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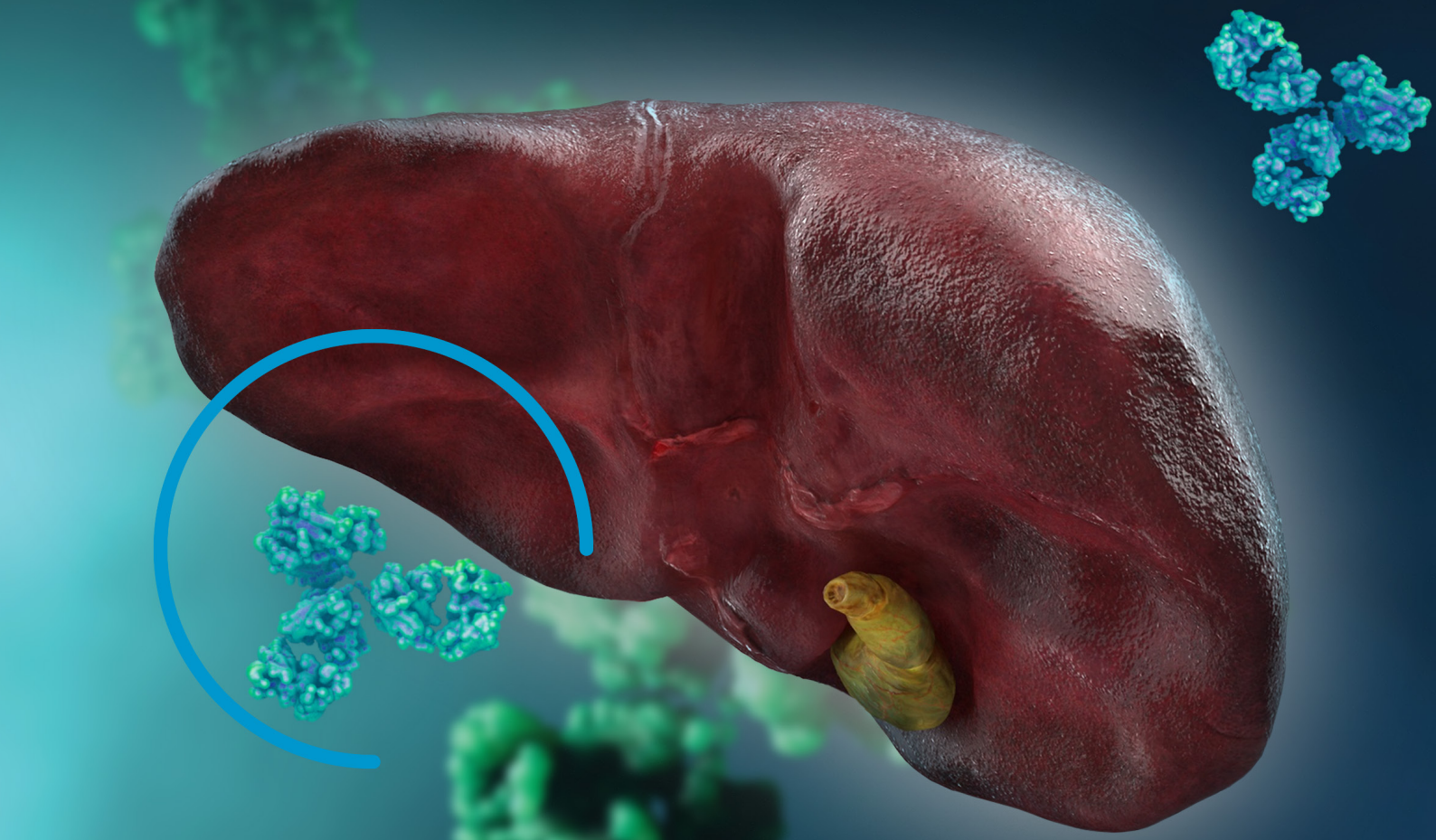

Transplantation with a positive X-match: The liver doesn't care (much)

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Brief Research Report

Building a vision for the future of organ transplantation

ESOT Congress
THE INTERNATIONAL TRANSPLANT CONGRESS®
ATHENS | 17-20 SEPTEMBER 2023

The world is still reeling from the greatest health challenge in modern history. A challenge with a huge human toll. A challenge which exposed deficiencies in every health system across the globe and revealed our unpreparedness to deal with major disasters. The pandemic also had an indirect impact on the delivery of other health services, including organ transplantation, by concentrating personnel and resources on the task in hand. As such, the long-term impact of the COVID-19 pandemic is yet to be fully understood.

However, every cloud has a silver lining as the pandemic has brought communities and countries closer than ever. We demonstrated the immense power of collaboration that allowed the rapid development of disruptive solutions to a global challenge. We were pushed to innovate, to re-think how services were delivered, how interactions with patients were conducted, how we could deliver healthcare services remotely and in the face of adversity, all whilst maintaining patients' trust in a high-quality care.

Even in the midst of this global challenge, our field took a major leap in xeno-transplantation, organ perfusion and preservation technology, and has built the foundation for bioengineering the organs of the future. We worked ever closer with patients in establishing shared care as the fundamental principle of transplant medicine and removed barriers in access to what is now a global resource and field. Now, as we emerge from the aftermath of the pandemic, we have an opportunity to radically re-shape the delivery of organ transplant care and to ensure preparedness for future challenges. We need to learn from the challenges of recent years and press on with the innovative spirit characteristic of our field to push disruptive innovations and developments.

Against this background, the transplant community will gather in Athens for the 21st ESOT Congress to discuss, debate and identify the transplant strategies of the future.

Technology was an essential component of the pandemic response, and it is widely believed that the pandemic has accelerated the digitalisation of healthcare. So, there is little doubt that the future of transplantation will be technological in almost every aspect of clinical care and research. At this year's congress, we will discuss how to integrate these disruptive technologies in routine care, what the potential societal, ethical and individual patient impacts are and how we can ensure accessibility for every single one of our patients. As such, the opening plenary session will set the scene for what disruptive innovation may look like in the future, explore how technology can enhance the human aspect of transplant care and how it can help us build the trusted care that we aim to achieve. These are big challenges that can only be achieved through collaboration.

In true ESOT spirit, we will think outside the box to find the most innovative solutions to the organ shortage and explore the realm of bioengineering and artificial intelligence, manipulating the very nature of our being by reprogramming our blood groups or making use of genetically modified organs, transcending the boundaries of species.

Each topic will be explored further in state-of-the-art conversations and then debated and discussed in solution rooms and fishbowl sessions, where experts will interact with the audience in open conversations. It is this level of interaction and dialogue that this congress aims to promote, allowing every participant to consider how these advances can be implemented and improve their practice.

This year's programme is not only about the cutting-edge technologies or the innovative solutions coming out of the research labs. It is also about the day-to-day practice, the interdisciplinary interactions, and the ability to share best practices to improve access to transplantation and reduce inequities in care across Europe. We have listened to the feedback from ESOT members that the congress should enable such conversations about what may appear routine issues in parts of the continent but are still a challenge in others, such as organ donation, living donation, research and consent. A significant number of sessions are designed to be interactive (dare to ask, let's talk about it, solution rooms), and we count on every participant to share their challenges, successes and ideas to improve transplant care and learn from each other in the most collaborative way.

The ESOT Congress 2023 marks a unique moment in transplant history, placing the people in need of a transplant at its very heart. For the first time in the field of transplantation, the Honorary Congress Chair is a patient, demonstrating ESOT's commitment to patient-centric science and clinical care. Many of the sessions are designed to inform about best practice in shared decision making, how patients can help democratise healthcare, how we can work closely to reduce non-adherence and improve long-term follow-up and outcomes. These interactions will be reflected in the stories we will hear from patients and pioneers in the field.

In a world still facing many unknowns and crises, we will openly discuss challenging issues such as caring in times of crisis, migration, organ trade and explore ways to build resilience in our systems. We will conclude the congress on an aspirational note, taking a moonshot towards a unified transplant healthcare and building a vision for the future of transplant care and health policies.

The congress will have something for everyone, or at least we hope so! Beyond the state-of-the-art science, the disruptive innovation and the trusted clinical care side of the meeting, we have the opportunity to connect, interact, hug old friends and make new ones. What better place to do this than the cradle of European civilisation and democracy?

It is time to book your travel to Athens!

Gabriel C Oniscu
ESOT Congress 2023 Co-Chair
ESOT President Elect

ESOTCongress

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ATHENS | 17-20 SEPTEMBER 2023

Disruptive Innovation, Trusted Care

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TRANSPLANT TRIAL WATCH
published: 21 March 2023
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Transplant Trial Watch

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Keywords: randomised controlled trial, kidney transplant, vaccine, COVID-19, immune response

To keep the transplantation community informed about recently published level 1 evidence in organ transplantation ESOT and the Centre for Evidence in Transplantation have developed the Transplant Trial Watch. The Transplant Trial Watch is a monthly overview of 10 new randomised controlled trials (RCTs) and systematic reviews. This page of Transplant International offers commentaries on methodological issues and clinical implications on two articles of particular interest from the CET Transplant Trial Watch monthly selection. For all high quality evidence in solid organ transplantation, visit the Transplant Library: www.transplantlibrary.com.

RANDOMISED CONTROLLED TRIAL 1

Benefits of Switching Mycophenolic Acid to Sirolimus on Serological Response after a SARS-CoV-2 Booster Dose among Kidney Transplant Recipients: A Pilot Study.

by Banjongjit, A., et al. Vaccines 2022; 10 (10): 09.

Aims

The aim of this study was to compare the immune response to the booster dose of BNT162b2 in renal transplant patients who remain on the standard immunosuppressive regimen [tacrolimus (TAC), MPA, and prednisolone] versus those who switch to the mammalian target of rapamycin inhibitor (mTORi), TAC, and prednisolone regimen.

Interventions

Participants were randomised to either continue the standard regimen or switch to a sirolimus (an mTORi), TAC, and prednisolone regimen.

Participants

28 kidney transplant recipients.

Outcomes

The main outcomes of interest were change in anti-SARS-CoV-2 S antibody level pre- and post-BNT162b2 vaccination, and adverse events.

Follow-Up

6 months.

CET Conclusion

This is a very interesting pilot study on vaccine responses on different immunosuppressive regimens. For 2 weeks prior and 2 weeks after vaccination (ChAdOx-1), recipients were randomised to switch

Transplant Trial Watch

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Mycophenolate to Sirolimus, or stay on usual immune suppression. The study was conducted in a single centre, and patients were randomly assigned by computer algorithm in an unblinded manner. Only 28 recipients met the inclusion criteria. Whilst the anti-SARS-CoV-2 S antibody levels increased significantly in both groups, the switching group had a significantly higher level in comparison. It is critical to consider adverse events, and in this study 2 patients in the sirolimus group experienced mouth ulcers that healed after returning to mycophenolate at the end of study. There were no significant changes in serum creatinine, urine albumin or any other significant symptoms. This study shows that a very short conversion window from mycophenolate to sirolimus can significantly improve vaccine antibody responses in kidney transplant recipients.

Jadad Score
2.

Data Analysis
Per protocol.

Allocation Concealment
Yes.

Trial Registration
TCTR20220404001.

Funding Source
Non-industry funded.

RANDOMISED CONTROLLED TRIAL 2

Alternative Strategies to Increase the Immunogenicity of COVID-19 Vaccines in Kidney Transplant Recipients not Responding to Two or Three Doses of an mRNA Vaccine (RECOVAC): A Randomised Clinical Trial.

by Kho, M. M. L., et al. The Lancet Infectious Diseases 2022 [record in progress].

Aims
This study aimed to compare the immunogenicity of a double dose vaccine, heterologous vaccination, and temporary discontinuation of mycophenolate mofetil or mycophenolic acid to that of a control single dose mRNA-1273 vaccination, in kidney transplant recipients who do not respond to two or three doses of an mRNA vaccine.

Interventions
In the first cohort, participants were randomised to receive a single dose of mRNA-1273, two doses of mRNA-1273, or the Ad26.COV2-S vaccine. In the second cohort, patients receiving triple immunosuppressive therapy were randomised to either continue mycophenolate mofetil or mycophenolic acid, or discontinue mycophenolate mofetil or mycophenolic acid, from 1 week before until 1 week after being vaccinated with a single 100 μg dose of mRNA-1273.

Participants
230 kidney transplant recipients were randomised in the first cohort and 103 kidney transplant recipients were randomised in the second cohort.

Outcomes
The primary endpoint was the percentage of participants with a spike protein (S1)-specific IgG concentration ≥10 BAU/mL 28 days following vaccination. Secondary endpoints included the presence of virus neutralising antibodies, serum concentration of S1-specific IgG, and SARS-CoV-2 specific T-cell response and safety.

Follow-Up
28 days.

CET Conclusions
This is another very interesting study on vaccine responses in kidney transplant recipients. In this complex study kidney transplant recipients were randomised to receive either an mRNA vaccine of 100 or 200 μg (mRNA-1273) versus a viral vector vaccine (Ad26.COV2-s). A small group was also randomised to continue or discontinue mycophenolate for 1 week before and 1 week after. The study showed again, as has been seen elsewhere, that a significant proportion of transplant recipients do not seroconvert after two or even three doses of SARS-CoV-2 vaccine (34% and 20%). Vaccination with 200 μg mRNA vaccine was not significantly better than 100 μg or the viral vector vaccine. Stopping mycophenolate for 1 week and before and 1 week after did not have any significant impact on vaccine response either. The study was adequately randomised and powered, however it was not blinded. Given the objective nature of the results this is not of significant concern for systematic bias in the reporting of the results. The study was funded by The Netherlands Organization for Health Research and Development and the Dutch Kidney Foundation.

Jadad Score
3.

Data Analysis
Per protocol.

Allocation Concealment
Yes.

Trial Registration
ClinicalTrials.gov—NCT05030974.

Funding Source
Non-industry funded.

CLINICAL IMPACT SUMMARY

For this month's clinical impact summary we have selected two related clinical trials. Transplant recipients do not have the same initial response to COVID-19 vaccines as other members of the population, leaving them at increased risk. Hence a strategy to improve vaccine response is critical.

The first study by Bangonjit et al., from Bangkok, Thailand is a relatively small pilot study from a single centre. Transplant patients in this study had previously received two doses of ChAdOx-1 vaccine (viral vector) and one dose of BNT162b2 (mRNA) vaccine (n = 28). Patients received a booster dose of BNT162b2 vaccine, but were randomised to switch from mycophenolic acid to sirolimus for 2 weeks prior and up to 2 weeks post-vaccination. The COVID-19 antibody levels post-vaccination were significantly higher in the sirolimus group than the mycophenolate group, without a significant number of adverse events. However, the study was very small and hence less common, although potentially very severe, events related to switching immune suppression may not have been revealed. There was only one seronegative patient, who remained seronegative after the booster dose.

The results from this study support those found by the team from the OPTIMIZE trial in the Netherlands, published earlier this year (1). Although there are some slight differences in patient group and immune suppression. They also echo the results of another study also published earlier this year (2).

The second study this month, by Kho et al, is from four centres in Netherlands, and includes 345 patients, although the study was complex with multiple subdivisions and randomisations. Kidney transplant recipients who remained seronegative after two, or three, doses of mRNA vaccine were included. Patients were randomised to receive an mRNA vaccine of 100 or 200 μg (mRNA-1273) versus a viral vector vaccine (Ad26.COV2-s). In addition, a small group receiving 100 μg mRNA vaccine was also randomised to continue or discontinue mycophenolate for 1 week before until 1 week after the third vaccine dose (n = 108).

A significant proportion of these recipients still did not seroconvert after two or even three doses of SARS-CoV-2 vaccine (34% and 20%). There was no significant difference in seroconversion rate comparing 200 μg mRNA vaccine to 100 μg, or the viral vector vaccine. Stopping mycophenolate for 1 week before and 1 week after did not have any significant impact on vaccine response either.

This study highlights the need for the third and even fourth COVID-19 booster vaccines to improve seroconversion in transplant recipients. Whilst this second study did not show an improved vaccine response when stopping mycophenolate, it was for a relatively short period only. Stopping mycophenolate for a longer period may be necessary to improve the immune response, however it may require the addition of another immune suppressant (such as sirolimus) to therapy during the switch period. Published studies assessing this concept have so far been small and therefore not reliable in assessing safety.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

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

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2. Schrezenmeier E, Rincon-Arevalo H, Jens A, Stefanski AL, Hammett C, Osmanodja B, et al. Temporary Antimetabolite Treatment Hold Boosts SARS-CoV-2 Vaccination-specific Humoral and Cellular Immunity in Kidney Transplant Recipients. *JCI Insight* (2022) 7(9):e157836. doi:10.1172/jci.insight.157836

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ORIGINAL RESEARCH
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Liver Transplantation in Recipients With a Positive Crossmatch: A Retrospective Single-Center Match-Pair Analysis

*Felix J. Krendl[1], Margot Fodor[1], Franka Messner[1], Agnes Balog[2], Anja Vales[2], Benno Cardini[1], Thomas Resch[1], Manuel Maglione[1], Christian Margreiter[1], Marina Riedmann[3], Hanno Ulmer[3], Dietmar Öfner[1], Rupert Oberhuber[1], Stefan Schneeberger[1] and Annemarie Weissenbacher[1]**

[1]Department of Visceral, Transplant and Thoracic Surgery, Center of Operative Medicine, Medical University of Innsbruck, Innsbruck, Austria, [2]Blood Transfusion Center, Innsbruck, Austria, [3]Department of Medical Statistics, Informatics, and Health Economics, Medical University of Innsbruck, Innsbruck, Austria

A positive crossmatch (XM+) is considered a contraindication to solid abdominal organ transplantation except liver transplantation (LT). Conflicting reports exist regarding the effects of XM+ on post-transplant outcomes. The goal of this retrospective single-center analysis is to evaluate the influence of XM+ on relevant outcome parameters such as survival, graft rejection, biliary and arterial complications. Forty-nine adult patients undergoing LT with a XM+ between 2002 and 2017 were included. XM+ LT recipients were matched 1:2 with crossmatch negative (XM–) LT recipients based on the balance of risk (BAR) score. Patient and graft survival were compared using Kaplan-Meier survival analysis and the log-rank test. Comparative analysis of clinical outcomes in XM+ and XM– groups were conducted. Patient and graft survival were similar in XM+ and XM– patients. Rejection episodes did not differ either. Recipients with a strong XM+ were more likely to develop a PCR+ CMV infection. A XM+ was not associated with a higher incidence of biliary or arterial complications. Donor age, cold ischemia time, PCR+ CMV infection and a rejection episode were associated with the occurrence of ischemic type biliary lesions. A XM+ has no effects on patient and graft survival or other relevant outcome parameters following LT.

Keywords: liver transplantation, biliary complications, graft survival, crossmatch, CMV

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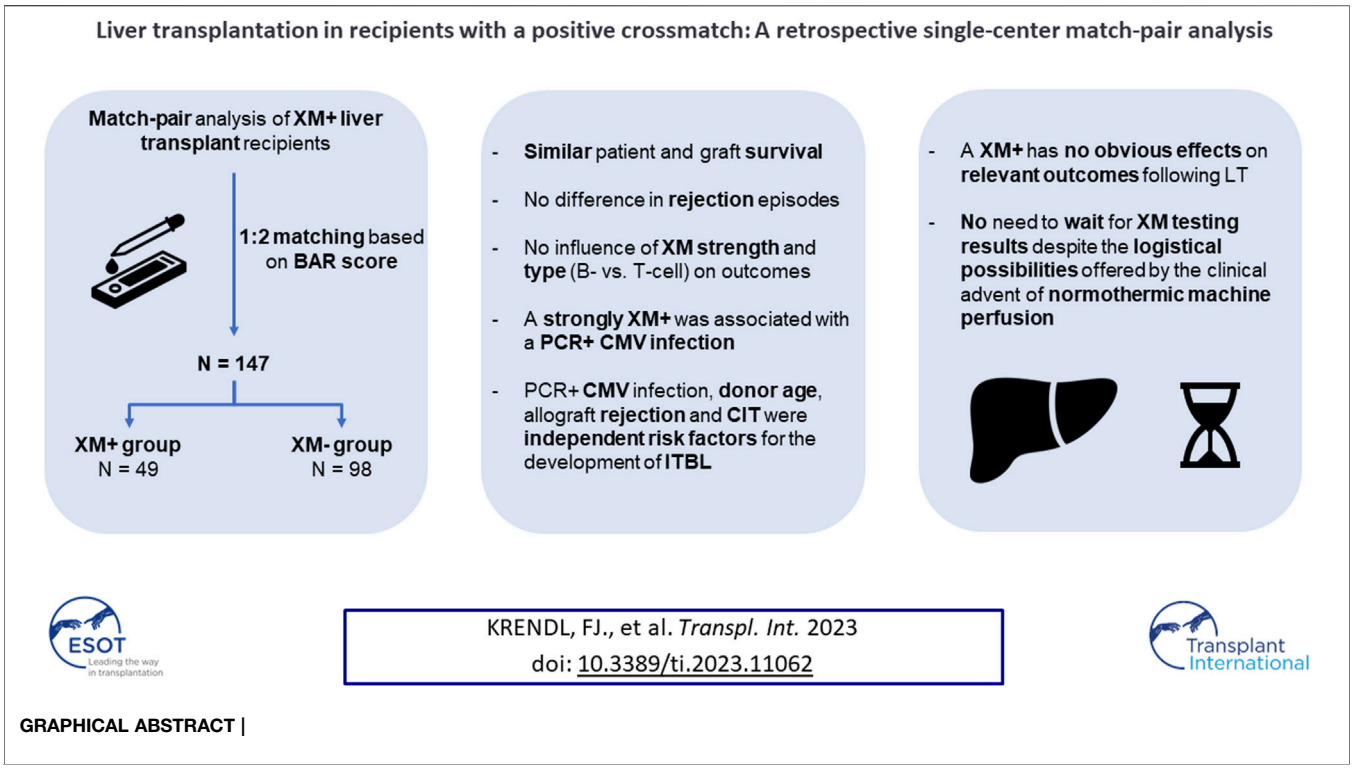
INTRODUCTION

A positive crossmatch (XM+) is usually considered a contraindication to all solid abdominal organ transplantations except liver transplantation (LT) (1, 2). Therefore, crossmatch testing is mandatory before pancreas, intestinal and kidney transplantation (3). However, in the context of LT the effect of a XM+ on post-transplant outcomes remains ill-defined and LT is commonly performed regardless of the crossmatch testing results, often even before these results become available (3–7).

Compared to other abdominal organs, the liver seems to be in a privileged immunological situation due to its dual afferent blood supply, its unique antigenic sinusoidal vasculature line by Kupffer cells and its ability to absorb preformed donor specific antibodies (DSAs) by



GRAPHICAL ABSTRACT |

secreting soluble antigens (8–10). Reports of combined liver and kidney transplantations in the presence of a XM+ in which the recipient became XM− within hours following transplantation underline the liver's impressive immunologic capabilities (9, 11, 12).

Still, some authors suggest a link between inferior patient and graft survival and a higher rate of postoperative complications following LT in the presence of a XM+ (8, 13–18). Others, however, were not able to duplicate those findings (6, 10, 12, 19–25). Yet, focusing on a XM+ alone might not tell the full story as XM strength (26) and type (T cell vs. B cell) may play a significant role concerning post-transplant outcomes (3, 5, 17, 18). Fittingly, a T cell but not B cell dependent XM+ was reported to be associated with impaired graft survival (3). Historically, LT was essentially an emergency surgical procedure in order to keep cold ischemia time (CIT) short. While it seemed unthinkable to postpone a LT until crossmatch testing results become available only a few years ago, the advent of machine perfusion has changed clinical practice (27). Machine preservation offers the possibility to optimize transplant conditions including immunologic risk stratification pre-transplant. Considering these implications, it seems worthwhile to explore whether a XM+ influences post-transplant outcomes. Previous studies on this subject were hampered by a small number of patients and mostly lacked adequate controls and comparisons (15, 16, 26, 28, 29).

The aim of this match-pair analysis is to evaluate the influence of a XM+, including XM strength and type, on relevant clinical outcome parameters such as patient and graft survival, rejection episodes, biliary and arterial complications.

PATIENTS AND METHODS

Study Population and Study Design

At the Medical University of Innsbruck, crossmatch testing is routinely performed for LT recipients. All adult patients who underwent XM+ deceased donor LT from donation after brain dead (DBD) donors between 2002 and 2017 were included. A 1:2 match-pair analysis was conducted, with patients who underwent LT with a negative crossmatch (XM−) serving as controls. Matching was performed based on the balance of risk (BAR) score (30, 31).

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board; protocol code 1034/2022. The results were reported according to the STROBE guidelines (32).

Immunosuppression and Postoperative Care

The standard immunosuppressive (IS) regimen for LT recipients at our center consisted of the following: Induction therapy with an intra-operative bolus of 500 mg methylprednisolone. As part of the PROTECT (33) and DIAMOND (34) trials, some patients received induction therapy with an interleukin 2 (IL2) antibody. Postoperatively patients received tacrolimus (Tac) (initial trough

TABLE 1 | Recipient characteristics in the matched cohort.

	All N = 147	XM+ n = 49	XM− n = 98	*p*-value
Age (years)	59.0 (54.0–65.0)	58.0 (53.5–64.0)	59.0 (54.0–65.0)	0.526
Sex				*0.022*
Female	37 (25.2)	18 (36.7)	19 (19.4)	
Male	110 (74.8)	31 (63.3)	79 (80.6)	
BMI (kg/m^2)	25.9 (22.9–28.8)	23.8 (21.6–27.1)	26.5 (23.9–29.1)	*0.003*
MELD score	16.0 (9.0–18.0)	16.0 (12.0–21.0)	13.5 (8.8–17.0)	*0.014*
Indication for LT				
AFLD	58 (39.5)	16 (32.7)	42 (42.9)	0.233
NAFLD	21 (14.3)	8 (16.3)	13 (13.3)	0.617
PBC	7 (4.8)	3 (6.1)	4 (4.1)	0.686
PSC	4 (2.7)	0 (0.0)	4 (4.1)	0.302
AIH	4 (2.7)	3 (6.1)	1 (1.0)	0.108
Tumor	62 (42.2)	13 (26.5)	49 (50.0)	*0.007*
Re - Tx	11 (7.5)	7 (14.3)	4 (4.1)	*0.042*
Induction (yes/no)	91 (61.9)	30 (61.2)	61 (62.2)	0.904
IL2	83 (57.2)	24 (49.0)	59 (61.5)	0.151
ATG	2 (1.4)	2 (4.1)	0 (0.0)	0.110
Alemtuzumab	5 (3.4)	4 (8.2)	1 (1.0)	*0.042*
Missing	1 (0.7)	0 (0.0)	1 (1.0)	
ABO blood group				0.769
A	58 (39.5)	22 (44.9)	36 (36.7)	
B	14 (9.5)	4 (8.2)	10 (10.2)	
0	61 (41.5)	18 (36.7)	43 (43.9)	
AB	14 (9.5)	5 (10.2)	9 (9.2)	
CMV mismatch				0.228
D+/R-	35 (23.8)	9 (19.6)	26 (26.8)	
D-/R+	38 (25.9)	10 (21.7)	28 (28.9)	
D+/R+	54 (36.7)	23 (50.0)	31 (32.0)	
D-/R-	16 (10.9)	4 (8.7)	12 (12.4)	
Missing	4 (2.7)	3 (6.1)	1 (1.0)	
Median follow-up (months)	60.2 (25.0–98.6)	70.7 (33.1–108.0)	57.9 (23.8–96.8)	0.304

Values are presented as medians or absolute numbers with IQRs and percentages in parentheses. Italic values show significant p*-values. AFLD, alcoholic fatty liver disease; AIH, autoimmune hepatitis; ATG, anti-thymocyte globulin; BAR, balance of risk; BMI, body mass index; CMV, cytomegalovirus; COD, cause of death; CVA, cerebrovascular accident; ET-DRI, Eurotransplant donor risk index; MELD, model for end-stage liver disease; NAFLD, non-alcoholic fatty liver disease. IL2, interleukin 2. IQR, interquartile range; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; PCR, polymerase chain reaction. Re-Tx, re-transplantation. SAB, subarachnoid hemorrhage; XM, crossmatch.*

levels 6–8 ng/mL, gradually decreased to 6 ng/mL at 6 months, and 4–6 ng/mL at 12 months) and either mycophenolate mofetil (MMF) (1,000 mg twice daily) or mycophenolic acid (MPA) (720 mg twice daily). Steroids were gradually tapered to 5 mg prednisolone per day as part of the maintenance therapy. Complete steroid withdrawal was considered on an individual basis considering the side effect profile as well as the patient's immunologic risk. Reasons to divert from our standard protocol were related to recipient factors. Conversion from Tac to cyclosporine A (CsA) was considered in case of long-QT syndrome, or tacrolimus associated neurotoxicity. MMF/MPA was switched to azathioprine (Aza) in case of gastrointestinal side effects or to avoid the teratogenic potential in female patients wishing to conceive.

Definitions

Crossmatch

All recipient sera were tested for cytotoxic antibodies against donor lymphocytes (CDC crossmatch). For the XM to be deemed positive more than 15% cytolysis had to be present. Additionally, a XM was defined as weakly positive when cytolysis ranged between 15% and 50% and strongly positive when cytolysis exceeded 50%. Cytotoxic cross-matching activity was tested before and after treatment with dithiothreitol (DTT) which inactivates IgM antibodies (35, 36). For XM strength analysis the post DTT treatment value was employed. In addition to XM strength, the XM type (T cell dependent vs. B cell dependent) was recorded.

Graft Loss and Graft Dysfunction

Graft loss was defined as patient death or the need for liver re-transplantation. Primary non-function was defined as peak AST ≥3000 IU/L plus at least one of the following criteria: INR ≥2.5, serum lactate ≥4 mmol/L and total bilirubin ≥10 mg/dL (values measured on postoperative day 3, biliary obstruction being excluded). Early allograft dysfunction (EAD) was defined according to the Olthoff criteria (37).

Rejections

Acute rejection was defined as biopsy proven rejection which required steroid bolus treatment (38). Steroid bolus treatment consisted of an intravenous steroid pulse of 500 mg methylprednisolone for three consecutive days. Chronic rejection was defined based on persistent laboratory abnormalities and histological confirmation (38).

TABLE 2 | Donor characteristics and operative data in the matched cohort.

	All N = 147	XM+ *n* = 49	XM− n = 98	*p*-value
Age (years)	53.0 (42.0–62.3)	55.0 (41.5–65.5)	52.0 (43.0–62.0)	0.639
Sex				*0.036*
Female	72 (49.0)	30 (61.2)	42 (42.9)	
Male	75 (51.0)	19 (38.8)	56 (57.1)	
BMI (kg/m^2)	25.7 (22.9–29.0)	24.2 (22.6–26.2)	26.8 (23.9–29.8)	*0.001*
COD				0.132
Trauma	37 (25.3)	16 (32.6)	21 (21.4)	
Anoxia	11 (7.5)	1 (2.0)	10 (10.2)	
CVA	96 (65.3)	31 (63.2)	65 (66.3)	
Other	2 (1.3)	0 (0.0)	2 (2.0)	
Missing	1 (0.7)	1 (2.0)	0 (0.0)	
ECD	109 (74.7)	33 (68.8)	76 (77.6)	0.251
Preservation				*0.018*
UW	37 (25.3)	18 (37.5)	19 (19.4)	
HTK	109 (74.7)	30 (62.5)	79 (80.6)	
Missing	1 (0.7)	1 (2.0)	0 (0.0)	
Anhepatic time (min)	54.0 (46.0–63.0)	51.0 (43.3–57.8)	57.0 (47.8–65.3)	*0.007*
WIT (min)	46.0 (39.0–55.0)	41.5 (36.0–51.0)	47.5 (41.0–56.0)	*0.008*
CIT (h)	8.6 (7.5–10.0)	8.8 (7.5–10.7)	8.4 (7.5–9.8)	0.316
ET-DRI	1.64 (1.40–1.88)	1.67 (1.40–1.91)	1.57 (1.39–1.86)	0.659

Values are presented as medians or absolute numbers with IQRs and percentages in parentheses; Italic values show significant p-*values. BMI, body mass index; COD, cause of death; CVA, cerebrovascular accident; ECD, extended criteria donor; ET-DRI, Eurotransplant donor risk index; HTK, histidine-tryptophan-ketoglutarate. IQR, interquartile range; SAB, subarachnoid hemorrhage; UW, University of Wisconsin; WIT, warm ischemia time; XM, crossmatch.*

Biliary Complications

Biliary complications were classified as bile duct leaks, biliary cast syndrome, anastomotic stenosis (AS) and non-anastomotic stenosis (NAS). Ischemic type biliary lesions (ITBL) were defined as NAS with or without biliary cast formation in the absence of hepatic artery stenosis or thrombosis (39–41).

Extended Criteria Donors

ECDs were defined according to the Eurotransplant Manual, Chapter 9: The Donor (42).

Outcomes

The primary outcome was patient and graft survival. Secondary outcomes included incidence and risk factors for rejection episodes as well as incidence, risk factors and type of biliary and arterial complications.

Statistical Analysis

A 1:2 optimal pair matching was performed with the goal of minimizing the absolute pairwise distances in the matched sample (median BAR score values XM+ 8.0 vs. XM− 7.5). For descriptive analysis, categorical variables were summarized with the help of absolute and relative (percentages) frequencies, continuous variables were summarized with means and standard deviation (SD) or medians and interquartile range (IQR) as appropriate. Comparative analysis of clinical outcomes in the XM+ and XM− group was conducted using the Chi-square or Fisher's exact test (if one or more cells had an expected count of less than five) for categorical variables. The Mann–Whitney U test was used to compare continuous, not normally distributed variables. Any variable having a significant univariate test (p-value cut-off point of 0.25 based on the Wald test) was selected as a candidate for the multivariate analysis (43). Uni- and multivariate analyses were performed for the primary and secondary endpoints starting with a univariate analysis of each variable. Kaplan-Meier survival analysis was performed to compare patient and graft survival between XM+ patients and XM− patients using the log-rank test. Multivariate analysis for patient and graft survival endpoints was performed with Cox proportional hazards regression analysis. Logistic regression analysis was used to assess the effects of clinical parameters on secondary endpoints. Statistical analysis was conducted with SPSS (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp).

RESULTS

Recipient Characteristics

Forty-nine patients undergoing LT with a XM+ were matched 1: 2 with XM− patients. Matching was performed based on the BAR score. The indications for LT and recipient demographics are presented in **Table 1**. The median recipient age was 58.0 years in the XM+ group compared to 59.0 years in the XM− group (p = 0.526). Patients in the XM+ group were more likely to be female [XM+ 36.7% (18 of 49) vs. XM− 19.4% (19 of 98), p = 0.022], have a lower BMI [XM+ 23.8 (21.6–27.1) vs. XM− 26.5 (23.9–29.1), p = 0.003] and a higher MELD score [XM+ 16.0 (12.0–21.0) vs. XM− 13.5 (8.8–17.0), p = 0.014] compared to patients in the XM− group. The groups were similar in terms of AB0 blood groups (p = 0.769), CMV mismatching (p = 0.228) and median follow-up (p = 0.304). Patients in the XM+ group had more commonly received a previous LT [XM+ 14.3% (7 of 49) vs. XM− 4.1% (4 of 98), p = 0.042]. The overall use of induction therapy was similar between

TABLE 3 | Clinical outcomes and complications.

	All N = 147	XM+ n = 49	XM− n = 98	*p*-value
EAD	49 (33.3)	12 (24.5)	37 (37.8)	0.138
Rejection	17 (11.6)	7 (14.3)	10 (10.2)	0.466
Acute	12 (8.2)	5 (10.2)	7 (7.1)	0.535
Chronic	5 (3.4)	2 (4.1)	3 (3.1)	1.000
Biliary complications	61 (41.5)	21 (42.9)	40 (40.8)	0.813
Bile duct leaks	22 (15.0)	5 (10.2)	17 (17.3)	0.252
AS	37 (25.2)	14 (28.6)	23 (23.5)	0.502
NAS	15 (10.2)	6 (12.2)	9 (9.2)	0.563
ITBL	15 (10.2)	6 (12.2)	9 (9.2)	0.563
Casts	20 (13.6)	6 (12.2)	14 (14.3)	0.734
Arterial complications	13 (8.8)	2 (4.1)	11 (11.2)	0.220
Stenosis	2 (1.4)	0 (0.0)	2 (2.0)	0.553
Thrombosis	6 (4.1)	1 (2.0)	5 (5.1)	0.664
Dissection	6 (4.1)	1 (2.0)	5 (5.1)	0.664
CMV PCR +	31 (20.7)	14 (28.6)	17 (17.3)	0.116

Values are presented as absolute numbers with percentages in parentheses. AS, anastomotic stricture. XM+, positive crossmatch. XM−, negative crossmatch. CMV, cytomegalovirus; EAD, early allograft dysfunction; ITBL, ischemic type biliary lesion; NAS, non-anastomotic stricture; PCR, polymerase chain reaction; PNF, primary non-function.

TABLE 4 | Factors influencing ITBL - Multivariate analysis.

	OR	95% CI	*p*-value
Rejection	7.773	1.878–32.169	0.005
Donor Age	1.076	1.021–1.135	0.006
CMV PCR +	4.096	1.180–14.219	0.026
CIT	1.315	1.032–1.676	0.027

CI, confidence interval; CIT, cold ischemia time; CMV, cytomegalovirus; ITBL, ischemic type biliary lesions; OR, odds ratio; PCR, polymerase chain reaction.

the groups [XM+ 61.2% (30 of 49) vs. XM− 62.2% (61 of 98), p = 0.904]. However, XM+ patients more often received antibody induction with ATG [XM+ 4.1% (2 of 49) vs. XM− 0% (0 of 98), p = 0.110], although not statistically significant, and alemtuzumab [XM+ 8.2% (4 of 49) vs. XM− 1.0% (1 of 98), p = 0.042]. Yet, in a subgroup analysis, antibody induction had no significant influence on any of the explored outcome parameters including patient and graft survival.

Donor Characteristics

Donor age [XM+ 55.0 (41.5–65.5) vs. XM− 52.0 (43.0–62.0), p = 0.639], and ET-DRI [XM+ 1.67 (1.40–1.91) vs. XM− 1.57 (1.39–1.86), p = 0.659] were similar between groups. The overall ET-DRI was 1.64, suggesting that very good quality grafts were used in this cohort. XM+ recipients more commonly received a graft from a female donor [XM+ female 61.2% (30 of 49) vs. XM− 42.9% (42 of 98), p = 0.036] and donor BMI was significantly lower in the XM+ group compared to the XM− group [XM+ 24.2 (22.6–26.2) vs. XM− 26.8 (23.9–29.8), p = 0.001]. Donor BMI and liver steatosis correlated directly with each other (p = 0.001). Anhepatic time [XM+ 51.0 (43.3–57.8) vs. XM− 57.0 (47.8–65.3), p = 0.007] and warm ischemic time (WIT) [XM+ 41.5 (36.0–51.0) vs. XM− 47.5 (41.0–56.0), p = 0.008] were significantly shorter in the XM+ group. University of Wisconsin (UW) solution was more commonly used as a preservation solution in the XM+ compared to the XM− group [XM+ 37.5% (18 of 49) vs. XM− 19.4% (19 of 98), p = 0.018] (**Table 2**).

Early Allograft Dysfunction

The EAD rate was similar in XM+ and XM− patients [XM+ 24.5% (12 of 49) vs. XM− 37.8% (37 of 98), p = 0.138] (**Table 3**). XM strength or type had no influence on EAD rates. EAD, however, was associated with a positive CMV PCR. Univariate analysis showed recipient BMI, graft steatosis, donor gGT and XM type to be risk factors for the development of EAD. Considering these factors for multivariate analysis, only donor gGT remained as a statistically significant factor for the development of EAD (p = 0.045).

Rejection Episodes

Rejection episodes did not differ significantly between XM+ and XM− recipients [XM+ 14.3% (7 of 49) vs. XM− 10.2% (10 of 98), p = 0.466]. XM strength (p = 0.400) and type (p = 0.282) had no influence on the incidence of rejection episodes. Acute and chronic rejection rates were similar between groups [acute: XM+ 10.2% (5 of 49) vs. XM− 7.1% (7 of 98), p = 0.535; chronic: XM+ 4.1% (2 of 49) vs. XM− 3.1% (3 of 98), p = 1.000] (**Table 3**). Patients with a documented episode of allograft rejection tended to have more biliary complications than those without a rejection episode but that difference proved not to be statistically significant [58.8% (10 of 17) vs. 39.2% (51 of 130), p = 0.123]. Neither a CMV mismatch at LT (p = 0.546) nor a positive CMV PCR (p = 0.758) following LT was associated with the occurrence of rejection episodes.

Biliary Complications

Of 147 patients, 61 (41.5%) developed biliary complications (**Table 3**). There was no significant difference in overall biliary complications between the XM+ and XM− group [XM+ 42.9% (21 of 49) vs. XM− 40.8% (40 of 98), p = 0.813]. Bile duct leaks occurred in 10.2% (XM+ 5 of 49) vs. 17.3% (XM− 17 of 98), (p = 0.252), anastomotic strictures in 28.6% (XM+ 14 of 49) vs. 23.5% (XM− 23 of 98), (p = 0.502), non-anastomotic strictures in 12.2% (XM+ 6 of 49) vs. 9.2% (XM− 9 of 98), (p = 0.563) and biliary casts in 12.2% (XM+ 6 of 49) vs. 14.3% (XM− 14 of 98), (p = 0.734). In all NAS cases the hepatic artery was patent without stenosis or thrombosis and therefore, according to the pre-specified definition, these cases were all recorded as ITBL. Recipients with ITBL received organs from older donors [donor age median 64.0 years (48.0–76.0) vs. 52.0 years (42.0–61.0), p = 0.027] and the duration of the CIT was longer [CIT median 9.8 h (8.3–11.4) vs. 8.5 h (7.5–9.8), p = 0.038]. ET-DRI, a score incorporating donor age and CIT, was also significantly higher for recipients with ITBL [ET-DRI median 2.00 (1.74–2.30) vs. 1.57 (1.38–1.84), p = 0.002]. An episode of active CMV replication was associated with the occurrence of ITBL (p = 0.018). Univariate analysis revealed donor age, CIT, ET-DRI, allograft rejection and active CMV replication as risk factors for the development of ITBL. Considering these parameters for multivariate analysis (except for ET-DRI, as this a composite parameter) the most

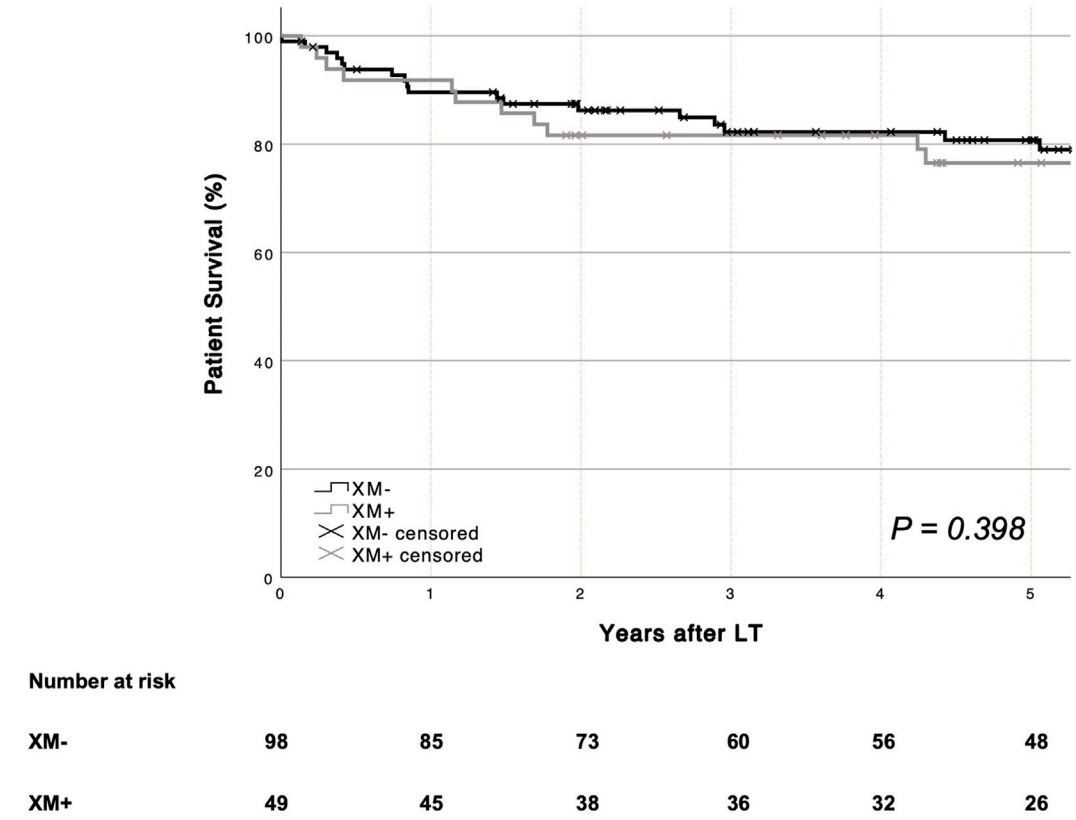


FIGURE 1 | Overall patient survival was similar for recipients with and without a positive XM (log-rank $p = 0.398$). *LT, liver transplantation. XM, crossmatch.*

independent significant factor was allograft rejection [OR 7.773 (95% CI 1.878–31.169), $p = 0.005$] followed by donor age [OR 1.076 (95% CI 1.021–1.135), $p = 0.006$], active CMV replication [OR 4.096 (95% CI 1.180–14.219), $p = 0.026$] and duration of CIT [OR 1.315 (95% CI 1.032–1.676), $p = 0.027$] (**Table 4**). Out of 61 patients with a biliary complication, 50 patients (82.0%) required an endoscopic retrograde cholangiopancreatography, 15 patients (24.6%) underwent a re-operation while 13 patients (21.3%) required a re-transplantation. Patients with an ITBL were more likely to require a re-transplantation [33.3% (5 of 15) vs. 12.1% (16 of 132), $p = 0.042$]. Overall, patients with biliary complications had a significantly higher graft loss rate compared to patients without biliary complications [47.5% (29 of 61) vs. 27.9% (24 of 86), $p = 0.015$]. Neither XM strength nor XM type were associated with the development of biliary complications or ITBL.

Arterial Complications

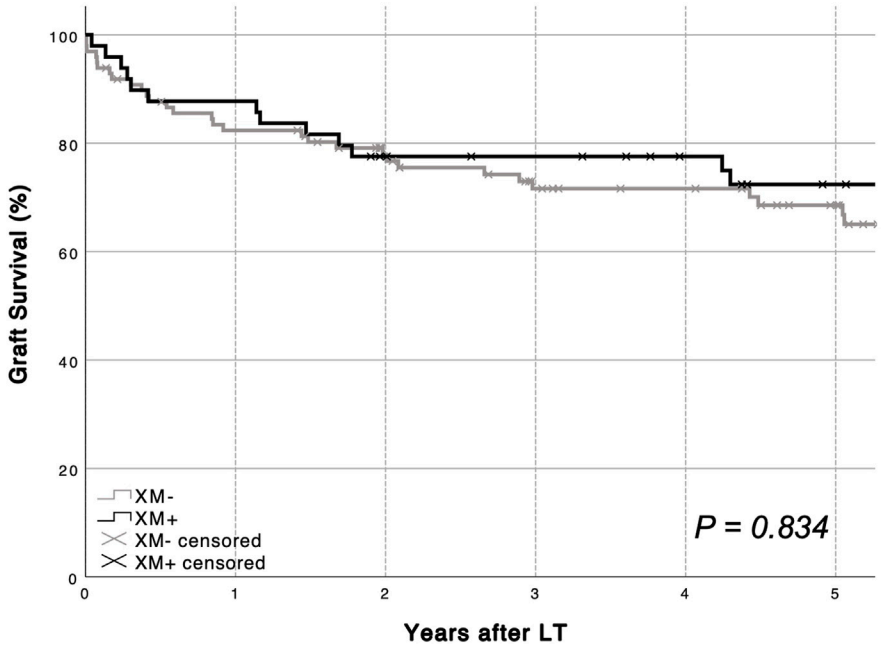
In total, 13 patients (8.8%) developed arterial complications. The incidence of arterial complications did not differ between patients with and those without a positive crossmatch [XM+ 4.1% (2 of 49) vs. XM− 11.2% (11 of 98), $p = 0.220$]. No difference regarding the incidence of hepatic artery thrombosis (HAT) was noted between groups [XM+ 2.0% (1 of 49) vs. XM− 5.1% (5 of 98), $p = 0.664$].

CMV Infection

Overall, 20.7% of recipients developed a CMV infection (CMV PCR+). XM status was not associated with CMV PCR+ [XM+ 28.6% (14 of 49) vs. XM− 17.3% (17 of 98), $p = 0.116$]. Neither was XM type ($p = 0.312$). However, XM strength was associated with a CMV PCR+ [XM strong 50% (9 of 18) vs. XM weak 16.7% (5 of 30), $p = 0.022$]. CMV mismatch status at LT was associated with a subsequent CMV infection (D-/R- 0, D+/R- 4, D-/R+ 9, D+/R+ 17, $p = 0.019$).

Patient and Graft Survival

Mean patient survival was similar in patients with (XM+) and those without (XM−) a positive crossmatch [XM+ 134.7 months (95% CI 107.5–161.9) vs. XM− 117.2 months (95% CI 105.5–128.9), $p = 0.398$]. One- and five-year patient survival rates are shown in **Figure 1**. Mean graft survival was comparable between groups [XM+ 114.4 months (95% CI 90.4–138.5) vs. XM− 97.8 months (95% CI 84.5–111.2), $p = 0.834$]. One- and five-year graft survival rates are shown in **Figure 2**. No single parameter, including XM strength or type, was found to affect patient or graft survival in univariate Cox regression analysis. Re-transplantation rates [XM+ 8.2% (4 of 49) vs. XM− 17.3 (17 of 98), $p = 0.234$] did not differ significantly between groups. One primary non-function (PNF) was recorded in the XM+ group, whereas no PNF occurred in the XM− group.



Number at risk						
XM-	98	78	65	53	49	41
XM+	49	43	36	34	30	25

FIGURE 2 | Overall graft survival according to XM status is shown. No difference in graft survival was seen based on recipient XM status (log-rank $p = 0.834$). *LT, liver transplantation. XM, crossmatch.*

Cause of Death

Overall, 37 out of 147 patients (25.2%) died during the observation period. Of those 37 patients, 13 (35%) died due to post-transplant malignancies, eight (22%) due to septic complications, six (16%) had recurrence of disease, six (16%) died of unknown causes, two (5%) died due to graft vs. host disease, one (3%) due to cardiovascular events and one (3%) due to other, non-specified reasons. Overall, 28 patients (76%) died with a functioning graft [XM+ 63% (10 of 16) vs. XM− 86% (18 of 21), $p = 0.136$].

DISCUSSION

This analysis comparing XM+ and XM− LT recipients over the course of a 16-year period demonstrated that a XM+ has no obvious effects on patient and graft survival and does not appear to influence any of the relevant clinical outcome parameters following LT such as rejection episodes, biliary or arterial complications. Furthermore, neither XM type nor strength had any influence on post-transplant outcomes.

Known risk factors for XM+ are female recipient sex, previous LT as well as immunologic indications for LT such as autoimmune hepatitis (AIH) (6, 14, 24). In contrast to an analysis by Ruiz et al. (6), patients with AIH were not at risk for a XM+ in our study. Considering that only four patients in our cohort underwent LT for AIH this finding needs to be viewed cautiously. However, similar to results reported by Ruiz et al. and others (8, 13, 24, 44, 45), we found a higher number of female recipients and re-transplantations in the XM+ group; attributable to previous pregnancies, blood transfusions during or in the aftermath of the primary transplant operation and sensitization caused by the initial graft itself. We also found the recipient BMI to be lower in XM+ recipients, which is in accordance with the finding that the XM+ group encompassed more female recipients.

A high rate of antibody induction (61.9%) was observed in the study cohort. This can be explained by the fact that our center took part in two IL2 antibody induction studies (PROTECT (33) and DIAMOND (34)) during the study period. While the overall antibody induction rate did not differ between XM+ and XM− negative patients, XM+ patients were more likely to receive alemtuzumab (although the absolute number was small). Interestingly, XM strength did not correlate with the use of antibody induction. However, XM strength did correlate with subsequent PCR+ CMV infections.

