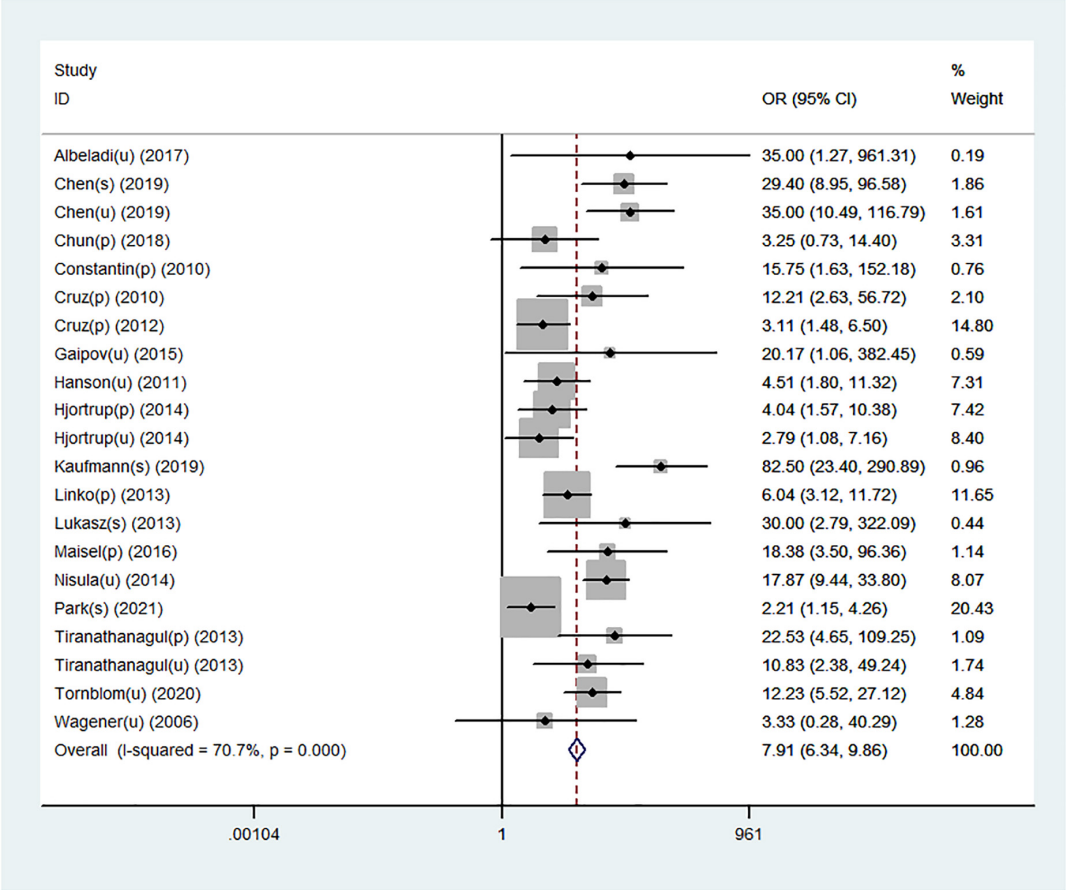


FIGURE 4
Forest plot for the predictive value of NGAL for AKI requiring RRT from plasma/serum and urine samples.

differentiation, proliferation, and survival in renal epithelial cells, where it helps maintain the tubular structure and limits apoptosis (16). In addition, exogenous NGAL has been shown to have a dramatic renoprotective effect in the mouse models of renal ischemia–reperfusion injury (47). Generally, NGAL is among the most extensively studied biological markers for early prediction of AKI in both urine and blood specimens. A systematic review was performed by Haase-Fielitz et al. wherein they included 58 studies collectively encompassing >16,500 patients and reported that both plasma and urine NGAL were predictive of AKI, with overall AUCs ranging from 0.79 to 0.87 in different clinical settings (48). The predictive accuracy of plasma and urine NGAL for the prediction of AKI was systematically reviewed, and the pooled results indicated that plasma and urine NGAL precisely predicted AKI with sepsis (AUC = 0.86 and 0.90, respectively) (49). In addition, NGAL is considered to play a vital role in early prediction of AKI as its level can rapidly increase after contrast medium exposure (27). However, it remains controversial whether NGAL is predictive of AKI requiring RRT because of the lack of pertinent statistical data in this regard, and it remains unclear when and whether RRT should be commenced to improve the outcome of patients with AKI on the basis of NGAL levels. Currently, the information on NGAL for the prediction of RRT in patients with AKI is extremely limited.

Several studies have evaluated the predictive value of NGAL for AKI of different etiologies that requires RRT, and a wide range of predictive values of NGAL levels for AKI has been reported in observational cohort studies. However, few studies considered patients after AKI administration for RRT (50). Since it is still unclear whether and when to commence RRT, standards for prediction of AKI requiring RRT are considered as a major limitation of biomarker studies (51). Recently, two trials employed a preset NGAL threshold as an inclusion criterion for RRT prediction and used NGAL to guide the early initiation of RRT (9, 52). Nevertheless, NGAL was found to detect patients with AKI in the ELAIN trial, whereas it was universally elevated in the STARRT-AKI pilot trial and showed weak discriminative value between patients requiring and not requiring RRT (9). The results of our study were consistent with those of previous

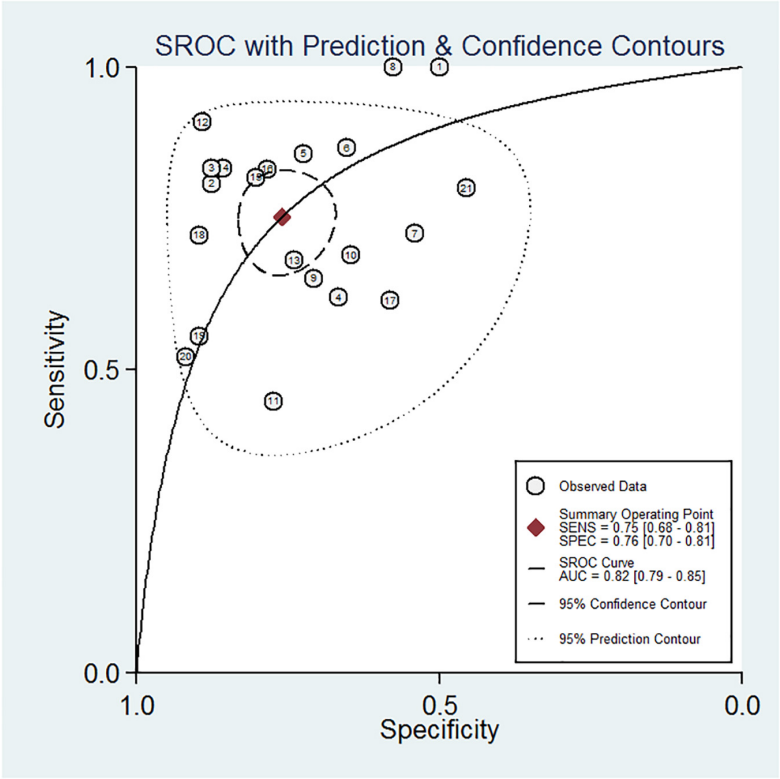


FIGURE 5
Summary receiver operating characteristic (SROC) curve of plasma/serum and urine NGAL for AKI requiring RRT.

TABLE 3 Subgroup analysis on the basis of different standards.

Studies	Number		Sensitivity	Specificity	PLR	NLR	DOR	AUC-ROC
Biological material	12	Plasma/serum	0.75 (0.68–0.81)	0.76 (0.70–0.81)	2.9 (2.1–4.1)	0.34 (0.25–0.46)	9 (5–16)	0.82 (0.79–0.85)
	9	Urine	0.78 (0.61–0.90)	0.77 (0.65–0.85)	3.4 (2.4–4.8)	0.28 (0.15–0.52)	12 (6–24)	0.84 (0.80–0.87)
Definition of AKI	15	Traditional	0.78 (0.70–0.84)	0.79 (0.72–0.85)	3.7 (2.7–5.1)	0.28 (0.21–0.39)	13 (8–22)	0.85 (0.82–0.88)
	6	Non-traditional	0.69 (0.54–0.81)	0.63 (0.51–0.73)	1.9 (1.5–2.3)	0.49 (0.35–0.69)	4 (2–6)	0.70 (0.66–0.74)
Geographic location	14	Occidental	0.75 (0.66–0.83)	0.74 (0.67–0.81)	2.9 (2.2–3.9)	0.33 (0.24–0.46)	9 (5–15)	0.81 (0.78–0.85)
	7	Oriental	0.75 (0.67–0.82)	0.82 (0.70–0.89)	4.1 (2.3–7.3)	0.30 (0.20–0.45)	14 (5–35)	0.85 (0.81–0.87)
NGAL assay method	10	ELISA	0.78 (0.67–0.87)	0.80 (0.70–0.87)	3.9 (2.6–5.7)	0.27 (0.18–0.41)	14 (8–26)	0.86 (0.83–0.89)
	11	Others	0.73 (0.65–0.81)	0.73 (0.65–0.79)	2.7 (2.0–3.7)	0.37 (0.26–0.52)	7 (4–14)	0.79 (0.75–0.83)
Causes	6	ICU	0.80 (0.63–0.90)	0.70 (0.52–0.83)	2.6 (1.7–3.9)	0.29 (0.17–0.49)	9 (5–16)	0.82 (0.78–0.85)
	8	Sepsis/heart failure	0.75 (0.64–0.84)	0.77 (0.68–0.85)	3.3 (2.1–5.2)	0.32 (0.20–0.51)	10 (4–25)	0.83 (0.80–0.86)
	7	Critically ill	0.73 (0.64–0.80)	0.77 (0.65–0.85)	3.1 (2.0–4.9)	0.36 (0.26–0.49)	9 (4–18)	0.80 (0.76–0.83)

studies, and we found NGAL to be a useful early predictor of AKI requiring RRT; our sensitivity analyses also revealed that the findings were robust. The association between NGAL and AKI requiring RRT is further highlighted by a sensitivity of 75% and a specificity of 76%. By contrast, several studies have reported sensitivities of 40%–60% and specificities of 40%–55%. The observed differences may be attributed to variations in the definitions of AKI, the etiology of AKI, the NGAL assay, and the geographic location of patients. To evaluate the predictive value of the NGAL level in various conditions, our subgroup analysis had included these parameters.