Overall, the number of rejection episodes was similar between our XM+ and XM− recipients. Previous studies have reported

higher rejection rates in XM+ recipients (13, 14, 17, 46, 47). However, almost all of these studies used different definitions of what constitutes a positive XM. Charco et al. (13) and Bathgate et al. (14) defined a XM+ as cytolysis greater than 20%, while Takaya et al. (44) defined a XM+ as cytolysis of 50% or more. Furthermore, IS regimens differed between study centers (14, 17, 46, 47), and most of these studies were conducted decades ago when IS regimens were less intensive with lower CsA and Tac target levels. While originally reporting a higher complication rate in recipients with a XM+ Takaya et al. showed, in a follow-up study, that comparable outcomes can be achieved with a more intense IS regimen (48). The more intense IS regimen used in the follow-up study constitutes the standard IS regimen today at most transplant centers including ours (45). This might explain why, in more recent studies with more intense IS regimens, a XM+ had no influence on the occurrence of rejection episodes, patient and graft survival as well as overall complications (24, 25, 45, 49), which is in accordance with our observations. To the contrary: in a recent study by Ünlü et al. (50) LT recipients perceived to be at an increased immunologic risk received more intense IS leading to higher infectious complications without providing any graft or patient survival benefit. Considering the liver's privileged immunologic status, a more intense IS for XM+ recipients might be unnecessary and even harmful. Accordingly, when analyzing their 20-year experience with XM+ LT recipients Ruiz et al. (6) found no association between a XM+ and graft complications as well as patient and graft survival.

Compared to previous studies (44, 51, 52), we were unable to find any association between a XM+, including XM strength and type, and the occurrence of biliary complications. Unsurprisingly, patients with biliary complications had a higher graft loss rate and patients with ITBL required re-transplantation more often. ITBL remain one of the most worrisome complications following LT. Immunologic factors have been implicated in the pathogenesis of ITBL in addition to ischemia reperfusion injury and bile salt toxicity (39). While a XM+ had no influence on ITBL development in our study, allograft rejection as well as a positive CMV PCR were associated with an increased risk for the development of ITBL in uni- and multivariate analysis; as were older donor age and prolonged CIT, both well known risk factors for the development of ITBL. Furthermore, XM strength was positively associated with subsequent PCR+ CMV infections. Previous clinical studies have shown acute rejection and active CMV replication to be immunologic risk factor for the development of biliary complications in the context of LT (53-56). Interestingly, a PCR+ CMV infection in immunocompromised HIV positive patients has been known to cause destruction in the biliary tree for a long time, a condition termed AIDS cholangiopathy (57). In a study examining the effects of a CMV infection on rat liver grafts Martelius et al. provided experimental data supporting the role of CMV in the pathogenesis of bile duct injury (58). CMV infection leads to upregulation of MHC antigens and expression of vascular adhesion molecules such as VCAM-1 and ICAM-1 through secretion of pro-inflammatory cytokines (58, 59). Similarly, allograft rejection is thought to induce an inflammatory state at the local level leading to endothelial injury (60, 61). Since viability of the biliary tree depends on the oxygen rich arterial blood supply, an immune-mediated micro-vasculopathy may result in ischemic type injury to the bile ducts, providing a possible pathophysiological explanation for our findings (52, 62, 63).

Strengths and Limitations

The study compared XM+ with XM− LT using a 1:2 match-pair design. Matching was performed based on the BAR score which has shown to correlate best with post-transplant outcomes compared to other published risk scores (30, 31). Strengths of our study include the prospectively maintained LT database at our center, the match-pair analysis and the relatively long follow-up. Limitations of the present study include the retrospective design and a possible bias concerning the selection of participants beyond the data displayed in the demographics. Despite performing a match-pair analysis in order to guarantee a homogenous comparison group, differences in donor and recipient characteristics did exist between the XM+ and XM− group. The donor BMI was significantly lower, and anhepatic as well as WIT were significantly shorter in the XM+ group compared to the XM− group. This may introduce a bias as a lower donor BMI and shorter ischemia times could imply favorable outcomes. Furthermore, the recipients' MELD score was found to be higher in the XM+ group. However, the BAR score which, among other factors, includes the MELD score and correlates with relevant outcome parameters following LT was used for match-pair analysis to mitigate potential biases. None of these factors had any significant influence on patient or graft survival in our cohort when performing univariate Cox proportional hazards regression analysis (**Supplementary Tables S1, S2**) as well as when adjusting for these differences in baseline characteristics in a multivariate Cox regression model (**Supplementary Tables S3, S4**). Also, University of Wisconsin (UW) solution was more commonly used than Histidine-Tryptophan-Ketoglutarate (HTK) solution as a preservation solution in the XM+ group. UW used to be the gold standard for static cold storage perfusion of liver grafts but preservation with HTK is reported to be clinically equivalent (64, 65). Concerns regarding the higher viscosity of UW leading to an incomplete flush of the peribiliary glands and an increase in ITBL have been voiced. However, these concerns have not materialized (66). Moreover, the type of preservation solution had no significant influence on the development of ITBL in our recipients in univariate binary logistic regression analysis (**Supplementary Table S5**).

CONCLUSION

In the present era of LT, a XM+ has no effects on graft and patient survival as well as postoperative complications. Therefore, our center policy will not change, and we will continue to transplant patients without waiting for XM testing results

despite the logistical possibilities offered by the advent of normothermic machine perfusion. A PCR+ CMV infection was more likely to occur in recipients with a strongly positive XM. Together with allograft rejection, donor age and CIT, a PCR+ CMV infection was among the strongest independent predictor for the development of ITBL. Patients with ITBL had higher re-transplantation rates than patients without ITBL.

DATA AVAILABILITY STATEMENT

Data is available upon reasonable request form the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethikkommission der Medizinischen Universität Innsbruck. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

FJK: Study design, data acquisition, data analysis, interpretation of the data, drafting and revising the manuscript; MF: Data acquisition, interpretation of the data, drafting and revising the manuscript; FM: Interpretation of the data, drafting and revising the manuscript; AB: Data acquisition, interpretation of the data, drafting and revising the manuscript; AV: Data acquisition, interpretation of the data, drafting and revising the manuscript; BC: Interpretation of the data, drafting and revising the manuscript; TR: Interpretation of the data, drafting and revising the manuscript; MM: Interpretation of the data, drafting and revising the manuscript; CM: Interpretation of the data, drafting and revising the manuscript; MR: Study design, data analysis, interpretation of the data, drafting and revising the manuscript; HU: Study design, data analysis, interpretation of the data, drafting and revising the manuscript; DÖ: Interpretation of the data, drafting and revising the manuscript; RO: Interpretation of the data, drafting and revising the manuscript; SS: interpretation of the data, drafting and revising the manuscript; AW: Study design, data acquisition, data analysis, interpretation of the data, drafting and revising the manuscript.

CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11062/full#supplementary-material

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GLOSSARY

AIH autoimmune hepatitis
AS anastomotic stenosis
AST aspartate transferase
ATG anti-thymocyte globulin
BAR score balance of risk score
BMI body mass index
CDC cytotoxic dependent cytotoxicity
CIT cold ischemia time
CMV cytomegalovirus
COD cause of death
CsA cyclosporine A
CVA cerebrovascular accident
DCD donation after cardiocirculatory death
DSA donor specific antibody
DTT dithiothreitol
EAD early allograft dysfunction
ECD extended criteria donor
ET-DRI Eurotransplant donor risk index
gGT gamma-glutamyltransferase
HAT hepatic artery thrombosis
HTK histidine-tryptophan-ketoglutarate
IL2 interleukin 2
IS immunosuppressive
ITBL ischemic type biliary lesion
INR international normalized ratio
IQR interquartile range
LT liver transplantation
MELD model of end-stage liver disease
MMF mycophenolate mofetil
MPA mycophenolic acid
NAFLD non-alcoholic fatty liver disease
NAS non-anastomotic stenosis
PBC primary biliary cirrhosis
PCR polymerase chain reaction
PSC primary sclerosing cholangitis
Re-Tx re-transplantation
SAB subarachnoid hemorrhage
SD standard deviation
Tac tacrolimus
UW University of Wisconsin
WIT warm ischemia time
XM crossmatch
XM+ positive crossmatch
XM− negative crossmatch

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ORIGINAL RESEARCH
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A Pilot Single-Blinded, Randomized, Controlled Trial Comparing BNT162b2 vs. JNJ-78436735 Vaccine as the Third Dose After Two Doses of BNT162b2 Vaccine in Solid Organ Transplant Recipients

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Solid Organ Transplant (SOT) recipients are at significant higher risk for COVID-19 and due to immunosuppressive medication, the immunogenicity after vaccination is suboptimal. In the previous studies, booster method showed significant benefit in this population. In the current study, we compared using a mix-and-match method vs. same vaccine as a third dose in SOT recipients. This was a patient-blinded, single center, randomized controlled trial comparing BNT162b2 vs. JNJ-78436735 vaccine as the third dose after two doses of BNT162b2 vaccine. We included adult SOT recipients with functional graft who had received two doses of BNT162b2 vaccine. Participants were randomly assigned to receive either BNT162b2 or JNJ-78436735 in one-to-one ratio. Primary outcome was SARS-CoV-2 IgG positivity at 1 month after the third dose. Sixty SOT recipients, including 36 kidney, 12 liver, 2 lung, 3 heart, and 5 combined transplants, were enrolled, and 57 recipients were analyzed per protocol. There were no statistically significant differences between the two vaccine protocols for IgG positivity (83.3% vs. 85.2% for BNT162b2 and JNJ-78436735, respectively, p = 0.85, Odds Ratio 0.95, 95% Confidence Interval 0.23–4.00). Comparison of the geometric mean titer demonstrated a higher trend with BNT162b2 (p = 0.09). In this pilot randomized controlled trial comparing mix and match

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Abbreviations: COVID-19, Coronavirus disease 2019; GMT, Geometric mean titer; RBD, Receptor binding domain; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SOT, Solid Organ Transplant.

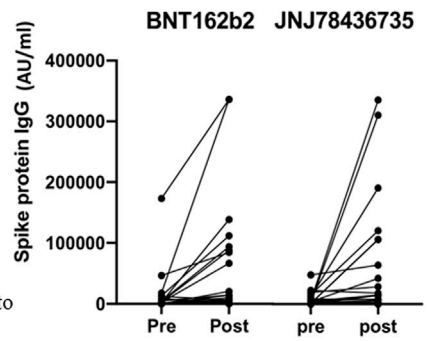
method vs. uniform vaccination in SOT recipients, both vaccines were safely used. Since this was a small sample sized study, there was no statistically significant difference in immunogenicity; though, the mix and match method showed relatively lower geometric mean titer, as compared to uniform vaccine. Further studies need to be conducted to determine duration of this immunogenicity.

Clinical Trial Registration: https://clinicaltrials.gov/ct2/show/NCT05047640?term=20210641&draw=2&rank=1, identifier 20210641.

Keywords: COVID-19, solid organ transplant, vaccine, booster, randomized controlled trial

A Pilot Single-Blinded, Randomized, controlled trial comparing BNT162b2 vs JNJ-78436735 vaccine as the third dose after two doses of BNT162b2 vaccine in Solid Organ Transplant Recipients

- Patient-blinded, single center, randomized controlled trial comparing BNT162b2 vs JNJ-78436735 vaccine as the third dose after two doses of BNT162b2 vaccine
- Adult SOT recipients with functional graft
- 60 (36 kidney, 12 liver, 2 lung, 3 heart, and 5 combined) transplants reicipients were enrolled.
- IgG positivity; BNT162b2(83.3%) vs JNJ-78436735 (85.2%) (p=0.85).
- Geometric mean titer demonstrated a higher trend with BNT162b2 (p=0.09).
- The mix and match method showed relatively lower geometric mean titer, as compared to uniform vaccine.



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GRAPHICAL ABSTRACT |

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), known as the etiology behind the coronavirus disease 2019 (COVID-19) worldwide pandemic, has resulted in significant mortality rates worldwide. Solid organ transplant (SOT) recipients, not unexpectantly, are more likely to experience poor outcomes after SARS-CoV-2 infection including higher hospital admission rates and increase mortality (1). In this context, there is an urgent need to provide robust protection in this vulnerable population in addition to standard preventive strategies including wearing mask and hand hygiene.

Other than the natural immunological response against infections, vaccination and monoclonal antibody therapy are the other pathways available to augment the immune systems response to this infection. The United States Food and Drug Administration provided emergency use authorization for ticagevimab/cilgavimab as primary prophylaxis in high-risk patients such as immunocompromised recipients including SOT recipients (2). However, as different variants of concern including Omicron have emerged, the efficacy of some of the monoclonal antibody product has been challenged (3, 4). Thus, the importance of vaccination in this population continues to be a foundation of an effective preventive strategy.

Although the high efficacy of COVID-19 vaccines is well documented in the general population (5), the immunogenicity and efficacy of SARS-CoV-2 vaccination is suboptimal in SOT recipients, something that has been seen in with other vaccines (6). There have been several attempts to improve vaccine efficacy and/or immunogenicity in this vulnerable population, especially with boosted doses. A randomized controlled trial comparing placebo vs. other mRNA vaccine as a third dose study demonstrated significant benefit

(7). Furthermore, while this study was being conducted, the addition of a fourth dose has shown to have been beneficial (8), leading to the recommendation of a second booster in the immunosuppressed population. Even with the boosted dose strategy, reports of breakthrough infection in SOT recipients with COVID-19 exist (9).

We hypothesized that the mix and match method, i.e., using the different type of vaccine as a booster, would provide higher immunogenicity in SOT recipients. However, there are two studies comparing the mix and match method vs. uniform method in SOT recipients: one multicenter prospective, non-randomized, study and one randomized controlled trial (10, 11). The former vaccine series of Schwaighofer et.al. cohort differed from our study by utilizing various vaccines such as mRNA-1273 and BNT162b2 prior to administration of the third dose of AD26COVS1(10). Chiang et.al. conducted a prospective observation study, which cannot avoid selection bias (11). To study this concept more carefully, we conducted a single center randomized controlled trial comparing BNT162b2 (mRNA vaccine) vs. JNJ-78436735 (viral vector) as a third dose after completion of two doses of BNT162b2 vaccine in SOT recipients.

PATIENTS AND METHODS

Patient Selection and Study Design

This was a patient-blinded, superiority, randomized controlled trial, conducted at the Miami Transplant Institute, Jackson Health System, Miami, Florida, USA. The Miami Transplant Institute is one of the biggest SOT centers in North America, providing comprehensive care to all SOT recipients.

We included SOT recipients with a functional graft, whose age was 18 years and older at the time of enrollment. Inclusion for enrollment consisted of recipients with a minimum of 1 month post-transplant and having received two doses of BNT162b2 vaccine. Of note, the prior vaccines could have been administered any time pre or post transplantation. The third dose should have been given at least 28 days from the second dose of BNT162b2 vaccination and at least 1-month post-transplant. Exclusion criteria included any significant side effects due to previous SARS-CoV-2 vaccination, people unable to consent, receipt of more than or equal to three doses of SARS-CoV-2 vaccination, pregnancy and patients who previously received monoclonal antibody treatment that are specifically directed against the spike protein for SARS-CoV-2 such as Bamlanivimab plus Etesevimab, Casirivimab plus Imdevimab, and Sotrovimab at any time prior to the trial. Of note, at the time of enrollment, Ticagevimab/Cilgavimab was not available in USA.

This study was approved by local research ethics board and was given NCT05047640.

Blinding, Unblinding, Randomization and Follow up

After obtaining written informed consent, adult SOT recipients were randomized in one to one ratio to receive either BNT162b2 vs. JNJ-78436735. BNT16b2 uses nucleoside-modified mRNA encoding the viral spike glycoprotein for SARS-CoV-2 as an ingredient. On the other hand, JNJ-78436735 uses recombinant, replication-incompetent Adenovirus 26 vector, encoding a stabilized variant of SARS-CoV-2 spike protein, as an ingredient. A randomization schedule was created electronically and simple randomization was performed. The participants' blood specimens were collected to analyze anti-spike protein SARS-CoV-2 IgG. The patients were contacted by phone at day 3 and 7 post vaccination to monitor for adverse events. Follow-up blood test was planned between 21 and 35 days after the third dose of the vaccine to measure anti-spike protein SARS-CoV-2 IgG. We measured IgG titer to the SARS-CoV-2 spike protein receptor binding domain using enzyme-linked immunosorbent assay as described elsewhere (12). Briefly, the SARS-CoV-2 enzyme-linked immunosorbent assays were performed following a 2-step enzyme-linked immunosorbent assay protocol and results were interpreted in accordance with the manufacturer's cutoff calculations. Anti-spike protein SARS-CoV-2 IgG was reported as receptor binding domain (RBD) (13). At that time, we also questioned the adverse events. The vaccine given at the time of enrollment was unblinded at the time of follow up blood test to the participant. However, if an emergency ensued, the vaccine could be unblinded immediately for the patient and caring team.

Of note, this study was not observer blinded. However, the laboratory members were not notified of the randomization results.

Statistical Analysis and Sample Size Calculation

The primary outcome of the study was anti-spike protein SARS-CoV-2 IgG positivity after 28 (21–35) days of the booster dose with either vaccine. Secondary outcomes included side effect, graft rejection, and SARS-CoV-2 infection. The follow-up period of the current study was 28 (21–35) days, up to the follow-up blood collection. We set alfa of 0.05 and beta of 0.2. For pre-specified outcome analysis, based on our hypothesis, we compared IgG positivity between two vaccines. As an ancillary analysis, we tried to identify the risk factors to develop or not to develop IgG positivity in this cohort. We assumed the anti-spike protein SARS-CoV-2 IgG positivity in JNJ-78436735 as 80% and BNT162b2 as 60% (7). The number of subjects required for this analysis was 93 per each arm, or a total of 186. We assumed 5%–10% of patients would be lost to follow-up. Therefore, we planned to enroll 200 patients in total, to achieve statistical significance per protocol sample.

Demographics were analyzed using descriptive statistics. Pre- and post-vaccination anti-spike protein SARS-CoV-2 IgG titers were compared using Wilcoxon rank-sum test. Univariate analyses were performed to determine significant factors affecting seroconversion using chi-squared or Fisher's exact test for categorical variables and Mann–Whitney U for continuous variables. For multivariate analysis, we planned to construct a model using variables whose p-value were less than 0.2 on univariate analysis. Multivariate analysis was performed using logistic regression with stepwise backward elimination. Statistical significance was defined as a p-value of less than 0.05. Statistical analysis was performed using SPSS version 26.0 (Chicago) and GraphPad Prism version 8.0 (La Jolla, CA, USA).

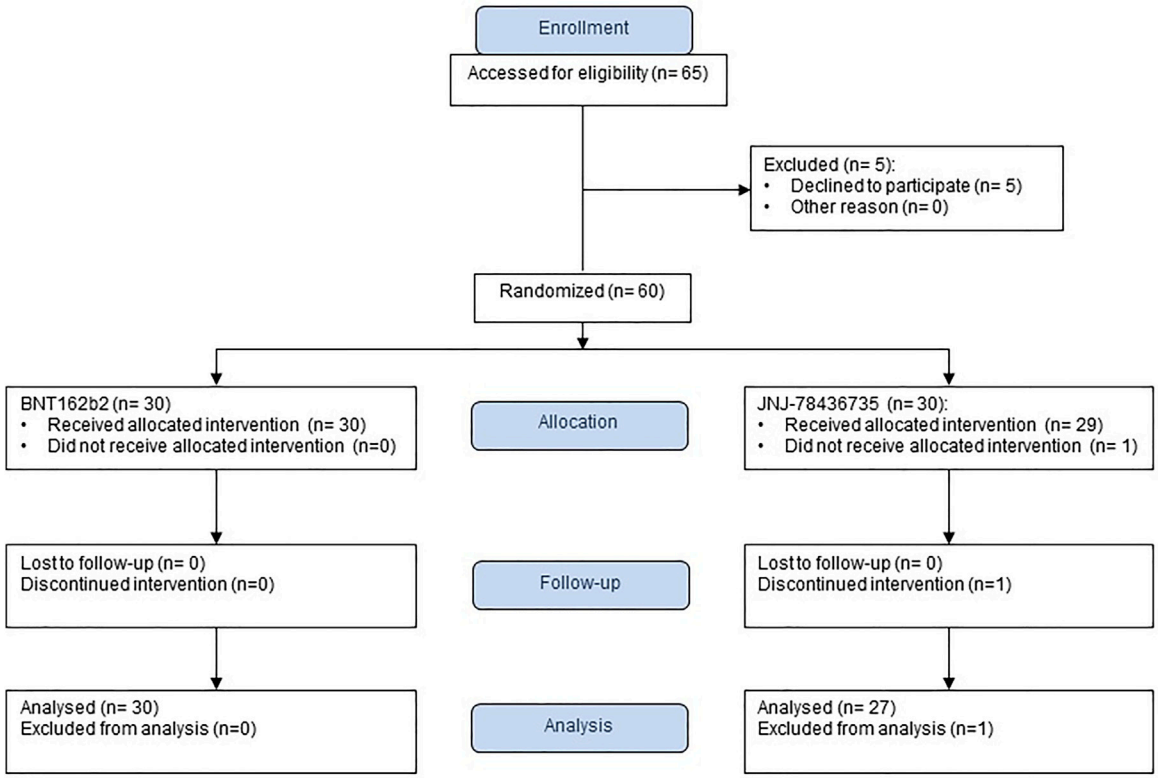

FIGURE 1 | Study flow chart.

TABLE 1 | Patient characteristics at enrollment.

Characteristic	All (n = 58)	BNT162b2 (n = 30)	JNJ-78436735 (n = 28)
Age, median (range)	57.5 (26–79)	59.5 (27–76)	54.5 (26–79)
Male sex (%)	38 (65.5)	21 (70)	17 (60.7)
Time from transplantation to vaccination (months), median (interquartile range)	11.5 (3–27)	10.7 (4.7–38.4)	12.5 (2.8–25.7)
Within 1 year of transplantation (%)	30 (51.7)	16 (53.3)	14 (50.0)
History of documented COVID-19(%)	7 (12.1)	4 (13.3)	3 (10.7)
Receipt of Anti-thymocyte globulin[a] (%)	17 (29.3)	8 (26.6)	9 (32.1)
Recent Rejection (%)	14 (24.1)	7 (23.3)	7 (25.0)
Type of transplant (%)			
Kidney	36 (62.0)	19 (63.3)	17 (60.7)
Liver	12 (20.7)	3 (10)	9 (32.1)
Lung	2 (3.4)	2 (6.7)	0 (0)
Heart	3 (5.2)	3 (10.0)	0 (0)
Combined	5 (8.6)	3 (10.0)	2 (7.1)
Immunosuppression			
Prednisone (%)	25 (43.1)	14 (46.7)	11 (39.2)
Prednisone dose, mg/day, median (range)	5 (2.5–80)	5 (2.5–80)	7.5 (4–40)
Tacrolimus (%)	52 (89.7)	26 (86.7)	26 (92.9)
Mycophenolate mofetil/mycophenolate sodium (%)	46 (79.3)	25 (83.3)	21 (75.0)

[a]*Within 6 months prior to the third dose of vaccination.*

RESULTS

Patient Population

From September to December 2021, we enrolled 60 SOT recipients and 59 of them received a study vaccine as one patient withdrew after obtaining the consent, prior to vaccination (30 BNT162b2, 29 JNJ-78436735) (**Figure 1**). We could not enroll the number of recipients because the majority of them had already received the third dose. The termination was not due to the interim analysis. After enrollment, one patient

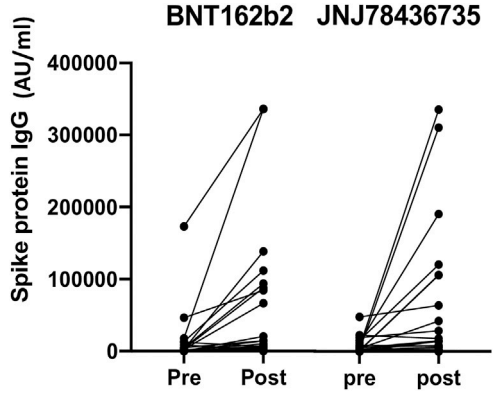

FIGURE 2 | Quantitative anti-spike protein IgG titer pre and post third dose of either BNT162b2 or JNJ78436735. Each dot represents each participant's IgG titer at pre or 1 month post third dose of vaccination.

TABLE 2 | Adverse Events after vaccination.

	BNT162b2 (n = 30)	JNJ- 78436735 (n = 28)
Local		
Arm Pain	8 (26.7)	6 (21.4)
Erythema	1 (3.3)	0 (0)
Any local reaction	9 (30.0)	6 (21.4)
Systemic		
Headache	3 (10.0)	2 (7.1)
Fatigue	5 (16.7)	2 (7.1)
Muscle aches/Joint pain	0 (0)	0 (0)
Gastrointestinal symptoms	0 (0)	0 (0)
Fever/Chills	1 (3)	1 (3.5)
Thrombosis	0 (0)	0 (0)
Any systemic reaction	7 (23)	5 (17)

declared that he had received monoclonal antibody, (resulting in the withdrawal of that participant (30 BNT162b2, 28 JNJ-78436735). Finally, we enrolled 36 kidney, 12 liver, 2 lung, 3 heart, and 5 combined. Baseline characteristics of 58 enrolled patients were shown in **Table 1**. The overall median time from transplant and the second dose of BNT162b2 to study vaccination was 10.7 [IQR] (4.7–38.4) and 7.8 (IQR 6.6–8.3) months, respectively. Of note, 20/58 (34.5%) of the recipients received the prior two doses prior to transplant. Only ethnicity was different between both groups ($p = 0.02$). Other demographic characteristics including type of transplant, presence of recent rejection, and immunosuppression at the time of vaccination were well balanced in the two groups.

Vaccine Immunogenicity

Of the 58 patients who were successfully vaccinated, one recipient that had received JNJ-78436735 was not included for the immunogenicity analysis due to acquiring SARS-CoV-2 infection prior to the second blood draw (**Figure 1**). The remainder of the recipients completed pre- and post-vaccination sera collection. Therefore, 57 patients were available for the immunogenicity analysis (30 BNT162b2, 27 JNJ-78436735) (**Figure 1**).

Post vaccination immunogenicity rates, which is the primary outcome, for BNT162b2 and JNJ-78436735 were 83.3% and 85.2% respectively ($p = 0.85$, Odds Ratio 0.95, 95% Confidence Interval 0.23–4.00).

The baseline anti-spike protein SARS-CoV-2 IgG positive rate was 36.9% among all cohort and there was no statistically significant difference between BNT162b2 and JNJ-78436735. Median quantitative SARS-CoV-2 IgG titers at the time of enrollment for BNT162b2 and JNJ-78436735 were 719 (range 11–173057) AU/mL and 2385 (range 101–48296) AU/mL, respectively.

Quantitative anti-spike protein SARS-CoV-2 IgG increased significantly post third dose vaccination compared to baseline ($p < 0.001$) in entire cohort (**Figure 2**).

Median geometric mean titer (GMT), analyzed as the absolute fold-increase of titer from pre- to post- third dose of the vaccination, for BNT162b2 and JNJ-78436735 was 9.51 (range 0.18–284.54) and 1.64 (range 0.24–170.2), respectively and there was a trend towards BNT162b2 showing higher response ($p = 0.09$).

When proceeding to analyze factors affecting vaccine IgG positivity after vaccination, we found in the univariate analysis that none of the variables could be identified as risk factors since all p values were greater than 0.2. Of note, we have analyzed age, gender, race, transplanted organ, duration between transplant and vaccination, recent rejection, usage of immunosuppressive medication including prednisone, tacrolimus, mycophenolate and anti-thymocyte globulin. Hence, we did not conduct multivariate analysis.

Vaccine Adverse Events

Vaccine-related adverse events were assessed in the 58 patients who received study vaccine (**Figure 1**). During follow-up, there were no statistically significant differences for local and systemic side effects in both groups (**Table 2**). The most common adverse event reported was localized injection site pain (14/58, 24.1%), which were seen within 7days after the vaccination. None of the 58 patients were diagnosed with new onset of rejection during the follow up. Mild SARS-CoV-2 infection was diagnosed in one patient at 31 days after JNJ-78436735 vaccination.

DISCUSSION

This was a randomized controlled trial comparing BNT162b2 vs. JNJ-78436735 as a third dose after completion of two doses of BNT162b2 in SOT recipients. Similar to previous randomized controlled trial (10) and non-randomized large observational study (11), these two vaccines were safely used in this population with similar immunogenicity as shown. Due to small sample size, not only the primary outcomes but also the secondary analysis, including risk factor analysis, may be inconclusive. However, although not statistically significant, we observed slightly higher immunogenicity following vaccination with mRNA vaccine.

At the time of our trial, there were two studies assessing the immunogenicity of mixing method in SOT recipients. One single center randomized controlled trial, conducted by Schwaighofer et al. (10), compared mRNA vaccine (either BNT162b2 or mRNA-1273) vs. Ad26COVS1 in 197 kidney transplant recipients with negative responses after two doses of mRNA vaccine. The positive antibody responses against SARS-CoV-2 spike protein after mRNA vaccine vs. Ad26COVS1 were 35% and 42%, respectively, not statistically significant. The other trial by Chiang et. al. concluded that mixing method did provide higher rate of seroconversion at 3- and 6-months post third dose vaccination in contrast to our study where GMT was higher in uniform method group. As a hypothesis, there might be an additive synergistic effect accompanying the administration of the same vaccine in contrast to the results seen using the mixing method. Of note, currently, JNJ-78436735 COVID-19 vaccine is authorized for adults only in certain limited situations due to risk of thrombosis with thrombocytopenia syndrome.

There are several limitations in this current study. Sample size was never achieved due to the challenges of persuading patients to possibly receive different vaccines based on randomization. Of note, the majority of our recipients had received the third dose at the time of enrollment. In addition, the prior vaccines could have been administered pre- or post transplantation; 34% of participants were vaccinated before transplant. Thus, we cannot conclude whether results are comparable between those vaccinated pre- and post-transplantation. In this study, we are limited to the use of surrogate marker, not the incidence itself. We included not only seronegative but also seropositive recipient at the time of the third dose vaccination in order to most accurately reflect our current population. We tried to address this limitation by calculating GMT. Lastly, our follow up consisted of 1 month duration making challenging to capture late occurring adverse events, along with concluding that IgG positivity 30 days post third vaccine dose properly reflect long term immunogenicity in transplant recipients. This warrants longer follow up for future studies.

In conclusion, we conducted a patient-blinded, randomized controlled trial comparing BNT162b2 vs. JNJ-78436735 vaccine for the third dose after two doses of BNT162b2 COVID-19 vaccines in SOT recipients. We found similar immunogenicity using both vaccination strategies. Even though the primary outcome was not achieved due to small sample size being underpowered, larger studies will need to be performed to draw conclusion. Further investigation is needed to understand the optimal method of COVID-19 vaccination in this vulnerable group of patients. Also, further studies need to be conducted to determine duration of this immunogenicity.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Human Subject Research Office at the University of Miami. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YN and GG designed the study. YN, EM, AM, and GG conceived the study. YN and SP analyzed the data. All the authors participated in the generation of the data and interpretation of results. YN and GG wrote the first version of the article. All the authors approved the final version of the manuscript.

CONFLICT OF INTEREST

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

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ORIGINAL RESEARCH
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Tremor, Daily Functioning, and Health-Related Quality of Life in Solid Organ Transplant Recipients

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Solid organ transplant recipients (SOTR) frequently report tremor. Data concerning tremor-related impairment and its potential impact on health-related quality of life (HRQoL) are lacking. This cross-sectional study assesses impact of tremor on activities of daily living and HRQoL using validated questionnaires among SOTR enrolled in the TransplantLines Biobank and Cohort Study. We included 689 SOTR (38.5% female, mean [±SD] age 58 [±14] years) at median [interquartile range] 3 [1–9] years after transplantation, of which 287 (41.7%) reported mild or severe tremor. In multinomial logistic regression analyses, whole blood tacrolimus trough concentration was an independent determinant of mild tremor (OR per µg/L increase: 1.11, 95% CI: 1.02 to 1.21, $p = 0.019$). Furthermore, in linear regression analyses, severe tremor was strongly and independently associated with lower physical and mental HRQoL ($\beta = -16.10$, 95% CI: −22.23 to −9.98, $p < 0.001$ and $\beta = -12.68$, 95% CI: −18.23 to −7.14, $p < 0.001$ resp.). SOTR frequently report tremor-related impairment of activities of daily living. Tacrolimus trough concentrations appeared as a main determinant of tremor among SOTR. The strong and independent association of tremor-related impairment with lower HRQoL warrants further studies into the effects of tacrolimus on tremor.

Clinical Trial Registration: ClinicalTrials.gov, Identifier NCT03272841.

Keywords: tremor, solid organ transplant recipient, calcineurin inhibitors, health-related quality of life, ADL impairment

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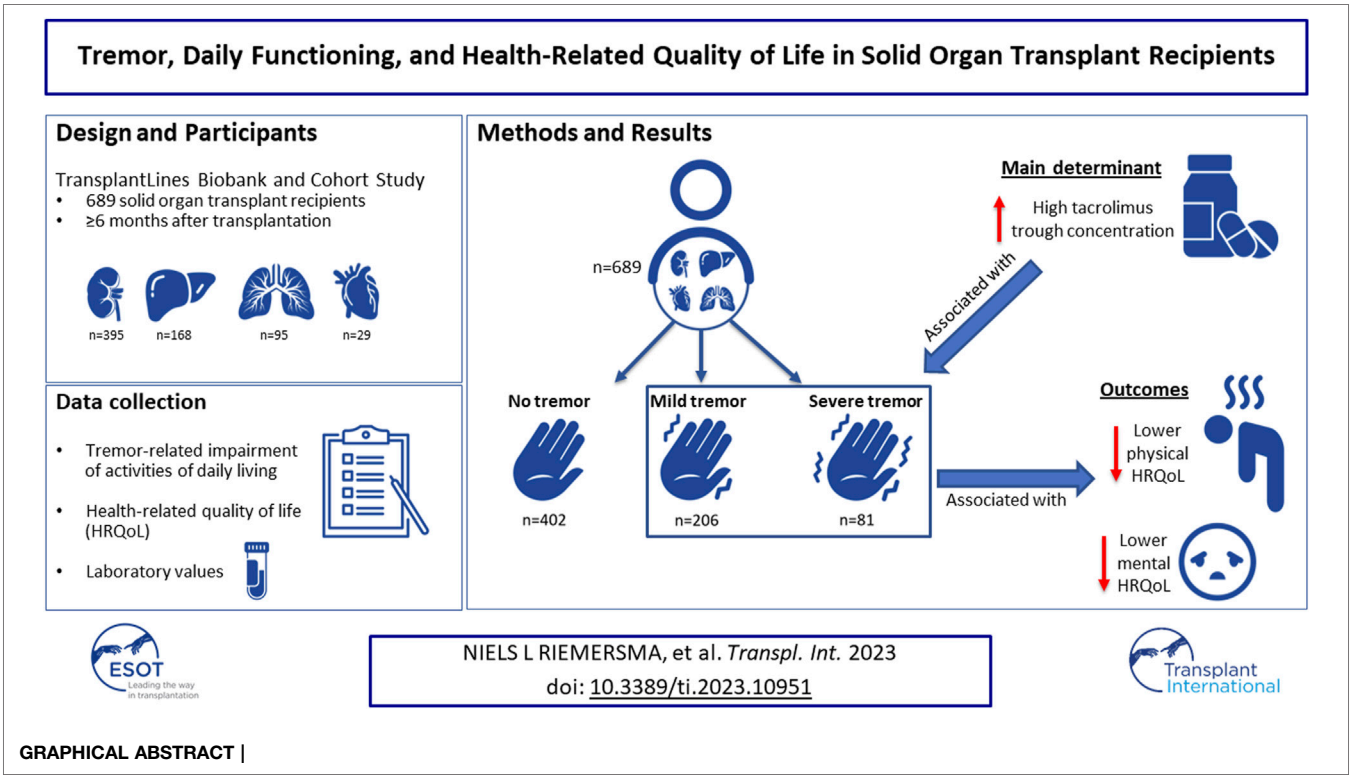
Abbreviations: ADL, Activities of daily living; CNI, Calcineurin inhibitor; FTM TRS-C, Fahn-Tolosa-Marin tremor rating scale C; HRQoL, Health-related quality of life; MCS, Mental component summary; PCS, Physical component summary; SOTR, Solid organ transplant recipients.



GRAPHICAL ABSTRACT |

INTRODUCTION

Solid organ transplantation has evolved from a scientific novelty to the preferred treatment for end-stage organ failure. For example, kidney, liver, lung, and heart transplantations strongly improve long-term survival in otherwise untreatable diseases (1–7). Unfortunately, even after successful transplantation, solid organ transplant recipients (SOTR) continue to have reduced health-related quality of life (HRQoL) compared to the general population (8–10). A suggested cause for this limited HRQoL is the need to adhere to a strict immunosuppressive maintenance regime, generally including calcineurin inhibitors (CNIs) (11). These CNIs, including cyclosporine and tacrolimus, are essential to prevent graft rejection, and are a cornerstone in current post-transplantation care (12). However, CNI-use is associated with multiple side effects, including nephrotoxicity and neurotoxicity (13, 14).

One of the most frequently reported side effects of CNIs is the development of tremor: rhythmic, sinusoidal oscillations of the limbs, head, or trunk (15). Medication-induced tremor may limit SOTR in performing activities of daily living (ADL), such as eating, writing, and personal hygiene, much like tremor interferes with ADL in other populations (16).

CNI-induced tremor generally occurs soon after initiation of CNI maintenance therapy (17), and occurs in up to half of SOTR using CNIs (18). The occurrence of CNI-induced tremor is reportedly dose-dependent, yet some patients experience tremor even at low dosages (18, 19). Currently, we lack the knowledge to what extent tremor leads to impairments in ADL, and how this may affect HRQoL among SOTR. In-depth investigation of the impact of tremor on ADL and HRQoL is therefore warranted and may add valuable information to previous studies that abstained from using tremor-related ADL impairment as primary outcome measurement (18).

We therefore aimed to assess the prevalence and severity of tremor-related ADL impairment among SOTR. Additionally, we aimed to identify clinical, biochemical, and pharmaceutical factors that may predispose SOTR for the development of tremor-related impairment. Finally, we assessed associations of tremor-related impairment with HRQoL.

MATERIALS AND METHODS

Design and Study Population

For this cross-sectional study, data from the TransplantLines Biobank and Cohort Study (ClinicalTrials.gov Identifier: NCT03272841) were used (20). This ongoing cohort study includes SOTR and donors (aged ≥18 years) visiting the University Medical Center Groningen (UMCG, Groningen, The Netherlands). More detailed information on the study design, inclusion and exclusion criteria has been described previously (20). The study was conducted in accordance with the guidelines laid down in the Declarations of Helsinki and Istanbul and approved by the Institutional Review Board (METc 2014/077). The study protocol was reviewed and approved by the TransplantLines Scientific Committee (TxL 2022/004).

TABLE 1 | Immunosuppressive target trough concentration per transplant type in the UMCG.

Time after transplantation (months)	Tacrolimus trough concentration (μg/L)			Cyclosporine trough concentration (μg/L)		
	7–12	13–24	>24	7–12	13–24	>24
Kidney transplantation	4–6	4–6	4–6	75–125	75–125	75–125
Liver transplantation						
[a]Dual therapy	7–10	7–10	7–10	*	*	*
[b]Triple therapy	5–7	5–7	5–7	*	*	*
Lung transplantation	7–10	7–10	7–10	150	100–150	100
Heart transplantation	7–9	7–9	7–9	150	125	125
Small intestine transplantation	10–15	10–15	10–15	~	~	~

[a]Dual therapy consists of tacrolimus and prednisone, and [b]triple therapy additionally includes mycophenolate mofetil.
*~ target trough concentrations are not listed in UMCG protocols. *Cyclosporine is not used for this type of transplantation.*

For the current study, all enrolled SOTR with a functioning allograft for at least 6 months, with available data on tremor influence on ADL between September 2016 and November 2020, were included. A consort flow diagram is shown in **Supplementary Figure S1**.

Immunosuppressive Regimen

All included SOTR attended the outpatient clinic of the UMCG and were treated according to standard immunosuppressive therapies, with revision of therapy effectiveness at least once per year. Immunosuppressive maintenance therapies were generally tacrolimus-based, with addition of mycophenolate mofetil (MMF), and prednisolone. Other immunosuppressive drugs used less frequently were cyclosporine, everolimus, sirolimus, and azathioprine. Although immunosuppressive regimens may be adapted on an individual basis, standard protocol target trough concentrations of tacrolimus and cyclosporine in the UMCG are shown in **Table 1**.

Laboratory Methods

Blood was drawn in the morning after an overnight fasting period of at least 8 h, including no medication use. All tacrolimus and cyclosporine whole blood trough concentrations were determined by validated liquid chromatography mass-spectrometry analyses at the UMCG (21). Other laboratory parameters were measured using routine laboratory methods (Roche Diagnostics, Basel, Switzerland).

Tremor Rating

Tremor severity was assessed using a Dutch translation of the Fahn-Tolosa-Marin (FTM) tremor rating scale part C (TRS-C) (22). This questionnaire is recommended for use in clinical practice (23). The TRS-C consists of eight questions to assess patient-perceived tremor occurrence during ADL, including writing, speaking, and bringing food or liquids to the mouth, and is provided in **Supplementary Table S1**. Furthermore, the questionnaire assesses the influence of tremor on personal hygiene, dressing, working, and social activities. As the TRS-C is designed to evaluate the impact of tremor on ADL, by nature, this questionnaire assesses severity over the previous couple of days of the patient's life. The interviewers were trained to conduct the questionnaire during routine follow-up visits, and to interpret the answers of patients. To every question, patients graded the impairment tremor has on ADL with a score ranging from 0 (no influence of tremor on ADL) to 4 (severe influence of tremor on ADL). A total score was calculated by summing the individual scores, with a theoretical maximum score of 32 points. Patients with a TRS-C total score of 0, or with a TRS-C total score of 1 acquired because of mild disability of speech (i.e., shaky voice only when nervous), were classified as having no tremor. For data visualisation, and statistical analyses, tremor severity was rated according to severity: no tremor (defined as 0 points or 1 point on speech ("mild tremor only perceived when nervous" on the total TRS C score)), mild tremor (1–3 points), and severe tremor (≥4 points).

Health-Related Quality of Life

HRQoL was assessed using the 36-Item Short Form health survey, which is a validated questionnaire to assess several health domains, including physical functioning, role limitations due to physical health, role limitations due to emotional problems, vitality, emotional wellbeing, social functioning, pain, and general health perceptions (24). Subsequently, physical component summary (PCS) and mental component summary (MCS) scores were derived, with a higher score meaning a higher HRQoL. The PCS includes items from physical functioning, role limitations due to physical health, vitality, social functioning, pain, and general health perceptions, whereas the MCS includes items from role limitations due to emotional problems, vitality, emotional wellbeing, social functioning, and general health perceptions.

Additional Data Collection

Blood pressure and heart rate were measured with a semi-automatic device (Philips Suresign VS2+, Andover, Massachusetts, USA). Body weight and height were measured with participants wearing indoor clothing without shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Information on patients' medical history was extracted from electronic patient records, and medication use was extracted from patient records and

TABLE 2 | Characteristics of 689 SOTR without tremor, with mild tremor, or with severe tremor, based on the TRS-C total score.

Variables	No tremor (*n* = 402)	Mild tremor (*n* = 206)	Severe tremor (*n* = 81)	*p*-value
Tremor rating scale-C score	0 [0-0]	2.0 [1.0–2.0]	5.0 [4.0–7.0]	-
Recipient				
Female sex	158 (39.3%)	75 (36.4%)	32 (39.5%)	0.7
Age at visit (years)	54.4 ± 13.6	54.9 ± 14.2	58.1 ± 13.0	0.1
Transplant type*				
Kidney (n = 395)	238 (59.2%)	112 (54.4%)	45 (55.6%)	0.5
Liver (n = 168)	**111 (27.6%)**	**44 (21.4%)**	**13 (16.0%)**	**0.042**
Lung (n = 95)	**40 (10.0%)**	**40 (19.4%)**	**15 (18.5%)**	**0.002**
Heart (n = 29)	13 (3.2%)	9 (4.4%)	7 (8.6%)	0.1
Small intestine (n = 2)	0 (100.0%)	1 (0.5%)	1 (1.2%)	0.1
Polypharmacy (>4 drugs)	**340 (84.6%)**	**183 (88.8%)**	**77 (95.1%)**	**0.025**
Diabetes	101 (25.1%)	54 (26.2%)	21 (25.9%)	1.0
Anaemia	81 (24.7%)	52 (30.4%)	25 (35.7%)	0.1
Body mass index (kg/m^2)	27.0 ± 4.8	26.6 ± 4.7	27.0 ± 5.3	0.5
Kidney transplant characteristics				
Donor age (years)	51.0 [39.0–59.0]	52.0 [40.0–62.0]	51.0 [34.8–61.0]	0.2
Living donor	134 (33.3%)	71 (34.5%)	24 (29.6%)	0.7
Time after transplantation (years)	3.0 [1.0–10.0]	2.0 [0.0–9.0]	2.0 [1.0–11.0]	0.1
Laboratory measurements				
eGFR creatinine (mL/min/1.73m^2)	**59.6 ± 22.4**	**55.9 ± 20.3**	**53.8 ± 24.2**	**0.041**
Creatinine (μmol/L)	112.0 [89.3–139.8]	118.0 [98.0–140.0]	116.0 [88.0–168.0]	0.1
Haemoglobin (mmol/L)	8.3 ± 1.1	8.1 ± 1.2	8.1 ± 1.2	0.1
HbA1c (mmol/mol)	38.0 [34.0–43.0]	39.0 [35.0–44.8]	39.0 [35.0–44.3]	0.2
Glucose (mmol/L)	**5.6 [5.1–6.5]**	**5.8 [5.2–6.8]**	**5.8 [5.3–7.0]**	**0.030**
Vitamin B12 (pmol/L)	305.0 [245.0–418.5]	307.5 [239.5–422.0]	313.0 [223.0–467.5]	0.9
Folic acid (nmol/L)	13.8 [9.9–18.1]	12.8 [10.0–17.9]	12.3 [9.6–20.7]	0.4
[a]Tacrolimus (μg/L)	**5.7 ± 2.4**	**6.3 ± 2.5**	**6.3 ± 2.1**	**0.022**
[b]Cyclosporine (μg/L)	65.0 [49.0–98.0]	104.0 [83.5–111.0]	88.0 [36.5–120.3]	0.1
Medication				
Calcineurin inhibitor use	341 (84.8%)	183 (88.8%)	70 (86.4%)	0.4
No use	61 (15.2%)	23 (11.2%)	11 (13.6%)	0.4
Tacrolimus	**290 (72.1%)**	**170 (82.5%)**	**64 (79.0%)**	**0.014**
Cyclosporine	48 (11.9%)	13 (6.3%)	7 (8.6%)	0.1
mTOR inhibitor	21 (5.2%)	13 (6.3%)	5 (6.2%)	0.8
Proliferation inhibitor	309 (76.9%)	165 (80.1%)	67 (82.7%)	0.4
Prednisolone or prednisone	322 (80.1%)	172 (83.5%)	72 (88.9%)	0.1
Beta blockers	161 (40.0%)	84 (41.0%)	35 (43.2%)	0.9
Short or long acting bronchodilators	20 (5.0%)	10 (4.9%)	6 (7.4%)	0.6

**Percentages were calculated by dividing the number of patients in each transplant type by the total number of all solid organ transplant patients with no/mild/severe tremor. Bold type indicates significance of results.*

eGFR, estimated glomerular filtration rate as calculated using CKD-EPI formula. Normally distributed data are presented as mean ± standard deviation, skewed data as median [interquartile range], and categorical data as number (valid percentage). p-values represent significance of differences between tremor severity groups as assessed with Analyses of Variance, Kruskal-Wallis or Chi-squared tests, depending on distribution. Data were available in [a]514 and [b]62 patients.

verified with the patient by a trained researcher. Diabetes was defined based on use of antidiabetic drugs, fasting glucose ≥7.0 mmol/L, and/or HbA1c ≥6.5%. Kidney glomerular filtration rate was estimated with the creatinine-based CKD-EPI equation (25). Anaemia was defined as a haemoglobin concentration <8.1 mmol/L for men and <7.5 mmol/L for women according to WHO criteria (26).

Statistical Analyses

Dispersion of TRS-C scores in total, and per transplant type, were visualized by means of pie charts and bar charts. Patient characteristics are presented and compared in patients without tremor, with mild tremor, and with severe tremor. Continuous variables are summarized as mean ± SD or median [interquartile range], depending on distribution, whereas categorical or dichotomous variables are presented as count (%). To assess differences between tremor severity groups, Analyses of Variance were used for normally distributed variables, Kruskal-Wallis tests for non-normally distributed variables and Chi-squared tests for categorical variables. To assess associations of tremor severity with clinical, biochemical and pharmacotherapeutic parameters, multinomial logistic regression analyses, adjusted for sex, age, and $\log_2$ time after transplantation were performed. Sensitivity analyses with additional adjustment for tacrolimus trough concentrations were performed. Also, exploratory subgroup analyses were performed to compare non-CNI users with no, mild, or severe tremor.

Differences in PCS and MCS between grades of tremor severity were visualised with boxplots. Analyses of variance were performed for testing significance of differences between the different grades. Furthermore, bivariable linear regression

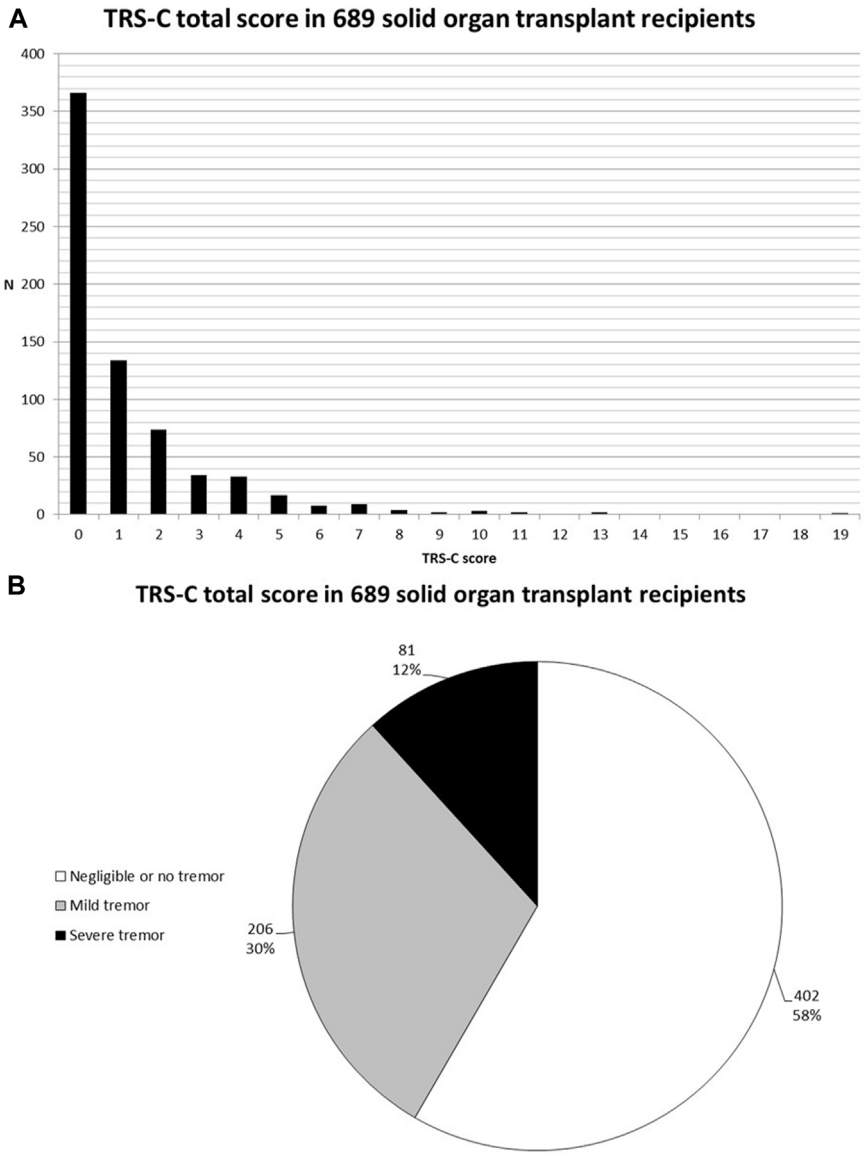


FIGURE 1 | (A) Bar chart of TRS-C total score in 689 SOTR. **(B)** Pie-chart of tremor severity in 689 SOTR.

analyses with PCS and MCS as dependent variable were performed to assess associations of mild and severe tremor with HRQoL. In multivariable linear regression analyses we assessed the association of mild and severe tremor with HRQoL, while adjusting for potential confounders including age, sex, type of transplantation, $\log_2$ time after transplantation, polypharmacy, diabetes, anaemia, eGFR, use of tacrolimus, use of cyclosporine, employment status, educational level, and the presence of a partner. Potential presence of effect modification by age and sex was assessed by adding interaction terms to the linear regression models.

Scatterplots and QQ-plots were visually evaluated to assess data distribution. Non-normally distributed variables were transformed using a binary logarithm ($\log_2$) when necessary to meet the assumptions for regression. All statistical analyses were performed with IBM SPSS software (version 23.0, SPSS Inc., Chicago, IL, USA) and R (version 3.5.1, Vienna, Austria). In all analyses, a two-sided p-value <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics

In total, 689 SOTR were included in the current study, including kidney (n = 395, 57.3%), liver (n = 168, 24.4%), lung (n = 95, 13.8%), heart (n = 29, 4.2%), and small intestine (n = 2, 0.3%) transplant recipients with a mean ± SD age of 58.0 ± 13.7 years, of whom 38.5% were female. A detailed

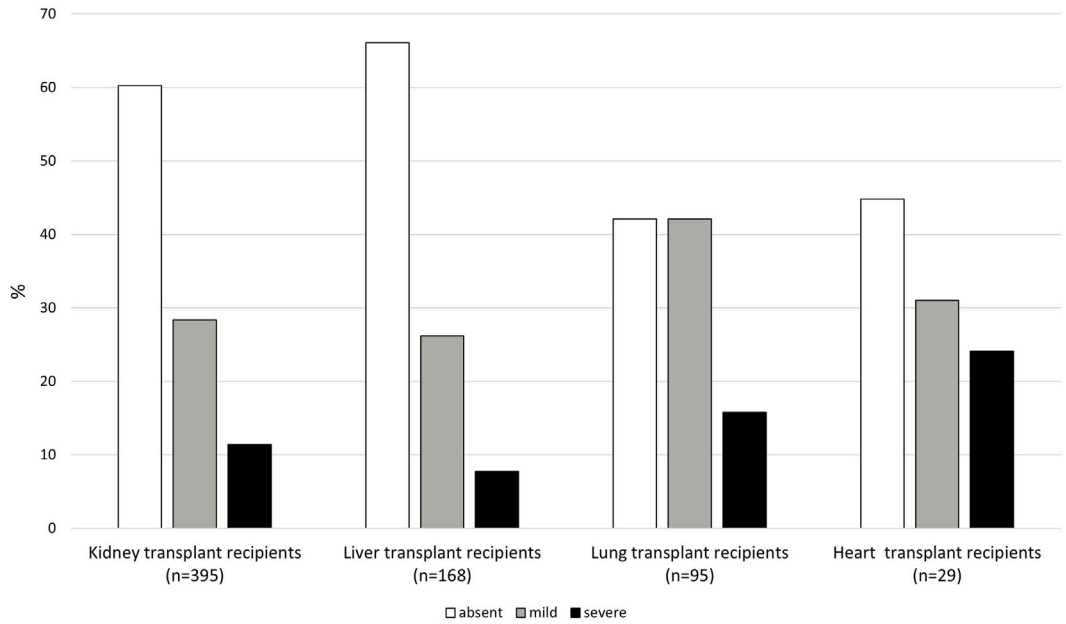


FIGURE 2 | Bar charts of tremor severity group distribution per transplant type.

overview of baseline characteristics in patients with either no, mild, or severe tremor, is presented in **Table 2**. In brief, patients with more tremor-related impairment were more often lung transplant recipients, less often liver transplant recipients, more frequently used tacrolimus and tended to have higher tacrolimus trough concentrations. Furthermore, tremor-related impairment was more frequently reported in patients with polypharmacy, patients with lower eGFR, and patients with higher fasting glucose concentrations. Notably, patients with mild or severe tremor tended to have higher cyclosporin trough concentrations, although differences between the groups were not statistically significant.

Occurrence of Tremor and Impact on Activities of Daily Life

206 (29.9%) SOTR reported mild tremor and 81 (11.8%) reported severe tremor. Mild or severe tremor was reported in 39.7% among kidney transplant recipients, 33.9% among liver transplant recipients, 57.9% among lung transplant recipients, and 55.1% among heart transplant recipients. TRS-C scores of all SOTR and per organ type are shown in **Figures 1A, B** and **2** respectively. Additional bar charts of TRS-C total score per organ type are shown in **Supplementary Figures S2A–D**. Among patients that reported impairment because of tremor, median TRS-C score was 2.0 [1.0–4.0] [range 0–19]. Notably, patients reported that tremor-related impairment was most pronounced during writing and bringing food to the mouth. Furthermore, 18 (2.6%) patients reported changes in social activities because of tremor. Scores per TRS-C question are visualized by means of bar charts in **Supplementary Figures S3A–H**.

Determinants of Tremor-Related Impairment

Results of multinomial logistic regression analyses adjusted for age, sex, and $\log_2$ time after transplantation with presence of mild or severe tremor as dependent variable are shown in **Table 3**. Whole blood tacrolimus trough concentration was a determinant of mild tremor, independent of age, sex, and $\log_2$ time after transplantation (OR per μg/L increase: 1.11, 95% CI: 1.02 to 1.21, $p = 0.019$). Notably, higher whole blood tacrolimus trough concentration also tended to be associated with more severe tremor, although this observation was not statistically significant (OR per μg/L increase: 1.11, 95% CI: 0.98 to 1.26, $p = 0.1$). Furthermore, lung transplantation was associated with mild tremor; heart transplantation, polypharmacy and higher creatinine were associated with severe tremor. These associations remained similar after adjustment for tacrolimus trough concentrations (**Supplementary Table S2**). Notably, of the 95 patients who did not use calcineurin inhibitors, 34 (36%) reported mild or severe tremor. Exploratory subgroup analyses showed that non-CNI users with tremor more often had diabetes (**Supplementary Table S3**). Use of concomitant medication that may affect tremor, such as beta blockers and bronchodilators, was not associated with mild of severe tremor.

Health-Related Quality of Life

SOTR that reported mild or severe tremor had significantly lower physical and mental HRQoL, as visualized in **Figure 3** ($p < 0.001$ for both). In bivariable linear regression analyses, both mild and severe tremor were associated with a lower physical HRQoL, with the strongest association for severe tremor (PCS: $\beta = -5.64$, 95% CI:

TABLE 3 | Multinomial logistic regression analyses of tremor severity with adjustment for age, sex, and log_2 time after transplantation in 689 solid organ transplant recipients.

Baseline variables	No tremor (n = 402)	Mild tremor (*n* = 206)		Severe tremor (*n* = 81)		N
		OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	
Recipient						
*Female sex	*Ref.*	0.91 (0.64–1.29)	0.6	1.05 (0.64–1.71)	0.9	689
*Age at visit (per 10 years)	*Ref.*	1.03 (0.91–1.16)	0.7	**1.23 (1.02–1.48)**	**0.029**	689
Transplant type						
Kidney (*n* = 269)	*Ref.*	*Ref.*	*Ref.*	*Ref.*	*Ref.*	-
Liver (*n* = 154)	*Ref.*	0.98 (0.63–1.53)	0.9	0.64 (0.32–1.27)	0.2	689
Lung (*n* = 75)	*Ref.*	**2.21 (1.34–3.63)**	**0.002**	1.96 (1.00–3.87)	0.1	689
Heart (*n* = 29)	*Ref.*	1.63 (0.67–3.96)	0.3	**2.74 (1.02–7.38)**	**0.047**	689
Polypharmacy (>4 drugs)	*Ref.*	1.26 (0.73–2.15)	0.4	**3.13 (1.08–9.08)**	**0.036**	689
Diabetes	*Ref.*	1.08 (0.73–1.59)	0.7	0.94 (0.54–1.65)	0.8	689
Anaemia	*Ref.*	1.30 (0.86–1.96)	0.2	1.70 (0.98–2.97)	0.1	569
Body mass index (kg/m^2)	*Ref.*	0.98 (0.94–1.01)	0.2	0.99 (0.94–1.04)	0.8	686
Kidney transplant characteristics						
log_2 donor age (years)	*Ref.*	1.23 (0.90–1.68)	0.2	0.87 (0.59–1.27)	0.5	642
Living donor	*Ref.*	0.95 (0.66–1.38)	0.8	0.85 (0.50–1.46)	0.6	689
*log_2 time after transplantation (years)	*Ref.*	**0.90 (0.81–0.99)**	**0.036**	0.96 (0.83–1.11)	0.6	689
Laboratory measurements						
eGFR creatinine ($mL/min/1.73m^2$)	*Ref.*	0.99 (0.99–1.00)	0.1	0.99 (0.98–1.00)	0.2	650
log_2 creatinine (μmol/L)	*Ref.*	1.24 (0.88–1.76)	0.2	**1.71 (1.08–2.70)**	**0.022**	650
Haemoglobin (mmol/L)	*Ref.*	0.87 (0.74–1.02)	0.1	0.82 (0.65–1.03)	0.1	649
log_2 HbA1c (mmol/mol)	*Ref.*	1.70 (0.97–2.97)	0.1	1.39 (0.62–3.12)	0.4	645
log_2 glucose (mmol/L)	*Ref.*	1.54 (0.92–2.56)	0.1	1.64 (0.82–3.29)	0.2	624
log_2 vitamin B12 (pmol/L)	*Ref.*	1.02 (0.78–1.32)	0.9	1.12 (0.78–1.60)	0.6	636
log_2 folic acid (nmol/L)	*Ref.*	0.97 (0.75–1.25)	0.8	1.08 (0.75–1.55)	0.7	577
Tacrolimus (μg/L)	*Ref.*	**1.11 (1.02–1.21)**	**0.019**	1.11 (0.98–1.26)	0.1	514
log_2 cyclosporine (μg/L)	*Ref.*	2.12 (0.94–4.81)	0.1	1.32 (0.46–3.75)	0.6	62
Medication						
Calcineurin inhibitor use	*Ref.*	1.17 (0.66–2.06)	0.6	1.11 (0.51–2.40)	0.8	689
No use	*Ref.*	*Ref.*	*Ref.*	*Ref.*	*Ref.*	-
Tacrolimus	*Ref.*	1.49 (0.83–2.68)	0.2	1.38 (0.62–3.08)	0.4	689
Cyclosporine	*Ref.*	0.74 (0.34–1.62)	0.5	0.82 (0.29–2.28)	0.7	689
mTOR inhibitor	*Ref.*	1.17 (0.57–2.40)	0.7	1.17 (0.42–3.23)	0.8	689
Proliferation inhibitor	*Ref.*	1.10 (0.72–1.68)	0.7	1.42 (0.75–2.68)	0.3	689
Prednisolone or prednisone	*Ref.*	1.16 (0.74–1.81)	0.5	1.92 (0.91–4.05)	0.1	689
Beta blockers	*Ref.*	0.97 (0.68–1.38)	0.9	1.04 (0.64–1.71)	09	689
Short or long acting bronchodilators	*Ref.*	0.95 (0.43–2.08)	0.9	1.39 (0.54–3.62)	0.50	689

*Bold type indicates significance of results. log_2, the binary logarithm; eGFR, estimated glomerular filtration rate as calculated using CKD-EPI formula. Intestinal transplant recipients are excluded in analyses due to low number of participants (n = 2). *Presented numbers represent regression coefficients of the concerned variable in a model including age, sex, and log_2 time after transplantation.*

−9.34 to 1.94, $p = 0.003$ and β = −15.87, 95% CI: −21.26 to −10.48, $p < 0.001$ resp.). A similar pattern was found for mental HRQoL (MCS: β = −3.50, 95% CI: −6.68 to −0.31, $p = 0.032$ and β = −11.22, 95% CI: −15.86 to −6.57, $p < 0.001$ resp.). These associations remained similar after adjustment for age, sex, type of transplantation, log_2 time after transplantation, polypharmacy, diabetes, anaemia, eGFR, use of tacrolimus, and use of cyclosporine (**Table 4**). When we additionally adjusted for employment status, educational level, and the presence of a partner, results remained generally similar, although the association between mild tremor and physical HRQoL was no longer statistically significant. No interactions of age and sex were present for the association of tremor severity with HRQoL.

DISCUSSION

This study shows that mild or severe tremor frequently impairs daily life activities of SOTR. Tacrolimus trough concentration was a main determinant of tremor. Importantly, mild and severe tremor-related impairments were strongly associated with lower HRQoL, independent of other known determinants of tremor.

Our results confirm that SOTR frequently experience tremor-related impairment of ADL. A previous study reported similar tremor prevalence compared to our study (18). However, the current study provides additional insights, because we assessed prevalence based on impact of tremor on ADL. The pathophysiology of CNI-induced tremor remains unknown. However, the transplant population tends to show lower TRS-C scores compared to patients with essential tremor (27). Our results show that tacrolimus trough concentrations are associated with tremor-related impairment among SOTR. This is further corroborated by the finding that tremor-related impairment is higher among SOTR with higher target trough concentrations (e.g., lung and heart transplant recipients). Also, patients with severe tremor had higher creatinine blood concentrations, which is consistent with the notion that tacrolimus is both nephrotoxic

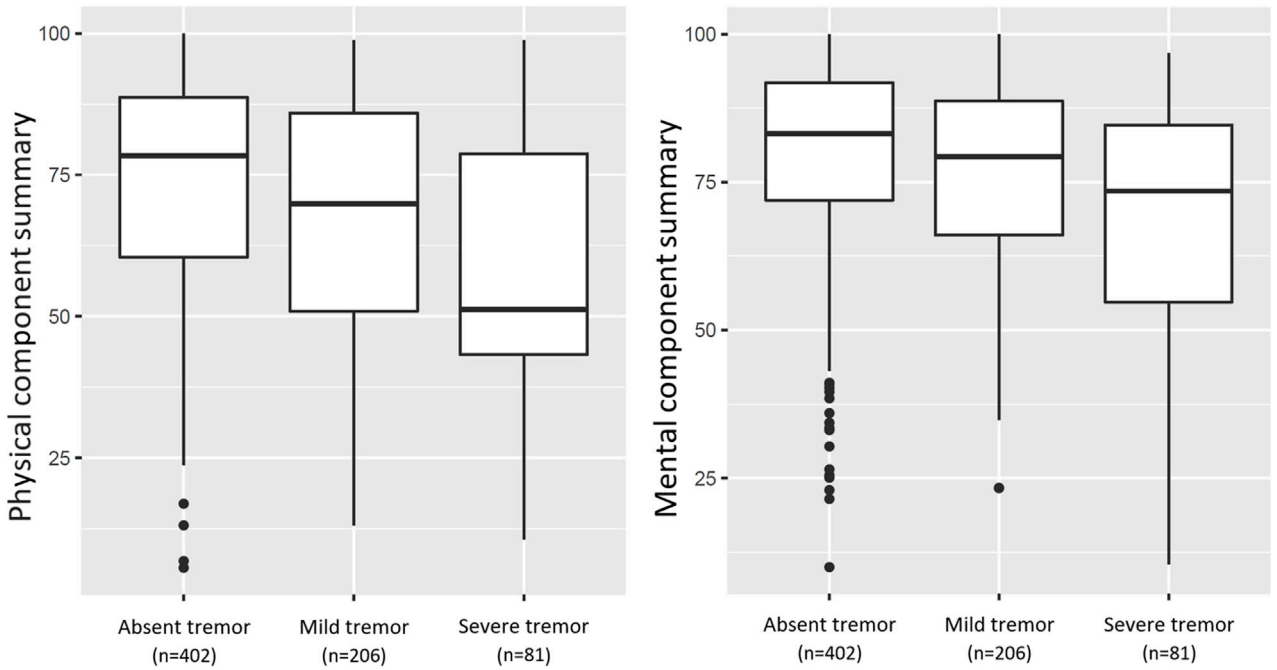


FIGURE 3 | Boxplots of PCS, and MCS for HRQoL in 689 SOTR (mild and severe tremor present in 206 (29.9%) and 81 (11.8%) SOTR, respectively). The PCS, and MCS differed significantly between groups as calculated using Analyses of Variance ($p < 0.001$; $p < 0.001$, resp.).

and neurotoxic (18, 19). However, alternatively, renal impairment may also predispose patients to tremor, so tacrolimus use does not necessarily relate to the occurrence of both nephro- and neurotoxicity in the same patient and claims regarding causality cannot be drawn from the current study. Nevertheless, our findings support the previously suggested notion that the occurrence of CNI side effects is dose-dependent (18, 19). Generally, factors including vitamin B12, HbA1c and glucose are regarded as key determinants of tremor in different populations (28), and concomitant medication such as bronchodilators and betablockers are known to affect tremor. Our results highlight that these factors appear to be less important among SOTR, and that CNI use appears to be the main cause of tremor in this population.

The notion that tacrolimus trough concentrations are independently associated with tremor-related impairment among SOTR is important for treating physicians to consider during treatment regimens. Furthermore, this notion highlights the need for alternative immunosuppressive treatment regimens that are less likely to cause tremor, while maintaining low risks of rejection. Since dose-dependency seems to be key in the pathophysiology of medication-induced tremor, extended-release preparations may be able to reduce tremor prevalence by reducing peak-to-trough variability (19). Langone et al. have reported promising results in patients using extended-release tacrolimus, showing a significantly lower peak-to-trough variability, lower tremor prevalence, and a higher quality of life (19). However, this study included a small number of only kidney transplant recipients. Therefore, future interventional studies are required for strengthening these results.

The potential impact of tremor on the lives of SOTR is further highlighted by the associations of severe tremor with both lower mental and physical HRQoL. These results are generally in line with findings in previous smaller studies. For example, Langone et al. reported an improved quality of life after a reduction in tremor severity, in a small cohort of 38 kidney transplant recipients (19).

A major strength of this study is the large number of included SOTR with available data regarding subjective tremor and HRQoL. Additionally, we included kidney, liver, lung, heart, and small intestine transplant recipients, allowing for evaluation of tremor prevalence among these patient groups. In addition, the cohort was well-characterized, allowing us to adjust for many potential confounders, including extensive clinical data, laboratory measurements, and treatment regimens. A limitation of the current study is that the participants mainly used tacrolimus-based treatment regimens, and associations of cyclosporine and other immunosuppressive drugs with tremor could therefore be incompletely studied. In addition, since patients were not evaluated before transplantation, the presence and exacerbation of preconditions which may cause tremor could not be assessed. Non-CNI users with tremor more frequently had diabetes compared to those without diabetes, which may partly explain the high prevalence of tremor in this subgroup. Nevertheless, this observation cannot fully explain the tremor occurrence in this subgroup, and future studies are needed to gain insights into the tremor susceptibility of some SOTR without CNI-use. Lastly, due to the observational design of the study, we cannot draw conclusions regarding causality of our findings. Moreover, the current cross-sectional study cannot identify trajectories of

TABLE 4 | Linear regression analyses of tremor severity with physical and mental HRQoL.

Independent variable	Physical component summary		Mental component summary	
	β (95% CI)	*p*-value	β (95% CI)	*p*-value
Crude				
No tremor	*Ref.*	*n/a*	*Ref.*	*n/a*
Mild tremor	−5.64 (−9.34 to −1.94)	0.003	−3.50 (−6.68 to −0.31)	0.032
Severe tremor	−15.87 (−21.26 to −10.48)	<0.001	−11.22 (−15.86 to −6.57)	<0.001
Model 1				
No tremor	*Ref.*	*n/a*	*Ref.*	*n/a*
Mild tremor	−5.76 (−9.42 to −2.10)	0.002	−3.70 (−6.86 to −0.54)	0.022
Severe tremor	−15.14 (−20.50 to −9.79)	<0.001	−11.54 (−16.16 to −6.93)	<0.001
Model 2				
No tremor	*Ref.*	*n/a*	*Ref.*	*n/a*
Mild tremor	−5.18 (−8.88 to −1.49)	0.006	−3.58 (−6.79 to −0.38)	0.028
Severe tremor	−14.36 (−19.76 to −8.97)	<0.001	−11.41 (−16.09 to −6.73)	<0.001
Model 3				
No tremor	*Ref.*	*n/a*	*Ref.*	*n/a*
Mild tremor	−4.97 (−8.62 to −1.31)	0.008	−3.52 (−6.72 to −0.31)	0.032
Severe tremor	−13.83 (−19.17 to −8.48)	<0.001	−11.24 (−15.93 to −6.56)	<0.001
Model 4				
No tremor	*Ref.*	*n/a*	*Ref.*	*n/a*
Mild tremor	−4.89 (−8.78 to −1.01)	0.014	−3.70 (−7.21 to −0.20)	0.038
Severe tremor	−15.08 (−20.61 to −9.56)	<0.001	−11.89 (−16.87 to −6.90)	<0.001
Model 5				
No tremor	*Ref.*	*n/a*	*Ref.*	*n/a*
Mild tremor	−4.86 (−8.75 to −0.96)	0.015	−3.74 (−7.25 to −0.23)	0.037
Severe tremor	−15.05 (−20.59 to −9.52)	<0.001	−11.92 (−16.91 to −6.92)	<0.001
Model 6				
No tremor	*Ref.*	*n/a*	*Ref.*	*n/a*
Mild tremor	−5.03 (−8.92 to −1.13)	0.012	−3.80 (−7.32 to −0.28)	0.034
Severe tremor	−15.35 (−20.88 to −9.81)	<0.001	−12.06 (−17.06 to −7.06)	<0.001
Model 7				
No tremor	*Ref.*	*n/a*	*Ref.*	*n/a*
Mild tremor	−4.09 (−8.42 to 0.24)	0.06	−4.05 (−7.97 to −0.13)	0.043
Severe tremor	−16.10 (−22.23 to −9.98)	<0.001	−12.68 (−18.23 to −7.14)	<0.001

95% CI, 95% confidence interval; PCS, physical component summary; MCS, mental component summary; eGFR, estimated glomerular filtration rate; HRQoL, health-related quality of life. ***Model 1****: adjusted for age and sex;* ***model 2****: model 1 + type of transplantation and log_2 time after transplantation;* ***model 3****: model 2 + polypharmacy;* ***model 4****: model 3 + diabetes and anaemia;* ***model 5****: model 4 + eGFR;* ***model 6****: model 5 + use of tacrolimus and use of cyclosporine;* ***model 7****: model 6 + employment status, education level, and presence of a partner.*

tremor before and after transplantation, and therefore longitudinal studies assessing the determinants of tremor are warranted. Such studies may also help to account for potential tacrolimus dosage adaptations that clinicians may conduct in patients with severe tremor.

SOTR frequently report tremor-related impairment of ADL. Tacrolimus trough concentrations appeared a main determinant of tremor among SOTR. The strong and independent association of tremor-related impairment with lower HRQoL warrants further studies into the effects of tacrolimus on tremor.

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DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because public participant data sharing is not included in the TransplantLines informed consent forms. Data requests are only legally allowed after approval by the TransplantLines Scientific Committee upon reasonable request, in accordance with the medical ethical committee allowance and the UMCG Biobank Regulations. Requests to access the datasets should be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by METc University Medical Centre Groningen 2014/077. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

NR and DK wrote the draft manuscript. NR and DK performed data analyses. DK, AG-N, and ME performed the research and contributed to data collection. TK contributed to data collection, interpretation, and manuscript revisions. CG, SN, HB, VM, KD, GD, JE, SB, and DT were responsible for the research design and manuscript revisions. SB and AS were responsible for the research design, contributed to data interpretation and manuscript revisions. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

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.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.10951/full#supplementary-material

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Management and Outcome After Early Renal Transplant Vein Thrombosis: A French Multicentre Observational Study of Real-Life Practice Over 24 Years

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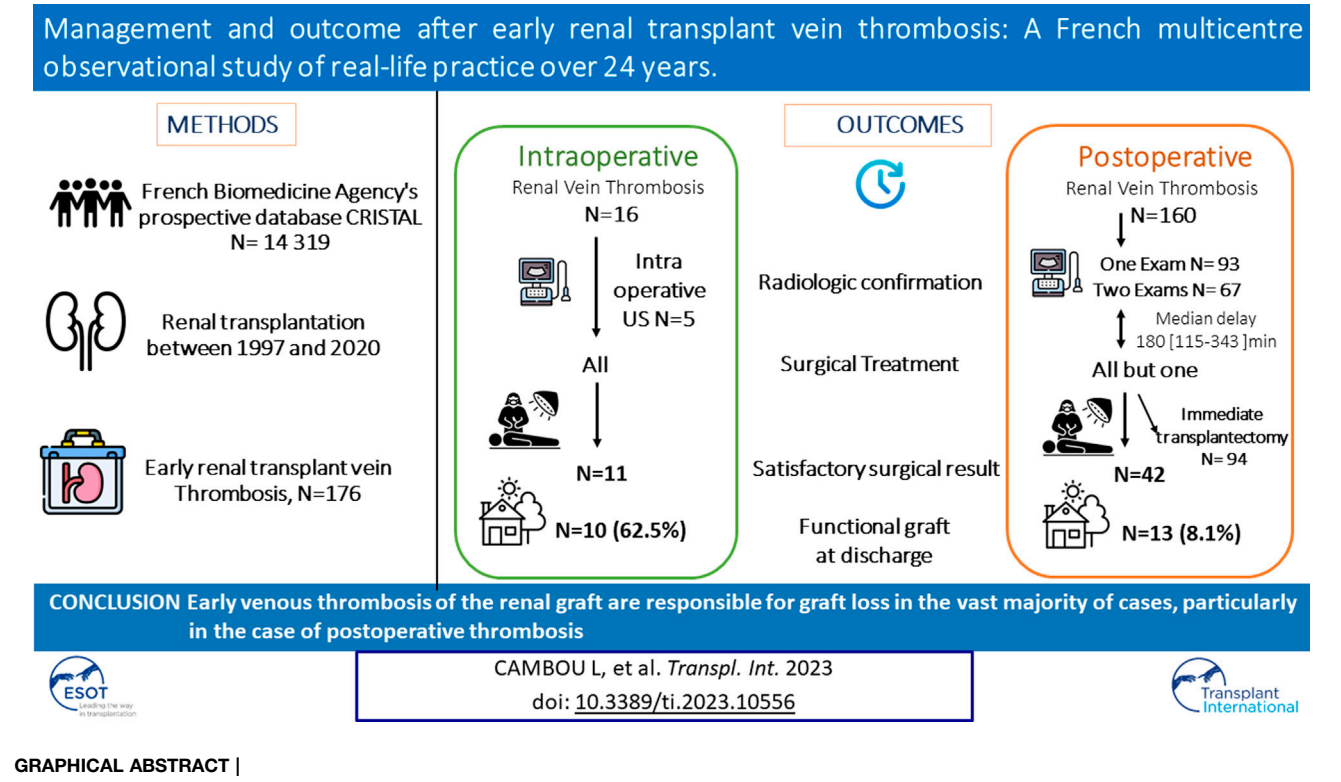
Early (<14 days) renal transplant vein thrombosis posttransplant (eRVTPT) is a rare but threatening complication. We aimed to assess eRVTPT management and the rate of functional renal transplantation. Of 11,172 adult patients who had undergone transplantation between 01/1997 and 12/2020 at 6 French centres, we identified 176 patients with eRVTPT (1.6%): 16 intraoperative (Group 1, G1) and 160 postoperative (Group 2, G2). All but one patient received surgical management. Patients in group G2 had at least one imaging test for diagnostic confirmation (N = 157, 98%). During the operative management of the G2 group, transplantectomy for graft necrosis was performed immediately in 59.1% of cases. In both groups, either of two techniques was preferred, namely, thrombectomy by renal venotomy or thrombectomy + venous anastomosis repair, with no difference in the functional graft rate (FGR) at hospital discharge (p = NS). The FGR was 62.5% in G1 and 8.1% in G2 ($p < 0.001$). Numerous complications occurred during the initial hospitalization: 38 patients had a postoperative infection (21.6%), 5 experienced haemorrhagic shock (2.8%), 29 exhibited a haematoma

Abbreviations: AST, Aspartate aminotransferase; CK, Creatinine kinase; eRVTPT, Early (<14 days) renal transplant vein thrombosis posttransplant; FGR, Functional graft rate; LDH, Lactate dehydrogenase; MRI, Magnetic resonance imaging; RT, Renal transplant.

(16.5%), and 97 (55.1%) received a blood transfusion. Five patients died (2.8%). Our study confirms the very poor prognosis of early renal graft venous thrombosis.

Keywords: kidney transplant, vein thrombosis, early vein thrombosis, outcome, management



GRAPHICAL ABSTRACT |

INTRODUCTION

Early renal vein thrombosis posttransplantation (eRVTPT) is a serious complication occurring during the first 14 days of renal transplantation (1), and its frequency is estimated to be between 0.1% and 5.5% (2–6). It is very often accompanied not only by graft loss (7) due to an absence of collaterality with venous flow coming only from the renal vein of the transplant (8) but also by embolic and/or haemorrhagic complications that can lead to death. eRVTPT should be suspected in the presence of pain that is not relieved by the usual analgesic treatments, the occurrence of oligoanuria, an excessively productive drainage or even an increase in macroscopic haematuria and a deterioration of renal function (2,7). Clinical suspicion can be confirmed by renal Doppler ultrasound (9), computed tomography angiography, or magnetic resonance angiography (10,11).

To date, there is no recommendation concerning the management of eRVTPT. Indeed, the data on such management in the literature are based on case series or small cohorts. It is reported to require surgical revision, with thrombectomy by renal venotomy (12), anastomotic repair, or explantation, flushing with preservative solution and reimplantation (13), and more rarely endovascular treatment (14) or thrombolysis alone (15). Regardless of the reported management, the rate of functional grafts at discharge is extremely low (5–7,16).

The aim of our study was to investigate different management strategies during the occurrence of eRVTPT and the outcome of the renal graft.

PATIENTS AND METHODS

Patients and General Data

This retrospective multicentre observational study was conducted at 6 French adult renal transplantation centres: Gabriel Montpied Hospital, Clermont-Ferrand University Hospital; Michallon Hospital, Grenoble University Hospital; Necker-Enfants Malades Hospital, AP-HP; Edouard Herriot Hospital, Lyon University Hospital; Rangueil Hospital, Toulouse University Hospital; and Nouvel Hospital Civil, Strasbourg University Hospital.

The inclusion criteria were patients aged more than 18 years who had undergone renal transplantation between 01/01/1997 and 31/12/2020 complicated by venous thrombosis of the

graft during the initial hospitalization (<14 days). To avoid selection bias, we submitted a request to the Biomedicine Agency database with the following terms: "vascular complications" and/or "no primary function." We then checked all medical records and included only patients with early vein thrombosis of the allograft.

We collected the following demographic and clinical characteristics of the donor from the Biomedicine Agency's prospective database CRISTAL and possibly from the patient's file: type of donor (living or deceased), age, so-called "marginal" donor with extensive selection criteria (17), presence of thromboembolic risk factors, year of transplantation, laterality of the kidney, possible anatomical abnormalities, and conditions of retrieval. We also collected the following demographic, clinical and biological data of the recipient: age, sex, body mass index, thromboembolic history, haematological pathologies, history of miscarriage, smoking, diabetes, initial renal disease, presence of pretransplant anticoagulant or antiaggregant treatments, and induction immunosuppressive treatments.

Intraoperative graft data were collected from operative reports. We distinguished the type of graft (bitransplant, multiorgan transplant, or renal transplant alone) and the duration of cold and warm ischaemia; from the operative reports, we identified any difficulties that occurred during vascular anastomoses and the flushing or non-flushing of the vessels intraoperatively. We also noted signs suggestive of renal graft vein thrombosis, whether clinical and/or biological, and imaging studies allowing us to confirm this, as well as the management, the functional results in the long term, and the complications secondary to this management.

Definition of Groups

Of the 14,319 renal transplants (RTs) performed at these 6 centres in the period from 01/01/1997 to 12/31/2020, 182 (1.3%) patients presented with renal graft vein thrombosis during the initial transplant hospitalization. We excluded 2 patients with partial thromboses, 2 patients for whom the diagnosis was uncertain, and 2 patients because of a lack of data (**Figure 1**).

Patients were divided into 2 groups. Group 1 (G1; N = 16) included patients with intraoperative renal vein thrombosis. Group 2 (G2; N = 160) included patients with a postoperative diagnosis of eRVTPT.

Statistical Analysis

Statistical analysis was essentially exploratory to describe management strategies in the event of eRVTPT. Data are presented as the mean and standard deviation or the median and interquartile range. The assumption of normality distribution was studied with the Shapiro–Wilk test. The chi-square test or, if appropriate, Fisher's exact test was used to compare independent groups concerning graft outcomes, such as the proportion of patients with a functional graft rate at hospital discharge or the time to thrombosis. Statistical analysis was performed using the Stata software (version 15, StataCorp, College Station, Texas, US). The statistical tests were two-sided, with type I error set at 0.05. We performed a study of the factors associated with functional grafts at discharge using generalized linear mixed models with a logit link function to model between- and within-centre variability (as random effects). For multivariate analyses, we performed a multiple mixed logistic regression that considered covariables in terms of their significant results in univariate analysis ($p < 0.10$) (**Table 1**) as well as their clinical relevance (2,18–20). The results are expressed in terms of odds ratios (ORs) and 95% confidence intervals (95% CIs).

RESULTS

Characteristics of Patients With Early RT Vein Thrombosis

The characteristics of the patients are given in **Table 1**. The 176 patients included in our study were predominantly male (56.2%), aged 56.5 ± 10.0 years at transplantation, and had a mean body mass index of 26.2 ± 3 kg/m^2. In this cohort, thirty-one patients were diabetic (17.2%), and 16 (9.5%) were active smokers. A history of thrombosis was reported in 34 patients (19.0%), 12 of whom had multiple thromboses (6.8%). Haematological, haemostasis or immunological pathology posing a risk of thrombosis was found in 9.1% of cases (N = 16). Thirty-eight patients (21.6%) were receiving antiaggregation therapy, and 13 (7.3%) were receiving anticoagulant therapy (**Table 1**).

In the vast majority of cases, patients received a first kidney transplant (N = 144, 84.2%) from a deceased brain-dead donor (N = 166, 94.3%). The median donor age was 60 [46–71] years; 51.2% of the grafts (85/166) were considered marginal (**Table 2**).

Among the patients who received a kidney transplant from a living donor, 1 received a right kidney, 3 others received a left kidney with a short and thin vein and 1 with 2 veins that had been ligated. eRVTPT was more frequent in patients who received a right kidney than in those who received a left kidney during the interest period (101/6421 (1.57%) patients vs. 74/7797 (0.95%) patients, $p = 0.002$).

Initial Renal Transplantation Surgery

The median cold ischaemia time was 918 [714–1259] minutes. The median warm ischaemia time to perform vascular anastomosis was 45 [34–55] minutes. In 23 recipients (13.4%), the venous anatomy of the graft was abnormal (11 multiple veins, 10 venous wounds at harvesting, and 2 dysplastic veins). In addition, seven operators had to redo the venous anastomosis during the transplantation procedure (4.4%). During the initial operative procedure, 37 patients received a flush solution with heparinized saline or normal saline (27.4%), and 13 patients (9.4%) received heparin therapy (**Table 2**).

Diagnosis of Intraoperative RT Vein Thrombosis (Group 1)

Half of the diagnoses of intraoperative RT vein thrombosis were made at one centre. All patients with intraoperative eRVTPT at this centre had a functional graft at discharge. This team frequently used intraoperative ultrasound during kidney transplant procedures to assess graft vascular anastomoses and

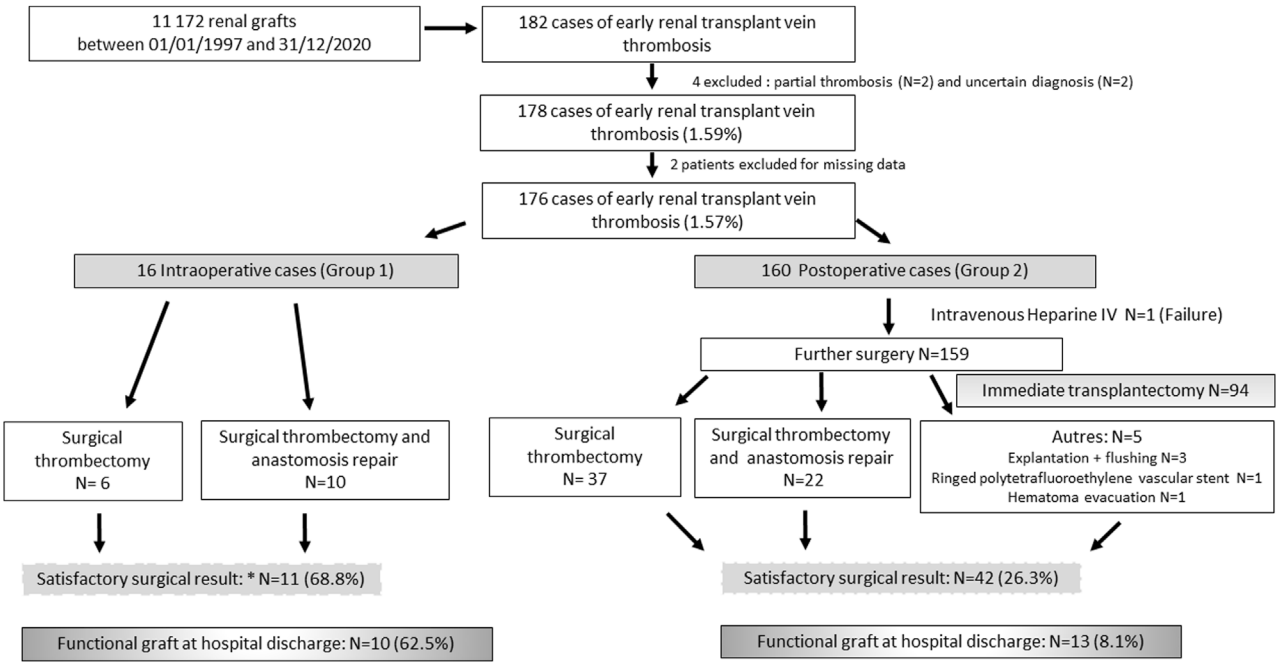


FIGURE 1 | Flow Chart.

flow. Recently, another centre introduced this technique and diagnosed one case of intraoperative eRVTPT with a favourable outcome.

Diagnosis of Early Postoperative RT Vein Thrombosis (Group 2)

In the majority of cases, venous thrombosis was symptomatic (86.2%). It manifested as oligoanuria (63.1%), abnormal pain in the renal pelvis (26.9%) and frank haematuria (17.5%). Venous thrombosis was revealed by haemodynamic disorders, such as the use of vasopressor amines in 14 patients (8.6%) or haemorrhagic shock in 7 other recipients (4.3%). The main biological criterion leading to the diagnosis was increased creatinine levels (81/160; 50.6%). The vast majority of patients underwent imaging to confirm graft vein thrombosis: 68/160 (42.5%) by graft Doppler ultrasound, 22 (13.8%) by abdominopelvic computed tomography angiography, 61 (38.1%) by both aforementioned modalities, and 6 (3.7%) by ultrasound combined with MRI (**Table 3**).

Therapeutic Management

In group 1, 10/16 (62.5%) intraoperative venous thromboses were treated surgically by repair of the venous anastomosis (**Figure 1**), and 6/16 (37.5%) were treated by venotomy for thrombectomy. Four (25.0%) patients underwent intraoperative transplantectomy, and 1 (6.3%) underwent secondary transplantectomy (**Figure 1**).

In group 2, the time to onset of venous thrombosis was 48 [24–120] hours (**Table 3**) after transplant surgery. Management was almost exclusively surgical (159/160, 99.4%), with the exception of one patient who received heparin therapy alone (**Figure 1**). In 94/159 patients (59.1%), transplantectomy was performed immediately because of a necrotic renal graft. For the other 65 patients (40.9%), the 2 main surgical techniques used were venotomy and thrombectomy (N = 37) or thrombectomy added to venous anastomosis repair (N = 22). For one patient, the type of surgical revision was not specified (**Figure 1**). Surgical revision was accompanied by primary procedure failure in 23/159 (14.5%) cases with intraoperative transplantectomy. For 42 patients (42/159; 26.4%), surgical revision was said to be satisfactory because of macroscopically satisfactory revascularization of the graft (**Figure 1**). However, 22/42 (52.4%) patients required a secondary transplantectomy during the initial stay, and only 13/42 (31.0%) had a functional graft vs. 10/11 (90.9%) in G1 ($p = 0.001$). Among the 7 patients discharged with a non-functional graft in place, 2 died in the weeks that followed, 3 benefited from a transplantectomy several months after the transplant for graft intolerance syndrome, 1 benefited from graft embolization, and the last patient kept the graft in place.

In 96 patients (62.8%), a pathology report of the allograft nephrectomies was available for analysis and confirmed the renal infarction due to renal thrombosis. None of the patients had signs of acute rejection.

Complications of Early Postoperative RT Vein Thrombosis

The majority of patients in our cohort had significant blood loss defined as the need for at least one red blood cell transfusion (N = 131; 74.4%). Among them, 5 patients (2.8%) presented

TABLE 1 | Characteristics of the kidney transplant recipients (N = 176).

	All (N = 176)	G1 (N = 16)	G2 (=160)	*p*
Cardiovascular risk factors				
Age	56.5 [45.5–65.0]	61.5 [45–70.5]	55.0 [45.5–64.5]	0.33
Male	99 (56.2)	6 (37.5)	93 (58.1)	0.11
BMI at transplant. kg/m^2	25.8 [22.6–29.3]	28.7 [25.5–31.4]	25.7 [22.4–29.2]	**0.03**
Diabetes	31 (17.6)	5 (31.2)	26 (16.3)	0.30
Smoking	29 (16.4)	2 (13.3)	27 (16.7)	0.67
Risk factors for venous thrombosis				
History of venous thrombosis	34 (19.3)	6 (37.5)	28 (17.5)	**0.05**
Vein thrombosis on a previous graft	3 (1.7)	0	3 (1.8)	0.52
Arteriovenous fistula thrombosis	15 (8.5)	3 (18.7)	12 (7.5)	0.12
Deep vein thrombosis and/or pulmonary embolism	34 (19.3)	6 (37.5)	28 (17.5)	0.05
Central line thrombosis	1 (0.6)	0	1 (0.6)	1.00
Systemic pathologies	16 (9.1)	1 (6.3)	15 (9.3)	1.00
Systemic autoimmune diseases	2 (1.1)	0	2 (1.3)	
Coagulation disorders	9 (5.1)	1 (6.3)	8 (5)	
Haematological pathologies	5 (2.8)	0	5 (3.1)	
Surgery within previous 3 months	6 (3.6)	3 (18.7)	3 (1.8)	**0.01**
Initial Nephropathy				0.18
Glomerulopathy	53 (30.1)	4 (25.0)	48 (27.7)	
Vascular nephropathy	25 (14.2)	6 (37.5)	19 (10.7)	
Polycystic kidney disease	23 (13.1)	2 (12.5)	21 (13.1)	
Diabetic nephropathy	21 (11.9)	3 (18.7)	18 (11.2)	
Unknown	18 (10.2)	0	18 (11.2)	
Malformative uropathies	14 (7.9)	0	14 (7.9)	
Chronic Interstitial Nephropathy	14 (7.9)	1 (6.25)	13 (7.3)	
Vasculitis/Connectivities	8 (4.6)	0	8 5)	
Usual treatment at Transplantation Day				
Anticoagulants	13 (7.3)	0	13 (8.1)	0.23
Antiaggregants	38 (21.6)	4 (25)	34 (21.2)	0.72
Induction immunosuppressive regimen	N = 146	N = 16	N = 130	
Thymoglobulin	91 (62.3)	10 (62.5)	81 (62.3)	0.71
Basiliximab	51 (34.9)	5 (31.2)	46 (32.3)	0.89
Unknown	4 (2.7)	1 (6.2)	3 (2.3)	

Data are presented as the number of patients (associated percentages), as the mean ± standard deviation, or as the median [interquartile range]. Bold values denote statistical significance at the p< 0.05 level.

Abbreviations: BMI, body mass index; G1, Group 1 included patients with intraoperative renal vein thrombosis; G2, Group 2 included patients with a postoperative diagnosis of eRVTPT.

haemorrhagic shock, and 29 developed large haematomas (29/176; 16.5%). Thirty-one patients presented concomitant deep vein thrombosis and/or pulmonary embolism (**Table 3**). All received curative anticoagulant treatment that may contribute to significant blood loss. Thirty-eight patients presented a postoperative infection (**Table 4**).

Five patients (5/176; 2.8%) died during initial management, including 4/176 (2.3%) within the first 15 days of transplantation. In G1, 1 patient died on day 4 from haemorrhagic shock. Four patients died in G2: 1 in the operating room from haemorrhagic shock during transplantectomy, 1 on day 6 from anaemia and hyperkalaemia, and the other 2 on day 11 and day 61 because of multivisceral failure, preceded by multiple repeat operations (**Table 4**).

Patient Outcome After Hospital Discharge

Overall, the proportion of patients with a functional graft at discharge was 13.1% (N = 23). The proportion of patients with a functional graft at discharge from the hospital in the G1 group compared with the G2 group was 10/16 (62.5%) and 13/160 (8.1%), respectively ($p < 0.001$). A sensitivity analysis excluding patients who underwent dual kidney transplantation (N = 8) or multiorgan transplantation (N = 5) exhibited a similar rate of remission (data not shown). In multivariate analysis, patients who had a postoperative diagnosis of eRVTPT had a lower probability of having a functional graft at discharge (OR = 0.016, 95% CI [0.002; 0.119], $p < 001$).

All these grafts were also functional at 1 year. The median serum creatinine at 1 year was 155 [130–207] μmol/L, with similar values in the 2 groups (data not shown).

At 5 years, 16 patients had a functional graft, 2 patients were dialysed, 1 patient died, and 4 were lost to follow-up. In addition, 44/153 (29.1%) of the patients who lost their graft were able to receive a new transplant. None of the patients had thrombosis of their new graft (**Table 4**).

DISCUSSION

To our knowledge, this study is the largest series describing the management of eRVTPT (<14 days) and the first to describe the

TABLE 2 | Transplantation characteristics (N = 176).

	All (N = 176)	G1 (N = 16)	G2 (N = 160)	*p*
Donors				
Age. median (IQR)	60 [46–71]	64.2 [48–78]	57.9 [45–70]	0.16
Deceased donor with expanded criteria	86/170 (50.6)	9/16 (56.2)	77/154 (50)	0.31
Living donor	10 (5.6)	0	10 (6.25)	0.60
Disseminated intravascular coagulation	2 (1.1)	0	2 (1.25)	0.65
Cold ischaemic time. min (N = 161)	918 [714–1259]	868 [780–1534]	919 [615–1255]	0.47
Difficult cannulation	4/155 (2.6)	2 (12.5)	2 (1.25)	**0.004**
Transplant				
First kidney transplantation	144/171 (84.2)	15 (93.7)	129 (83.2)	0.71
Single kidney	163 (93)	16 (100)	147 (91.8)	0.24
Dual kidney	8 4)	0	8 (4.5)	0.36
Multiorgan transplantation	5 3)	0	5 (3.13)	0.47
Right kidney	101/175 (57.7)	8 (50)	93 (58.4)	0.51
Immunology (N = 124)				
DSA	7 (5.6)	1/16 (6.3)	6/108 (5.6)	0.91
Panel-reactive antibody ≥85%	16 (12.9)	2/16 (12.5)	14/108 (12.9)	0.95
Intraoperative data				
Warm ischaemic time. min (N = 161)	45 [34–55]	47 [42–55]	475 [33–55]	0.60
Vein anatomy abnormality	23/172 (13.4)	1 (6.2)	22/156 (14.1)	0.58
Multiple veins with at least one sacrificed	11 (6.4)	0	11/156 (7.1)	
Vascular wounds at retrieval	10 (5.8)	0	9/156 (5.7)	
Fibromuscular dysplasia	2 (1.2)	1 (6.2)	1/156 (0.6)	
Venous anastomosis revision	19/159 (11.9)	9/16 (56.2)	10/143 (6.9)	**<0.001**
Renal vein twist	1 (0.6)	0	1/143 (0.7)	
Partial thrombus	2 (1.3)	0	2/143 (1.4)	
Strangulation of the iliac vein	1 (0.6)	0	1/143 (0.7)	
Not specified	3 (1.9)	0	3/143 (2.1)	
Arterial anatomy abnormality	55/170 (32.3)	4/16 (25.0)	51/144 (35.4)	0.41
Multiple arteries	37 (2.8)	0	37/144 (25.7)	
Atherosclerotic plaque	14 (8.2)	2/16 (12.5)	12/144 (8.3)	
Vascular wounds at retrieval	2 (1.2)	2/16 (12.5)	0	
Not specified	2 (1.2)	0	2/144 (1.3)	
Intraoperative heparin therapy	13/138 (9.4)	4/14 (28.5)	9/124 (7.3)	**0.01**
Vessel flushing	37/176 (21.0)	4/16 (25.0)	33/160 (20.6)	0.63

Data are presented as the number of patients (associated percentages). as the mean ± standard deviation. or as the median [interquartile range]. Bold values denote statistical significance at the p< 0.05 level.

DSA, donor-specific antigen; G1, Group 1 included patients with intraoperative renal vein thrombosis; G2, Group 2 included patients with a postoperative diagnosis of eRVTPT.

prognosis of intraoperative thrombosis (G1) and postoperative (G2) thrombosis. We reported an incidence of venous thrombosis of 1.4%, which is a rate comparable to that in the literature (6,16). In our cohort, venous thrombosis was responsible for graft loss in 86.4% of cases, a rate close to that in the literature (5,16). Only intraoperative thrombosis is associated with better graft survival (63.5%), which is probably due to the possibility of immediate management (13). Indeed, thrombosis of the RT vein is responsible for a decrease in blood flow at the microvascular level, resulting in renal ischaemia lesions. In the case of "surgical recovery," the RT has undergone new ischaemia–reperfusion lesions with the consequences of a delay in the resumption of function due to tubular necrosis or even cortical necrosis, chronic dysfunction of the graft due to endothelial-mesenchymal transition and acute or chronic rejection lesions (21–23). Thus, at discharge, the graft was functional after "satisfactory" revascularization in 10/11 patients with intraoperative thrombosis (G1) and in 13 of 42 patients with postoperative thrombosis (G2) ($p < 0.001$). Therefore, a major challenge is to preserve or remove the kidney at the time of salvage surgery in G2 patients to avoid complications, i.e., haemorrhage or infection, or a new nephrectomy surgery. To help the surgical decision-making process related to this emergency surgery (24–26), further tools must be investigated. Ultrasound, a first-line imaging examination, in particular contrast-enhanced ultrasound (27,28), may be helpful in verifying macrovessel vascularisation but also parenchymal perfusion. Contrast-enhanced ultrasound can be easily performed intraoperatively to assist in decision making in case of doubt during initial surgery (G1) (27,29) or to assess viability (27,30) of the RT during rescue surgery but also at the bedside (28) to confirm the diagnosis. In our cohort, the diagnosis of intraoperative RT vein thrombosis was made by intraoperative ultrasound in 5 patients with favourable outcomes in all cases.

eRVTPT management was almost exclusively surgical. Indeed, in our cohort, only one patient was treated with curative-dose heparin therapy, without success. In the literature, only one case of curative dose heparin therapy with preservation of graft function has been reported (31) in a patient with late-onset

TABLE 3 | Postoperative diagnosis of graft vein thrombosis (Group 2. N = 160).