Recently, plasma and urine NGAL were reported to have relatively low predictive values for the requirement of RRT in ICU patients with severe sepsis and without CKD, and the AUCs were 0.73 (95% CI: 0.61–0.85; $P = 0.64$) and 0.68 (95% CI: 0.53–0.83; $P = 0.64$), respectively (33). In addition, in a study of 126 patients with sepsis, 23 of 58 patients with septic AKI received RRT (53). The results showed that the peak urine NGAL was

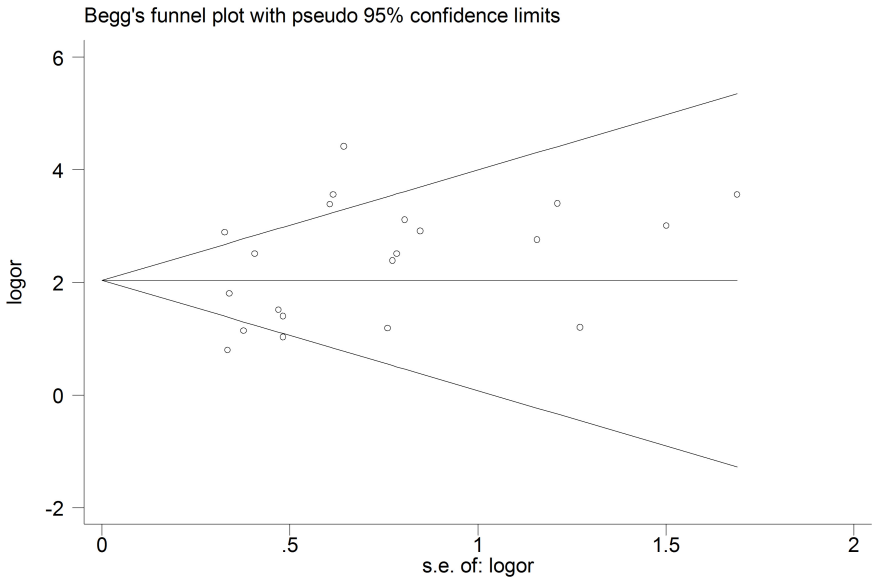


FIGURE 6
Begg's funnel plot for testing publication bias.

higher in patients receiving hemodialysis than in those not receiving hemodialysis (median, 456 ng/mL vs. 341 ng/mL, $P < 0.0001$). The AUC of the peak urine NGAL for predicting the need for hemodialysis was 0.77 (95% CI: 0.64–0.83), with a cutoff value of 494 ng/mL; the sensitivity and specificity were 0.89 and 0.71, respectively. In addition, a study performed a subgroup analysis of AKI patients with community-acquired pneumonia who met the RIFLE-F criteria and showed that plasma NGAL was a poor predictor of the requirement of RRT (AUC, 0.62; 95% CI: 0.45–0.81) (54). Another study reported that the peak plasma NGAL showed fair discriminatory power for the prediction of AKI (AUC = 0.71) and need for RRT (AUC = 0.78); however, urine NGAL did not perform equally well for prediction of AKI (AUC = 0.70) and need for RRT (AUC = 0.70). Together, these findings are not comparable with our findings in terms of both plasma/serum NGAL and urine NGAL in the present meta-analysis for predicting AKI requiring RRT (55). Furthermore, a recent study conducted by Albert C. et al. also showed that urinary and plasma NGAL concentrations may identify patients at high risk for AKI and the associated need for dialysis therapy (56).

Currently, NGAL is the only biomarker that has been investigated in both plasma/serum and urine for its early predictive value of AKI and AKI requiring RRT. Therefore, herein, we assessed the predictive value of plasma/serum and urine NGAL and further examined the studies that conducted comparisons of both plasma/serum NGAL and urine NGAL for subgroup analysis. Regarding plasma/serum NGAL, many studies showed unsatisfactory results, and our subgroup analysis also showed that plasma/serum NGAL may have inferior performance to urine NGAL in prediction of AKI requiring RRT. Typically, plasma/serum NGAL is considered an indicator of systemic inflammation and not of renal injury. However, urine sample collection is non-invasive, and urine has reduced interfering proteins, thus making it an ideal fluid for kidney biomarker discovery. The present meta-analysis confirmed the superiority of urine NGAL for the prediction of AKI requiring RRT, suggesting urine NGAL levels should be quantified before plasma/serum NGAL levels. Conversely, it may be difficult to obtain urine samples from AKI patients with severe oliguria, which is common in cardiac surgery. Therefore, even though urine NGAL has a higher predictive value (DOR, 12; AUC, 0.84) than plasma/serum NGAL (DOR, 9; AUC, 0.82) for early predictive value of AKI requiring RRT, plasma/serum NGAL may be an alternative to urine NGAL in case urine is unobtainable. Subsequently, in the present study, subgroup analyses showed the definition of AKI as the main source of heterogeneity. Compared with the traditional definition of AKI (DOR, 13; AUC, 0.85), the non-traditional definition of AKI was found to be associated with an inferior predictive value of NGAL (DOR, 4; AUC, 0.70). To our knowledge, few studies have conducted a comparison of research-based assays. Therefore, prospective studies with geographic location or multiple RRT settings involving head-to-head comparisons may contribute to a more comprehensive evaluation of NGAL in prediction of AKI requiring RRT.

Nevertheless, this study has several limitations. First, the literature search strategy applied in this study was limited to open-access publications. In this case, the studies that may have met the inclusion criteria of this meta-analysis but were

not published on open-access platforms were missed. Second, different cutoff values for NGAL were applied in the included studies. Furthermore, cutoff values were corrected by urine creatinine, and it was difficult to determine the optimized overall cutoff value for patients with AKI (57–59). Moreover, deeper investigations should be pursued incorporating a wide range of clinical settings of AKI. Third, the pooled ORs were calculated by the numbers of genotypes or alleles of controls and cases; however, no adjustment was performed for other confounding factors. Fourth, because of the limitation of statistical power, the results from subgroups analysis should be interpreted with caution. Finally, the included studies in the present meta-analysis had different match variables, and this may have affected the pooled results.

Conclusion

The present systematic review and meta-analysis showed a significant association between NGAL and AKI requiring RRT. Therefore, NGAL could be considered a useful marker with a high predictive value of AKI requiring RRT. Despite these encouraging findings, in further studies, a larger sample of homogeneous patients should be used, and different NGAL assay methods need to be unbiased to clarify this issue. Furthermore, similar relevant studies incorporating long-term follow-up studies are needed to confirm the role played by NGAL in the progression and development of AKI.

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.

Author contributions

CX, SL, and ZL extracted the data and performed the initial analysis. CX and SL wrote the first draft, which has been carefully reviewed and edited by ZL. CX, SL, LM, and ZL performed further review and subsequent revisions. All authors have contributed to the conception and design of the study and agreed to the submission for publication.

Funding

This research was funded by the National Natural Science Foundation of China (82100706 and 81972366), Postdoctoral Science Foundation of China (2021M692219), Special Fund for Economic and Technological Development of Longgang District of Shenzhen City (LGKCYLWS2020064 and LGKCYLWS2020068), the National Science Foundation Projects of Guangdong Province (2022A1515012606), and the Special Project of Science and Technology for Sustainable Development, Shenzhen Municipal Science and Technology Innovation Committee (KCXFZ20211019143336002, JCYJ2018 0228163012046, and JCYJ20200109120208018).

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.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Peerapornratana S, Manrique-Caballero CL, Gomez H, Kellum JA. Acute kidney injury from sepsis: current concepts, epidemiology, pathophysiology, prevention and treatment. *Kidney Int.* (2019) 96:1083–99. doi: 10.1016/j.kint.2019.05.026

2. Ostermann M, Bellomo R, Burdmann EA, Doi K, Endre ZH, Goldstein SL, et al. Controversies in acute kidney injury: conclusions from a kidney disease: improving global outcomes (KDIGO) conference. *Kidney Int.* (2020) 98:294–309. doi: 10.1016/j.kint.2020.04.020

3. Maggiore U. Incomplete recovery from COVID-19-associated acute kidney injury in kidney transplant recipients: prior graft injury matters the most. *Transpl Int.* (2021) 34:1002–4. doi: 10.1111/tri.13896

4. Joannidis M, Druml W, Forni LG, Groeneveld ABJ, Honore PM, Hoste E, et al. Prevention of acute kidney injury and protection of renal function in the intensive care unit: update 2017. *Intensive Care Med.* (2017) 43:730–49. doi: 10.1007/s00134-017-4832-y

5. Gaudry S, Hajage D, Schortgen F, Martin-Lefevre L, Pons B, Boulet E, et al. Initiation strategies for renal-replacement therapy in the intensive care unit. *N Engl J Med.* (2016) 375:122–33. doi: 10.1056/NEJMoa1603017

6. Trongtrakul K, Sawawiboon C, Wang AY, Chitsomkasem A, Limphunudom P, Kurathong S, et al. Acute kidney injury in critically ill surgical patients: epidemiology, risk factors and outcomes. *Nephrology (Carlton).* (2019) 24:39–46. doi: 10.1111/nep.13192

7. Hoste EA, Bagshaw SM, Bellomo R, Cely CM, Colman R, Cruz DN, et al. Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. *Intensive Care Med.* (2015) 41:1411–23. doi: 10.1007/s00134-015-3934-7

8. Wang L, Wang T, Rushton SN, Parry G, Dark JH, Sheerin NS. The impact of severe acute kidney injury requiring renal replacement therapy on survival and renal function of heart transplant recipients - a UK cohort study. *Transpl Int.* (2020) 33:1650–66. doi: 10.1111/tri.13675

9. Zarbock A, Kellum JA, Schmidt C, Van AH, Wempe C, Pavenstädt H, et al. Effect of early vs delayed initiation of renal replacement therapy on mortality in critically ill patients with acute kidney injury: the ELAIN randomized clinical trial. *JAMA.* (2016) 315:2190–9. doi: 10.1001/jama.2016.5828

10. Chaudhuri D, Herritt B, Heyland D, Gagnon LP, Thavorn K, Kobewka D, et al. Early renal replacement therapy versus standard care in the ICU: a systematic review, meta-analysis, and cost analysis. *J Intensive Care Med.* (2019) 34:323–9. doi: 10.1177/0885066617698635

11. Forni LG, Darmon M, Ostermann M, Straaten OV, Pettilä V, Prowle JR, et al. Renal recovery after acute kidney injury. *Intensive Care Med.* (2017) 43:855–66. doi: 10.1007/s00134-017-4809-x

12. Beitland S, Joannidis M. Biomarkers of acute kidney injury – a mission impossible? *Acta Anaesthesiol Scand.* (2018) 62:2–5. doi: 10.1111/aas.13010

13. Klein SJ, Brandtner AK, Lehner GF, Ulmer H, Bagshaw SM, Wiedermann CJ, et al. Biomarkers for prediction of renal replacement therapy in acute kidney injury: a systematic review and meta-analysis. *Intensive Care Med.* (2018) 44:323–36. doi: 10.1007/s00134-018-5126-8

14. Nisula S, Yang R, Kaukonen KM, Vaara ST, Kuitunen A, Tenhunen J, et al. The urine protein NGAL predicts renal replacement therapy, but not acute kidney injury or 90-day mortality in critically ill adult patients. *Anesth Analg.* (2014) 119:95–102. doi: 10.1213/ANE.0000000000000243

15. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Campo S, Fazio MR, et al. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. *Clin J Am Soc Nephrol.* (2009) 4:337–44. doi: 10.2215/CJN.03530708

16. Macdonald SPJ, Bosio E, Neil C, Arendts G, Burrows S, Smart L, et al. Resistin and NGAL are associated with inflammatory response, endothelial activation and clinical outcomes in sepsis. *Inflamm Res.* (2017) 66:611–9. doi: 10.1007/s00011-017-1043-5

17. Bagshaw SM, Darmon M, Ostermann M, Finkelstein FO, Wald R, Tolwani AJ, et al. Current state of the art for renal replacement therapy in critically ill patients with acute kidney injury. *Intensive Care Med.* (2017) 43:841–54. doi: 10.1007/s00134-017-4762-8

18. Siddiqui WJ, Alvarez C, Aslam M, Bakar A, Khan MH, Aslam A, et al. Meta-analysis comparing outcomes and need for renal replacement therapy of transcatheter aortic valve implantation versus surgical aortic valve replacement. *Am J Cardiol.* (2018) 122:468–76. doi: 10.1016/j.amjcard.2018.04.030

19. Ragland C, Ochoa L, Hartjes T. Early mobilisation in intensive care during renal replacement therapy: a quality improvement project. *Intensive Crit Care Nurs.* (2019) 52:22–7. doi: 10.1016/j.iccn.2018.12.005

20. Moher D, Liberati A, Tetzlaff J, Altman D. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J Clin Epidemiol.* (2009) 62:1006–12. doi: 10.1016/j.jclinepi.2009.06.005

21. McInnes MDF, Moher D, Thombs BD, McGrath TA, Bossuyt PM, Clifford T, et al. Preferred reporting items for a systematic review and meta-analysis of diagnostic test accuracy studies: the PRISMA-DTA statement. *JAMA.* (2018) 319:388–96. doi: 10.1001/jama.2017.19163

22. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med.* (2011) 155:529–36. doi: 10.7326/0003-4819-155-8-201110180-00009

23. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol.* (2010) 25:603–5. doi: 10.1007/s10654-010-9491-z

24. Wu H, Zhang H, Li P, Gao T, Lin J, Yang J, et al. Association between dietary carbohydrate intake and dietary glycemic index and risk of age-related cataract: a meta-analysis. *Invest Ophthalmol Vis Sci.* (2014) 55:3660–8. doi: 10.1167/iovs.13-13695

25. Dong F, Yang G, Pan HW, Huang WH, Jing LP, Liang WK, et al. The association of PTPN22 rs2476601 polymorphism and CTLA-4 rs231775 polymorphism with LADA risks: a systematic review and meta-analysis. *Acta Diabetologica.* (2014) 51:691–703. doi: 10.1007/s00592-014-0613-z

26. Xia C, Amador C, Huffman J, Trochet H, Campbell A, Porteous D, et al. Pedigree- and SNP-associated genetics and recent environment are the major contributors to anthropometric and cardiometabolic trait variation. *PLos Genetics.* (2016) 12:e1005804. doi: 10.1371/journal.pgen.1005804

27. Macdonald SP, Stone SF, Neil CL, van Eeden PE, Fatovich DM, Arendts G, et al. Sustained elevation of resistin, NGAL and IL-8 are associated with severe sepsis/septic shock in the emergency department. *PLoS One.* (2014) 9:e110678. doi: 10.1371/journal.pone.0110678

28. Cantoni V, Green R, Acampa W, Petretta M, Bonaduce D, Salvatore M, et al. Long-term prognostic value of stress myocardial perfusion imaging and coronary computed tomography angiography: a meta-analysis. *J Nucl Cardiol.* (2016) 23:185–97. doi: 10.1007/s12350-015-0349-3

29. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics.* (1994) 50:1088–101. doi: 10.2307/2533446

30. Constantin JM, Futier E, Perbet S, Roszyk L, Lautrette A, Gillart T, et al. Plasma neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in adult critically ill patients: a prospective study. *J Crit Care.* (2010) 25:e1–6. doi: 10.1016/j.jcrc.2009.05.010

31. Cruz DN, De MC, Garzotto F, Perazella MA, Lentini P, Corradi V, et al. Plasma neutrophil gelatinase-associated lipocalin is an early biomarker for acute kidney injury in an adult ICU population. *Intensive Care Med.* (2010) 36:444–51. doi: 10.1007/s00134-009-1711-1

32. Cruz D, Geus HRD, Ronco C, Groeneveld ABJ, Kulkarni OP, Hagemann JH, et al. New advances in the pathophysiology of AKI. *Nephrol Dial Transplant.* (2012) S2:ii31. doi: 10.1093/ndt/gfs190

33. Hjortrup PB, Haase N, Treschow F, Møller MH, Perner A. Predictive value of NGAL for use of renal replacement therapy in patients with severe sepsis. *Acta Anaesthesiol Scand.* (2015) 59:25–34. doi: 10.1111/aas.12427

34. Linko R, Pettilä V, Kuitunen A, Korhonen A-M, Nisula S, Alila S, et al. Plasma neutrophil gelatinase-associated lipocalin and adverse outcome in critically ill patients with ventilatory support. *Acta Anaesthesiol Scand.* (2013) 57:855–62. doi: 10.1111/aas.12112

35. Kaufmann M, Schlossbauer M, Hubauer U, Stadler S, Fischer M, Wallner S, et al. N-acety-b-D-glucosaminidase: a potential biomarker for early detection of acute kidney injury in acute chest pain. *Nephrology (Carlton).* (2020) 25:135–43. doi: 10.1111/nep.13664

36. Tornblom S, Nisula S, Petaja L, Vaara ST, Haapio M, Pesonen E, et al. Urine NGAL as a biomarker for septic AKI: a critical appraisal of clinical utility-data from the observational FINNAKI study. *Ann Intensive Care.* (2020) 10:51. doi: 10.1186/s13613-020-00667-7

37. Abduzhappar G, Yalcin S, Kultigin T, Aysun T, Ahmet Nihat B, Humeyra C, et al. Serum uric acid may predict development of progressive acute kidney injury after open heart surgery. *Ren Fail.* (2015) 37:96–102. doi: 10.3109/0886022X.2014.976130

38. Lukasz A, Beneke J, Menne J, Vetter F, Schmidt BM, Schiffer M, et al. Serum neutrophil gelatinase-associated lipocalin (NGAL) in patients with Shiga toxin mediated haemolytic uraemic syndrome (STEC-HUS). *Thromb Haemost.* (2014) 111:365–72. doi: 10.1160/TH13-05-0387

39. Maisel AS, Wettersten N, van Veldhuisen DJ, Mueller C, Filippatos G, Nowak R, et al. Neutrophil gelatinase-associated lipocalin for acute kidney injury during acute heart failure hospitalizations: the AKINESIS study. *J Am Coll Cardiol.* (2016) 68:1420–31. doi: 10.1016/j.jacc.2016.06.055

40. Wagener G, Jan M, Kim M, Mori K, Barasch JM, Sladen RN, et al. Association between increases in urinary neutrophil gelatinase-associated lipocalin and acute renal dysfunction after adult cardiac surgery. *Anesthesiology.* (2006) 105:485–91. doi: 10.1097/00000542-200609000-00011

41. Hanson J, Hasan MMU, Royakkers AA, Alam S, Charunwatthana P, Maude RJ, et al. Laboratory prediction of the requirement for renal replacement in acute falciparum malaria. *Malar J.* (2011) 10:217. doi: 10.1186/1475-2875-10-217

42. Albeladi FI, Algethamy HM. Urinary neutrophil gelatinase-associated lipocalin as a predictor of acute kidney injury, severe kidney injury, and the need for renal replacement therapy in the intensive care unit. *Nephron Extra.* (2017) 7:62–77. doi: 10.1159/000477469

43. Chen X, Chen Z, Wei T, Li P, Zhang L, Fu P. The effect of serum neutrophil gelatinase-associated lipocalin on the discontinuation of continuous renal replacement therapy in critically ill patients with acute kidney injury. *Blood Purif.* (2019) 48:10–7. doi: 10.1159/000499026

44. Chun W, Kim Y, Yoon J, Lee S, Yim H, Cho YS, et al. Assessment of plasma neutrophil gelatinase-associated lipocalin for early detection of acute kidney injury and prediction of mortality in severely burned patients. *J Burn Care Res.* (2018) 39:387–93. doi: 10.1097/BCR.0000000000000605

45. Park Y, Ban TH, Kim HD, Ko EJ, Lee J, Kim SC, et al. Mortality prediction of serum neutrophil gelatinase-associated lipocalin in patients requiring continuous renal replacement therapy. *Korean J Intern Med.* (2021) 36:392–400. doi: 10.3904/kjim.2019.446

46. Tiranathanagul K, Amornsuntorn S, Avihingsanon Y, Srisawat N, Susantitaphong P, Praditpornsilpa K, et al. Potential role of neutrophil gelatinase-associated lipocalin in identifying critically ill patients with acute kidney injury stage 2-3 who subsequently require renal replacement therapy. *Ther Apher Dial.* (2013) 17:332–8. doi: 10.1111/1744-9987.12004

47. Hong SN, Medeiros P, Menezes P, Bridi R, Balbi A, Ponce D. Sepsis and AKI in clinical emergency room patients: the role of urinary NGAL. *Biomed Res Int.* (2015) 2015:413751. doi: 10.1155/2015/413751

48. Haase-Fielitz A, Haase M, Devarajan P. Neutrophil gelatinase-associated lipocalin as a biomarker of acute kidney injury: a critical evaluation of current status. *Ann Clin Biochem.* (2014) 51:335–51. doi: 10.1177/0004563214521795