Time from transplant to diagnosis. hours	**48 [24–120]**
Clinical signs suggestive of venous thrombosis	
Asymptomatic	22 (13.8)
Oligoanuria	101 (63.1)
Abnormal pain	43 (26.9)
Macroscopic haematuria	28 (17.5)
Haemodynamic disorders	14 (8.6)
Haemorrhagic shock	7 (4.3)
Productive Redon catheter (blood)	6 (3.7)
Fever	3 (1.9)
Others (oedema. testicular pain)	3 (1.9)
Biological criteria suggestive of venous thrombosis	
None	37 (23.1)
Increased serum creatinine	81 (50.6)
Increased LDH levels	17 (10.6)
Hyperlactatemia	10 (6.3)
Thrombopenia	6 (3.7)
Anaemia	5 (3.1)
Inflammatory syndrome	3 (1.8)
Increased CK levels	2 (1.2)
Hyperkalaemia	2 (1.2)
Increased AST levels	1 (0.6)
Radiological examinations	
None	3 (1.9)
Doppler ultrasound	69 (42.5)
Computed tomography angiography	22 (13.8)
Doppler ultrasound and MRI	6 (3.8)
Doppler ultrasound + computed tomography angiography	61 (3.1)
Time between first radiological examination and second surgery. min (*n* = 75) 180 [115–342]	
1 exam (N = 41)	180 [106–300]
2 exams (N = 34)	215 [118–344]
Concomitant thrombosis. yes	44 (27.5)
Deep vein thrombosis	13 (8.1)
Thrombosis of the graft artery	13 (8.1)
Pulmonary embolism	10 (6.3)
Arteriovenous fistula thrombosis	8 (5.0)

Data are presented as the number of patients (associated percentages) or as the median [interquartile range].
AST, aspartate aminotransferase; CK, creatinine kinase; LDH, lactate dehydrogenase; MRI, magnetic resonance imaging.

venous graft thrombosis more than 9 years after transplantation. The use of heparin in our cohort was infrequent, both at the time of surgery (15.0%) and as a curative measure after surgery (6.3%). These data are probably explained by the fear of bleeding risk immediately posttransplantation (17), urging caution by medical and surgical teams. Exceptionally, other therapies have been reported in cases of early thrombosis, such as thrombolysis (15,32) or thromboaspiration followed by heparin therapy in curative doses (14). These techniques are most often proposed in cases of late venous thrombosis (33–35).

It should be noted that an immediate transplantectomy was performed in nearly 3 out of 5 cases when the graft was necrotic at the time of the revision surgery (G2). In the case of a viable graft, the two most common revascularization techniques were thrombectomy by venotomy and anastomotic repair. In the face of intraoperative venous thrombosis (G1), anastomotic repair is the most favoured technique. This is most often justified by a surgical imperfection at the origin of this thrombosis, requiring complete repair of the venous anastomosis: a twist in the vein, strangulation of the iliac vein, folding of the vein during positioning of the graft, folding over a long vein, a disparity in calibre between the vessels of the graft and those of the recipient, or external compression (3). The 2 complete explantations of the graft with flushing and reimplantation were not effective, contrary to the results reported in a retrospective series of 5 patients with venous complications. However, only 1 patient had vein thrombosis (36).

Morbidity remains high following the occurrence of venous thrombosis. Indeed, in our data collection, we observed 5 deaths, 4 of which occurred within the first 15 days of the transplant. The other complications observed were 5 cases of haemorrhagic shock, 38 (21.6%) postoperative infections, and a requirement for blood transfusion in more than half of the patients (55.1%). This may limit access to a new transplant due to immunization against the human leukocyte antigen system (37,38). However, 44 patients who lost their graft (29.1%) received a new kidney transplant after a median waiting time of 1 year. Indeed, the French Biomedicine Agency takes into account list seniority on the transplant waiting list in cases of early loss of graft function below 3 months.

Our work has several limitations. First, we report the results of a retrospective cohort. Thus, some difficulties during kidney retrieval or transplantation may have been overlooked. Second, at the time of revision surgery (G2), 94 (59.7%) transplantectomies were performed on a necrotic graft. There may have been a delay in diagnosis and/or management. Indeed, the clinical signs of venous thrombosis are aspecific (pain, oligoanuria, macroscopic haematuria) but must evoke the diagnosis (2). Serum LDH monitoring can aid in the diagnosis of thrombosis and should be measured daily during initial hospitalization (39). On the other hand, when the diagnosis was highly suspicious on ultrasound (40), 67/157 (42.7%) patients underwent another imaging procedure, which may have increased the delay in management. Therefore, the median time between the first radiological examination and salvage surgery was 180 [115–342] minutes (**Table 3**). In our study, 5/67 (7.5%) patients who benefited from two radiological examinations had a functional graft at discharge compared with 8/93 (8.6%) who had only one or none, p = NS. Third, we cannot exclude that some patients had abdominal compartment syndrome manifested by profuse bleeding (N = 13). All but one had immediate transplantectomy. The last patient underwent haematoma evacuation with a favourable outcome. In the case of suspected renal compartment syndrome, placing the graft intraperitoneally during salvage surgery may be proposed. Another limitation of our study is the absence of a control group, which prevents us from comparing medical (thrombophilia) and surgical aetiologies. Indeed, many of the following risk factors were identified (41): the occurrence of a perioperative haemodynamic disorder in the recipient, a history of thrombosis and/or diabetes in the recipient, and deceased donors aged less than 6 years or more than 60 years. This last factor remains controversial (42). In our series, grafts from marginal donors (18) represented approximately 51% of our cohort, which is comparable to the data from the French Biomedicine Agency (43). It has also been reported that there is an increased risk of thrombosis in the case of a right kidney, as in our study (44,45).

TABLE 4 | Complications associated with early RT vein thrombosis (N = 176).

	All (N = 176)	G1 (N = 16)	G2 (N=160)	*p*
Mortality	5 (2.8)	1 (6.2)	4 (2.5)	0.39
Intraoperative death during revision surgery	1 (0.6)	0	1 (0.6)	1.00
Between Day 0 and Day 15	3 (1.7)	1 (6.2)	2 (1.2)	0.25
Between Day 15 and Day 30	0	0	0	NA
Between Day 30 and Day 90	1 (0.6)	0	1 (0.6)	1.00
Complications				
Blood transfusion	97 (55.1)	9 (56.2)	88 (55)	0.92
Haematoma	29 (16.5)	1 (6.2)	28 (17.5)	0.48
Haemorrhagic shock	5 (2.8)	2 (12.4)	3 (1.8)	0.07
Postoperative infection	38 (21.6)	1 (6.2)	37 (23.1)	0.20
Urinary tract infection	11 (6.2)	0	11 (6.9)	0.60
Surgical site infection	9 (5.1)	1 (6.2)	8 (5.0)	0.59
Pneumonia	8 (4.5)	0	8 (5.0)	1.00

Data are presented as the number of patients (associated percentages) or as the median [interquartile range].

In conclusion, our study confirms the extreme severity of early venous thrombosis of the renal graft, which is responsible for graft loss in the vast majority of cases, particularly in the case of postoperative thrombosis. Although the prognosis is poor, its management is mostly surgical and relies on immediate intraoperative venotomy for thrombectomy or thrombectomy and anastomotic repair. Further studies should allow us to better identify patients at risk of venous thrombosis to ensure close monitoring and to facilitate the development of appropriate thromboprophylaxis protocols.

COLLABORATORS FOR THIS STUDY

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DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: General Data Protection Regulation - CNIL- https://www.cnil.fr. Requests to access these datasets should be directed to the corresponding author.

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

LC collected data, analysed data, and wrote the paper. CM designed the research/study, analysed data, and wrote the paper. NT collected data. PM collected data. M-OT collected data. DA collected data. LB collected data. EM collected data. TP collected data. NK collected data. AL collected data. PP collected data. CU-C collected data. BP, analysed data and contributed important reagents. AH collected data. CG designed the research/study, analysed data, and wrote the paper. LG designed the research/study and wrote the paper.

CONFLICT OF INTEREST

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

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Predictors and Adverse Outcomes of Acute Kidney Injury in Hospitalized Renal Transplant Recipients

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Data about in-hospital AKI in RTRs is lacking. We conducted a retrospective study of 292 RTRs, with 807 hospital admissions, to reveal predictors and outcomes of AKI during admission. In-hospital AKI developed in 149 patients (51%). AKI in a previous admission was associated with a more than twofold increased risk of AKI in subsequent admissions (OR 2.13, $p < 0.001$). Other major significant predictors for in-hospital AKI included an infection as the major admission diagnosis (OR 2.93, $p = 0.015$), a medical history of hypertension (OR 1.91, $p = 0.027$), minimum systolic blood pressure (OR 0.98, $p = 0.002$), maximum tacrolimus trough level (OR 1.08, $p = 0.005$), hemoglobin level (OR 0.9, $p = 0.016$) and albumin level (OR 0.51, $p = 0.025$) during admission. Compared to admissions with no AKI, admissions with AKI were associated with longer length of stay (median time of 3.83 vs. 7.01 days, $p < 0.001$). In-hospital AKI was associated with higher rates of mortality during admission, almost doubled odds for rehospitalization within 90 days from discharge and increased the risk of overall mortality in multivariable mixed effect models. In-hospital AKI is common and is associated with poor short- and long-term outcomes. Strategies to prevent AKI during admission in RTRs should be implemented to reduce re-admission rates and improve patient survival.

Keywords: acute kidney injury, calcineurin inhibitors, readmission, renal transplant recipients, mortality abbreviations

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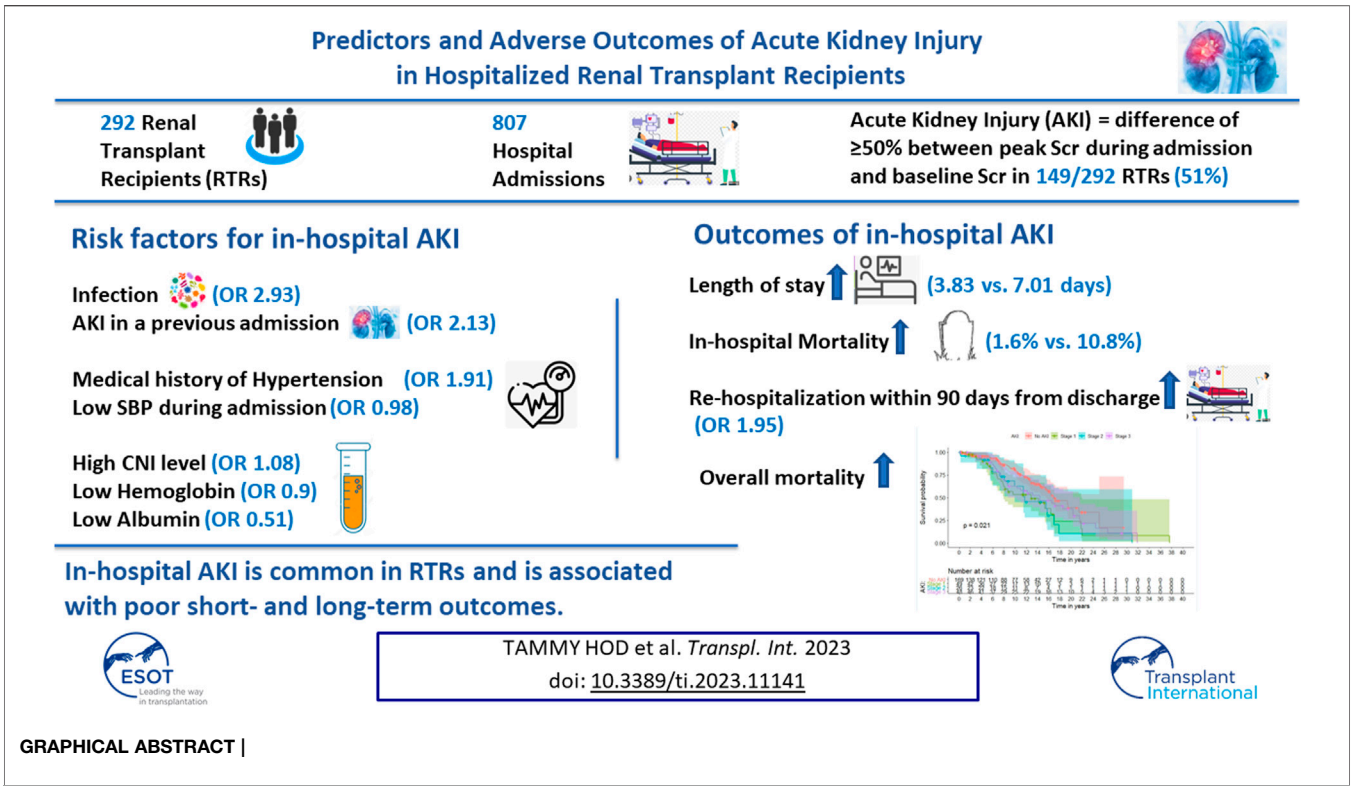
Abbreviations: BMI, body mass index; CHF, congestive heart failure; CI, confidence interval; CNI, calcineurin inhibitor; DBP, diastolic blood pressure; DM, diabetes; eGFR, estimated glomerular filtration rate; ESRD, end stage renal disease; HTN, hypertension; ICU, intensive care unit; IHD, ischemic heart disease; KDIGO, Kidney Disease Improving Global Outcomes; LOS, length of stay; MPA, mycophenolic acid; OR, odds ratio; RTR, renal transplant recipients; SBP, systolic blood pressure; Scr, serum creatinine; SD, standard deviation.



GRAPHICAL ABSTRACT |

INTRODUCTION

The prevalence of chronic kidney disease is increasing, accounting for more than 10% of hospital admissions in the adult population. The parallel increase in the rates of in-hospital acute kidney injury (AKI) may reach as much as 50% of intensive care unit (ICU) admissions (1, 2). The consequences of AKI during hospitalization are dismal (3, 4): Even modest changes in serum creatinine (Scr) (an increase >0.5 mg/dL) have been associated with a 6.5-fold increase in the odds of death and a 3.5-day increase in the length of stay (LOS) (5). Small changes in Scr have been also associated with increased mortality and prolonged hospitalizations in elderly patients admitted with congestive heart failure (6).

With the aim of preventing this serious complication, different studies have sought to establish predictors for AKI during hospitalization, both in the general population (7–11) and particularly for renal transplant recipients (RTRs). Unfortunately, however, information on predictors for AKI during hospitalization of RTRs is still lacking, although 11% of RTRs develop in-hospital AKI during the first three post-transplant years, which is associated with transplant failure and death (12). For RTRs, studies to date have focused mostly on delayed graft function, which is a form of AKI in the immediate peri-transplant period (13, 14).

RTRs constitute a unique population with an inherent increased risk vs. the general population for in-hospital AKI secondary to different etiologies related to subclinical and chronic rejection, higher risk of infections and immunosuppressive therapy. As a result, strategies to prevent or minimize the occurrence and consequences of AKI during hospitalization in this population would necessarily be more complex than those for the general population.

Renal allograft survival has improved significantly in the short term, with one-year graft survival rates reaching 98.4% (15). However, ensuring long-term graft survival still poses a very significant challenge in renal transplantation. For RTRs, a better understanding of the risk factors for AKI during admission would form the basis for developing preventive therapeutic measures aimed at reducing the rate of in-hospital AKI, resulting in improved long-term renal allograft survival.

In this study, we sought to pinpoint the risk factors for AKI during hospitalization of RTRs in a non-intensive care setting. In addition, we examined the implications of in-patient AKI for in-hospital mortality, duration of hospitalization, subsequent in-hospital AKI, re-hospitalizations, and overall mortality in this vulnerable population.

MATERIALS AND METHODS

Study Population and Design

Clinical and biochemical parameters were collected retrospectively from the MdClone system, the data acquisition tool at Sheba Medical Center. Additional data was collected from clinical records, as needed. The study was approved by the local ethics committee (IRB approval number: SMC-70-5320). Data was collected for up to 12 admissions post "Renal Transplant"

diagnosis for admission dates falling between July 2007 and November 2020.

The initial dataset included 1405 hospitalizations for 399 transplant recipients. We then excluded from the analysis admissions post graft loss (baseline eGFR<15 mL/min per 1.73 m^2), post chronic dialysis initiation, and/or admissions ≤30 days from transplant to eliminate the effect of changes in immunosuppressive medications or in renal allograft function early post-transplant secondary to slow and/or delayed graft function, infections and early rejections. Hospitalizations with no Scr or only one Scr measured during admission were also excluded (**Figure 1**). The final study cohort included 292 RTRs who had undergone kidney transplantation between June 1982 and June 2000, with a total of 807 non-ICU admissions.

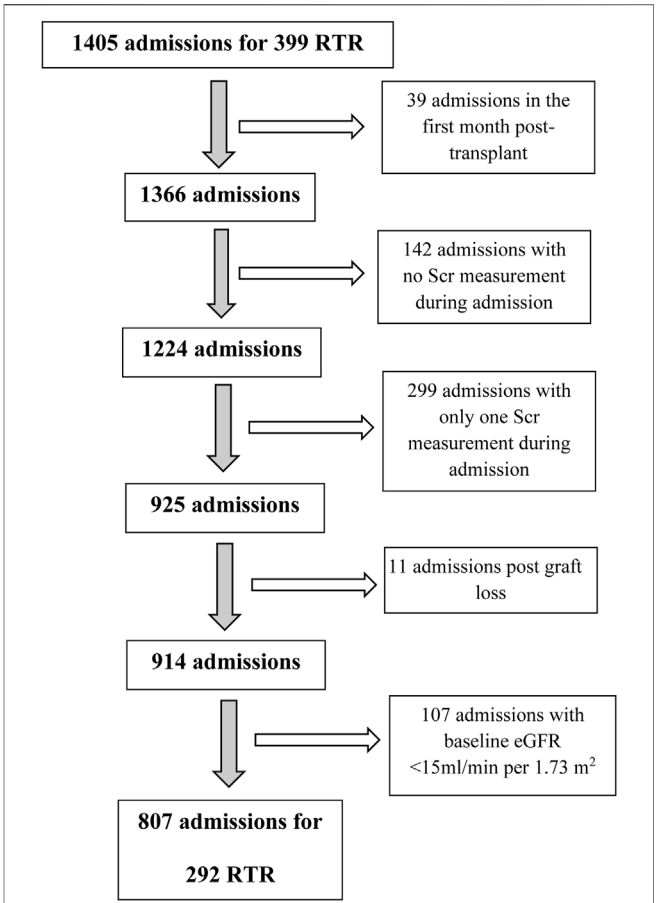


FIGURE 1 | Consort diagram. RTR, renal transplant recipients; Scr, serum creatinine.

Primary Outcome

The primary outcome was AKI during admission, which was defined as a difference of ≥50% between peak Scr during admission and baseline Scr, according to the Kidney Disease Improving Global Outcomes (KDIGO) definition. AKI staging was based on the KDIGO definitions for SCr increases, i.e., a difference between peak Scr during admission and baseline Scr that was: Stage 1, ≥1.5–1.9 times the baseline Scr; Stage 2, ≥2–2.9 times the baseline Scr; and Stage 3, >3 times the baseline Scr or a peak Scr during admission ≥4.0 mg/dL or initiation of renal replacement therapy.

To determine baseline eGFR, we chose the minimum Scr in the 120 days to 1 day prior to admission. For admissions without Scr measurements in the 1- to 120-day period, we used minimum Scr during admission. To avoid misjudgment of baseline renal allograft function affected by frequent changes in Scr early post-transplant, we used minimum Scr during admission for baseline eGFR assessment in admissions of less than 150 days from transplant.

Data Extraction and Study Assessments

The following information was extracted from electronic patient records: age, gender, etiology of end stage renal disease (ESRD), dialysis pre-transplant (yes/no), transplant number, donor type, transplant date and relevant medical history, specifically hypertension, congestive heart failure (CHF), ischemic heart disease (IHD) and pre-transplant diabetes. All diagnoses during admissions were obtained from MDClone. After a manual review of in-patient diagnoses, the main hospitalization etiology was selected, and diagnoses were grouped into five categories: infectious disease, cardiovascular disease, disease of the gastrointestinal system, neoplasm, and all others.

The following biochemical parameters during hospitalization were retrieved in an automated fashion from MDClone: average and maximum tacrolimus trough level, average total white blood cell count, average and minimum absolute lymphocyte count, average hemoglobin, average albumin, maximum and minimum glucose, maximum globulins, and average C-reactive protein. The following additional clinical and biochemical information during admission was also retrieved from MDClone: average and minimum systolic and diastolic blood pressures, average weight and body mass index (BMI), fever, maximum pulse, and minimum oxygen saturation. Use and average dose administered during admission for the following medications was automatically obtained from MDClone: tacrolimus, cyclosporine, mycophenolic acid (MPA) (for 238 admissions, mycophenolate dose was converted to the equivalent MPA dose by dividing mycophenolate dose by 1.388) and steroids (steroid derivatives used during admission, such as hydrocortisone, dexamethasone and methylprednisolone, were converted to the equivalent prednisone dose). Other medications administered during admission were also recorded.

Statistical Analysis

All demographic, clinical and biochemical covariates of interest were tabulated and compared between patients for AKI during admission (yes/no) and between admissions (with and without AKI, and partitioned into AKI stage for AKI patients). Categorical variables were compared using the Chi-squared test (or Fisher's test where the numbers were small), while continuous variables were first tested for normality using the Shapiro-Wilks Test (and for equality of variances), and were then compared using a t-test (or Anova) for normally distributed variables or a-parametric tests for non-normally distributed

variables. An FDR [false discovery rate (Benjamini and Hochberg)] procedure was then carried out to correct for multiple comparisons.

For the primary outcome of AKI during admission, logistic mixed models were used, with RTR being a random effect, and other variables being fixed effects. Univariate models were considered first; variables that were significant ($p = 0.05$) and/or those with clinical importance were entered into multivariate models.

For the secondary outcomes, LOS was examined using a linear mixed model, with RTR as a random effect and the other variables being fixed effects. LOS (which is inherently right-tailed) was log-transformed to normalize it. Mortality during admission was modeled using the Cox proportional hazards model, which modeled the time from transplant to death or end of follow-up, taking into account the number of admissions per person. Overall mortality was modeled using Kaplan-Meier estimation, with AKI in different staging groups. Thereafter, the Cox proportional hazards model was used to model the time from transplant to death or end of follow-up. Readmission within 90 days was calculated. Logistic mixed models were used to predict the cause of readmission within 90 days, with RTR being a random effect, and other variables being mixed effects. For all secondary outcomes, univariate models were considered first. Significant variables and/or those with clinical importance were entered into multivariate models.

All statistical analyses were carried out using R-3.4.1 [R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/].

RESULTS

Characteristics of the RTRs Cohort

A total of 292 RTRs comprised our study cohort (**Table 1**). Median transplant age was 54.9 (IQR, 46.2–64.2); 98 (33.6%) were females. Median time from transplant to first admission was 6.33 years (IQR, 2.5–11.8). Of the RTR cohort, 64 (21.9%), 39 (13.4%), 59 (20.2%) and 26 (8.9%) patients had ESRD secondary to diabetic nephropathy, autosomal dominant polycystic kidney disease (APCKD), glomerulonephritis and nephrosclerosis, respectively; 158 (54.1%) were on renal replacement therapy before the transplant; and 140 (47.9%) had a living donor renal transplant. Forty RTRs (13.7%) died during admission, and the overall mortality rate for the duration of the study was 41.1%. The total number of admissions ranged between 1 and 10.

Univariate Comparison of RTRs Without Any AKI vs. With Any AKI During Admission

Of the 292 RTRs, 149 (51%) had 1 to 8 AKI admission events. For patients with any AKI during admission, the number of admissions per person, the death rate during admission, and the overall mortality were all higher ($p < 0.001$). ESRD secondary to APCKD and renal living donor transplant were more common in RTRs without vs. with any AKI during admission as the difference between the groups approached statistical significance. All other comparisons of characteristics, including age, sex, time from transplant to first admission, transplant number and dialysis pretransplant, are shown in **Table 1**.

Characteristics of Total Admissions

Our cohort of 292 RTR had a total of 807 non-ICU admissions. Median age during admission was 66.75 (IQR 57.16–73.12); 266 (33%) were females. Median time from transplant to admission was 7.65 years (IQR 4.22–12.75). The most prevalent admission etiology [312 (38.7%) admissions] was an infection. Forty (4.96%) admissions resulted in death during admission. Median LOS was 4.65 days (IQR 2.67–9). For 302 (37.4%) admissions, patients were readmitted within 90 days (**Table 2**).

Univariate Comparison of Admissions Without vs. With AKI During Admission

An AKI during admission was recorded for 297 of 807 (36.8%) admissions. In admissions with AKI vs. admissions without AKI, ESRD secondary to APCKD was less prevalent, while nephrosclerosis was more common ($p = 0.06$). RTRs with at least one AKI had higher rates of hypertension and IHD (67% and 43.1% compared to 58.2% and 32.2% of admissions without AKI, $p = 0.037$ and 0.006, respectively). The main admission diagnosis was an infection in 142 (47.8%) of admissions with AKI vs. 170 (33.3%) in admissions without AKI ($p < 0.001$). Significant differences in the various vital signs monitored during admission [maximum temperature, maximum, minimum and average pulse, minimum and average systolic blood pressure (SBP) and diastolic blood pressure (DBP), and O_2 saturation] were observed between admissions with and without AKI (**Table 2**). Steroids use and average dose during admission were significantly higher in admissions with vs. without AKI [216 (72.2%) vs. 333 (65.3%), $p = 0.06$ (after adjustment for multiple comparisons) and 22.1 (SD 41.01) vs. 12.27 (SD 26.52) mg, $p < 0.001$ respectively]. The use of loop diuretics and of proton pump inhibitors was also significantly higher in admissions with vs. without AKI, as opposed to the use of renin-angiotensin-aldosterone system (RAAS) inhibitors, which was lower in admissions with vs. without in-hospital AKI. Laboratory results also differed significantly between admissions with and without AKI. Total white blood cell count, maximum glucose, maximum globulins and C-reactive protein levels were higher in admissions with vs. without AKI, whereas lymphocytes (absolute minimum), average and minimum hemoglobin, minimum glucose and average and minimum albumin were lower in admissions with vs. without AKI. Maximum tacrolimus 12-h trough level was significantly higher in admissions with vs. without AKI [7.1 (IQR 4.05–10.9) vs. 5.7 (4.05–8.5), $p = 0.028$]. Rates of death during admission and readmission within 90 days as well as LOS were significantly higher for those with vs. without AKI (**Table 2**).

Univariate Comparison of AKI Stages 1, 2 and 3 During Admission

Of 297 admissions with AKI during admission, 134 (45.1%), 70 (23.6%) and 93 (31.3%) presented with AKI Stages 1, 2 and 3, respectively. The rate of female RTRs fell as AKI progressed

TABLE 1 | Demographic and clinical characteristics of RTRs, stratified by AKI during admission.

Variable	Total cohort (*n* = 292)	Without AKI (*n* = 143)	With any AKI (*n* = 149)	*p*
RTR characteristics				
Transplant age, years [median (IQR)]	54.98 (46.2, 64.2)	55.53 (47.5, 64.51)	54.21 (45.2, 64.2)	0.89
Female sex, n (%)	98 (33.56)	48 (33.57)	50 (33.56)	1
Transplant to 1st admission, years [median (IQR)]	6.33 (2.45, 11.81)	6.7 (1.9, 11.2)	6.3 (3.2, 12.2)	0.35
ESRD etiology, *n* (%)				
ADPCKD	39 (13.36)	28 (19.58)	11 (7.38)	0.07
Diabetic nephropathy	64 (21.92)	31 (21.68)	33 (22.15)	
Glomerulonephritis	59 (20.21)	26 (18.18)	33 (22.15)	
Nephrosclerosis	26 (8.9)	12 (8.39)	14 (9.4)	
Other	69 (23.63)	29 (20.28)	40 (26.85)	
Unknown	35 (11.99)	17 (11.89)	18 (12.08)	
Pre-transplant dialysis				
Yes	158 (54.11)	72 (50.35)	86 (57.72)	0.11
No	46 (15.75)	29 (20.28)	17 (11.41)	
Unknown	88 (30.14)	42 (29.37)	46 (30.87)	
Transplant type, *n* (%)				
Kidney only	280 (95.89)	138 (96.5)	142 (95.3)	1
Liver kidney	4 (1.37)	2 (1.4)	2 (1.34)	
Heart kidney	7 (2.4)	3 (2.1)	4 (2.68)	
Pancreas kidney	1 (0.34)		1 (0.67)	
Transplant number, *n* (%)				
1	262 (89.73)	127 (88.81)	135 (90.6)	0.66
2	26 (8.9)	13 (9.09)	13 (8.72)	
3	4 (1.37)	3 (2.1)	1 (0.67)	
Donor type, *n* (%)				
Living	140 (47.9)	82 (57.34)	66 (44.3)	0.08
Deceased	85 (29.11)	36 (25.17)	49 (32.89)	
Unknown	59 (20.21)	25 (17.48)	34 (22.82)	
Number of admissions, *n* (%)				
1	116 (39.73)	86 (60.14)	30 (20.13)	**<0.001****
2	55 (18.84)	29 (20.28)	26 (17.45)	
3	33 (11.3)	10 (6.99)	23 (15.44)	
4	31 (10.62)	9 (6.29)	22 (14.77)	
5	22 (7.53)	3 (2.1)	19 (12.75)	
6	15 (5.14)	3 (2.1)	12 (8.05)	
7	7 (2.4)	2 (1.4)	5 (3.36)	
8	9 (3.08)		9 (6.04)	
9	3 (1.03)	1 (0.7)	2 (1.34)	
10	1 (0.34)		1 (0.67)	
Number of AKI (per person), *n* (%)				
0		143 (100)		
1			76 (26.03)	
2			30 (10.27)	
3			25 (8.56)	
4			11 (3.77)	
5			2 (0.68)	
6			4 (1.37)	
8			1 (0.34)	
Death during admission, *n* (%)	40 (13.7)	5 (3.5)	35 (23.49)	**<0.001****
Overall mortality, *n* (%)	120 (41.1)	40 (27.97)	80 (53.69)	**<0.001****

*p < *0.05*; **p < *0.01*.

ADPKD, autosomal dominant polycystic kidney disease; ESRD, end stage renal disease, RTR, renal transplant recipient. Bold values are the p *values which are significant (<0.05 or <0.01).*

(39.6%, 31.4% and 23.7% in AKI stages 1, 2 and 3 respectively, $p = 0.13$). Time from transplant to admission increased from 6.95 (IQR 3.8–11.5) to 8.05 (IQR 4.65–12.3) to 8.7 years (IQR 5.9–16.5) as in-hospital AKI stage increased from 1 to 2 to 3, respectively ($p = 0.04$). Vital signs monitored during admission, such as maximum pulse, increased, whereas minimum SBP and minimum oxygen saturation decreased with worsening AKI stage. MPA use and average dose decreased, whereas average steroid dose increased with progression of AKI. Minimum glucose and average albumin decreased as in-hospital AKI progressed. LOS increased as AKI stage increased, but this was not statistically significant, possibly due to the low number of patients in each group. Rates of death during admission and readmission within 90 days increased as AKI worsened (**Table 3**).

TABLE 2 | Demographic, clinical and biochemical characteristics of total admissions stratified by the presence or absence of AKI during admission.

Variable	Total admissions (*n* = 807)	Admissions without AKI (*n* = 510)	Admissions with AKI (*n* = 297)	*p*[a]
RTR characteristics				
Admission age, years [median (IQR)]	66.75 [57.2, 73.1]	66.9 [58, 73.2]	66.5 [56, 73]	0.65
Female sex, *n* (%)	266 (33)	169 (33.1)	97 (32.7)	0.95
Transplant to admission, years [median (IQR)]	7.65 [4.2, 12.8]	7.6 [3.8, 12.6]	7.7 [4.8, 13.5]	0.43
ESRD etiology, *n* (%)				
ADPCKD	81 (10)	63 (12.4)	18 (6.1)	0.06
Diabetic nephropathy	187 (23.2)	119 (23.3)	68 (22.9)	
Glomerulonephritis	154 (19.1)	102 (20.0)	52 (17.5)	
Nephrosclerosis	73 (9)	40 (7.8)	33 (11.1)	
Other	212 (26.3)	124 (24.3)	88 (29.6)	
Unknown	100 (12.4)	62 (12.2)	38 (12.8)	
Pre-transplant dialysis				
Yes	449 (55.6)	289 (56.7)	160 (53.9)	0.75
No	125 (15.5)	79 (15.5)	46 (15.5)	
Unknown	233 (28.9)	142 (27.8)	91 (30.6)	
Transplant type, *n* (%)				
Kidney only	775 (96)	487 (95.5)	288 (97.0)	0.76
Liver kidney	7 (0.9)	5 (1.0)	2 (0.7)	
Heart kidney	23 (2.8)	17 (3.3)	6 (2.0)	
Pancreas kidney	2 (0.2)	1 (0.2)	1 (0.3)	
Transplant number, *n* (%)				
1	735 (91.1)	461 (90.4)	274 (92.3)	0.71
2	67 (8.3)	45 (8.8)	22 (7.4)	
3	5 (0.6)	4 (0.8)	1 (0.3)	
Donor type, *n* (%)				
Living	426 (52.8)	267 (52.4)	159 (53.5)	0.96
Deceased	239 (29.6)	152 (29.8)	87 (29.3)	
Unknown	142 (17.6)	91 (17.8)	51 (17.2)	
Medical history, *n* (%)				
Diabetes mellitus	273 (33.8)	169 (33.1)	104 (35.0)	0.7
Hypertension	496 (61.5)	297 (58.2)	199 (67.0)	**0.037***
IHD	292 (36.2)	164 (32.2)	128 (43.1)	**0.006****
CHF	170 (21.1)	98 (19.2)	72 (24.2)	0.19
Admission etiology, *n* (%)				
ID	312 (38.7)	170 (33.3)	142 (47.8)	**<0.001****
CV	174 (21.6)	132 (25.9)	42 (14.1)	
GI	64 (7.9)	48 (9.4)	16 (5.4)	
CA	38 (4.7)	23 (4.5)	15 (5.1)	
Others	219 (27.1)	137 (26.9)	82 (27.6)	
Vital signs and other clinical parameters during admission, [median (IQR)]				
Fever max, °C	37.2 [36.9, 37.9]	37.2 [36.9, 37.6]	37.4 [37, 38.4]	**<0.001****
Pulse max	96 [84, 110]	93 [81, 102]	103 [89, 120]	**<0.001****
Pulse min	61 [54, 69]	61 [55, 70]	60 [53, 67]	**0.04***
Pulse average	75.9 [68.1, 83.7]	75 [67, 82.5]	78 [70.1, 85.8]	**<0.001****
SBP min mmHg	103 [89, 117]	108 [96, 120]	95 [80, 108]	**<0.001****
SBP average mmHg	131.2 [118.8, 145.8]	132.6 [121.2, 147.1]	128.8 [115.5, 144.1]	**0.008***
DBP min mmHg	54 [46, 62]	56 [50, 63]	50 [40, 59]	**<0.001****
DBP average mmHg	70.7 [65.2, 76.6]	71 [66.4, 76.6]	69.8 [63.1, 76.6]	**0.04***
O_2 saturation min	93 [89, 95]	94 [90, 95]	91 [85, 94]	**<0.001****
Weight average	75 [64.5, 87.4]	75 [65, 90]	73.8 [64, 85.1]	0.34
BMI average	26.4 [23.1, 30.5]	26.5 [23.4, 30.8]	26.1 [22.8, 30.3]	0.46
Medications during admission				
Tacrolimus, *n* (%)	386 (47.8)	232 (45.5)	154 (51.9)	0.17
Tacrolimus average dose mg [mean (SD)]	1.49 (0.96)	1.46 (0.92)	1.53 (1.03)	0.7
Cyclosporine, *n* (%)	97 (12.0)	63 (12.4)	34 (11.4)	0.79
Cyclosporine average dose, mg [mean (SD)]	63.51 (30.15)	60.60 (23.68)	68.90 (39.30)	0.3
MPA, *n* (%)	321 (39.8)	212 (41.6)	109 (36.7)	0.28
MPA average dose, mg [mean (SD)]	157.8 (217.4)	164.1 (218.7)	146.9 (215.1)	0.4
Steroids, *n* (%)	549 (68.0)	333 (65.3)	216 (72.7)	0.06
Steroids average dose, mg [mean (SD)]	16.01 (33.13)	12.27 (26.52)	22.10 (41.01)	**<0.001****
mTOR inhibitors, *n* (%)	66 (8.2)	50 (9.8)	16 (5.4)	0.06
Azathioprine, *n* (%)	45 (5.6)	31 (6.1)	14 (4.7)	0.66
Loop diuretics, *n* (%)	339 (42)	193 (37.8)	146 (49.2)	**0.005***
Thiazides, *n* (%)	44 (5.4)	29 (5.7)	15 (5.1)	0.79

(Continued on following page)

TABLE 2 | (*Continued*) Demographic, clinical and biochemical characteristics of total admissions stratified by the presence or absence of AKI during admission.

Variable	Total admissions (*n* = 807)	Admissions without AKI (*n* = 510)	Admissions with AKI (*n* = 297)	*p*[a]
Calcium channel blockers, *n* (%)	314 (38.9)	209 (41.0)	105 (35.4)	0.2
Beta blockers, *n* (%)	530 (65.7)	338 (66.3)	192 (64.6)	0.75
RAAS inhibitors, *n* (%)	247 (30.6)	176 (34.5)	71 (23.9)	**0.005***
Aldosterone antagonists, *n* (%)	37 (4.6)	28 (5.5)	9 (3.0)	0.2
Statins, *n* (%)	387 (47.9)	249 (48.8)	138 (46.5)	0.69
NSAIDs, *n* (%)	5 (0.6)	2 (0.4)	3 (1.0)	0.48
PPIs, *n* (%)	550 (68.1)	327 (64.1)	223 (75.1)	**0.004***
Laboratory results during admission [median (IQR)]				
White blood cell average (K/μL)	8.8 [6.5, 11.9]	8.3 [6.2, 10.8]	9.9 [7.3, 14.6]	**<0.001****
Lymphocyte absolute average (K/μL)	1.1 [0.7, 1.6]	1.1 [0.8, 1.7]	1.1 [0.7, 1.5]	0.05
Lymphocyte absolute min (K/μL)	0.7 [0.4, 1.2]	0.8 [0.5, 1.3]	0.6 [0.3, 0.9]	**<0.001****
Hemoglobin average (g/dL)	10.5 [9.3, 12]	11 [9.7, 12.3]	9.9 [9, 10.98]	**<0.001****
Hemoglobin min (g/dL)	9.9 [8.3, 11.4]	10.5 [8.9, 11.9]	8.96 [7.66, 10.24]	**<0.001****
Creatinine (mg/dL)	1.4 [1.0, 1.96]	1.3 [1.0, 1.8]	1.63 [1.13, 2.37]	<0.001
eGFR baseline (CKD-EPI)**	59.9 [41.3, 80.98]	61.2 [43.7, 81.1]	58.18 [35.81, 80.34]	0.34
Glucose max (mg/dL)	205 [137, 321.5]	188 [130, 282]	244 [152, 380]	**<0.001****
Glucose min (mg/dL)	86 [71, 106]	90 [76, 112]	78 [64, 93]	**<0.001****
Albumin average (g/dL)	3.2 [2.8, 3.6]	3.4 [3.0, 3.8]	2.9 [2.6, 3.3]	**<0.001****
Albumin min (g/dL)	2.9 [2.5, 3.3]	3.1 [2.7, 3.5]	2.6 [2.3, 3]	**<0.001****
Globulins max (g/dL)	2.9 [2.6, 3.3]	2.9 [2.5, 3.2]	3 [2.6, 3.6]	**<0.001****
Globulins min (g/dL)	2.5 [2.1, 2.9]	2.5 [2.2, 2.9]	2.5 [2, 2.9]	0.47
Tacrolimus trough level average (μg/L)	5.5 [3.7, 8.2]	5.1 [3.6, 7.9]	6.0 [3.8, 8.6]	0.25
Tacrolimus trough level max (μg/L)	6.2 [4, 9.6]	5.7 [4.1, 8.5]	7.1 [4.1, 10.9]	**0.028***
C-reactive protein average (mg/L)	61.3 [19.1, 113.3]	50.1 [14.8, 101.5]	79.8 [36.1, 137.8]	**<0.001****
Death during admission, *n* (%)	40 (4.96)	8 (1.6)	32 (10.8)	**<0.001****
LOS, days [median (IQR)]	4.6 [2.7, 9.0]	3.8 [2.1, 7.0]	7.01 [3.62, 15.34]	**<0.001****
Readmission in 90 days, *n* (%)	302 (37.4)	148 (29.0)	154 (51.9)	**<0.001****

[a]*After adjustment for multiple comparisons.*

*p < *0.05*; **p < *0.01*.

****eGFR was calculated according to the following CKD-EPI formula: eGFR = 141* min (Scr/k, 1)α * max (Scr/k, 1)-1.209 * 0.993Age * 1.018 * 1.159 (if black) (where Scr - standardized serum creatinine; k = 0.7 if female, 0.9 if male; α = –0.329 if female, –0.411 if male; min = the minimum of Scr/k of 1; max = the maximum of Scr/k or 1).*

ADPKD, autosomal dominant polycystic kidney disease; BMI, body mass index; CA, cancers; CHF, congestive heart failure; CV, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; ESRD, end stage renal disease; GI, gastrointestinal; ID, infectious diseases; IHD, ischemic heart disease; LOS, length of stay; MPA, mycophenolic acid; NSAID, non-steroidal anti-inflammatory drug; RAAS, renin-angiotensin-aldosterone system; RTR, renal transplant recipients; SBP, systolic blood pressure. Bold values are the p *values which are significant (<0.05 or <0.01).*

Multivariable Analysis for AKI During Admission in RTRs

A mixed-effect logistic model (including admission age, sex, time from transplant to admission, ESRD etiology, medical history of diabetes, hypertension, IHD, CHF and AKI in a previous admission, admission etiology, vital signs, medications during admission, maximum tacrolimus trough level and other laboratory results during admission) revealed that the odds for an AKI during admission increased by 93% (OR 1.93, 95% CI 1.13–3.32, $p = 0.017$) for AKI in a previous admission and by 91% (OR 1.91, 95% CI 1.07–3.41, $p = 0.028$) for a medical history of hypertension. In addition, for every increase in minimum SBP of 1 mm Hg, the odds for in-hospital AKI decreased by 2% (OR 0.98, 95% CI 0.97–0.99, $p = 0.002$). Tacrolimus maximum trough level and albumin level during admission were also found to be associated with AKI during admission (OR 1.08, 95% CI 1.02–1.13, $p = 0.005$ and OR 0.51, 95% CI 0.29–0.92, $p = 0.025$, respectively). When tacrolimus maximum trough level was excluded to increase the number of patients and admissions included in the analysis, the odds for in-hospital AKI was more than twofold higher in the case of AKI in a previous admission (OR 2.13, 95% CI 1.44–3.14, $p < 0.001$) and almost three times higher when the major diagnosis upon admission was an infection (OR 2.93, 95% CI 1.23–6.98, $p = 0.015$). For every increase in minimum hemoglobin of 1 g/dL, the odds for AKI during admission decreased by 10% (OR 0.9, 95% CI 0.82–0.98, $p = 0.016$). Minimum SBP and albumin level during admission were also found to be independent predictors for in-hospital AKI (**Table 4**).

Outcomes of AKI During Admission in RTRs

We examined four outcomes of AKI during admission: readmission within 90 days, mortality during admission, overall mortality, and LOS.

Readmission in 90 Days

In a mixed effect logistic regression analysis, in-hospital AKI increased the odds for readmission within 90 days by 95% (OR 1.95, 95% CI 1.35–2.81, $p < 0.001$). For every increase in minimum hemoglobin of 1 g/dL, the odds for readmission in 90 days decreased by 8% (OR 0.92, 95% CI 0.85–0.99, $p = 0.02$; **Table 5**).

Mortality During Admission

In a mixed effect logistic regression analysis, admission age, AKI stage 3 vs. no AKI, minimum SBP and minimum albumin during

TABLE 3 | Demographic, clinical and biochemical characteristics of patients admitted with AKI, stratified by AKI stage during admission.

Variable	Admissions with AKI (*n* = 297)	AKI stage 1 (*n* = 134)	AKI stage 2 (*n* = 70)	AKI stage 3 (*n* = 93)	*p*[a]
RTR characteristics					
Admission age, years, [median (IQR)]	66.5 [55.98, 73]	67.3 [58.6, 73.7]	66.1 [55.5, 75.5]	64.9 [53.4, 72]	0.4
Female sex, *n* (%)	97 (32.7)	53 (39.6)	22 (31.4)	22 (23.7)	0.13
Transplant to admission, years [median (IQR)]	7.7 [4.77, 13.48]	6.95 [3.8, 11.5]	8.05 [4.65, 12.30]	8.7 [5.9, 16.5]	**0.04***
ESRD etiology, n (%)					
ADPCKD	18 (6.1)	10 (7.5)	5 (7.1)	3 (3.2)	0.29
Diabetic nephropathy	68 (22.9)	37 (27.6)	17 (24.3)	14 (15.1)	
Glomerulonephritis	52 (17.5)	25 (18.7)	9 (12.9)	18 (19.4)	
Nephrosclerosis	33 (11.1)	18 (13.4)	5 (7.1)	10 (10.8)	
Other	88 (29.6)	29 (21.6)	27 (38.6)	32 (34.4)	
Unknown	38 (12.8)	15 (11.2)	7 (10.0)	16 (17.2)	
Pre-transplant dialysis					
Yes	160 (53.9)	77 (57.5)	34 (48.6)	49 (52.7)	0.82
No	46 (15.5)	18 (13.4)	14 (20.0)	14 (15.1)	
Unknown	91 (30.6)	39 (29.1)	22 (31.4)	30 (32.3)	
Transplant type, *n* (%)					
Kidney only	288 (97.0)	130 (97.0)	68 (97.1)	90 (96.8)	0.86
Liver kidney	2 (0.7)	1 (0.7)	1 (1.4)	0 (0.0)	
Heart kidney	6 (2.0)	2 (1.5)	1 (1.4)	3 (3.2)	
Pancreas kidney	1 (0.3)	1 (0.7)	0 (0.0)	0 (0.0)	
Transplant number, *n* (%)					
1	274 (92.3)	124 (92.5)	67 (95.7)	83 (89.2)	0.6
2	22 (7.4)	9 (6.7)	3 (4.3)	10 (10.8)	
3	1 (0.3)	1 (0.7)	0 (0.0)	0 (0.0)	
Donor type, *n* (%)					
Living	159 (53.5)	72 (53.7)	36 (51.4)	51 (54.8)	0.55
Deceased	87 (29.3)	45 (33.6)	19 (27.1)	23 (24.7)	
Unknown	51 (17.2)	17 (12.7)	15 (21.4)	19 (20.4)	
Medical history, *n* (%)					
Diabetes mellitus	104 (35.0)	49 (36.6)	26 (37.1)	29 (31.2)	0.78
Hypertension	199 (67.0)	90 (67.2)	44 (62.9)	65 (69.9)	0.78
IHD	128 (43.1)	54 (40.3)	34 (48.6)	40 (43.0)	0.71
CHF	72 (24.2)	26 (19.4)	16 (22.9)	30 (32.3)	0.24
Admission etiology, *n* (%)					
ID	142 (47.8)	68 (50.7)	37 (52.9)	37 (39.8)	0.37
CV	42 (14.1)	22 (16.4)	5 (7.1)	15 (16.1)	
GI	16 (5.4)	7 (5.2)	6 (8.6)	3 (3.2)	
CA	15 (5.1)	5 (3.7)	2 (2.9)	8 (8.6)	
Other	82 (27.6)	32 (23.9)	20 (28.6)	30 (32.3)	
Vital signs and other clinical parameters during admission, [median (IQR)]					
Fever max, ˚C	37.4 [37, 38.40]	37.3 [37, 38.4]	37.4 [37, 38.25]	37.5 [37.1, 38.5]	0.49
Pulse max	103 [89, 120]	98 [87, 111]	103.5 [90.8, 128.5]	110 [94, 130]	**0.01***
Pulse min	60 [53, 67]	60 [53.2, 67]	59.5 [53, 65]	61 [52, 68]	0.8
Pulse average	78 [70, 85.84]	76.6 [68, 84.6]	77.9 [71.5, 84.6]	79.2 [72.9, 87.1]	0.24
SBP min mmHg	95 [80, 108]	99 [86, 110.7]	91.5 [72, 102.8]	89 [70, 110]	**0.01***
SBP average mmHg	128.8 [115.5, 144]	130 [118.7, 146.4]	128.4 [114.1, 137.5]	126.5 [112, 145.8]	**0.04***
DBP min mmHg	50 [40, 59]8	50 [45, 58]	44 [39, 56.5]7	49 [36, 63]	0.24
DBP average mmHg	69.8 [63.1, 76.6]	70.5 [64.8, 75.3]	68.7 [62.6, 75.1]	70.6 [61.8, 81.1]	0.6
O_2 saturation min	91 [85, 94]	93 [88, 95]	91 [84.3, 93]	90 [81, 94]	**0.04***
Weight average, kg	73.8 [64, 85.1]	72.9 [63.2, 85]	72.7 [63.2, 91.2]	76.4 [65, 85.6]	0.78
BMI average	26.1 [22.8, 30.3]	26.1 [23.3, 29.5]	25.7 [22.1, 30.8]	26.6 [22.8, 30.5]	0.91
Medications during admission					
Tacrolimus, *n* (%)	154 (51.9)	68 (50.7)	47 (67.1)	39 (41.9)	**0.03***
Tacrolimus average dose [mean (SD)]	1.53 (1.03)	1.49 (0.91)	1.50 (0.97)	1.62 (1.30)	0.88
Cyclosporine, *n* (%)	34 (11.4)	17 (12.4)	7 (10.0)	10 (10.8)	0.94
Cyclosporine average dose [mean (SD)]	68.90 (39.30)	71.81 (40.30)	64.03 (41.24)	67.35 (40.04)	0.92
MPA, *n* (%)	109 (36.7)	61 (45.5)	26 (37.1)	22 (23.7)	**0.02***
MPA average dose [mean (SD)]	146.86 (215.11)	186.40 (237.78)	140.91 (192.53)	94.35 (185.05)	**0.039***
Steroids, *n* (%)	216 (72.7)	92 (68.7)	53 (75.7)	71 (76.3)	0.59
Steroids average dose [mean (SD)]	22.10 (41.01)	14.47 (20.03)	24.98 (38.47)	30.72 (59.23)	0.06
mTOR inhibitors, *n* (%)	16 (5.4)	8 (6.0)	3 (4.3)	5 (5.4)	0.91
Azathioprine, *n* (%)	14 (4.7)	7 (5.2)	3 (4.3)	4 (4.3)	0.93
Loop diuretics, *n* (%)	146 (49.2)	61 (45.5)	34 (48.6)	51 (54.8)	0.59
Thiazides, *n* (%)	15 (5.1)	9 (6.7)	1 (1.4)	5 (5.4)	0.47

(Continued on following page)

TABLE 3 | (*Continued*) Demographic, clinical and biochemical characteristics of patients admitted with AKI, stratified by AKI stage during admission.