49. Zhang A, Cai Y, Wang PF, Qu JN, Luo ZC, Chen XD, et al. Diagnosis and prognosis of neutrophil gelatinase-associated lipocalin for acute kidney injury with sepsis: a systematic review and meta-analysis. *Critical Care.* (2016) 20:41. doi: 10.1186/s13054-016-1212-x

50. Bouman CSC, Forni LG, Joannidis M. Biomarkers and acute kidney injury: dining with the Fisher King? *Intensive Care Med.* (2010) 36:381–4. doi: 10.1007/s00134-009-1733-8

51. Parikh CR, Heather TP. Key concepts and limitations of statistical methods for evaluating biomarkers of kidney disease. *J Am Soc Nephrol.* (2014) 25:1621–9. doi: 10.1681/ASN.2013121300

52. Wald R, Adhikari NK, Smith OM, Weir MA, Pope K, Cohen A, et al. Comparison of standard and accelerated initiation of renal replacement therapy in acute kidney injury. *Kidney Int.* (2015) 88:897–904. doi: 10.1038/ki.2015.184

53. Fan H, Zhao Y, Zhu J, Song F. Urine neutrophil gelatinase-associated lipocalin in septic patients with and without acute kidney injury. *Ren Fail.* (2014) 36:1399–403. doi: 10.3109/0886022X.2014.945184

54. Srisawat N, Murugan R, Lee M, Kong L, Carter M, Angus DC, et al. Plasma neutrophil gelatinase-associated lipocalin predicts recovery from acute kidney injury following community-acquired pneumonia. *Kidney Int.* (2011) 80:545–52. doi: 10.1038/ki.2011.160

55. Bagshaw SM, Michael B, Michael H, Anja HF, Moritoki E, Hiroshi M, et al. Plasma and urine neutrophil gelatinase-associated lipocalin in septic versus non-septic acute kidney injury in critical illness. *Intensive Care Med.* (2010) 36:452–61. doi: 10.1007/s00134-009-1724-9

56. Albert C, Zapf A, Haase M, Rover C, Pickering JW, Albert A, et al. Neutrophil gelatinase-associated lipocalin measured on clinical laboratory platforms for the prediction of acute kidney injury and the associated need for dialysis therapy: a systematic review and meta-analysis. *Am J Kidney Dis.* (2020) 76:826–41.e1. doi: 10.1053/j.ajkd.2020.05.015

57. Che M, Xie B, Xue S, Dai H, Qian J, Ni Z, et al. Clinical usefulness of novel biomarkers for the detection of acute kidney injury following elective cardiac surgery. *Nephron Clin Pract.* (2010) 115:c66–72. doi: 10.1159/000286352

58. Liu S, Che M, Xue S, Xie B, Zhu M, Lu R, et al. Urinary L-FABP and its combination with urinary NGAL in early diagnosis of acute kidney injury after cardiac surgery in adult patients. *Biomarkers.* (2013) 18:95–101. doi: 10.3109/1354750X.2012.740687

59. Noto A, Cibecchini F, Fanos V, Mussap M. NGAL and metabolomics: the single biomarker to reveal the metabolome alterations in kidney injury. *Biomed Res Int.* (2013) 2013:612032. doi: 10.1155/2013/612032

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 20 October 2022
DOI 10.3389/fmed.2022.903090

OPEN ACCESS

EDITED BY
Kathrin Eller,
Medical University of Graz, Austria

REVIEWED BY
Zeid Khitan,
Marshall University, United States
Neftali Eduardo Antonio-Villa,
Universidad Nacional Autónoma de México, Mexico

*CORRESPONDENCE
Edgar Denova-Gutierrez
edgar.denova@insp.mx

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 23 March 2022
ACCEPTED 07 October 2022
PUBLISHED 20 October 2022

CITATION
Colli VA, González-Rocha A, Canales D, Hernández-Alcáraz C, Pedroza A, Pérez-Chan M, Barquera S and Denova-Gutierrez E (2022) Chronic kidney disease risk prediction scores assessment and development in Mexican adult population. *Front. Med.* 9:903090. doi: 10.3389/fmed.2022.903090

Chronic kidney disease risk prediction scores assessment and development in Mexican adult population

Victor A. Colli[1,2], Alejandra González-Rocha[2], David Canales[3], Cesar Hernández-Alcáraz[2], Andrea Pedroza[3], Manuel Pérez-Chan[1], Simón Barquera[2] and Edgar Denova-Gutierrez[2]*

[1]División Académica de Ciencias de la Salud, Universidad Juárez Autónoma de Tabasco, Villahermosa, Mexico, [2]Centro de Investigación en Nutrición y Salud, Instituto Nacional de Salud Pública, Cuernavaca, Mexico, [3]Centro de Investigación en Evaluación y Encuestas, Instituto Nacional de Salud Pública, Cuernavaca, Mexico

Background: Chronic kidney disease (CKD) is a major public health problem, with considerable growth in prevalence and mortality in recent years. Screening of CKD at primary care is crucial for the implementation of prevention strategies. The aims of this study are to assess CKD risk prediction scores and to develop a risk prediction score for the Mexican adult population.

Methods: Data from the Mexican National Health and Nutrition Survey 2016 was utilized and 3463 participants $\geq$ 20 years old were included. Reduced renal function with Glomerular filtration rate and/or the presence of albuminuria was defined as CKD. Multiple logistic regression models were performed for the creation of a training and validation model. Additionally, several models were validated in our Mexican population.

Results: The developed training model included sex, age, body mass index, fast plasma glucose, systolic blood pressure, and triglycerides, as did the validation model. The area under the curve (AUC) was 0.78 (95% CI: 0.72, 0.79) for training model, and 0.76 (95% CI: 0.71, 0.80) in validation model for Mexican adult population. Age, female gender, presence of diabetes and hypertension, elevated systolic and diastolic blood pressure, serum and urinary creatinine, and higher HbA1c were significantly associated with the prevalent chronic kidney disease. Previous CKD risk predictive models were evaluated with a representative sample of the Mexican adult population, their AUC was between 0.61 and 0.78.

Conclusion: The designed CKD risk predictive model satisfactorily predicts using simple and common variables in primary medical care. This model could have multiple benefits; such as, the identification of the population at risk, and prevention of CKD.

KEYWORDS

risk score, chronic kidney disease, Mexican, prediction, validation

Background

Chronic kidney disease (CKD) is a major public health problem associated with major adverse health events (e.g., greater cognitive impairment, higher prevalence of anemia, hypertension, and metabolic bone disease), progression to kidney failure, and death (1, 2).

Worldwide, in 2017, the prevalence of CKD was 9.1% (95% uncertainty interval [UI] 8.5–9.8), which was roughly 700 million cases. There were 7.3 million (95% UI 5.4–9.2) years of healthy life lost due to disability (YLDs), 28.5 million (95% IU 27.6–29.3) years of life lost (YLLs) and 35.8 million (95% IU 33.7–38.0) disability-adjusted life years (DALYs). There were 1.2 million (95% IU 1.2–1.3) deaths as a result of CKD. In addition, 1.4 million (95% UI 1.2–1.6) deaths from cardiovascular disease (CVD) were attributable to impaired kidney function (7.6% of deaths from CVD) (1, 3). For Mexico, in 2017, the prevalence of CKD was 12.2% (14.5 million cases) (1), 210.9 thousand YLDs, 1.5 million YLLs, 1.7 million DALYs, and 51.4 deaths per 100,000 inhabitants (65 thousand deaths); thus, made this disease the second leading cause of death in that year. From 1990 to 2017, CKD mortality rate increased by 102.3% (2).

Excessive growth in prevalence has been related to accelerated demographic and epidemiological changes (4), such as the high prevalence of Type 2 diabetes (T2D) (15.7%) (5), hypertension (49.4%) (6), overweight, and obesity (75.2%) (7), which are the main risk factors for developing non-communicable diseases and also contribute to deaths from CKD, accounting for half of these deaths.

Up to 98% of people with CKD due to T2D in Mexico are in stages one to three where the disease process can be delayed and controlled, while 2% will require complex and expensive treatments such as peritoneal dialysis, hemodialysis and/or kidney transplantation as replacement and restitutive therapies to survive (stages 4 and 5, considered irreversible) (8). In Mexico CKD is having a significant impact on the finances of the institutions and family economy. In 2014, the average annual health expenditure per person for this disease was US$ 8,966 in the Ministry of Health, and US$ 9,091 in the Mexican Institute of Social Security (9).

Therefore, the first level of medical care is crucial for the implementation of primary prevention strategies aimed at the early and timely control of cardiovascular risk factors, strategies for a stricter control of glycemia and blood pressure, promotion of healthy eating habits, health education, rationalization of the use of potentially nephrotoxic drugs and preventive treatment of hyperfiltration (10). One of the strategies for the timely CKD diagnosis is CKD risk predictive scores, these should be simple but precise and the variables included should be easily accessible in routine clinical settings (11).

The use of risk scores holds promise for large-scale CKD risk stratification and would allow the identification of all segments of the population that would benefit from CKD detection. To date, several studies have shown CKD risk predictive scores, and the predictive capacity of the scores ranges between 0.63 and 0.91 area under the receiver operating characteristic curve (AUROC). However, none have been evaluated in the healthy Mexican population.

Thus, the aims are: (1) to assess previous designed CKD risk prediction scores in Mexican adult population, (2) to develop a training and validation risk prediction score based on the Mexican National Health and Nutrition Survey 2016 (ENSANUT-MC 2016, by its acronym in Spanish).

Abbreviations: AIC, Akaike information criterion; AUROC, area under the receiver operating characteristic curve; BMI, body mass index; CKD, chronic kidney disease; CKD-EPI, chronic kidney disease epidemiology collaboration; CVD, cardiovascular disease; DALYs, disability-adjusted life years; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; ENSANUT, National Health and Nutrition Survey; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HOMA-IR, homeostasis model assessment-estimated insulin resistance; IDMS, isotope dilution mass spectrometry; SBP, systolic blood pressure; T2D, type 2 diabetes; UACR, urinary albumin-creatinine ratio; WHO, World Health Organization; YLDs, years of healthy life lost due to disability; YLLs, years of life lost.

Materials and methods

Design and study population

Data from ENSANUT-MC 2016 was used, a cross-sectional, multistage, stratified, and clustered probabilistic sample of the Mexican population, with national, regional, and urban-rural

representation. Detailed survey's design, sample size calculation and methodology were previously described (12, 13).

For the current study, individuals with at least 8 h of fasting at blood sample collection time were included. In addition, complete serum, urinary biomarker data, and complete survey.

Sociodemographic variables

This variables and risk factors included were based on self-reported information in the applied survey, such as age, sex, education level (Illiterate, elementary school, high school, and bachelor's degree), socioeconomic level, place of residence (rural or urban), indigenism and region (center, Mexico City, south and north). Trained and standardized personnel applied the interviews.

Clinical variables

The trained personnel performed two blood pressure measurements with 30 s difference between each one, with 5 min of rest before the first measurement with the patient seated, an automatic device (Omron HEM-907 XL) was used and the mean of the two measurements was chosen (14). The mean of the two measurements was chosen. Hypertension was considered according to the new criteria of the ISH 2020: systolic blood pressure (SBP) was 140 mmHg or higher and/or diastolic blood pressure (DBP) was 90 mmHg or higher, or when hypertension was self-reported (15). History of kidney stone was measured by self-reporting kidney stone.

Biochemical variables

Prediabetes was considered when fasting serum glucose was between 100 mg/dl – 125 mg/dl or HbA1c between 5.6 and 6.4%, T2D when fasting serum glucose was 126 mg/dl or greater, or HbA1c was 6.5% or greater, or T2D was self-reported, or use of glucose lowering drugs, and low control of T2D when HbA1c was higher than 7% (16).

The homeostasis model assessment-estimated insulin resistance (HOMA-IR) was used to calculate insulin resistance, it was calculated multiplying fasting plasma insulin (FPI) by fasting plasma glucose (FPG), then dividing by the constant 22.5 [HOMA-IR = (FPI × FPG)/22.5]. The cut-off point (17) to define insulin resistance was higher than 3.80.

Urine albumin was measured with the immunoturbidimetric assay. Urine creatinine was measured using the isotope dilution mass spectrometry (IDMS) standardized methodology, and serum creatinine was measured with the Jaffe method (13). Urinary albumin-creatinine ratio (UACR) was computed and reported in milligrams per gram. Albuminuria was considered when the UACR was 30 mg/g or higher.

Definition of chronic kidney disease

Reduced renal function [estimated glomerular filtration rate (eGFR) less than 60 mL/min/1.73 m^2] and/or the presence of albuminuria (UACR) was 30 mg/g or higher were used to define CKD (18).

Glomerular filtration rate (GFR) was estimated using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (19).

$$GFR = 141 \times min(S_{cr}/\kappa, 1)^{\alpha} \times max(S_{cr}/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018[iffemale] \times 1.159[ifblack]$$

For this equation: S_{cr}: serum creatinine (mg/dL), κ: 0.7 for females and 0.9 for males, α: −0.329 for females and −0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

Anthropometric variables

Trained personnel measured height, weight, and waist circumference for all participants. The body mass index (BMI) was calculated from the data of height (m^2) and weight (kg), and the criteria of the World Health Organization (WHO) was used to classify underweight (<18.5), normal weight (18.5–24.9), overweight (25–29.9) and obesity (higher than 30.0). We define abdominal obesity as; 88 cm or higher waist circumference for men and 102 cm or higher for women (20).

Chronic kidney disease risk predictive scores

After a review of the literature, scores that had AUROC greater than 0.70 were selected, and included variables to be applied in the first level of medical care, shown in **Table 3**.

For the creation of the novel prediction score the TRIPOD guidelines were followed (Verification Checklist **Supplementary material**).

Statistical analysis

Participant's characteristics are described in percentages if categorical, or in mean and standard deviation if numerical and are compared between subjects with and without CKD using a *t*-test if numerical or using $\chi^{2;}$ test if categorical.

Multivariable logistic regression models were fitted to assess how each risk factor contributes to the probability of developing CKD. The data was split into training and validation on an 80/20 ratio. The training dataset was used to fit the predictors on the outcome and the latter to provide an unbiased evaluation of the final models fitted on the validation dataset.

In the training dataset, we fitted different logistic regression models including CKD as the outcome of interest and combinations of age, sex, fasting plasma glucose, SBP, triglycerides, and BMI as predictors. These variables were chosen because we considered them as the most clinically relevant features in order to predict CKD. We selected the model to build our proposed score using the Akaike Information Criterion (AIC).

For training and internal validation of the risk prediction equations among the testing dataset, the AUROC was used, which measures how well the model differentiates those individuals at higher risk of having an event from those at lower risk, a property known as discrimination. Hosmer–Lemeshow χ^2 tests were also calculated to compare the predicted number of events with the number of events seen. All analyses were done with Stata for windows version 13.0.

Results

Main characteristics of the study population

A total of 3,463 subjects from ENSANUT 2016 MC were included in the final analysis to derive the CKD risk prediction scores (Figure 1). The main baseline characteristics of participants, sociodemographic, clinical, and biochemical, according to the CKD presence or absence, are shown in Table 1. The mean age was 45.8 years, 65.0% were females, 67.3% had elementary school, and 54.9% lived in a rural area. According to anthropometric and clinical measures, those with CKD had a higher mean of BMI compared to

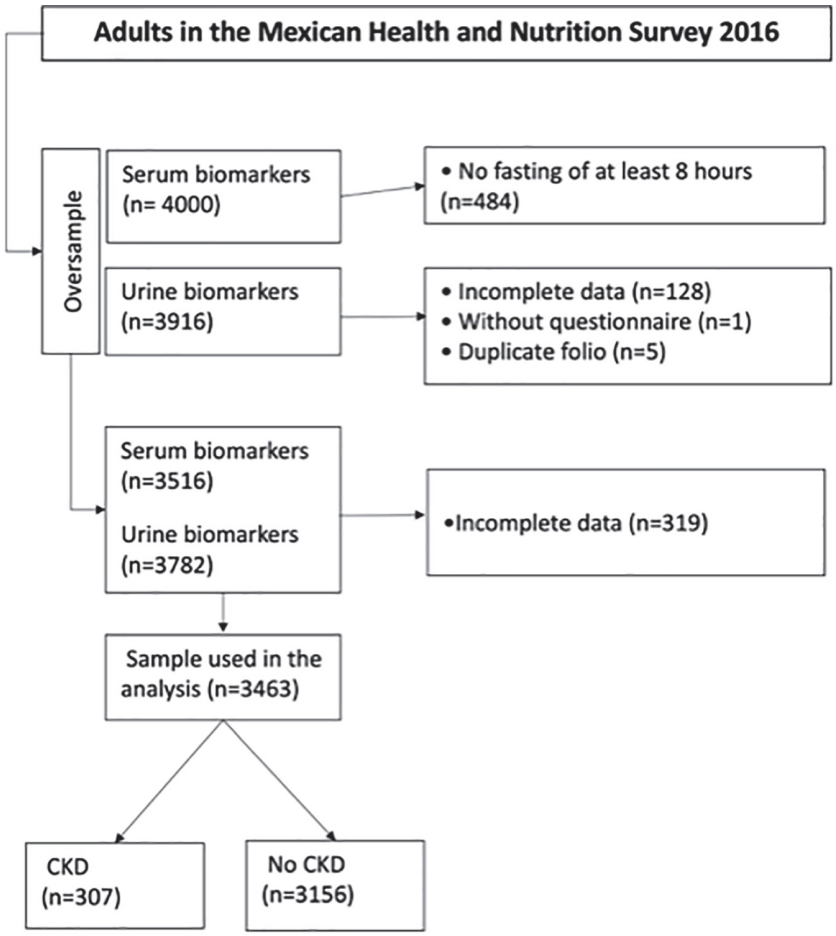


FIGURE 1
Flowchart of the sample selection.

TABLE 1 Main characteristics of Mexican National Health and Nutrition Survey 2016, according to the presence of chronic kidney disease.

Variable	Total		No CKD		CKD		*P*-value
	Mean	*SD*	Mean	*SD*	Mean	*SD*	
Age (year)	45.8	17.0	44.7	16.7	56.3	16.3	<0.001
Sex							
Male, %	35.0		35.5		29.9		0.05
Female, %	65.0		64.5		70.1		
Education level							
Illiterate, %	12.9		12.1		20.1		<0.001
Elementary school, %	67.3		67.1		69.1		
High school, %	13.3		14.0		7.0		
Bachelor's degree, %	6.5		6.8		3.8		
Socioeconomic level							
Low, %	38.1		38.3		37.3		0.387
Medium, %	33.3		33.4		30.1		
High, %	28.6		28.3		31.6		
Region							
North, %	21.2		20.6		27.4		0.020
Center and Mexico City, %	42.9		43.3		39.8		
South, %	35.9		36.1		32.8		
Place of residence (urban and rural)							
Urban, %	45.1		44.5		50.6		0.038
Rural, %	54.9		55.5		49.4		
Indigenism, %							
Yes	12.9		13.3		8.9		0.024
Anthropometric variables							
Weight, kg	70.1	15.6	70.1	15.4	70. 3	17.7	0.791
Height, cm	156.2	9.3	156.4	9.2	154.5-	9.6	<0.01
Body mass index, kg/m^2	28.7	6.2	28.7	6.2	29.3	6.1	0.092
Waist circumference, cm	95.4	13.0	95.2	12.9	98.4	14.1	<0.001
Clinics and biochemistry variables							
Systolic blood pressure, mmHg	121.4	19.4	119.7	18.6	132.8	25.7	<0.001
Diastolic blood pressure, mmHg	72.8	10.7	72.6	10.5	75.4	12.8	<0.001
Hemoglobin, mg/dL	14.1	1.9	14.1	1.8	13.3	2.1	<0.001
Serum creatinine, mg/dL	0.74	0.63	0.70	0.15	1.12	2.01	<0.001
Serum total cholesterol, mg/dL	188.8	40.1	188.1	38.7	196.4	52.0	<0.001
HDL-cholesterol, mg/dL	39.2	10.9	39.2	10.8	38.9	11.8	0.729
LDL-cholesterol, mg/dL	112.3	32.1	112.2	31.7	113.9	35.9	0.372
Serum triglycerides, mg/dL	196.5	123.5	194.4	121.0	218.6	144.4	0.001
Fasting plasma glucose, mg/dL	108.4	46.4	105.2	41.0	140.8	76.3	<0.001
HbA1c, %	5.8	1.4	5.7	1.3	6.9	2.2	<0.001
Insulin, mcU/ml	11.2	10.3	11.2	10.5	11.5	8.5	0.617
HOMA-IR[a] > 3.80	3.1	3.3	3.0	3.3	3.9	3.2	<0.001
Urine creatinine, mg/dL	138.6	80.8	110.6	77.9	1.41.4	80.5	<0.001
Albumin-to-creatinine ratio, mg/g	42.6	93.7	4.3	5.3	436.4	258.1	<0.001
eGFR, ml/min/1.73 m^2	105.8	27.0	107.6	24.7	87.3	39.4	<0.001
Family history variables							
Diabetes, %	35.2		35.1		36.3		0.693
Hypertension, %	39.2		39.6		35.7		0.206
Cardiovascular disease, %	14.0		14.3		11.0		0.137

[a]HOMA-IR, homeostasis model assessment-estimated insulin resistance, HOMA-IR equation = (FPI × FPG)/22.5; FPI, fasting plasma insulin; FPG, fasting plasma glucose.