Variable	Admissions with AKI (*n* = 297)	AKI stage 1 (*n* = 134)	AKI stage 2 (*n* = 70)	AKI stage 3 (*n* = 93)	*p*[a]
Calcium channel blockers, *n* (%)	105 (35.4)	41 (30.6)	29 (41.4)	35 (37.6)	0.47
Beta blockers, *n* (%)	192 (64.6)	83 (61.9)	43 (61.4)	66 (71.0)	0.53
RAAS inhibition, *n* (%)	71 (23.9)	35 (26.1)	15 (21.4)	21 (22.6)	0.82
Aldosterone antagonists, *n* (%)	9 (3.0)	4 (3.0)	1 (1.4)	4 (4.3)	0.72
Statins, *n* (%)	138 (46.5)	64 (47.8)	35 (50.0)	39 (41.9)	0.72
NSAIDs, *n* (%)	3 (1.0)	0 (0.0)	0 (0.0)	3 (3.2)	0.16
PPIs, *n* (%)	223 (75.1)	96 (71.6)	54 (77.1)	73 (78.5)	0.65
Laboratory results during admission [median (IQR)]					
White blood cell average (K/μL)	9.92 [7.27, 14.57]	9.5 [6.9, 14.1]	11.9 [7.7, 17.1]	10.2 [7.3, 13.5]	0.42
Lymphocyte absolute average (K/μL)	1.09 [0.67, 1.52]	1.1 [0.71, 1.5]	1.1 [0.7, 1.6]	1 [0.6, 1.35]	0.42
Lymphocyte absolute min (K/μL)	0.58 [0.28, 0.94]	0.6 [0.3, 1.1]	0.65 [0.3, 0.96]	0.5 [0.2, 0.8]	0.24
Hemoglobin average (g/dL)	9.91 [9.00, 10.98]	9.9 [8.98, 11]	10.1 [9.2, 11.1]	9.7 [8.8, 10.8]	0.59
Creatinine (mg/dL)	1.63 [1.13, 2.37]	1.4 [1.1, 1.9]	1.4 [1.1, 2]	2.5 [1.6, 3.5]	**<0.001****
eGFR baseline (CKD-EPI)**	58.2 [35.8, 80.3]	59.6 [44, 77.8]	72.1 [54.5, 88.7]	34.3 [23.6, 72.6]	**<0.001****
Glucose max (mg/dL)	244 [152, 380]	231 [152, 380]	268 [150, 405]	225 [168, 350]	0.78
Glucose min (mg/dL)	78 [64, 93]	81 [68, 103]	77 [65.25, 90]	74 [57, 85]	**0.018***
Albumin average (g/dL)	2.9 [2.6, 3.3]	3.1 [2.7, 3.35]	2.8 [2.4, 3.1]	2.7 [2.4, 3.8]	**0.003***
Globulins max (g/dL)	3 [2.6, 3.6]	3 [2.7, 3.6]	3.2 [2.8, 3.5]	3 [2.5, 3.6]	0.61
Tacrolimus trough level average (μg/L)	5.97 [3.8, 8.6]	6.5 [4.4, 9.1]	5.5 [3.5, 7.1]	4.95 [3.6, 8.1]	0.11
Tacrolimus trough level max (μg/L)	7.1 [4.1, 10.9]	8.1 [4.6, 11.2]7	6.2 [3.7, 8.5]	6.95 [3.9, 11.7]	0.39
C-reactive protein average (mg/L)	79.8 [36.1, 137.8]	62.7 [31.7, 119]	104.2 [42.9, 158.3]	85.5 [43.7, 140.5]	0.11
Death during admission, *n* (%)	32 (10.8)	3 (2.2)	9 (12.9)	20 (21.5)	**<0.001****
LOS, days, [median (IQR)]	7 [3.6, 15.3]	6.31 [3.28, 13.11]	7.34 [4.3, 12.5]	8.17 [3.62, 19.95]	0.42
Readmission in 90 days, *n* (%)	154 (51.9)	65 (48.5)	31 (44.3)	58 (62.4)	**0.042***

[a]*After adjustment for multiple comparisons.*

*p < *0.05*; **p < *0.01*.

****eGFR was calculated according to the following CKD-EPI formula: eGFR = 141* min (Scr/k, 1)α * max (Scr/k, 1)-1.209 * 0.993Age * 1.018 * 1.159 (if black) (where Scr - standardized serum creatinine; k = 0.7 if female, 0.9 if male; α = –0.329 if female, –0.411 if male; min = the minimum of Scr/k of 1; max = the maximum of Scr/k or 1).*

ADPKD, autosomal dominant polycystic kidney disease; BMI, body mass index; CA, cancers; CHF, congestive heart failure; CV, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; ESRD, end stage renal disease; GI, gastrointestinal; ID, infectious diseases; IHD, ischemic heart disease; LOS, length of stay; MPA, mycophenolic acid; NSAID, non-steroidal anti-inflammatory drug; PPI, protein pump inhibitor; RAAS, renin-angiotensin-aldosterone system; RTR, renal transplant recipients; SBP, systolic blood pressure. Bold values are the p *values which are significant (<0.05 or <0.01).*

admission were found to be independent predictors for in-hospital mortality. The odds of mortality during admission were four times higher in RTRs with AKI stage 3 vs. RTRs with no AKI during admission (OR 4.0, 95% CI 1.38–11.6, $p = 0.01$; **Table 6**).

Overall Mortality

Figure 2 shows Kaplan–Meier curves for time to death according to the presence and severity of AKI in the last admission for each patient. In a multivariable Cox proportional hazards regression model for long-term mortality, transplant age, diabetic nephropathy vs. all other ESRD etiologies, and presence of AKI vs. no AKI in any admission were associated with a 1.08-fold (95% CI 1.06–1.1), 1.95-fold (95% CI 1.27–2.99) and 1.51-fold (95% CI 1.01–2.25) increased risk of death, respectively (**Table 7**).

Length of Stay

Figure 3 shows a box-plot diagram for in-hospital LOS according to the presence and severity of in-hospital AKI. In a multivariable linear mixed model for LOS, a major admission diagnosis of a cancer significantly prolonged the LOS compared to all other admission etiologies. For every 1 mm Hg increase in minimum SBP, LOS was shortened by 1% (0.99, 95% CI 0.98–0.99, $p < 0.001$). Use of a calcineurin inhibitor (tacrolimus or cyclosporine) during admission increased the LOS by 41% (1.41, 95% CI 1.26–1.58, $p < 0.001$). Loop diuretic use, minimum hemoglobin, maximum glucose and minimum albumin during admission were independently associated with LOS. AKI during admission was not found to be an independent predictor for hospital LOS (**Table 8**).

DISCUSSION

In this study of 292 RTRs, with a total of 807 non-ICU admissions, we found a 51% rate (149/292) of any AKI over multiple hospital admissions. AKI during admission was observed in 36.8% (297/807) of total admissions. Of 297 admissions with AKI, stages 1, 2 and 3 were recorded for 134 (45.1%), 70 (23.6%) and 93 (31.3%) admissions, respectively. Multivariable mixed effect models for AKI during admission revealed that an AKI in a previous admission doubled the odds for AKI in the subsequent admission. The odds for AKI during an admission were almost three times higher with major diagnosis of infectious etiology during admission. In addition, a medical history of hypertension, minimum SBP, minimum hemoglobin, albumin and tacrolimus maximum trough level were significantly associated with AKI during admission. AKI during

TABLE 4 | Univariate and multivariate stepwise mixed effect logistic regression analysis for AKI during admission in RTRs.

Effect	Univariate logistic regression		Logistic regression (*n* = 193 patients, 385 admissions)		Logistic regression (*n* = 283 patients, 767 admissions)	
	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*
RTR characteristics						
Admission age, per 1 year increase	0.99 (0.98, 1.01)	0.85	0.99 (0.97, 1.02)	0.45	0.99 (0.97, 1.01)	0.21
Female vs. male	0.93 (0.59, 1.48)	0.77	1.17 (0.67, 2.04)	0.59	0.99 (0.65, 1.49)	0.95
Transplant to admission, years	1.02 (0.99, 1.06)	0.11	1.02 (0.97, 1.07)	0.47	1.00 (0.97, 1.03)	0.78
ESRD etiology						
APCKD	1		1		1	
Diabetic nephropathy	2.33 (1.00, 5.41)	**0.049***	1.09 (0.34, 3.51)	0.88	1.06 (0.43, 2.57)	0.91
Glomerulonephritis	2.22 (0.94, 5.24)	0.07	1.7 (0.53, 5.51)	0.37	1.6 (0.7, 3.69)	0.27
Nephrosclerosis	3.41 (1.26, 9.22)	**0.016***	1.87 (0.52, 6.73)	0.34	2.05 (0.77, 5.44)	0.15
Other	2.89 (1.26, 6.59)	**0.012***	1.41 (0.48, 4.15)	0.53	1.72 (0.77, 3.82)	0.19
Unknown	2.36 (0.93, 6.00)	0.07	1.54 (0.43, 5.52)	0.51	1.7 (0.68, 4.23)	0.26
Medical history						
Diabetes mellitus	1.3 (0.82, 2.05)	0.27				
Hypertension	2.02 (1.27, 3.22)	**0.003***	1.91 (1.07, 3.41)	**0.028***	1.36 (0.88, 2.1)	0.16
IHD	1.95 (1.25, 3.05)	**0.003***	1.23 (0.67, 2.23)	0.51	1.17 (0.75, 1.81)	0.49
CHF	1.59 (0.98, 2.57)	0.05	1.28 (0.64, 2.54)	0.49	1.35 (0.81, 2.24)	0.24
Previous AKI	2.88 (2.06, 4.03)	<**0.001****	1.93 (1.13, 3.32)	**0.017****	2.13 (1.44, 3.14)	<**0.001****
Admission etiology						
CA	1		1		1	
CV	0.47 (0.19,1.14)	0.1	1.03 (0.26, 4.02)	0.97	1.5 (0.58, 3.9)	0.4
GI	0.51 (0.18, 1.42)	0.19	0.68 (0.16, 3.2.98)	0.61	0.82 (0.28, 2.39)	0.72
ID	1.34 (0.59, 3.05)	0.49	1.9 (0.55, 6.61)	0.31	2.93 (1.23, 6.98)	**0.015***
Others	0.9 (0.39, 2.1)	0.81	2.2 (0.6, 8.14)	0.24	2.73 (1.11, 6.68)	**0.03***
Vital signs and other clinical parameters during admission						
Pulse max, per 1/min increase	1.028 (1.02, 1.037)	<**0.001****	1.00 (0.99–1.02)	0.64	1.01 (1.00, 1.02)	**0.03***
SBP min, per 1 mm Hg increase	0.97 (0.96, 0.98)	<**0.001****	0.98 (0.97, 0.99)	**0.002***	0.98 (0.97, 0.99)	<**0.001****
DBP min, per 1 mm Hg increase	0.95 (0.94, 0.97)	<**0.001****				
Sat O_2 min per 1% increase	0.97 (0.95, 0.99)	<**0.001****	1.00 (0.98, 1.02)	0.95	1.01 (0.99, 1.02)	0.55
Medications during admission						
Steroids average dose, per 1 mg of prednisone increase	1.011 (1.005, 1.016)	<**0.001****				
Loop diuretics use	1.52 (1.04, 2.22)	**0.03***	1.22 (0.66, 2.26)	0.53	1.1 (0.71, 1.7)	0.66
PPI use	1.83 (1.2, 2.77)	**0.0047****	1.05 (0.56, 1.94)	0.89	0.99 (0.64, 1.51)	0.95
Laboratory results during admission						
Lymphocyte absolute average per 1 K/μL increase	0.94 (0.82, 1.07)	0.36				
Lymphocyte absolute min per 1 K/μL increase	0.76 (0.6, 0.95)	**0.015***	1.02 (0.87, 1.19)	0.79	1.0 (0.87, 1.15)	0.99
Hemoglobin average per 1g/dL increase	0.74 (0.67, 0.82)	<**0.001****				
Hemoglobin min per 1g/dL increase	0.75 (0.69, 0.81)	<**0.001****	0.95 (0.84, 1.08)	0.41	0.9 (0.82, 0.98)	**0.016**
eGFR baseline (CKD-EPI)** per 1 mL/min increase	0.999 (0.99, 1.01)	0.8	1.01 (0.99, 1.02)	0.44	1.00 (0.99, 1.01)	0.42
Glucose max per 1 mg/dL increase	1.005 (1.00, 1.01)	<**0.001****	1.00 (1.00, 1.01)	**0.028**	1.00 (1.00, 1.01)	<**0.001****
Glucose min per 1 mg/dL increase	0.98 (0.98, 0.99)	<**0.001****				
Albumin average per 1 g/dL increase	0.59 (0.45, 0.8)	<**0.001****				
Albumin min per 1 g/dL increase	0.2 (0.14, 0.28)	<**0.001****	0.51 (0.29, 0.92)	**0.025**	0.42 (0.27, 0.64)	<**0.001****
Globulins max per 1 g/dL increase	1.87 (1.36, 2.56)	<**0.001****				
Globulins min per 1 g/dL increase	0.72 (0.5, 1.04)	**0.08**	1.02 (0.62, 1.69)	0.94	1.05 (0.72, 1.51)	0.81
Tacrolimus trough level max per 1 μg/L increase	1.065 (1.02, 1.11)	**0.0065***	1.08 (1.02, 1.13)	**0.005***		

*p < *0.05*; **p < *0.01*.

****eGFR was calculated according to the following CKD-EPI formula: eGFR = 141* min (Scr/k, 1)α * max (Scr/k, 1)-1.209 * 0.993Age * 1.018 * 1.159 (if black) (where Scr - standardized serum creatinine; k = 0.7 if female, 0.9 if male; α = –0.329 if female, –0.411 if male; min = the minimum of Scr/k of 1; max = the maximum of Scr/k or 1).*

ADPKD, autosomal dominant polycystic kidney disease; CA, cancers; CHF, congestive heart failure; CV, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; ESRD, end stage renal disease, GI, gastrointestinal; ID, infectious diseases; IHD, ischemic heart disease; PPI, protein pump inhibitor; RTR, renal transplant recipients; SBP, systolic blood pressure. Bold values are the p *values which are significant (<0.05 or <0.01).*

admission was associated with adverse events, i.e., for patients who developed AKI, LOS and mortality during admission rose and rates of readmission within 90 days increased with worsening outcomes as AKI severity increased. The overall mortality risk was also higher in RTRs with any AKI vs. no AKI during admission.

The overall incidence of AKI developing 3 months or later after kidney transplantation, excluding RTRs with deceased donor transplants and recipients of second or third transplants, was 20.4% (16). In seeking to compare this finding with values in the literature, we found that there are only very few studies dealing with the subject. In pediatric

TABLE 5 | Multivariate mixed effect logistic regression analysis for readmission within 90 Days in RTRs.

Effect	Odds ratio (95% CI)	*p*
Admission age	1.01 (0.99–1.03)	0.17
Gender, F vs. M	0.93 (0.62–1.38)	0.72
Hypertension, yes vs. no	1.34 (0.91–1.97)	0.14
In-hospital AKI, yes vs.no	1.95 (1.35–2.81)	**<0.001****
SBP min (for every increase of 1 mm Hg)	1.0 (0.99–1.01)	0.93
Albumin min per 1g/dL increase	0.76 (0.53–1.1)	0.15
Glucose max per 1 mg/dL increase	1.0 (0.99–1.0)	0.94
Hemoglobin min per 1 g/dL increase	0.92 (0.85–0.99)	**0.02***

*p < *0.05*; **p < *0.01*.

RTR, renal transplant recipients; SBP, systolic blood pressure. Bold values are the p *values which are significant (<0.05 or <0.01).*

TABLE 6 | Multivariate mixed effect logistic regression analysis for mortality during admission in RTRs.

Effect	Odds ratio (95% CI)	*p*
Admission age (for every increase in 1 year)	1.05 (1.01–1.09)	**0.01***
Gender, F vs. M	0.88 (0.35–2.18)	0.77
Reference- no AKI during admission		
AKI stage 1	0.65 (0.16–2.67)	0.55
AKI stage 2	2.76 (0.86–9.98)	0.09
AKI stage 3	4.00 (1.38–11.6)	**0.01***
SBP min (for every increase of 1 mm Hg)	0.97 (0.95–0.98)	**<0.001****
Albumin min per 1 g/dL increase	0.21 (0.08–0.55)	**0.001****
Glucose max per 1 mg/dL increase	1.0 (0.99–1.0)	0.56
Hemoglobin min per 1 g/dL increase	0.99 (0.85–1.17)	0.94

*p < *0.05*; **p < *0.01*.

RTR, renal transplant recipients; SBP, systolic blood pressure. Bold values are the p *values which are significant (<0.05 or <0.01).*

kidney transplant recipients, the incidence of AKI was 37% over a study period of 12 years (17). A very much lower value – 3,066 of 27,232 transplant recipients (11.3%) – was reported in the only study focused on in-hospital AKI (4181 hospitalizations) during the first three post-transplant years. In that study, AKI was identified by the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM), which has limited sensitivity, and therefore the overall incidence of AKI was probably underestimated (12). Based on Scr levels pre and post admission, we detected AKI in a median time from transplant of 7.7 years (IQR 4.77–13.48) in 149/292 (51%) of RTRs, with a total of 513 admissions (1-10 admissions per person). Our analysis provides a more accurate assessment of the higher incidence of in-hospital AKI in RTRs compared to the non-transplant population, in which AKI occurs in 4%–20% of hospitalized patients (16, 18, 19). Similarly to our findings, a higher rate of AKI following cardiac surgery (46% vs. 28%) was observed in RTRs compared with non-RTRs (2).

In a study of 11,683 patients developing in-hospital AKI, 2954 (25%) were re-hospitalized with recurrent AKI within 12 months of discharge (20), with each episode of recurrence conferring an increased risk for progression to chronic kidney disease (21). Analysis of a large database of about 150,000 patients revealed that approximately 20% were readmitted with AKI and about 10% were seen in an emergency room within 30 days of discharge (22). We found the readmission rate within 90 days to be 51.9% vs. 29% in admissions without in-hospital AKI ($p < 0.001$). Moreover, the severity of AKI also affected the 90-day readmission rate, which reached 62.4% in stage 3 as opposed to 48.5% in stage 1 AKI. Our study is the first to show the negative effects of in-hospital AKI on the readmission rate and subsequent AKI events in RTRs.

It is not surprising that a major diagnosis of an infection was associated with in-hospital AKI; for example, in a study conducted in Italy the incidence of in-hospital AKI was 31.7% during the COVID-19 pandemic compared to 25.9% during the pre-COVID-19 period (23). RTRs are prone to infections and complications of infections, given the immunosuppressive agents they receive to prevent rejection. Infections are commonly complicated by AKI secondary to sepsis associated with hemodynamic instability, volume depletion, and the nephrotoxicity of antibiotics, among other factors. A medical history of hypertension, associated with oxidative stress and endothelial dysfunction (24), was also found to be an independent predictor for in-hospital AKI in our population, as previously described in patients with AKI following surgical resection of malignant pleural mesothelioma (25).

Given the large number and extensive variety of the components of our dataset (different vital signs, clinical parameters, medications and laboratory results during admission) that were retrieved as possible confounders, we were able to demonstrate associations of SBP, hemoglobin, albumin and maximum tacrolimus trough level with in-hospital AKI. CNI nephrotoxicity, a well-known complication of CNI use (26, 27), remains the leading cause of renal failure after transplantation of a non-renal organ (28, 29). Similar abnormalities have been found when CNIs are used in other settings, for example, in patients with psoriasis (30). The pathophysiology of acute CNI nephrotoxicity is related to profound alterations in renal vascular resistance and blood flow in the afferent and efferent arterioles and even a reduced diameter of the afferent arterioles (27). In line with our findings, higher vs. lower preoperative CNI trough levels (73% vs. 36%) have been associated with higher rates of AKI following cardiac surgery in RTRs (2).

The pathophysiology of low SBP and a low hemoglobin level associated AKI is related to the reduction in perfusion pressure and oxygenation, leading to ischemic injury. Renal ischemia-reperfusion injury in kidney transplantation leads to AKI, delayed graft function, and even graft loss (31). Kidney transplantation involves implantation of denervated kidneys, with impairment of blood flow autoregulation, rendering the renal allograft highly susceptible to ischemic injury and subsequent inflammation and cell death. A reduced nephron mass in RTRs may also increase their susceptibility to ischemic injury. Furthermore, renal allograft ischemia may exacerbate immune-related mechanisms of allograft injury, as manifested by the effect of cold and warm ischemia times on graft function and rejection (32).

AKI is common in RTRs and confers a high risk for graft failure and death (12, 17, 33). In populations other than RTRs, AKI has been associated with increased LOS and higher mortality (25, 34). We are the first to describe the association of in-hospital AKI in RTRs with

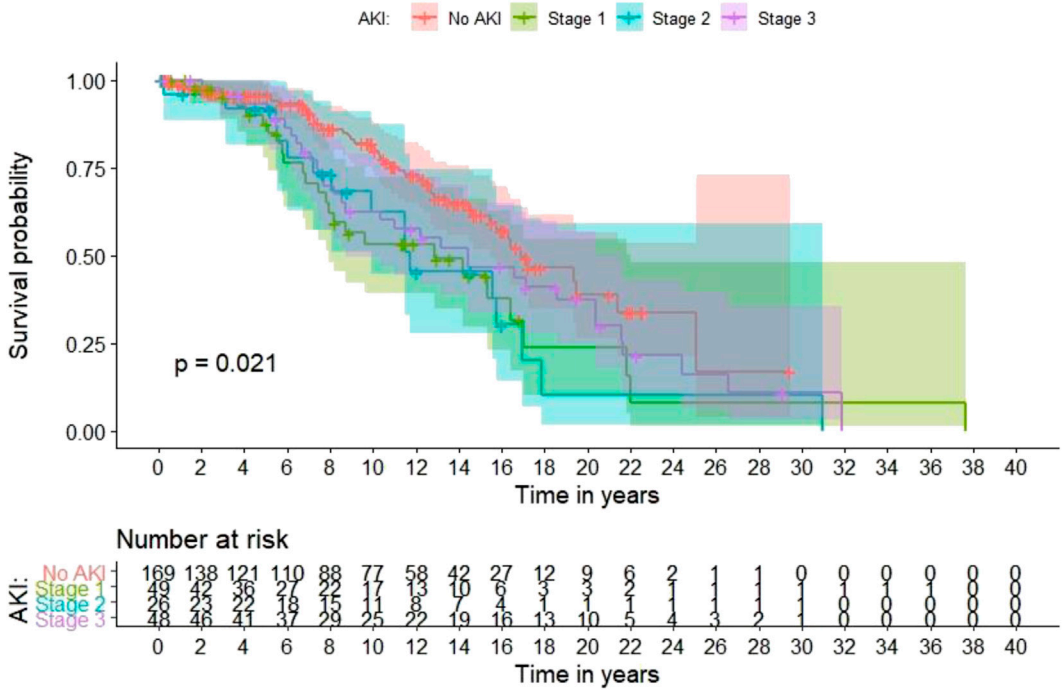


FIGURE 2 | Long-term mortality based on the occurrence and severity of in-hospital AKI in the last admission for each patient.

TABLE 7 | Multivariate cox regression hazard model for overall mortality in RTRs.

Effect	Hazard ratio (95% CI)	*p*
Transplant age	1.08 (1.06–1.1)	<**0.001****
Gender, F vs. M	0.93 (0.61–1.4)	0.72
Diabetic nephropathy vs. all other ESRD etiologies	1.95 (1.27–2.99)	**0.002****
AKI, ever vs. never	1.51 (1.01–2.25)	**0.04***

*p < *0.05;*
**p < *0.01.*
RTR, renal transplant recipients; ESRD, end stage renal disease. Bold values are the p *values which are significant (<0.05 or <0.01).*

TABLE 8 | Multivariate linear mixed model for LOS during admission in RTRs.

Effect	Hazard ratio (95% CI)	*p*
Admission age (for every increase in 1 year)	0.99 (0.99–1.00)	**0.003***
Gender, F vs. M	0.93 (0.81–1.06)	0.25
Diabetic nephropathy vs. all other ESRD etiologies	1.13 (0.96–1.33)	0.13
IHD, yes vs. no	1.1 (0.97–1.26)	0.13
AKI during admission yes vs. no	1.04 (0.92–1.18)	0.5
Major admission diagnosis		
CA	References	
CV	0.58 (0.44–0.76)	<**0.001****
GI	0.57 (0.42–0.77)	<**0.001****
ID	0.69 (0.53–0.89)	**0.005***
SBP min (for every increase in 1 mm Hg)	0.99 (0.98–0.99)	<**0.001****
Loop diuretics use	1.27 (1.12–1.43)	<**0.001****
PPI use	1.05 (0.93–1.20)	0.4
CNI use	1.41 (1.26–1.58)	<**0.001****
Hemoglobin min per 1 g/dL increase	0.97 (0.94–0.99)	**0.01***
Glucose max per 1 mg/dL increase	1.00 (1.00–1.00)	<**0.001**
Albumin min per 1 g/dL increase	0.65 (0.57–0.73)	<**0.001****

*p < *0.05;* **p < *0.01.*
CA, cancers; CNI, calcineurin inhibitors; CV, cardiovascular disease; GI, gastrointestinal disease; ID, infectious diseases; IHD, ischemic heart disease; PPI, protein pump inhibitor; RTR, renal transplant recipients; SBP, systolic blood pressure. Bold values are the p *values which are significant (<0.05 or <0.01).*

increased LOS and mortality during admission. Our study is probably underpowered to detect an association between in-hospital mortality and milder AKI, found in studies of non-transplant patients (25). In addition, we found a strong association of AKI with overall mortality over a period of more than 30 years.

Several limitations should be mentioned, including the retrospective study design. In addition, minimum Scr during admission used as baseline Scr in recipients with no Scr within 120 days prior to admission or within 150 days from transplant may not reflect baseline Scr as it could be elevated due to AKI prior to admission, there is no information about the exact timing of maximum Scr during admission, rejection, use of erythropoietin stimulating agents, admissions to other hospitals, transplant loss, renal replacement therapy or recovery from AKI, mortality (death with a functioning graft) and death-censored graft loss. Urine output criteria were not used. In addition, the use of serum creatinine levels to estimate GFR has limitations in assessing kidney function. The

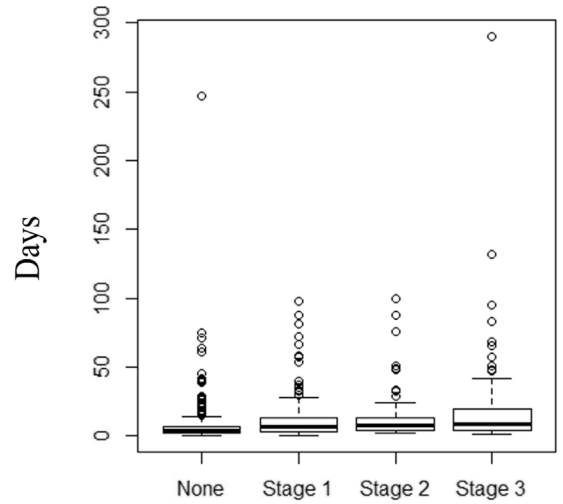


FIGURE 3 | Box-plot diagram for in-hospital length of stay (days) in RTR with no AKI, and AKI stages 1,2 and 3.

strengths of this study include the large size of the cohort, creatinine-based definitions to capture index and recurrent AKI, and the power to examine multiple potential confounders. Nonetheless, we cannot exclude potential residual confounding as in-hospital AKI may be a surrogate for disease severity.

In conclusion, in-hospital AKI in RTRs is an independent risk factor associated with poor short- and long-term outcomes. RTRs with an AKI during admission should be followed up closely, with specific monitoring after discharge to reduce the risk of rehospitalization and death. Efforts should be made to identify patients at high risk for AKI, to develop strategies to prevent AKI during admission, and to minimize adverse outcomes. RTRs should be closely monitored during admission to prevent hypotension, anemia, and hypoalbuminemia. The association of CNI with in-hospital AKI further emphasizes the importance of individualized tailoring of immunosuppressive therapy based on rejection vs. infection risk to prevent complications associated with over immunosuppression, including infections, which are independently associated with in-hospital AKI and AKI itself.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The study was approved by the local ethics committee in Sheba medical center (IRB approval number: SMC-70-5320). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

TH: Conception and design, data acquisition, data interpretation, writing, revising; BO: Data analysis; NS: Data acquisition; LL: Data interpretation; GS: Conception and design; PB: Data acquisition; KC-H: Data interpretation; EM: Revising; EG: Revising; EZ: Conception and design, data interpretation; MS: conception and design, data interpretation, revising. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

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External Validation of the Toulouse-Rangueil Predictive Model to Estimate Donor Renal Function After Living Donor Nephrectomy

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A predictive model to estimate post-donation glomerular filtration rate (eGFR) and risk of CKD at 1-year was developed from a Toulouse-Rangueil cohort in 2017 and showed an excellent correlation to the observed 1-year post-donation eGFR. We retrospectively analyzed all living donor kidney transplants performed at a single center from 1998 to 2020. Observed eGFR using CKD-EPI formula at 1-year post-donation was compared to the predicted eGFR using the formula eGFR (CKD-EPI, mL/min/1.73 m^2) = 31.71+ (0.521 × preoperative eGFR) − (0.314 × age). 333 donors were evaluated. A good correlation (Pearson r = 0.67; $p < 0.001$) and concordance (Bland-Altman plot with 95% limits of agreement −21.41–26.47 mL/min/1.73 m^2; $p < 0.001$) between predicted and observed 1-year post-donation eGFR were observed. The area under the ROC curve showed a good discriminative ability of the formula in predicting observed CKD at 1-year post-donation (AUC = 0.83; 95% CI: 0.78–0.88; $p < 0.001$) with optimal cutoff corresponding to a predicted eGFR of 65.25 mL/min/1.73 m^2 in which the sensibility and specificity to predict CKD were respectively 77% and 75%. The model was successfully validated in our cohort, a different European population. It represents a simple and accurate tool to assist in evaluating potential donors.

Keywords: external validation, predictive model, living donor renal function, kidney transplantation, chronic kidney disease

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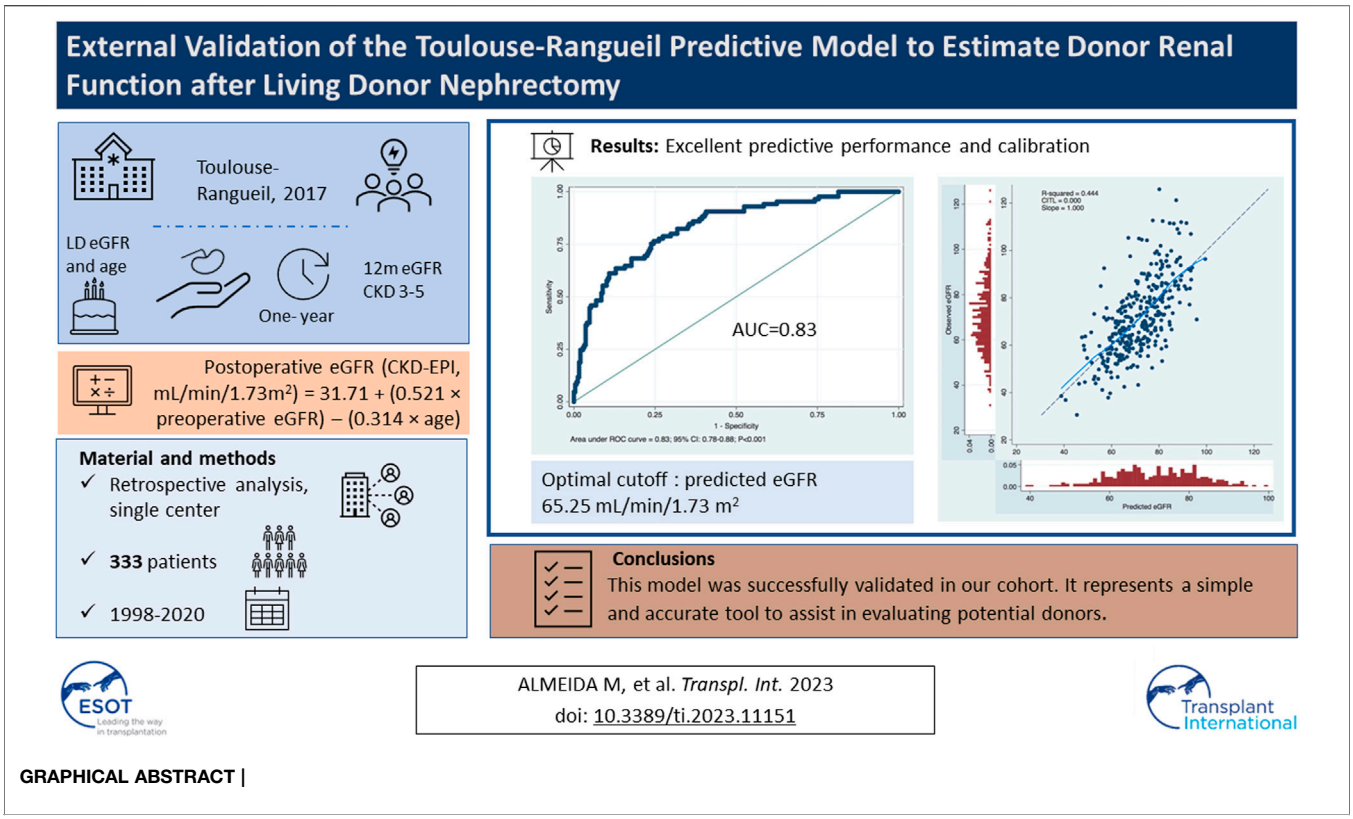
Abbreviations: AUC, area under the curve; BMI, body mass index; CI, confidence interval; CITL, calibration in the large; CKD, chronic kidney disease; CKD-EPI, chronic kidney disease epidemiology collaboration; ESRD, end-stage renal disease; eGFR, estimated glomerular filtration rate; GFR, glomerular filtration rate; LKD, living kidney donor; MDRD, modification of diet in renal disease; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; SD, standard deviation.



GRAPHICAL ABSTRACT |

INTRODUCTION

Living donor kidney transplant is the best treatment for ESRD patients eligible for transplant (1, 2). Living donation increases organ availability, decreases time on the waiting list, allows pre-emptive transplantation, and improves graft and patient survival (1–3).

The evaluation of a living donor candidate is a multidisciplinary task to minimize the risk for the donor while ensuring the organ's suitability for the recipient (4, 5). Despite being the only surgical indication that grants no direct medical benefit to a healthy patient, a living nephrectomy is considered a safe procedure for the donor (5–7). Long-term follow-up data, however, have shown that donors are at an increased risk of CKD and, rarely, ESRD compared to healthy non-donors (6–9) As such, these patients would be subjected to the cardiovascular and global morbidity and mortality of CKD (10). Furthermore, the increasing acceptance of donors with increasing age or with minor medical changes that were previously declined (6), makes the issue of kidney donors' safety of utmost importance (6). Moreover, the scarcity of good-quality studies on their long-term follow-up must be acknowledged (6, 7).

Current Clinical practice guidelines on the evaluation and care of living kidney donors from Kidney Disease Improving Global Outcomes (KDIGO) recommend a comprehensive approach to risk assessment that should replace decisions based on assessments of single risk factors evaluation (4). Transplant programs should provide each donor candidate with individualized quantitative risks from donation and communicate them clearly to donor candidates (4). Furthermore, each donor candidate's risk should be compared to predetermined thresholds for acceptance and declined if the risk exceeds the acceptable limit for the Transplant Unit (4). Nevertheless, precise tools to quantify individualized donor risks are lacking.

A predictive model to estimate the donor 1-year post-donation estimated glomerular filtration rate (eGFR) and risk of CKD was developed from a Toulouse-Rangueil cohort in 2017 (11). Benoit et al. retrospectively evaluated a single-center French cohort of 202 living donors and identified age and preoperative eGFR as independent predictors of postoperative eGFR. A formula using multiple linear regression was designed for clinical application and the authors described a good statistical performance (11). This model was then externally validated in a German center by Kullik et al. (12) and in a different French cohort (13) and was shown to have a good correlation to the observed 1-year post-donation eGFR.

We sought to externally validate this predictive tool in a different, large European cohort of patients who underwent a living donor kidney transplant at our center.

MATERIAL AND METHODS

This external validation study was conducted according to the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnostics (TRIPOD) guidelines (14).

TABLE 1 | Patients' characteristics of the 333 living donors.

	N = 333
Age, mean ± SD	47.3 ± 10.6
Sex F:M, n (%)	236 (71):97 (29)
BMI, mean ± SD (Kg/m^2)	25.3 ± 3.4
Smoking habits, n (%)	51 (15)
Hypertension, n (%)	50 (15)
Pre-donation SCr, mean ± SD (mg/dL)	0.75 ± 0.16
Pre- donation eGFR, mean ± SD	100.3 ± 14.7
1-year postdonation SCr, mean ± SD (mg/dL)	1.05 ± 0.23
1-year postdonation eGFR, mean ± SD	71.4 ± 16.2
Predicted 1-year postdonation eGFR, mean ± SD	69.1 ± 10.0

eGFR: mL/min/1.73 m^2.

We retrospectively reviewed the clinical data of all (n = 366) the donors who underwent nephrectomy for living donor kidney transplantation at our institution between 1998 and December 2019. After excluding 33 donors, in whom eGFR at 1 year was missing, the remaining 333 donors were included in this study.

Following international guidelines, all donors were subjected to a standard evaluation protocol. Baseline demographic, anthropomorphic, analytical, and clinical data were collected from the living kidney donors. Serum creatinine Serum creatinine-based CKD-EPI equation (15) was used to predict eGFR. Split renal function was evaluated by Nuclear Renography and renal anatomy by a Computed Tomography scan.

Hypertension was defined by blood pressure in the consultation >140/90 mmHg, ABPM > 135/85 mmHg, and past diagnosis of hypertension or antihypertensive medication. Uncontrolled hypertension or evidence of end-organ damage were criteria of exclusion. Potential donors with a history of malignancy, obesity, or diabetes were excluded. Although a lower limit of eGFR was not established by Unit protocol, potential donors with eGFR below 80 m mL/min/1.73 m^2 were usually discarded. The final approval for kidney donation was reviewed in a multidisciplinary meeting and the ethical approval was mandatory.

Left-side procurement was preferred for anatomical reasons except for complex vessels anatomy or when a significant renal asymmetry was found, and the right kidney had the lower clearance. A transperitoneal laparoscopic approach was performed in most donors. Lifetime annual follow-up appointments are available for all donors.

For validation of the predictive model, eGFR was calculated using the CKD-EPI Chronic Kidney Disease Epidemiology pre-donation and 1 year (±30 days) after donation.

Statistical Analysis

Data are presented as mean (and standard deviations for continuous variables and frequency (and percentages) for categorical variables.

Observed eGFR using CKD-EPI formula at 1-year post-donation was compared to the predicted eGFR using the formula developed in Toulouse-Rangueil: postoperative eGFR (CKD-EPI, mL/min/1.73 m^2) = 31.71 + (0.521 × preoperative eGFR) − (0.314 × age).

The ability of this formula to predict the observed GFR was analyzed by Pearson correlation, and agreement was explored by the Bland-Altman plot. The discriminative ability to predict CKD3-5 was evaluated by the area under the receiver operating characteristic (ROC) curve and using sensitivity, specificity, and positive, or negative predictive values (PPV or NPV). Furthermore, the accuracy of the predictive model was depicted by constructing a calibration plot and assessed through the calibration slope and the calibration in the large.

A 2-sided p-value < 0.05 was considered as statistically significant. Statistical calculations were performed using STATA/MP, version 15.1 (Stata Corp, College Station, TX, United States).

RESULTS

Baseline Characteristics

The baseline donors' characteristics for the cohort of 333 patients are presented in **Table 1**. The mean donor age was 47.3 ± 10.6 years old (age range 20.7–76.2 years old), and most were female (71%). The mean body mass index was 25.3 ± 3.4 Kg/m^2. Fifty donors (15%) were hypertensive pre-donation, and fifty-one (15%) had smoking habits. Pre-donation mean eGFR was 100.3 ± 14.7 mL/min/1.73 m^2, while the mean 1-year post-donation eGFR was 71.4 ± 16.2 mL/min/1.73 m^2. The mean predicted 1-year post-donation GFR was 69.1 ± 10.0 mL/min/1.73 m^2.

Eighty-five donors (25.5%) reached the definition of CKD at 1-year after donation as depicted in **Table 2**.

A significant correlation was observed between calculated and observed 1-year eGFR (p < 0.001; Pearson R = 0.67), as shown in **Figure 1**. The concordance is represented by the Bland-Altman plot with a mean difference of observed-predicted eGFR = +2.33 mL/min/1.73 m^2 (95% limits of agreement −21.41–26.47 mL/min/1.73 m^2; p < 0.001) (**Figure 2**).

Furthermore, the model showed a good discriminative ability of the formula in predicting observed CKD at 1-year post-donation, with the area under the receiver operating characteristic (ROC) curve of 0.83 (95% CI: 0.78–0.88; p < 0.001), as shown in **Figure 3**, with optimal cutoff (by Youden criteria) corresponding to a predicted eGFR of 65.25 mL/min/1.73 m^2 (5.25 mL above the equality cutoff), for which the sensibility and specificity to predict CKD were respectively 77% and 75% (**Table 2**). Overall, the model performance was similar in females and males (data not shown), although the optimal cutoff for the female sex corresponded to 62.23 mL/min/1.73 m^2 (2.23 mL above the equality cutoff), for which the sensibility and specificity to predict CKD were respectively 66% and 85%. For the male sex, the optimal cutoff was similar to the global cohort, for which the sensibility and specificity to predict CKD were 77% and 82%, respectively.

The Calibration curves illustrated the model's accuracy in the prediction of eGFR <60 mL/min/1.73 m^2 at 1 year. The calibration curve, shown in **Figure 4**, exhibited an excellent prediction with a slope = 1.000 and a Calibration In The Large (CITL) = 0.000.

TABLE 2 | ROC: McNemar's exact test for optimal cutoff and for CKD cutoff.

		Observed eGFR		Total
		<60	≥60	
Predicted eGFR	<65.25	65 (76)	61 (25)	126
	≥65.25	20 (24)	187 (75)	207
Total		85	248	333
McNemar's exact test $p < 0.001$, Sensitivity 77%, Specificity 75%, PPV 52%, NPV 90%				
Predicted eGFR	<60	40 (47)	17 (7)	57
	≥60	45 (53)	231 (93)	276
Total		85	248	333
McNemar's exact test $p < 0.001$, Sensitivity 47%, Specificity 93%, PPV 70%, NPV 84%				

eGFR: mL/min/1.73 m^2.

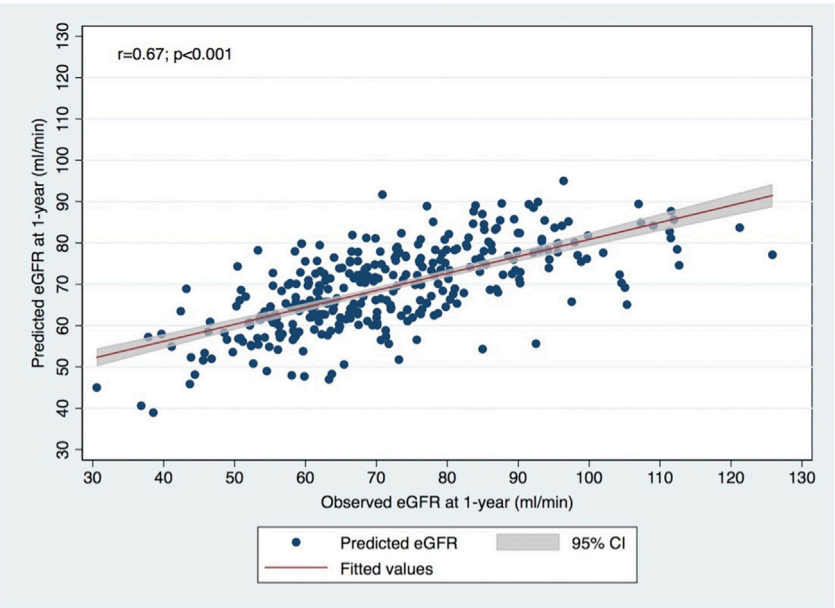


FIGURE 1 | Correlation between observed eGFR using CKD-EPI formula at 1-year post-donation and predicted eGFR using the formula developed in Toulouse-Rangueil.

DISCUSSION

In this study, the predictive model developed at Toulouse-Rangueil (11) was validated externally in our cohort of living kidney donors in concordance with other external validation studies in different European populations (12, 13). A significant correlation was observed between calculated and observed 1-year eGFR (Pearson $r = 0.67$), and for the prediction of CKD (eGFR values < 60 mL/min/1.73 m^2) at 1 year after donation, the model presented an AUROC of 0.83, which represents an excellent performance. Benoit et al. (13), in a population of 400 French living donors that performed nephrectomy at Necker Hospital, also described a significant correlation between predicted and observed 1-year eGFR (Pearson $r = 0.66$), and for the prediction of CKD at 1 year, the model presented an AUROC of 0.86. We must emphasize that the optimal value of predicted eGFR was around 5 mL/min higher than the equality cutoff for CKD detection at 1 year, an outcome that was correctly predicted (both its presence and absence) in every 3 out of 4 donors. This tool represents a non-invasive, low cost and readily available tool that can be joined to the living donor evaluation routine consultation, improving the living donor risk estimation and the informed consent process. The predicted eGFR value ≥ 65.25 mL/min was associated with a very high NPV (90%), identifying donors that are clearly admissible concerning renal function (**Table 2**). Otherwise, a predicted eGFR < 60 mL/min was associated with a high PPV (70%), identifying donors that probably should not be accepted, concerning their renal function. Anyway, a global risk assessment is mandatory (4). An older donor will have a lower 1-year eGFR, and the lower expected lifespan will mitigate a higher chance of CKD, but the expected risk of ESRD compared to a younger donor.

LDKT is considered safe, but some donors will develop CKD. And, rarely, ESRD. Two landmark studies in the living kidney donation (8, 9) made this discussion more pertinent. Furthermore,

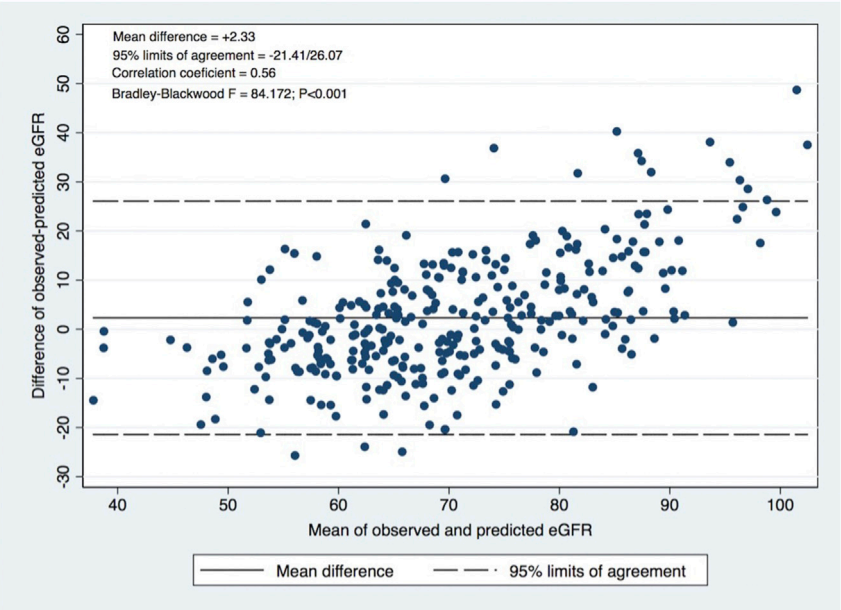


FIGURE 2 | Bland-Altman plot: Agreement evaluation, correlation coefficient between the difference and the mean of observed and predicted eGFR.

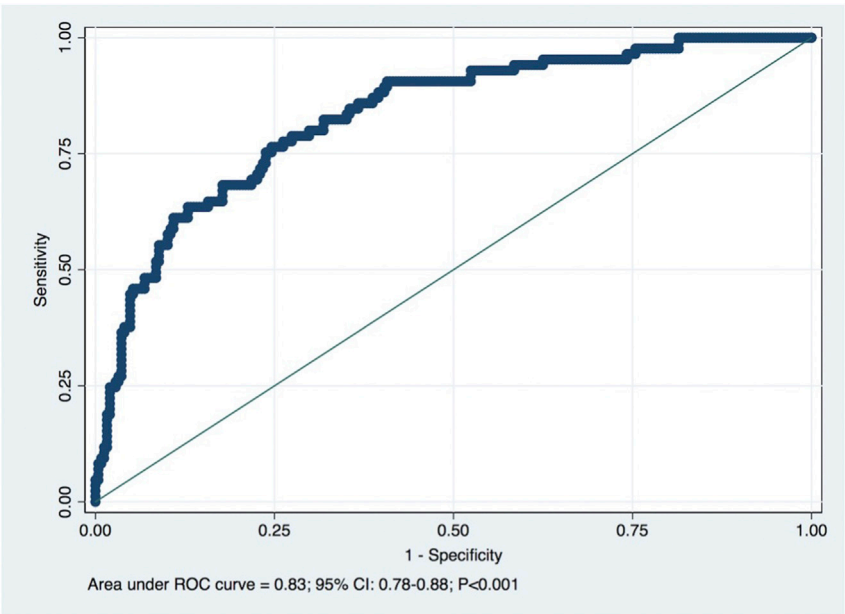


FIGURE 3 | Receiver operating characteristic (ROC) curve for predicted eGFR for the detection of CKD (eGFR < 60 mL/min/1.73 m^2). Diagonal line is the reference line: AUC = 0.83. Optimal cutoff: 65.25 mL/min/1.73 m^2.

the characteristics of our living donors are changing. We are facing a population increasingly older in dialysis, and their potential donors are also older, with an increasing chance of having borderline preoperative eGFR. In this tool, age, and preoperative CKD-EPI eGFR were shown to be independent predictors of 1-year postoperative renal function.

The evaluation of the glomerular filtration rate is a crucial point in LKD. We used eGFR based on serum creatinine determinations because it is feasible and is the most common method worldwide (4). More reliable methods of isotopic evaluation are not routinely available (4). In a large retrospective study, Stevens et al. (16) demonstrated that

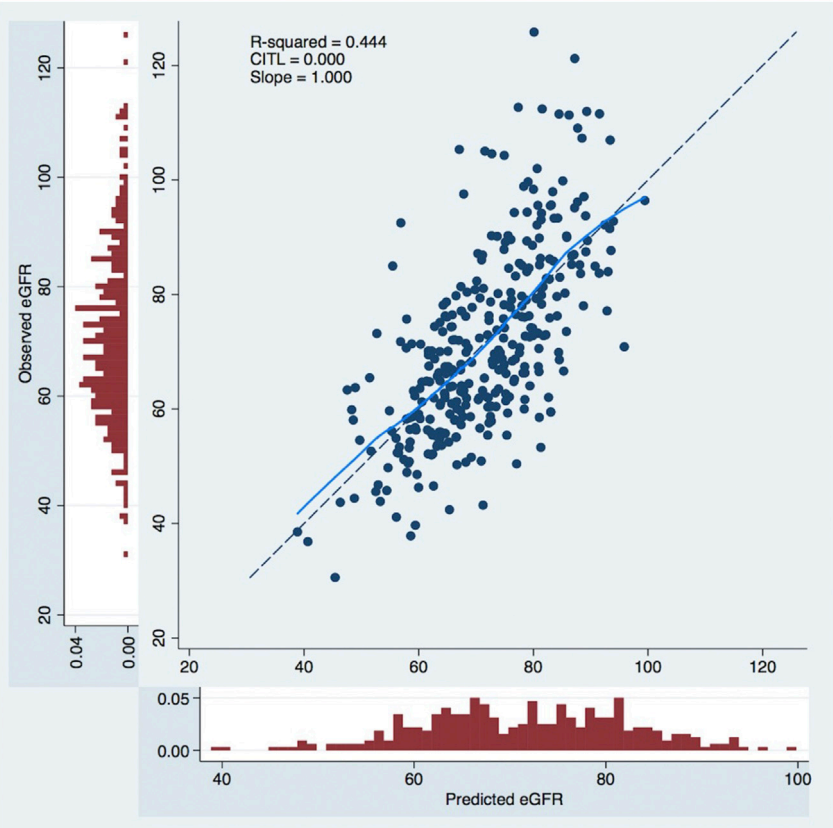


FIGURE 4 | Calibration curves to predict 1-year postoperative eGFR. The *x*-axis represents model predictions, the *y*-axis the observed eGFR at 1-year. CITL, calibration in the large; eGFR, estimated glomerular filtration rate, mL/min/1.73.

CKD-EPI estimates were more accurate than MDRD estimates considering the actual GFR measured by urinary or plasma clearance of exogenous filtration markers. It suggests that the CKD-EPI method must be preferred in the clinical practice (16). Most transplant centers use CKD-EPI equation eGFR in the initial assessment of renal function in potential living kidney donors (5), and it was the method used in the initial description of the model by Benoit et al. (11), although the external validation by Kulik et al. (12) used the MDRD formula to calculate the eGFR pre and after donation.

The risk of ESRD in living donors, although marginal, was evidenced in two studies in comparison with healthy controls (8, 9). As ESRD is a rare event, its surrogates have been pursued by several groups to improve living donor selection and donor safety. CKD, as defined by eGFR < 60 mL/min/1.73 m^2, was associated with an increased risk of death, cardiovascular events, and hospitalization in a large, community-based population (10). In a registry-based cohort study of 71,468 living kidney donors, reported an independent association of living kidney donor eGFR at postoperative 6 months and subsequent ESRD. A 10 mL/min/1.73 m^2 difference in early post-donation estimated glomerular filtration rate was significantly associated with a 28% higher risk of subsequent end-stage renal disease (17). However, no significant association has been found with the preoperative eGFR (17), and no marker could be identified in pre donation evaluation. One-year post-donation eGFR was assessed in this study, and it can be assumed as a surrogate of long-term renal function in the donor. We hypothesize that donors with lower eGFR 1 year after donation would benefit from increased surveillance and further preventive measures for renal health. Considering the global performance of this formula, we can go further and hypothesize that at pre-donation consultation, potential donors with predicted lower 1-year eGFR could be considered unfit to donate, after a global risk assessment, considering donor age and expected lifespan.