TABLE 2 Prevalence of comorbidities stratified by CKD in the study population.

Variable	Total	No CKD	CKD	*P*-value
BMI (kg/m^2), %				
<25.0	25.9	26.0	24.4	0.193
25.0–29.9	39.4	39.7	36.1	
≥30.0	34.7	34.2	39.1	
Abdominal obesity, %				
Yes	80.6	80.0	86.4	0.009
Diabetes (yes), %				
Yes	17.2	14.6	44.0	<0.001
Hypertension, %				
Yes	27.0	24.1	56.6	<0.001
Cardiovascular disease[b], %				
Yes	4.9	4.6	7.8	0.012
Kidney stone, %				
Yes	4.1	3.9	6.6	0.025
Hypercholesterolemia, %	35.3	34.8	40.1	0.065
Low HDL levels, %	57.9	57.8	58.6	0.777
High LDL levels, %	63.9	63.9	64.3	0.090
Hypertriglyceridemia, %	56.8	56.4	61.2	0.100
Insulin resistance, %				
High (3.8)	24.5	23.2	38.1	0.001
Albuminuria (UACR ≥ 30), %	7.1	0	81.1	<0.001
Smoker, %				
Never	53.0	53.37	49.5	0.071
Former smoker	11.4	11.6	9.1	
Current smoker	35.6	35.0	41.3	

[b]Cardiovascular disease: Acute myocardial infarction, angina pectoris, stroke, heart failure, and other heart diseases. BMI, body mass index.

those without CKD (29.3 vs. 28.7 kg/m^2), similar results were observed in the mean of waist circumference (98.4 vs. 95.2 cm), SBP (132.8 vs. 119.7 mmHg), DBP (75.4 vs. 72.6 mmHg), serum creatinine (1.12 vs. 0.70 mg/dL), total serum cholesterol (196.4 vs. 188.1 mg/dL), serum triglycerides (218.6 vs. 194.4 mg/dL), and FPG (140.8 vs. 105.2 mg/dL).

Most of the participants without CKD were overweight (39.7%), while in those with CKD, obesity (39.1%) and abdominal obesity (86.4%) predominated. 14.6% of the participants without CKD lived with T2D, 24.1% lived with hypertension and 23.2% had insulin resistance, while in those with CKD the prevalence was 44.0, 56.6, and 38.1%, respectively (Table 2).

Assessment of chronic kidney disease risk predictive models

Chronic kidney disease risk predictive models (21–27) were selected to be assessed with a representative sample of the Mexican adult population, characteristics are shown in Table 3 and the AUROC of the models in Figure 2. The external validation of the different predictive models performed in the Mexican adult population, the AUROC of Kwon et al. model (21) was 0.75; while, in the O'Seaghdha et al. clinical models (22) were 0.74, 0.76, and 0.77 for models 1, 2, and 3, respectively. Additionally, the external validation using Al-Shamsi et al. models (23) showed an AUROC of 0.78 for the first model, and 0.76 the second model. Finally, Lee et al. (24) for model 3 included sex, BMI, level of education, FPG, serum albumin, eGFR and proteinuria, with an AUROC of 0.77 and for model 4 the same variables were included plus Framingham risk score, with an AUROC of 0.77.

Development of a chronic kidney disease risk predictive model

A model to predict the CKD presence in Mexican adult population was computed. In this sense, the training (1) and validation model (2) included age, sex, BMI, FPG, SBP, and triglycerides. The AUROC for the validation model was 0.76 (95% CI: 0.71, 0.80) and for the training model was 0.78 (95% CI: 0.72, 0.79) (Table 4).

Chronic kidney disease risk prediction algorithms

Prediction equations for training and validation models for CKD are presented below:

Training model:

$$L_{Training} = \left(-7.3 + 0.3646\ if\ Women\right) + 0.0295\ x\ ln\left(Age\right)$$
$$+0.0099\ x\ ln\left(BMI\right) + 0.0198\ x\ ln\left(FPG\right) + 0.7030\ x\ ln(SPB)$$
$$+ 0.0099\ x\ ln\left(TG\right) * P\left(CKD\right)_{Training}$$
$$= 1 - exp(exp(L_{Training}))$$

Validation model:

$$L_{Validation} = \left(-7.5 + 0.4446\ if\ Women\right) + 0.0392 \cdot ln\left(Age\right)$$
$$+0.0099 \cdot ln\left(BMI\right) + 0.0198 \cdot ln\left(FPG\right) + 0.7080 \cdot ln(SPB)$$
$$+ 0.0099 \cdot ln\left(TG\right) * P(CKD)_{Validation}$$
$$= 1 - exp(exp(L_{Validation}))$$

*β values taken from ORs reported on Table 4

Discussion

A predictive risk model was designed for the Mexican adult population using data from the ENSANUT 2016 and it

TABLE 3 External models validation in Mexican adult population.

Authors (year)	Population	Model name/ type of model	Variables	Outcomes predicted	AUCROC	Sensitivity (%)	Specificity (%)	External validation in Mexican adult population	Sensitivity (%) in Mexican adult population	Specificity (%) in Mexican adult population
Kwon et al. (21)	Korea	Korean model (KM)/ BLRM	Age (year), sex (female), anemia (yes/no), hypertension (yes/no), diabetes (yes/no), CVD (yes/no), and proteinuria (yes/no).	CKD: eGFR < 60 ml/min/1.73 m^2 – MDRD and CKD-EPI equation	0.87 (0.84–0.89)	89.4 (84.4–93.2)	70.6 (68.9–72.3)	0.750	51.0	81.0
O'Seaghdha et al. (22)	USA	Model 1: clinical model/ BLRM	Age (year), diabetes (yes/no) and hypertension (yes/no).	CKD: eGFR < 60 mL/min/1.73 m^2 – MDRD and CKD-EPI equation	0.786	NR	NR	0.744	49.0	78.0
		Model 2: clinical model and baseline eGFR/ BLRM	Age (year), diabetes (yes/no), hypertension (yes/no) and baseline eGFR (mL/min/1.73 m^2)		0.812	NR	NR	0.762	53.0	85.0
		Model 3: Model 2 plus measure of proteinuria (M3) / BLRM	Age (year), diabetes (yes/no), hypertension (yes/no), baseline eGFR (mL/min/1.73 m^2), quantitative albuminuria (UACR > 30 or dipstick proteinuria +)		0.813	NR	NR	0.770	55.0	89.0
Al-Shamsi et al. (23)	United Arab Emirates	Full model (FM)/ FGRM	Age (year); sex (male); diabetes (yes/no), hypertension (yes/no), dyslipidemia (yes/no), smoking (yes/no), CVD (yes/no), SBP (mmHg), DBP (mmHg); total cholesterol (mmol/L); triglycerides (mmol/L); HbA1c (%), eGFR (mL/min/1.73 m^2).	CKD: eGFR < 60 mL/min/1.73 m^2 for ≥3 months – CKD-EPI equation	0.904 (0.853–0.945)	NR	NR	0.782	56.0	90.0
		Stepwise model (SM) / FGRM	eGFR (mL/min/1.73 m^2), diabetes (yes/no), cholesterol (mmol/L), and HbA1c (%).		0.918 (0.846–0.964)	NR	NR	0.769	53.0	81.0
Lee et al. (24)	Korea	Model 3 (M3)/ CPHRM	Sex (male), BMI (kg/m^2), education level, fasting glucose (mg/dL), serum albumin (mg/dL), eGFR (mL/min/1.73 m^2) and proteinuria (yes/no).	CKD: eGFR < 60 mL/min/1.73 m^2 for at least two consecutive measurements during follow-up – CKD-EPI equation	0.798 (0.784–0.813)	NR	NR	0.773	58.0	91.0

(Continued)

10.3389/fmed.2022.903090

TABLE 3 (Continued)

Authors (year)	Population	Model name/ type of model	Variables	Outcomes predicted	AUCROC	Sensitivity (%)	Specificity (%)	External validation in Mexican adult population	Sensitivity (%) in Mexican adult population	Specificity (%) in Mexican adult population
		Model 4 (M4)/ CPHRM	Sex (male), BMI (kg/m^2), education level, income, fasting glucose (mg/dL), serum albumin (mg/dL), eGFR (mL/min/1.73 m^2), proteinuria (yes/no), and Framingham risk score.		0.813 (0.798–0.827)	NR	NR	0.774	53.0	83.0
Nelson et al. (25)	Multinational	Model/ BLRM	Age (year), sex (female), race/ethnicity, eGFR (mL/min/1.73 m^2), history of CVD (yes/no), ever smoker (yes/no), hypertension (yes/no), BMI (kg/m^2), and albuminuria (yes/no).	CKD: eGFR < 60 mL/min/1.73 m^2 – CKD-EPI equation	0.845 (0.789–0.890)	NR	NR	0.757	51.0	82.0
Wen et al. (26)	China	Validation: Simple clinical model BLRM	Sex (female), Waist circumference (cm), Systolic blood pressure (mmHg), diabetes (yes/no), and education (Illiterate/primary school and above).	Predicting incident CKD: reduced renal function or the presence of albuminuria/Albuminuria (UACR ≥ 30 mg/g) and reduce renal function (eGFR < 60 mL/min/1.73m^2).	0.717 (0.689–0.744)	70.49 (63.30–77.00)	65.14 (61.90–68.30)	0.631	41.0	72.0
Saranburut et al. (27)	Thailand	Model 1 (Clinical) BLRM	Age (year), sex (male), diabetic mellitus (yes/no), systolic blood pressure (mmHg), waist circumference (cm).	Incident cases with decreased eGFR: subjects with preserved GFR (eGFR ≥ 60) at baseline who subsequently developed decreased GFR (eGFR < 60 mL/min/1.73 m^2) at the 10 years follow-up.	0.71 (0.68–0.74)	NR	NR	0.614	39.0	68.0
		Model 2 (Clinical + limited laboratory tests) BLRM	Age (year), sex (male), systolic blood pressure (mmHg), diabetic mellitus (yes/no), GFR category (ml/min/1.73 m^2)		0.75 (0.72–0.78)	NR	NR	0.66	38.0	74.0

AUCROC, area under the receiver operating characteristic curve; CVD, cardiovascular disease; SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure; BMI, body mass index; NR, not reported; m, male; f, female; Y, year; kg/m^2, kilogram per square meter; mmol/L, millimole per liter; mg/dL, milligrams per deciliter; BLRM, Binary Logistic Regression Model; FGRM, Fine and Gray regression model; CPHRM, Cox Proportional Hazard Regression Model.