Benoit et al. (11) developed a model to estimate the donor's 1-year post-donation eGFR. In this predictive model, Age and preoperative eGFR were shown to be independent predictors of 1-year postoperative renal function. Other donor characteristics like kidney size, gender, hypertension, obesity, dyslipidemia, and smoking were not found to influence the 1-year postoperative eGFR (11). In contrast, a recent retrospective study from Lam et al. (18) evaluated a Canadian cohort of living kidney donors and allowed a better understanding of kidney function over 5 years after living donor nephrectomy. In this study, changes in eGFR after donation varied by sex, percent decline in eGFR within the first 6 weeks after donation, and eGFR category at 1 year, but not by age category at donation, pre-

donation hypertension, pre-donation eGFR category, socioeconomic status, or distance to transplant center (18). Be it as it may, the predictive model developed from the Toulouse-Rangueil cohort unquestionably showed a good correlation between predicted and observed donor eGFR 1-year after donation in 3 different centers (11–13). These results, along with the fact that donor age was found to be a strong predictor of CKD after LDKT, may defer the wish to extend, without fair criticism, the age limit of donors, which has been advocated to expand the pool (19, 20). A global risk assessment must always guide the clinical decision.

At the original cohort (11), 22.4% of donors had CKD at 1-year after donation, meeting KDIGO criteria of CKD (21). Kullik et al. (12), in the external validation in a German cohort, found a surprisingly higher incidence of CKD in their LKD cohort: 70.8%. A careful interpretation is needed as eGFR was calculated using the MDRD formula and not CKD-EPI. Additionally, the authors refer that at least 30% of all living donors preferred external follow-up appointments and were not included in the study. In our population, 25.5% of donors (85 out of 333) reached the definition of CKD, although none had ESRD at long-term follow-up. These donors represent a population that deserves more careful long-term surveillance. Further studies are necessary to evaluate the different trajectories of the long-term evolution of kidney function in these donors. It is recognized that some groups of living donors have a higher long-term risk of ESRD than others. Massie et al. (22) used data from the Scientific Registry of Transplant Recipients of 133,824 living kidney donors in the United States between 1978 and 2015 to construct a risk calculator that includes sex, Age, race, BMI, and first-degree biological relationship. Male sex, black race, older Age in the non-black race, greater body mass index, and first-degree biological relationship to the recipient were associated with increased risk of ESRD (22). Although the predicted 20-year risk of ESRD for the median donor was only 34 cases per 10,000 donors, 1% of donors had predicted risk exceeding 256 cases per 10,000 donors (22). Ibrahim et al. (23) used data from the University of Minnesota from 3,956 White kidney donors between 1963 and 2013. Their calculator estimates ESRD risk in White donors using Age, BMI, and systolic blood pressure at the time of donation (23). ESRD was associated with older age, higher BMI, and higher systolic blood pressure in the donation (23).

Most of our living donors were females (71%). Women are more likely than men to become living kidney donors (24, 25). In a recently published review of country-specific sex disparities in living kidney donation (26), Kurnikowski et al., described a population size-weighted donor distribution consisting of 35.9% men and 64.1% women. This data cannot be explained by a comprehensive reason (24). Biological and sociocultural aspects must be considered. Biological reasons usually described include the sex distributions of some potential biological risk factors for disease, including smoking, and a higher incidence of hypertension and ischemic heart disease that can preclude the acceptance of male candidates more often. Although women have a higher prevalence of chronic kidney disease than men, end-stage renal disease incidence is higher in men (24). Socio-cultural aspects are very significant in most cultures. It is expected that increased altruism from women, is derived from the women's more traditional role as the caregiver in the family (25–27). The family expectations frequently remain on her to be a living donor, whether it remains on the man to keep working and support the entire family. This is still very common in Portuguese society nowadays, mainly in the rural and less favored communities. The predictive model performance did not differ when both sexes were considered separately, although the optimal cutoff for the prediction of CKD was slightly lower in women.

We must recognize the limitations associated with this study, beginning with its retrospective and observational design. Thirty-one donors were excluded from the study because 1-year serum creatinine was unavailable to calculate eGFR. Still, later creatinine values were available and were not different from the rest of the cohort. We assume it would not compromise the results of our validation cohort. All patients were Caucasians, but they were representative of the Portuguese population. Other races and ethnic origins are not represented. We used CKD-EPI to calculate eGFR and not an isotopic method. However, we must point out the unsuitability of the latter in clinical practice, as it is not recommended as a standard of care by current guidelines (4), and the model itself was developed using the CKD-EPI formula. Although we must be aware of the potential risk of analysis bias judgment of the original model, it should not preclude the results of this and the other external validation results.

The primary goal in assessing a living donor candidate must ensure minimal risk to the donor. Hence, the prediction of postoperative renal function is a critical point in their evaluation and, in our population, can be achieved with this tool. Furthermore, the required variables are low-cost and easily assessed, so its potential as a counseling tool is undeniable. We recall, however, that validation out of Europe is lacking and that further studies are necessary to validate prognostic models for longer-term prediction of donor kidney function.

CONCLUSION

The formula developed in Toulouse-Rangueil was successfully validated in our cohort, a different European population than previously described. We must, anyway, emphasize that the optimal value of predicted eGFR was around 5 mL/min higher than the equality cutoff for CKD detection at 1 year. This model represents a simple and accurate tool that may be used to assist in the evaluation of potential donors, particularly in the current setting of increasing donor age, donors with minor comorbidities, or renal function close to the accepted threshold.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics board of Centro Hospitalar Universitario

do Porto (CHUPorto) [Ref.: 147-21 (119-DEFI/122-CE)]. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MA, CC, and JM: Research design, data acquisition, data analysis, and paper writing. CS, CF, SV, JS, SC, and SP were engaged in the data acquisition and analysis. MR and LM were involved in the research design and data analysis. All the authors approved the submitted version.

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CONFLICT OF INTEREST

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

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ORIGINAL RESEARCH
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Simultaneous Heart-Kidney Transplant—Does Hospital Experience With Heart Transplant or Kidney Transplant Have a Greater Impact on Patient Outcomes?

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High institutional transplant volume is associated with improved outcomes in isolated heart and kidney transplant. The aim of this study was to assess trends and outcomes of simultaneous heart-kidney transplant (SHKT) nationally, as well as the impact of institutional heart and kidney transplant volume on survival. All adult patients who underwent SHKT between 2005–2019 were identified using the United Network for Organ Sharing (UNOS) database. Annual institutional volumes in single organ transplant were determined. Univariate and multivariable analyses were conducted to assess the impact of demographics, comorbidities, and institutional transplant volumes on 1-year survival. 1564 SHKT were identified, increasing from 54 in 2005 to 221 in 2019. In centers performing SHKT, median annual heart transplant volume was 35.0 (IQR 24.0–56.0) and median annual kidney transplant volume was 166.0 (IQR 89.5–224.0). One-year survival was 88.4%. In multivariable analysis, increasing heart transplant volume, but not kidney transplant volume, was associated with improved 1-year survival. Increasing donor age, dialysis requirement, ischemic times, and bilirubin were also independently associated with reduced 1-year survival. Based on this data, high-volume heart transplant centers may be better equipped with managing SHKT patients than high-volume kidney transplant centers.

Keywords: outcomes, kidney transplant, heart transplant, cardiac function, volume

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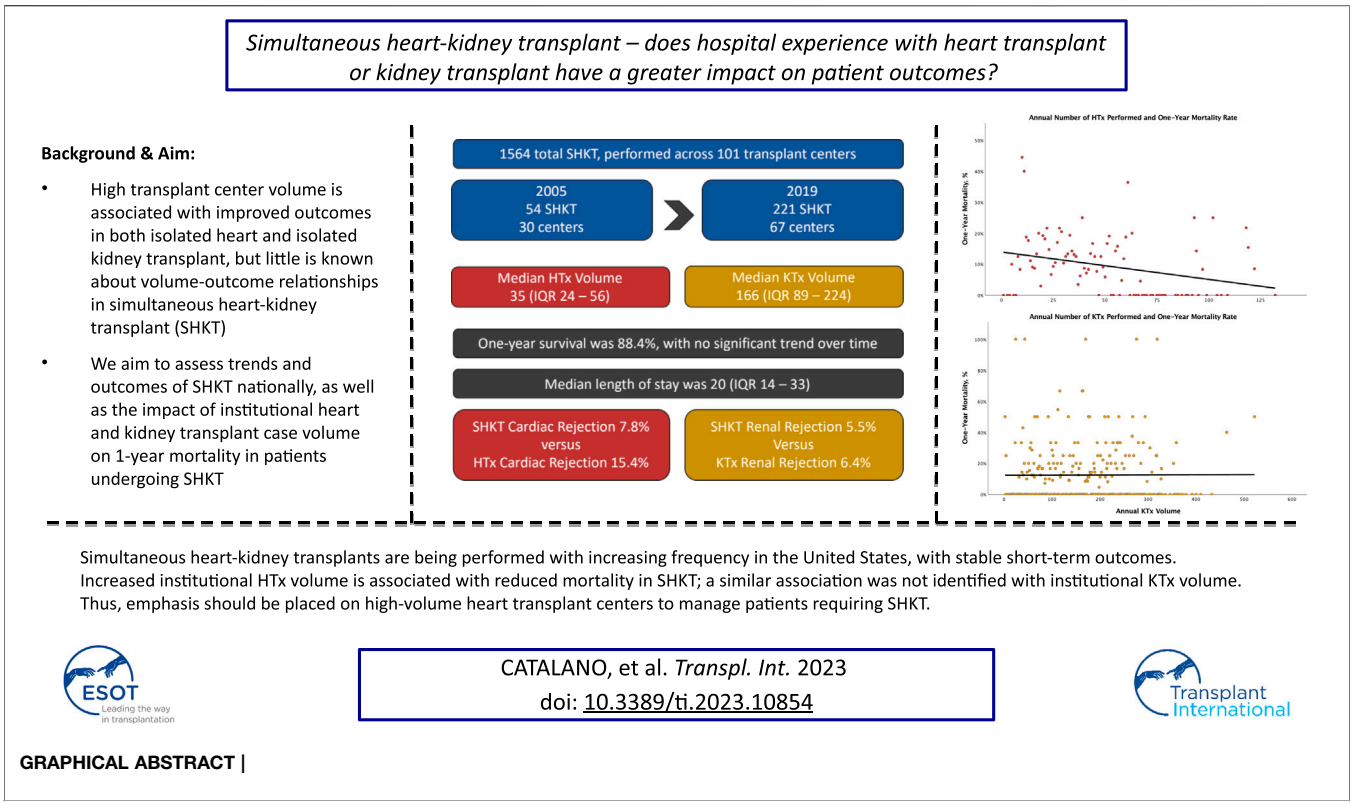
Abbreviations: ECMO, extracorporeal membrane oxygenation; HTx, heart transplant; IABP, intra-aortic balloon pump; KTx, kidney transplant; LVAD, left ventricular assist device; NDDRI, non-dialysis-dependent renal insufficiency; OR, odds-ratio; SHKT, simultaneous heart-kidney transplant; STAR, standard transplant analysis and research; UNOS, United Network for Organ Sharing.



GRAPHICAL ABSTRACT |

INTRODUCTION

Kidney disease and heart disease share common risk factors. Given these shared risk factors, as well as the renal impairment with abnormal hemodynamics associated with heart failure, end-stage heart and kidney disease frequently coexist. For that reason, as well as general overall improvement in organ transplant outcomes, there has been an increase in simultaneous heart-kidney transplant (SHKT) in the United States (1, 2). Small, single-center studies have demonstrated acceptable outcomes for this procedure (3–6), and large, national database studies have revealed improved outcomes relative to isolated heart transplant (HTx) in certain patient populations (1, 7–10). While a number of ethical and clinical questions remain regarding the utilization of SHKT (2, 11), its increasing utilization in the United States warrants further study. Specifically, it is important to assess which institutions may be best suited to care for this unique patient population.

Across surgical subspecialties, institutional experience with surgical procedures is associated with significantly improved clinical outcomes (12–15). This relationship has been demonstrated in both isolated HTx (16–23) and isolated kidney transplant (KTx) (24–28), as well as in lung and liver transplants (24, 29–32). However, little is known about the relationship between surgical volume and outcomes in SHKT.

The aim of this study was to evaluate contemporary trends and outcomes of SHKT nationally and to assess the impact of institutional HTx and KTx case volume on 1-year survival in patients undergoing SHKT.

MATERIALS AND METHODS

A retrospective analysis of the United Network for Organ Sharing (UNOS) Standard Transplant Analysis and Research (STAR) files was conducted for the years 2005–2019. This study was deemed exempt from review by an Institutional Review Board as the data provided by UNOS contains no patient identifiers.

In order to understand national trends in transplant volume, we first analyzed the total volume of isolated HTx, isolated KTx, and SHKT in adult patients (≥18 years old) performed in the United States each year. In order to avoid double-counting, SHKT patients were not included in our volume analysis of isolated HTx and KTx.

All adult patients who underwent SHKT were included in our analysis; patients undergoing sequential heart-kidney transplant were excluded. Patient-specific information collected included sex, age at transplant, body mass index (BMI), diabetes, total bilirubin at transplant, creatinine at transplant, and dialysis requirement at listing (as well as an indicator of hemodialysis *versus* peritoneal dialysis) and at transplant. Dialysis requirement was selected as the indicator of renal function to allow for more consistent comparison between patients—creatinine or eGFR measurements may vary significantly based on when drawn. The utilization of cardiovascular support at time of transplant, including extracorporeal membrane oxygenation (ECMO), intraaortic balloon pump (IABP), left-ventricular assist device (LVAD), and inotropic agents was also collected. These variables were utilized as primary indicators of global hemodynamic

compromise. Additionally, hemodynamics at time of transplant—including cardiac output, pulmonary artery pressures, and pulmonary capillary wedge pressures—were assessed; however, the use of quantitative measures of hemodynamics is limited given the possibility of transient fluctuations in these markers that may misrepresent the true overall hemodynamic picture based on when they were captured. Other variables included total days on waitlist, cardiac and renal ischemia time in hours, and age of heart donor.

Institutional experience in isolated heart transplant (HTx), isolated kidney transplant (KTx), and SHKT was assessed as the annual institutional transplant volume, by year. Thus, each institution is assigned a value for HTx volume, KTx volume, and SHKT volume for each year it participated in the dataset. This methodology was used in order to account for the dynamic changes in institutional experience over time, especially those that have recently opened and demonstrated rapid growth.

The primary outcome of interest was 1-year post-transplant survival. Secondary endpoints included length of stay, acute heart transplant rejection episodes requiring treatment within 1 year of transplant, and acute kidney rejection transplant episodes requiring treatment within 1 year of transplant. Length of stay was evaluated as the number of days from transplant to discharge or death. In evaluating 1-year post-transplant survival and rejection episodes requiring treatment, patients undergoing transplant in 2019 were excluded. This step was taken to avoid potential effects on survival of the COVID-19 pandemic in the year 2020.

In order to describe overall trends in utilization, the entire dataset was queried to identify all HTx and KTx over the selected timeframe, as well as changes over time. Trends were also assessed among the selected sample of SHKT. Next, descriptive analysis was conducted for the selected sample, including patient demographics, donor demographics, risk factors, organ ischemia time, and institutional experience. Each of these factors was also assessed as a predictor of 1-year survival in univariate and multivariable analysis. In univariate analysis, the Pearson chi-square test was used to analyze categorical variables, and Student's t-test was used to evaluate continuous variables. In multivariable analysis, binary logistic regressions were conducted, and odds-ratios (OR) and p-values are reported. Multivariable analysis was also conducted to assess predictors of secondary endpoints. Length of stay was assessed using multivariable linear regression, with coefficients and p-values reported. Acute transplant rejection episodes were assessed using binary logistic regression, with OR and p-values reported.

All statistical analyses were performed using SAS, version 9.4 (SAS Institute, Inc., Cary, NC). All P-values were 2-sided with a significance threshold of <0.05. A 95% confidence interval ($p < 0.05$) was defined as statistical significance for all analyses.

RESULTS

Trends in utilization of SHKT, HTx, and KTx are presented in **Figure 1**. Over the study period of 2005–2019, we identified 1564 SHKT, increasing from 54 procedures performed across 30 centers in 2005 to 221 procedures across 67 centers in 2019 (309.3% volume growth). While incidence of isolated HTx (1,841 in 2005, to 3,088 in 2019, 67.7% volume growth) and isolated KTx (16,489 in 2005, to 23,510 in 2019, 42.6% volume growth) also increased over the study period, the magnitude of growth was substantially lower. Utilization of SHKT increased from 2.9% of all heart transplants performed in 2005, to 7.2% in 2019. We observed a 1-year mortality of 11.5% for SHKT, with no significant change over time. Median length of stay was 20.0 days (IQR 14.0–33.0). Cardiac rejection episodes in the first-year post-transplant occurred in 7.8% of SHKT patients (*versus* 15.4% of isolated HTx), and kidney allograft rejection episodes in the first-year post-transplant occurred in 5.5% of SHKT patients (*versus* 6.4% of isolated KTx).

Baseline characteristics for patients undergoing SHKT and institutional transplant volume, and their association with 1-year survival, for the years 2005–2018, are presented in **Table 1**. Across the 1,343 patients, mean recipient age was 54.1 ± 11.5 years; mean donor age was 31.7 ± 11.4 years. Male patients made up 79.1% of the sample. There was no significant association between recipient age or sex and survival in univariate analysis; increasing donor age was associated with decreased survival ($p = 0.019$). Dialysis requirement was observed in 30.0% of patients at listing (including 27.0% of patients on hemodialysis and 3.0% of patients on peritoneal dialysis) and 38.2% of patients at time of transplant. Hemodialysis at listing trended towards an association with reduced survival ($p = 0.076$); any dialysis at transplant was associated with decreased survival ($p < 0.001$). Other patient and transplant factors associated with decreased survival on univariate analysis included elevated total bilirubin ($p < .001$), increased cardiac ischemia time ($p = 0.007$), and increased renal ischemia time ($p = 0.046$).

At the time of transplant, 603 (44.9%) patients were supported by inotropes, 275 (20.5%) were supported by an LVAD, 109 (8.1%) were supported by an IABP, and 17 (1.3%) were supported by ECMO. Utilization of inotropic or mechanical circulatory support was not associated with 1-year survival. While there was no significant association between mechanical circulatory support and survival, elevated pulmonary artery pressures and pulmonary capillary wedge pressures were associated with reduced 1-year survival (**Table 1**).

Median annual institutional HTx volume across the sample of institutions performing SHKT was 35.0 (IQR 24.0–56.0); median annual institutional KTx volume was 166.0 (IQR 89.5–224.0). Centers performing SHKT had greater annual experience with isolated HTx and KTx than centers which did not perform SHKT (**Figure 2**). In 2019, median HTx volume across all institutions was 23, compared to median HTx volume of 32 across institutions performing SHKT. Similarly, median KTx volume across all institutions was 70, compared to median KTx volume of 164 across institutions performing SHKT. On univariate analysis, transplant centers performing a higher volume of annual heart transplants had improved 1-year survial in their SHKT patients (annual volume of 44.2 ± 30.4 in patients who survived, vs. annual volume of 36.4 ± 24.2 in patients who died, $p = 0.002$). There was

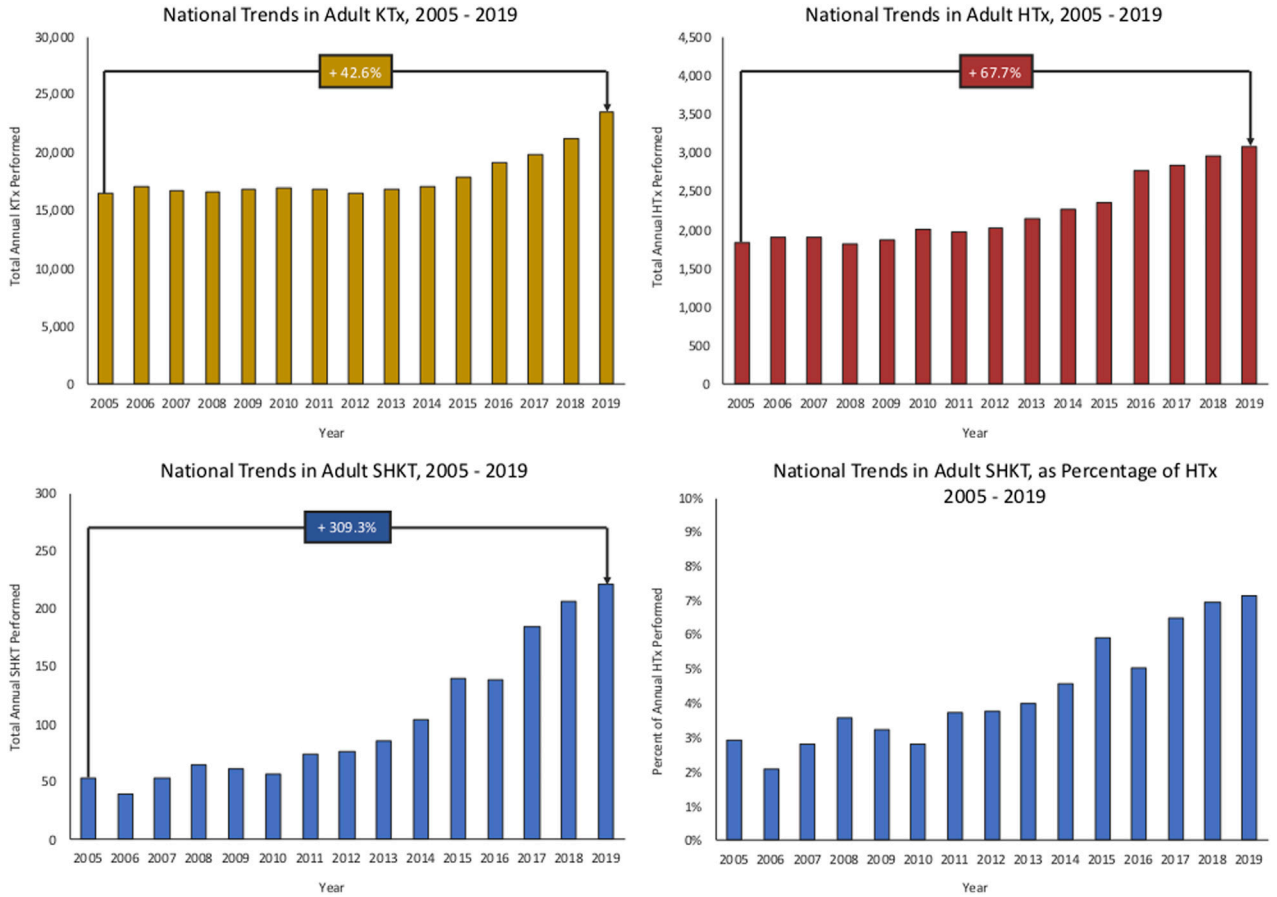


FIGURE 1 | National trends in adult isolated KTx, HTx, SHKT, and SHKT as a proportion of total HTx (2005–2019). HTx, heart transplant; KTx, kidney transplant; SHKT, simultaneous heart-kidney transplant.

no significant association between annual kidney transplant volume and survival (p = 0.121) (**Table 1**).

Multivariable analysis of factors associated with 1-year suvival in SHKT patients is shown in **Table 2**. Increased annual heart transplant volume remained associated with improved 1-year survival (OR 1.12 for every 10 heart transplants, p = 0.004). Other factors associated with decreased 1-year survival included increasing donor age, increasing recipient serum bilirubin, dialysis requirement at transplant, and increasing cardiac ischemia time. Annual kidney transplant volume was not associated with 1-year survival (p = 0.485).

Factors associated with prolonged length of stay after transplant in multivariable analysis included younger transplant recipient age, older heart donor age, higher recipient bilirubin, and longer renal ischemia time (**Table 3**). None of the assessed variables were associated with 1-year cardiac rejection episodes in multivariable analysis (**Table 3**). The presence of dialysis at transplant and reduced cardiac ischemia time was associated with increased risk of 1-year renal rejection episodes (**Table 3**).

DISCUSSION

Our study provides a contemporary assessment of the utilization and outcomes of SHKT, and is the first to assess the impact of institutional experience with HTx and KTx on SHKT outcomes. We identify a continued trend of increased SHKT utilization, increasing 309.3% over 14 years. We also observe a significant association between annual institutional HTx volume and 1-year survival in SHKT patients. A similar association between institutional KTx volume and SHKT outcomes was not observed. Further, we found that dialysis at transplant, increased donor age, increased bilirubin, and prolonged cardiac ischemia time are independently associated with reduced 1-year survival.

Our finding of increased utilization of SHKT, out-of-proportion to the increase in isolated HTx, is consistent with prior studies of SHKT in the United States. Karamlou et al., who assessed SHKT vs. isolated HTx in the United States from 2000–2010, found that national HTx volume increased 3.6% over time, while prevalence of SHKT increased 147% (1). Similarly, Melvinsdottir et al. found that, while staged heart-

TABLE 1 | Baseline characteristics as predictors of survival (2005–2018).

Variable	Total	Died	Survived	P-value
Total (%)	1,343	155 (11.5)	1,188 (88.5)	
Male Sex	1,062 (79.1)	123 (79.4)	939 (79.0)	0.927
Recipient Age, years	54.1 ± 11.5	54.3 ± 11.2	54.0 ± 11.6	0.763
Donor Age, years	31.7 ± 11.4	33.7 ± 11.5	31.4 ± 11.4	0.019
Recipient BMI	26.6 ± 4.9	27.3 ± 5.4	26.5 ± 4.8	0.062
Hemodynamics at Transplant				
Cardiac Output	4.9 ± 1.7	5.0 ± 1.8	4.9 ± 1.6	0.792
PA Systolic Pressure	43.9 ± 13.8	47.0 ± 13.6	43.5 ± 13.8	0.003
PA Diastolic Pressure	21.3 ± 7.9	23.3 ± 7.6	21.0 ± 7.9	0.001
Mean PA Pressure	29.9 ± 9.5	32.3 ± 9.1	29.6 ± 9.5	0.002
PCWP	19.8 ± 8.4	21.3 ± 7.7	19.6 ± 8.4	0.028
Dialysis at Listing				
Hemodialysis	362 (27.0)	51 (32.9)	311 (26.2)	0.076
Peritoneal Dialysis	40 (3.0)	6 (3.9)	34 (2.9)	0.487
Dialysis at Transplant	513 (38.2)	83 (53.5)	430 (36.2)	<0.001
Creatinine at Transplant	3.5 ± 2.6	4.0 ± 3.1	3.4 ± 2.5	0.019
Total Bilirubin, mg/dL	1.2 ± 3.4	2.1 ± 6.7	1.1 ± 2.7	<0.001
Waiting List Days	219.5 ± 351.9	199.3 ± 287.2	222.1 ± 359.6	0.448
Recipient Diabetes	580 (43.2)	72 (46.5)	508 (42.8)	0.382
ECMO at Transplant	17 (1.3)	4 (2.6)	13 (1.1)	0.119
IABP at Transplant	109 (8.1)	17 (11.0)	92 (7.7)	0.166
Inotropes at Transplant	603 (44.9)	61 (39.4)	542 (45.6)	0.140
LVAD at Transplant	275 (20.5)	30 (19.4)	245 (20.6)	0.712
Cardiac Ischemic Time, hours	3.1 ± 1.0	3.3 ± 1.1	3.1 ± 1.0	0.007
Kidney Ischemic Time, hours	14.6 ± 8.2	15.9 ± 8.5	14.4 ± 8.2	0.046
Annual HTx Volume	43.3 ± 29.8	36.4 ± 24.2	44.2 ± 30.4	0.002
Annual KTx Volume	162.8 ± 92.1	152.0 ± 90.4	164.2 ± 92.3	0.121

Pearson chi-square test was used for evaluation of categorical variables, with column percent in parentheses; Student's t-test was used for evaluation of continuous variables. BMI, body mass index; PA, pulmonary artery; PCWP, pulmonary capillary wedge pressure; ECMO, extracorporeal membrane oxygenation; HTx, heart transplant; IABP, intraaortic balloon pump; KTx, kidney transplant; LVAD, left ventricular asssit device.

kidney transplant utilization has decreased from 1990–2016, SHKT utilization has increased (2). We demonstrate that this trend has continued, as SHKT as a proportion of total HTx has increased from 2.9% in 2005% to 7.2% in 2019. The increase in utilization has likely been influenced by evolving literature demonstrating acceptable outcomes of patients undergoing SHKT. In 1997, Laufer et al. retrospectively assessed the clinical and immunologic outcomes of six patients who underwent SHKT at their institution. With a mean follow-up of 32 months, they identified 100% survival, with no episodes of renal transplant rejection. Further, in a comparison to isolated HTx patients, there was no difference in rates of cardiac rejection (5). Hermsen et al., similarly, reviewed patient and graft survival across 19 SHKTs performed at their institution from 1987–2006, comparing outcomes to isolated HTx, isolated KTx, and staged heart-kidney transplant. They found no difference in patient or graft survival; further, they identified reduced rates of coronary allograft vasculopathy and increased time to graft rejection episodes in SHKT patients, suggesting an immunologic benefit to simultaneous organ transplantation (4). Our finding of reduced cardiac and kidney allograft rejection episodes for SHKT patients, as compared with isolated HTx and KTx, supports this suggested immunologic benefit. Grupper et al., in their 2017 study of 35 SHKT patients, identified survival rates of 97% at 6 months, 91% at 1 year, and 86% at 3 years (3). This 1-year mortality rate of 9% is comparable to our finding of 11.5% 1-year mortality nationally.

As utilization of SHKT continues to increase nationally, it is vital to understand if there are centers that may be better suited to care for this unique patient population. Based on the existence of a volume-outcome relationship in organ transplantation (16–32) and other surgical fields (12–15), our focus was on identifying whether experience with one or both components of this particular multi-organ transplant has an impact on outcomes. Our finding that increased annual HTx volume is associated with improved SHKT survival is consistent with our hypothesis of the existence of a volume-outcome relationship, and it is consistent with prior isolated HTx literature. In their study of isolated HTx in Korea, Nam et al. assessed outcomes in 833 adult transplants across 17 centers, identifying in-hospital mortality of 3.7% in high-volume centers (>20 HTx/year), 10.1% in medium-volume centers (10–20 HTx/year), and 18.6% in low-volume centers (<10 HTx/year). This difference persisted in evaluation of 10-year survival (19). Differences in short-term and long-term HTx patient and graft survival have also been demonstrated using UNOS in both congenital (17, 18) and general adult populations (16, 21–23). In order to understand why a volume-outcome relationship may exist in HTx, Arnaoutakis et al. assessed institutional volume as an effect modifier on the relationship between patient risk and survival. In their analysis, low-volume centers (<7 HTx/year) had increased mortality relative to medium-volume (7–15 HTx/year) and high-volume (>15 HTx/year) centers. However, the difference in mortality was primarily driven by outcomes in high-risk patients; the effect

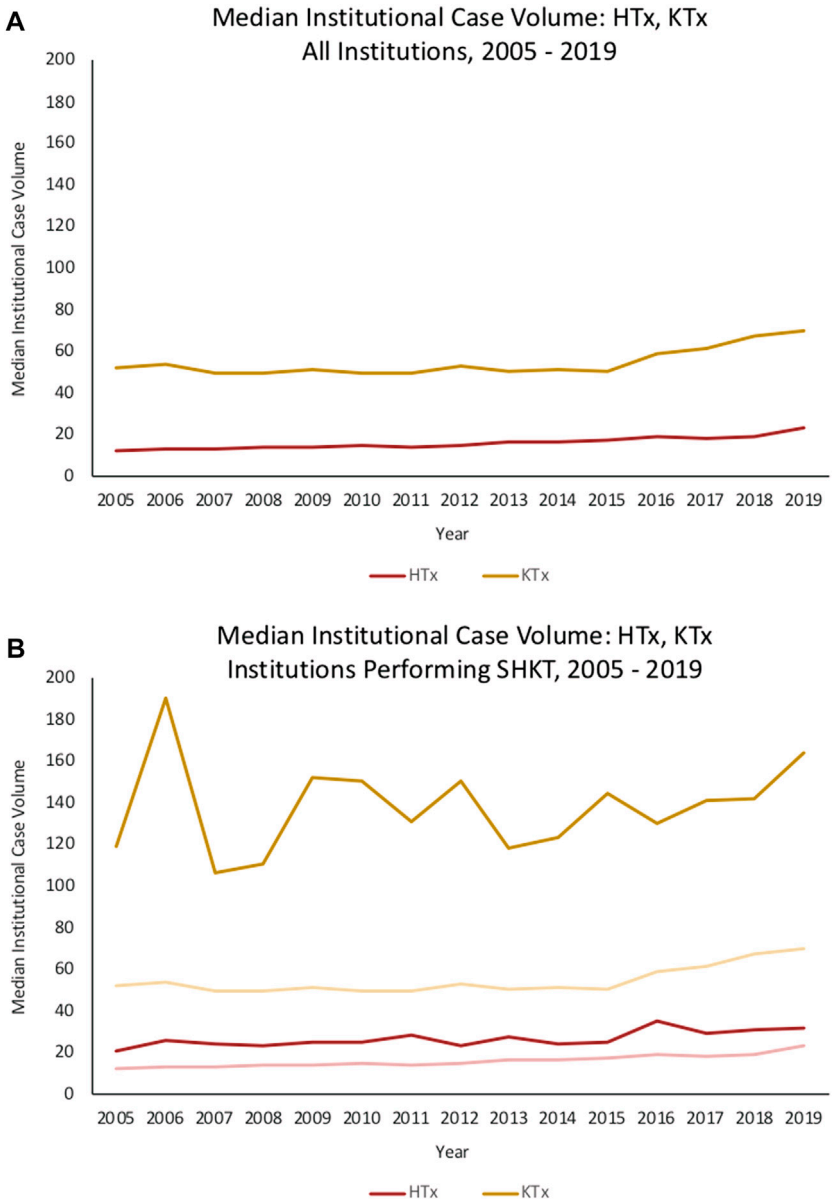


FIGURE 2 | Trends in median institutional case volume for HTx, KTx, and SHKT among **(A)** all institutions in the United States, 2005–2019, and **(B)** only institutions performing SHKT in the United States, 2005–2019. HTx, heart transplant; KTx, kidney transplant; SHKT, simultaneous heart-kidney transplant.

of center volume on outcomes in low-risk patients is minimal (16). This suggests that institutional experience in HTx may primarily play a role in caring for sicker, more complex patients. While we do not quantify risk in our study, SHKT patients tend to carry a greater burden of comorbidities than isolated HTx patients, potentially explaining why a volume-outcome relationship was observed. It is, indeed, possible that lower volume centers included in our sample were transplanting sicker patients; however, despite including comorbidities in our multivariable analysis, case volume remained a significant predictor of post-operative survival, suggesting that experience may be important across all risk groups. Another study that provides insight into the reason that experience in transplant affects outcomes is that by Kilic et al. In their study of isolated lung transplant, they found no association between center volume and occurrence of major post-operative complications. However, they found that in patients who do experience complications, risk of mortality is significantly greater at low-volume centers (29). This, similar to the results of our study, suggests that higher-volume institutions are better equipped to care for the most complex transplant patients.

In contrast to the HTx volume-outcome relationship, we observed no association between institutional isolated KTx experience and SHKT outcomes. This may be rationalized by

TABLE 2 | Multivariable predictors of 1-year survival in SHKT (2005–2018).

Variable	Odds ratio for mortality (95% CI)	p-value
Annual Heart Transplant Volume (+10)	1.12 (1.04–1.21)	0.004
Annual Kidney Transplant Volume (+10)	1.01 (0.99–1.03)	0.485
Recipient Male Sex	1.19 (0.75–1.88)	0.458
Recipient Age (+10)	0.89 (0.74–1.06)	0.221
Donor Age (+10)	0.83 (0.70–0.98)	0.031
Recipient BMI (+5)	0.87 (0.71–1.06)	0.187
Dialysis at Transplant	0.46 (0.31–0.68)	<0.001
Recipient Serum Bilirubin (+0.3)	0.93 (0.90–0.97)	<0.001
Total Days on Waiting List (+30)	1.01 (0.99–1.02)	0.537
Recipient Diabetes	1.08 (0.72–1.61)	0.690
ECMO at Transplant	0.46 (0.13–1.61)	0.228
Intraaortic Balloon Pump at Transplant	0.71 (0.38–1.33)	0.292
Inotropes at Transplant	1.19 (1.78–1.78)	0.370
Left Ventricular Assist Device at Transplant	1.17 (0.71–1.92)	0.534
Cardiac Ischemia Time (+1 h)	0.78 (0.66–0.92)	0.004
Kidney Ischemia Time (+10 h)	0.83 (0.68–1.02)	0.083

BMI, body mass index; ECMO, extracorporeal membrane oxygenation; SHKT, simultaneous heart-kindey transplant.

TABLE 3 | Multivariable predictors of LOS, 1-year HTx rejection, and 1-year KTx rejection (2005–2018).

Variable	LOS[a]		HTx rejection		KTx rejection	
	Coefficient	P	OR	P	OR	P
Annual HTx Volume (+10)	–0.5 (–1.2, 0.1)	0.119	0.95 (0.88, 1.03)	0.210	0.94 (0.85, 1.04)	0.210
Annual KTx Volume (+10)	–0.2 (–0.4, 0.0)	0.055	1.01 (0.99, 1.04)	0.239	1.01 (0.99, 1.04)	0.339
Recipient Male Sex	2.6 (–2.2, 7.3)	0.283	1.09 (0.64, 1.86)	0.750	0.96 (0.52, 1.77)	0.897
Recipient Age (+10)	–2.4 (–4.1, –0.7)	0.007	0.89 (0.74, 1.08)	0.246	0.83 (0.67, 1.04)	0.106
Donor Age (+10)	2.2 (0.4, 3.9)	0.014	1.01 (0.83, 1.22)	0.912	0.97 (0.77, 1.22)	0.793
Recipient BMI (+5)	0.2 (–1.8, 2.2)	0.828	0.92 (0.73, 1.16)	0.474	1.09 (0.84, 1.42)	0.506
Dialysis at Transplant	2.6 (–1.4, 6.6)	0.208	0.99 (0.64, 1.55)	0.976	2.19 (1.32, 3.65)	0.003
Recipient Bilirubin (+0.3)	0.6 (0.2, 1.1)	0.003	1.00 (0.96, 1.05)	0.878	1.00 (0.95, 1.05)	0.993
Days on Waiting List (+30)	–0.1 (–0.3, 0.1)	0.246	1.00 (0.98, 1.02)	0.937	1.02 (1.00, 1.03)	0.082
Recipient Diabetes	3.0 (–1.0, 7.0)	0.141	1.04 (0.66, 1.64)	0.864	1.18 (0.70, 2.02)	0.534
ECMO at Transplant	11.9 (–1.4, 25.3)	0.080	0.70 (0.09, 5.45)	0.733	0.98 (0.12, 7.95)	0.988
IABP at Transplant	–0.5 (–6.4, 5.5)	0.879	0.86 (0.38, 1.93)	0.712	0.57 (0.17, 1.87)	0.353
Inotropes at Transplant	–3.1 (–7.1, 0.9)	0.129	1.11 (0.71, 1.73)	0.646	1.58 (0.93, 2.66)	0.090
LVAD at Transplant	–1.5 (–6.7, 3.8)	0.581	0.79 (0.43, 1.45)	0.450	0.88 (0.43, 1.77)	0.711
HTx Ischemia (+1 h)	–0.5 (–2.4, 1.3)	0.582	1.00 (0.82, 1.22)	0.978	0.73 (0.56, 0.94)	0.016
KTx Ischemia (+10 h)	4.4 (2.1, 6.7)	<0.001	1.09 (0.86, 1.39)	0.461	0.78 (0.56, 1.10)	0.154

[a]*LOS analysis includes patients undergoing SHKT in 2005–2019; 1-year rejection episode analysis includes patients undergoing SHKT in 2005–2018.*
BMI, body mass index; ECMO, extracorporeal membrane oxygenation; HTx, heart transplant; IABP, intraaortic balloon pump; KTx, kidney transplant; LOS, length of stay; LVAD, left ventricular asssit device; OR, odds ratio.

the difference in expected short-term mortality in isolated HTx *versus* isolated KTx—given the substantially greater risk associated with the HTx component of the simultaneous procedure, it can be expected that strong experience with HTx drives outcomes in SHKT. Moreover, center selection bias may play a role. While median annual KTx volume across all institutions in the United States during our study period is approximately 60 KTx/year, the median annual KTx volume among the subset of institutions performing SHKT is 166 KTx/year. Thus, we are already selecting for relatively high-volume KTx institutions, which may explain why differences in volume have less of an impact on outcomes in our select population. The existing literature in isolated KTx also less consistently demonstrates the volume-outcome relationship observed in isolated HTx (28). Axelrod et al. identify a significantly increased risk of mortality and 1-year renal graft loss in isolated KTx at low-volume centers as compared to high-volume centers. On the other hand, Sonnenberg et al. found no association between KTx volume quartile (ranging from Q1 <66 KTx to Q4 >196 KTx) and 3-year graft or patient survival (33).

While we identified a volume-outcome relationship in patient survival, the same relationship was not observed between transplant center experience and 1-year cardiac and renal allograft rejection episodes. Interestingly, however, we did identify a higher rate of cardiac allograft rejection compared to renal allograft rejection among the population of SHKT patients (7.8% *versus* 5.5%); while it is challenging to ascertain

the cause of this difference, one likely explanation is the difference in identification of rejection episodes—while renal allograft may only be identified when clinical signs present, planned endomyocardial biopsies allow for the detection of subclinical rejection episodes. Another interesting finding in multivariable analysis was the significant association between cardiac ischemic time and renal allograft rejection, with prolonged cardiac ischemic time associated with lower rates of renal allograft rejection. Without knowing exactly when each renal allograft implantation began relative to cardiac allograft implantation, this is challenging to explain. However, a common critique of SHKT is that the hemodynamic instability and coagulopathy that occur immediately during and after heart transplant place the renal allograft at significant risk of dysfunction and early rejection. Thus, some advocate for a short period of hemodynamic recovery in the operating room prior to initiation of the renal allograft transplantation. It is, therefore, possible that reduced cardiac allograft ischemic time is associated with a more rapidly performed procedure overall, including rapid renal allograft implantation, greater early exposure of the renal allograft to hemodynamic instability, and greater risk of renal allograft compromise and early graft rejection.

In addition to understanding volume-outcome relationships, we also sought to identify comorbidities associated with 1-year survival. We found that dialysis-dependent patients undergoing SHKT have decreased 1-year survival and increased rates of renal allograft rejection relative to patients not requiring pre-transplant dialysis. Despite the increased risk identified, there is substantial literature that suggests that SHKT provides benefit relative to isolated HTx in patients with the most severe degrees of kidney dysfunction. For instance, Karamlou et al. compared 593 SHKT and 26,183 isolated HTx, assessing the impact of pre-operative renal function on benefit of SHKT relative to isolated HTx. They observed similar overall survival; however, when stratifying by eGFR quintiles, patients in the lowest quintile (eGFR <37 mL/min) undergoing isolated HTx had significantly worse survival than patients undergoing SHKT, suggesting a relative benefit of SHKT (1). The utilization of eGFR as a measure of renal function in UNOS studies is limited by the fact that it is based on a single creatinine measure, often that most proximal to the transplant date. Thus, other studies have attempted to expand upon the association between renal function and SHKT benefit by looking specifically at dialysis-dependence. Gill et al. assessed clinical outcomes in 263 SHKT patients relative to isolated HTx. Overall adjusted risk of death was found to be 44% lower with SHKT compared to isolated HTx, and this difference was driven by dialysis-dependent patients (8). Schaffer et al. compared outcomes of SHKT *versus* isolated HTx in patients with eGFR <50 mL/min, stratified by dialysis-dependence. Five-year posttransplant survival was improved in SHKT patients among dialysis-dependent patients (73% vs. 51%) as well as those with non-dialysis-dependent renal insufficiency (80% vs. 69%) (10). While kidney recovery for patients with non-dialysis-dependent renal insufficiency is possible following isolated HTx, these findings suggest that SHKT may provide a significant survival advantage in this patient population. Thus, while our results highlight that dialysis-dependence represents an independent risk factor for poor outcomes among SHKT patients, there exists strong evidence that SHKT remains beneficial as compared to isolated HTx in dialysis-dependent patients.

Our study is not without limitations. First, this is a retrospective study using a clinical database with inherent limitations. In the evaluation of a clinically complex patient population, nuances in pathology and management may not be captured by the database. Second, our study does not provide insight into why volume-outcome relationships are observed in SHKT. While we identify increased ischemic time as a predictor of decreased survival and high-volume centers are likely to have reduced ischemic times, further explanation is an important area of future study. Third, we do not include sequential heart-kidney transplant patients in our analysis; this is because the volume of sequential heart-kidney transplant is quite low in the United States, the patients undergoing sequential heart-kidney transplant are inherently different than SHKT patients (2), and this patient population has already been quite well described (2). Melvinsdottir et al. identify that sequential heart-kidney transplant may have improved outcomes relative to SHKT; however, they also show that sequential heart-kidney transplant volume in the United States is falling out of favor, with only 6 procedures performed in 2016 (2).

In summary, simultaneous heart-kidney transplants are being performed with increasing frequency in the United States, with stable short-term outcomes. Increased institutional HTx volume, but not KTx volume, is associated with improved 1-year survival in SHKT. Thus, emphasis should be placed on high-volume heart transplant centers to manage patients requiring SHKT.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study, available through the United Network for Organ Sharing (UNOS) and the Organ Procurement and Transplantation Network (OPTN), and they can be found here: https://optn.transplant.hrsa.gov/data/view-data-reports/request-data/.

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

MC conducted idea conception, data collection and initial analysis, manuscript drafting, and manuscript revision. SP conducted idea conception, manuscript drafting, and manuscript revision. KJ

conducted idea conception and manuscript revision. GS conducted idea conception and manuscript revision. AH conducted idea conception and manuscript revision. P-JY conducted idea conception, data collection and analysis, manuscript drafting, and manuscript revision.

CONFLICT OF INTEREST

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

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ORIGINAL RESEARCH
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Individual-Level Socioeconomic Position and Long-Term Prognosis in Danish Heart-Transplant Recipients

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Socioeconomic deprivation can limit access to healthcare. Important gaps persist in the understanding of how individual indicators of socioeconomic disadvantage may affect clinical outcomes after heart transplantation. We sought to examine the impact of individual-level socioeconomic position (SEP) on prognosis of heart-transplant recipients**.** A population-based study including all Danish first-time heart-transplant recipients (n = 649) was conducted. Data were linked across complete national health registers. Associations were evaluated between SEP and all-cause mortality and first-time major adverse cardiovascular event (MACE) during follow-up periods. The half-time survival was 15.6 years (20-year period). In total, 330 (51%) of recipients experienced a first-time cardiovascular event and the most frequent was graft failure (42%). Both acute myocardial infarction and cardiac arrest occurred in ≤5 of recipients. Low educational level was associated with increased all-cause mortality 10–20 years post-transplant (adjusted hazard ratio [HR] 1.95, 95% confidence interval [CI] 1.19–3.19). During 1–10 years post-transplant, low educational level (adjusted HR 1.66, 95% CI 1.14–2.43) and low income (adjusted HR 1.81, 95% CI 1.02–3.22) were associated with a first-time MACE. In a country with free access to multidisciplinary team management, low levels of education and income were associated with a poorer prognosis after heart transplantation.

Keywords: mortality, heart transplantation, prognosis, individual-level, socioeconomic position

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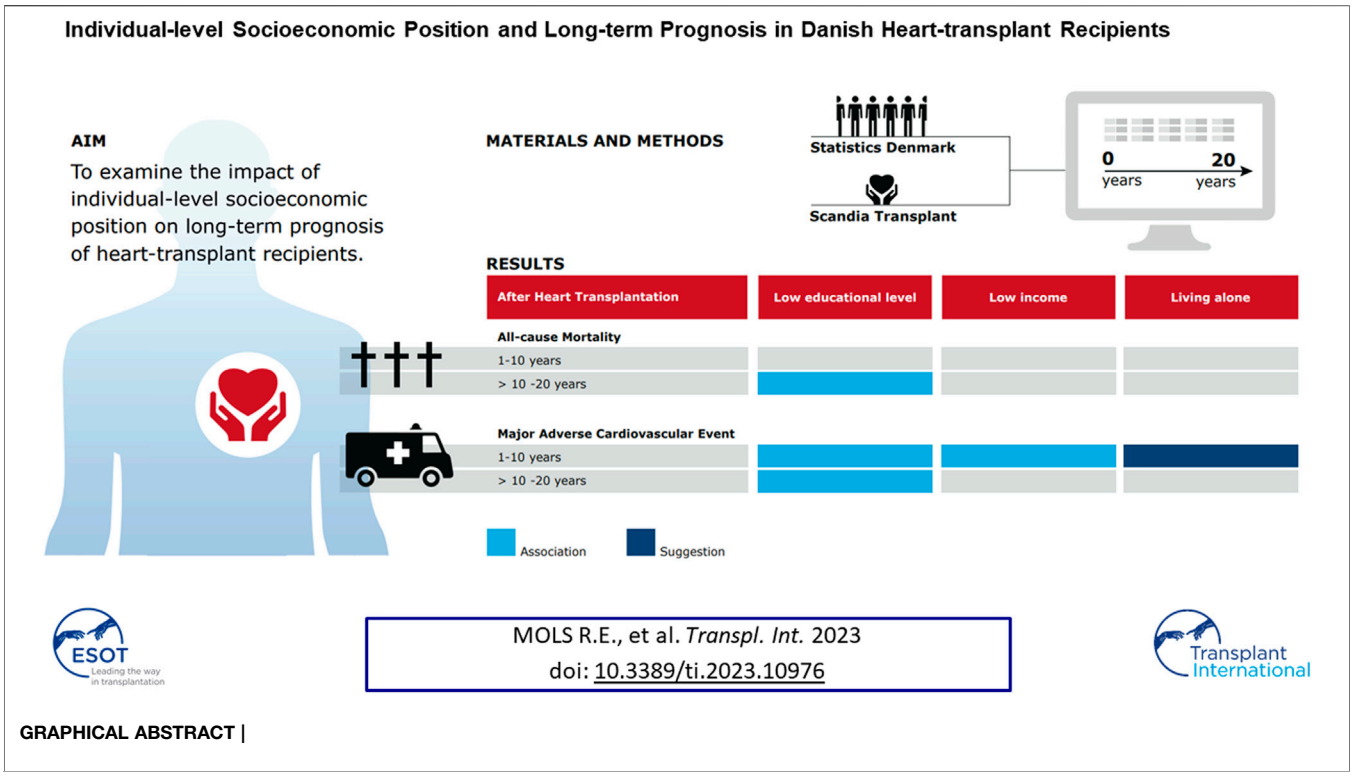
INTRODUCTION

Heart transplantation is a widely accepted procedure improving survival, quality of life, and physical capacity in patients with end-stage heart failure (1, 2). During the past 30 years, survival rates have increased significantly, despite high-risk and older recipients undergoing heart transplantation (1, 3). Currently, the 50% survival estimate after heart transplantation in adults is 12.5 years, and 14.8 years

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



GRAPHICAL ABSTRACT |

when conditional on 1-year survival (3). Advances in immunosuppressive treatment and perioperative care have improved 1-year survival to approximately 90% (4). The main causes of death immediately following heart transplantation are primary graft dysfunction, rejection, and infection; primary causes of long-term mortality are cardiac allograft vasculopathy, non-specific graft failure, renal dysfunction, and malignancy (3, 4). It is pivotal for follow-up of heart transplant recipients that transplant centers establish multidisciplinary team management programs, designed to improve survival (2, 5).

Studies in both the United States and the United Kingdom have shown that multiple indicators of index-based socioeconomic position (SEP) are associated with death, independent of baseline clinical characteristics of heart transplant recipients (6–8). Among American heart transplant survivors, low SEP (score) predicted an increased risk of rejection and graft loss (9). Earlier studies in the United States have suggested higher mortality in patients covered by Medicare compared with patients covered by private insurance at the time of heart transplantation (6, 10). Studies primarily conducted in the United States have also reported that depression before or early after heart transplantation is associated with higher post-transplant mortality (11–13). Mental health conditions often coexist with physical chronic diseases (14). Multimorbidity including chronic psychiatric disorder has been associated with higher mortality (14, 15). Moreover, data support a strong socioeconomic gradient in the onset of multimorbidity (16, 17). However, important gaps persist in the understanding of how individual indicators of socioeconomic deprivation and comorbidities affect clinical outcomes after heart transplantation in European universal healthcare systems with free access to multidisciplinary team management programs.

The structure and content of Danish healthcare registers ensure a unique and virtually complete individual-level linkage of data and long-term follow-up (18). Furthermore, the universal healthcare model in Denmark provides health service free of charge to all residents. We used the Scandiatransplant Database (STD) and nationwide health and administrative registers to examine the 20-year prognosis of all heart-transplant recipients in Denmark and the prognostic impact of individual-level SEP and comorbidities.

MATRIALS AND METHODS

Setting

The Danish national healthcare system provides tax-financed healthcare for all residents at general practitioners and hospitals as well as reimbursement of prescribed medical therapy. The Civil Registration System (CRS) can unambiguously link up-to-date national health and administrative register data using a unique 10-digit identifier assigned to all residents at birth or upon immigration (18). Denmark has two transplant centers at the University Hospital of Copenhagen and at Aarhus University Hospital.

Data Sources

This study was based on data from: 1) STD, which covers data on all Danish heart-transplant recipients and donors (19), 2) The Danish National Patient Registry (DNPR) (18) containing

information on discharge diagnosis according the International Classification of Diseases (ICD-8 and since 1994 ICD-10 codes), along with codes for diagnostic and surgical procedures (18), 3) The Psychiatric Central Research Register (PCRR) containing information on psychiatric diagnoses (18), 4) The Danish National Prescription Registry (NPR) (18), containing data on all redeemed prescriptions at Danish community pharmacies (18). Medical therapies were identified by substance level (Anatomical Therapeutic Chemical [ATC] Classification), 5) The Danish Causes of Death Registry (DCDR) (18), where causes of death are listed as the immediate, underlying, and contributing cause of death (18), 6) CRS including data on vital status, date of birth, gender, and marital status (18), and finally 7) Statistics Denmark (18) covering information from the Education Registry, the Income Statistics Register, and the Integrated Database for Labor Market Research.

This study was approved by the Danish Data Protection Agency (no: 1-16-02-656-18) and the Danish Patient Safety Authority, authorizing access to medical records (no: 3-3013-3173/1).

Study Population and Characteristics

We established a nationwide cohort study including Danish first-time heart-transplant recipients during 1994–2018 recorded in the STD by ICD-10 code (DZ94.1). The index date was defined as the date of the first surgical heart transplantation in the STD. Heart-transplant recipients were followed from index date until 31 December 2018, emigration, or death, whichever occurred first. Recipients undergoing re-transplantation identified in the DNPR (KFQA00, KFQA10) were not censored, since reoperation would be part of the causal pathway of long-term outcome. Information on age, gender, and vital status was retrieved from the CRS (18). Data on donor age and gender mismatch (donor/recipient) were extracted from the SDT.

Age at index date was categorized as 0-20, 21-40, 41-60, and ≥61 years, due to increasing complexities in early, middle, and long-term management post-surgery (20); follow-up time was defined as 0–1, >1–10, and >10 years. The number of recipients alive at end of follow-up was calculated. We collected information on clinically relevant comorbidities by ICD codes registered in the DNPR (18) and PCRR (18) 10 years prior to the index date: Myocardial infarction, angina pectoris, heart failure, heart valve disease, cardiac arrhythmia, congenital heart disease, cardiomyopathy, cardiac inflammation, aortic disease, peripheral arterial disease, cerebrovascular disease, cardiogenic shock and pulmonary edema, diabetes, hypertension, chronic obstructive pulmonary disease, obesity, and psychiatric disorder (**Supplementary Table S1**). Based on the definition of multimorbidity in other Danish studies (20, 21), we summarized the number of comorbidities 10 years prior to the index date. This Danish algorithm estimates multimorbidity as the co-occurrence of two or more chronic conditions included in the 11 comprehensive chronic disease groups (**Supplementary Table S3**). Medical treatment was defined as ≥1 redeemed prescription 6 months prior to the index date retrieved from the NPR (18). Polypharmacy was defined as redeeming at least one prescription for ≥5 different cardiovascular agents (18) (**Supplementary Table S3**).

Individual-Level Socioeconomic Position

Data on individual-level SEP were obtained from Statistics Denmark. Cohabitation status at index date was defined as living alone or cohabiting. We used the highest attained educational level in the calendar year prior to the index date (18) and categorized educational level into five groups: Low (primary and lower secondary education), medium (upper secondary education and academy profession degree), high (bachelor and above), not completed an education (patients age ≤16 years), and missing. We used personal income (pre-tax total) within the calendar year prior to the index date. Based on the annual percentiles in the Danish population, we classified income into percentiles and used the 25th percentile as the cut-off point for low (≤25th percentile) and medium-high (>25th percentile) personal income. Occupational status in the year prior to the index date (18) was grouped into working, non-working (no employment or early retirement), out-of-workforce (state pension, under education), and missing (**Supplementary Table S4**).

Outcomes

We used the CRS (18) to ascertain date on all-cause mortality during the years following the index date. We also examined cause of mortality using information from the DCDR (18). Cause of mortality was defined by underlying cause and possible cause (immediate cause when available, 1st contributory cause when immediate cause was missing, or 2nd contributory cause when immediate and 1st contributory cause was missing). We generated a list of all documented causes (ICD-10 codes) and divided these into twelve categories: Complications to heart transplantation, multiple organ failure, sudden death, cardiovascular disease, heart failure, cerebrovascular disease, infection, pulmonary disease, malignancy, kidney disease, diabetes, other specified, and not specified (**Supplementary Table S5**).

The first-time occurrence of hospital admission with a cardiovascular event after the index date was examined (acute myocardial infarction, peripheral arterial disease, cardia arrest, stroke, cardiac inflammation and infection, readmission due to heart failure, graft failure, percutaneous coronary intervention, radiofrequency ablation for atrial fibrillation, cardiac pacemaker, and valve surgery) (**Supplementary Table S6**). Information was retrieved from the DNPR by primary in-patient diagnosis and surgical procedure codes (18). We investigated the risk of first-time major adverse cardiovascular event (MACE). Composite MACE included readmission due to heart failure, graft failure, percutaneous coronary intervention, acute myocardial infarction, cardiac arrest, and all-cause mortality. To account for potential misclassification of first-time occurrence of hospital admission due to a MACE (especially graft failure due to standard biopsy controls in the first post-transplant year; heart failure, which could follow

TABLE 1 | Baseline characteristic in heart-transplant recipients.

	Total
	N = 649
Gender	
Male	503 (78)
Female	146 (22)
Age in years	
0–20	67 (10)
21–40	117 (18)
41–60	381 (59)
≥61	84 (13)
Donor	
Age, median (IQR)	41 (27–50)
Gender mismatch	118 (29)
Follow-up time in years	
0–1	97 (15)
>1–10	296 (46)
>10	256 (39)
Median (IQR)	7.4 (2.7–13.7)
Alive at end of follow-up	375 (58)
Comorbidities (10 years prior to the index date)	
Myocardial infarction	211 (33)
Angina Pectoris	272 (42)
Heart failure	547 (84)
Heart valve disease	71 (11)
Cardiac arrhythmia	307 (47)
Congenital heart disease	70 (11)
Cardiomyopathy	434 (67)
Cardiac inflammation	66 (10)
Aortic disease	–[a]
Peripheral arterial disease	10 (2)
Cerebrovascular disease	61 (9)
Cardiogenic shock and pulmonary edema	57 (9)
Diabetes	77 (12)
Hypertension	80 (12)
Chronic obstructive pulmonary disease	69 (11)
Obesity	21 (3)
Mental disease	–[a]
Multimorbidity (10 years prior to the index date)	
Number of chronic diseases, median (IQR)	1 (1–2)
Cardiovascular polypharmacy (6 months prior to the index date) [b]	
Prescribed medications ≥5	348 (54)
Cohabitation status	
Living alone	281 (43)
Cohabitation	368 (57)
Highest obtained educational degree	
Low (primary and lower secondary education)	193 (30)
Medium (upper secondary education and academy profession)	283 (44)
High (bachelor and above)	116 (18)
Not completed education (patients age ≤16 years)	40 (6)
Missing	17 (3)
Personal income group	
Low income (≤25th percentile)	134 (21)
Medium-high income (>25th percentile)	515 (79)
Occupational status	
Working	300 (46)
Non-working	27 (4)
Out-of- workforce (state pension, under education)	300 (46)
Missing	22 (3)

Values are n (%).
[a]*Due to data protection (<5 patients).*
[b]*Data available since 1995 in the Danish National Prescription Registry.*

from prior index date), we conducted a blanking period of 365 days after the index date (**Supplementary Table S7**).

Statistical Analyses

Baseline data were reported as mean and standard deviation (SD) if normally distributed and as median with 25th–75th interquartile range (IQR) if skewed continuous data. Categorical data were presented as prevalence (percentage).

Cause of mortality and first-time cardiovascular events were recorded in numbers and percentages. The Kaplan-Meier method was used to compute the risk of all-cause mortality (All). Conditional analyses were performed in recipients who survived the first year (1-year Post-surgery Survival). As supplementary, survival was stratified by time era (1994–2000, 2001–2010, 2011–2018). In addition, the Kaplan-Meier method was used to compute the risk of first-time MACE using the first year after the index date as a blanking period (1-year Post-surgery MACE). Due to Danish law on data protection, first-time acute myocardial infarction (≤5) and cardiac arrest (≤5) were not included in the MACE. However, sensitivity analysis including these events did not change the results. As supplementary, survival and first-time MACE were stratified by gender. To identify the most socially disadvantaged recipients, all socioeconomic factors were dichotomized by the worst quartile or lowest status. Recipients with low educational level (low-degree) were compared to those with medium-higher educational level (medium-high-degree). Recipients <16 years and with missing information on education were not included. Prognostic outcomes were assessed among unemployed (non-working) compared to employed (working, out-of-workforce) recipients. In case of missing information on occupational status, recipients were excluded. Due to the limited sample size, it was not possible to further categorize the exposure variables.

Based on the increasing complexity in long-term management after transplantation (20), we determined the impact of all exposure variables on prognostic outcomes within follow-up intervals: 0–1, >1–10, and >10–20 years. Crude and adjusted hazard ratios (HRs) were computed using Cox Proportional Hazards regression comparing recipients within the dichotomized socioeconomic groups. In multivariable analyses, we adjusted for age, gender, donor age, gender mismatch, hypertension, and diabetes. We evaluated the proportional hazards assumption by visual inspection of log-log plots. Since the median number of comorbidities at baseline was one and less than 2% of the recipients had a psychiatric disorder, these two covariates did not change the results and were thus not included in the regression. We found no indication of any difference between the two Danish transplant centers and transplantation site was not distinguished between in the analyses. A *post hoc* power analysis was not performed as the utility to inform outcome already observed seems analytically misleading (22). All statistical analyses were performed using SAS statistical software package (version 9.4) and R version 4.1.0 (2021-05-18).

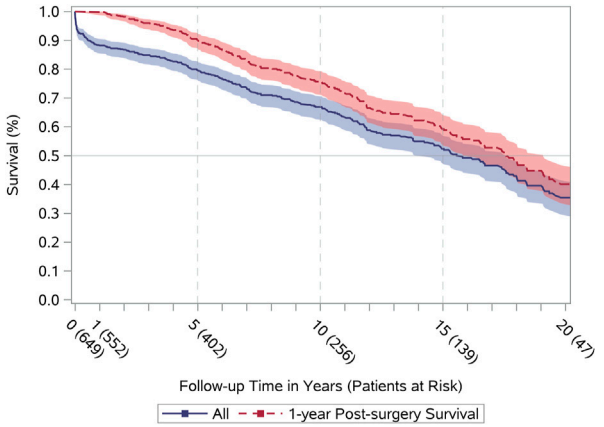


FIGURE 1 | Long-term survival. All (blue), All-cause mortality after surgery date (index date). 1-year Post-surgery Survival (red), Conditional all-cause mortality in recipients who survived first year after the index date.

TABLE 2 | Cause of mortality in heart-transplant recipients.

Overall (N = 649)	Underlying cause	Possible cause[a]
	n = 274	*n* = 274
Complications of heart transplantation	–[b]	16 (6)
Multiple organ failure	–[b]	19 (7)
Sudden deaths	–[b]	25 (9)
Cardiovascular disease	48 (18)	18 (7)
Heart failure	68 (25)	32 (12)
Cerebrovascular disease	–[b]	15 (5)
Infection	12 (4)	18 (7)
Pulmonary disease	–[b]	23 (8)
Malignancy	48 (18)	22 (8)
Kidney disease	–[b]	12 (4)
Diabetes	–[b]	–[b]
Other specified	–[b]	–[b]
Not specified	55 (20)	39 (14)

Recipients were followed after heart transplantation (index day) and until 31 December 2018, emigration, or mortality, whichever occurred first.
Values are n (%).
[a]*Immediate cause when it is available; 1st contributory cause when immediate cause is missing; 2nd contributory cause when immediate and 1st contributory cause is missing.*
[b]*Due to data protection (<5).*

TABLE 3 | First-time cardiovascular event in heart-transplant recipients.

Overall (N = 649)	*n* = 330
Acute myocardial infarction	–[a]
Peripheral arterial disease	11 (3)
Cardiac arrest	–[a]
Stroke	10 (3)
Cardiac inflammation and infection	–[a]
Readmission due to heart failure	47 (14)
Graft failure	140 (42)
Percutaneous Coronary Intervention	68 (21)
Radiofrequency ablation for atrial fibrillation	–[a]
Cardiac pacemaker	18 (6)
Valve surgery	11 (3)

Recipients were followed from day +365 after heart transplantation (index day) and until 31 December 2018, emigration, or death, whichever occurred first.
Values are n (%).
[a]*Due to data protection (<5 events).*

RESULTS

Between 1994 and 2018, first-time heart transplantation was performed in 649 recipients in Denmark (**Table 1**). Most recipients were male (78%) and 59% were between 41 and 60 years of age at surgery date. Diabetes and hypertension both occurred in 12% of recipients. The median (IQR) number of comorbidities within 10 years prior to transplantation was 1 (1–2). Psychiatric disorder was present in ≤5 of recipients. Median donor age was 41 (IQR, 27–50) and gender mismatch was present in 29%.

Outcomes

Twenty-year survival curves for all-cause mortality are displayed in **Figure 1**. The half-time survival was 15.6 years (95% confidence interval [CI] 13.8–17.5) and 17.6 years when conditional on 1-year survival (95% CI 16.2–19.1) (**Supplementary Figures S1–S4**). The leading underlying causes of mortality were heart failure (25%), cardiovascular disease (18%), and malignancy (18%) (**Table 2**). The three cardiovascular first-time events with the highest incidence (within 1–20 years post-surgery) were graft failure (42%), readmission due to heart failure (14%), and percutaneous coronary intervention (21%) (**Table 3**). Both acute myocardial infarction and cardiac arrest occurred in ≤5 of recipients. Approximately half of the heart transplant recipients were at risk of a first-time MACE within an 11-year period after the index date among those surviving to at least 1-year (**Figure 2**).

Individual-Level Socioeconomic Position

Adjusted HRs for all-cause mortality by socioeconomic factors and in different follow-up intervals are presented in **Figure 3**. Low educational level was associated with all-cause mortality within the period 10–20 years after heart transplantation (HR 1.95, 95% CI 1.19–3.19); otherwise we found no associations between socioeconomic factors and all-cause mortality (**Supplementary Table S8**). In contrast, we observed SEP-related associations with first-time MACE (**Figure 4**). During both >1–10 years and >10–20 years after the index date, low educational level was associated with first-time MACE. Low income was associated with first-time MACE within >1–10 years after the index date (HR 1.81, 95% CI 1.02–3.22). Cohabitation status was not significantly associated with first-time MACE during follow-up intervals. However, although it did not reach significance there was a suggestion that living alone was associated with a higher risk of first-time MACE within >1–10 years (HR 1.46, 95% CI 0.98–2.17). No associations between occupational status and first-time MACE were documented (**Figure 4**) (**Supplementary Table S9**).

DISCUSSION

In this nationwide cohort study comprising all Danish first-time heart-transplant recipients during a 20-year period, the half-time

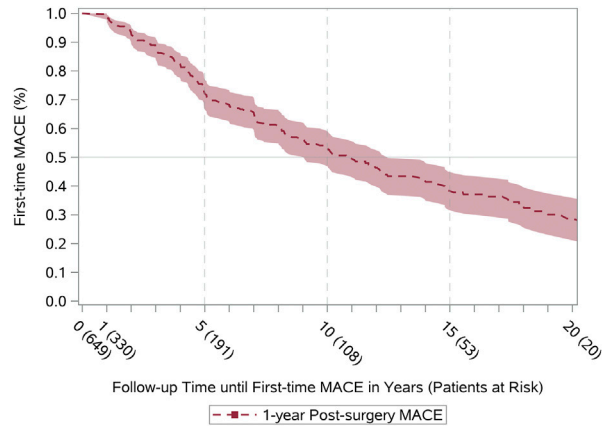


FIGURE 2 | Long-term first-time MACE. Conditional first-time MACE in recipients who survived first year after the index date (1-year Post-surgery MACE). MACE, Major Adverse Cardiovascular Event (composite of readmission due to heart failure, graft failure, percutaneous coronary intervention, and all-cause mortality).

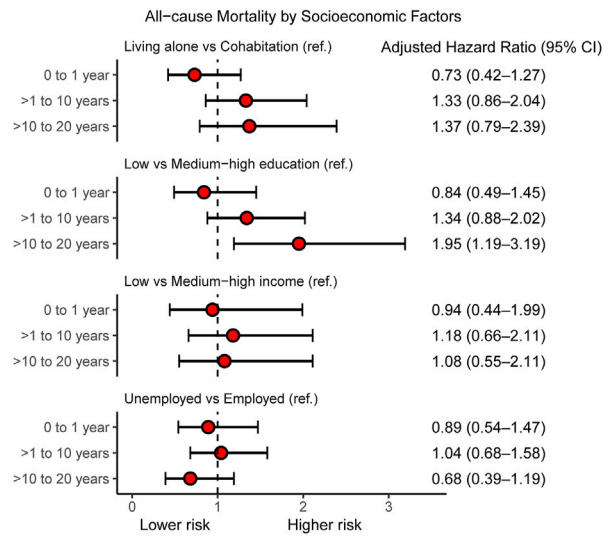


FIGURE 3 | Individual-level socioeconomic position and all-cause mortality. Cox Proportional Hazard models for adjusted hazard ratios for all-cause mortality within follow-up intervals: 0–1 year, >1–10 years, and >10–20 years after heart transplantation in Denmark (1994–2018) according to socioeconomic factors. In multivariate analyses, the hazard ratios are adjusted for age, gender, donor age, gender mismatch, hypertension, and diabetes. CI, confidence interval.

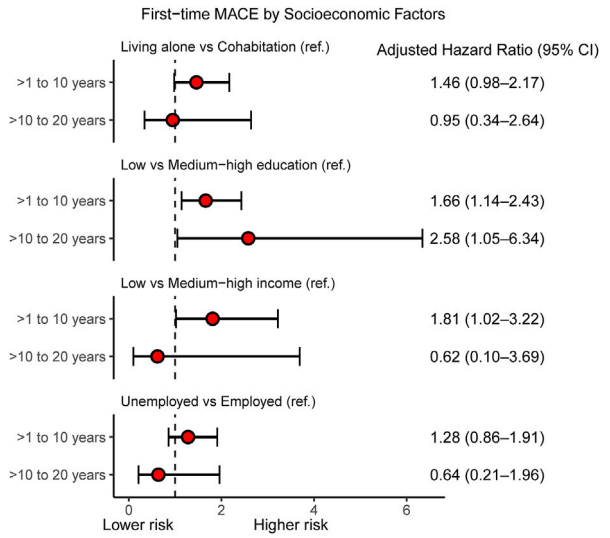


FIGURE 4 | Individual-level socioeconomic position and first-time MACE. Cox Proportional Hazard models for adjusted hazard ratios for first-time MACE within follow-up intervals: >1–10 years and >10–20 years after heart transplantation according to socioeconomic factors. In multivariate analyses, the hazard ratios are adjusted for age, gender, donor age, gender mismatch, hypertension, and diabetes. MACE, Major Adverse Cardiovascular Event (composite of readmission due to heart failure, graft failure, percutaneous coronary intervention, and all-cause mortality); CI, confidence interval.

survival estimate was 15.6 years. The highest prevalence of first-time cardiovascular events was graft failure. This study revealed two major findings: 1) low educational level at index date was associated with higher risk of all-cause mortality within 10–20 years after heart transplantation and 2) low educational level, low income, and a suggestion towards living alone were associated with higher risk of first-time MACE within 1–10 years post-transplant.

In a Scandinavian cohort (1983–2009) of heart-transplant recipients (n = 2293; 8% <18 years), the half-time survival was estimated to 13.2 years (19) and 15.3 years when conditional survival was set at 1-year. We demonstrated an excellent half-time survival (15.6 years) as well as 1-year conditional survival (17.6) when compared with internationally published data. This may be attributed to heart-transplant survival consistently improving over the last 30 years and has been described as the era effect. Heart transplantation in Denmark was initiated later than in the United States and other European countries (3, 19, 23). This is supported by our supplementary survival curves in Danish heart-transplant recipients stratified by time period (1994–2000, 2001–2010, 2011–2018). In the current study, we found that the three cardiovascular first-time events with the highest incidence were graft failure, readmission due to heart failure, and percutaneous coronary intervention. Approximately half of the heart-transplant recipients were at risk of a first-time MACE within 11 years after transplantation conditional on survival of at least 1-year. Our findings consolidate that graft failure and rejection remain the leading causes of mortality post-transplant (3, 4). Cardiac allograft vasculopathy is the main reason for allograft failure, and percutaneous coronary intervention is usually considered a palliative treatment because of the progressive nature of vasculopathy (24). In addition, a recent study based on the nationwide readmission database in the United States reported that heart failure is one of the main primary unplanned diagnoses causing readmission after heart transplantation (25). We were not able to establish whether

gender influence survival and first-time MACE curves since only 23% of the recipients were female (**Supplementary Figures S3, S4**). Scandinavian results on long-term follow-up after heart transplantation (n = 2293) have documented no significant difference in survival when stratified by gender (p = 0.44) (19). However, this issue warrants further investigation.

Several previous studies have linked SEP to prognostic outcomes in heart-transplant recipients. A nationwide follow-up study in England, including 2,384 adult heart transplant-recipients (1995–2014) demonstrated that the most socioeconomically disadvantaged recipients had a 27% higher risk-adjusted 19-year overall mortality (HR 1.27, 95% CI 1.04–1.55). The United Kingdom multiple deprivation index was used to measure SEP (7). Similarly, a study (6) using the UNOS database in 36,736 adult (≥18 years) first-time heart-transplant recipients (1994–2014) found that college educated patients had an 18% reduced rate of deaths. Moreover, lowest SEP (index of seven SEP indicators) confers higher unadjusted risk of post-transplant hospitalization (HR 1.13), rejection (HR 1.28), infection (HR 1.10), and ischemic event (HR 1.26) (6). Another UNOS-based study including 5,125 primarily pediatric heart transplant recipients (2000–2011) reflected that risk adjusted survival was poorer in groups with a low SEP (HR 1.41, 95% CI 1.10–1.80) (26). Findings from a single-center Boston study among first-time heart transplant recipients (n = 520) conducted between 1996 and 2005 supported that low SEP (score of six variables) was associated with higher adjusted risk of graft loss (HR 1.5, 95% CI 1.0–2.4) (9). Findings from a previous UNOS analysis in left ventricular assist devices (LVAD) recipient's ≥18 years (n = 3361) waiting for heart transplantation demonstrated that recipients with lower SEP (index of seven SEP indicators) had an early and sustained decreased adjusted post-transplant survival (lowest quartile: HR 0.57, 95% CI 0.39–0.82; highest quartile: HR 0.68, 95% CI 0.48–0.95 (8). Moreover, an analysis of the UNOS database including 33,893 adult heart-transplant recipients suggested an increased risk of mortality or re-transplantation (Adjusted p <0.001) associated with public health insurance status (Medicaid or Medicare versus private) (6). Research also based on the UNOS database studying a population (n = 20,676) of heart transplant recipients >17 years showed that Medicare and Medicaid insurances were associated with lower 10-year mortality risk (18%, 33%, respectively) than private insurance (10). In addition, multivariable analyses found that college-education decreased risk of mortality with 11% (10). In contrast to most previous studies using area-based social deprivation indexes or under-insurance status, we examined socioeconomic factors by individual and complete register-based single indicators of social vulnerability. Between 1 and 10 years post-surgery in particular, we observed a modest SEP gradient in the risk of a first-time MACE in heart transplant recipients. Remarkably, our results reflect that low educational attainment could be the most influential factor on both mortality and MACE, whereas personal income only influenced MACE. A recent single-center Danish study including 325 first-time heart transplant recipients (79% male and 69% between 41 and 60 years) described a lower median number of redeemed medical prescriptions during 15 years of follow-up in heart-transplant recipients within the lowest income group or if living alone (20). The association between income and prognosis could thus also be partly driven by an economic gradient in use of the prescribed medical treatment after heart transplantation. In line with the current understanding (6, 7, 9), it seems possible that even in a country with free access to multidisciplinary team management programs, educationally and economically disadvantaged heart transplant recipients could have an increased risk of non-adherence to immunosuppressive treatment, inadequate self-management skills, experience health disparities, and missed healthcare delivery; thus, graft failure and all-cause mortality are more likely in these recipients. However, our results indicate that the individual-level SEP impacts the middle follow-up period 1–10 years after transplantation. The most likely explanation for this is that socioeconomic disparities narrow over time after heart transplantation due to the role of the multidisciplinary team management identifying barriers to medical adherence and engaging patients to follow health recommendations. In accordance with the single-center Danish study, we believe that living alone may negatively influence on pharmalogical self-care. The lack of association between living alone and prognosis may be a result of the small sample size.

Our study also included information on comorbidities and chronic mental diseases 10 years prior to heart transplantation. Since the median number of comorbidities at baseline was one and less than 1% of the recipients had a psychiatric disorder, multimorbidity and psychiatric disorder were too rare to allow for further analyses of interactions. This may be explained by careful recipient selection based on pre- and post-transplant life expectancy, which reflects the recipient's pre-operative psychosocial status and comorbidity burden (1, 2).

Remarkably, a prior UNOS study (27) in the United States (2001–2014) investigated the effect of non-working of heart transplant recipients (n = 23.228, >18 years) on survival. An adjusted analysis demonstrated a 5% and 10% decrease in 5- and 10-year mortality, respectively. Our study did not reveal any influence of occupational status. The most likely explanation is that our cohort included recipients >65 years (age at receiving state pension in Denmark) as well as the early retirement status of chronic end-stage heart failure recipients.

Although the Danish healthcare system appears to ensure easy access to multidisciplinary team management programs and fully funded immunosuppressive and medical treatment, our results support that in mainly educationally and economically disadvantaged recipients, the long-term prognosis of heart-transplant recipients is affected. This study contributes with knowledge to target long-term healthcare strategies for socially disadvantaged heart-transplant recipients across the world (16, 17). Our data suggests the need to focus on socioeconomic factors and their influence on both adherence and rehabilitation to support adequate self-management, self-efficacy, and health literacy after heart transplantation (5). The development of new mobile health devises (mHealth) in the field of transplantation has immense potential to facilitate healthcare service and implement more individualized education and management programs (28, 29).

Further studies are needed to design and address delivery of more socially differentiated multidisciplinary team management programs for this patient group.

The setting in our study, including all heart transplant recipients in Denmark with long-term follow-up and individual accurate data linkage within a uniform healthcare system, reduced selection and recall bias. A critical limitation was that data in the DCDR (18) were not validated. Thus, the diagnosis of both underlying and contributory causes depends on the decision of the individual physician. We used a simple disease count algorithm to estimate the degree of multimorbidity. Thus, the relative severity of disease combinations was not assessed, and residual confounding could thus occur. Another limitation is the lack of precise temporality between baseline SEP and all-cause mortality or MACE, which does not allow inference from the identified observations. Notably, the combined MACE has not been validated. However, the component outcomes were validated in the general populations (18). Even though we adjusted our analysis for important confounding factors, residual confounding cannot be ruled out, since important clinical risk factors, blood sample measurements, and surgical procedure data were not available. Due to the small sample size, our reported associations should be supported in future large-scale observational studies.

We found that in first-time heart transplant recipients, the half-time survival was 15.6 years during a 20-year period. Low levels of education and income were associated with a poorer prognosis after surgery despite selection during the assessment process leading to heart transplantation.

DATA AVAILABILITY STATEMENT

Study data, statistical plan, and log-files can be made available through proposal to the Project Database (ID: 707738) at Statistics Denmark. https://www.dst.dk/en/TilSalg/Forskningsservice.

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 from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

RM, HE, and BBL designed the study. RM collected the data. RM, HE, and BL directed data management and analysis, which were carried out by IB and EH-P. All authors participated in the discussion and interpretation of results. RM, HE, and BL organized the writing and RM wrote the initial draft. All authors critically revised the manuscript and approved the final version.

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CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.10976/full#supplementary-material

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Risk of Bronchial Complications After Lung Transplantation With Respiratory *Corynebacteria*. Results From a Monocenter Retrospective Cohort Study

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Corynebacterium spp. are associated with respiratory infections in immunocompromised hosts. A link with bronchial complications after lung transplantation (LTx) has been suggested. We aimed to assess the link between respiratory sampling of *Corynebacterium* spp. and significant bronchial complication (SBC) after LTx. We performed a single center retrospective study. Inclusion of LTx recipients with at least one respiratory *Corynebacterium* spp. sample (July 2014 to December 2018). Subjects were matched to unexposed LTx recipients. Primary outcome was SBC occurrence after *Corynebacterium* spp. isolation. Secondary outcomes were *Corynebacterium* spp. persistent sampling, chronic lung allograft dysfunction (CLAD) onset and all-cause mortality. Fifty-nine patients with *Corynebacterium* spp. sampling with 59 without isolation were included. *Corynebacterium* spp. identification was not associated with SBC occurrence (32.4% vs. 21.6%, p = 0.342). Previous SBC was associated with further isolation of *Corynebacterium* spp. (OR 3.94, 95% CI [1.72–9.05]). Previous SBC and corticosteroids pulses in the last 3 months were the only factors associated with increased risk of *Corynebacterium* spp. isolation in multivariate analysis. *Corynebacterium* spp. sampling was significantly associated with CLAD onset (27.1% vs. 6.9%, p = 0.021). *Corynebacterium* spp. isolation was not associated with SBC but with higher risk of CLAD. Whether CLAD evolution is affected by *Corynebacterium* spp. eradication remains to be investigated.

Keywords: lung transplant, infection, chronic lung allograft dysfunction (CLAD), bronchial complications, corynebacteria

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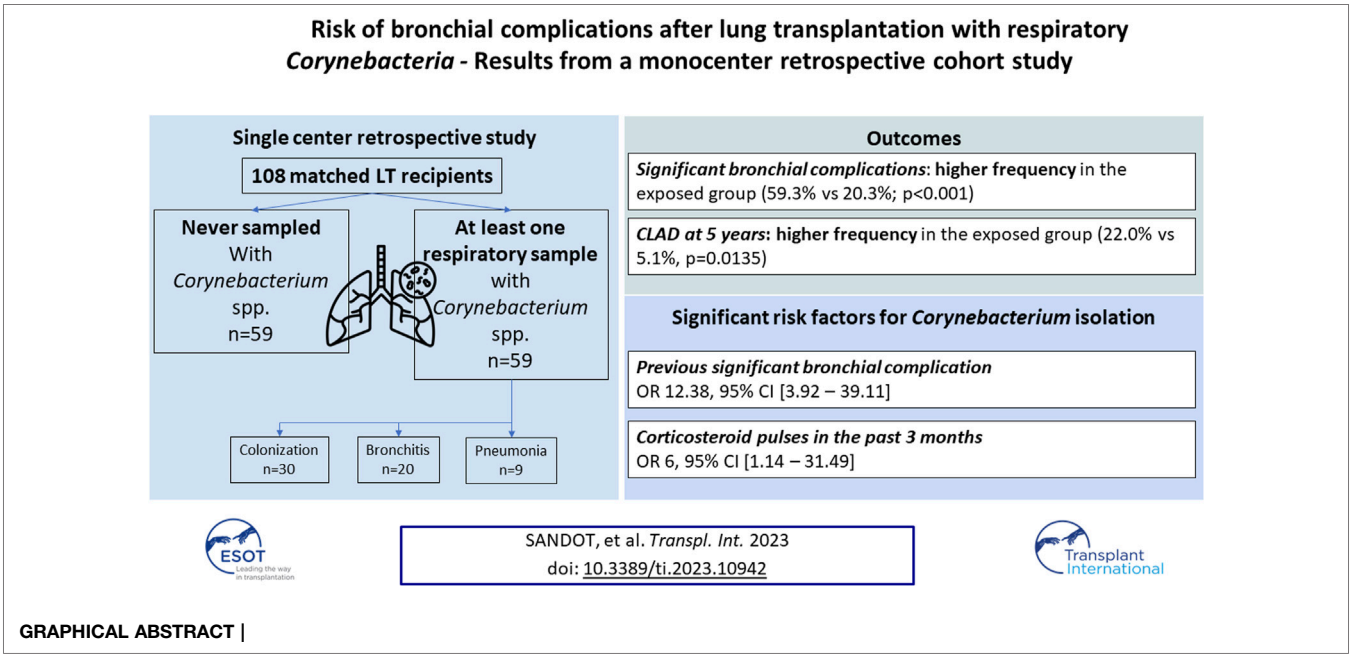

GRAPHICAL ABSTRACT |

INTRODUCTION

Bronchial complications after lung transplantation (LTx) are a major burden, leading to severe morbidity and mortality (1), occurring in nearly 10% of LTx recipients (LTRs) (2,3). The multiple risk factors might include characteristics of the harvested organ (duration of mechanical ventilation, previous bronchial colonization, duration of cold ischemia) or surgical issues (duration of surgery, anastomosis techniques) (2). Early post-operative complications have also been reported as risk factors for bronchial issues (2). Bronchial complications usually require close bronchoscopic assessment, and in up to 25% of cases (3), interventional bronchoscopy for bronchial stent placement, or balloon dilatation. In a longer perspective, these bronchial complications can lead to functional loss (1). Moreover, repeated respiratory infections and bronchial complications may be linked, as a cause or a consequence (2). For instance, isolation of *Pseudomonas aeruginosa* or *Staphylococcus aureus* has been found associated with bronchial stenosis (4,5). *Corynebacterium* spp. can induce various clinically significant respiratory infections, notably in immunocompromised patients or patients with severe respiratory diseases (6-8). In LTRs, being both immunocompromised and with structural bronchial abnormalities, *Corynebacterium* spp. have been suspected to be associated with bronchial complications (9). In this report, the presence of a bronchial stent was a significant risk factor for persistence of *Corynebacterium* spp. infection.

We aimed to unravel the possible link between respiratory isolation of *Corynebacterium* species and significant bronchial complications (SBCs) in a cohort of LTRs in the Paris-Bichat Lung Transplant Program, France. Our objectives were to investigate the association of *Corynebacterium* spp. isolation and the occurrence of an SBC and to describe the *Corynebacterium* spp. epidemiology, the course of *Corynebacterium* spp. infection and its risk factors and long-term prognosis.

PATIENTS AND METHODS

Patients and Settings

We retrospectively included all adult LTx recipients with at least one lower-respiratory-tract specimen in which a *Corynebacterium* spp. was isolated between July 2014 and December 2018 in the Paris-Bichat Lung Transplant Program. Cases were identified in the local microbiology department database, where all respiratory samples are recorded. Each case was matched to a non-exposed control, selected as the next LTx patient in chronological order. The matching was according to age at LTx ±5 years, mono- or bipulmonary status, underlying respiratory disease (defined in four categories: chronic obstructive pulmonary disease [COPD]/emphysema, interstitial lung disease, bronchial dilatation, miscellaneous). Data were collected anonymously, and the electronic files were used according to French law (*Informatique et Libertés*). The Evaluation Committee for observational research protocols of the French Respiratory Society (SPLF, CEPRO 2020-044) approved the study and waived informed consent.

Clinical and Microbiological Collected Data

All LTx candidates and recipients have been prospectively included in Paris-Bichat Lung Transplant database since 2006. This database includes demographical and anamnestic data,

details on LTx surgery and post-operative course, bronchoscopic findings, and respiratory function.

All lower-respiratory-tract samples (sputum, tracheal aspirate, bronchoalveolar lavage [BAL], or protected distal aspiration) taken during usual care were immediately sent to the bacteriology laboratory. They were inoculated onto routine agar plates incubated for 48 h at 35°C under aerobic and anaerobic conditions. Bacteria were identified at the species level by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) Microflex LT Biotyper (Bruker Daltonics, Bremen, Germany). Bacterial susceptibility to antibiotics was determined with the disk-diffusion method according to EUCAST guidelines (www.eucast.org).

From the bacteriology laboratory database, we retrieved all cases of LTRs in whom *Corynebacterium* spp. had been isolated in at least one lower-respiratory-tract specimen. In patients with sputum and tracheal aspirates, we included only specimens showing ≥25 leukocytes/field and ≤10 upper respiratory epithelial cells/field, as assessed by the scoring system of Murray and Washington (10). The usual thresholds were applied for interpreting quantitative cultures (i.e., $\geq 10^4$, $\geq 10^5$ and $\geq 10^7$ colony formation units/mL for BAL specimens, tracheal aspiration and sputum culture, respectively). Microbiological data are described in terms of the first *Corynebacterium spp*. isolation.

LTx Management

Usual management of LTx in our center is highly protocolized and reported elsewhere (11). In brief, intraoperative veno-arterial extra-corporeal membrane oxygenation (ECMO) was initiated according to hemodynamics and respiratory findings during surgery (12), with peripheral cannulation. All patients receive the same initial immunosuppressive regimen (mycophenolate mofetil, corticosteroids and tacrolimus). All patients receive life-long proton pump inhibitors. Antibiotic prophylaxis with cefazoline is administered for 48 h, then adapted to postoperative microbiological analysis. A first bronchoscopy is systematically performed within the first hours after LTx. During the post-operative course, surveillance bronchoscopy and BAL are performed in case of clinically suspected respiratory infection. In case of abnormalities in bronchial healing, these bronchoscopies are repeated, and microbiological samples are taken if an infection is suspected, thus allowing for longitudinal study of colonization.

Transbronchial biopsies are performed in case of clinically suspected acute cellular or antibody mediated rejection (AMR). Acute cellular rejection (ACR) was defined according to established criteria (13), as was AMR (14).

Study Definitions

We defined an SBC as the presence of a bronchial fistula diagnosed by chest CT-scan or bronchoscopy or the need for interventional bronchoscopy for dilatation or bronchial stenting (2). Persistent respiratory colonization was defined by isolation on a respiratory sample on at least three occasions at least 1 month apart in less than 1 year (15). Chronic lung allograft dysfunction (CLAD) was defined according to ISHLT recommendations (16) as a decline in forced expiratory volume per second (FEV1) ≥ 20% from baseline, persisting at 3-month intervals, excluding other causes. Baseline FEV1 was the mean of the 2 best post-transplant FEV1 measurements. The diagnosis of infection (pneumonia or bronchitis or colonization) was retrospectively defined according to Centers for Disease Control and Prevention definitions (17) by the review of all the medical files by a blinded adjudication committee.

Study Outcomes

The primary outcome was the occurrence of an SBC. The date of the event was the date of the first SBC. Secondary outcomes were 1) persistent respiratory colonization with *Corynebacterium* spp., 2) CLAD occurrence and its delay after transplantation, 3) all-cause mortality during follow-up, and 4) *Corynebacterium* species and their distribution.

Statistical Analysis

First, a descriptive analysis was performed in the entire cohort population and according to exposed or unexposed status. Categorical variables were summarized as counts (percentage) and frequency distributions were compared with the Mac Nemar test. Continuous variables were expressed as median (IQR) and differences were tested with the Wilcoxon test.

Second, we compared occurrence of SBC between exposed and unexposed patients in the subset of patients with respiratory *Corynebacterium* spp. isolation before the occurrence of SBC and their matched controls, using the Mac Nemar test. We also searched for factors associated with SBC among the following variables: *Corynebacterium* spp. isolation, underlying respiratory disease, invasive mechanical ventilation duration, ECMO necessity, by a univariate analysis. Third, factors associated with with *Corynebacterium spp*. isolation were investigated by univariate then multivariate logistic regression in the entire cohort population, using the same approach.

Time between LTx and occurrence of CLAD or death was compared between the exposed and unexposed groups by means of survival curves (Kaplan-Meier method) and tested by means of a log-rank test. If the patient was alive or without chronic rejection at the end of the study, the patient was censored at the study end date (December 31, 2018). Those analyses excluded patients with CLAD before isolation of *Corynbacterium* spp. Statistical tests were 2-sided with a significance level of 0.05. All analyses were performed using R software (version 4.0.3).

RESULTS

Characteristics of the Cohort

Over the study period, the cohort of LTx recipient represented 367 patients (**Figure 1**). *Corynebacterium* spp. were isolated in 60/367 LTRs (16.3% of the cohort) after a median of 128 days [interquartile range 38–503] after LTx. One patient was excluded from the analysis because *Corynebacterium* spp. had also been isolated before LTx on a systematic bronchoscopy, which left 59 LTR as the "exposed" cohort; these were matched to 59 non-exposed LTRs. Patient characteristics are in **Table 1**.

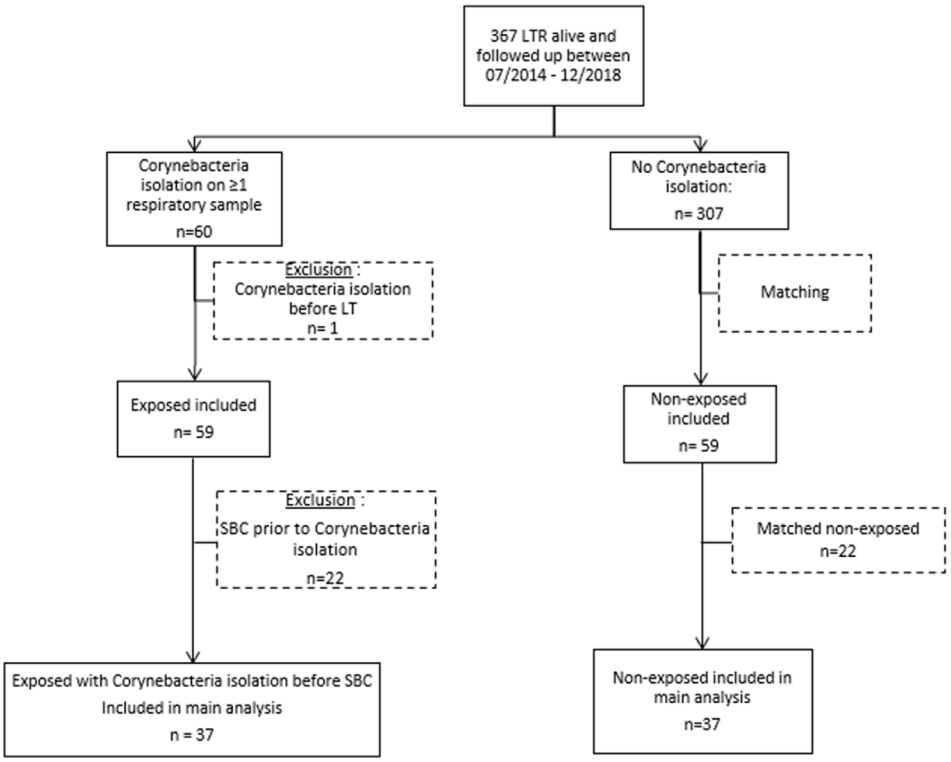

FIGURE 1 | Flowchart of the study. LTR, lung transplantation recipient; SBC, significant bronchial complication.

Strict matching for the underlying respiratory disease leading to transplantation was not possible for 3 cases with rare lung diseases (histiocytosis, lymphangioleiomyomatosis and pulmonary graft-versus-host disease) who were matched with patients transplanted for COPD. Matching on mono- or bipulmonary status was favored whenever possible, to eliminate the risk of confounding colonization or infection of the native lung on isolation of *Corynebacterium* spp.

All patients were receiving systemic corticosteroid therapy, with no difference between exposed and non-exposed patients in median dose of corticosteroids or use of antimetabolites or anticalcineurins. Patients with *Corynebacterium* spp. isolated significantly more frequently received other types of immunosuppressive therapies than non-exposed patients (7/59, 11.9% vs. 1/59, 1.8%, $p = 0.04$; mammalian target of rapamycin inhibitors for 4 patients, belatacept for 2, and rituximab in the previous 6 months for 1 vs. belatacept for 1) (**Table 1**).

A history of AMR was more common in the exposed than non-exposed group (16/59, 27.1% vs. 5/59, 8.47%, $p = 0.015$) (**Table 1**). None of the included patients underwent fundoplication surgery.

Risk Factor for Significant Bronchial Complication

Presence of a SBC was significantly more frequent in the exposed than non-exposed group: 35/59 (59.3%) and 12/59 (20.3%) ($p < 0.001$). Likewise, an interventional bronchoscopy procedure and placement of a bronchial stent were more frequently required in exposed patients (31/59, 52.5% vs. 10/59, 16.9% and 19/59, 32.2% vs. 2/59, 3.4%; both $p < 0.001$). Among the patients with bronchial stents, 12 in the exposed group had a mechanical stent obstruction requiring bronchoscopy and no patient in the non-exposed group.

We analyzed data for 74 patients (**Table 2**) to evaluate whether *Corynebacterium spp.* was a risk factor for SBC: 21 patients in the exposed group had an SBC before *Corynebacterium spp.* isolation and were excluded from the analysis with their matched control. One non-exposed patient had an SBC before the matched exposed counterpart had *Corynebacterium* spp. isolated. Therefore, he was excluded. The respiratory isolation of a *Corynebacterium* spp. was not associated with increased frequency of further SBC (OR 2.33, IC95 0.60–9.02, $p = 0.220$). The time to onset of SBC did not significantly differ between the two groups. None of each SBC type was significantly more frequent in patients with an *Corynebacterium* spp. isolated.

Risk Factors for the Isolation of *Corynebacterium* spp.

We compared data for exposed and unexposed patients by univariate then multivariate logistic regression (**Table 3**).

The presence of a previous SBC and history of corticosteroids pulses in the last 3 months were the only factors associated with an increased risk of *Corynebacterium* spp. isolation in multivariate analysis (OR 12.38, 95% CI [3.92–39.11]; $p <$

TABLE 1 | Baseline characteristics of lung transplantation (LTx) recipients with a lower-respiratory-tract specimen in which a *Corynebacterium* spp. was isolated (exposed) and non-exposed recipients.

	Exposed, *n* = 59	Non-exposed, *n* = 59	*p*-value[a]
Recipient characteristics			
Male sex, n (%)	46 (78.0)	43 (72.9)	0.60
Age (years)	56.1 [50.4–61.5]	56.5 [53.2–59.5]	0.057
Underlying respiratory disease, n (%)			
Emphysema/COPD	25 (42.4)	28 (47.5)	0.25
Interstitial lung disease	28 (47.5)	28 (47.5)	
Bronchiectasis	2 (3.4)	2 (3.4)	
Other	4 (6.9)	1 (1.7)	
Type of lung transplantation n (%)			
Right single-lung	15 (25.4)	17 (28.8)	0.719
Left single-lung	9 (15.3)	8 (13.6)	0.870
Double lung	35 (59.3)	34 (57.6)	1
Highly emergent LTx	7 (11.9)	9 (15.3)	0.75
Intraoperative veno-arterial extracorporeal membrane oxygenation, n (%)	42 (71.2)	31 (52.5)	0.03
Post-operative duration of mechanical ventilation (days)	6.0 [1.0–13.0]	1.0 [1.0–4.0]	0.117
Tracheostomy, n (%)	20 (33.9)	13 (22.0)	0.21
ICU length of stay (days)	16.0 [11.0–34.0]	14.0 [9.5–22.5]	0.521
Primary lung graft dysfunction, n (%)	15 (25.4)	12 (20.7)	0.68
CMV mismatch, n (%)	9 (15.3)	9 (16.1)	1
Immunosuppressive therapy		5	
Corticosteroids	59 (100)	9 (100)	
Corticosteroids dosage[a] (mg)	20.0 [7.5–30.0]	25.0 [10.0–37.5]	0.18
Mycophenolate mofetil	53 (89.8)	50 (87.7)	1.00
Calcineurins inhibitor	56 (94.9)	56 (98.2)	0.47
Other immunosuppressive therapy in the last 6 months	7 (11.9)	1 (1.8)	0.04
Antimicrobial therapy in the last 3 months	35 (59.3)	31 (52.5)	0.42
Immunological complications			
Acute cellular rejection	13 (22.0)	12 (20.3)	1
Corticosteroids pulses in the last 3 months	24 (40.7)	18 (30.5)	0.26
Allo-immunization or antibody mediated rejection	16 (27.1)	5 (8.5)	0.015

[a]*Prednisone equivalent.*

Data are median (interquartile range) unless otherwise indicated.

Baseline data were collected on the date of Corynebacterium *spp. isolation for exposed patients and on the date with equivalent time to transplantation for non-exposed patients. Primary lung graft dysfunction was diagnosed according to Snell et al. (43); Acute cellular rejection was diagnosed according to Stewart et al. (13); antibody-mediated rejection was diagnosed according to Levine et al. (18).*

Counts presented as n (%); medians presented with interquartile range for non-normally distributed data.

p-*value for the Wilcoxon or Mc Nemar non-parametric tests as appropriate—logistic regression for categorical variable with more than 2 modalities except for underlying respiratory disease.*

COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; CMV, cytomegalovirus.

0.001, and 6 [1.14–31.49]; $p = 0.0034$). Prior antibiotic therapy exposure and history of chronic allograft dysfunction were not associated with isolation of *Corynebacterium* spp.

Clinical Features of *Corynebacterium* spp. Isolation

The presence of *Corynebacterium* spp. in a respiratory sample was associated with a lower respiratory-tract-infection pattern or functional decline in 34/59 (57.6%) LTRs, including 9 (15.3%) with a monomicrobial isolation (**Supplementary Table S1**). Nine (15.3%) had radiological pneumonia, 2 (3.4%) with monomicrobial isolation. Overall, 20 (33.9%) patients had signs of bronchitis: cough in 14 (23.7%), sputum or bronchoscopic evidence of purulent secretion in 13 (22%) and both in 7 (11.9%). Five (8.5%) had functional decline associated with dyspnea. *Corynebacterium* spp. isolation was associated with functional decline in 14 (24.6%) patients, 5 (8.5%) with monomicrobial isolation. In total, 25 (42.4%) patients had no clinical or biological sign of infection and were therefore considered colonized.

In 42/59 (71%) patients, the first *Corynebacterium* spp. respiratory isolation occurred during the hospital stay, including 14 (24.7%) in the intensive care and 7 during the immediate post-LTx stay. Among the 7 patients admitted to the intensive care unit, 3 (21.4%) had acute respiratory failure and 4 (28.6%) respiratory-related sepsis. Oxygen therapy was needed for 19 (32.2%) patients, invasive mechanical ventilation for 11 (18.6%) patients, and non-invasive ventilation for one patient.

TABLE 2 | Factors associated with significant bronchial complication in univariate logistic regression.

	n/N[a]	OR (95% CI)	*p*-value
Previous *Corynebacterium* spp. isolation	37/74	2.33 (0.60–9.02)	0.220
URD - Pulmonary fibrosis	38/74	0.92 (0.17–5.13)	0.928
URD - COPD	34/74	0.88 (0.16–4.99)	0.888
URD - Bronchiectasis	2/74	4.64 (0.04–530.81)	0.526
Intubation length >24 h	63/74	1.01 (0.91–1.12)	0.814
Highly emergent transplantation	11/74	1.85 (0.18–18.91)	0.602
Intra-operative Extra corporeal membrane oxygenation	49/74	1.10 (0.23–5.19)	0.903

[a]*n, frequency; N, number observed; URD: underlying respiratory disease.*

TABLE 3 | Factors associated with *Corynebacterium* spp. sampling in univariate then multivariate analysis.

Covariates	n/N[a]	Univariate analysis			Multivariate analysis		
		Crude OR	CI 95%	*p*-value	Adjusted OR	CI 95%	*p*-value
Previous significant bronchial complication	30/118	10.83	3.47–33.80	<0.001	12.38	3.92–39.11	<0.001
Interstitial lung disease	56/118	1.00	0.49–2.06	1.000			
Emphysema/COPD	53/118	0.81	0.39–1.68	0.579			
Bronchiectasis	4/118	1.00	0.14–7.35	1.00			
CMV mismatch	18/115	0.94	0.34–2.57	0.904			
Tracheotomy	33/118	1.81	0.80–4.11	0.154			
Primitive lung graft dysfunction	27/117	1.31	0.55–3.10	0.544			
Parenteral corticosteroids <3 months	9/118	3.84	0.76–19.30	0.103	6.00	1.14–31.49	0.0034
Antibiotic treatment <3 months	69/118	1.63	0.78–3.42	0.192			
Previous CLAD	6/118	5.37	0.61–47.45	0.131			
Previous AMR	16/118	3.51	1.06–11.62	0.040			
Previous ACR	25/118	1.11	0.46–2.68	0.822			

[a]*n, Frequency; N, number of observation; OR: odds ratio; 95% CI, 95% confidence interval.*
COPD, chronic obstructive pulmonary disease; CMV, cytomegalovirus; CLAD, Chronic Lung Allograft Dysfunction (diagnosed accorded to Verleden et al. (16) ACR, acute cellular rejection (diagnosed according to Stewart et al. (13); AMR, antibody mediated rejection (diagnosed according to Levine et al. (18).
Primary lung graft dysfunction was diagnosed according to Christie et al. (19).

Description of First *Corynebacterium* spp. Isolation

C. striatum was the most frequently retrieved species, accounting for 71.2% of patients (n = 42), followed by *C. amycolatum* (14 patients, 23.7%) (**Supplementary Table S2**); *C. pseudodiphteriticum, C. accolens,* and *C. propinquum* were isolated from one patient each. In 41/59 (69.5%) patients, at least one other bacterium was isolated from the respiratory sample, with 25 (43.1%) above the significance threshold. *P. aeruginosa* was the main bacterium isolated from the plurimicrobial samples, in 18 (30.5%) patients.

Microbiological Outcomes

In total, 18 (30.5%) patients received effective antimicrobial therapy against *Corynebacterium* spp. infection based on antibiotics susceptibility testing.

Only 31 patients (52.5%) received antibiotic therapy on first isolation; only 18 (58.1%) of these received effective antibiotic therapy for *Corynebacterium* spp. based on antimicrobial susceptibility testing.

Forty-two patients (71.2%) had persistent *Corynebacterium* spp. colonization; among them, 16/18 (88.9%) patients who received an antibiotic course that was deemed effective on *in vitro* data. The antimicrobial therapies are detailed in the **Supplementary Table S3**. The median duration of *Corynebacterium* spp. carriage was 58 days [interquartile range 7–412]. We found no association between the administration of an effective antibiotic therapy and the presence of persistent colonization.

Long-Term Outcomes

The frequency of ACR episodes did not significantly differ between the exposed and non-exposed groups. An AMR episode occurred in 16/59 exposed patients, significantly more often than in the non-exposed patients (27.1% vs. 8.5%, p = 0.015). The frequency of CMV reactivations did not differ according to *Corynebacterium spp*. exposure (exposed vs. non-exposed 18.6% vs. 8.5%, p = 0.178). The length of follow-up did not significantly differ between the groups, but the occurrence of CLAD at 5 years of follow up was significantly higher in exposed than non-exposed patients (27.1% vs. 6.9%; p = 0.02122.0% vs. 5.1%, p = 0.0135). In the exposed group, CLAD was diagnosed after a median time of 497 days [346–888] of *Corynebacterium* spp. first isolation. Conversely, survival without CLAD differed significantly between the two groups (**Figure 2**; p = 0.012), with earlier onset in exposed versus non-exposed patients, but all-cause mortality did not significantly differ.

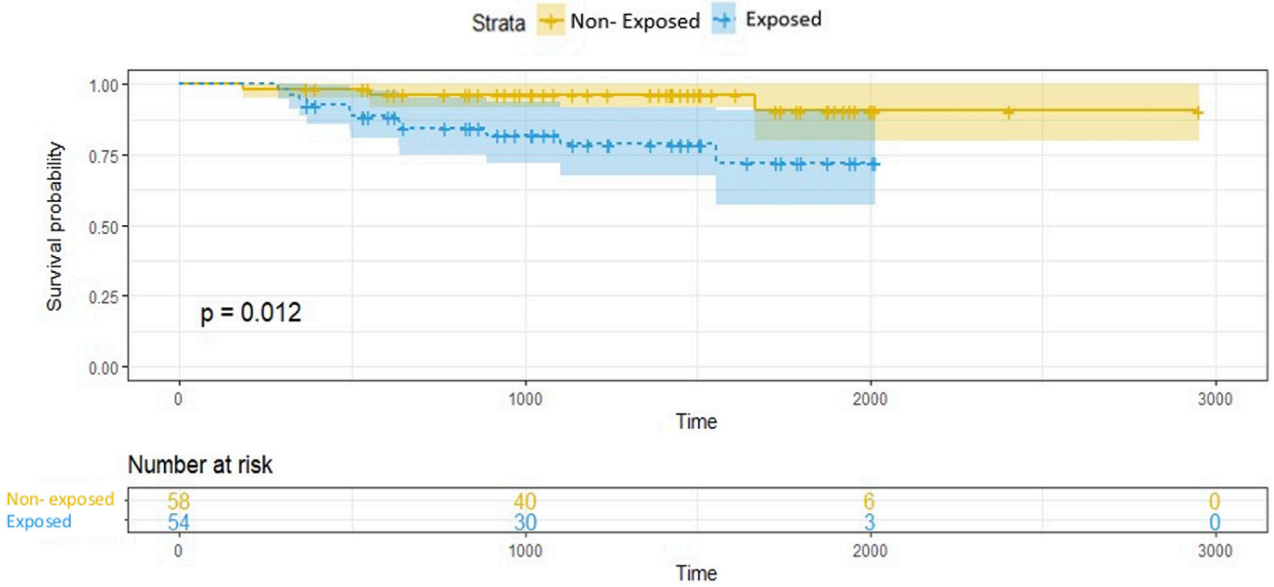

FIGURE 2 | Survival without chronic lung allograft dysfunction. Kaplan-Meier analysis. Survival was measured from the date of transplantation to the end of the collection period. Non-exposed = 0: not exposed to *Corynebacterium* spp.; exposed = 1: exposed to *Corynebacterium* spp.

DISCUSSION

In this retrospective case–control study, we aimed to investigate the suspected association between *Corynebacterium* spp. isolation in the respiratory tract and the occurrence of an SBC in 118 LTRs. Our findings can be summarized as follows: 1) although a pre-existing SBC was found an independent risk factor for detection of *Corynebacterium* spp., colonization by a *Corynebacterium* spp. was not associated with probability of a subsequent SBC; 2) the presence of a *Corynebacterium* spp. in the lower respiratory tract was associated with clinical manifestations of lower-respiratory-tract infection in 57.6% of cases, 68.6% being associated with another pathogen bacterial species; 3) although 18 (30.5%) patients received an antimicrobial course deemed effective by antimicrobial susceptibility testing, 16 (88.9%) of these had persistent colonization; and 4) survival was higher without CLAD in patients in whom a *Corynebacterium* spp. was never isolated.

The sole series of *Corynebacterium* spp. infection in LTRs reported the course and outcomes of 27 patients with *Corynebacterium* spp. isolation during a 2-year period (9). The low number of patients limits the relevance of the findings. In this series (9), more than half of the patients (53%) had a bronchial complication. The authors defined bronchial complication as the presence of mucosal plaques or purulent secretions at the bronchial suture. Despite guidelines for staging bronchial anomalies (20,21), the description of ischemic bronchitis and its extent is subjective and depends on the evaluator. In our center, fiberoptic bronchoscopies are performed by various physicians (namely LT pulmonologists, general pulmonologists, critical care physicians), not all of them skilled for reporting bronchial complications. A standardized assessment of bronchial complications (20,21) was therefore not available for all the patients. In our work, we deliberately chose an objective, and relevant endpoint, to limit a possible classification bias. Therefore, an SBC was defined as the occurrence of a bronchial fistula or the need for stenting or dilatation. This definition is undisputable as, during the study period, the bronchoscopists with skills on the evaluation of LT bronchial abnormalities were identical, indications for dilatation or bronchial stenting remained unchanged, and all the suspected dehiscence recorded by any physician were confirmed by a single skilled bronchoscopist. We do acknowledge the lack of formal guidelines on the timing or indication of interventional bronchoscopy or the procedure (dilatation or stenting), and the management of these complications may vary between centers. In our center, practices remained unchanged during the study period, with all indications performed by a single expert operator, which highly limits this classification bias. Using this definition, we report that *Corynebacterium* spp. isolation was associated with a pre-existing SBC in 21 (35.6%) patients.

Nevertheless, an interventional bronchoscopy procedure and the placement of a bronchial stent were more frequently needed in patients with *Corynebacterium* spp. isolation. Moreover, the presence of an SBC was an independent risk factor for *Corynebacterium* spp. isolation (OR 12.38 (3.92–39.11, $p < 0.001$).

In the recent study by Los-Arcos et al. (9), the clinical symptoms of lower-respiratory-tract infection were few. Only 12 (50%) patients had signs of respiratory infection (bronchitis, no pneumonia) and 9 when restricted to LTRs with exclusive isolation of *Corynebacterium* spp. and no other pathogen. Data concerning microbiological success or longer-term evolution, especially bronchial evolution, are not reported.

In our series, systemic infection was rare, with only 9 pneumonia (15.3%) cases and 20 (33.9%) with bronchitis.

The isolation of *Corynebacterium* spp. has been reported in immunocompromised hosts [solid-organ transplantation (8), connective tissue diseases under immunosuppressive therapy (22)], and patients with severe underlying respiratory disease (7,23,24). In these settings, infectious episodes related to *Corynebacterium* spp. have quite a silent course, more often appearing as bronchial or tracheobronchial than parenchymal infection (24). In a series of 18 patients with cystic fibrosis (23), 10 (76.9%) had worsening respiratory symptoms and none had pneumonia. In 10 hospitalized patients with COPD (7) 6 had pneumonia and 4 had exacerbations. Of note, in 6 of the 10 samples, the *Corynebacterium* spp. was the sole pathogen isolated and therefore responsible for the clinical symptoms.

In our series, the poor clinical picture may be explained by several combined factors. LTRs receive a high immunosuppressive regimen, thus impairing the T-cell and B-cell response (25-27). Moreover, owing to post-operative anatomical factors, the local host response to infection is decreased (28): modification of bronchial innervation secondary to the surgery decreasing cough reflex (29), impaired lymphatic drainage (30,31) etc.

Corynebacterium spp. are usually reported as skin and nasal mucosa commensal bacteria. Their isolation in a respiratory specimen is frequently considered a simple colonization, even though several recent studies suggest a varied pathogenicity at different sites (respiratory, endocarditis (32), brain abscess and meningal (33) infections) as well as the possibility of cross transmission of resistant strains between patients (34,35). These factors, associated with a limited number of symptomatic patients, might explain why *Corynebacterium* spp. isolation, even when reaching the microbiological threshold of significance, was not systematically considered to dictate antimicrobial therapy.

The finding of even moderate ischemic bronchitis frequently leads to the prescription of antibiotic therapy, potentially leading to the selection of antibiotic-resistant strains. Among the patients with available antimicrobial susceptibility testing, only 58% had received effective *in vitro* antibiotic therapy (cf. data in supplementary appendix).

In this series, we evidenced an association of *Corynebacterium* spp. isolation following corticosteroids pulses. These results, based on limited effectives (only 9 patients), should be taken carefully. However, these findings are consistent with evidence of increased occurrence of bacterial and fungal infection after immunosuppression intensification (36), and with higher frequency of *Corynebacterium* spp. isolation in LTRs receiving other types of immunosuppressive therapies than the conventional immunosuppressive regimen.

We found an association with a previous history of AMR in univariate analysis (OR 3.41 – IC 1.06–11.62), disappearing in multivariate analysis. To our knowledge, the association between respiratory infection or colonization and the occurrence of AMR has not been reported. AMR treatment relies on a heavy immunosuppression regimen (18), which is known to increase the risk of further infection.

Of note, increased risk of ACR has been described after a viral (37, 38) or bacterial (39) infection. In our series, *Corynebacterium* spp. isolation was not associated with more frequent occurrence of ACR, although ACR is suspected to promote bronchial complications (20).

In our series, the occurrence of CLAD was significantly higher in patients who had at least one positive *Corynebacterium* spp. respiratory sample (27.1% vs. 6.9% in non-exposed patients, $p = 0.021$). Some viral (37) or bacterial (39) lower-respiratory-tract infections or colonization have been reported as risk factors for CLAD (37-39). A single-center retrospective study (40) of 64 patients with post-LTx isolation of *P. aeruginosa* reported a higher frequency of CLAD occurrence within 2 years post-transplantation (23.4% vs. 7.7%, $p = 0.006$) in patients with *P. aeruginosa* colonization. Likewise, another study (41) included 95 LTRs with at least one *P. aeruginosa* isolation. CLAD-free survival was significantly higher in patients with successful eradication than in prolonged colonized patients ($p = 0.018$). These findings support the hypothesis of an inflammatory role of the bacteria, promoting airway damage, and leading to the generation of CLAD (42). Some evidence suggests that a similar mechanism may be involved in *Corynebacterium* spp. infection (42). Obviously, experimental evidence to support these hypotheses are necessary.

Although including a large number of LTRs with a positive *Corynebacterium* spp. lower-respiratory-tract sample, this work has several limitations. This was a single-center study, therefore limiting the significance of its conclusions in other centers. Indeed, in our center, the patients referred for LTx mostly have interstitial lung disease and emphysema. The findings might have been different in a center in which the main underlying respiratory condition would be cystic fibrosis, for example. Nevertheless, the single-center design allows for limiting the confounding factors: the perioperative management and post-LTx follow-up remained identical throughout the study period; the bronchoscopy findings and the indications for endoscopic management of bronchial complications remained unchanged; and the rigorous endoscopic and microbiological follow-up of patients with ischemic bronchitis allowed for reducing the classification bias. All the included LTRs were identified from our center's microbiology laboratory database. The possibility to have missed the identification of a LTR with a documented *Corynebacterium* spp. in a respiratory sample outside our hospital is unlikely because the management of LTR is highly centralized in our center, for infectious events or for bronchial issues. We decided to match cases and controls according to the underlying respiratory disease, and single or double LTx in order to limit the role of possible pre-existing colonization at LTx. In addition, we referred to published definitions (43) for the various other variables of interest, thus allowing for a homogeneous collection. Because of its retrospective design, neither the susceptibility profile of all Corynebacteria strains nor their phylogenetic relation could be extensively studied.

In conclusion, in this single-center series of 118 LTRs, the isolation of a *Corynebacterium* spp. was not associated with a subsequent SBC but occurred more frequently in patients who already had a complication. We found increased frequency and

earlier occurrence of CLAD in patients with *Corynebacterium* spp. respiratory isolation. Although we suggest the responsibility of chronic airway inflammation and an association with increased occurrence of AMR, the exact pathophysiology remains to be clarified. The impact of *Corynebacterium* spp. eradication on the occurrence of CLAD should be evaluated in future studies.

INVESTIGATORS OF THE PARIS-BICHAT LUNG TRANSPLANT PROGRAM

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DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by CEPRO 2020-044.

AUTHOR CONTRIBUTIONS

AS, NG, VB, PE, and JM participated in research design; AS, TR, and JM participated in the writing of the paper; all the authors participated in the performance of the research, data analysis and critically reviewed the manuscript.

CONFLICT OF INTEREST

VB received advisory board fees from Novartis and Takeda; GW received advisory board fees from CSLBehring; PhM received advisory board and speaking fees from Pfizer, MSD, and Menarini; JM received congress reimbursement fees from Biotest and CSLBehring.

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.10942/full#supplementary-material

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BRIEF RESEARCH REPORT
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Additional Benefits of Rituximab and Plasma Exchange on Top of Standard Induction Therapy in Kidney Transplant Recipients With a Negative CDC Crossmatch but High Preformed Donor Specific Antibody Titer

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Optimal induction strategy in highly sensitized kidney transplant recipients (KTRs) is still a matter of debate. The place of therapies, such as plasma exchange and rituximab, with potential side effects and high cost, is not clearly established. We compared two induction strategies with (intensive) or without (standard) rituximab and plasma exchange in KTRs with high levels of preformed DSA transplanted between 2012 and 2019. Sixty KTRs with a mean age of 52.2 ± 12.2 years were included, 36 receiving standard and 24 intensive induction. Mean fluorescence intensity of immunodominant DSA in the cohort was 8,903 ± 5,469 pre-transplantation and similar in both groups. DSA level decrease was similar at 3 and 12 months after transplantation in the two groups. An intensive induction strategy was not associated with better graft or patient survival, nor more infectious complications. The proportion of patients with rejection during the first year was similar (33% in each group), but rejection occurred later in the intensive group (211 ± 188 days, vs. 79 ± 158 days in the standard group, $p < 0.01$). Our study suggests that an intensive induction therapy including rituximab and plasma exchanges in highly sensitized kidney recipients is not associated with better graft survival but may delay biopsy-proven rejection.

Keywords: kidney transplant, DSA, donor-specific HLA antibodies, induction therapy, plasma exchange, rituximab

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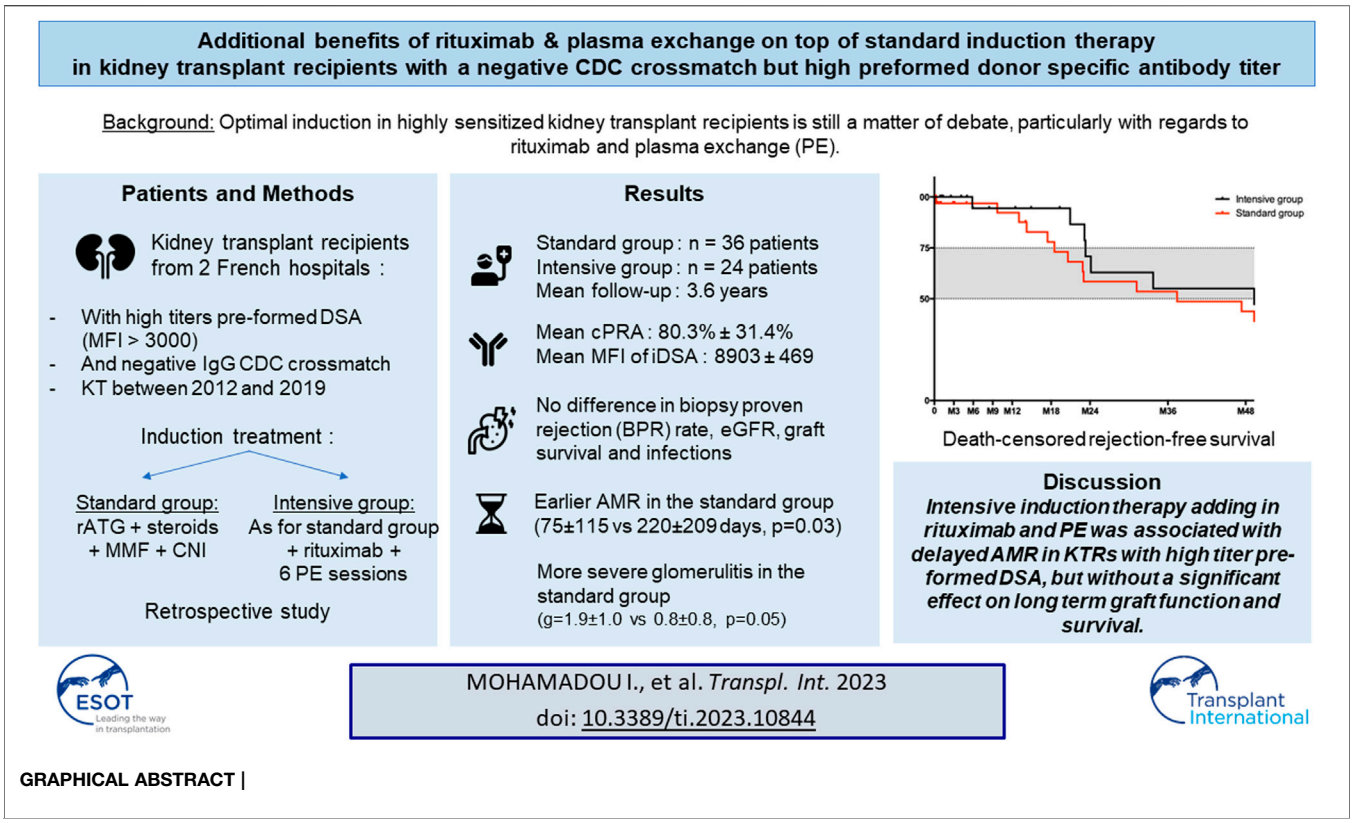
Abbreviations: AMR, antibody-mediated rejection; BPR, biopsy proven rejection; CDC, complement-dependent cytotoxicity; cPRA, calculated panel reactive antibodies; DSA, donor specific antibodies; EDTA, ethylenediaminetetraacetic acid; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; HS, highly sensitized; iDSA, immunodominant DSA; IVIg, intravenous immunoglobulin; KTR, kidney transplant recipients; MFI, mean fluorescence intensity; PE, plasma exchanges; rATG, rabbit anti-thymocyte globulin; SAFB, single antigen flow beads.



GRAPHICAL ABSTRACT |

INTRODUCTION

A crucial proportion of waitlisted patients are highly sensitized (HS) kidney transplant candidates. In the United States, in 2019, 12% of candidates had calculated panel reactive antibodies (cPRA) over 80% (1), and in France they represented 26% of waitlisted patients according to the 2019 report of the National French Biomédecine Agency. This group of patients is a challenge for kidney transplant teams: first, their access to transplantation is much lower when compared to naïve patients; second, the presence of anti-human leukocyte antigen (HLA) donor specific antibodies (DSA) is associated with a higher risk of antibody-mediated rejection (AMR) and long-term graft-loss (2–4). Many desensitization protocols have been proposed for these patients to improve their access to transplantation and limit AMR. These strategies for HLA-incompatible kidney transplantations have shown satisfactory results (5) and increased patient survival compared to remaining on the waiting list (6). However, the optimal induction therapy in HS patients is still a matter of debate, the goal being to limit the risk of graft rejection without over-immunosuppressing the recipients.

Indeed, it is now well established that rabbit anti-thymocyte globulin (rATG)-Thymoglobulin is a standard of induction therapy for HS recipients (7, 8), but it is not clear whether or not it should be complemented by other therapeutics, such as rituximab or plasma exchange (PE). Rituximab has been evaluated in randomized controlled trials as an induction therapy with inconsistent results. It failed to prove its superiority in a global recipient population (9, 10), but tended to reduce the rejection rate in a subgroup of HS patients (10), without increasing the infectious risk. However, the proportion of HS patients in these trials was low, and the estimation of the immunological risk was not as precise as current techniques permit. In kidney transplantation, PE have mostly been used in desensitization strategies (11, 12) and their additional benefit as an induction treatment has not been fully validated.

We conducted a retrospective analysis in HS KTRs, comparing rejection rates and graft survival according to the induction regimen they received, designed as standard (rATG-Thymoglobulin and steroids) or as intensive (rATG-Thymoglobulin, steroids, rituximab and PE).

MATERIALS AND METHODS

Study Design

We performed a retrospective study including all KTRs (January 2012 to September 2019) from two Paris (France) area transplant units (Tenon and Mondor hospitals) with at least one preformed Class I or Class II DSA and with a mean fluorescence intensity (MFI) above 3,000 between 3 months before transplantation and the day of transplantation. Immunodominant DSA (iDSA) was defined as the DSA with the highest pre-KT MFI. KT was only performed if complement-dependent cytotoxicity (CDC) crossmatch for IgG was negative on the day of transplantation, but IgM CDC crossmatch was not a contraindication to perform

KT. Patients who underwent ABO-incompatible transplantation or combined multiorgan transplantation were excluded. Follow-up ended December 1st 2020.

All patients received an induction treatment combining methylprednisolone (500 mg on the day of transplantation), rabbit-ATG (Thymoglobulin 1.5 mg/kg over four or 5 days, depending on center), mycophenolate mofetil (2–3 g/day), tacrolimus (target trough level 8–12 ng/mL during the first 3 months) and four intravenous immunoglobulin (IVIg) (Clairyg or Privigen, 2 g/kg) post-transplant courses (once every 3–4 weeks). Patients in the intensive group additionally received one rituximab 1,000 mg dose and six PE sessions (60 mL/kg, 100% plasma) after transplantation. The choice of standard or intensive induction regimen therapy was based on the center and the evaluation of the nephrologist before KT. Intensive induction protocol was based on previous studies with high immunological risk patients (13).

Maintenance therapy consisted of prednisone (20 mg/day during first month, followed by tapering of 2.5 mg every 2 weeks, reaching 10 mg/day at 3 months), calcineurin inhibitor (tacrolimus or ciclosporin with target trough level of 7–9 ng/mL and 150 ng/mL from 3 to 6 months after KT respectively, and 5–7 ng/mL and 100–150 ng/mL thereafter), and mycophenolate mofetil (2–3 g/day).

In case of T-cell mediated rejection, patients received 500 mg of methylprednisolone during 3 days, followed by prednisone 20 mg/day. Antibody mediated rejections were treated with methylprednisolone (500 mg during 3 days), PE, and IVIg according to the modified Marrakech-protocol (14).

Clinical and biological data were collected retrospectively and anonymously from computerized medical records.

Antibody Detection and Crossmatch Techniques

Luminex assay was used both for screening and single antigen flow beads (SAFB) identification of anti-HLA abs directed against HLA Class I and Class II antigens (LSM12 and LSA kits, One Lambda, Canoga Park, CA) in Saint-Louis Hospital Immunology Laboratory (Paris, France). Pre-transplant follow-up consisted of screening every 3 months when sera were available, and one Class I + Class II SAFB per year, with additional testing when screening positivity increased above doubling for the highest bead ratio in at least one Class. The day of transplant serum was tested in a SAFB assay. Post-transplant follow-up only relied on SAFB testing for all sera shipped to the laboratory. SAFB positivity threshold was set at a normalized MFI>500 according to the baseline formula calculated using Fusion software, after subtraction of the minimum MFI value for the corresponding locus to account for the non-specific binding observed, e.g., in the presence of IVIg. The DSA nature of the detected antibody was assigned at the antigenic level for antigens represented by a single bead or when all the beads for a given antigen were positive. For antigens with at least one negative bead, DSA was assigned when the bead bearing the donor allele was positive, the donor allele being either deduced from the emergency SSP typing (low resolution Olerup until end of 2016, Linkage Biosciences, thereafter) or retrospective high definition SSO typing (One Lambda), or when DNA was not available, deduced from common haplotypes using the HaploSTATS tool. Retained DSA MFI value was the average for the positive beads corresponding to the donor antigen. All sera were ethylenediaminetetraacetic acid (EDTA)-treated pre-testing since mid-September 2015, and for this study, retesting was performed with EDTA for anterior sera suspected of undergoing complement interference.

Kidney Biopsies

The kidney allograft biopsies were fixed in FAA (a solution of alcohol, formalin, and acetic acid), and subsequently embedded in paraffin. The biopsy sections (4 µm thick) were stained with periodic acid-Schiff, Masson's trichrome, Jones methenamine silver and hematoxylin and eosin. The allograft paraffin-embedded kidney biopsies were scored and graded according to the international Banff 2017 classification for kidney allograft transplantation by trained transplant pathologists (DB, AM). C4d staining was not performed with the same technique in the two centers (immunochemistery or immunofluorescence), and was therefore not included in the statistical analysis.

Statistical Analysis

Continuous variables were expressed as the mean ± SD. Categorical variables were reported as numbers and percentages. The intensive and standard groups were compared using the Mann-Whitney and Fisher exact tests for continuous and categorical variables, respectively. In survival analyses, Fine and Gray models were fitted, using death or loss of allograft function as a competitive event. To study the impact of day-0 DSA on ABMR occurrence, univariate models stratified on treatment regimen was used. Due to a log-linear type of association, sum of day-0 MFIs and MFI of day-0 iDSA were log-transformed. To study the impact of treatment regimen on the occurrence of ABMR, a multivariate model was fitted, using log(sum of day-0 MFIs) and the number of previous kidney transplantations (0 vs. 1 or more) as covariates. These covariates were chosen given their known prognostic value (based on the medical literature) on the risk of ABMR occurrence following transplantation. $p < 0.05$ was considered statistically significant and all tests were two-sided. Descriptive statistics were generated using Prism-6 (GraphPad). Survival analyses were performed using R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria) and package cmprsk.

Ethics Statement

The study was conducted in accordance with the ethical guidelines of the Assistance Publique – Hôpitaux de Paris. No institutional review board approval was necessary at the time of the study as it was a retrospective study involving no intervention. The study was conducted according to the ethical standards of the 2000 Declaration of Helsinki as well as the Declaration of Istanbul 2008.

RESULTS

Patients' Characteristics

The two centers performed 1,457 kidney transplantations between 1st January 2012 and 1st September 2019. Of these,

TABLE 1 | Patients' initial characteristics.

	Total (n = 60)	Intensive group (*n* = 24)	Standard group (*n* = 36)	p
Recipient's age (years, mean ± SD)	52.2 ± 12.2	49.7 ± 13.9	53.9 ± 10.7	0.26
Male (n, %)	29 (48.3%)	14 (58.3%)	15 (41.7%)	0.29
Causal nephropathy (n, %)				
Glomerulopathy	22 (36.7%)	8 (33.3%)	14 (38.9%)	
Hypertension	6 (10%)	0 (0%)	6 (16.7%)	
Uropathy	9 (15%)	4 (16.7%)	5 (13.9%)	
Genetic	7 (11.7%)	3 (12.5%)	4 (11.1%)	
Unknown	15 (25%)	8 (33.3%)	7 (19.4%)	
Other	1 (1.7%)	1 (4.7%)	0 (0%)	
Immunisation prior to transplantation (n, %)	50 (93.3%)	22 (91.7%)	34 (94.4%)	1
Previous transplantation (n, %)	37 (62%)	19 (79.2%)	18 (50%)	0.1
Pregnancy (n, %)	22 (71%)	8 (80%)	14 (67%)	0.67
Donor's age (years, mean ± SD)	57.5 ± 15.7	54.8 ± 18.5	59.3 ± 13.5	0.31
Deceased donor (n, %)	56 (93.3%)	23 (95.8%)	33 (91.7%)	0.64
Cold ischemia time (hours, mean ± SD)	17.4 ± 4.7	17.1 ± 4.0	17.7 ± 5.2	0.61
Follow-up (months, mean ± SD)	43.1 ± 29.3	52.4 ± 28.8	36.9 ± 28.4	0.03
Number of DSA at day 0 (mean ± SD)	2.7 ± 1.7	2.5 ± 1.2	2.7 ± 2.0	0.97
Class I	1.2 ± 1.0	0.92 ± 0.8	1.4 ± 1.1	0.15
Class II	1.5 ± 1.3	1.6 ± 1.2	1.4 ± 1.4	0.40
Mean MFI at day 0 (mean ± SD)	13,943 ± 11,764	12,290 ± 8,235	15,090 ± 13,690	0.69
MFI of iDSA at day 0 (mean ± SD)	8,903 ± 469	8,435 ± 4,574	8,935 ± 5,726	0.93
iDSA class class I (n, %)	24 (40%)	8 (33.3%)	16 (44.4%)	0.43
iDSA class II (n,%)	36 (60%)	16 (66.7%)	20 (55.6%)	

DSA, donor specific antibody; MFI, mean fluorescence intensity; iDSA, immunodominant DSA.

60 hypersensitized patients were included in the study. Thirty-six patients received a standard induction and 24 patients an intensive induction including PE and rituximab. Fifteen patients in the standard group also received a limited number of PE (1.5 ± 2.1 sessions) during the post-transplant period. **Table 1** shows patients' initial characteristics. Fifty-two percent ($n = 31$) of the patients were women and 93.3% ($n = 56$) had prior history of sensitization. Sixty two percent of the patients ($n = 37$) underwent at least one previous transplantation (standard group: $n = 18$ [50%]; intensive group: $n = 19$ [79.2%], $p = 0.1$), and 71% of women ($n = 22$) had at least one pregnancy before KT. Both groups were similar regarding mean recipient age (52.2 ± 12.2 years), donor age (57.5 ± 15.7 years), cold ischemia time (mean: 17.4 ± 4.7 h), pre-transplant iDSA MFI level (intensive group: 8,435 ± 4,574; standard group: 8,935 ± 5,726; $p = 0.93$) and mean number of DSA (intensive group: 2.5 ± 1.2; standard group: 2.7 ± 2.0; $p = 0.97$). The iDSA was Class II in 36 patients (60%). The mean time on the waiting list was 1,660 ± 1,058 days (intensive group: 1,701 ± 1,130 days; standard group: 1,023 ± 1,632 days; $p = 0.8$), and the mean calculated panel reactive antibody (cPRA) was 80.3% ± 31.4% (intensive group: 79.7% ± 29.9%; standard group: 80.7% ± 32.8%; $p = 0.9$). Day 0 CDC IgM crossmatch was positive in eight patients (13.5%) (intensive group: $n = 3$ [12.5%]; standard group: $n = 5$ [13.9%]). Mean follow-up was 52.4 ± 28.8 months in the intensive group and 36.9 ± 28.4 months in the standard cohort ($p = 0.03$).

Biopsy Proven Rejections

A total of 37 biopsy-proven rejection (BPR) episodes occurred in 24 patients during the follow-up (intensive group: $n = 11$ patients; standard group: $n = 13$ patients, p = ns), with 12 patients experiencing more than one BPR (intensive group: $n = 5$; standard group: $n = 7$). Twenty-four BPR (64%) occurred during the first year post-transplant (**Table 2**). The proportion of patients with BPR during the first year was not significantly different between the two groups ($n = 8$ [33.3%] in the intensive group and 12 [33.3%] in the standard group; $p = 1$).

The most frequent type of rejection was acute AMR, representing 71% of BPR in the first year. 78.5% of these BPR were AMR in the standard group and 60% in the intensive group ($p = 0.39$). AMR was associated with MFI of iDSA (HR = 2.07 [IQR: 1.04–4.1], $p = 0.037$), and with the sum of MFI at day 0 (HR = 1.92 [IQR: 1.12–3.29], $p = 0.017$), but not with the number of DSA at day 0 (HR = 1.2 [IQR: 0.964–1.50], $p = 0.1$). AMR-related microvascular inflammation tended to be more severe in the standard cohort, with a mean glomerulitis score of 0.8 ± 0.8 in the intensive group vs. 1.9 ± 1.0 in the standard group ($p = 0.05$), without any difference in peritubular capillaritis (1.07 ± 1.15 in the standard group and 1.14 ± 0.9 in the intensive group ($p = 0.89$)). Of note, three patients in the intensive group had a T-cell mediated rejection diagnosis during the first year. Histological BPR features are detailed in **Table 2**.

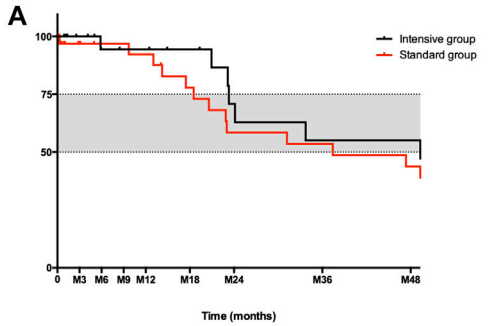
BPR was diagnosed earlier after transplantation in the standard group, as shown in **Figure 1A** (79 ± 158 days in the standard group vs. 211 ± 188 days in the intensive group ($p = 0.005$)). Mean time to AMR diagnosis was also shorter in the standard (75 ± 115 days) compared to the intensive group (220 ± 209 days, $p = 0.03$). However, global survival without rejection was not significantly different in the multivariate analysis (HR of BPR in the intensive group = 0.794 [0.34–1.9], $p = 0.6$).

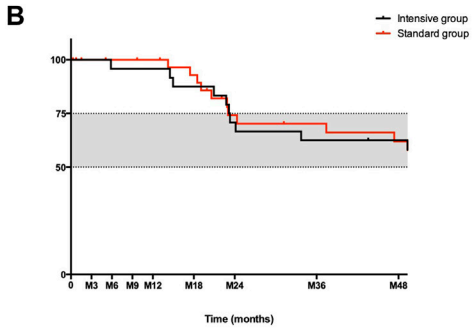
Forty patients had a systematic kidney biopsy performed at 3 months post-transplantation (intensive group: $n = 17$ [70.8%];

TABLE 2 | Clinical and biological endpoints.

	Total (n = 60)	Intensive group (n = 24)	Standard group (n = 36)	p
Patients with rejection during first year (n, %)	20 (33.3%)	8 (33.3%)	12 (33.3%)	1
Total number of rejection during first year	24	10	14	
Patients with AMR (acute or chronic)	19 (79.2%)	6 (60%)	13 (92.8%)	
Patients with acute AMR	17 (70.9%)	6 (60%)	11 (78.6%)	0.76
Patients with chronic AMR	2 (8.3%)	0	2 (14.3%)	0,5
Patients with acute cellular rejection	3 (12.5%)	3 (30%)	0	0.07
Patients with mixed rejection	2 (8.3%)	1 (10%)	1 (7.1%)	1
Histological data regarding BPR (mean ± SD)				
g	1.5 ± 1.1	0.8 ± 0.8	1.9 ± 1	0.05
i	0.4 ± 0.8	0.5 ± 1.1	0.5 ± 0.7	0.68
t	0.2 ± 0.5	0.3 ± 0.7	0.1 ± 0.3	0.61
v	0.2 ± 0.5	0.3 ± 0.7	0.1 ± 0.3	0.61
cpt	1.0 ± 1.0	0.7 ± 0.8	1.4 ± 1.1	0.39
cg	0 ± 0	0 ± 0	0 ± 0	NC
mm	0.1 ± 0.2	0 ± 0	0.1 ± 0.3	0.55
ci	0.3 ± 0.8	0.7 ± 1.1	0.2 ± 0.4	0.4
ct	0.4 ± 0.6	0.8 ± 0.8	0.2 ± 0.4	0.04
cv	0.8 ± 0.9	1.0 ± 1.2	0.7 ± 0.8	0.72
ah	0.3 ± 0.5	0.7 ± 0.5	0.2 ± 0.4	0.05
Patients with rejection during follow-up (n, %)	24 (40%)	11 (45.8%)	13 (36.1%)	0.78
Delayed graft function (n,%)	23 (39.6%)	8 (33.%)	15 (44.1%)	0.43
eGFR (CKD-EPI, mean ± SD)				
M1	39.9 ± 23.8	50.9 ± 27.0	31.0 ± 16.4	0.003
M3	44.9 ± 23.9	49.1 ± 24.5	31.7 ± 23.3	0.17
M12	42.2 ± 18.2	48.1 ± 19.1	37.5 ± 16.3	0.065
M24	43.9 ± 18.1	47.8 ± 21.9	40.2 ± 12.9	0.4
M36	42.7 ± 15.2	47.6 ± 18.3	37.5 ± 9.0	0.09
Proteinuria ($g \cdot mmol^{-1}$, mean ± SD)				
M3	0.07 ± 0,09	0.07 ± 0.1	0.07 ± 0.08	0.43
M12	0.1 ± 0.2	0.12 ± 0.2	0.13 ± 0.2	0.81
M24	0.04 ± 0.07	0.02 ± 0.01	0.06 ± 0.1	0.12
M36	0.1 ± 0.18	0.05 ± 0.04	0.11 ± 0.21	0.87
Lymphocytes ($G \cdot L^{-1}$, mean ± SD)				
day 0	1.5 ± 0.8	1.4 ± 0.7	1.6 ± 0.9	0.37
day 5	0.3 ± 0.6	0.12 ± 0.2	0.4 ± 0.8	0.0006
M12	0.9 ± 0.4	0.7 ± 0.3	1.0 ± 0.5	0.37
Tacrolimus residual levels ($ng \cdot mL^{-1}$)	8.6 ± 4.3	7.8 ± 2.6	9.1 ± 5.2	0.5
M1	9.8 ± 11.1	10.9 ± 16.3	8.9 ± 4.4	0.4
M3	6.3 ± 2.1	5.9 ± 1.6	6.5 ± 2.4	0.4
M12				
MFI of iDSA (mean ± SD)				
day 0	8,903 ± 5,469	8,435 ± 4,574	8,935 ± 5,726	0.93
M3	5,282 ± 5,660	4,878 ± 5,805	5,605 ± 5,620	0.40
M12	5,061 ± 6,152	4,709 ± 5,645	5,348 ± 6,630	0.96
Mean MFI (mean ± SD)				
day 0	13,943 ± 11,764	12,290 ± 8,235	15,090 ± 13,690	0.69
M3	9,478 ± 12,055	8,319 ± 11,919	10,411 ± 12,283	0.55
M12	7,799 ± 11,415	7,156 ± 9,236	8,292 ± 12,975	0.93
Patients with *de novo* DSA (compared to day 0)	25 (47.2%)	14 (58.3%)	11 (37.9%)	0.17
M3	17 (33.3%)	6 (27.3%)	11 (37.9%)	0.06
M12				
Patients with infection during first year of follow-up (n, %)	39 (65%)	16 (66.7%)	23 (63.9%)	1

AMR, antibody mediated rejection eGFR, estimated glomerular filtration rate; day 0, day of transplantation; M1, 1-month post-transplantation; M3, 3 months post-transplantation; M6, 6 months post-transplantation; M12, 12 months post-transplantation.





Number at risk	Day 0	M3	M6	M9	M12	M18	M24	M48
Intensive group	24	21	17	16	16	14	10	8
Standard group	36	24	22	22	21	17	13	11

	Intensive group (n=24)	Standard group (n=36)	p
Time between transplantation and graft rejection (days, mean ± SD)	211 ± 188	79 ± 158	0.0045

Number at risk	Day 0	M3	M6	M9	M12	M18	M24	M48
Intensive group	24	24	23	23	24	22	18	15
Standard group	36	32	31	31	30	27	20	17

	Intensive group (n=24)	Standard group (n=36)	p
Death-censored graft survival (n, %)			
M12	24 (100%)	29 (80.6%)	0.035
End of follow-up	20 (83.3%)	23 (63.9%)	0.15

FIGURE 1 | (A) Death-censored rejection free survival. The time between kidney transplantation and the first episode of biopsy proven rejection is shown. **(B)** Death-censored graft survival. The time between kidney transplantation and death-censored graft loss is shown.

standard group: n = 23 [63.9%]). Amongst these, 3 biopsies (7.5%) were also performed for cause (intensive group: n = 2, cause: control of anterior BPR; standard group: n = 1, cause: acute kidney injury). Glomerulitis score was significantly higher in the standard group (0.4 ± 0.8, vs. 0.06 ± 0.2) compared to the intensively treated patients (p = 0.04).

DSA

Both sets of patients experienced a significant decrease of iDSA MFI at 3 and 12 months when compared to day 0. In the standard group, mean MFI of iDSA dropped from 8,935 ± 5,726 at day 0, to 5,605 ± 5,620 at 3 months (−37%, p = 0.002) and remained stable thereafter at 5,348 ± 6,630 at 12 months (decrease of −40% when compared to day 0, p = 0.0003). In the intensive group, mean MFI of iDSA dropped from 8,435 ± 4,574 at day 0, to 4,878 ± 5,805 at 3 months (−42%, p = 0.002) and remained stable thereafter at 4,709 ± 5,645 at 12 months (−44% when compared to day 0, p = 0.004). There was no significant difference in iDSA MFI reduction and mean MFI at 3 and 12 months after transplantation between both groups (**Table 2**). Mean MFI of DSA did not significantly differ at 3 and 12 months. The number of patients with *de novo* DSA was similar in the two groups at 3 and 12 months after transplantation (**Table 2**).

eGFR and Proteinuria

Kidney function was significantly better in the intensive group at 1-month post-KT (estimated glomerular filtration rate (eGFR) in the intensive group: 50.9 ± 27.0 mL min^{-1}.1.73 m^2 versus 31.0 ± 16.4 0 mL min^{-1}.1.73 m^2 in the standard group; p = 0.003), but this difference was not observed afterwards. Estimated glomerular filtration rate (eGFR) was 48.8 ± 19.1 mL min^{-1}.1.73 m^2 (CKD-EPI) and 47.6 ± 18.3 mL min^{-1}.1.73 m^2 (CKD-EPI) at 12 and 36 months after transplantation in the intensive group, and 37.5 ± 16.3 mL min^{-1}.1.73 m^2 (CKD-EPI) and 37.5 ± 9 mL min^{-1}.1.73 m^2 (CKD-EPI) in the standard group (p = 0.65 at month 12 and 0.09 at month 36) (**Table 2**). Proteinuria was also not significantly different at 3 or 12 months.

Mean eGFR was not different in the subgroup of patients who experienced BPR during first year post-transplantation (41.4 vs. 42.1 mL/min/1.73 m^2; p = 0.91).

Graft and Patient Survival

Global patient survival at the end of follow-up (1,316 ± 895 days) was 75% in the intensive group and 77% in the standard cohort, with six and eight deceased patients, respectively (p = 1). Death-censored graft survival at 12 months was better in the intensive group (100% in the intensive group vs. 80.6% in the standard group, p = 0.035), but this difference did not persist to the end of follow-up (83.3% in the intensive group and 63.9% in the standard group, p = 0.15) as shown in **Figure 1B**. Excluding death of the recipient, six patients, all from the standard group, lost their graft during the first year of follow-up. Mean time to graft loss was 80 ± 121 days. Causes of graft loss were acute AMR for one patient, reduction of immunosuppressive regimen in the context of severe infection for two patients, and acute peri-operative graft ischemia without evidence of macroscopic or histologic arterial or venous thrombosis in three patients.

A total of 14 deaths happened during follow-up (intensive group: n = 6, standard group: n = 8, p = 1). Infection was the most frequent cause of death, representing 57% of the total of deaths during follow-up (intensive group: n = 2, standard group: n = 6), the most frequent lethal pathogen being SARS-CoV-2 (n = 4, one in the intensive group and three in the standard cohort). Other deaths were due to cardiovascular events (n = 3, intensive group) and cancer (n = 1, standard group). The cause of death was not specified in two patients.

Infections

During first year of follow-up, 39 patients (65%) were diagnosed with at least one infection (intensive group: n = 16 [66.7%], standard group: n = 23 [63.9%], p = 1). For six of these patients (10% of total), hospitalization in an intensive care unit (intensive group: n = 1 [4.2%]; standard group: 5 [13.9%], p = 0.3) was

required. The most frequent type of infection during the first year was pyelonephritis, representing 40.4% of infections. Mean time to first infection was 244 ± 351 days in the standard group and 215 ± 298 days in the intensive group ($p = 0.76$). There was no difference in the frequency of viremia at 3 and 12 months for cytomegalovirus, Epstein-Barr virus and BK virus.

DISCUSSION

Our study shows that an intensive induction strategy combining rATG-Thymoglobulin, rituximab and PE in patients with high MFI preformed DSAs is associated with a delayed occurrence of BPR and may minimize the microvascular injury burden but with no beneficial effect on post-transplantation DSA levels, long-term death-censored graft survival or graft function. The intensive therapy was noteworthy for not being associated with a higher rate of infections. The global graft survival was good in this HS population (death censored graft survival at 1 year: 90%), proving that HLA-incompatible transplantation can be performed with good results in this group of patients. In addition, AMR rate at 1 year was 35.1%, which is similar to that found in previous studies including HS kidney recipients (15, 16). Our study shows that in an immunologically well-characterized kidney recipient population, high-cost additional therapies such as rituximab or PE may not be efficient in terms of benefit for long-term graft survival and graft function. The initial immunological characterization and the follow-up of DSA was homogeneous and rigorous: DSA were analyzed in the same laboratory, with the same technique, and used an interpretation algorithm that considered false positive signals caused by IVIg interference.

Concerning induction therapy in HS recipients, previous randomized controlled studies (17) have shown a benefit of rATG-Thymoglobulin over basiliximab in terms of post-transplant rejection and long-term graft survival. Since these trials, rATG-Thymoglobulin has been widely used for HS kidney recipients in preference to Atgam (18) or alemtuzumab (19), and recommended by 2009 KDIGO consensus guidelines (20). However, the optimal dosage (usually 1.5 mg/kg for three to 5 days as used in our study) may remain a matter of debate (21, 22). Translated from post-rejection anti-HLA desensitization protocols, other supplemental therapies such as rituximab or PE have been added to the induction strategy and analyzed in retrospective studies since 2010 (13) in order to decrease anti-HLA antibody MFI and reduce the risk of AMR. In a seminal study, (13) compared an intensive strategy combining rATG, PE, rituximab and IVIg to a standard induction strategy with rATG and IVIg only in a historical cohort. Although they found no difference in the rate of AMR at 1 year (19.6% vs. 16.6%), patients who received induction therapy including rituximab and PE had lower histological AMR-related features such as glomerulitis, peritubular capillaritis or transplant glomerulopathy in the 1-year post-transplant follow-up biopsies. No prospective study has since been performed to validate these conclusions and some centers have added these supplementary but expensive therapies to their induction protocol for HS kidney recipients. Our study, which more recently compares these two similar strategies using modern and more accurate techniques of immunological risk assessment, also fails to demonstrate a benefit in terms of AMR occurrence with an intensive therapy using PE and rituximab. Although additional B-cell depletion by rituximab seemed to be effective, as illustrated by the differences between lymphocytes counts at Day 5 (0.12 ± 0.2 vs. 0.4 ± 0.8, $p = 0.0006$), the DSA levels, upstream regulator of AMR risk, were not further modified with the intensive strategy. Moreover, we showed that histological AMR-related parameters such as glomerulitis were decreased in the intensive group and, interestingly, the occurrence of AMR was delayed, showing a potential short-term effect of these additional therapies. The lower rate of lymphocytes in this intensive group could explain the delayed occurrence of AMR, despite the similarity of DSA levels between the two groups. The differences observed in eGFR at 1 and 3 months post-KT could be due to the delayed occurrence of BPR in the intensive group, as 3 months corresponds to the main timing between KT and BPR in the standard group (79 ± 158 days). However, there is a question over the cost-effectiveness of these additional techniques such as rituximab or PE since a longer delay to the occurrence of rejection episodes was not associated with a difference in more robust criteria such as long-term graft survival. Due to the techniques used and number of hospitalizations, the intensive strategy is obviously associated with increased costs that were not analyzed here. We observed no increase in possible side effects such as infectious complications, but these data should be interpreted with caution given the small size samples and a possible lack of power. Infections were the main cause of death in both groups (57% of total deaths during follow-up), which may urge clinicians to question the increase of immunosuppression without evidence of a clear benefit in this HS population, that will *de facto* be heavily immunocompromised. Clinicians should also note that the global survival rate at the end of follow-up was high (23.3%), and that COVID-19 took a heavy toll on our patients' mortality.

The potential limitations of the study should be acknowledged, including the small number of patients, its retrospective design, the absence of systematic flow cytometry crossmatch in our center, and the impossibility of evaluating the effect of each therapy separately. Finally, as previously stated, 15 patients under standardized treatment also received a small number of plasmapheresis treatments, but this subgroup showed a similar rate of BPR compared to the rest of the patients of the group.

In HS patients with preformed high-level DSA and negative CDC crossmatch, an intensive induction treatment using PE and rituximab in addition to rATG was associated with delayed AMR, but without a significative effect on long-term graft survival and graft function. The use of these additional therapies for induction immunosuppressive therapy should be carefully analyzed in randomized prospective studies as any additional value is still not clear.

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

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

AUTHOR CONTRIBUTIONS

IM, MM, J-LT, and YL: Substantial contributions to the conception or design of the work, the acquisition, analysis, and interpretation of data for the work; drafting the work and revising it critically for important intellectual content. SM: Substantial contributions to the conception or design of the work, the acquisition, analysis, and interpretation of data for the work. DB: Substantial contributions to the interpretation of data for the work. AM: Substantial contributions to the interpretation of data for the work; revising the work critically for important intellectual content. MJ, NO, CR, HF, CP-H, ER, LM, and PG: Substantial contributions to the interpretation of data for the work.

CONFLICT OF INTEREST

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

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