10.3389/fmed.2022.903090

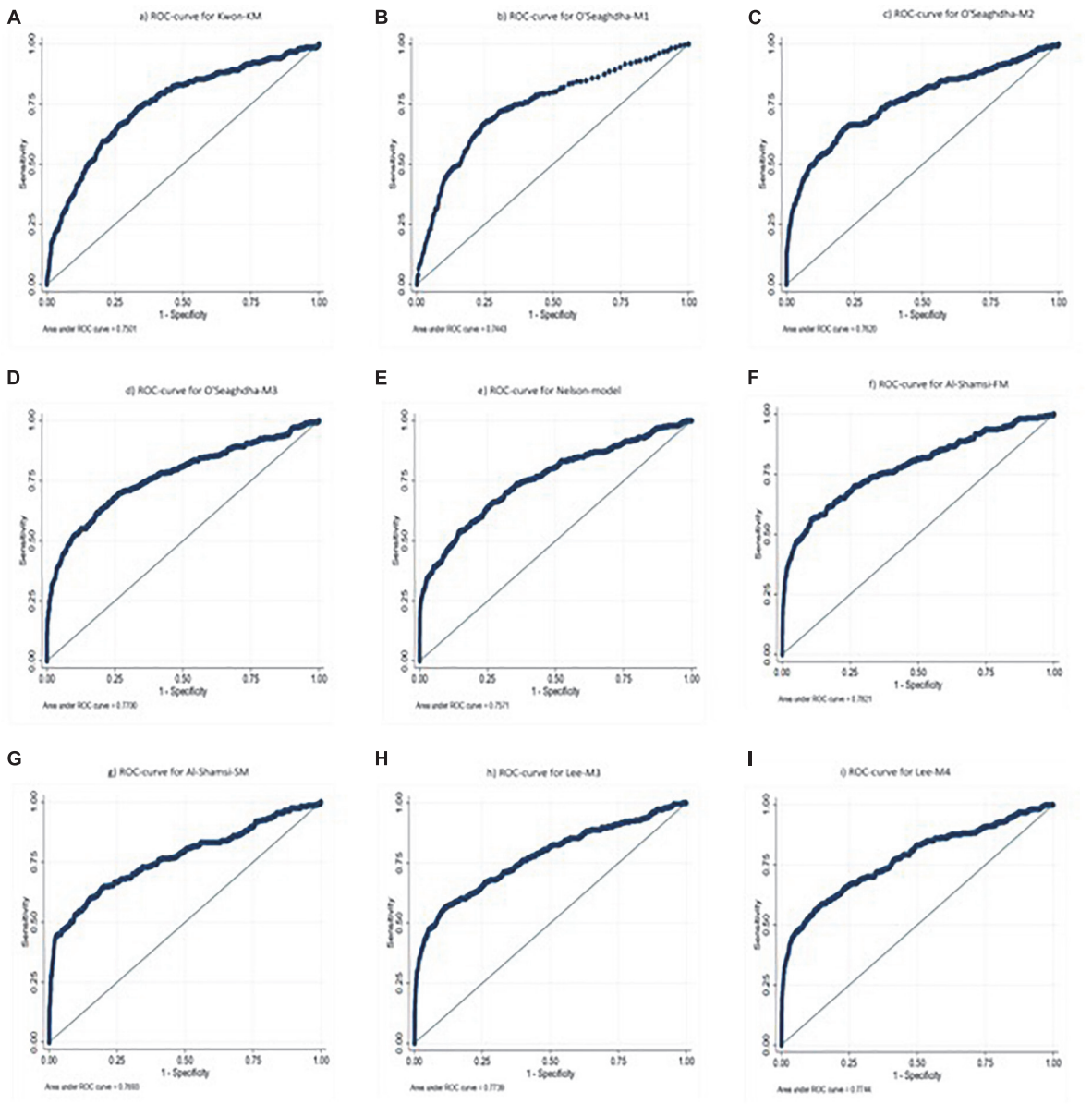


FIGURE 2
Area under the curve of risk predictive models of chronic kidney disease previously described in the Mexican population, using data of the National Health and Nutrition Survey 2016: **(A)** Kwon Korean model, **(B)** O'Seaghdha model 1, **(C)** O'Seaghdha model 2, **(D)** O'Seaghdha model 3, **(E)** Nelson model, **(F)** Al-Shamsi full model, **(G)** Al-Shamsi stepwise model, **(H)** Lee model 3, and **(I)** Lee model 4.

was observed that sex, age, BMI, FPG, SBP, and triglycerides variables predict the CKD risk with the 0.78 AUROC value.

Kwon et al. (21), Al-Shamsi et al. (23), and Lee et al. (24), as the study showed that CKD patients were older, prevalence was higher in T2D and hypertension, mean was higher in serum creatinine and HbA1c, compared to patients without CKD. Two studies (21, 24) reported that the mean total cholesterol, triglycerides and FPG were higher in those with CKD, as was found in this study's population. The CKD population in Kwon et al. (21) and this study's, present a higher mean waist circumference and a lower mean hemoglobin compared to those without CKD. For their part, Lee et al. (24) showed that elevated SBP and DBP was significant in CKD patients, as in this study, but not in Al-Shamsi et al. (23). Only this study evaluated HOMA-IR, the mean was 3.0 in patients without CKD and 3.9 in those with CKD, 38.1% of the patients with CKD had insulin resistance.

External validation of all CKD risk predictive models (21–27) was carried out with a representative sample of the Mexican National Health and Nutrition Survey, all of

TABLE 4 Training and validation risk scores for the development of CKD[c].

	Training model	Validation model
	OR (95% CI)	OR (95% CI)
Female	1.44 (1.09, 1.90)	1.56 (1.16, 2.10)
Age	1.03 (1.01, 1.04)	1.04 (1.01, 1.06)
BMI	1.01 (1.00, 1.03)	1.01 (1.00, 1.03)
FPG (mg/dL)	1.02 (1.01, 1.03)	1.02 (1.01, 1.04)
SBP (mmHg)	2.02 (1.54, 2.82)	2.03 (1.51, 2.80)
Triglycerides (mg/dL)	1.01 (1.01, 1.03)	1.01 (1.00, 1.03)
AUC	0.78 (0.72, 0.82)	0.76 (0.71, 0.80)
Sensitivity	75.0	77.1
Specificity	99.2	91.2
Positive predictive value	88.3	89.5
Negative predictive value	93.1	92.1

CI, confidence interval; BMI, body mass index; FPG, fasting plasma glucose; SBP, systolic blood pressure. [c]CKD defined as: eGFR < 60 ml/min/1.73 m^2 and albuminuria > 30 mg/g.

them were population-based, except Al-Shamsi et al. which was clinic-based. The CKD risk predictive models had a fair to good AUROC in their population; however, when replicating these models in our population, the predictive capacity was diminished.

These authors agree with Echouffo-Tcheugui et al. (28) that strategies for early identification and treatment of people with CKD are needed worldwide, and although the model equations incorporate several risk factors that are independently associated with the occurrence of CKD, these should be easily evaluable in routine clinical settings. In this sense, this study's model for the Mexican adult population was developed with variables which are easily available at the first medical care level. The validation model included sex, age, BMI, FPG, SBP, and triglycerides.

In agreement with the previous studies (21–27), older age, and presence of T2D and hypertension are the main risk factors for developing CKD in stages 3–5. This study's model uses variables which has been previously used in other predictive models, i.e., age, sex, T2D, and hypertension.

To our knowledge, the present study is the first to assess CKD risk predictive models in an apparently healthy Mexican adult population. The AUROC for this study's training model was 0.78 and for the validation model was 0.76, compared to Kwon et al. model (21), the AUROC was 0.87 in its primary population and 0.75 in the external validation. O'Seaghdha et al. (22) developed three models, AUROC was between 0.78 and 0.81 in its population, 0.76 in its external validation, and in our validation, it was between 0.74 and 0.77. The AUROC in Al-shamsi et al. (23) two models was 0.90 (multivariate full model) and 0.92 (multivariate stepwise model). They performed very well in their population, however, in the Mexican adult population AUROC was 0.78 and 0.76, respectively. Stepwise model had a lower AUROC than full model, this may be because the stepwise model includes eGFR, T2D, Cholesterol, and HbA1c, but not age, hypertension, triglycerides, and CVD variables, which does include full model and were more significant in Mexican adult population. Finally, Nelson et al. (25) used the CKD Prognosis Consortium (PC), which includes study cohorts from around the world, collecting more than five million patients, and developed two CKD predictive models, one for patients with T2D and the other without T2D. The median C statistic was 0.84 in the cohort without T2D and in his study's validation, AUROC was 0.75.

It was observed that CKD risk predictive models are characterized by including the main risk factors for developing CKD (older age, T2D, and hypertension); in addition to renal variables (eGFR, proteinuria, albuminuria) that usually improve predictive capacity, and other variables (dyslipidemia, CVD, BMI, sex, etc.). It was considered that the inclusion or not of this group of variables can increase or decrease the predictive capacity depending on the population because they are not always significant.

The objective of creating CKD risk predictive models is to prevent, applied mainly in populations with risk factors for CKD susceptibility, initiation, or progression. The prevalence of this disease has been increasing in Mexico and Latin America, for this reason the need to evaluate CKD risk predictive models, to be used in Mexican adult population and facilitate surveillance of groups susceptible to risk for developing CKD (8).

There are numerous strengths to this study, including the representative based sample, rigorous and detailed assessment of risk factors including measures of renal function and proteinuria. The parsimonious list of variables in the final model is also a significant strength, enhancing the score's utility and applicability. Some limitations should also be acknowledged. A very high coefficient of variation in creatinine concentrations was observed because creatinine was measured on a single occasion; however, multiple measurements in cross-sectional studies are not feasible. Furthermore, eGFR was estimated using the CKD-EPI equation, which may underestimate eGFR in both healthy individuals and those with CKD. However, a comparison of definitions of incident CKD in the setting of epidemiological research demonstrates that the present definition is the most sensitive (29), which is desirable in view of the potential application of the risk score for population screening. Finally, the cross-sectional design of the study.

Conclusion

The aims of this study were to evaluate different CKD risk predictive models in the Mexican adult population and develop our predictive risk model. The models evaluated showed a fair to good predictive capacity, however, adjusted in the Mexican adult population, this predictive capacity was diminished. The study's

model is a reliable tool for predict CKD risk among apparently healthy population. It was observed that the variables sex, age, BMI, FPG, SBP, and triglycerides satisfactorily predict the CKD risk, these variables are simple and common in the primary care attention. So, this model could help physicians to identify population at risk. The implementation of CKD risk predictive models will allow the prevention and control of CKD, applied in populations with risk factors for susceptibility, initiation, or progression of CKD. Communication and awareness of the risk to patients is the first step for prevention, it could motivate them to improve their lifestyle and adhere to prescribed therapies. Prevention by identifying patients at risk could also have an economic benefit in our health care system.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics statement

The ENSANUT-MC 2016 protocols were approved by the research, ethics and biosafety committees of the National Institute of Public Health, strictly adhering to the principles set forth in the Declaration of Helsinki. The voluntary nature of participation was recorded in the informed consent and assent forms. The patients/participants provided their written informed consent to participate in this study.

Author contributions

VC wrote the manuscript (Introduction, Results, and Discussion). AG-R wrote the manuscript (Materials and methods and Discussion). DC contributed the analysis tools. CH-A conceived and designed the analysis. AP wrote the manuscript (Materials and methods) and contributed the data. MP-C contributed the analysis tools. SB conceived and designed the analysis. ED-G conceived and designed the analysis, contributed the analysis tools, and performed the analysis. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the CONACYT-PRONASE-SALUD study (project number 2256, CI:1767).

Acknowledgments

We would like to thank the survey's team from the National Institute of Public Health for the elaboration of ENSANUT 2016 survey and database.

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.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.903090/full#supplementary-material

References

1. GBD Chronic Kidney Disease Collaboration. Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the global burden of disease study 2017. *Lancet.* (2020) 395:709–33. doi: 10.1016/S0140-6736(20)30045-3

2. Agudelo-Botero M, Valdez-Ortiz R, Giraldo-Rodríguez L, González-Robledo MC, Mino-León D, Rosales-Herrera MF, et al. Overview of the burden of chronic kidney disease in Mexico: secondary data analysis based on the global burden of disease study 2017. *BMJ Open.* (2020) 10:e035285. doi: 10.1136/bmjopen-2019-035285

3. Cockwell P, Fisher L-A. The global burden of chronic kidney disease. *Lancet.* (2020) 395:662–4. doi: 10.1016/S0140-6736(19)32977-0

4. Gómez-Dantés H, Fullman N, Lamadrid-Figueroa H, Cahuana-Hurtado L, Darney B, Avila-Burgos L, et al. Dissonant health transition in the states of Mexico, 1990–2013: a systematic analysis for the global burden of

disease study 2013. *Lancet.* (2016) 388:2386–402. doi: 10.1016/S0140-6736(16)31773-1

5. Basto-Abreu A, López-Olmedo N, Rojas-Martínez R, Aguilar-Salinas CA, De la Cruz-Góngora V, Rivera-Dommarco J, et al. Prevalence of diabetes and glycemic control in Mexico: national results from 2018 and 2020. *Salud Publica Mex.* (2021) 63:725–33. doi: 10.21149/12842

6. Campos-Nonato I, Hernández-Barrera L, Oviedo-Solís C, Ramírez-Villalobos D, Hernández B, Barquera S. Epidemiología de la hipertensión arterial en adultos mexicanos: diagnóstico, control y tendencias. Ensanut 2020. *Salud Publica Mex.* (2021) 63:692–704. doi: 10.21149/12851

7. INSP. *Encuesta Nacional de Salud y Nutrición 2018 - Presentación de resultados*. (2020). Available online at: https://ensanut.insp.mx/encuestas/ensanut2018/doctos/informes/ensanut_2018_presentacion_resultados.pdf [accessed on Jul 2021, 31]. .

8. Tamayo y Orozco J, Lastiri Quirós HS. *La enfermedad renal crónica en México. Hacia una politica nacional para enfrentarla. Intersistemas*. México: Academia Nacional de Medicina de México (2016) 82.

9. Figueroa-Lara A, Angel Gonzalez-Block M, Alarcon-Irigoyen J. Medical expenditure for chronic diseases in Mexico: the case of selected diagnoses treated by the largest care providers. *PLoS One.* (2016) 11:e0145177. doi: 10.1371/journal.pone.0145177

10. Aldrete-Velasco JA, Chiquete E, Rodríguez-García JA, Rincón-Pedrero R, Correa-Rotter R, Peña-García R, et al. Mortalidad por enfermedad renal crónica y su relación con la diabetes en México. *Med Interna Mex.* (2018) 34:536–50.

11. Ávila-Saldivar MN. Enfermedad renal cronica: prevencion y deteccion temprana en el primer nivel de atencion. *Med Interna Méx.* (2013) 29:148–53.

12. Romero-Martínez M, Shamah-Levy T, Cuevas-Nasu L, Gómez-Humarán IM, Gaona-Pineda EB, Gómez-Acosta LM, et al. Diseño metodológico de la encuesta nacional de salud y nutrición de medio camino 2016. *Salud Publica Mex.* (2017) 59:299–305. doi: 10.21149/8593

13. Hernández-Alcaraz C, Shamah-Levy T, Romero-Martínez M, Sepúlveda-Amor J, Aguilar-Salinas CA, Rivera-Dommarco J, et al. Submuestra para el análisis de enfermedades crónicas con biomarcadores, encuesta nacional de salud y nutrición 2016. *Salud Publica Mex.* (2020) 62:504–10. doi: 10.21149/11306

14. Campos-Nonato I, Hernández-Barrera L, Pedroza-Tobías A, Medina C, Barquera S. Hypertension in Mexican adults: prevalence, diagnosis and type of treatment. Ensanut MC 2016. *Salud Publica Mex.* (2018) 60:233–43. doi: 10.21149/8813

15. Unger T, Borghi C, Charchar F, Khan NA, Poulter NR, Prabhakaran D, et al. 2020 international society of hypertension global hypertension practice guidelines. *Hypertension.* (2020) 75:1334–57.

16. American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes—2018. *Diabetes Care.* (2018) 41(Suppl 1.):S13–27.

17. Qu HQ, Li Q, Rentfro AR, Fisher-Hoch SP, McCormick JB. The definition of insulin resistance using HOMA-IR for Americans of Mexican descent using machine learning. *PLoS One.* (2011) 6:e21041. doi: 10.1371/journal.pone.0021041

18. Levey AS, Eckardt K-U, Dorman NM, Christiansen SL, Hoorn EJ, Ingelfinger JR, et al. Nomenclature for kidney function and disease: report of a kidney disease: improving global outcomes (KDIGO) consensus conference. *Kidney Int.* (2020) 97:1117–29. doi: 10.1016/j.kint.2020.02.010

19. Arreola-Guerra J, Rincón-Pedrero R, Cruz-Rivera C, Belmont-Pérez T, Correa-Rotter R, Niño-Cruz J. Performance of MDRD-IDMS and CKD-EPI equations in Mexican individuals with normal renal function. *Nefrologia.* (2014) 34:591–8. doi: 10.3265/Nefrologia.pre2014.jun.12538

20. Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome. *Circulation.* (2009) 120:1640–5.

21. Kwon KS, Bang H, Bomback AS, Koh DH, Yum JH, Lee JH, et al. A simple prediction score for kidney disease in the Korean population. *Nephrology.* (2012) 17:278–84. doi: 10.1111/j.1440-1797.2011.01552.x

22. O'Seaghdha CM, Lyass A, Massaro JM, Meigs JB, Coresh J, D'Agostino R, et al. A risk score for chronic kidney disease in the general population. *Am J Med.* (2012) 125:270–7. doi: 10.1016/j.amjmed.2011.09.009

23. Al-Shamsi S, Oulhaj A, Regmi D, Govender RD. Use of estimated glomerular filtration rate to predict incident chronic kidney disease in patients at risk of cardiovascular disease: a retrospective study. *BMC Nephrol.* (2019) 20:325. doi: 10.1186/s12882-019-1494-8

24. Lee C, Yun HR, Joo YS, Lee S, Kim J, Nam KH, et al. Framingham risk score and risk of incident chronic kidney disease: a community-based prospective cohort study. *Kidney Res Clin Pract.* (2019) 38:49–59. doi: 10.23876/j.krcp.18.0118

25. Nelson RG, Grams ME, Ballew SH, Sang Y, Azizi F, Chadban SJ, et al. Development of risk prediction equations for incident chronic kidney disease. *JAMA J Am Med Assoc.* (2019) 322:2104–14. doi: 10.1001/jama.2019.17379

26. Wen J, Hao J, Zhang Y, Cao K, Zhang X, Li J, et al. Risk scores for predicting incident chronic kidney disease among rural Chinese people: a village-based cohort study. *BMC Nephrol.* (2020) 21:120. doi: 10.1186/s12882-020-01787-9

27. Saranburut K, Vathesatogkit P, Thongmung N, Chittamma A, Vanavanan S, Tangstheanphan T, et al. Risk scores to predict decreased glomerular filtration rate at 10 years in an Asian general population. *BMC Nephrol.* (2017) 18:240. doi: 10.1186/s12882-017-0653-z

28. Echouffo-Tcheugui JB, Kengne AP. Risk models to predict chronic kidney disease and its progression: a systematic review. Remuzzi G, editor. *PLoS Med.* (2012) 9:e1001344. doi: 10.1371/journal.pmed.1001344

29. Tonna SJ. Invited commentary: defining incident chronic kidney disease in epidemiologic study settings. *Am J Epidemiol.* (2009) 170:425–7. doi: 10.1093/aje/kwp148

Frontiers in Medicine

Translating medical research and innovation into improved patient care

A multidisciplinary journal which advances our medical knowledge. It supports the translation of scientific advances into new therapies and diagnostic tools that will improve patient care.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact