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**Kidneys from Older Living Donors:
a Precious Gift**



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DOI: 10.3389/ti.2024.12330

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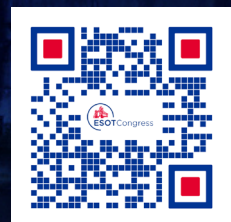


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Transplant Trial Watch

John M. O'Callaghan^{1,2*} and John Fallon²

¹University Hospitals Coventry and Warwickshire, Coventry, United Kingdom, ²Centre for Evidence in Transplantation, Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom

Keywords: randomised controlled trial, kidney transplantation, liver transplantation, hypothermic oxygenated machine perfusion, immunosuppression

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

Preventing Kidney Transplant Failure by Screening for Antibodies Against Human Leucocyte Antigens Followed by Optimised Immunosuppression: OuTSMART RCT.

by Stringer, D., et al. *Efficacy and Mechanism Evaluation* 2023; 10(5).

Aims

This study aimed to assess the cost-effectiveness of preventing kidney allograft failure by optimising immunosuppression in human leucocyte antigen Ab+ patients.

Interventions

Participants were randomised to receive either blinded standard care (SC) or unblinded biomarker-led care (BLC).

Participants

2037 kidney transplant recipients >1 year post-transplantation.

Outcomes

The primary outcome was time to graft failure following 43 months follow-up.

Follow-Up

Up to 64 months.

CET Conclusion

by John O'Callaghan

This is an extensive paper from 13 centres in the UK where anti-HLA antibodies were monitored after renal transplantation and immune suppression adapted in response. The study could not be



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*Correspondence

John M. O'Callaghan,

✉ ocallaghan.john@gmail.com

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blinded, clearly, for the purposes of monitoring and reviewing patients in the intervention arm. The study was large, including 2035 patients, also this number was required for the trial to be adequately powered to detect HR = 0.49. Patients were excluded if they received an HLA-incompatible transplant requiring desensitization. The prevalence and incidence rates of HLA Antibody positive patients were less than expected when the trial was planned. The primary outcome was therefore changed from transplant failure rate over 3 years to time to graft failure. The presence of donor-specific antibodies was associated with a higher risk of graft failure. However, the study found no evidence that biomarker-led care, with optimised immunosuppression in HLA antibody positive patients, delayed renal transplant failure. There was a significant reduction in rejection in the study group with biomarker-led care, but this did not carry through to improved graft survival. The development of non-donor specific antibodies was not associated with graft failure. The health economic analysis included in the paper demonstrates the biomarker-led care to be cost-ineffective.

Trial Registration

EudraCT—2012-004308-36; ISRCTN—46157828.

Funding Source

Non-industry funded.

RANDOMISED CONTROLLED TRIAL 2

Portable Hypothermic Oxygenated Machine Perfusion for Organ Preservation in Liver Transplantation (PILOTTM): A Randomized, Open-Label, Clinical Trial.

by Panayotova, G. G., et al. *Hepatology* 2023 [record in progress].

Aims

To assess if HMP-O2 improves liver transplant outcomes compare to cold storage.

Interventions

Livers were randomised to intervention, which was HMP-O2 on the Lifeport Liver Transporter device, perfused with Vasosol, or control, which was static cold storage.

Participants

179 adult whole liver transplant recipients.

Outcomes

The primary outcome was early allograft dysfunction (EAD) as defined by the Olthoff criteria. Secondary outcome measures were PNF, AKI, graft survival, biliary complications. Vascular complications and death. Additional exploratory outcomes were hospital LOS, ICU LOS, lactate clearance, bleeding, incisional hernia and SAEs.

Follow-Up

12 months.

CET Conclusion

by John Fallon

This large open labelled multi-centre randomised control trial is an exciting development in the field of liver HMP. The key strength of this work is that 43% ($n = 27$) of the HMP-O2 livers had continuous perfusion, having been placed on device at the donor. This is the first trial in liver HMP to do this and is an important development. Made possible by Organ Recovery Systems portable Lifeport Liver device, especially considering 81% travelled by air, a current limitation of the portable NMP devices. They demonstrated a nonsignificant reduction in EAD with 11% in HMP-O2 and 16% in SCS, while the finding is not significant it is in keeping with the 5 other published RCTs on HMP liver. The lack of significance may derive from the fact that within the intervention group only 24% were ECDs (including 5 DCD), upon sub-group analysis of these ECDs they find the reduction of EAD to be significant (20% in HMP-O2 and 33.3% in SCS $p = 0.004$). This is in keeping with previous large RCTs that the beneficial effects of HMP-O2 are amplified in the ECD cohort, especially in DCDs seen in Rijn et al's 2021 trial published in the *New England Journal* who perfused only DCD livers. None of their secondary outcomes reach significance, but with PNF only occurring in the SCS group with 3 patients and a further 2 ($n = 5$ 6.8%) went on to require re-transplant also due to ischaemic cholangiopathy. In HMP-O2 only 1 required retransplant, this was due to HAT. Biliary complications were nearly double in the SCS group (26.4% vs. 12.7%) which is impressive, but again this failed to reach significance. The trends are encouraging, but the lack of significance is disappointing, the trial having not been powered for overall EAD rates. An increase cohort size and a focus on EADs could have led to more dramatic results with potentially significance in many of the outcomes. An interesting note is the preservation fluid used in HMP-O2 was Vasosol, a UW-like solution with the addition of nitric oxide donors and vasodilators, this is the first HMP RCT across all organs to utilise this solution and could, in part be responsible for some of the beneficial trends. Unfortunately, the study was not sufficiently powered to compare continuous HMP-O2 with end-ischaemic HMP-O2 and SCS, the overall storage duration being comparable, but the percentage of that time being perfusion obviously being highest in the continuous group. They demonstrate safety and non-inferior efficacy of a novel portable device, which as it becomes more popular and people become more familiar with placing livers on device at retrieval more data should emerge on continuous HMP-O2, this trial was an important step.

Jadad Score

3.

Data Analysis

Per protocol analysis.

Allocation Concealment

Yes.

Trial Registration

Clinicaltrials.gov—NCT03484455.

Funding Source

Industry funded.

CLINICAL IMPACT SUMMARY

by John O'Callaghan

This is a very interesting randomised controlled trial in liver transplantation, and an important step in the clinical implementation of a new device (the Lifeport Liver Transporter from Organ Recovery Systems). Hypothermic machine perfusion (HMP) with oxygenation was compared to standard static cold storage prior to transplant. The study was set up as a non-inferiority trial, and hence was smaller than it may have been if designed to demonstrate superiority of one treatment. The non-inferiority design was done specifically to obtain 510 (k) device clearance in the United States. Randomisation was stratified for MELD score and DCD status to maintain a distribution between study arms. Primary outcome was Early Allograft Dysfunction (EAD).

Approximately 40% of grafts in the HMP arm were put on the pump immediately at retrieval, demonstrating the portability of the device and safety in travel. Statistical analysis of the primary outcome proved non-inferiority of oxygenated HMP, but did not demonstrate superiority

either. However, the rate of EAD in the control arm was far better than was expected; in the trial it was only 16%, when 30% had been used for the power calculation. When conducting a subgroup analysis of Extended Criteria Donor (ECD) livers, there was a significant benefit of oxygenated HMP, given the higher baseline risk of 33% EAD with static cold storage in this subgroup.

This trial report gives very reassuring information regarding the implementation of oxygenated HMP using this device, its ease of use, portability and safety. The benefit is seen in the ECD livers, and there is the possibility of benefits for standard criteria livers as well (for example PNF and biliary strictures) that may have been statistically significant and more clearly demonstrated in a larger trial.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

CONFLICT OF INTEREST

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

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Edited by Reshma Rana Magar.

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Survival Advantage Comparing Older Living Donor Versus Standard Criteria Donor Kidney Transplants

Kamlesh Patel¹, Anna Brotherton¹, Daoud Chaudhry², Felicity Evison³, Thomas Nieto¹, Dilan Dabare¹ and Adnan Sharif^{1,4*}

¹Department of Nephrology and Transplantation, University Hospitals Birmingham, Birmingham, United Kingdom, ²School of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom, ³Data Science Team, Research Development and Innovation, University Hospitals Birmingham, Birmingham, United Kingdom, ⁴Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, United Kingdom

The aim of this analysis was to explore mortality outcomes for kidney transplant candidates receiving older living donor kidneys (age ≥ 60 years) versus younger deceased donors or remaining on dialysis. From 2000 to 2019, all patients on dialysis listed for their first kidney-alone transplant were included in a retrospective cohort analysis of UK transplant registry data. The primary outcome was all-cause mortality, with survival analysis conducted by intention-to-treat principle. Time-to-death from listing was modelled using nonproportional hazard Cox regression models with transplantation handled as a time-dependent covariate. A total of 32,978 waitlisted kidney failure patients formed the primary study cohort, of whom 18,796 (58.5%) received a kidney transplant (1,557 older living donor kidneys and 18,062 standard criteria donor kidneys). Older living donor kidney transplantation constituted only 17.0% of all living donor kidney transplant activity (overall cohort; $n = 9,140$). Recipients of older living donor kidneys had reduced all-cause mortality compared to receiving SCD kidneys (HR 0.904, 95% CI 0.845–0.967, $p = 0.003$) and much lower all-cause mortality versus remaining on the waiting list (HR 0.160, 95% CI 0.149–0.172, $p < 0.001$). Older living kidney donors should be actively explored to expand the living donor kidney pool and are an excellent treatment option for waitlisted kidney transplant candidates.

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*Correspondence

Adnan Sharif,
✉ adnan.sharif@uhb.nhs.uk

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Keywords: kidney transplantation, mortality, survival, older living donor, standard criteria donor

INTRODUCTION

Living donor kidney transplantation is the optimal treatment of choice for kidney failure patients deemed suitable for surgery. In a systematic review and meta-analysis of 48 published cohort studies, any recipient of a living donor kidney had superior all-cause mortality compared to recipients of other kidney allografts or remaining waitlisted on dialysis [1]. This mirrors national registry data, with superior ten-year patient and graft survival reported after living donor kidney transplantation versus deceased donor kidney transplantation [2].

Despite these benefits, living donor kidney transplant rates have stagnated over the last decade in many kidney transplant programs. In the United Kingdom, living donor transplant rates have dropped by a quarter over the last decade, from a peak of 1,036 adult living donor kidney transplants in the year 2013/2014 to 789 in the last available year of 2021/2022 [3]. While some of this may relate to recovery processes post pandemic, it is notable that living donor kidney

Survival advantage comparing older living donor versus standard criteria donor kidney transplants.

PURPOSE

Recipients of living donor (LD) kidneys have better survival compared to recipients of other kidney allografts.

However, it is unclear if receiving an older LD kidneys (aged ≥ 60 years) still has better survival outcomes compared to a younger standard criteria donor (SCD) deceased kidney allograft.

We compared survival from waitlisting for kidney transplant candidates receiving older LD kidneys (aged ≥ 60 years) or SCD kidneys versus other kidney allografts and remaining wait-listed.

METHODS

Study design

Retrospective cohort study.

Study cohort

All patients listed for their first kidney-alone transplant between 2000-2019 in the United Kingdom ($n=47,917$)

Data

UK Transplant Registry from NHS Blood and Transplant.

Primary outcome

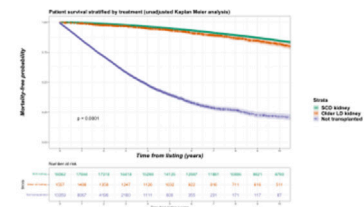
All-cause mortality from listing analyzed using time-dependent nonproportional Cox regression models.

RESULTS

Older living donor kidney transplantation constituted only 17.0% of all living donor kidney transplant activity (overall cohort; $n=9,140$).

Recipients of older LD kidneys have \downarrow mortality versus receiving SCD kidneys (see Figure of unadjusted Kaplan Meier plot).

After adjustment, recipients of older living donor kidneys had **reduced all-cause mortality compared to receiving SCD kidneys** (HR 0.904, 95% CI 0.845-0.967, $p=0.003$) and much lower all-cause mortality versus remaining on the waiting list (HR 0.160, 95% CI 0.149-0.172, $p<0.001$).



CONCLUSION

Receiving an older LD kidney has survival benefits versus receiving a SCD kidney.

Older living kidney donors should be actively explored to expand the living donor kidney pool and are an excellent treatment option for waitlisted kidney transplant candidates.



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GRAPHICAL ABSTRACT

transplant rates pre-pandemic in 2019/2020 were only 954. Therefore, a key component of the latest NHS Blood and Transplant (NHSBT) strategy document encourages expansion of living donor kidney transplantation activity [4]. To that effect, promoting living kidney donation among older individuals is very attractive. Bailey et al. report the number of living kidney donors aged ≥ 65 years has risen from 4% to 10% between 2006 and 2017 respectively [5]. However, numbers appear to have plateaued since then. According to national registry data, while 18% of all living donor kidney donors were aged ≥ 60 years between 2010 and 2016 [6], this has remained static at 20% between 2016 and 2022 [3].

The literature regarding survival outcomes for kidney transplant candidates receiving older living donor kidneys is not clear. In a systematic review of published studies, living donor age stratified at 60 years was associated with 1-year graft loss for recipients but no significant findings were observed for either 1- or 3-year recipient mortality or graft loss at a lower donor age stratification of 50 years [7]. However, the meta-analysis for mortality was conducted on three small studies for publications between 1989 and 2010, which severely limits its utility and interpretation. Other work has associated older living donor age as a risk factor for graft loss and/or mortality when compared to a younger living donor [8, 9]. However, this is not a useful comparison as many kidney transplant candidates will not have a choice between an older or younger living donor. More relevant is whether survival outcomes differ when comparing

receipt of an older living donor kidney versus receiving a standard criteria donor (SCD) kidney. This is an important question which kidney transplant candidates may be faced in the real-world and there is a paucity of contemporary literature to guide counselling on this matter. Therefore, the aim of this analysis was to explore this question using UK transplant registry data, with older living donors defined as any donor aged 60 years and above.

MATERIALS AND METHODS

Study Cohort

A retrospective cohort study was undertaken of prospectively collected registry data related to all waitlisted kidney failure patients receiving dialysis in the United Kingdom. From January 1, 2000 until September 30, 2019 inclusive, all patients who were either listed and received a first kidney-alone transplant in the United Kingdom versus those who were listed but never received a kidney transplant were included in the study. No formal sample size estimate was conducted as all eligible patient records were used. December 31, 2020 was considered the study end. The study is reported as per STROBE guidance [10].

Study Variables

The following study variables were available for all patients; age (at listing and at transplantation), sex, ethnicity (classified as white, black, Asian [Indo-Asian], other, known), primary cause of

kidney failure (classified as diabetes, glomerulonephritis, hypertension, other separate, polycystic kidney disease, pyelonephritis/reflux nephropathy, unknown/missing), year of listing, and waiting time.

Donor kidneys were stratified into living donors (with older living donors defined at an age ≥ 60 years) or SCD. Donors after brain and circulatory death (DBD and DCD respectively) were handled the same way. The primary cohort was obtained by excluding any expanded criteria donor (ECD) kidney recipients from the deceased donor cohort if they fulfilled the following criteria: 1) deceased donor aged ≥ 60 years, or 2) deceased donor aged between 50 and 59 years with any two from the following three additional criteria; hypertension; raised creatinine and/or death from stroke). However, secondary analyses were conducted with the inclusion of ECD kidney transplant recipients. The remaining waitlisted kidney transplant candidates did not proceed for transplantation and remained on dialysis.

Outcomes

The primary outcome of interest was all-cause mortality. The survival analysis was conducted according to the intention-to-treat principle; therefore, patients were not dropped from the analysis if they were removed from the waiting list or if transplantation subsequently failed. Secondary outcomes explored include death-censored graft loss.

Statistical Analysis

For baseline demographics, continuous variables were reported as medians and interquartile ranges (IQRs) and compared between groups using Mann-Whitney tests. Ordinal factors were also compared using Mann-Whitney tests, whilst nominal factors were analysed using Fisher's exact tests or Chi-square tests for those with two or more than two categories, respectively. Missing data underwent list-wise deletion and complete case analysis was undertaken.

Survival was analysed as time from initial placement on the waiting list to death, with data censored at loss of follow up or on December 31, 2020. Unadjusted survival-free probability was analysed by generation of Kaplan-Meier curves. After testing for violations of the proportional hazard assumption, time-to-death was modelled using nonproportional hazard Cox regression models with transplantation handled as a time-dependent covariate. Using this approach, all patients contribute data for time at risk (and death if it occurs) to the non-transplant group starting at study entry before some switch and contribute time at risk (and death if it occurs) to the transplant group starting at the time of transplantation (this forms the time-dependent transplant covariate in the model). Mortality hazard ratios were computed for the transplant recipients compared with those on the waiting list. We explored adjusted models factoring for age, sex, ethnicity, cause of kidney failure and year of placement on the waiting list. Time to graft loss models were conducted using survival/censoring-weighted Cox regression models and adjusted for age at listing, sex, ethnicity, cause of kidney failure, year of placement of the waiting list, level of HLA mismatches, delayed graft function and 1-year rejection.

Due to heterogeneous statistical methods used for reported transplant studies, as reported in **Supplementary Table D** from

the systematic review by Chaudhry et al. [1], complementary survival analyses were undertaken to investigate the robustness of our primary model. These included; 1) survival/censoring-weighted Cox regression, which is a parsimonious alternative to a standard Cox regression model and provides interpretable average effects in the either the presence or absence of non-proportional hazards [11], 2) re-analysis to overcome immortality bias by comparing time from transplant versus time from waitlisting for transplant versus non-transplant cohorts respectively, 3) weighted Cox regression of a propensity score matched cohort after nearest neighbour 1:1 matching (for age at listing, sex, ethnicity, cause of kidney failure and waiting time), and 4) extended nonproportional hazard Cox regression model with transplantation and graft loss handled as a time-dependent variables. Furthermore, subgroup analyses with different older living donor age stratifications were undertaken versus both SCD and ECD kidneys.

All analyses were done using R 4.0.4 (R Foundation for Statistical Computing, Vienna, Austria), with packages including *coxphw* (survival analyses) [11] and *MatchIt* (propensity-score matching).

Approvals

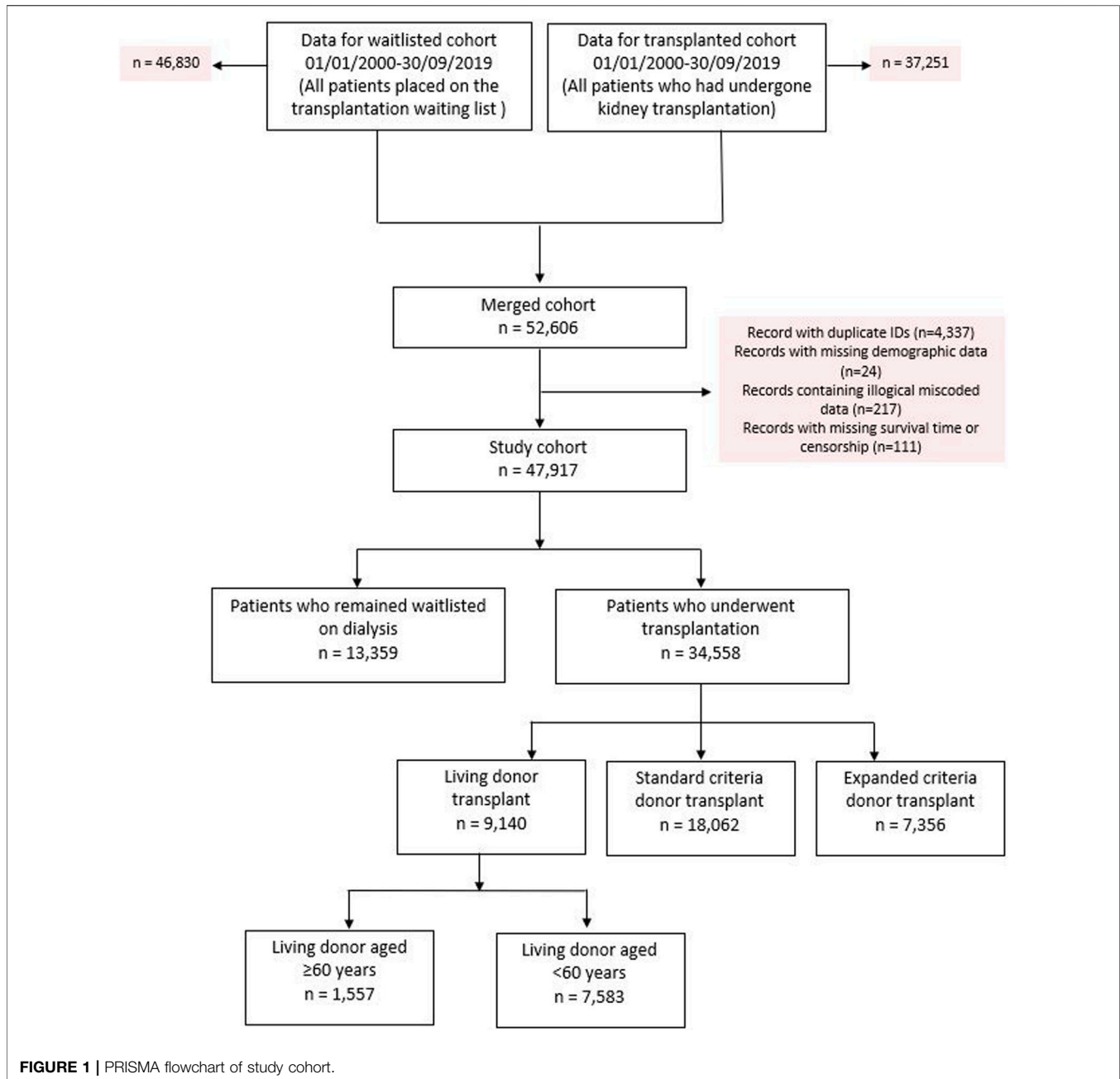
National Health Service Blood and Transplant (NHSBT) in the United Kingdom obtains informed consent from all patients undergoing solid organ transplantation for data collection and subsequent analyses. Study proposals are reviewed and approved by the kidney advisory group on behalf of NHSBT before data dissemination.

RESULTS

Study Cohort

The original cohort obtained from NHSBT contained records from two datasets between January 1, 2000 until September 30, 2019; kidney failure patients listed who received a kidney transplant ($n = 37,251$) and kidney failure patients listed for transplantation ($n = 46,830$). After combining both datasets, duplicated records and/or cases with missing demographic data were excluded. This left 47,917 kidney failure patients to form our total study cohort, of whom 34,558 (72.1%) subsequently received their first kidney transplant after waitlisting (living donors; $n = 9,140$, SCD; $n = 18,062$ and ECD; $n = 7,356$). For the primary analysis, we excluded recipients of ECD and living donor kidneys aged < 60 years ($n = 7,583$), which left a primary study cohort of 32,978. Observation time for the study cohort involved a total of 222,896 patient-years, with median follow up 5.8 years. See **Figure 1** for the PRISMA flowchart.

Table 1 shows baseline demographics at the time of listing for the study cohort and identifies significant differences in baseline demographics between those that received different types of kidney allografts versus those that remained without transplantation. **Table 2** compares waitlisted kidney transplant candidates who received older versus younger living donors, showing very different demographics between the recipients of both kidneys. **Supplementary Figure S1** shows the evolution of age demographics among living kidney donors over the study



cohort period, highlighting the increase in proportion of living donor donors aged ≥ 60 years from the beginning of the study period but static percentages in recent years.

Mortality Events

In the primary study cohort, waitlisted kidney failure patients who did not receive kidney transplants had 4,003 deaths (30.0% of dialysis cohort) versus 3,

701 deaths in the SCD group (20.5% of cohort) versus 257 deaths in the older living donor group (16.5% of older living donor cohort).

For the living donor transplant group, 257 deaths in the older living donor cohort compares with 870 deaths (11.5% of total deaths)

of the younger living donor cohort. Unadjusted Kaplan-Meier plot for mortality stratified by older living donor kidneys versus alternative treatments from listing is shown in **Figure 2**, while in **Figure 3** an unadjusted mortality comparison is made between older versus younger living donor kidney transplants from surgery.

Unadjusted and Adjusted Graft Survival (Death-Censored) Using Weighted Cox Regression

Death-censored graft losses over the follow up period were compared between older living kidney, younger living kidney

TABLE 1 | Baseline demographics of waitlisted kidney failure patients.

Variable		LD kidney	SCD kidney	ECD kidney	Dialysis	p-Value
Percentage (n)		19.1% (9,140)	37.7% (18,062)	15.4% (7,356)	27.9% (13,359)	—
Median Age at waitlisting in years (IQR)		43 (23)	45 (19)	57 (15)	53 (21)	<0.001
Sex	Male	61.4% (5,611)	62.7% (11,326)	64.2% (4,719)	61.0% (8,143)	<0.001
	Female	38.6% (3,529)	37.3% (6,736)	35.8% (2,637)	39.0% (5,216)	
Ethnicity	White	82.6% (7,550)	75.3% (13,593)	75.2% (5,532)	71.6% (9,564)	<0.001
	Asian	8.8% (808)	13.4% (2,418)	13.5% (990)	15.5% (2,072)	
	Black	4.8% (436)	7.7% (1,383)	7.5% (554)	9.0% (1,198)	
	Other	2.8% (252)	2.7% (496)	3.0% (219)	3.1% (416)	
	Unknown	1.0% (94)	1.0% (172)	0.8% (61)	0.8% (109)	
Cause of kidney failure	Diabetes	7.2% (659)	7.5% (1,351)	12.3% (903)	27.6% (3,681)	<0.001
	Glomerulonephritis	6.6% (602)	6.8% (1,231)	6.3% (462)	3.8% (511)	
	Hypertension	4.7% (431)	5.3% (950)	6.7% (491)	4.7% (633)	
	Other Separate	31.8% (2,905)	27.2% (4,911)	24.7% (1,815)	20.9% (2,787)	
	Polycystic Kidney	8.9% (810)	11.5% (2,072)	12.4% (909)	6.3% (845)	
	Pyelonephritis/reflux	6.9% (629)	7.8% (1,411)	5.9% (431)	4.4% (592)	
	Unknown/Missing	34.0% (3,104)	34.0% (6,136)	31.9% (2,345)	32.3% (4,310)	
Waiting time in days (IQR)		230 (576)	791 (1,016)	896 (988)	475 (614)	<0.001

LD, living donor; SCD, standard criteria donor; ECD, expanded criteria donor; IQR, interquartile range.

TABLE 2 | Characteristics of recipient receiving living donor kidneys.

Recipient variables		All LD kidney	Old LD (aged ≥60 years)	Young (aged <60 years)	p-Value
Percentage (n)		100.0% (9,140)	17.0% (1,557)	83.0% (7,580)	—
Median Age at waitlisting in years (IQR)		43 (23)	51 (25)	42 (22)	<0.001
Median Age at transplantation in years (IQR)		44 (23)	53 (26)	45 (22)	<0.001
Sex	Male	61.4% (5,611)	60.4% (940)	61.6% (4,669)	0.366
	Female	38.6% (3,529)	39.6% (617)	38.4% (2,911)	
Ethnicity	White	82.6% (7,550)	87.5% (1,363)	81.6% (6,184)	<0.001
	Asian	8.8% (808)	6.6% (102)	9.3% (706)	
	Black	4.8% (436)	2.6% (40)	5.2% (396)	
	Other	2.8% (252)	2.3% (36)	2.8% (216)	
	Unknown	1.0% (94)	1.0% (16)	1.0% (78)	
Cause of kidney failure	Diabetes	7.2% (659)	8.5% (133)	6.9% (526)	<0.001
	Glomerulonephritis	6.6% (602)	6.4% (99)	6.6% (503)	
	Hypertension	4.7% (431)	5.3% (83)	4.6% (348)	
	Other Separate	31.8% (2,905)	32.8% (510)	31.6% (2,395)	
	Polycystic Kidney	8.9% (810)	11.0% (171)	8.4% (638)	
	Pyelonephritis/reflux	6.9% (629)	6.2% (96)	7.0% (533)	
	Unknown/Missing	34.0% (3,104)	29.9% (465)	34.8% (2,637)	
Waiting time in days (IQR)		230 (576)	275 (632)	223 (558)	<0.001
Time period	2010 onwards	4,330	52.8% (822)	46.3% (3,507)	<0.001
	Pre 2010	4,808	47.2% (735)	53.7% (4,073)	

LD, living donor; IQR, interquartile range.

and SCD kidney transplant recipients. Overall, 3,658 graft losses occurred in the SCD cohort (20.4% of SCD group) versus 249 graft losses in the older living donor cohort (16.0% of older living donor group). In younger living donor kidney recipients, a total of 1,189 graft losses occurred (15.7% of younger living donor group). Unadjusted Kaplan-Meier plots for death-censored graft loss stratified by older living donor, younger living donor and SCD kidneys is shown in **Figure 4**.

In adjusted models, compared to receiving a SCD kidney, receiving an older living donor kidney was associated reduced risk for graft loss (HR 0.872, 95% CI

0.761–1.000, $p = 0.050$) independent of other variables. No significant difference in risk for graft loss was observed comparing older to younger living donor kidneys (HR 1.273, 95% CI 0.956–1.695, $p = 0.098$).

Adjusted Mortality Analyses Nonproportional Hazards Cox Regression Model With Transplantation a Time-dependent Covariate

In a non-proportional hazard Cox regression model using a time-dependent analysis, with transplantation handled as a time-dependent covariate, recipients of older living donor

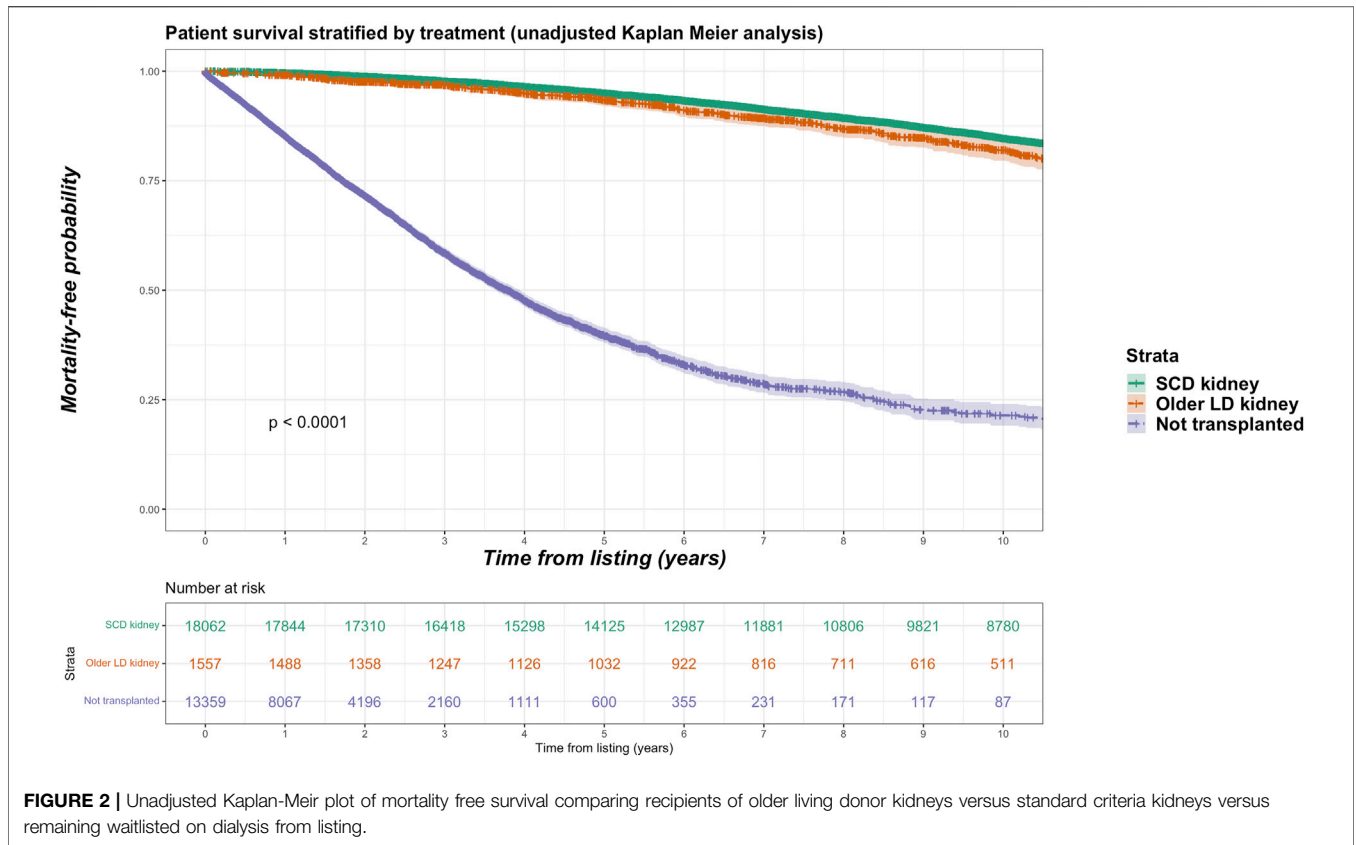


FIGURE 2 | Unadjusted Kaplan-Meier plot of mortality free survival comparing recipients of older living donor kidneys versus standard criteria kidneys versus remaining waitlisted on dialysis from listing.

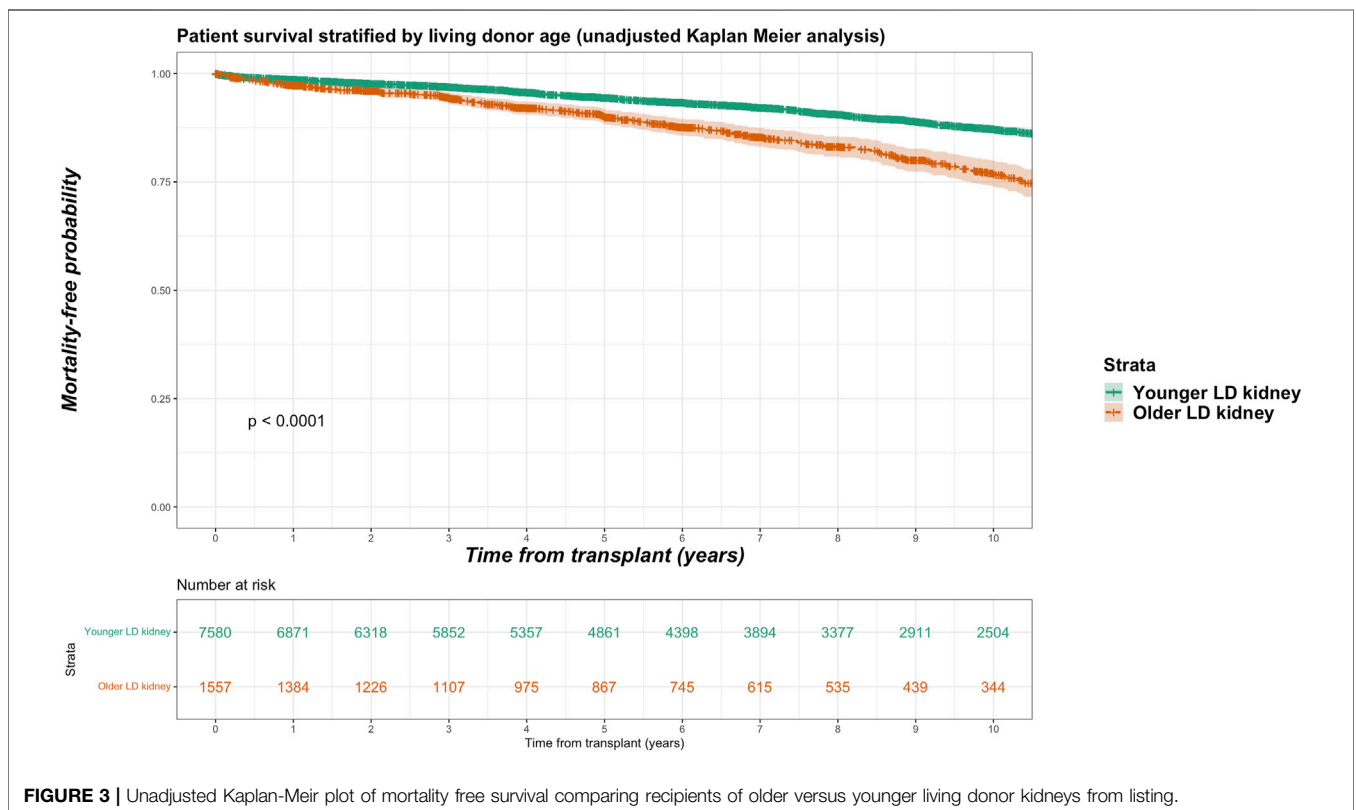


FIGURE 3 | Unadjusted Kaplan-Meier plot of mortality free survival comparing recipients of older versus younger living donor kidneys from listing.

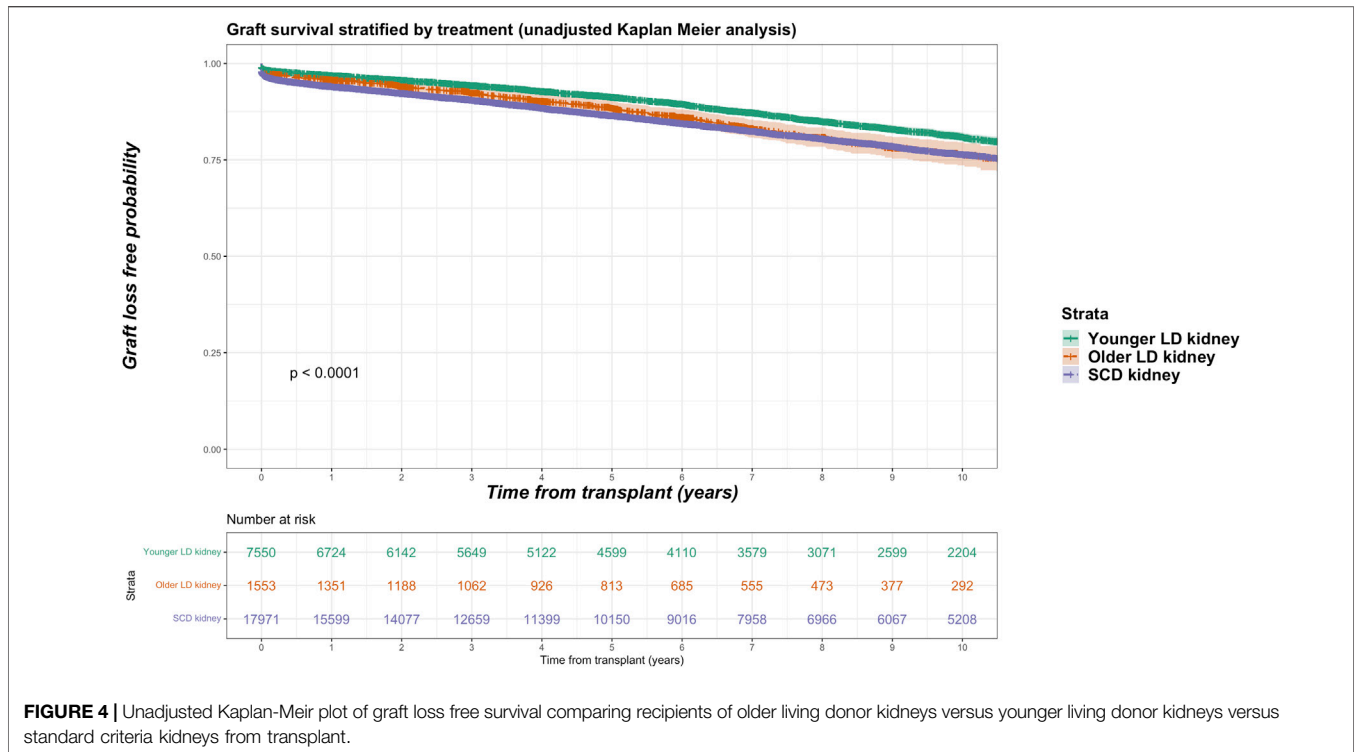


FIGURE 4 | Unadjusted Kaplan-Meier plot of graft loss free survival comparing recipients of older living donor kidneys versus younger living donor kidneys versus standard criteria kidneys from transplant.

TABLE 3 | Non-proportional hazard Cox model of predictors for mortality after kidney transplantation with either dialysis or SCD as reference [fully adjusted model with transplantation (Model 1) or transplantation + graft loss (Model 2) handled as a time varying covariate].

Variable		HR (95% CI)	Variable		HR (95% CI)
Treatment (Model 1)	Dialysis	1.000	Treatment	SCD	1.000
	SCD	0.177 (0.171–0.184)		Dialysis	5.641 (5.445–5.844)
	LD	0.160 (0.149–0.172)		LD	0.904 (0.845–0.967)
Treatment (Model 2)	Dialysis	1.000	Treatment	SCD	1.000
	SCD	0.166 (0.161–0.172)		Dialysis	6.021 (5.827–6.221)
	LD	0.149 (0.141–0.158)		LD	0.897 (0.851–0.946)

LD, living donor; SCD, standard criteria donor; HR, hazard ratio; CI, confidence interval.

kidneys had reduced all-cause mortality compared to receiving SCD kidneys (HR 0.904, 95% CI 0.845–0.967, $p = 0.003$) and much lower all-cause mortality versus remaining on the waiting list (HR 0.160, 95% CI 0.149–0.172, $p < 0.001$) independent of other variables. We conducted a non-proportional Cox regression analysis with both transplantation and graft loss factored as time-dependent covariates. In this extended model, receiving older living kidneys still had reduced risk for all-cause mortality versus receiving SCD kidneys (HR 0.897, 95% CI 0.851–0.946, $p < 0.001$) or remaining on dialysis (HR 0.149, 95% CI 0.141–0.158, $p < 0.001$) independent of other variables. This is shown in Table 3.

Alternate Survival Models

In a survival/censoring-weighted Cox regression model, compared to SCD kidney recipients, older living donor kidney

recipients had equivalent all-cause mortality after waitlisting (Hazard Ratio 0.902, 95% CI 0.774–1.051, $p = 0.187$) but lower all-cause mortality compared to dialysis (HR 0.100, 95% CI 0.085–0.118, $p < 0.001$). In a model that overcomes immortal time bias for pre-transplant survival on the waiting list, recipients of older living donor kidneys had lower all-cause mortality compared to SCD kidneys (HR 0.804, 95% CI 0.701–0.923, $p = 0.002$) versus remaining on the waiting list (HR 0.163, 95% CI 0.141–0.189, $p < 0.001$). In a propensity score matched cohort comparing older living donor kidney recipients with SCD (balance plot shown in **Supplementary Material S2**), older living donor kidney recipients had reduced all-cause mortality from listing (HR 0.690, 95% CI 0.547–0.872) or from transplant (HR 0.733, 95% CI 0.597–0.899, $p = 0.003$). **Figure 5** summarizes the comparative Hazard ratios from the different models.

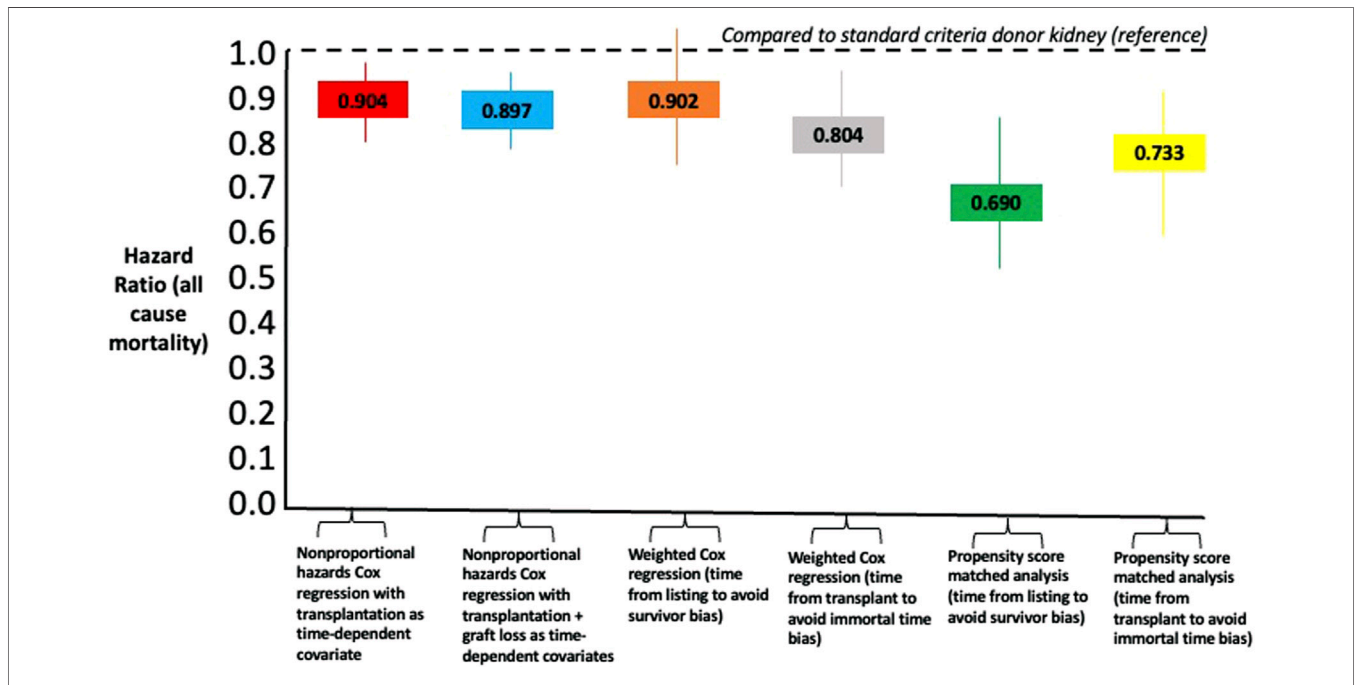


FIGURE 5 | Comparison of Hazard Ratios for all-cause mortality using different statistical models comparing recipients of older living donor kidneys versus standard criteria kidneys as reference point.

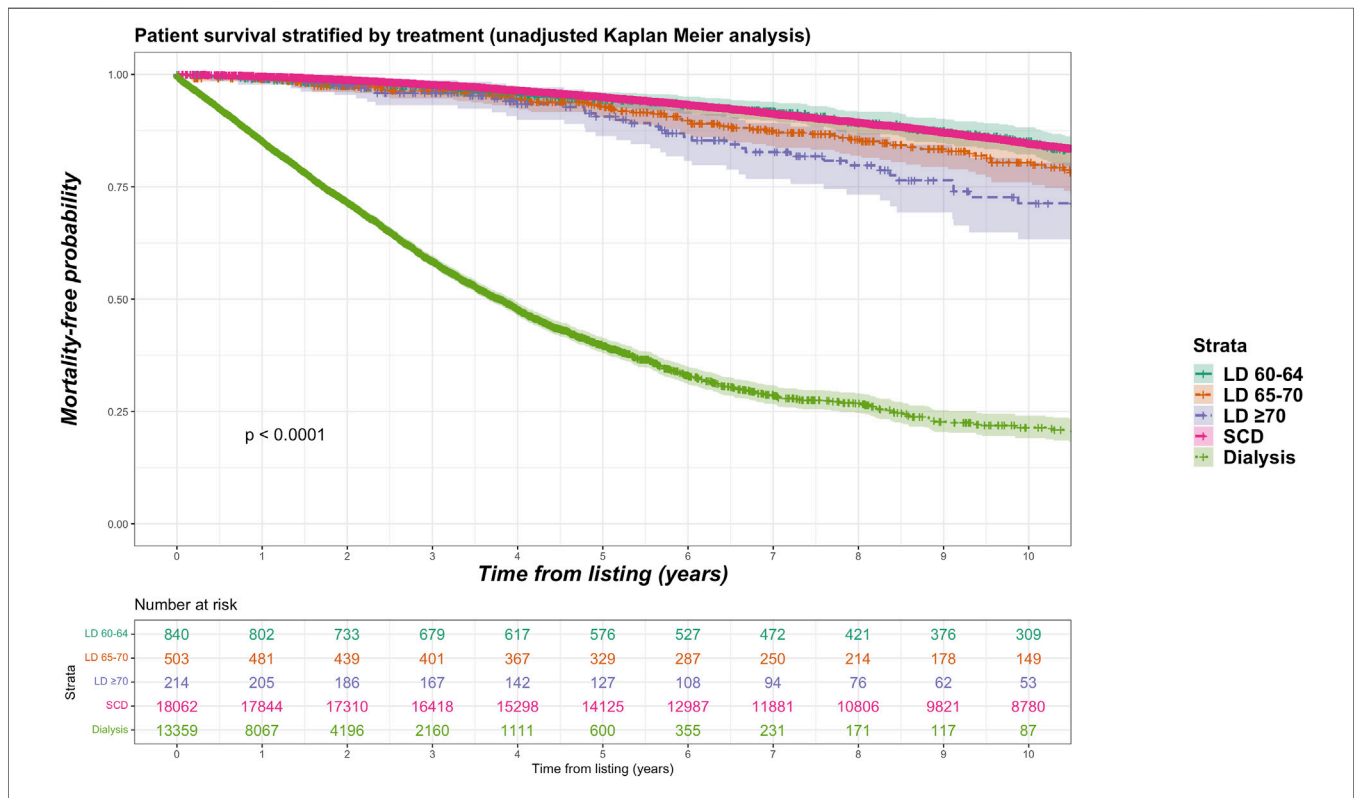


FIGURE 6 | Unadjusted Kaplan-Meier plot of mortality free survival comparing recipients of older living donor kidneys stratified by age groups (60–64 years, 65–69 years, ≥70 years) versus standard criteria donor kidneys versus remaining waitlisted on dialysis from listing.

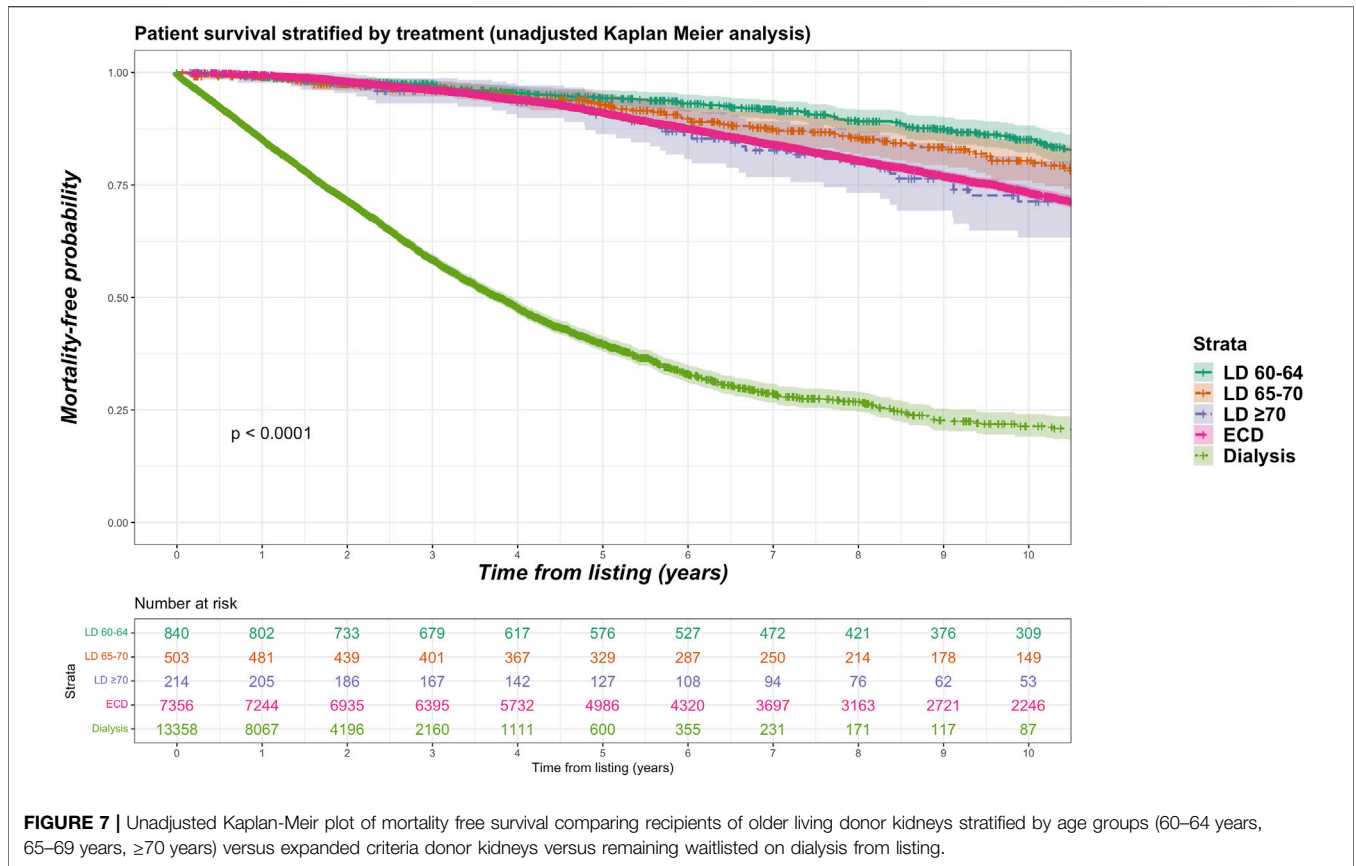


FIGURE 7 | Unadjusted Kaplan-Meier plot of mortality free survival comparing recipients of older living donor kidneys stratified by age groups (60–64 years, 65–69 years, ≥70 years) versus expanded criteria donor kidneys versus remaining waitlisted on dialysis from listing.

TABLE 4 | Non-proportional hazard Cox model of predictors for mortality after kidney transplantation with SCD or ECD as reference (fully adjusted model with transplantation handled as a time varying covariate).

Variable		Hazard ratio (95% CI)	
		SCD as reference	ECD as reference
Treatment	LD aged 60–64	0.857 (0.782–0.940)	0.697 (0.634–0.765)
	LD aged 65–69	0.857 (0.762–0.963)	0.724 (0.644–0.815)
	LD aged ≥70	1.232 (1.052–1.443)	1.066 (0.910–1.249)
	Dialysis	5.646 (5.450–5.850)	4.811 (4.628–5.001)
Median Age at waitlisting in years (IQR)		1.044 (1.043–1.045)	1.033 (1.031–1.034)
Sex	Female	REF	REF
	Male	1.111 (1.081–1.143)	1.230 (1.187–1.275)
Ethnicity	White	REF	REF
	Asian	0.797 (0.764–0.833)	0.758 (0.720–0.799)
	Black	0.754 (0.711–0.800)	0.698 (0.639–0.739)
	Other	0.637 (0.572–0.710)	0.676 (0.600–0.762)
	Unknown	0.992 (0.873–1.127)	1.128 (0.916–1.388)
Cause of kidney failure	Diabetes	REF	REF
	Glomerulonephritis	0.426 (0.400–0.454)	0.434 (0.400–0.470)
	Hypertension	0.504 (0.471–0.539)	0.461 (0.424–0.501)
	Other Separate	0.446 (0.426–0.467)	0.456 (0.431–0.481)
	Polycystic Kidney	0.376 (0.355–0.397)	0.405 (0.378–0.433)
	Pyelonephritis/reflux	0.508 (0.478–0.540)	0.514 (0.475–0.555)
Year of listing	Unknown/Missing	0.479 (0.460–0.500)	0.487 (0.464–0.512)
		0.926 (0.923–0.929)	0.940 (0.937–0.943)

LD, living donor; SCD, standard criteria donor; ECD, expanded criteria kidney; CI, confidence interval.

Sub-analyses (Older Living Donor Age Stratified by Age Groups)

Sub-group analyses were undertaken with different stratifications for older living donor age. We identified 840 living donors aged between 60 and 64 years (9.2% of total living donor cohort, median 61 years), 503 living donors aged between 65 and 70 (5.5% of total living donor cohort, median 66 years) and 214 donors aged 70 years and over (2.3% of total living donor cohort, median 72 years). Mortality rate was 15.2%, 16.7% and 21.0% for recipients of kidneys from living donor age groups 60–64, 65–69 and ≥ 70 years respectively. **Figure 6** shows unadjusted Kaplan-Meier plots of all-cause mortality for recipients of the different older living donor age groups versus SCD kidneys versus remaining on dialysis. **Figure 7** shows unadjusted Kaplan-Meier plots of all-cause mortality for recipients of the different older living donor age groups versus ECD kidneys versus remaining on dialysis.

Table 4 summarizes the output from a non-proportional time-dependent hazard Cox regression model, with transplantation handled as a time-dependent covariate, comparing all-cause mortality for recipients of older living kidney stratified by age groups. The comparator is versus SCD or ECD kidney transplant recipients, with remaining on dialysis also included. In comparison to receiving a SCD kidney, recipients of older living donor kidneys from anyone aged 60–64 years or 65–69 years had lower all-cause mortality while higher all-cause mortality was observed for recipients of living donor kidneys aged ≥ 70 years. In comparison to receiving a ECD kidney, recipients of older living donor kidneys from anyone aged 60–64 years or 65–69 years had lower all-cause mortality but equivalent all-cause mortality was observed for recipients of living donor kidneys aged ≥ 70 years.

DISCUSSION

The literature reports heterogeneous outcomes for recipients of older living donor kidneys, dependent upon whether comparisons are made with different types of deceased donor allografts or younger living donors. From a practical perspective, the key question is whether waitlisted kidney transplant candidates likely to receive SCD kidneys have any survival advantage or disadvantage to proceed with an older living kidney donor versus a SCD kidney. In our contemporary population cohort study, our findings suggest receiving an older living donor kidney (aged ≥ 60 years) is associated with lower mortality and risk of graft loss versus receiving a SCD kidney. On sensitivity analyses with older living donor age stratified, all older living donor age groups provide a mortality benefit except receiving a kidney from a living donor aged ≥ 70 years, which was associated with higher mortality compared to receiving a SCD kidney (but equivalent mortality when compared to receiving an ECD kidney).

Disparate outcomes from previous studies reflect era effects, variable definitions, diverse study populations, methodological differences, and different study comparators (e.g., recipients of

younger living donor or SCD kidneys). Favorable outcomes are reported in a 1990–2010 cohort from the United States, where 219/97,782 (0.2%) of all living kidney donors were identified as aged older than ≥ 70 years [9]. No statistically significant difference in recipient survival was seen between those who received kidneys from living kidney donors aged ≥ 70 years versus matched recipients of kidneys from younger living kidney donors aged 50–59 years (HR 1.31, 95% CI 0.95–1.69). When compared to matched recipients of SCD kidneys from deceased donors aged 50–59 years, no statistically significant difference in patient survival was seen (HR 0.79, 95% CI 0.60–1.03) [9]. Although not statistically significant, the effect sizes are clinically significant and likely to reflect type 2 statistical errors in view of low sample size. A subsequent registry analysis using data from the United Network for Organ Sharing (UNOS) dataset between 1994 and 2012 was undertaken by Englem et al., with 4.4% of the living donor cohort (4,186/92,646) aged ≥ 60 years (3.2% aged 60–64 years; 1.0% aged 65–69 years; 0.2% aged ≥ 70 years) [12]. Compared to SCD recipients, no difference in overall graft survival was observed between living donors aged 65 years or older but risk for death-censored graft loss was higher. Transplant recipients with older living donor kidneys had significantly lower graft and overall survival compared to younger living donor recipients.

Examining a contemporary cohort is important, as era effects may be present. Iordanous et al. identified inferior patient and graft survival for recipients of older (aged 60–85 years) versus younger (aged 30–55 years) living donor kidneys in a systematic review and meta-analysis of study cohorts published between 1980 and 2008, although survival differences dissipated in the 2000s [13]. In subsequent work by the same group using data from Ontario, Canada between 2000 and 2008, no significantly increased risk for death (HR 1.83, 95% CI 0.96–3.48, $p = 0.07$) or graft-censored graft loss (HR 0.71, 95% CI 0.32–1.56, $p = 0.39$) was observed with median follow up 4 years for older living kidney donors (aged ≥ 60 years) versus SCD kidney recipients [14]. However, the hazard ratio was not proportional and increased with time, meaning uncertainty for longer outcomes. This is consistent with data from the Scientific Registry of Transplant Recipients (SRTR), which demonstrate 10-year adjusted hazard ratios for death or graft loss among recipients increase in a non-linear fashion with increasing living donor age and is highest among the ≥ 60 years group (compared to the reference of living donors aged 18–30 years) [15].

When compared to published data, our results provide reassurance that older living kidney donors provide a survival advantage for kidney transplant candidates versus receiving a SCD (but survival disadvantage if the living donor is aged ≥ 70 years). For candidates more likely to receive ECD kidneys, there is survival advantage using an older living kidney donor (and survival equivalence if the living donor is aged ≥ 70 years). Utilization of living donors aged ≥ 70 years, while a small proportion of the overall living donor cohort, requires careful matching of donors and recipients to facilitate optimized outcomes. One suggestion is to avoid extreme age differences when considering living donors aged ≥ 70 years. In a small single-center study, Hiramitsu et al.

observed living donor kidney transplantation from donors aged 70–89 years to recipients with a donor-recipient age difference of 10–15 years was an independent risk factor for graft loss and recipient mortality [8]. This complements our analyses and suggest living kidney donors aged ≥ 70 years are an appropriate choice for kidney transplant candidates likely to receive ECD kidneys but not SCD kidneys (or any candidate if compared to dialysis).

This is an important and topical question, especially as countries strive to expand living donor numbers. In the United States, data from the SRTR show living kidney donors aged ≥ 55 years have been the fastest growing cohort among all living kidney donor activity and are now the second commonest age group between 40 and 54 years (which has been slowly declining) [16]. If living donor activity can successfully increase, especially among older adults as potential donors, then our data can influence decision making for optimized patient counselling. Parallel to discussions about recipient survival outcomes are the safety outcomes associated with using older living kidney donors. Although low among living kidney donors, cumulative 15-year incidence of end-stage kidney disease per 10,000 varies significantly by age and is highest for donors aged ≥ 60 years 70.2 (95% CI 30.4–161.8, $p < 0.001$) [17]. Some of this risk may be due to an age-related sluggish physiological response by the contralateral kidney after donor nephrectomy. In a retrospective single-center analysis, Bellini et al. observed slower recovery of kidney function for living donors aged ≥ 60 years and higher percentual difference in estimated glomerular filtration rate (eGFR) post-donation [18]. This was consistent with findings from a systematic review and meta-analysis of 31 published studies [19]. While low eGFR is an independent risk factor for cardiovascular disease and all-cause mortality, any increased risk for these outcomes has reassuringly not been observed among older living donors. Analyzing UNOS data, Segev et al. observed no increase in mortality for living kidney donors versus age-matched “healthy” nondonors when stratified by age [3,017/80,347 (3.8%) living donors were aged ≥ 60 years] [20]. Reese et al., specifically matched older living kidney donors (mean age 59 years) from the UNOS dataset to healthy older individuals in the Health and Retirement Study, finding no difference in risk for cardiovascular disease or death. In summary, older age *per se* should not be considered a contra-indication to being a living kidney donor [21]. However, rigorous selection criteria is warranted and careful donor-recipient matching necessary for optimized outcomes.

Our study has many strengths in comparison to the available published literature. Firstly, our cohort of 2000–2019 is more contemporary than previous studies, reflecting current clinical practice and selection criteria. Many allocation systems aim to match like-for-like for donors and recipients like the United Kingdom, which should make these results translatable to other countries with similar allocation policies. Secondly, we have utilized different statistical approaches to test for robustness. It is reassuring to observe the take-home messages from our analyses are generally consistent across all statistical models used and reinforces our primary study findings. Limitations of this

study must also be appreciated for accurate interpretation of the results. As an intention-to-treat analysis, we did not factor for waitlisted kidney failure patients who were suspended or removed from the waiting list due to lack of fitness. This could lead to informative-treatment bias, i.e., where the pool of transplants recipients is systematically different from the remaining-on-dialysis comparator group. Censoring patients at delisting would have yielded an overestimation of survival on dialysis as data from the United Kingdom confirms increased mortality associated for waitlisted kidney failure patients who experience any period of suspension [22]. This analysis comprised waitlisted kidney transplant candidates who either had their primary transplant or remained on dialysis; therefore it provides no targeted evidence in the setting of advanced chronic kidney disease or a failed kidney transplant exploring repeat transplantation. Lack of data relating to medical comorbidities or dialysis vintage limits interpretation of survival probabilities in the setting of specific health burdens, which may tip the balance of more borderline risk versus benefit calculations for recipients of older living kidney donors. Residual confounding is an important but inevitable limitation of retrospective registry analyses despite adjusted statistical analyses. This is certainly the case in this analysis due to unavailable data and unmeasured confounders. Finally, this analysis has focused solely upon survival benefits associated with transplant surgery for kidney failure patients and overlooks the importance of quality of life which was beyond the scope of this study but is under investigation elsewhere [23].

To conclude, in this contemporary national cohort study of kidney failure patients listed for transplantation, proceeding with an older living donor kidney transplant affords a survival benefit to kidney transplant candidates when compared to receiving a standard criteria donor kidney or remaining on dialysis. While our data is reassuring, the caveat remains that survival benefits at a population-level must be translated to individual kidney transplant candidates with personalized risk counselling (e.g., using living donors aged ≥ 70 years). However, our data provides reassurance to clinicians involved in the care of kidney failure patients that older living donor candidates are an untapped pool of potential kidney donors that should be actively pursued.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: UK transplant registry.

ETHICS STATEMENT

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

KP, AB, and AS wrote the manuscript. DC and FE performed analyses. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2024.12559/full#supplementary-material>

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Updates in Skin Cancer in Transplant Recipients and Immunosuppressed Patients: Review of the 2022–2023 Scientific Symposium of the International Immunosuppression and Transplant Skin Cancer Collaborative

Catherine Pisano^{1*}, Justin J. Leitenberger², Melissa Pugliano-Mauro³ and Bryan T. Carroll⁴

¹Department of Dermatology, Brigham and Women's Hospital, Boston, MA, United States, ²Department of Dermatology, Oregon Health & Science University, Portland, OR, United States, ³Department of Dermatology, University of Pittsburgh Medical Center, Pittsburgh, PA, United States, ⁴Department of Dermatology, Case Western Reserve University School of Medicine, Cleveland, OH, United States

OPEN ACCESS

*Correspondence

Catherine Pisano,
✉ cpisano1@bwh.harvard.edu

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The International Immunosuppression and Transplant Skin Cancer Collaborative (ITSCC) and its European counterpart, Skin Care in Organ Transplant Patients-Europe (SCOPE) are comprised of physicians, surgeons, and scientist who perform integrative collaborative research focused on cutaneous malignancies that arise in solid organ transplant recipients (SOTR) and patients with other forms of long-term immunosuppression. In October 2022, ITSCC held its biennial 4-day scientific symposium in Essex, Massachusetts. This meeting was attended by members of both ITSCC and SCOPE and consisted of specialists including Mohs micrographic and dermatologic oncology surgeons, medical dermatologists, transplant dermatologists, transplant surgeons, and transplant physicians. During this symposium scientific workshop groups focusing on consensus standards for case reporting of retrospective series for invasive squamous cell carcinoma (SCC), defining immunosuppressed patient status for cohort reporting, development of multi-institutional registry for reporting rare tumors, and development of a KERACON clinical trial of interventions after a SOTRs' first cutaneous SCC were developed. The majority of the symposium focused on presentation of the most up to date research in cutaneous malignancy in SOTR and immunosuppressed patients with specific focus on chemoprevention, immunosuppression regimens, immunotherapy in SOTRs, spatial transcriptomics, and the development of cutaneous tumor registries. Here, we present a summary of the most impactful scientific updates presented at the 2022 ITSCC symposium.

Keywords: solid organ transplant (SOT), immunosuppression, chemoprophylaxis, skin cancer, checkpoint inhibitors

INTRODUCTION

The International Immunosuppression and Transplant Skin Cancer Collaborative (ITSCC) was established by dermatologic surgeons in 2000, and its European counterpart, Skin Care in Organ Transplant Patients-Europe (SCOPE), was established by dermatologists and transplant physicians in 2001. Both ITSCC and SCOPE are now comprised of physicians, surgeons, and scientist who perform integrative collaborative research focused on cutaneous malignancies that arise in solid organ transplant recipients (SOTR) and patients with other forms of long-term immunosuppression. In October 2022, ITSCC held its biennial 4-day scientific symposium in Essex, Massachusetts. This meeting was attended by members of both ITSCC and SCOPE and consisted of 36 specialists including Mohs micrographic and dermatologic oncology surgeons, medical dermatologists, transplant dermatologists, transplant surgeons, transplant physicians, fellows-in-training, and residents-in-training. The symposium attendees were from all over the United States as well as several different European countries, and included physicians from large academic institutions as well as private practices. During this symposium a multi-disciplinary tumor-board for complex clinical cases was held, a keynotes lecture by Dr. Matthew Bottomley from the University of Oxford on the development of a secondary malignancy in SOTRs with cutaneous SCC was given, and scientific workshop groups focusing on consensus standards for case reporting of retrospective series for invasive squamous cell carcinoma (SCC), defining immunosuppressed patient status for cohort reporting, development of multi-institutional registry for reporting rare tumors, and development of a KERACON clinical trial of interventions after a SOTRs' first cutaneous SCC were developed. The majority of the symposium focused on presentation of the most up to date research in cutaneous malignancy in SOTR and immunosuppressed patients with specific focus on chemoprevention, immunosuppression regimens, immunotherapy in SOTRs, spatial transcriptomics, and the development of cutaneous tumor registries. Here, we present a summary of the most impactful scientific updates presented at the 2022 ITSCC symposium.

CHEMOPREVENTION

Chemoprevention, defined as the use of prophylactic medical management to prevent the development of malignancies, specifically in the context of cutaneous SCC in SOTRs was a largely discussed topic during the ITSCC 2022 symposium. While it is well-known that SOTRs have a 20 to 200 times higher risk of developing cutaneous SCC and increased mortality compared to non-SOTR patients, primary prevention of cutaneous SCC is quickly becoming one of the most important roles of dermatologic care in the overall health of SOTR [1]. However, no consensus guidelines on chemoprevention of cutaneous SCC in SOTRs previously existed, until the 2021 publication of the Delphi Consensus Statement by Massey et al. [2].

At the biennial ITSCC symposium in September 2018, several experts in transplant dermatology began the process of

developing a Delphi study to provide consensus-based recommendations for the prevention of cutaneous SCC in SOTRs, of which the results were published in *JAMA Dermatology* by Massey et al. in 2021. The panel of experts involved in this Delphi study represented 13 countries with 56% of those panelists located in the United States. Additionally, this Delphi study used a threshold of 80% or higher to define consensus. The results of this study showed that there is consensus recommendation for routine skin cancer surveillance exams in all SOTRs [2]. A 2019 Delphi consensus recommended all high-risk Caucasian SOTRs should be screened within 2 years of the solid organ transplant whereas all non-high-risk Caucasians, Asian, Hispanic, and high-risk African American patients should be screened within 5 years of solid organ transplantation. High-risk transplant patients were defined in this Delphi as thoracic organ transplant recipients, age 50 or above at time of solid organ transplantation, and male SOTRs [3]. Additionally, in keeping with previously published studies, regular use of sunscreen and sun-protective behaviors was recommended by this expert panel [2–4].

In regards to topical treatment of precancerous lesions, specifically actinic keratoses, the Delphi study resulted in full consensus for lesion-directed therapy using cryotherapy for scattered actinic keratoses and the use of field therapy (with or without the adjuvant use of cryotherapy for thicker lesions) for actinic keratoses confined to a single anatomic location. While no full-consensus was reached in regards to which topical agent should be used for field therapy in this setting, this study had a near-consensus (70% to less than 80% agreement) in favor of using topical fluorouracil. Additionally, while 74% of the group reported that photodynamic therapy (PDT) had the best adherence, only 4% considered PDT to be the most effective field agent.

In regards to oral chemoprevention, the only agent that had a consensus recommendation in this study was acitretin in the setting of high-rate of development of cutaneous SCC (>10 tumors per year) or development of high-risk cutaneous SCC (AJCC8 T3 or above or Bringham and Women's Hospital stage T2b or above) in SOTR, which is supported by prior findings in randomized controlled trials involving renal transplant recipients [5, 6]. However, no consensus was reached in regards to chemoprevention after the development of a first low-risk SCC in SOTR, regardless of specific organ transplanted (i.e., abdominal vs. thoracic). Additionally, no consensus recommendation for use of oral nicotinamide or capecitabine was reached.

This study also had a consensus recommendation to discuss change in SOTRs' immunosuppression regimen with the transplant team in the setting of advanced cutaneous SCC, defined in this study as multiple invasive low risk cutaneous SCCs (>10 tumors per year) or development of a high-risk cutaneous SCC. No consensus recommendation was made in regards to which specific immunosuppression regimen should be used to provide the lowest risk for development of cutaneous SCC.

The findings of the Massey et al. Delphi study detailing consensus-based recommendations for the prevention of

cutaneous SCC in SOTR were heavily discussed during the ITSCC 2022 symposium, and provide the most up-to-date findings supporting preventative dermatologic care in this patient population. Additional presentations at this symposium discussed ongoing research into the use of acitretin, capecitabine, and nicotinamide for chemoprevention in SOTR, emphasizing the importance of continued work in this area to find the most effective prevention for cutaneous SCC in transplant recipients given the increased risk of morbidity and mortality in this patient population.

IMMUNOSUPPRESSION

Chronic immunosuppression is an important part of long-term medical treatment of SOTRs, and the increased risk of developing cutaneous malignancies in this setting is well documented and an area in which ongoing research is aiming to minimize. The role of immunosuppression, development of cutaneous malignancies, and possible alterations of immunosuppression therapy was a heavily discussed topic at the ITSCC 2022 symposium.

A recent publication polled expert transplant dermatologist to determine which immunosuppression regimen was clinically correlated with the development of the most cutaneous malignancies, and 88% of respondents reported azathioprine was associated with development of the most cutaneous malignancies following solid organ transplantation. Additionally, 69% reported that sirolimus was the immunosuppressant least associated with the development of cutaneous malignancies following transplant [2]. These clinical findings are supported by transitional research showing that cutaneous SCC in SOTRs taking azathioprine show unique mutational signatures caused by UVA absorption by DNA [7, 8]. While azathioprine is now less commonly used as a primary immunosuppression regimen for SOTRs, it is still used in patients who are intolerant to mycophenolate therapy or who may be planning to become pregnant [9].

More modern immunosuppression regimens appear to have a decreased risk of developing cutaneous malignancies when compared to azathioprine. A recent large retrospective control-matched cohort study by Gibson et al. published in 2021 found a significant reduction in skin cancer development with the use of tacrolimus and mycophenolate when compared to cyclosporine and azathioprine, respectively [10]. This study found an incidence rate ratio (IRR) of 0.44 ($p = 0.03$, 95% CI = 0.21–0.92) when SOTRs were switched from cyclosporine to tacrolimus, as well as an IRR of 1.66 ($p = 0.01$, 95% CI = 1.16–2.36%) with azathioprine compared to an IRR of 0.78 with mycophenolate ($p = 0.18$, 95% CI = 0.54–1.12) [9]. Additionally, transition from azathioprine to mycophenolate appears to reduce the risk of developing a first cutaneous SCC post solid organ transplantation with mycophenolate having an IRR of 0.49 ($p = 0$, 95% CI = 0.32–0.75) compared to azathioprine in this setting [9–11].

Another point of discussion regarding immunosuppressive regimens was the more recent use of belatacept as a primary immunosuppressant or adjuvant immunosuppressant used with calcineurin inhibitors in kidney transplant recipients and the

correlation to the development of skin cancer. Given the more recent incorporation of belatacept in SOTR medical management, there is currently limited evidence, but thus far, small single-center studies show that use of belatacept in the place of calcineurin inhibitors leads to a lower risk of developing skin cancers post solid organ transplantation [12].

As discussed above in the Massey et al. publication, the development of multiple invasive low risk cutaneous SCC or the development of a high-risk cutaneous SCC should prompt discussion of alteration of immunosuppression regimen with the patient's transplant team. The two secondary prevention strategies used in this situation are to change the immunosuppressive regimen or change the immunosuppressive intensity, and individual patient assessment must be used to determine the best course of action [9]. As immunosuppression regimens continue to evolve, more research is needed to determine best therapy and dose to balance maintaining the function of the transplanted organ and decreasing the risk of secondary malignancies.

CHECKPOINT INHIBITOR THERAPY IN SOTR

Given the increased risk of SOTRs to development high-risk cutaneous malignancies that may require systemic treatment, the use of immune checkpoint inhibitors (ICIs) in this population is a highly discussed topic at this time. The use of ICIs in SOTR with high-risk cutaneous malignancies, including melanoma, Merkel cell carcinoma, and high-risk SCC, presents a significant challenge as ICIs place transplant recipients at risk of acute allograft rejection. Historically, SOTRs have been excluded from ICI clinical trials for treatment of advanced skin cancers as retrospective studies have shown acute allograft rejection rates between 10% and 65% with ICI use in this population [13]. Of these SOTRs who experience acute allograft rejection with ICI therapy, 24%–81% subsequently lose their allograft, which may lead to death [13, 14].

Careful deliberation and risk-benefit assessment is needed on an individualized basis when considering ICI treatment in SOTRs. While ICIs are the only approved systemic treatments for locally advanced or metastatic cutaneous SCC and Merkel cell carcinoma, the risk of acute leading to fulminant rejection of the allograft is a major factor in oncologic management of SOTR [15, 16]. Given the high risk of allograft rejection, kidney transplant recipients are the primary SOTRs that may be considered for ICI treatment in the setting of advanced cutaneous malignancy as transplant rejection can be managed by dialysis in most cases and rarely leads to fatality. However, thoracic transplant patients (i.e., heart or lung) are less commonly considered for ICI therapy in this setting as risk of allograft failure is more life-threatening [13]. While retrospective and systematic reviews are helpful in assessing the risk of ICI use in SOTR, there is now a major focus on prospective and randomized controlled trials in this area to better elucidate the role of ICI in SOTRs. The ongoing CASE (Cemiplimab-rwlc Survivorship and Epidemiology) study is a longitudinal prospective multicenter study evaluating the safety

and effectiveness of cemiplimab used to treat advanced cutaneous SCC in SOTRs. Preliminary results from the CASE study appear to be similar to those from prior ICI trials that excluded SOTRs [15, 16]. Additionally, an active phase I trial studying the efficacy and risk of tacrolimus, nivolumab, and ipilimumab in treating kidney transplant recipients with selected unresectable or metastatic cancers (including cutaneous SCC) was discussed at the ITSCC 2022, and importance of such trials was emphasized [17, 18].

The use of ICI in advanced cutaneous malignancies is a mainstay of therapy in treating non-immunocompromised patients, and is now playing a more prominent role in the treatment of such malignancies in SOTR. More prospective and randomized controlled studies are needed to elucidate the role of ICI in SOTR, specifically in regards to which SOTRs are good candidates for ICI therapy, patient factors that may help predict allograft rejection, immunosuppressive regimens that may be protective of the allograft during ICI treatment, and ideal ICI dosing to provide oncologic benefit while reducing risk of allograft rejection.

SPATIAL TRANSCRIPTOMICS

Spatial transcriptomics, particularly in regards to cutaneous malignancies, was another highly discussed topic at the 2022 ITSCC symposium given the increase in popularity of spatial transcriptomics in cancer research as of late. In brief, spatial transcriptomics allows for the measure of gene activity in a tissue sample while allowing mapping of where the activity is occurring without disrupting the anatomic structure of the sampled tissue [19, 20]. Previously, bulk and single-cell RNA sequencing were used to better understand cell to cell interactions in cancer; however, these techniques did not allow for retention of spatial orientation in the tissue specimens [19]. Given the importance of the tumor microenvironment, tumor heterogeneity, and tumor interface in cancer, spatial transcriptomics have provided significant advances in the microscopic understanding of malignancies and is an exciting advancement in oncologic research. With better understanding of spatial histology of tumors, there is greater potential to improve pathologic diagnosis, understanding of prognostic factors, understanding of tumorigenesis and progression, and prediction of treatment response [19, 20].

Spatial transcriptomics is now being used in the research of high-risk and advanced cutaneous malignancies, including those seen in SOTRs. Specifically, Dr. Matthew Bottomley of the University of Oxford is using spatial transcriptomic profiling to explore immunosuppression and immunosenescence-driven skewing of immune response in cutaneous SCCs in kidney transplant recipients, which may explain the enhanced predisposition to cutaneous SCC in these cohorts [21]. Additionally, Dr. John Carucci of New York University presented his lab's research on the transcriptomic profile of CDS+ tumor infiltrating lymphocytes (TIL) in aggressive basal cell carcinomas, and the subsequent effects on T-cell trafficking, clonal expansion, and T-cell exhaustion. This work is an exciting

advancement in the further understanding of high-risk cutaneous malignancies on a microscopic and genetic level, and encourages further research in the area of high-risk cutaneous malignancies using spatial transcriptomics.

TUMOR REGISTRIES

Tumor registries specific to cutaneous malignancies was also a prominent topic at the 2022 ITSCC symposium. While melanoma is often included in national tumor registries such as the National Cancer Institute's Surveillance, Epidemiology, and End Result Program, other primary cutaneous malignancies are not. This lack of tumor registry, specifically for rare cutaneous tumors, inhibits the further understanding of incidence, prognosis, natural history of disease, and treatment response. While several academic institutions in the United States and Europe have individual tumor registries, there has yet to be a national or international registry to collect combined information on these tumors. One of the focus groups at the ITSCC 2022 symposium was dedicated to initiating a multi-institution registry for rare cutaneous tumors. This registry plans to collect information about the tumors as well as patient characteristics including history of solid organ transplants and immunosuppression status. Not only will a national, and perhaps 1 day international, tumor registry of rare cutaneous malignancies provide a larger sample size of such tumors to allow a better understanding of innate characteristics of these cancers, but it will also elucidate the relationship between SOTR, immunosuppression, and the development and outcome of these rare tumors.

FUTURE DIRECTION

The ITSCC 2022 symposium was a great success, and provided an opportunity for experts in the field of transplant and immunosuppression dermatology to discuss the most recent scientific advancements in this area and collaborate on ongoing research. Through the workgroups developed during this meeting, longitudinal projects including development of a multi-institutional registry for reporting rare tumors, consensus definition of immunosuppressed patient status for cohort reporting, consensus standards for case reporting of retrospective series for invasive SCC, and KERACON clinical trial of interventions after a SOTR first cutaneous SCC are currently underway. Additionally, ITSCC is now offering a 2-year academic mentorship program which connects junior members of ITSCC with more established senior members of ITSCC to assist in career and academic development, particularly in regards to establishing transplant and immunosuppressed patient clinics as well as performing clinical and/or translation research in these areas. Further discussion and presentation of scientific work in the dermatologic care in SOTRs and the immunosuppressed patient population are scheduled for the annual ITSCC meeting at the American Academy of Dermatology annual meeting as well as the SCOPE symposium planned for Fall 2023, and the next ITSCC biennial symposium in Fall 2024.

AUTHOR CONTRIBUTIONS

All authors attended and were involved in the ITSCC scientific symposium. MP-M and BC organized the speakers for the symposium and were in charge of the scientific agenda, JL is the president of ITSCC and also helped to organize the scientific agenda and oversaw consensus opinions during symposium. CP attended the symposium and wrote the manuscript.

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CONFLICT OF INTEREST

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

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Harnessing Immune Cell Metabolism to Modulate Alloresponse in Transplantation

Johan Noble^{1,2,*†}, Zuzana Macek Jilkova^{2,3}, Caroline Aspod^{2,4}, Paolo Malvezzi¹, Miguel Fribourg⁵, Leonardo V. Riella^{6,7} and Paolo Cravedi⁵

¹Nephrology, Hemodialysis, Apheresis and Kidney Transplantation Department, University Hospital Grenoble, Grenoble, France, ²Inserm U 1209, CNRS UMR 5309, Team Epigenetics, Immunity, Metabolism, Cell Signaling and Cancer, Institute for Advanced Biosciences Grenoble, University Grenoble Alpes, La Tronche, France, ³Hepato-Gastroenterology and Digestive Oncology Department, University Hospital Grenoble, Grenoble, France, ⁴Établissement Français du Sang Auvergne-Rhône-Alpes, R&D-Laboratory, Grenoble, France, ⁵Translational Transplant Research Center, Icahn School of Medicine at Mount Sinai New York, New York, NY, United States, ⁶Center for Transplantation Sciences, Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States, ⁷Division of Nephrology, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States

Immune cell metabolism plays a pivotal role in shaping and modulating immune responses. The metabolic state of immune cells influences their development, activation, differentiation, and overall function, impacting both innate and adaptive immunity. While glycolysis is crucial for activation and effector function of CD8 T cells, regulatory T cells mainly use oxidative phosphorylation and fatty acid oxidation, highlighting how different metabolic programs shape immune cells. Modification of cell metabolism may provide new therapeutic approaches to prevent rejection and avoid immunosuppressive toxicities. In particular, the distinct metabolic patterns of effector and suppressive cell subsets offer promising opportunities to target metabolic pathways that influence immune responses and graft outcomes. Herein, we review the main metabolic pathways used by immune cells, the techniques available to assay immune metabolism, and evidence supporting the possibility of shifting the immune response towards a tolerogenic profile by modifying energetic metabolism.

Keywords: solid organ transplantation, immune cells, metabolism, rejection, glycolysis

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*Correspondence

Johan Noble,
✉ jnoble@chu-grenoble.fr,
✉ johan.noble@mssm.edu

†Present address:

Johan Noble,
Translational Transplant Research
Center, Icahn School of Medicine at
Mount Sinai New York, New York, NY,
United States

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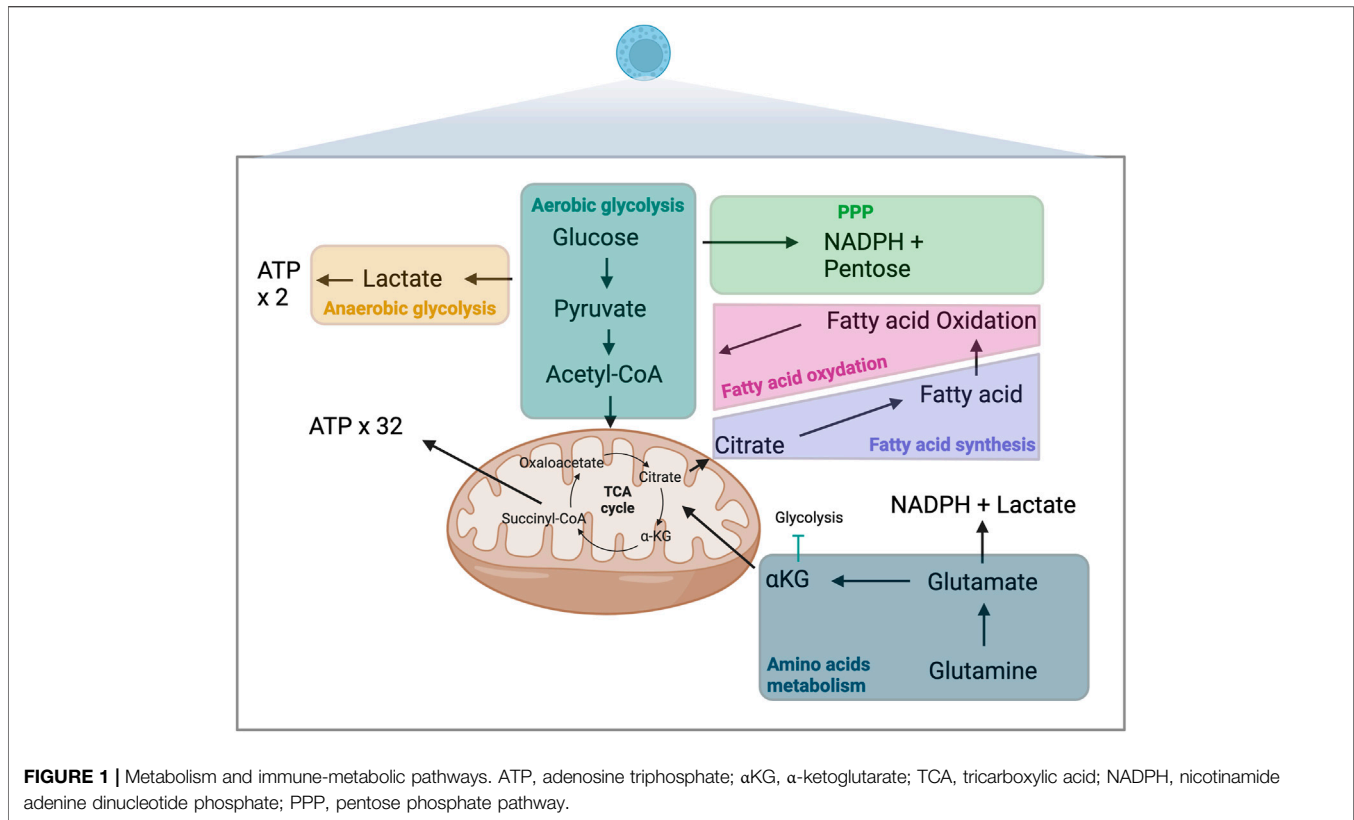
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INTRODUCTION

For many decades, transplant immunology has focused on the mechanisms of organ rejection and developing strategies to prevent graft injury by blocking key activation pathways in the recipient's immune system [1]. Changes in the metabolism of the alloimmune cells have been regarded as the downstream effect of their effector function. More recently, it has become apparent that changes in

Abbreviations: αKG, α-ketoglutarate; ACC1, acetyl-CoA carboxylase 1; AMPK, adenosine monophosphate-activated protein kinase; ATP, adenosine triphosphate; ECAR, extracellular acidification rate; FAO, fatty acid oxidation; HLA, human leukocyte antigen; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IL, interleukin; mTORC1, mammalian target of rapamycin complex 1; NADPH, nicotinamide adenine dinucleotide phosphate; OXPHOS, oxydative phosphorylation; OCR, oxygen consumption rate; PPP, pentose phosphate pathway; PBMC, peripheral blood mononuclear cells; SCENITH, single-cell energetic metabolism by profiling translation inhibition; SOT, solid-organ transplantation; TCA, tricarboxylic acid; TCR, T cell receptor; Tex, exhausted T cells; TLR, toll-like receptor; Treg, regulatory T cells.



immune cell metabolism can, by themselves, drive immune cell fate. The advent of novel technologies has allowed the collection of detailed data to decipher the plasticity of the metabolic state of immune cells [2]. These findings highlight the metabolic pathways in immune cells as a potential novel therapeutic approach to reprogramming immune responses and preventing transplant rejection [3].

Herein, we review the current knowledge on the importance of metabolic changes in immune responses, recent technologies to study immune metabolism, and how targeting immune cell metabolism could improve outcomes in SOT recipients.

CELLULAR METABOLIC PATHWAYS IN IMMUNE CELLS

Cellular metabolism is divided into anabolism and catabolism and both anabolic and catabolic reactions are essential for immune cell function and survival.

Anabolic reactions involve chain biosynthetic reactions that generates cell materials such as proteins and polypeptides from amino acids, DNA, RNA, lipids from fatty acid (FA). Anabolism require energy, typically provided in the form of adenosine triphosphate (ATP) molecules. Fatty acid synthesis (FAS) is a major anabolic reaction closely linked to immune cell function changes, differentiation, and proliferation [4]. Catabolic reactions involve the breakdown of complex molecules into simpler ones resulting in the release of energy such as proteins becoming

amino acids or triglycerides breaking up into FA. Glycolysis and oxidative phosphorylation (OXPHOS) are the two main metabolic pathways that provide ATP for cells. Glycolysis refers to glucose oxidation to obtain ATP. OXPHOS refers to oxidation of nutrients within the mitochondria to generate ATP. Catabolic reactions are essential to support the high energetic requirements of immune cells, such as for cytokine production, rapid proliferation, and migratory activities (Figure 1).

Sugars

Glycolysis, the breakdown of glucose, occurs in the cytosol of cells and is one of the primary catabolic processes contributing to the production of ATP [5]. The efficacy of the process depends not exclusively on oxygen availability and the mitochondrial capacity of immune cells. Aerobic glycolysis is the primary metabolic process contributing to energy generation in most immune cells. This highly efficient multi-step process starts with glucose molecule broken into two pyruvate molecules. In the presence of oxygen, which is required to re-oxidize nicotinamide adenine dinucleotide (NADH) to NAD^+ , pyruvate moves in the mitochondria and is converted to acetyl-CoA via pyruvate dehydrogenase. Acetyl-CoA enters in tricarboxylic acid (TCA) cycle and undergoes OXPHOS, leading to the production of 32 ATP molecules. In the absence of oxygen, glucose is metabolized in an anaerobic glycolysis process, through which pyruvate is converted into lactate, which yields only 2 ATP molecules. This process of lactate production can occur

TABLE 1 | Drug targets that modify the metabolism of immune cells.

Metabolism pathway	Name	Targeted molecule	Effect on metabolism	Origin
OXPHOS	Oligomycin [15, 16]	ATP synthase	Inhibition	<i>Streptomyces diastatochromogenes</i>
OXPHOS	Rotenone [17]	Mitochondrial Complex I	Inhibition	Roots
OXPHOS	Antimycin A [18, 19]	Mitochondrial Complex III	Inhibition	<i>Streptomyces kitazawensis</i>
OXPHOS	Myxothiazol [20]	Mitochondrial Complex III	Inhibition	<i>Myxococcus fulvus</i>
OXPHOS and FAO	Metformin [21–23]	AMP Kinase Complex I FAO	Activation Inhibition Increase	<i>Galega officinalis</i>
Glycolysis	2-DG [24–26]	Hexokinase	Inhibition	<i>De novo</i> synthesis
Glycolysis	Galactose [27, 28]	Pyruvate	Inhibition	Milk
Glycolysis	3-bromopyruvate [29]	Hexokinase II	Inhibition	<i>Escherichia coli</i>
Glycolysis	Ritonavir [30]	GLUT1 and 4	Inhibition	<i>De novo</i> synthesis
Glycolysis	FX11 [31]	LDHA	Inhibition	<i>De novo</i> synthesis
Glycolysis	DCA [32]	PDK2	Inhibition	<i>De novo</i> synthesis
Glycolysis	4-CIN [33]	Monocarboxylate transporter	Inhibition	<i>De novo</i> synthesis
Glycolysis	TEPP-46 [34–36]	PKM2	Inhibition	<i>De novo</i> synthesis
Glutamine	DON [37, 38]	Glutaminase	Inhibition	<i>Streptomyces</i>
Glutamine	BPTES [39]	Glutaminase	Inhibition	<i>De novo</i> synthesis
Glutamine	CK [40]	Glutaminase	Inhibition	<i>Escherichia coli</i>
FAO	Etomoxir [41, 42]	CPT1a	Inhibition	<i>De novo</i> synthesis
FAO	AICAR [43]	AMP kinase	Increase	<i>Escherichia coli</i>
FAS	C75 cerulenin, C75, orlistat, C93 [44]	Fatty acid synthase	Inhibition	<i>De novo</i> synthesis
FAS	TOFA [45]	Acetyl CoA carboxylase	Inhibition	<i>De novo</i> synthesis

AICAR, 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside; ATP, Adenosine triphosphate; BPTES, bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide; CPT1a, Carnitine palmitoyl-transferase 1a; CK, L-2-amino-4-oxo-5-chloropentanoic acid; DON, 6-Diazo-5-oxo-L-Norleucine; DCA, dichloroacetic acid; FAO, Fatty acid oxidation; FAS, Fatty acid synthesis; FX11, 3-dihydroxy-6-methyl-7-(phenylmethyl)-4-propylnaphthalene-1-carboxylic acid; GLUT, Glucose transporter; PKM2, pyruvate kinase M2; LDHA, Lactate dehydrogenase-A; OXPHOS, Oxidative phosphorylation; PDK2, Pyruvate dehydrogenase kinase 2; TEPP, thieno-pyrrole-pyridazinone; TOFA, 5-tetradecyloxy-2-furoic acid; 2-DG, 2-deoxyglucose; 4-CIN, α -cyano-4-hydroxycinnamate.

despite the presence of oxygen and fully functioning mitochondria (Warburg effect). Lactate also increases the NADH/NAD⁺ ratio. The pentose phosphate pathway (PPP) is an alternative pathway for glucose metabolism that generates nicotinamide adenine dinucleotide phosphate (NADPH) and pentoses (5-carbon sugars), essential moieties for synthesizing nucleotides. This pathway is crucial for effector functions of innate immune cells, including removing apoptotic cells including tolerogenic cells or activating and generating an oxidative burst of neutrophils [6, 7]. 5' AMP-activated protein kinase (AMPK) is a metabolic sensor able to induce glycolysis through the mammalian target of rapamycin complex (mTOR) pathway.

Amino acids

Glutamine is an essential substrate for immune cell metabolism. This non-essential amino acid can be transformed into glutamate and then into α -ketoglutarate (α KG), which like glucose-derived acetyl-CoA, is an essential fuel for the TCA cycle [8]. The second fate of glutamine-derived glutamate involves its transformation into lactate and NADPH through a truncated TCA cycle in which succinyl-CoA is converted into succinate, fumarate and then malate [9]. Outside the mitochondria, malate will be converted into pyruvate and then lactate. Amino acids other than glutamine have also been shown to play essential roles in immune metabolism [10]. Tryptophan derived metabolites such as kynurenine or kynurenic acid have also emerged as a major

pathway involved in regulatory T cells generation, in autoimmune diseases and in tolerance [11].

Fatty acids

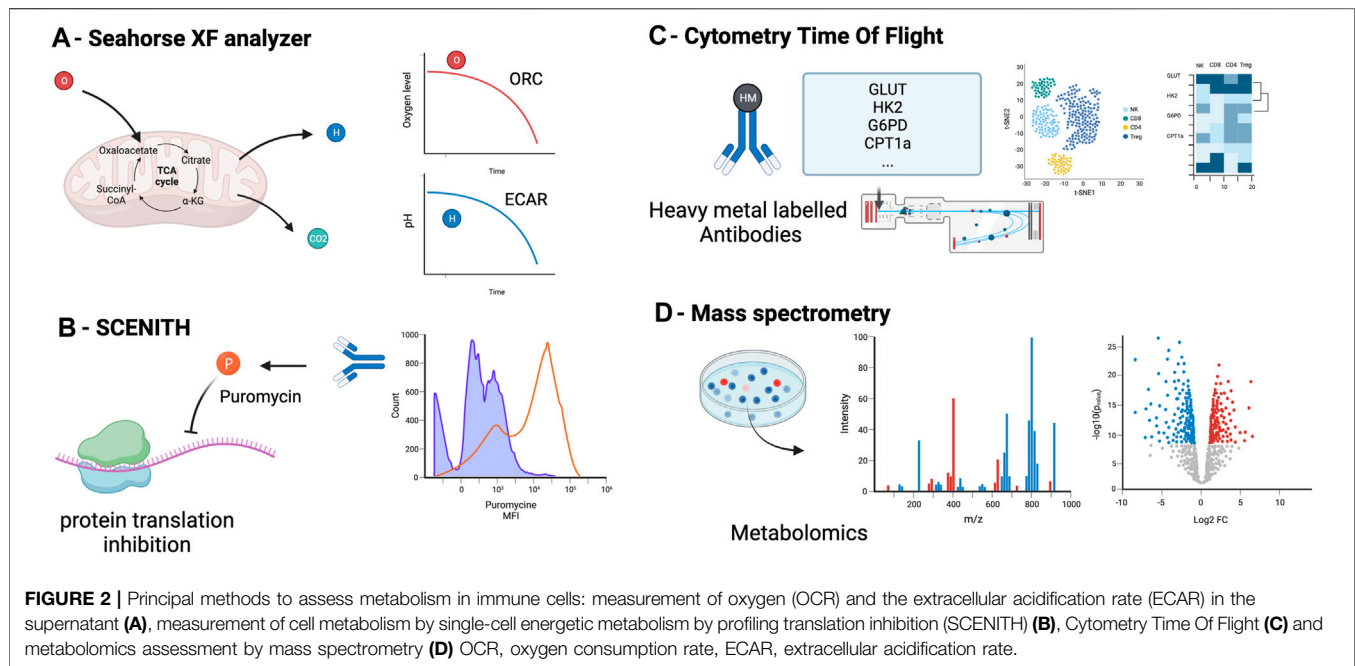
Fatty acids (FAs) can fuel cellular metabolism through FA oxidation (FAO), another source of acetyl-CoA, which can then be shuttled to the TCA cycle. This metabolic pathway is of particular importance in adaptive immune responses [12]. AMPK is a metabolic sensor able to induce FAO.

TECHNIQUES TO STUDY IMMUNE CELL METABOLISM

Different methods to measure cell metabolism have been used [13]. Each technique represents a different approach and has advantages and limitations [14]. **Table 1** summarizes the available tools to block or activate the different metabolic pathways.

Global Oxygen and Acidification Measurement

For the last decade, OXPHOS and glycolysis have been measured using the Seahorse[®] XF Analyzers (**Figure 2A**). This technique infers the oxygen consumption rate (OCR) through measuring the oxygen concentration in the supernatant of a cell culture over time, a surrogate marker of OXPHOS [46, 47]. Similarly, it



estimates the extracellular acidification rate (ECAR) by measuring the changes in proton concentration in the supernatant over time, and using it as a surrogate marker of glycolysis [46]. OCR and ECAR can be assessed in parallel 96-well plate to perform replicates and multiple conditions.

This approach requires prior cell purification and it is unable to assess the metabolism at a single-cell level [48]. This method does not allow to perform simultaneous cell phenotyping nor cell sorting. Cells need to be incubated from 12–18 h which can induce variability between wells in the number of cells and results.

Single-Cell Energetic Metabolism by Profiling Translation Inhibition (SCENITH)

The SCENITH method has recently provided an interesting additional tool to assess immune metabolism at a single-cell level using flow cytometry [49]. This method assumes that most of the cell's energy is employed for protein synthesis [50, 51]. By using the ability of puromycin to incorporate into protein during synthesis and our ability to detect it with an anti-puromycin antibody, the SCENITH method utilizes the level of puromycin incorporation as a marker of protein synthesis and thus, a surrogate marker of cell global metabolic activity (Figure 2B). The advantages of this technique include the possibility to study metabolism at a single cell level, the simultaneous study of multiple cell subtypes, the lack of sensitivity to metabolic modifications induced by the media, and the requirement of only a low number of cells (~2000 cells) without purification. An additional advantage is the possibility of assessing cell phenotyping and other functions concomitantly [52]. The main limitation of the SCENITH method is its reliance on protein synthesis, which is only an indirect marker of cell metabolism, and lacks relevance for cells with low levels of protein synthesis, such as quiescent cells.

Flow Cytometry and Cytometry by Time of Flight

By using a panel of key enzymes, flow cytometry can assess metabolic state. The “Met-Flow” panel includes 10 metabolic enzymes and transporters, including Hexokinase 1 for glycolysis, Carnitine palmitoyl-transferase 1A for FAO, Glucose-6-phosphate dehydrogenase [53]. This panel allows single-cell and phenotypic analysis of cell metabolism and does not require prior cell purification but the number of antibody needed may be a challenge.

Cytometry by time of flight (CyTOF) is another technique that can assess immune cell metabolism (Figure 2C). Instead of using fluorescent-labeled antibodies as in regular flow cytometry, cells are stained with antibodies conjugated to heavy metal isotopes [54], increasing the capacity to multiplex and reducing spectral overlap. About 110 metabolism-associated antibodies are available [55]. Recent studies reported a subset of 41–45 antibodies to target the important regulators (transporters, enzymes, signaling molecules, transcription factors) of metabolic pathways [56, 57]. CyTOF main limitation are the cost of the CyTOF equipment and the fact that this technique does not allow to recover living cells after analysis and thus only static measurement of single cells.

Metabolomics

Metabolomics encompasses methods to detect and measure the cell metabolite levels and modifications (mass spectrometry combined with chromatography or ion mass, protein weight, ionization, and magnetic resonance) [58]. Carbon-labeled tracers can be added to mass spectrometry to specifically interrogate metabolic enzyme activities. Isotope tracers allow to quantify metabolomic flux on top of metabolic concentrations [59]. Mass spectrometry allow to detect and quantify even low concentration metabolites. Metabolites are detected according to their mass and

charge. Classical separation techniques are liquid or gas chromatography. These methods require the least amount of material (about 200 cells without purification) and can target specific metabolites or all the metabolome (Figure 2D). Because of unbiased analysis, it also allows the discovery of new or uncharacterized metabolites. Limitations of this technique are the potential impact of the handling (medium and storage) on metabolite levels, the impossibility of combining with phenotypic analyses, the cost of the equipment and the variability resulting of the metabolites quenching and purification that can change rapidly the level of metabolites.

HOW METABOLISM AFFECTS IMMUNE CELLS

Cellular metabolism does not only constitute a way to provide energy for immune cell survival and function, but it also regulates immune cell signaling pathways [60]. Metabolites have emerged as critical regulators of immune cell survival, differentiation, activation and function [5]. The metabolic network and its plasticity shape the fate and functions of both innate and adaptive immune cells [61].

Innate Immune Cells

Myeloid Cells: Dendritic Cells and Macrophages

Activation of DC through Toll-like receptors (TLRs) is a crucial step for DC activation, maturation, as well as antigen processing and presentation. TLR engagement is associated with an increased level of glycolysis and a decreased level of OXPHOS [62–64]. Interestingly, DC can switch to OXPHOS metabolism when deprived of glucose due to competitive glucose uptake by T cells in the context of antigen presentation and T cell activation. Notably, glucose deprivation increases the capacity of DC to present and stimulate T cells [65].

An increase in glycolysis is critical in the initial phase of DC proliferation and differentiation, but then specific inflammatory or tolerogenic metabolic reprogramming follows [66]. This has been illustrated through SCENITH and CyTOF analyses of DC metabolism which show an increase of AMPK pathway and a decrease of mTOR pathway in tolerogenic DC as compared to inflammatory DCs [67]. Tolerogenic DC have been shown to highly increase the ECAR level in the presence of glucose, to produce more lactate and have a higher lactate dehydrogenase activity as compared to other DC, suggesting a strong glycolytic profile of those cells [68].

In addition, many studies demonstrate that tumors promote specific metabolic pathways and nutrient uptake as compared to innate immune cells to reduce their effector functions and escape immuno-surveillance [69].

Different metabolic profiles characterize macrophage subsets. Pro-inflammatory macrophages (M1) have a higher succinate dehydrogenase in the TCA cycle which results in an increase of succinate which stabilizes Hypoxia Inducible Factor 1 α (HIF1 α) and in turn, promotes and sustains glycolysis activity [70]. Conversely, anti-inflammatory macrophages (M2) exhibit enhanced FAO and OXPHOS activity with an intact TCA

cycle. Interestingly, increasing glutamine concentration *in vitro* culture medium drives mouse macrophage polarization into M2 profile, providing support to the notion that it is possible to orient the immune response through metabolism modifications [71]. α KG, a product of glutaminolysis, acts as a sensor of pro-anti-inflammation signals in mouse macrophages and can promote M2 polarization, but the role of glutamine in human macrophage is unknown [71].

Natural Killer Cells

The NK cell metabolic profile and effector functions depend on the context and the microenvironment. Cytokine-driven NK cell activation is associated with increased mitochondrial OXPHOS and glycolysis [72]. The relationship between metabolism and NK cell function was shown in tumor models, in which reduced availability of glucose and amino acids (leucine, arginine, glutamine) results in NK cell function impairment [73, 74]. Tryptophan pathway induction by indole 2,3-diamine oxygenase (IDO) in tumors results in NK cell apoptosis to promote survival of cancer cells [75].

T cells

Naive T cells

Metabolic program and T-cell activation are closely linked [8]. Before T Cell Receptor activation (TCR), naïve T cells are quiescent and have low ATP requirements. In their naïve state, their principal source of ATP is OXPHOS fueled by the oxidation of pyruvate and FAO, with a low glycolysis-based metabolism [8]. TCR engagement results in the activation, proliferation, and differentiation of the naïve T cells into effector, memory, and central memory T cells. This is paralleled by the transcription of key metabolic enzymes including the glucose transporter GLUT1 and the acetyl-CoA carboxylase 1 (ACC1) translation [76].

Activated T cells

T cell activation leads to major metabolic changes that favor glycolysis over OXPHOS [77].

Upon activation, CD8⁺ T cells undergo a first metabolic shift consisting of shuttling pyruvate to lactate metabolism [78], followed by a full switch from anaerobic to aerobic glycolysis [79]. The increase in glycolysis activity in activated CD8⁺ T cells is underpinned by an increase of glycolytic enzymes and an expression of glucose transporters such as GLUT1 [80]. Glycolysis inhibition results in cytokine and proliferation impairments in activated CD8⁺ T cells [81].

Effector CD4⁺ T cells, T helpers 1 (Th1), T helpers 2 (Th2), and T helpers 17 (Th17) cells are highly dependent on aerobic glycolysis, which is under HIF1 α - mTOR regulation [82]. Although OXPHOS is more efficient in producing ATP, glycolysis gives the cells an advantage by rapidly providing the required energy for effector functions and proliferation. Interestingly, aerobic glycolysis is not needed for T cell activation, but it is strictly required for T effector functions such as cytokine production (IL-2, IFN- γ mRNA translation and secretion) [77]. After TCR engagement and CD28 co-stimulation, the glucose uptake is increased by the upregulation of the cell surface glucose transporter GLUT-1 [83].

Interestingly, pharmacological blockade of glycolysis impairs Th1 and Th17 survival and function [84].

OXPPOS is also increased in activated CD4⁺ T cells even though the ratio OXPPOS/glycolysis is lower during T cell activation than in naïve CD4⁺T cells. The energy provided by OXPPOS seems to be mainly required during the first step of T cell activation, acting as an impulse [77]. CD4 T cells deficient for the mitochondrial complex III-derived Reactive oxygen Species (ROS) cannot activate and proliferate upon antigen presentation [85].

Amino acids are fundamental for activated T cells. Glutamine, a non-essential amino acid, constitutes an important energy source through glutaminolysis in activated T cells [86]. This is illustrated by the increase of glutamine transporters in activated T cells, and the reduced proliferation and cytokine secretion by T cells during glutamine starvation [87, 88]. Leucine have also been described to be of significant importance in the proliferation and differentiation of T cells trough [89].

T cell proliferation requires lipid synthesis to generate cell membranes for daughter cells. Blocking ACC1, a major enzyme for FAS, impairs Th1, Th2, and Th17 proliferation [90]. Interestingly, during Th17 cell development, but not regulatory T cells, FAS depends on ACC1, and blockade of this glycolytic-lipogenic pathway selectively impaired TH17 generation [90, 91].

Memory T cells

Memory T cells have a metabolic profile close to that of naïve T cells (lower glycolysis compared to OXPPOS) but with notable differences: they have a higher mitochondrial mass and a higher spare respiratory capacity which allow them to respond faster in case of antigen re-exposure [92]. Glycolysis in memory T cells is higher than in naïve T cells despite the similar ratio of glycolysis/OXPPOS [93]. As in regulatory T cells (Treg), FA constitute the principal fuel of OXPPOS for memory T cells [92]. FA come preferentially from *de novo* synthesis via the glycolytic-lipogenic pathway via mitochondrial citrate transformation and not from exogenous FA uptake, as in Tregs [94, 95].

AMPK is a regulator of FAO and glycolysis and the inability to generate memory T cells in AMPK-deficient mice is associated with deficient mitochondrial FAO [21]. Similarly, AMPK deficient CD8⁺ T cells are enable to generate memory CD8⁺ T cells [96–99]. In summary, metabolism signature of memory T cells remains uncertain while they exhibit an elevated profile of glycolysis and OXPPOS.

Exhausted T cells

In the context of persisting antigen and TCR stimulation, effector T cells progressively modify their phenotype to slowly become exhausted T cells, a low functional state phenotypically characterized by specific markers including programmed-death 1 (PD-1) [100]. In case of a high energy demand (glycolysis) sustained over time, glucose deprivation progressively drives a metabolic modification on effector T cells. These modifications are driven by the PD-1 pathway [101], resulting in a decrease in T cell glucose uptake and in OXPPOS [102], while FAO is upregulated [103].

Regulatory T cells

The metabolic profiles described above for other CD4⁺ T cells do not seem to apply to regulatory T cells (Treg), whose energy demands are not met through glycolysis but through OXPPOS and FAO [104].

Their independence from aerobic glycolysis has been shown *in vivo* in GLUT-1 deficient mice [105]. GLUT-1 deficiency was associated with impaired growth, proliferation, and survival of mature effector T cells, but did not affect either natural or induced Treg generation and expansion. The rate of glycolysis in Tregs is similar to that of naïve T cells but lower than in Th1 and Th17 cells [104, 105]. Inhibition of glycolysis using dichloroacetate increases Treg differentiation and promotes IL-10 production and FOXP3 expression [106]. Similarly, blocking glycolysis with 2-DG promotes Treg differentiation at the expense of Th17 [82].

In contrast, blocking OXPPOS results in Treg differentiation impairment [84]. In Treg, OXPPOS is fueled through FAO, blocking FAS has been shown to promote Treg generation, and FAO activity associates with an increase in AMPK activity. Adoptive transfer of modified OXPPOS or FAO deficient Tregs, resulted in a reduction of graft survival compared to wild-type [107]. Consistently, dysfunction of mitochondrial proteins (complex III, transcription factor A) is associated with Treg loss of function [108, 109].

B cells

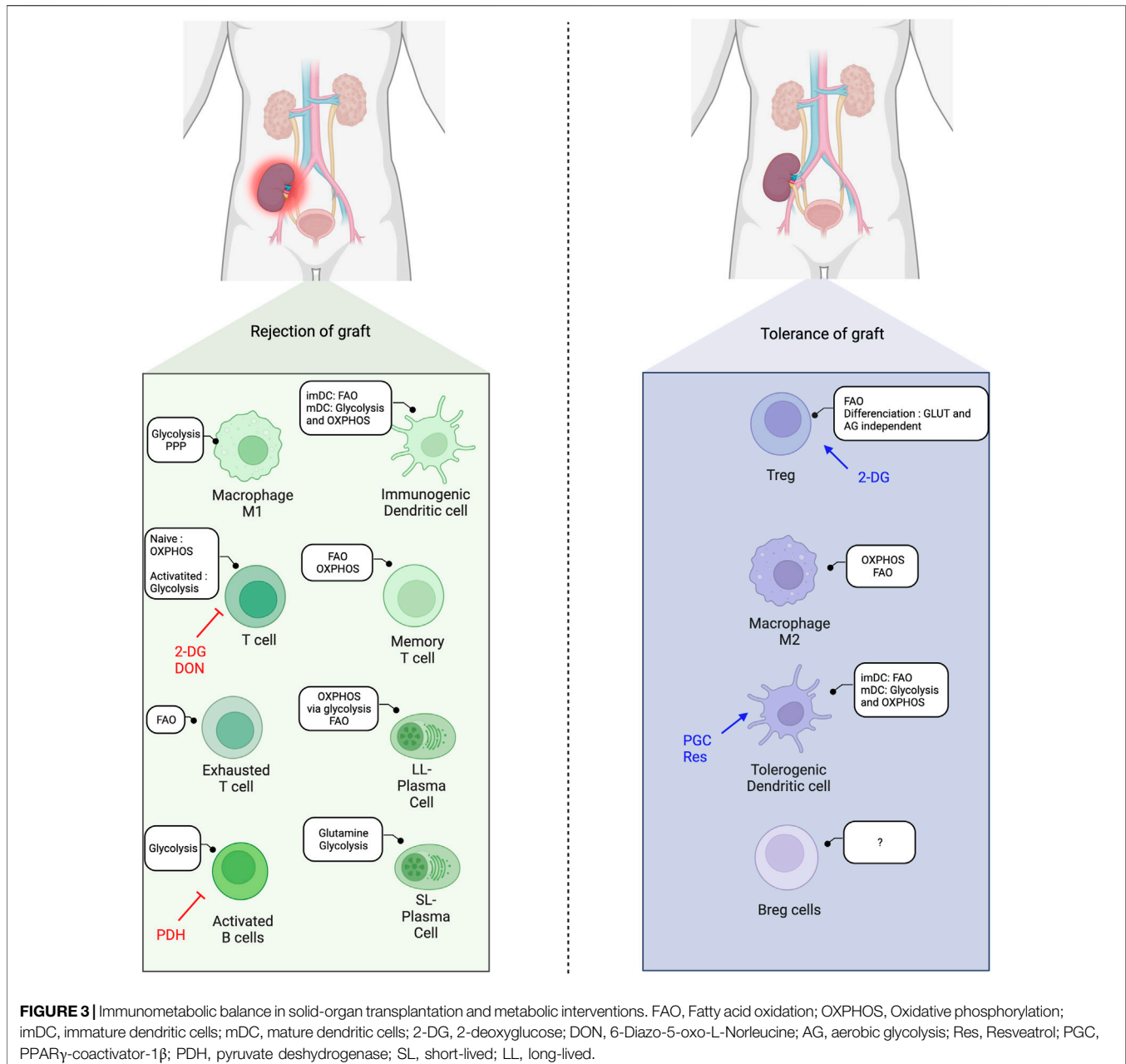
Activation of B cells trough B cell receptor (BRC) increases glucose and amino-acid uptake [110, 111]. However, glucose is not used for glycolysis, but for PPP and nucleotide synthesis [112]. OXPPOS and TCA cycle are augmented in activated B cells, but they are fueled by other source of energy than glucose, such as FAs [112, 113].

Following antigen activation, naïve germinal center B cells migrate into the follicle, where somatic hypermutation and antibody affinity maturation occur. During this process, B cells display a significant increase in OXPPOS activation [114].

Thereafter, B cells are transformed into short-lived plasma cells outside the lymphoid follicle and then into long-lived plasma cells and memory B cells inside the follicle. In plasma cells, the production of antibodies requires a high production of glutamate from glutamine pathway and a lower rate of glycolysis [115]. However, T-dependent long-lived plasma cells are characterized by a higher glucose and amino-acid uptake as compared to short-lived plasma cells [116].

IMMUNOMETABOLISM IN SOLID ORGAN TRANSPLANTATION

As the metabolism impacts the development and function of immune cells, alterations in metabolic pathways can modulate immune cell differentiation and subsequently affect the balance between pro-inflammatory and regulatory cells, and thus influence transplantation outcomes (Figure 3).



Modulation of Immune Metabolism in SOT

Few but very promising studies in murine transplant models highlight the impact of metabolic reprogramming on the alloimmune response [14].

In 2015, Lee *et al.* were able to modulate the alloimmune response in a fully mismatched murine model of skin and heart allograft transplantation by targeting metabolic pathways [117]. The authors showed that glycolysis inhibition using 2-DG and metformin hindered proliferation and cytokine production in activated T cells. Combination of 2-DG with the glutamine inhibitor 6-diazo-5-oxo-l-norleucine (DON) resulted in an even more important inhibition of alloreactive CD4⁺ T cell

proliferation and cytokine production, a decrease of acetyl-CoA levels and associated lipid synthesis, and a reduction of mTORC1 activation. The authors showed that in mice treated with 2-DG, metformin, and DON, CD4⁺ T cell kept their ability to differentiate into antigen-specific Foxp3⁺ CD4 T cells (Treg). Finally, the triple anti-metabolic therapy (2-DG, metformin, and DON), prolonged graft survival in a model of allogeneic skin and heart transplantation, while discontinuation of treatment led to rapid graft rejection.

Immune metabolism may be modulated at the translational level. Quiescent CD4⁺ T cells accumulate a large amount of non-translated mRNA encoding key metabolic enzymes, which can be

rapidly translated to activate aerobic glycolysis and FAO [76]. The engagement of the TCR triggers the translation of key proteins (GLUT1 and ACC1) for T cell activation and differentiation. Therefore, therapeutics that interfere with mRNA translation may affect alloimmunity by changing T cell metabolism.

Nian *et al.* showed that advanced age negatively impacts OXPHOS and glycolysis in naïve CD4⁺ T cells, and that glutaminolysis becomes the major source of ATP production later in life [118]. When the authors blocked OXPHOS with oligomycin, only CD4⁺ T cells from young mice were able to compensate for the metabolic loss with an increase in glycolysis. Interestingly, DON (glutaminolysis inhibitor) was able to inhibit IL-2, IFN- γ secretion, and cell proliferation in naïve CD4⁺ T cells from aged mice but not in naïve CD4⁺ T cells from young mice, which highlights the increasing reliance of naïve T cells on glutaminolysis with age. This approach prolonged graft survival and increased Tregs in a skin transplantation model in old mice. The results of DON on CD4⁺ T cells from aged mice were confirmed in human PBMC, suggesting its potential as a new age-dependent metabolic-mediated immunosuppression therapy [118].

Immune Metabolism Modulation in Combination With Costimulation Blockade

In 2020, Lee *et al.* used the combination of their triple anti-metabolic therapy (metformin, 2DG and DON) in association with a co-stimulatory blocker (CTLA4-Ig) [119]. Their model showed that CTLA4-Ig and metabolic inhibition have distinct but synergic effects on immune cells. They first showed that metabolic inhibition resulted in a higher inhibition of proliferation and promotion of apoptosis than CTLA4-Ig. Interestingly, in a model of skin allograft acute rejection in mice, they also showed prolonged graft survival with anti-metabolic drugs compared to CTLA4-Ig and controls. This may be explained by the costimulation-independent activation of memory T cells that CTLA4-Ig did not block. When metabolic inhibitors were added to CTLA4-Ig, there was an additive

inhibiting effect on T cell proliferation, T-bet expression, and cytokine secretion. Finally, CTLA4-Ig and metabolic inhibition were synergic in preventing skin and heart allograft loss in their mice transplantation model.

Moreover, CTLA4-Ig addition to metabolic inhibitors allowed long-term acceptance of heart allograft, which was not possible when anti-metabolic therapy was given alone [117]. Priyadharshini *et al.* proposed that a sequential with first metabolism blockade (2DG) associated with CTLA4-Ig may induce tolerance phase that could be maintained by adding secondarily mTOR inhibitors. This strategy could specifically increase the Treg and tolerogenic DC in the context of SOT [120].

CONCLUSION

Recent studies and newly developed technologies have paved the way for modifying cell metabolism to influence the immune cell response. A better understanding of metabolic pathways in immune cells in the context of transplantation may offer the possibility to modulate the alloimmune response by reprogramming their metabolism to reshape specific immune cell subsets toward tolerogenic profiles. Further insights into metabolic dysregulation in SOT hold great promise to design novel therapies to improve graft and patient outcomes.

AUTHOR CONTRIBUTIONS

JN wrote the first draft under ZM and PC's guidance. CA, PM, LR, and MF provided comments and edited the manuscript. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

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Characteristics of Delayed Graft Function and Long-Term Outcomes After Kidney Transplantation From Brain-Dead Donors: A Single-Center and Multicenter Registry-Based Retrospective Study

Amanda Ahlmark*, Ville Sallinen, Verner Eerola, Marko Lempinen and Ilkka Helanterä

Department of Transplantation and Liver Surgery, Abdominal Center, Helsinki University Hospital and University of Helsinki, Helsinki, Finland

Delayed graft function (DGF) after kidney transplantation is common and associated with worse graft outcomes. However, little is known about factors affecting graft survival post-DGF. We studied the association of cold ischemia time (CIT) and Kidney Donor Profile Index (KDPI) with the long-term outcomes of deceased brain-dead donor kidneys with and without DGF. Data from Finland ($n = 2,637$) and from the US Scientific Registry of Transplant Recipients (SRTR) registry ($n = 61,405$) was used. The association of KDPI and CIT with the graft survival of kidneys with or without DGF was studied using multivariable models. 849 (32%) kidneys had DGF in the Finnish cohort. DGF and KDPI were independent risk factors for graft loss, [HR 1.32 (95% CI 1.14–1.53), $p < 0.001$, and HR 1.01 per one point (95% CI 1.01–1.01), $p < 0.001$, respectively], but CIT was not, [HR 1.00 per CIT hour (95% CI 0.99–1.02), $p = 0.84$]. The association of DGF remained similar regardless of CIT and KDPI. The US cohort had similar results, but the association of DGF was stronger with higher KDPI. In conclusion, DGF and KDPI, but not CIT, are independently associated with graft survival. The association of DGF with worse graft survival is consistent across different CITs but stronger among marginal donors.

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*Correspondence

Amanda Ahlmark,
✉ amanda.ahlmark@helsinki.fi

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Keywords: kidney donor profile index, long-term outcome, delayed graft function, kidney transplant, cold ischemia time


INTRODUCTION


Delayed graft function (DGF) is still encountered in 20%–40% of all deceased donor kidney transplants, with higher frequencies being associated with expanded criteria donors [1–4]. DGF is considered to be the result of an ischemic-reperfusion injury, which arises during the procurement and subsequent cold storage of the graft as well as the reperfusion during implantation [5, 6]. DGF

Abbreviation: DBD, donation after brain death; DCD, donation after circulatory death; DGF, delayed graft function; EF, early function; CIT, cold ischemia time; KDPI, Kidney Donor Profile Index; HLA-MM, human leukocyte antigen mismatch; PRA, panel reactive antibodies; SRTR, Scientific Registry of Transplant Recipients.


Characteristics of Delayed Graft Function and Long-Term Outcomes after Kidney Transplantation from Brain-Dead Donors

Kidney Transplantations

 N = 2,637

 N = 61,405

Median Kidney Donor Profile Index

 Early graft function 54
Delayed graft function 63


Early graft function 43
Delayed graft function 53

Delayed Graft Function

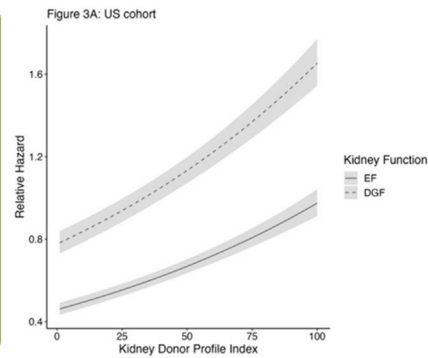
 32%

 27%

Median Cold ischemia time (h)

 Early graft function 19.6
Delayed graft function 21.5

Early graft function 15.6
Delayed graft function 18



The harmful association of delayed graft function with worse graft survival is increased with higher Kidney Donor Profile Index values but not with longer cold ischemia time.



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GRAPHICAL ABSTRACT

has been linked to worse graft survival rates [6, 7] and higher rates of acute rejection [6], although contradicting results also exist [8]. A meta-analysis found increased risk of graft failure, acute rejection, and mortality associated with DGF [9]. The most significant risk factors for DGF are increased donor age, increased kidney donor profile index (KDPI), and increased cold ischemia time (CIT) [3, 10–13].

The increasing demand for kidneys and the growing use of extended criteria kidneys underscores the importance of understanding the complex nature of DGF and factors affecting the long-term outcomes of kidneys with DGF, as the rate of DGF is reportedly increasing over time [3]. However, conclusive evidence on factors affecting the long-term outcomes among kidney transplants with DGF is still lacking. Furthermore, as most studies have a regional cohort that affects both donor and recipient characteristics, universal conclusions are difficult to reach. While the effect of acute rejection might have little cumulative effect on the outcomes of kidneys with DGF [14], it remains unclear whether the association of DGF with graft survival is similar among patients with longer CIT or higher KDPI. Some transplant programs, such as the Eurotransplant senior program, aim to minimize CIT among older kidney donors. It has been suggested that longer CIT would be more harmful in older donor kidneys or kidneys with poor quality [15], especially due to the occurrence of DGF. However, our recent study suggested that the effect of longer CIT is not more harmful among older donors or donors with high KDPI [16]. The role of pretransplantation biopsies has also been discussed in literature. The histologic findings might affect the allocation process, and a single-center study found that both the rate of DGF was higher,

and the graft survival was worse among kidneys with a suboptimal histological score [17].

This study aims to examine the association of DGF with graft survival using a national cohort from Finland and to study whether the association of DGF with graft survival differs in subgroups based on KDPI and CIT. Furthermore, the aim is to confirm these findings in a larger US cohort using data from the Scientific Registry of Transplant Recipients (SRTR).

MATERIALS AND METHODS

Study Population and Data Collection

This study was a retrospective observational registry analysis. The initial study population consisted of all adult (age >16 years) patients receiving deceased donor kidney transplants performed at Helsinki University Hospital (HUH), Finland from 12 May 2004 to 31 December 2019. HUH is the only transplantation center in Finland. Patients with primary nonfunction or graft loss within the first week after transplantation ($n = 73$, 2%) were excluded. In addition, living donor kidneys, pediatric recipients (age <16 years), and recipients of multiorgan transplants (total $n = 565$, 17%) were excluded. All donors were brain-dead donors, as donation after circulatory death (DCD) was not implemented in Finland during the study period. Machine perfusion was not used in Finland during the study period. Due to the definition of DGF (need for dialysis during the first post-transplant week), and because patients were not accepted to the waiting list pre-emptively in Finland during the study period, only patients who were on dialysis pretransplantation were included.

Patients and their pre-and post-transplant data were collected from the Finnish Kidney Transplant Registry, which is a national registry for the follow-up of kidney transplant patients obliged by law. Patients were followed until death, graft loss, or 31 December 2020.

In addition, this study used data from the Scientific Registry of Transplant Recipients (SRTR). The SRTR data system includes data on all donors, waitlisted candidates, and transplant recipients in the United States, submitted by the members of the Organ Procurement and Transplantation Network (OPTN). The Health Resources and Services Administration, US Department of Health and Human Services, provides oversight to the activities of the OPTN and SRTR contractors. To create a dataset similar to the Finnish data, only deceased brain-dead donor kidney-only transplant recipients between 01 January 2014 and 09 September 2019 with pretransplant dialysis treatment were included, i.e., donation after circulatory death (DCD) kidneys were excluded. Patients were followed until death, graft loss, or 9 September 2020.

Cases with missing data were excluded from the analyses, due to the low number of missing data (<3% in both cohorts).

This study was approved by the institutional review board of Helsinki University Hospital (HUS/115/2020) and SRTR. The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the Declaration of Istanbul on Organ Trafficking and Transplant Tourism.

Definitions

DGF was defined as the need for dialysis during the first seven postoperative days [18].

KDPI was calculated as described on the Organ Procurement and Transplantation Network website [19]. The KDPI values were calculated using 2019 KDPI reference values. For donors with unknown status of diabetes and/or hypertension, KDPI was calculated as instructed on the OPTN website [19].

Statistical Analyses

Categorical variables are described as the number of cases and percentages. As the distributions within either dataset were not normal, continuous variables are described as median and interquartile range. Mann-Whitney-U-test was used to assess statistical significance for differences in the continuous variables and Chi Square test was used for categorical data.

Kaplan-Meier survival curves were used to analyze graft survival, with both death with functioning graft and return to dialysis as outcomes. Differences between the studied groups were analyzed with the log-rank test. Multivariable Cox regression analysis was used to examine risk factors for graft loss. Sensitivity analyses were performed using death-censored graft loss as the binary outcome. Two-sided p -values <0.05 were considered statistically significant. Variables chosen for the multivariable model were earlier confirmed risk factors for DGF or variables significant in univariable models. When KDPI was included in the models, all the other donor factors used to calculate KDPI were left out of the model due to possible multi-collinearity (age, race, body mass index (BMI), history of hypertension, history of diabetes, and cause of death). Interactions

between CIT and DGF as well as KDPI and DGF were used to analyze whether the risk associated with DGF differed according to cold ischemia time or KDPI value. To account for clustered data due to the relationship between kidneys from the same donor, the Huber-White method served to adjust the standard errors of the regression coefficients and provide robust standard errors of the coefficients [20].

We assessed the validity of the Cox model by plotting the scaled Schoenfeld residuals for testing the proportional hazards assumption, using visualization of deviance residuals for checking influential outliers and testing for non-linearity. Restricted cubic splines were used to determine the nonlinearity of the associations and for plotting nonlinear associations between covariates and the outcome, as regression models require the assumption of linearity. Variables plotted by restricted cubic splines are reported as figures and p -values, and other variables are reported as hazard ratios with 95% confidence intervals.

Statistical analysis was performed using R (R Core Team, 2023), RStudio (Posit Team, 2023), and the R packages survival (Thernau, 2023), survminer (Kassambara, Kosinski, Biecek, 2021), ggplot2 (Wickham, 2016), gtsummary (Sjoberg, Whiting, Curry, Labery, Larmarange, 2021), and rms (Harrell, 2023).

RESULTS

Finnish Study Population

A total of 3,275 kidney transplants were performed during the period 12 May 2004 to 31 December 2019 in Finland. After excluding grafts that were lost during the first week, pediatric recipients, living donor kidney recipients, and recipients of multiorgan transplants, the final study population consisted of a total of 2,637 patients receiving kidney transplants, of which 865 (32%) had DGF. Demographic characteristics of the Finnish study population grouped by early function (EF) and DGF are presented in **Table 1**. DGF was more frequent among male recipients, recipients receiving kidneys from male donors, older recipients, and recipients receiving kidneys from older donors. KDPI was higher and CIT was longer among recipients with DGF. Recipients with DGF more often had one or several previous kidney transplants compared to recipients with EF.

Early Function vs. Delayed Graft Function in Finland

Graft survival estimates were significantly lower among recipients with DGF compared to recipients with EF in unadjusted analyses ($p < 0.001$, **Figure 1A**), with 10-year survival among patients with EF being 66% (CI 95% 63%–69%), and 51% (CI 95% 46%–55%) among patients with DGF. Additionally, the hazard ratio (HR) for graft loss or death for DGF in the univariable analysis was 1.53 (CI 95% 1.33–1.77, $p < 0.001$). In multivariable analysis (adjusted for CIT, KDPI, peak PRA >30%, previous kidney transplant, recipient age and sex, and recipient pre-transplant diabetes as well as accounting for clustering), DGF was independently associated with worse graft

TABLE 1 | Finnish cohort characteristics.

Variable	Kidney function		p-value ^b
	EF	DGF	
	N = 1,788 (68%) ^a	N = 849 (32%) ^a	
Donor age (years)	55 (44,64)	58 (50,65)	<0.001
Donor sex			0.019
Female	817 (46%)	346 (41%)	
Male	971 (54%)	503 (59%)	
Kidney Donor Profile Index	54.0 (31.0, 77.0)	63.0 (44.0, 82.0)	<0.001
Cold ischemia time (hours)	19.6 (16.2, 22.8)	21.5 (18.3, 24.3)	<0.001
HLA mismatch	3.0 (2.0, 3.0)	3.0 (2.0, 3.0)	0.282
Recipient age at transplant (years)	54 (43,62)	55 (46,63)	0.002
Recipient sex			0.013
Female	665 (37%)	273 (32%)	
Male	1,123 (63%)	576 (68%)	
Recipient diabetes	407 (23%)	220 (26%)	0.084
Recipient previous kidney transplant	182 (10%)	134 (16%)	<0.001
Recipient peak PRA	0.0 (0.0, 20.0)	0.0 (0.0,33.0)	0.001
Follow-up time (months)	61.3 (28.3,114.3)	73.7 (39.3,109.8)	0.004
Donor cause of death			<0.001
Anoxia	61 (3%)	18 (2%)	
Cerebral hemorrhage	1,027 (56%)	495 (57%)	
Stroke	64 (3%)	50 (6%)	
Trauma	432 (23%)	167 (19%)	
Other	258 (14%)	137 (16%)	

^aMedian (25%, 75%); n (%).

^bMann-Whitney test for continuous, Chi-square for categorical variables EF, early function; DGF, delayed graft function; HLA, human leukocyte antigens; PRA, panel reactive antibodies.

survival (HR 1.32, 95% CI 1.14–1.53; **Table 2**). The unadjusted analyses were also performed with death-censored graft loss as outcome, and the results remained similar (**Figure 1B**).

In the Finnish cohort, all variables met the proportional hazards assumption, and the associations of all continuous variables were linear except for recipient age. The association of KDPI was plotted as non-linear, even if the non-linearity *p*-value was non-significant, as the plotted model visualizes the association of KDPI better than an HR value.

Cold Ischemia Time in Finland

Longer CITs were not independently associated with worse graft survival in multivariable analysis (**Table 2**). In a plotted association of DGF with graft survival, the association remained similar regardless of CIT (**Figure 2A**). There was no significant interaction between CIT and DGF, *p* = 0.824. The survival rates of EF and DGF kidneys with CITs longer and shorter than 18 h can be found in **Supplementary Table S1**.

Kidney Donor Profile Index in Finland

KDPI was an independent risk factor for graft loss in multivariable analyses (**Table 2**). In a plotted prediction of the association of DGF with graft survival, the difference between EF and DGF kidneys remained similar regardless of KDPI value. The association of higher KDPI was similar in both graft function groups (**Figure 2B**). There was no significant interaction between KDPI and DGF, *p* = 0.217. DGF kidneys with KDPI values ≥85 had the worst survival rates, while EF kidneys with KDPI ≥85 and DGF kidneys with KDPI <85 had similar survival

rates; the survival rates of EF and DGF kidneys based on KDPI can be found in **Supplementary Table S1**.

Validations of Results With SRTR Data

Altogether, 94,154 kidney-only transplantations were performed from deceased donors in the US between 01 January 2014 and 09 September 2019. From these the following groups were excluded: pre-emptive transplantations (*n* = 10,782), <20 years old (*n* = 3,812), primary non-function (*n* = 312), DCD donors (*n* = 17,840) and cases with missing data (*n* = 3), resulting in a final cohort of 61,404 kidney transplantations. The characteristics of the SRTR cohort are presented in **Table 3**.

DGF occurred in 26,674 recipients (27%). Graft survival estimates were significantly lower among recipients with DGF compared to recipients with EF in unadjusted analyses (**Figures 1C, D**). In a multivariable model, (adjusted for CIT, KDPI, previous kidney transplant, recipient sex, recipient age, recipient diabetes and use of machine perfusion), DGF was an independent risk factor for graft loss (HR 1.63, 95% CI 1.48–1.80; **Table 4**).

The association of recipient age were nonlinear and thus modeled with restricted cubic splines. Other continuous variable associations were linear.

As there was a violation of the proportionality assumption of the onset of graft function (*p* < 0.001), CIT (*p* = 0.01) and recipient diabetes (*p* = 0.007), Schoenfeld residuals and Kaplan-Meier curve were assessed and deemed acceptable; the plotted Schoenfeld residuals can be found in **Supplementary Figure S1**. The association of KDPI with worse graft survival was stronger in

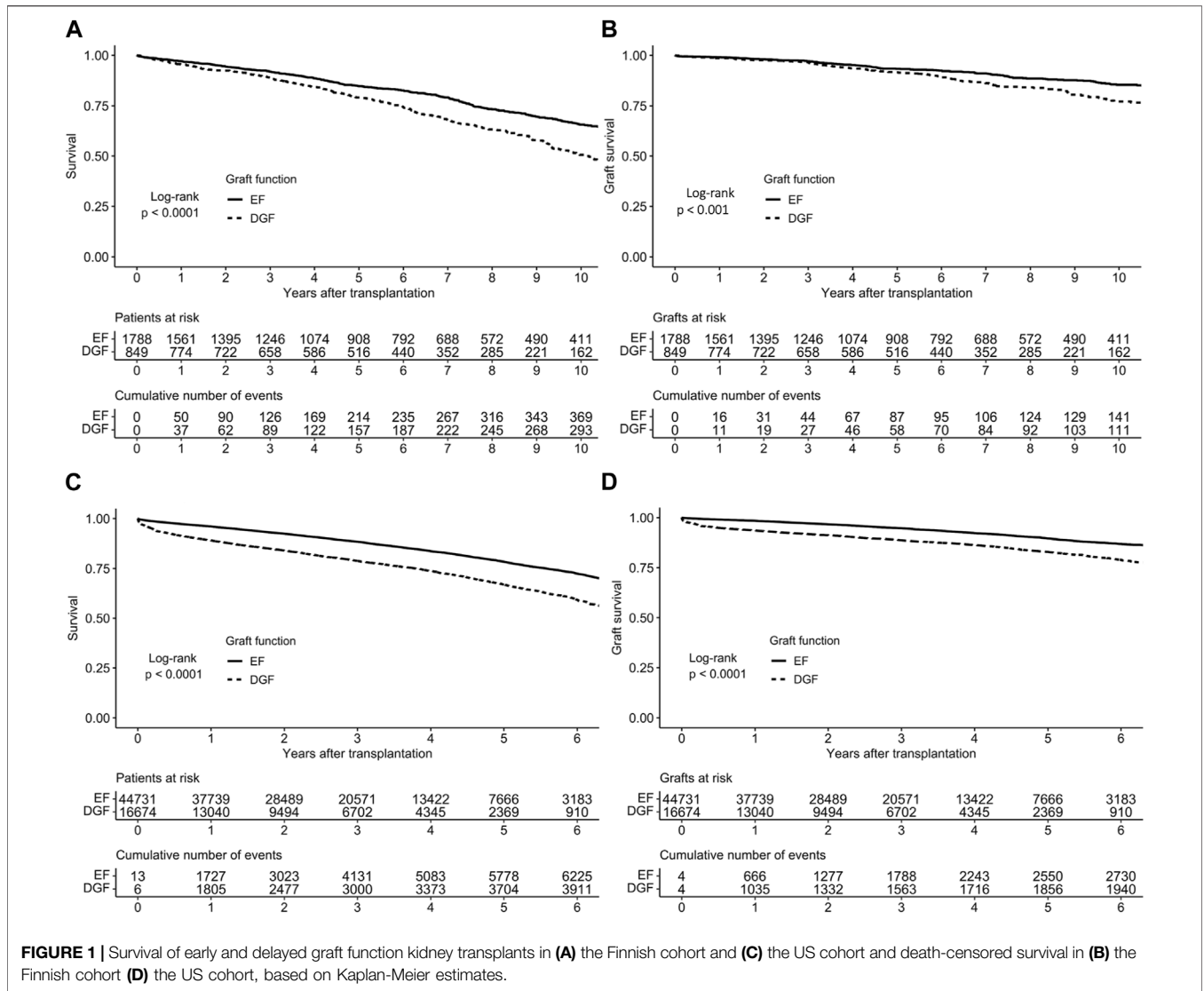


TABLE 2 | Multivariable Cox regression results for time to graft loss, Finnish cohort (N = 2,637).

Characteristic	HR	95% CI	p-value
Delayed graft function	1.32	1.14, 1.53	<0.001
Cold ischemia time (per hour)	1.00	0.99, 1.02	0.84
Kidney Donor Profile Index (per one point)	1.01	1.01, 1.01	<.001
Recipient diabetes	1.96	1.69, 2.28	<0.001
Recipient age (per year)	Not applicable ^a		0.015 ^b
Recipient sex (male)	1.17	1.00, 1.36	0.05
Recipient peak PRA over 30%	1.08	0.86, 1.35	0.52
Previous kidney transplant	1.61	1.24, 2.08	<0.001

HR, hazard ratio; CI, confidence interval; PRA, panel reactive antibodies.

^aNot applicable due to non-linearity.

^bp-value for non-linearity.

DGF kidneys with high KDPI values, compared to EF kidneys (Figure 3A). There were no significant interactions between CIT and DGF ($p = 0.051$), KDPI and DGF ($p = 0.571$) or machine

perfusion and DGF ($p = 0.814$). Although linear, the associations of KDPI and CIT on graft survival were also plotted as the plotted model visualizes the associations better than an HR value (Figures 3A, B).

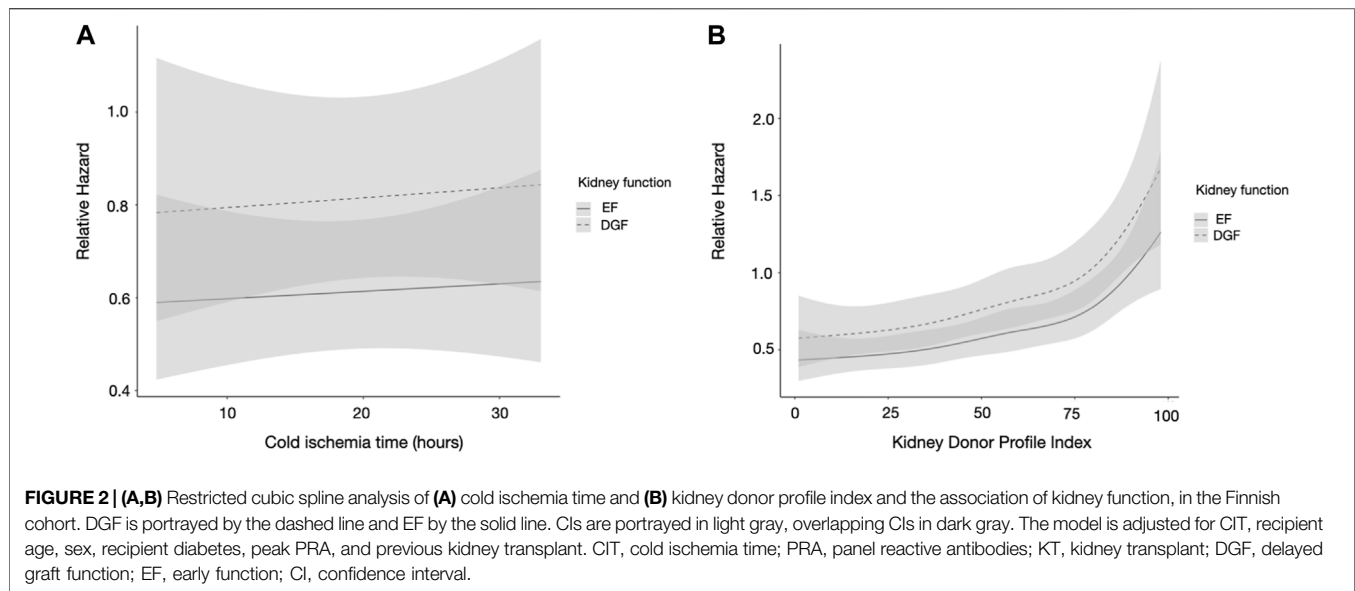
Survival rates of EF and DGF kidneys in the US cohort based on KDPI values and CIT can be found in **Supplementary Table S2**.

Death-Censored Graft Survival Analyses

Multivariable regression analyses were also performed with death-censored graft loss as the outcome, and the results remained similar in the Finnish cohort (Supplementary Table S3; Figure 2), which suggests that other causes of death are not large confounders. Similar results were found in the SRTR cohort (Supplementary Table S4; Supplementary Figures S3, S4).

Regression Model Based on Time-Splitting

A time-splitting was also made because of the violations of the proportional hazards assumption in the US cohort, and the



associations of CIT and KDPI on graft survival were assessed during the first follow-up year as well as the time after the first year separately. No large differences were found (**Supplementary Figures S5–S8**).

DISCUSSION

In our study, we found that DGF kidneys have worse graft survival compared to EF kidneys, as expected. The harmful association of DGF with graft survival remained similar regardless of CIT length. This, along with the non-significant interactions between CIT and DGF, suggests that the harmful effect of DGF is not increased when CIT increases. These findings are also supported by an earlier study [21].

KDPI was found to be an independent risk factor for graft loss in multivariable analyses, as expected. In plotted predictions of the association of DGF with graft survival as a function of KDPI, the association of DGF remained similar. No significant interaction between DGF and KDPI could be found in the Finnish cohort.

The findings of the US cohort support the findings from the Finnish cohort that the risk of graft loss associated with DGF is similar in various CIT lengths. However, in the US cohort, the association of DGF on graft survival as a function of KDPI shows a stronger association of DGF with high KDPI. No significant interaction between DGF and KDPI could be found. The difference seen is not as noticeable in the Finnish cohort, which partly could be explained by the smaller cohort.

Many transplanted kidneys to this day still suffer from DGF and thus it is essential that the causes of DGF are understood and that routines to minimize other factors affecting the long-term outcomes, such as acute rejection, are used. With a greater understanding of the process of DGF, transplantation procedures, and pre- and post-operative care can be planned most beneficially.

Previous studies have concluded that DGF is associated with worse graft survival [6, 7] and increased mortality [9, 22]. In both our cohorts we recorded worse graft survival for DGF kidneys.

Longer CIT has been identified as a risk factor for DGF [3, 10, 11, 16], and increased CIT has also been associated with higher risks for graft failure in some studies [23, 24], but not in others [21]. In our current study, CIT was not an independent risk factor for graft loss. The differences between study results remains somewhat unclear, possibly different analytical strategies (CIT as a continuous variable or categorized) might explain some of the discrepancies. However, as CIT has been recognized as a risk factor for DGF, protocols designed to reduce the CIT are also beneficial to reduce the risk of graft loss, as well as the size of costs and length of hospital stays, since DGF has been associated with poor graft survival, higher costs, and longer hospital stays [25]. One study also described DGF leading to a more complex post-operative course for the patient [6].

Higher KDPI values have been associated with increased risk for DGF [3, 10–12]. The effects of KDPI on graft survival have been studied using mixed cohorts of both EF and DGF kidneys, and a few studies could be found where the impact of KDPI had been studied on a DGF population [7, 26]. One of these studies showed that KDPI >85% was associated with worse outcomes in both EF and DGF kidneys which was confirmed in our study of a larger cohort [7]. As donors with KDPI >85% have been compared to the earlier used designation extended criteria donors [12], a worse graft survival estimate of these kidneys is in line with earlier research. Another study showed increased risk of graft loss in DGF kidneys with kidney donor risk index >1 [26]. One study examined the risk of DGF and graft loss in standard vs. extended criteria donors from both brain-dead donors and DCD donors. This study found that the DGF risk was increased in extended criteria DCD donations compared to standard criteria DBD donations, but did not find a difference in the risk of graft loss in any group compared to standard criteria DBD donations [27].

TABLE 3 | US cohort characteristics.

Variable	Kidney function		p-value ^b
	EF	DGF	
	N = 44,731 (73%) ^a	N = 26,674 (27%) ^a	
Donor age (years)	37 (25,50)	42 (30,53)	<0.001
Donor sex			0.034
Female	18,030 (40%)	6,563 (39%)	
Male	26,701 (60%)	10,111 (61%)	
Kidney Donor Profile Index	43.0 (22.0, 66.0)	53.0 (33.0, 73.0)	<0.001
Cold ischemia time (hours)	15.6 (10.4, 21.5)	18.0 (12.4, 24.2)	<0.001
HLA mismatch	4.0 (3.0, 5.0)	4.0 (4.0, 5.0)	<0.001
Recipient age at transplant (years)	54 (42,63)	56 (46,64)	<0.001
Recipient sex			<0.001
Female	18,728 (42%)	5,431 (33%)	
Male	26,003 (58%)	11,243 (67%)	
Recipient diabetes	15,152 (34%)	7,252 (44%)	<0.001
Recipient previous kidney transplant	5,883 (13%)	2,233 (13%)	0.442
Follow up time (months)	35.2 (17.7,53.2)	29.4 (12.3,48.2)	<0.001
Donor cause of death			<0.001
Anoxia	17,378 (39%)	7,064 (42%)	
Cerebrovascular/stroke	11,443 (26%)	5,179 (31%)	
Head trauma	14,648 (33%)	3,998 (24%)	
CNS Tumor	207 (0%)	62 (0%)	
Other	1,055 (2%)	371 (2%)	
Donor serum creatinine	0.9 (0.7–1.3)	1.2 (0.8–2.2)	<0.001
Donor ethnicity			<0.001
Asian	1,114 (2%)	519 (3%)	
Black	7,255 (16%)	2,639 (16%)	
Multi	175 (0%)	85 (1%)	
Native	284 (1%)	89 (1%)	
Pacific	157 (0%)	62 (0%)	
White	35,756 (80%)	13,280 (80%)	
Recipient ethnicity			<0.001
Asian	3,318 (7%)	1,239 (7%)	
Black	15,399 (34%)	6,653 (40%)	
Multi	337 (1%)	115 (1%)	
Native	436 (1%)	205 (1%)	
Pacific	236 (1%)	83 (0%)	
White	25,005 (56%)	8,379 (50%)	

^aMedian (25%, 75%); n (%).

^bMann-Whitney test for continuous, Chi-square for categorical variables EF, early function; DGF, delayed graft function; HLA, human leukocyte antigens.

TABLE 4 | Multivariable Cox regression results for time to graft loss, US cohort (N = 60,919).

Characteristic	HR	95% CI	p-value
Delayed graft function	1.63	1.48, 1.80	<0.001
Cold ischemia time (per hour)	1.00	1.00, 1.00	0.228
Kidney donor profile index (per one point)	Not applicable ^a		< 0.001 ^b
Recipient diabetes	1.29	1.24, 1.35	<0.001
Recipient age (per year)	Not applicable ^a		<0.001 ^b
Recipient sex (male)	1.10	1.05, 1.14	<0.001
Machine perfusion	1.08	1.04, 1.12	<0.001
Previous kidney transplant	1.14	1.08, 1.21	<0.001

HR, hazard ratio; CI, confidence interval.

^aNot applicable due to non-linearity.

^bp-value for non-linearity.

As the study is retrospective, there are several limitations to the study. Data that was missing was excluded, instead of using imputation, as the number of cases with missing data

was very low. The Finnish cohort is smaller than the US cohort, which could lead to a risk of under-powered results in the Finnish cohort. We chose to include two different cohorts for better generalizability, as studies have shown that differences exist in graft survival between different countries [28–30], and also the deceased donor characteristics are different between the US and Europe, with older donors increasingly utilized in Europe. Furthermore, relating to the retrospective nature of the study, the cause and effect cannot be proven, and only associations between DGF and CIT as well as KDPI could be shown. Efforts to minimize potential bias and confounding were made, by using two different cohorts as well as analyzing the data with multivariable regression models. The associations studied are complex, and DGF is not a clean confounder and can work as a mediator as well. Using a large cohort helps with both minimizing bias and confounding. Graphical visualization aids in showcasing these complex

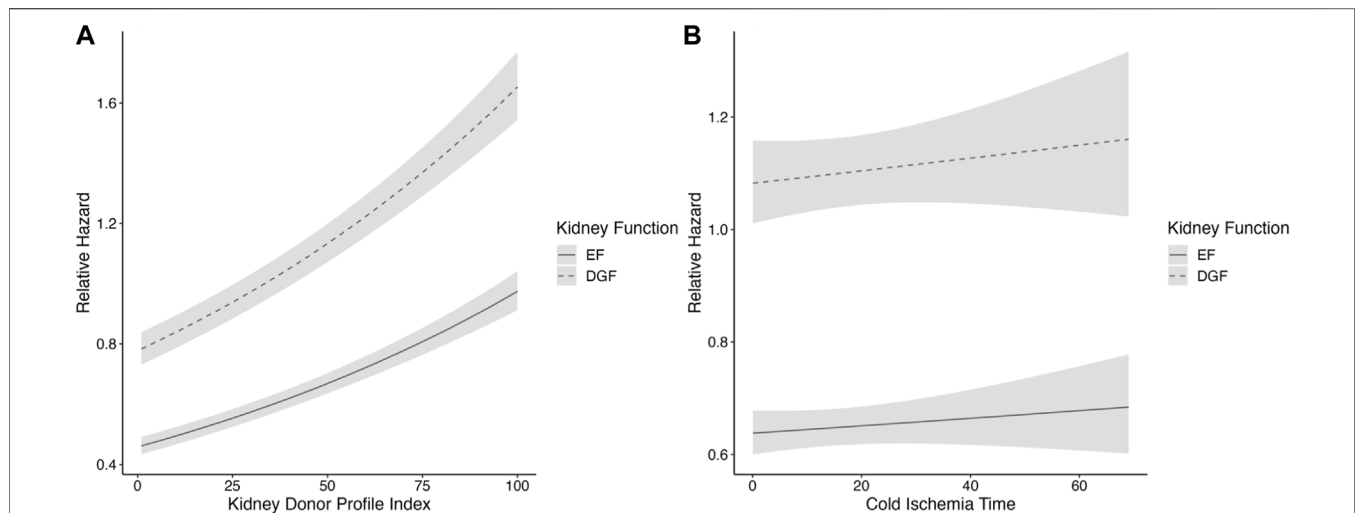


FIGURE 3 | (A,B) Restricted cubic spline analysis of **(A)** kidney profile index and **(B)** cold ischemia time and the association of kidney function, in the SRTR cohort. DGF is portrayed by the dashed line and EF by the solid line. CIs are portrayed in light gray. The model is adjusted for CIT, recipient age, sex, recipient diabetes, machine perfusion, and previous kidney transplant. CIT, cold ischemia time; PRA, panel reactive antibodies; KT, kidney transplant; DGF, delayed graft function; EF, early function; CI, confidence interval.

associations. In our study we focused on DBD kidneys as they are still the majority of transplantations, but it is also noteworthy that DCD donations are increasing in clinical practice and the risk of DGF is much higher among DCD kidneys. Studying and understanding the risks regarding DGF and graft survival in DCD kidney transplantation would be highly important in the future.

Since the increasing demand for kidneys drives allocation processes to use extended criteria donors, knowledge of potential increased risks is important. Although higher KDPI values are associated with a greater risk for graft loss, the risk of graft loss associated with DGF remained similar in a wide range of KDPI values in our study, suggesting that other aspects of the transplantation process play a role in the long-term outcomes of kidney transplants as well.

In conclusion, our study shows that the association of DGF with graft survival does not change with CIT length and that the association of DGF is higher among kidneys with higher KDPI values.

To meet the future demand for kidneys and make the most of the available kidneys in the allocation process, further knowledge of the nature of DGF and factors affecting the long-term outcomes of kidneys with DGF is necessary. For example, understanding the histological and molecular changes in kidneys with DGF could help in understanding the risks following DGF, and could further facilitate the use of marginal kidneys for the benefit of wait-listed patients.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Access to the Finnish datasets is not readily available

as it is limited by national regulations and restrictions regarding sharing transplant patient data. Access to the US dataset is subject to limitations outlined in the current data use agreements with SRTR. Requests to access these datasets should be directed to SRTR <https://www.srtr.org/contact-us/contact-form/>.

ETHICS STATEMENT

The studies involving humans were approved by the institutional review board of Helsinki University Hospital (HUS/115/2020) and Scientific Registry of Transplant Recipients. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

All authors contributed to the design of the study. AA and VE collected data. AA conducted data analysis, contributed to the discussion, wrote, and revised the manuscript. AA, VS, VE, and IH wrote, contributed to the discussion, reviewed, and revised the manuscript. ML reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

IH holds research grants from Hansa Biopharma, and MSD and has received consultancy fees from AstraZeneca, Hansa Biopharma, Takeda, and MSD.

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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The data reported here have been supplied by the Hennepin Healthcare Research Institute (HHRI) as the contractor for the Scientific Registry of Transplant Recipients (SRTR). The interpretation and reporting of these data are the responsibility of the author(s) and in no way should be seen as an official policy of or interpretation by the SRTR or the U.S. Government. The graphical abstract was created with Biorender.com.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2024.12309/full#supplementary-material>

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Metabolic Syndrome and Heart Transplantation: An Underestimated Risk Factor?

Sandro Sponga^{1,2*†}, Igor Vendramin^{2†}, Veronica Ferrara¹, Michela Marinoni¹, Giulia Valdi¹, Concetta Di Nora², Chiara Nalli², Giovanni Benedetti², Daniela Piani², Andrea Lechiancole², Maria Parpinel¹, Uberto Bortolotti² and Ugolino Livi^{1,2}

¹Department of Medicine (DAME), University of Udine, Udine, Italy, ²Cardiothoracic Department, University Hospital of Udine, Udine, Italy

Metabolic Syndrome (MetS), a multifactorial condition that increases the risk of cardiovascular events, is frequent in Heart-transplant (HTx) candidates and worsens with immunosuppressive therapy. The aim of the study was to analyze the impact of MetS on long-term outcome of HTx patients. Since 2007, 349 HTx patients were enrolled. MetS was diagnosed if patients met revised NCEP-ATP III criteria before HTx, at 1, 5 and 10 years of follow-up. MetS was present in 35% of patients pre-HTx and 47% at 1 year follow-up. Five-year survival in patients with both pre-HTx (65% vs. 78%, $p < 0.01$) and 1 year follow-up MetS (78% vs 89%, $p < 0.01$) was worst. At the univariate analysis, risk factors for mortality were pre-HTx MetS (HR 1.86, $p < 0.01$), hypertension (HR 2.46, $p < 0.01$), hypertriglyceridemia (HR 1.50, $p=0.03$), chronic renal failure (HR 2.95, $p < 0.01$), MetS and diabetes at 1 year follow-up (HR 2.00, $p < 0.01$; HR 2.02, $p < 0.01$, respectively). MetS at 1 year follow-up determined a higher risk to develop Coronary allograft vasculopathy at 5 and 10 year follow-up (25% vs 14% and 44% vs 25%, $p < 0.01$). MetS is an important risk factor for both mortality and morbidity post-HTx, suggesting the need for a strict monitoring of metabolic disorders with a careful nutritional follow-up in HTx patients.

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*Correspondence

Sandro Sponga,
✉ sandro_sponga@yahoo.com

[†]These authors have contributed equally to this work

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Keywords: heart transplantation, metabolic syndrome, cardiac allograft vasculopathy, long-term mortality, long-term outcome

INTRODUCTION

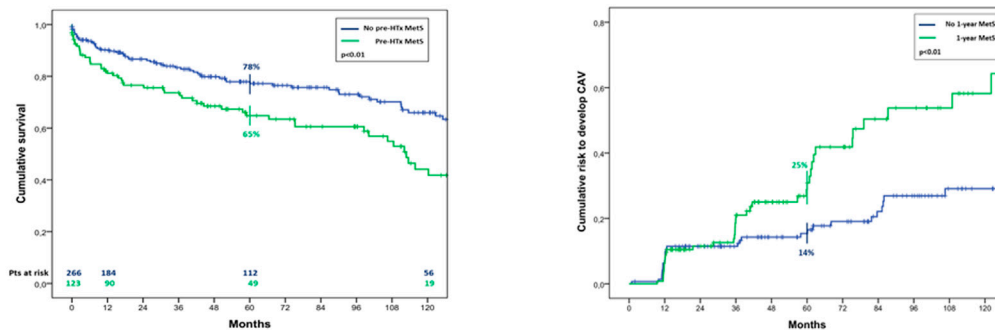
The Metabolic Syndrome (MetS) is a multi-factorial condition, explained as the association of several cardiovascular risk factors, including elevated glucose, hypertension, abdominal obesity and dyslipidemias that cluster in the same subject. The physiopathological process of its development is complex, but insulin resistance and abdominal obesity play a key role [1]. The prevalence of MetS in the general population varies from 18% to 39%, depending on the diagnostic criteria used, demographic, and racial differences, and this condition is correlated to an enhanced risk to develop chronic related diseases, such as cancer, neurological disorders and cardiovascular diseases [2, 3]. In particular, the presence of MetS has been associated with a twofold increase in the

Abbreviations: BMI, Body Mass Index; CAV, Coronary Allograft Vasculopathy; CKD, Chronic Kidney Disease; CMV, Cytomegalovirus; COPD, Chronic Obstructive Pulmonary Disease; Cys, Cyclosporine; DCM, Dilated Cardiomyopathy; DM, Diabetes Mellitus; HDL, High-Density Lipoprotein; HTN, Hypertension; HTx, Heart Transplantation; ICM, Ischemic Cardiomyopathy; MetS, Metabolic Syndrome; MMF, Mycophenolate Mofetil; TGL, Triglyceride.

Metabolic syndrome and heart transplantation: an underestimated risk factor?

Metabolic syndrome (MetS), a multifactorial condition that increases the risk of cardio-vascular events, is frequent in heart-transplant (HTx) candidates and worsens with immunosuppressive therapy

Since 2007, 349 HTx patients were enrolled. MetS was present in 123 (35%) of patients pre-HTx and in 131 (47%) at 1-year follow-up



At univariable analysis pre-HTx MetS was a risk factor for mortality (HR 1.86, 1.29-2.69, $p < 0.01$) and for CAV (HR 1.86, 1.16-2.99, $p = 0.01$)

MetS is an important risk factor for both mortality and morbidity post-HTx, suggesting in addition the need for a strict monitoring of metabolic disorders in HTx patients during follow-up.



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GRAPHICAL ABSTRACT

risk of development of cardiovascular disease, cardiovascular mortality, and nonfatal acute myocardial infarction and stroke, and a 1.5-fold increase in all-cause mortality [4].

Despite many advances in patients' management and pharmacological treatment, MetS represents a real burden in heart transplanted (HTx) patients, mainly due to the side effects of immunosuppressive therapy, which severely affects their long-term outcomes. Moreover, the biochemical features of MetS have been strongly related to the presence and progression of the cardiac allograft vasculopathy (CAV), a peculiar complication after HTx, characterized by a diffuse intimal hyperplasia and fibrosis related to chronic rejection, but also to cardiovascular risk factors, including hypertension, diabetes or dyslipidemia [2, 5]. In literature, the prevalence of MetS in HTx patients is reported to be around 40%, but these previous studies involved a limited number of patients in limited follow-ups [4].

The aim of the present study was to assess the prevalence of MetS in HTx patients of University Hospital of Udine over 10 years of follow-up, and to evaluate the impact on the long-term outcome in terms of morbidity and mortality.

MATERIALS AND METHODS

Study Design, Patient Population and Data Collection

From January 2007 to September 2021, 349 subjects underwent HTx at the University Hospital of Udine and were enrolled in this

retrospective observational study. Data were collected from clinical informatic system and patient charts, considering 4 timepoints: before HTx surgery (baseline); at 1, 5 and 10 years of follow-up after HTx.

At baseline timepoint, demographic and clinical pre-HTx data were collected.

At the follow-up timepoints, long-term outcome and mortality, laboratory tests parameters, including a complete blood count, fasting blood glucose, lipid profile, renal function, echocardiogram exam parameters, drugs therapy, anthropometric measures and blood pressure values were collected.

The present study was approved by the local Institutional Review Board (code 17_2020) and informed consent was obtained as required by the study authorizing entity.

Follow-Up and Immunosuppression Therapy

The postoperative and long-term follow-up protocol for HTx patients included endomyocardial biopsies made every week during the first month, every 15 days in months 2 and 3, and monthly or bimonthly up to 12 months, and if required thereafter. Coronary angiography was performed at the first year and every 2 years afterwards or on clinical requirement. Clinical follow-up was conducted by a dedicated team including a cardiac surgeon, a cardiologist, a nurse and a psychologist every 15 days during the first 3 months, every month between months 3 and 12, every 3 months between 1 and 3 years, every 4 months between 3 and

5 years and every 6 months after 6 years from transplantation [6]. At each postoperative control, right and left ventricular function and morphology were evaluated by transthoracic 2D-Echo.

During clinical evaluation, adherence to immunosuppressive treatment was also verified, and therapy modified or titrated according to case-specific conditions. The first-line immunosuppression included cyclosporine (Cys) or Tacrolimus, mycophenolate mofetil (MMF), and corticosteroids in all patients. Everolimus was administered instead of MMF in case of patients with diagnosis of CAV. All recipients received induction therapy with antithymocyte globulins, whenever possible. A standardized protocol for corticosteroid withdrawal, within 6 months after HTx, and Cys serum concentration lowering was applied guided by serial endomyocardial biopsies coupled with clinical and laboratory findings [7].

Metabolic Syndrome Diagnosis

According to modified, revised NCEP-ATP III (Third Report of the National Cholesterol Education Program) criteria [1], diagnosis of MetS was made when the patient met at least three of the following criteria:

- Triglycerides (TGL) levels ≥ 150 mg/dL or drug treatment for hypertriglyceridemia
- High-density lipoprotein (HDL)-C < 40 mg/dL in men and < 50 mg/dL in women or drug treatment to raise HDL-C levels
- Diabetes mellitus (DM) and treatment for elevated glucose or fasting glucose levels ≥ 100 mg/dL
- Blood pressure $\geq 130/85$ mmHg or antihypertensive drug treatment
- Waist circumference > 102 cm in men and > 88 cm in women.

This latter parameter was substituted with body mass index (BMI) > 30 as the cut-off point for obesity. This substitution was already used also in other papers [4].

The diagnostic criteria used for MetS in this study have been used in many different studies associating MetS with cardiovascular disease in both the general population and in HTx recipients [8, 9].

Definitions

Cardiac allograft vasculopathy (CAV) was diagnosed by angiography and defined according to the ISHLT classification [10]. Infections were registered as any episodes requiring antibiotic treatment. Malignancies included both hematological or involving solid organs. Rejection grade were calculated as described by Stewart et al. [11].

Chronic kidney disease (CKD) was defined as stage 4 CKD according to an eGFR < 30 mL/min/1.73mq, calculated through EPI-CKD equations.

Statistical Analysis

Categorical variables were expressed as absolute frequency and percentage and quantitative variables as mean \pm standard or

TABLE 1 | Baseline recipients' data.

N. patients	349
Mean Age, years	56 \pm 11
Male/Female, n (%)	283 (81)/66 (19)
Aetiology	
DCM, n (%)	168 (48)
ICM, n (%)	97 (28)
Other, n (%)	79 (23)
Re-HTx, n (%)	19 (5)
Previous CCH, n (%)	127 (36)
Smoking, n (%)	137 (39)
Active smoker, n (%)	62 (18)
Former smoker, n (%)	75 (21)
COPD, n (%)	13 (4)
Chronic Renal Failure, n (%)	112 (32)
Atrial fibrillation, n (%)	57 (16)
PM/ICD, n (%)	110 (32)
MCS, n (%)	72 (21)
Median LVEF, %	27 (20–35)
Mean sPAP, mmHg	43 \pm 1

CCH, cardiac surgery; COPD, chronic obstructive pulmonary disease; DCM, dilated cardiomyopathy; ICD, implantable cardioverter-defibrillator; ICM, ischemic cardiomyopathy; HTx, heart transplantation; LVEF, Left Ventricular Ejection Fraction; MCS, Mechanical Circulatory Support; PM, pacemaker; sPAP, systolic pulmonary artery pressure.

median (interquartile range) according to data distribution, after performing the Kolmogorov-Smirnov test for normality.

Overall survival was estimated using the Kaplan–Meier method (log-rank test). Cox-regression model estimated factors independently associated with long-term mortality and grade CAV. A difference was considered statistically significant if $p < 0.05$. All statistics were performed using the Statistical Package for Social Sciences (SPSS) program (Chicago, IL, USA).

RESULTS

During the study period, 349 patients underwent HTx at our center. Baseline recipients' data about the period before HTx are reported in **Table 1**. The mean age was 56 \pm 11 years and 81% were men. The primary indication for HTx was dilated cardiomyopathy (DCM) in 48% of patients, followed by ischemic cardiomyopathy (ICM) in 28%, and other diseases in 23%. Smoking was present in 39% of patients, with 18% active smoker and 21% formers. Thirteen percent had chronic obstructive pulmonary disease (COPD), 32% had chronic renal failure and 16% atrial fibrillation at the time of surgery.

During a median follow-up of 53 (16–112) months, late mortality was 30%. Most common complications were infection episodes in 32% of patients, acute rejection grade ≥ 2 in 24%, malignancies in 19%, Cytomegalovirus (CMV) infection in 17%, renal failure grade ≥ 4 in 15% and CAV grade ≥ 2 in 9% (**Table 2**).

Figure 1 and **Table 3** shows the patients' immunosuppressive treatment during follow-up. At the first year after HTx the most frequent combination of immunosuppression medications was

TABLE 2 | Long-term outcome.

N. patients	332
Median follow-up, months	53 (16–112)
Late mortality, n (%)	100 (30%)
Acute rejection grade ≥ 2 , n (%)	80 (24%)
Infection, n (%)	106 (32%)
CMV infection, n (%)	55 (17%)
Malignancies, n (%)	63 (19%)
CAV grade ≥ 2 , n (%)	30 (9%)
Renal failure grade ≥ 4 , n (%)	48 (15%)

CAV, cardiac allograft vasculopathy; CMV, Cytomegalovirus.

Cys + MMF with corticosteroids (31%) or without (29%). At 5 and 10 years of follow-up, the Cys + MMF combination therapy remained the most prescribed treatment (44% and 48% at 5 and 10 year follow-up, respectively), followed by a progressive increase in the Cys + Everolimus prescription (29% and 33% at 5 and 10 year follow-up, respectively). The use of corticosteroids decreased over the time, according to our center protocol, shifting from a 53% of patients at the first year after HTx, in combination with the other immunosuppressive drugs, to a 19% and a 15% at 5 and 10 years of follow-up, respectively.

Metabolic Syndrome Prevalence

As regard the prevalence of MetS, 35% of patients already satisfied the criteria for the diagnosis before HTx. During the follow-up, this percentage steadily grew, with 47% of patients at the first year after HTx, 52% at 5 years and 46% at 10 years of follow-up. In particular, among the 131 patients with MetS at 1 year after HTx, only 60 (46%) had MetS before HTx too.

Focusing on the singular criteria, half of the patients (50%) had TGL ≥ 150 mg/dL or was prescribed with treatment for

hypertriglyceridemia before HTx, while this number increased during the follow-up, with 92% of patients at 1 year of follow-up, 89% at 5 years and 93% at 10 years. Similarly, 34% of patients had hypertension (HTN) or took an anti-HTN treatment prior to HTx, but the percentage reached 86% at 1 year of follow-up, 90% at 5 years, and 91% at 10 years. As regard obesity, 12% of patients had a BMI >30 before HTx, while within the first year after HTx obese patients were 19%, 25% at 5 years and 20% at 10 years. DM and glucose blood level appeared, instead, to be halved: while 61% of patients had DM or fasting hyperglycemia pre-HTx, at 1, 5 and 10 years of follow-up the frequencies were respectively 35%, 43% and 38%. Finally, also low HDL blood level, presented in 34% of patients before HTx, resulted decreased during the follow-up, with 18% of patients at 1 year, 20% at 5 years and 16% at 10 years (Table 4).

Mortality and Morbidity Predictors

The overall survival in patients with MetS before HTx appeared significantly worst, resulting of $81\% \pm 4\%$ vs. $90\% \pm 2\%$, $65\% \pm 5\%$ vs. $78\% \pm 3\%$ and $44\% \pm 6\%$ vs. $66\% \pm 4\%$ ($p < 0.01$) at 1, 5, and 10 years of follow up in patients with and without pre-HTx MetS, respectively (Figure 2). Similar results were found also in patients with MetS at the first year of follow-up, with a survival of $78\% \pm 4\%$ vs. $89\% \pm 3\%$ and $57\% \pm 6\%$ vs. $75\% \pm 5\%$ ($p < 0.01$) at 5 and 10 years of follow up in patients with and without MetS at 1 year follow-up, respectively (Figure 3).

At the univariate analysis, risk factors for mortality were recipient age (HR 1.07, 1.04–1.09, $p < 0.01$), pre-HTx MetS (HR 1.86, 1.29–2.69, $p < 0.01$), pre-HTx HTN (HR 2.46, 1.70–3.55, $p < 0.01$), pre-HTx hypertriglyceridemia (HR 1.50, 1.04–2.18, $p = 0.03$), chronic renal failure (HR 2.95, 2.03–4.27, $p < 0.01$), MetS and DM at 1 year follow-up (HR 2.00, 1.25–3.19, $p < 0.01$; HR 2.02, 1.27–3.23, $p < 0.01$, respectively). The last two

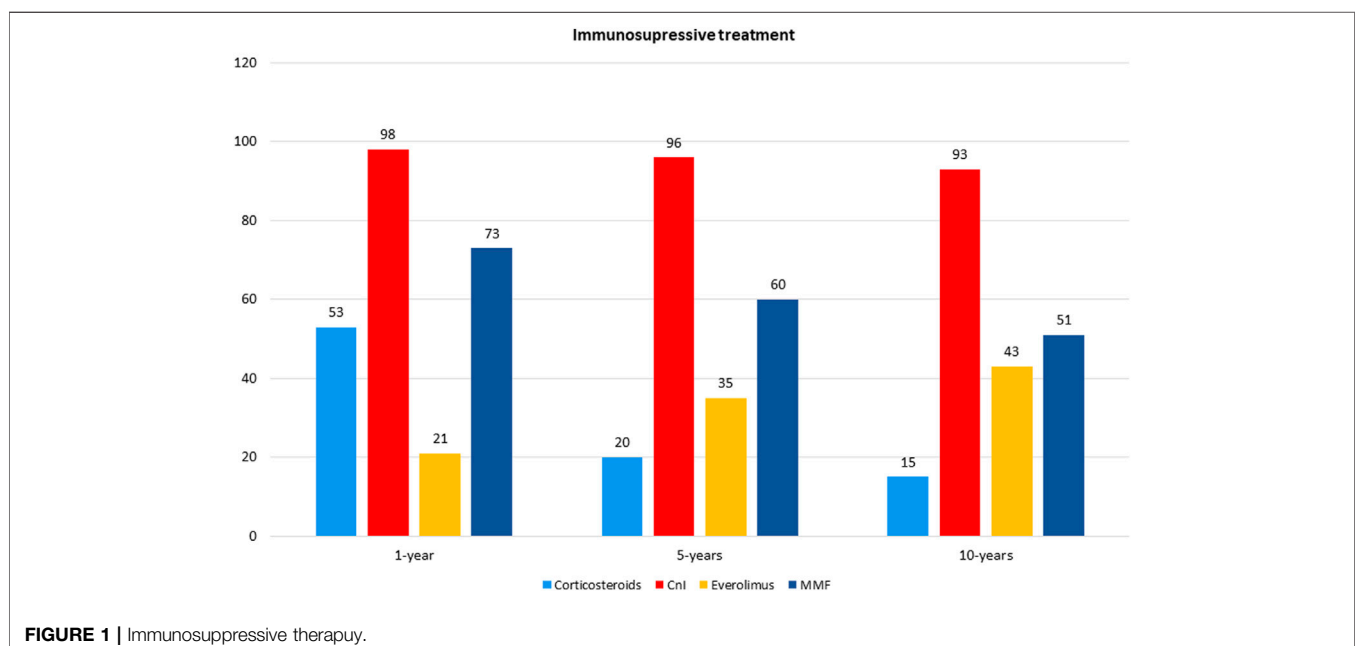


TABLE 3 | Immunosuppressive treatment during study period.

	1 year f-up	5 year f-up	10 year F-up
N. of patients	280	166	80
Cys + MMF + Corticosteroids	86 (31)	12 (7)	2 (3)
Cys + Eve + Corticosteroids	23 (8)	6 (4)	3 (4)
Cys + AZA + Corticosteroids	5 (2)	0	0
Tacrolimus + MMF + Corticosteroids	18 (6)	4 (2)	1 (1)
Tacrolimus + Everolimus + Corticosteroids	2 (0.7)	0	1 (1)
Cys + MMF	82 (29)	73 (44)	38 (48)
Cys + Everolimus	29 (10)	48 (29)	26 (33)
Cys + Corticosteroids	10 (4)	6 (4)	1 (1)
Tacrolimus + MMF	19 (7)	11 (6)	2 (3)
Tacrolimus + Corticosteroids	1 (0.4)	1 (0.6)	0
Tacrolimus + Everolimus	1 (0.4)	0	1 (1)
MMF + Corticosteroids	1 (0.4)	0	1 (1)
Everolimus + Corticosteroids	3 (1)	5 (3)	3 (4)
Cys + AZA	0	0	1 (1)

AZA, azathioprine; Cys, Cyclosporine; MMF, mycophenolate mofetil.

TABLE 4 | Prevalence of Metabolic syndrome before and after Heart Transplantation.

Prevalence of MetS	Pre-HTx	1 year f-up	5 year f-up	10 year F-up
N. of patients	349	280	166	80
TGL ≥150 mg/dL or hypertriglyceridemia drugs, n (%)	173 (50)	257 (92)	147 (89)	74 (93)
HDL <40 mg/dL in men or <50 mg/dL in women, n (%)	120 (34)	49 (18)	33 (20)	13 (16)
DM or glucose ≥100 mg/dL	211 (61)	97 (35)	72 (43)	30 (38)
Blood pressure ≥ 130/85 mmHg or HTN drugs, n (%)	120 (34)	242 (86)	149 (90)	73 (91)
BMI > 30, n (%)	43 (12)	53 (19)	41 (25)	16 (20)
MetS, n (%)	123 (35)	131 (47)	86 (52)	37 (46)

BMI, body mass index; DM, diabetes mellitus; HDL, high density lipoprotein cholesterol; HTN, hypertension; MetS, metabolic syndrome; TGL, triglyceride.

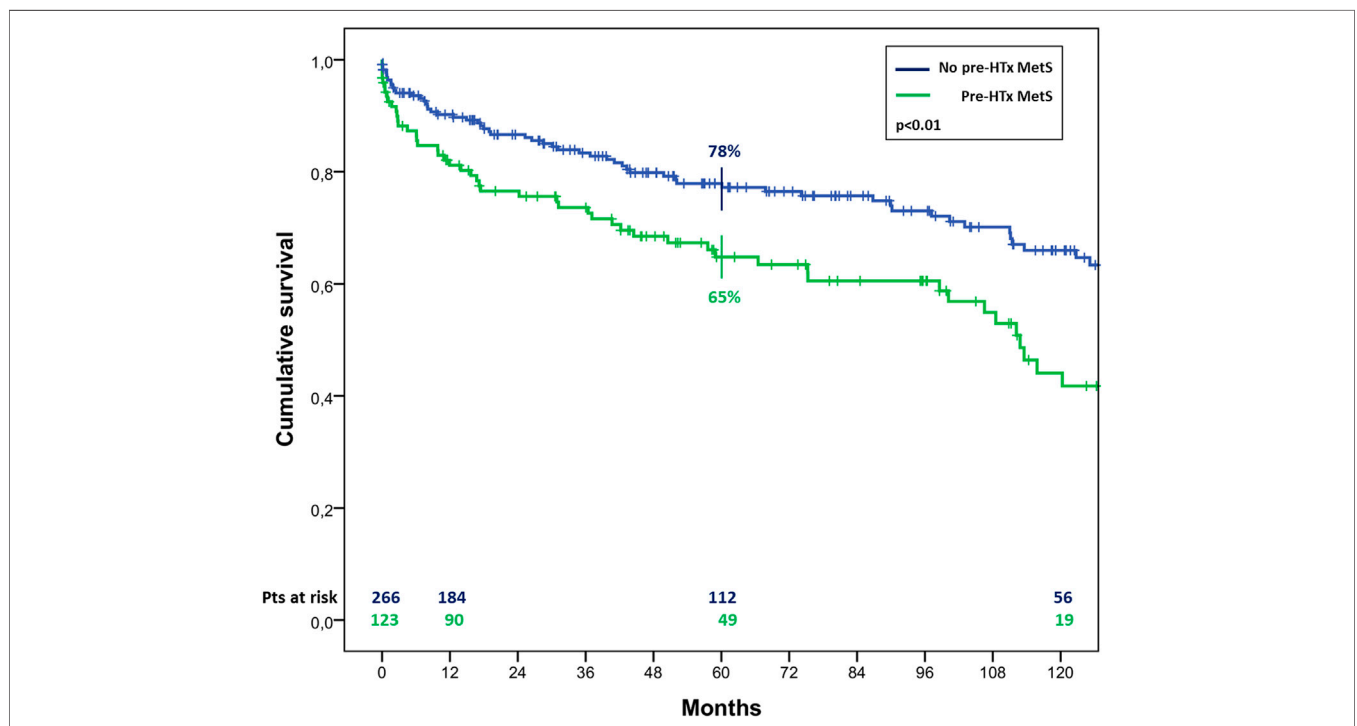


FIGURE 2 | Cumulative survival in cardiac transplanted patients with or without MetS before HTx.

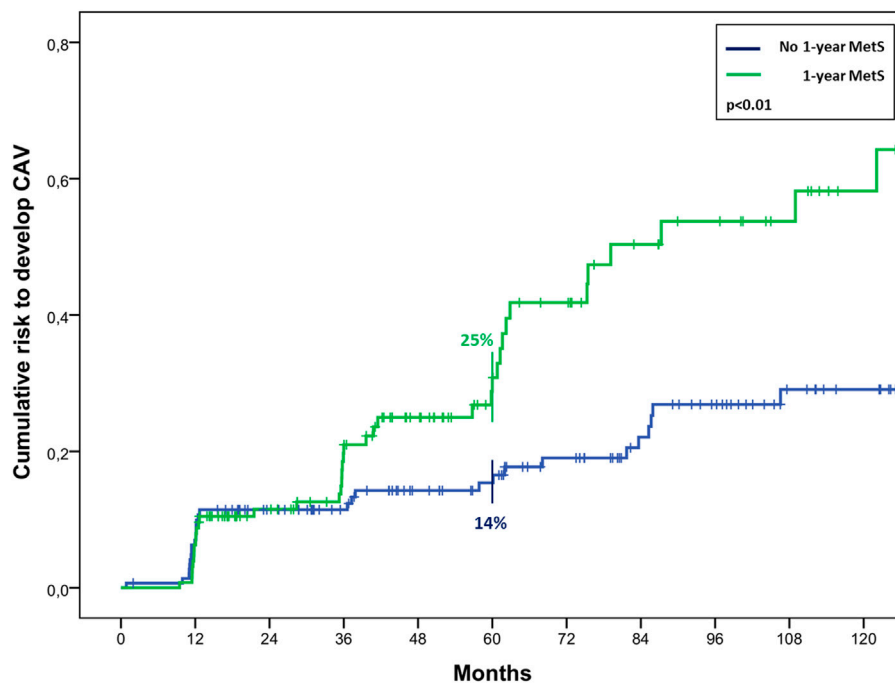


FIGURE 3 | Cumulative survival in cardiac transplanted patients with or without MetS at 1 year after HTx.

resulted also risk factors for CAV (HR 1.86, 1.16–2.99, $p = 0.01$; HR 1.67, 1.03–2.69, $p = 0.04$, respectively). In particular, MetS at 1-year follow-up determined a significant higher risk to develop CAV, resulting in a risk of $25\% \pm 4\%$ vs. $14\% \pm 3\%$ at 5 years after HTx, and $44\% \pm 6\%$ vs. $25\% \pm 4\%$ at 10 years ($p < 0.01$) (Figure 4).

DISCUSSION

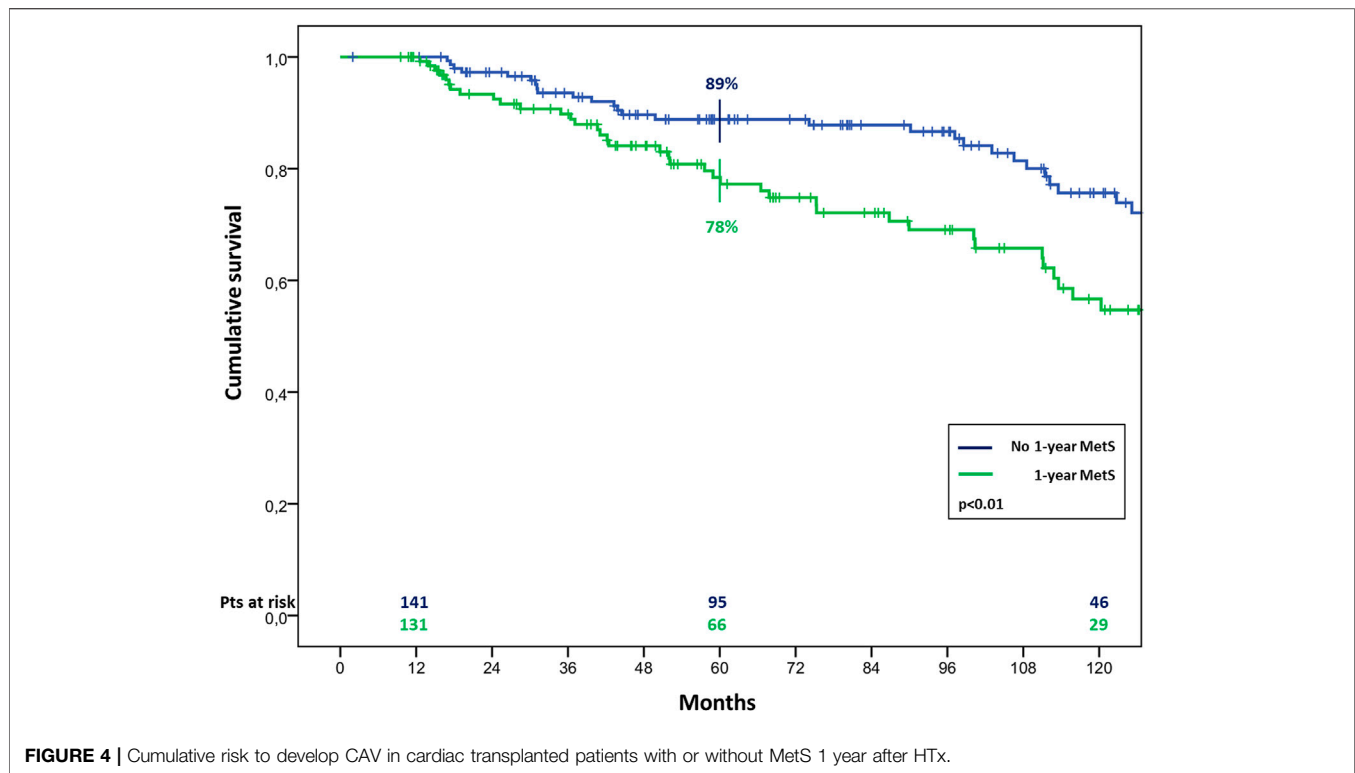
The main findings of this study were a) MetS was highly prevalent in HTx patients of our center, with hypertriglyceridemia and hypertension being the most common increased metabolic factors; b) both MetS before and at 1 year after HTx determined a significant worst survival, resulting also as risk factors for mortality at the univariate analysis; c) MetS at 1 year after HTx determined a significant higher risk to develop CAV.

MetS is a multi-factorial condition, a cluster of metabolic risk factors (abdominal obesity, dyslipidemia, high blood glucose, high blood pressure), frequently observed in clinical practice, especially after HTx. In these patients, in fact, MetS represents a burden that strongly affect their long-term outcome, mainly correlated to the side effects of the lifelong immunosuppressive therapy.

In this study, we firstly focused on analyzing the prevalence and the evolution of MetS in HTx patients of our center over 10 years of follow-up. Only a limited number of studies, to date, have discussed this topic, considering different timepoints. Martinez-Dolz et al., for instance, evaluated the prevalence of early MetS (pre-HTx or in the first 3 months post-HTx), which

resulted to be 41.9%, in line with other studies concerning liver or renal transplantation [8, 12, 13]. A similar percentage was also found by Cordero et al., who reported a 43% prevalence of MetS in 111 HTx patients after 8 ± 6 years from transplant [2]. However, the prevalence reported in our study was even higher, with 47% of patients being affected by this condition at 1 year after HTx and more than half of them (52%) at 5 years of follow-up. The prevalence seemed to increase over the follow-up period, suggesting a possible association with greater exposure time to immunosuppressive treatment.

Analyzing the parameters involved in the MetS diagnosis, it was observed a surge in the hypertension/anti-hypertensive treatment criterion and in the hypertriglyceridemia/treatment for hypertriglyceridemia criterion over the three timepoints considered, as well as an increase in BMI. Several studies have shown an attitude to the development of hypertension, dyslipidemia and obesity in transplant population during the follow-up, mostly correlated to the side effects of immunosuppressive therapy [14–16]. Data from the International Society for Heart and Lung Transplantation (ISHLT) showed that hypertension is present in 50%–90% of transplant patients and is associated with increased cardiovascular morbidity and mortality [17]. It has been shown that patients receiving cyclosporine develop new-onset hypertension requiring pharmacological treatment in 82% of cases [15]. Other metabolic side effects related to cyclosporine use are hyperlipidemia and *de novo* diabetes mellitus at 1 year, which is present as many as 10% of patients, as long as a higher risk to develop osteoporosis [14]. Weight gain and obesity,



instead, are mainly correlated with the use of glucocorticoids [18], with an approximately 10 kg gain, on average, in the first year after HTx [16].

As regard results about mortality, the patients in this series who met early MetS criteria, showed a significantly worst long-term survival, with a 5 year survival of 65% vs. 78% for patients with or without MetS before HTx, and 78% vs. 89% for patients with or without MetS at 1 year after HTx, respectively. These results are a confirmation of the hypothesis stated by Martinez-Dolz et al., according to whom the chronological development of MetS is a relevant concern regarding its prognostic value [8]. Interestingly, among the MetS criteria analyzed, three were found to be independent risk factors for mortality: HTN and hypertriglyceridemia prior to HTx and DM at 1 year follow-up. The latest report of the ISHLT registry identified the recipient history of diabetes as an independent risk factor for mortality after both 5 and 10 years after HTx [17]. In particular, new onset DM after HTx has been reported to be associated to an increased risk of cardiovascular incidents resulting in death and other diseases. Other adverse effects included infection, rejection, and early graft loss [19].

In HTx patients, different metabolic abnormalities have been associated with the development of CAV or chronic rejection, which is one of the main causes of graft failure and death over the long-term follow-up after HTx [5, 20] CAV is considered a rapid form of atherosclerosis confined to the graft, caused by an endothelial dysfunction of multifactorial origin. Since MetS is characterized by a chronic systemic inflammation which induces endothelial dysfunction [21], it is reasonable to expect an impact

of MetS on the development of CAV. Indeed, in this study the univariate analysis showed MetS and DM at 1 year after HTx to be associated to the development of CAV. A similar association was also found by Sanchez-Gomez et al., where 67% of patients with MetS developed CAV, being the presence of MetS an independent predictor with an OR of 7.97 [4]. At the univariate analysis, they found the MetS components hypertriglyceridemia, high BMI and low HDL-C levels to be associated with the CAV. In our study only DM resulted associated, but considering that insulin resistance (IR) is a known cause of endothelial cells dysfunction and one of the main player involved in triggering MetS [21], a consequent correlation between all the other associated metabolic components appears clear. Moreover, an association between IR and CAV was already been described in a study by Valantine et al [22]. They showed that metabolic markers of IR are significantly correlated with coronary artery intimal thickening in the transplanted heart subjects and that this metabolic abnormality significantly predicted the development of CAV and death during the subsequent 5 years of follow-up. Another interesting confirmation comes from a prospective, cross-sectional study by Raichlin et al, in which, evaluating blood samples from HTx patient on average nearly 5 years after transplantation, markers of IR and systemic inflammation independently identified patients at higher risk for subsequent angiographic CAV and cardiovascular events [23].

All these results underline the importance to keep monitored the metabolic alterations after HTx, during the follow-up of the patients.

Patients with MetS diagnosis before or within the first year of HTx should be followed closer, as they are more prone to develop cardiovascular events. Indeed, immunosuppressive therapy plays a primary role in the development and progression of MetS and the associated components. However, also inadequate dietary habits and physical inactivity might strongly affect the metabolic status of these patients and could represent two important tools to monitor the onset or evolution of this multifactorial condition. Adding a nutritional support and a physical activity program in the standard follow-up care of HTx patients might outline a valid strategy to limit the prevalence of MetS.

This study has some limitations related to its single-center retrospective nature and the results may not be as representative as multi-center reports, but may add valuable data in a topic, as MetS after HTx, not frequently described in literature. Moreover, concerning a specific pathological population, the sample size resulted automatically limited compared to other studies about MetS in general population. Another limitation was the failure to establish the actual contribution of immunosuppressive therapy on the development of the factors associated with MetS, because of its gradually modification over time and among patients during the study period. Further analysis, possibly through a multi-center study, are needed to better explore the impact and features of MetS after HTx.

In conclusion, this study confirmed the high prevalence of MetS in the sample of HTx patients of our center, and the presence of early MetS, both before and at 1 year after HTx, resulted in a significant worst outcome in terms of survival and development of CAV.

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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 Institutional Review Board of University of Udine. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SS and UL contributed to conception and design of the study. VF organized the database. VF, SS, and AL performed the statistical analysis. All authors contributed to manuscript revision, read, 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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Impact of a Public Health Emergency on Behavior, Stress, Anxiety and Glycemic Control in Patients With Pancreas or Islet Transplantation for Type 1 Diabetes

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*Correspondence

Eelco J. P. de Koning,
✉ e.j.p.de_koning@lumc.nl

†ORCID:

Cyril P. Landstra
orcid.org/0000-0003-2927-8447

Merel M. Ruissen
orcid.org/0000-0002-5720-0674

Hannah Regeer
orcid.org/0000-0003-3013-1602

Michiel F. Nijhoff
orcid.org/0000-0002-5131-2237

Bart E. P. B. Ballieux
orcid.org/0000-0002-8928-3849

Paul J. M. van der Boog
orcid.org/0000-0002-8178-1413

Aiko P. J. de Vries
orcid.org/0000-0002-9284-3595

Sasja D. Huisman
orcid.org/0000-0002-9176-9215

Eelco J. P. de Koning
orcid.org/0000-0002-1232-7022

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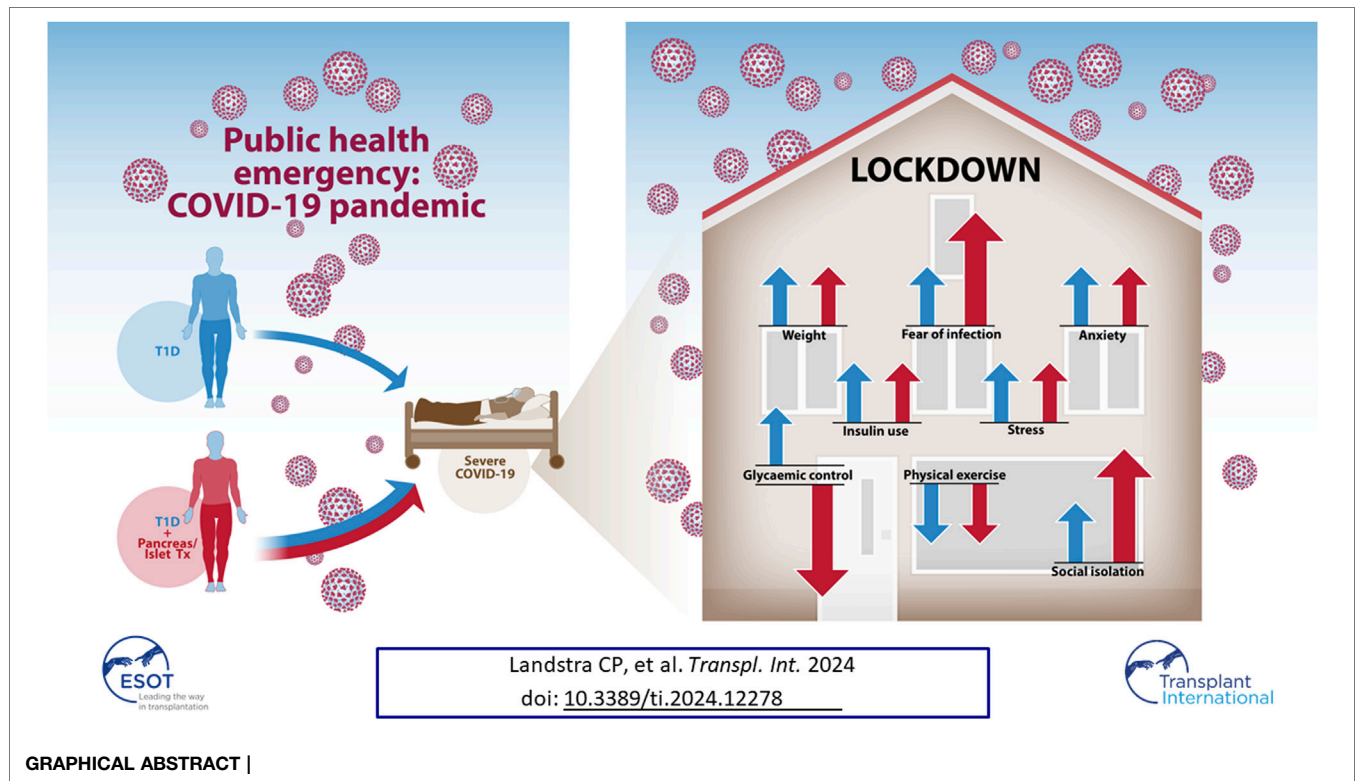
Cyril P. Landstra^{1†}, Merel M. Ruissen^{1,2†}, Hannah Regeer^{1†}, Michiel F. Nijhoff^{1,3†}, Bart E. P. B. Ballieux^{4†}, Paul J. M. van der Boog^{1,3†}, Aiko P. J. de Vries^{1,3†}, Sasja D. Huisman^{1†} and Eelco J. P. de Koning^{1,3*†}

¹Department of Internal Medicine, Leiden University Medical Center, Leiden, Netherlands, ²Department of Biomedical Data Sciences, Section Medical Decision Making, Leiden University Medical Center, Leiden, Netherlands, ³Transplantation Center, Leiden University Medical Center, Leiden, Netherlands, ⁴Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, Netherlands

A public health emergency such as the COVID-19 pandemic has behavioral, mental and physical implications in patients with type 1 diabetes (T1D). To what extent the presence of a transplant further increases this burden is not known. Therefore, we compared T1D patients with an islet or pancreas transplant (β -cell Tx; $n = 51$) to control T1D patients ($n = 272$). Fear of coronavirus infection was higher in those with β -cell Tx than without (Visual Analogue Scale 5.0 (3.0–7.0) vs. 3.0 (2.0–5.0), $p = 0.004$) and social isolation behavior was more stringent (45.8% vs. 14.0% reported not leaving the house, $p < 0.001$). A previous β -cell Tx was the most important predictor of at-home isolation. Glycemic control worsened in patients with β -cell Tx, but improved in control patients (Δ HbA1c $+1.67 \pm 8.74$ vs. -1.72 ± 6.15 mmol/mol, $p = 0.006$; Δ Time-In-Range during continuous glucose monitoring -4.5% (-6.0% – -1.5%) vs. $+3.0\%$ (-2.0% – -6.0%), $p = 0.038$). Fewer patients with β -cell Tx reported easier glycemic control during lockdown (10.4% vs. 22.6%, $p = 0.015$). All T1D patients, regardless of transplantation status, experienced stress (33.4%), anxiety (27.9%), decreased physical activity (42.0%), weight gain (40.5%), and increased insulin requirements (29.7%). In conclusion, T1D patients with β -cell Tx are increasingly affected by a viral pandemic lockdown with higher fear of infection, more stringent social isolation behavior and deterioration of glycemic control.

This trial has been registered in the clinicaltrials.gov registry under identifying number NCT05977205 (URL: <https://clinicaltrials.gov/study/NCT05977205>).

Keywords: COVID-19, type 1 diabetes, islet transplantation, pancreas transplantation, behavior, stress, anxiety, glycemic control



INTRODUCTION

As the most recent public health emergency of international concern, as declared by the World Health Organization, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic has resulted in a rapid increase in morbidity and mortality. Up until now, almost 775 million cases have been reported worldwide, resulting in more than 7 million deaths [1]. In an attempt to control the outbreak, governments of many countries implemented different quarantine strategies, varying from a complete lockdown and curfews to social distancing measures and a ban on public and social events. The predominant incentives for these measures were to relieve the extraordinary pressure put on already strained healthcare systems and to protect vulnerable patient groups from contracting COVID-19 [2, 3]. These restrictive measures required major adaptation in behavior and resulted in considerable disruptions in daily life, known to influence both mental and physical health [4, 5].

Some patients are at particularly high risk of a more severe course of the disease and mortality. These include patients with older age, obesity, hypertension, cardiovascular disease (CVD), chronic kidney disease, a history of organ transplantation and diabetes mellitus [6–8]. These risk groups, including patients with type 1 diabetes mellitus (T1D), were warned to be particularly stringent in adhering to quarantine measures [9, 10]. Therefore, an increased adverse effect on mental and physical health may be expected in these patients. Moreover, emotional distress, anxiety

and change in daily structures are known to influence glycemic control [11–13]. Indeed, we recently reported a significant impact on psychological outcomes including stress and anxiety in patients with T1D [14]. Unexpectedly, however, glycemic control showed improvement in patients with T1D in different countries during the COVID-19 lockdown, underpinning the multitude of factors that influence glycemic control [14–18].

A specific subgroup of patients with T1D are those with severely complicated diabetes who have received a pancreas or islet transplantation (β -cell Tx) [19, 20]. In addition to the higher risk of severe COVID-19 in T1D alone, these patients use immunosuppression, providing another key risk factor for a severe course of COVID-19 [7, 21–23]. Therefore, we not only expected an even greater impact of COVID-19 and the subsequent lockdown on mental and physical health in these patients, but also more stringent social isolation behavior which could adversely affect glycemic control [24]. As there is a lack of data on these aspects in patients with a previous pancreas or islet transplantation, we studied the impact of a COVID-19 nationwide partial lockdown in this patient group.

PATIENTS AND METHODS

Design and Patients

For this single-center observational cross-sectional study, patients with T1D in care at the Leiden University Medical Center (LUMC) were asked to participate in the study. From the

LUMC Transplant Center, patients with T1D who had received an islet or pancreas [including simultaneous pancreas-kidney (SPK)] transplantation were included. This group is referred to as the “ β -cell Tx” group in this manuscript. In the Netherlands, the government implemented the first partial lockdown on March 15th, 2020, which is considered the start of the lockdown period in this study. Social distancing measures were implemented, gatherings were banned and people were strongly advised to stay at home, with the exception of individuals working in vital areas of society. Public spaces, non-essential shops, restaurants, bars, and schools were closed [25].

The patients with T1D without β -cell transplantation were part of a larger study into the effects of the COVID-19 lockdown in patients with type 1 and type 2 diabetes mellitus [14]. All islet transplant recipients as well as pancreas transplant recipients with less than optimal transplant function according to Igl's criteria were eligible, in order to determine the effect of the lockdown period on glycemic control [26]. Information on the Igl's criteria and how they score graft function can be found in our **Supplementary Material (Supplementary Table S1)**. Further inclusion criteria included adult age (≥ 18 years), sufficient understanding of the Dutch language, the ability to perform a fingerstick HbA1c measurement and the ability to complete an online questionnaire [14]. Exclusion criteria included pregnancy, recent diagnosis with any malignancy (≤ 6 months), current immuno- or chemotherapy and admission at a hospital or rehabilitation center. Additionally, for this analysis, patients with T1D without a previous β -cell Tx that used steroids and/or other immunosuppressive agents at the time of inclusion were excluded from the analyses, as well as patients with a previous β -cell Tx that did not use steroids and/or other immunosuppressive at the time of inclusion. Reasons for (not) using immunosuppressive agents and more information on the exclusion of these patients can be found in the flowchart in **Supplementary Figure S1**. Since a recent start of flash glucose monitoring (FGM) or CGM can improve glycemic control, all patients (i.e., both T1D with as well as without β -cell Tx) that started using FGM or CGM within 2 months of the start of the lockdown were excluded from flash or continuous glucose sensor data analysis as well as HbA1c analysis.

Prior to the start, this study was approved by the Medical Ethical Committee of Leiden—Den Haag—Delft under the Medical Research Involving Human Subjects Act, under reference number NL73778.058.20. The study was registered in the clinicaltrials.gov registry under identification number NCT05977205. Written informed consent was provided by all participants.

Data Collection

Digital questionnaires were sent out and data was collected using Castor (Castor Electronic Data Capture, Ciwit BV, Amsterdam, the Netherlands). For HbA1c measurements, a validated capillary blood sampling set containing a small tube, lancet and medical return envelope was sent to each participant to prevent unnecessary visits to the hospital [27]. Patients were instructed to fill the tube with a few drops of blood and return it by mail to the LUMC, where HbA1c was analyzed on the day of arrival using

a Tosoh G8 HbA1c analyzer. Other patient data (including other laboratory values and patient medical history) were extracted from electronic patient records. These data were all collected during the lockdown period, 8–11 weeks after the start of the lockdown.

Outcome Measures

The primary outcome measure was the difference in HbA1c that was measured before and during the lockdown. Several secondary outcome measures were defined. For patients with FGM or CGM, glucose monitoring data were assessed for two different two-week time periods, before and at the end of the lockdown (February 24th—March 8th, 2020 and April 23rd—May 7th, 2020, respectively). For these two-week time frames, time in range (TIR; % of time between 3.9–10.0 mmol/L), time above range (TAR; % of time ≥ 10.0 mmol/L), time below range (TBR; % of time < 3.9 mmol/L), the coefficient of variation (CV), time of active use (% of time) and for patients with FGM also the average amount of scans per day (n) were evaluated. Level of education was categorized as low (primary education), middle (practical training and education; lower and senior preparatory vocational education; senior general secondary education), and high [higher professional education; (pre-) university and (post-) doctoral studies]. Psychological distress was assessed by the Perceived Stress Scale (PSS), in which a score of ≥ 14 indicates moderate stress [28]. The online questionnaire also included items on daily routines, physical activity and reported (changes in) glycemic control, medication use and stress and anxiety regarding COVID-19 (**Supplementary Material S1**). For patients with β -cell Tx, graft function was assessed using the Igl's criteria to be optimal, good, marginal or failed. Treatment success was determined as optimal or good graft function, while treatment failure was determined as marginal or failed graft function [26]. As is the case in any similar study, selection bias may have played a role as the approached potential participants could voluntarily decide to participate or not. Recall bias may have been present, but is minimized due to the relatively short interval between the start of the lockdown and the questionnaires.

Data Analysis

Statistical analyses were performed using IBM® SPSS® Statistics version 25 (IBM Corporation, Armonk, New York, United States of America). Normality of distribution was assessed by the Kolmogorov-Smirnov test of normality as well as through visual histogram distribution evaluation. An unpaired *t*-test was used for comparing normally distributed numerical variables in patients with T1D with and without β -cell Tx. A paired *t*-test was used for comparing normally distributed numerical variables in patients before and during the lockdown period. Mann-Whitney U test was used for comparing non-parametric numerical variables in patients with versus without β -cell Tx and Wilcoxon Signed Rank test for non-parametric numerical variables in patients before and during the lockdown period. For categorical variables, χ^2 test was used for comparing unpaired and Wilcoxon Signed Rank test for paired variables. One-way ANOVA was used for comparing normally distributed numerical variables, and Kruskal-Wallis

TABLE 1 | Baseline characteristics.

	β-cell Tx	T1D	p-value
n	51	272	NA
Age, years (median, Q1–Q3)	55 (48–59)	53 (37–62)	0.103
Sex, female (n, %)	20/51 (39.2%)	126/272 (46.3%)	0.349
BMI, kg/m ² (median, Q1–Q3)	23.3 (20.9–27.4)	25.2 (23.0–28.0)	0.016
Level of education (n, %) ^a			0.002
<i>Low</i>	3/47 (6.4%)	9/256 (3.5%)	
<i>Middle</i>	29/47 (61.7%)	95/256 (37.1%)	
<i>High</i>	15/47 (31.9%)	152/256 (59.4%)	
Living situation (n, %)			0.988
<i>Alone</i>	8/48 (16.7%)	37/257 (14.4%)	
<i>Co-habitant</i>	40/48 (83.3%)	220/257 (85.6%)	
Duration of diabetes, years (median, Q1–Q3)	42 (34–48)	38 (15–39)	<0.001
Antihyperglycemic therapy (n, %)			<0.001
<i>None</i>	0/51 (0.0%)	0/255 (0.0%)	
<i>Oral antihyperglycemic agents only</i>	6/51 (11.8%)	0/255 (0.0%)	
<i>Insulin: long-acting only</i>	9/51 (17.6%)	9/255 (3.5%)	
<i>Insulin: basal-bolus therapy</i>	36/51 (70.6%)	246/255 (96.5%)	
Glucose monitoring (n, %)			<0.001
<i>None</i>	6/48 (12.5%)	3/257 (1.2%)	
<i>Blood glucose monitoring only</i>	19/48 (39.6%)	61/257 (23.7%)	
<i>Flash or continuous glucose monitoring</i>	23/48 (47.9%)	193/257 (75.1%)	
Complications (n, %)			0.007
<i>Retinopathy</i>	44/51 (86.3%)	182/269 (67.7%)	
<i>Lasercoagulation</i>	40/50 (80.0%)	57/268 (21.3%)	<0.001
<i>eGFR ≥ G2^b</i>	49/50 (98.0%)	116/262 (44.3%)	<0.001
<i>Albuminuria (A2–A3)</i>	29/41 (70.7%)	24/49 (49.0%)	0.090
<i>Peripheral neuropathy</i>	35/50 (70.0%)	64/264 (24.2%)	<0.001
<i>Cardiovascular complications^c</i>	35/51 (68.6%)	44/272 (16.2%)	<0.001
Immunosuppressive regimen (n, %)		NA	NA
<i>None</i>	0/51 (0.0%)		
<i>Tac + MMF</i>	8/50 (16.0%)		
<i>Tac + pred</i>	5/50 (10.0%)		
<i>Tac + MMF + pred</i>	28/50 (56.0%)		
<i>Tac + pred + other</i>	5/50 (10.0%)		
<i>Other</i>	4/50 (8.0%)		
Type of β-cell transplantation		NA	NA
<i>Islets</i>	19/51 (37.3%)		
<i>Solitary pancreas</i>	5/51 (9.8%)		
<i>SPK</i>	27/51 (52.9%)		
IgIs score (n, %)		NA	NA
<i>Failure</i>	13/51 (25.5%)		
<i>Marginal</i>	16/51 (31.4%)		
<i>Good</i>	21/51 (41.2%)		
<i>Optimal</i>	1/51 (1.9%)		
Blood pressure, mmHg (median, Q1–Q3)			<0.001
<i>Systolic blood pressure</i>	146 (134–160)	130 (121–140)	
<i>Diastolic blood pressure</i>	80 (77–83)	78 (73–82)	0.085
Anti-hypertensive medication (n, %) ^d	37/51 (72.5%)	102/272 (37.5%)	<0.001
LDL cholesterol, mmol/mol (median, Q1–Q3)	2.22 (1.91–2.51)	2.30 (1.84–2.89)	0.306
Lipid lowering medication (n, %)			0.009
<i>Statins</i>	30/51 (58.8%)	105/269 (39.0%)	
<i>Ezetimibe</i>	2/51 (3.9%)	10/269 (3.7%)	0.944

(Continued on following page)

TABLE 1 | (Continued) Baseline characteristics.

	β -cell Tx	T1D	p-value
Smoking (n, %) ^e			
No	47/51 (92.2%)	231/259 (89.2%)	0.491
Occasional	0/51 (0.0%)	7/259 (2.7%)	
Regular	4/51 (7.8%)	21/259 (8.1%)	
Pulmonary comorbidities (n, %) ^f	2/51 (3.9%)	16/270 (5.9%)	0.568

β -cell Tx, β -cell transplantation; T1D, type 1 diabetes; BMI, body mass index; eGFR, estimated glomerular filtration rate; Tac, tacrolimus; MMF, mycophenolate mofetil; pred, prednisolone; SPK, simultaneous pancreas-kidney transplantation; LDL, low-density lipoprotein.

Bold p-values are considered statistically significant ($p < 0.05$).

^aLevel of education: low (primary education); middle (practical training and education); lower and senior preparatory vocational education; senior general secondary education); and high [higher professional education]; (pre-) university and (post-) doctoral studies].

^bChronic Kidney Disease Guideline classification: eGFR \geq G2 = eGFR \leq 89 mL/min/1.73 m².

^cHistory of myocardial infarction, percutaneous coronary intervention, peripheral vascular disease, stroke, transient ischemic attack, heart failure, amputation of limbs (toe/foot/leg).

^dUse of any or more of the following anti-hypertensive medication: angiotensin converting enzyme (ACE) inhibitor, angiotensin receptor blocker (ARB), calcium antagonist, alpha blocker, beta blocker, thiazide diuretics, spironolactone.

^eOccasional smoking \geq 1x/week; regular smoking \geq 1x/day.

^fHistory of pulmonary comorbidities: asthma, chronic obstructive pulmonary disease (COPD) or fibrosis.

for comparing non-parametric numerical variables in patients with islet transplantation (ITx), solitary pancreas transplantation (PTx) and SPK. Univariable linear regressions were used to assess associations for univariable numerical outcomes of interest (including potential confounders), all variables that reached statistical significance in the univariable linear regression analysis were added to the multivariable linear regression model. Univariable logistic regressions were used for univariable categorical outcomes, all variables that reached statistical significance in the univariable logistic regression analysis were added to the multivariable logistic regression model. Missing data were considered to be missing at random, cases with missing data were excluded from the particular analysis where data was missing and were not excluded from all analyses. Normally distributed numerical variables are expressed as mean \pm standard deviation (SD), non-parametric numerical variables as median [first quartile (Q1)—third quartile (Q3)]. Calculated differences (Δ) are expressed as mean difference \pm standard error of the difference. Categorical variables are expressed as number of cases (percentage of patient population). A p -value of <0.05 was considered statistically significant.

RESULTS

Patients and Characteristics

A total of 51 patients with T1D with a previous β -cell Tx and 272 patients with T1D without β -cell Tx were eligible for this study and provided signed written informed consent. Of the 51 β -cell Tx recipients, 19 (37.3%) received islets, 5 (9.8%) solitary pancreas, and 27 (52.9%) SPK transplants. Baseline characteristics of patients with T1D with and without β -cell Tx are described in **Table 1**. Patients with β -cell Tx had a lower BMI, longer diabetes duration with more diabetes-related complications, and higher blood pressure with more anti-hypertensive medication. Other important risk factors for a severe course of COVID-19, such as age, sex, smoking and pulmonary comorbidities, were not different between the groups. A total of 33/50 (66.0%) of patients with β -cell Tx receive triple

immunosuppression, mostly consisting of tacrolimus, mycophenolate mofetil and prednisolone (5 mg/d). Data from FGM/CGM were available in a total of 99/323 (30.7%) patients (12/51 (23.5%) of patients with β -cell Tx and 87/272 (32.0%) of patients with T1D). A total of 305/323 (94.4%) patients (48/51 (94.1%) of patients with β -cell Tx and 257/272 (94.5%) of patients with T1D) completed the online questionnaire on daily routines, physical activity, stress and anxiety, glycemic control and medication.

COVID-19 Fear and Social Isolation Behavior

Patients with β -cell Tx had significantly higher fear of COVID-19 infection as compared to patients with T1D alone (**Figure 1A**; VAS 5.0 (3.0–7.0) vs. 3.0 (2.0–5.0), $p = 0.004$). They also behaved differently with regard to social isolation and adherence to the lockdown measures, with 52.1% vs. 18.3% ($p < 0.001$) reporting not going out for groceries and 45.8% vs. 14.0% ($p < 0.001$) reporting not leaving the house at all (**Figure 1B**). Univariate analysis demonstrated that a β -cell transplantation, a history of CVD and higher fear of infection with COVID-19 were univariable significant predictors of not leaving the house (**Table 2**). The only significant independent predictor that remained after multivariable regression analysis was a previous β -cell transplantation, with an odds ratio (OR) of 4.275 (95% CI 1.919–9.537, $p < 0.001$). Age, sex, BMI, level of education, pre-lockdown HbA1c, blood pressure, and pulmonary comorbidities were not found to be associated with isolating at home.

Impact on Glycemic Control

HbA1c during lockdown (measured at a median of 9.3 (8.7–10.3) weeks after the initiation of the lockdown) was compared to the last known pre-lockdown HbA1c (measured at a median of 9.1 (4.4–21.0) weeks before the lockdown). In patients with β -cell Tx, HbA1c increased by 1.7 ± 8.7 mmol/mol Hb (54.3 mmol/mol Hb pre-lockdown to 56.0 mmol/mol Hb during the lockdown). In patients with T1D HbA1c decreased by -1.7 ± 6.1 mmol/mol Hb (60.5 mmol/mol Hb pre-lockdown to 58.8 mmol/mol Hb during

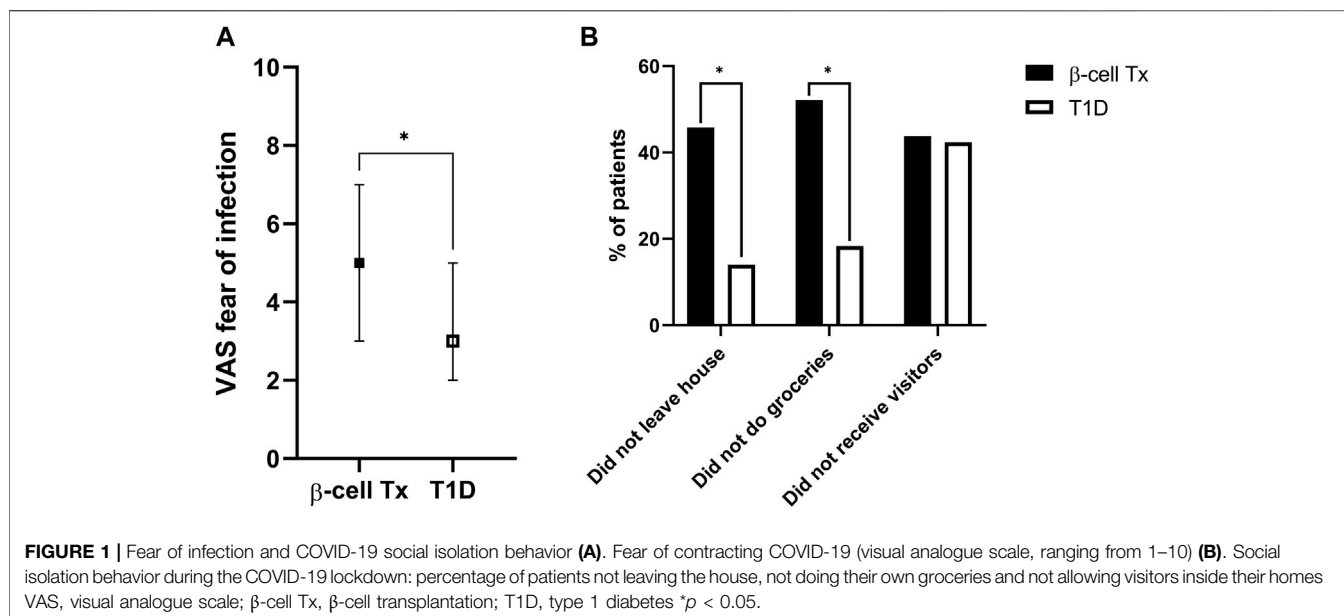
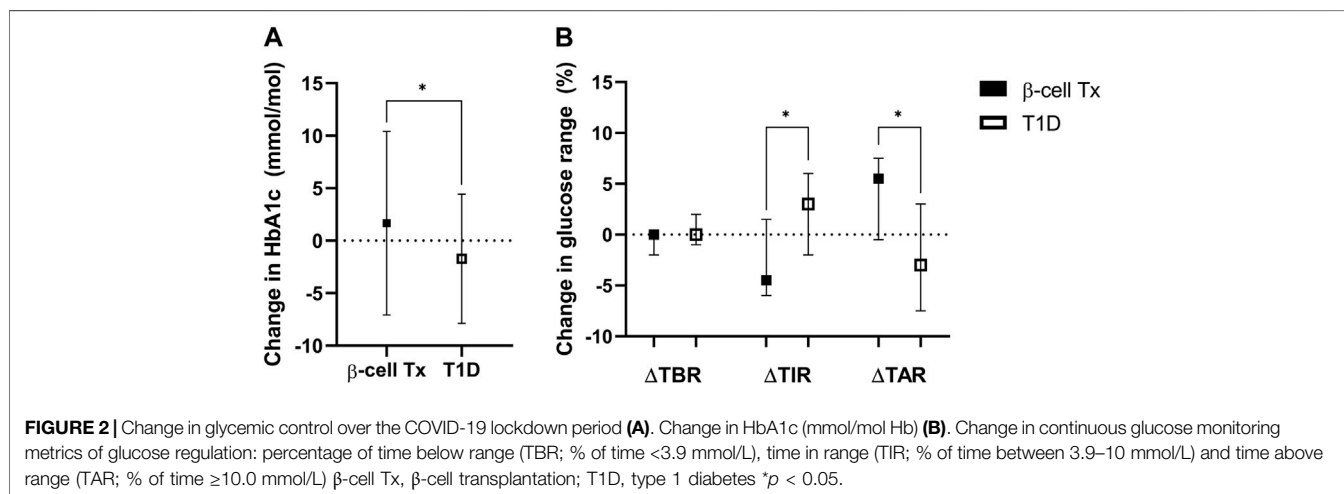
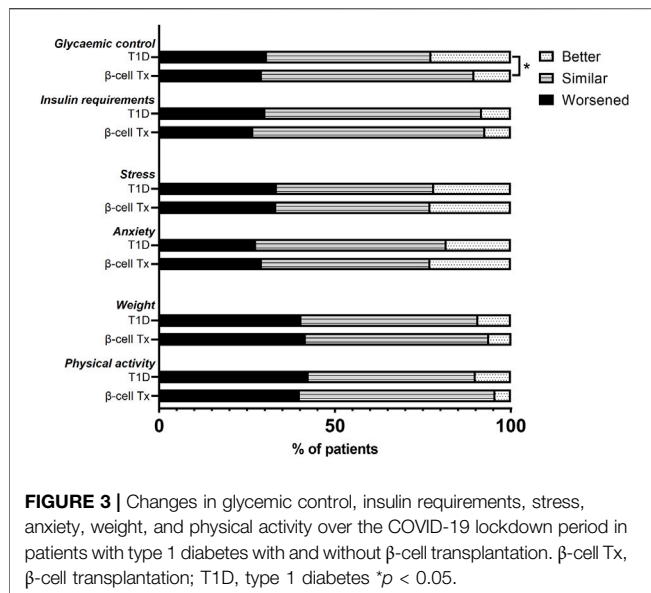


TABLE 2 | Univariable and multivariable predictors of not leaving the house in patients with type 1 diabetes with and without β -cell transplantation.

	Univariable logistic regression			Multivariable logistic regression		
	B	R ² (95% CI)	p-value	B	R ² (95% CI)	p-value
Age, years	0.009	1.009 (0.989–1.030)	0.384			
Sex (ref. female)	–0.239	0.787 (0.444–1.395)	0.412			
BMI, kg/m ²	–0.078	0.925 (0.852–1.004)	0.061			
Level of education (ref. middle)						
Low	–0.182	0.833 (0.171–4.055)	0.821			
High	–0.012	0.988 (0.548–1.780)	0.967			
HbA1c pre-L, mmol/mol Hb	0.004	1.004 (0.981–1.026)	0.758			
β -cell Tx (ref. T1D)	1.648	5.194 (2.663–10.133)	<0.001	1.453	4.275 (1.919–9.537)	<0.001
Pulmonary comorbidities	–0.529	0.589 (0.130–2.668)	0.492			
Cardiovascular disease	0.879	2.408 (1.302–4.451)	0.005	0.183	1.201 (0.559–2.580)	0.639
Systolic blood pressure	0.015	1.015 (1.000–1.030)	0.055			
VAS fear of infection	0.137	1.147 (1.019–1.291)	0.023	0.092	1.096 (0.968–1.241)	0.148

Ref, reference; BMI, body mass index; Hb, hemoglobin; pre-L, pre-lockdown; β -cell Tx, β -cell transplantation; T1D, type 1 diabetes mellitus; VAS, visual analogue scale. Bold p-values are considered statistically significant ($p < 0.05$).





the lockdown) (Figure 2A; $p = 0.006$). These findings were reflected in glucose monitoring data showing a reduction in time in range (TIR) and an increase in time above range (TAR) in patients with β -cell Tx, while patients with T1D showed an increase in TIR and reduction in TAR (Figure 2B; Δ TIR β -cell Tx -4.5% ($-6.0\% - 1.5\%$) vs. T1D 3.0% ($-2.0\% - 6.0\%$), $p = 0.038$; Δ TAR β -cell Tx 5.5% ($-0.5\% - 7.5\%$) vs. T1D -3.0% ($-7.5\% - 3.0\%$), $p = 0.025$). There was no significant difference in Δ HbA1c or CGM outcomes between patients with different types of β -cell transplantation (i.e., ITx, PTx or SPK; Supplementary Table S2). In patients with T1D with and without β -cell Tx, 26.8% vs. 30.2% ($p = 0.871$) reported administration of more insulin compared to before the COVID-19 lockdown (Figure 3; Supplementary Table S3). In terms of reported glucose regulation, a similar number of patients (29.2% vs. 30.7%) reported more difficulty with glycemic control, but in patients with β -cell Tx compared to T1D alone, less patients reported that they found it easier to regulate their blood glucose levels over the lockdown period (10.4% vs. 22.6%; $p = 0.015$; Figure 3; Supplementary Table S3). Within the group of patients with β -cell Tx, these outcomes did not differ between different β -cell replacement modalities (Supplementary Table S4). Univariate analysis showed that pre-lockdown HbA1c (OR 0.918; 95% CI 0.858–0.983; $p = 0.014$) and treatment success, as determined by the Igl score (OR 5.571; 95% CI 1.297–23.934; $p = 0.021$), were significant predictors for a deterioration in HbA1c for patients with β -cell Tx. However, in a multivariable model, both variables lost statistical significance (Supplementary Table S5).

Impact on Stress, Anxiety, Weight and Physical Exercise

In patients with T1D with and without β -cell Tx, increased anxiety (29.2% vs. 27.6%, $p = 0.677$) and stress (33.3% vs.

33.5%, $p = 0.984$) during the COVID-19 lockdown as compared to before the lockdown was reported in similarly high numbers (Figure 3; Supplementary Table S3), with a PSS of 14.04 ± 7.05 vs. 13.69 ± 6.16 ($p = 0.722$), indicating moderate perceived stress in patients with β -cell Tx and low perceived stress in patients with T1D alone. Furthermore, 41.7% vs. 40.5% ($p = 0.787$) of patients with T1D with and without β -cell Tx reported weight gain and 40.0% vs. 42.5% ($p = 0.399$) reported less physical activity during the lockdown, with only 6.3% vs. 9.3% reporting weight loss and 4.4% vs. 10.0% increased physical activity (Figure 3; Supplementary Table S3). The changes in stress, anxiety, weight and physical activity in patients with β -cell Tx were not statistically significantly different between patients with different modalities of β -cell transplantation (Supplementary Table S4).

DISCUSSION

In this study, we show that islet or pancreas transplantation (β -cell Tx) in patients with type 1 diabetes leads to additional fear of infection, more stringent social isolation behavior and deterioration of glycemic control during the COVID-19 pandemic and the subsequent lockdown. In fact, having had a β -cell transplantation was the most important determinant of not leaving the house during the COVID-19 lockdown. In addition, patients with T1D both with and without β -cell Tx experience high rates of stress and anxiety, decreased physical activity and weight gain.

The COVID-19 pandemic is the most relevant recent example of a public health emergency of international concern. Patients with diabetes mellitus are considered a high-risk population during these situations. Indeed, diabetes mellitus is a key independent risk factor for a severe course of COVID-19. Patients with T1D have a high risk of developing severe COVID-19, with evidence pointing to an even higher risk as compared to patients with type 2 diabetes [6, 29–32]. Patients who are eligible for β -cell replacement therapy through either islet or whole pancreas transplantation usually have severely complicated T1D [19]. With glucose dysregulation and poor glycemic control significantly associated with severe COVID-19, this further increases their risk [7, 33]. In addition, although data on the specific risks in patients with β -cell Tx are currently scarce, patients with (solid) organ transplants have been marked as an essential risk group for a more severe course of COVID-19 as well. This higher risk for COVID-19 severity and mortality has been extensively linked to the use of immunosuppression [7, 8, 22, 34, 35]. Thus, patients who have received β -cell Tx for complicated T1D have multiple important factors adding up to an increasingly higher risk of severe COVID-19 and can therefore be considered a very vulnerable patient group.

These vulnerable patients at high risk for a severe course of COVID-19 were continuously warned to be particularly stringent in observing lockdown measures and practicing social isolation [9, 10]. Importantly, staying at home was strongly advised, but not mandatory during the lockdown in the Netherlands [25]. Nonetheless, almost half of the

patients with β -cell Tx reported not leaving the house at all, which was almost three times higher than patients with T1D alone. This was associated with a significantly higher fear of COVID-19 infection in patients with β -cell Tx as compared to T1D. A history of CVD, as an additional risk factor for severe COVID-19, was also associated with not leaving the house. Other known risk factors for severe COVID-19, such as older age, male sex, a higher BMI, worse glycemic control, hypertension and pulmonary comorbidities were not found to be associated with isolating at home. Furthermore, we found high rates of stress and anxiety in this vulnerable patient group. This is in line with previous studies that have also described extensive psychological influences of the COVID-19 pandemic and lockdown measures in both patients with type 1 diabetes, and (solid) organ transplant recipients [4, 5, 10, 36–40].

Apart from the psychological impact, we also found a strong impact on physical outcomes with high rates of weight gain and decreased physical exercise during the COVID-19 lockdown. These unfavorable findings have been reported in the general population as well, and health effects appear to persist after lifting the lockdown [41–44]. Patients with β -cell Tx also showed a slight deterioration in glycemic control over the lockdown period. This could be related to the reported changes in daily structures, behavior, emotional distress, anxiety, weight gain and the limited possibility for physical exercise during the lockdown, as all of these factors are well-known to influence glycemic control [11–13, 37]. However, our analyses did not show these effects. Additionally, altered healthcare access due to a shift towards COVID-19-related care put greater emphasis on patients' self-management [45], which may have complicated glycemic control. Patients with a higher pre-lockdown HbA1c were more likely to experience deterioration of HbA1c over the lockdown. Also, β -cell Tx recipients with a successful graft function were much more likely to experience deterioration of HbA1c as compared to patients with a failed graft. This may point to patients with a successful graft losing diabetes self-management skills as a result of their glycemic stabilization after transplantation, while patients with failed grafts continue to endure complicated diabetes [20].

Interestingly, in contrast to patients with β -cell Tx, patients with T1D showed a small overall improvement in glycemic control over the lockdown period [14]. This finding was supported by other smaller studies from Italy and Spain, which also found an improvement in glycemic control during lockdown in patients with T1D [15–18]. The difference in glycemic control between patients with T1D with and without β -cell Tx may be related to differences in self-management skills and social isolation behavior [24], the higher general impact of the pandemic and subsequent lockdown, and the increased (feeling of) vulnerability in patients with β -cell Tx, since in addition to having complicated T1D and using immunosuppression, these patients also more often have other (cardiovascular) comorbidities.

There are several strengths and limitations to our study. To our knowledge, this is the first study describing the psychological and physical impact of the COVID-19 pandemic and subsequent lockdown in patients with β -cell Tx, assessing a wide variety of

outcomes including behavior, anxiety and stress, physical activity and weight. We determined the effect on glycemic control using both HbA1c as well as glucose monitoring data and included a large control group of patients with T1D without β -cell Tx. HbA1c measurements were conducted at median 9.3 weeks after the start of the lockdown. With HbA1c reflecting glycemic control over the preceding 8–12 weeks, interference of pre-lockdown glycemic control may be present [46]. Because of COVID-19 restrictions and altered healthcare access, we had to rely on patient-reported data for certain outcomes. This included weight change over the lockdown period. However, since reported weight change is often an underestimation of the actual weight change [47], the proportion of patients with weight gain may even be larger than reported in this current study. We used validated questionnaires for e.g., perceived stress, but could not compare them with pre-lockdown outcomes, because these questionnaires were not regularly used before the COVID-19 lockdown. For this reason, we asked patients in our additional questionnaire to compare outcomes like stress during the lockdown to before the lockdown. Using this approach, we were able to report changes in outcomes over the COVID-19 lockdown period. The COVID-19 pandemic has brought much attention to health inequalities, as certain groups were found to have been more highly impacted during the pandemic than others [48, 49]. In this study, we found no influence of sex or level of education, but we have no information on whether living in urban, suburban or rural communities or access to exercise possibilities may have played a role in our findings. We did not include a control group of people without risk factors for severe COVID-19, which could have provided us with information on a potential stepwise increasing impact of the lockdown with increasing vulnerability.

Lockdown measures are implemented to shield vulnerable population groups—including patients with β -cell Tx for T1D—from contracting COVID-19 in an attempt to prevent a severe course and mortality. However, during the lockdown this group experienced weight gain and deterioration of glycemic control. With both of these factors independently associated with a severe course of COVID-19 [6, 33], this pinpoints a complex problem wherein the effects of the lockdown may contribute to an even further increased risk of severe COVID-19. However, since poor glycemic control and increased weight are both modifiable risk factors, emphasis can be put on better (self-) management (support) as well as a healthy lifestyle.

This study was conducted during the first COVID-19 lockdown, when vaccines were not yet available. However, it is known that immunosuppressed transplant recipients often lack adequate antibody responses [50, 51]. A study in kidney transplant recipients showed similarly high rates of depression, anxiety and lower health-related quality of life after vaccination as compared to before vaccination, with only a small improvement in psychological distress [52]. These findings underline the continuing vulnerability of transplant recipients after vaccination, and may point to continued psychological, physical and behavioral impact.

In summary, patients with type 1 diabetes and a previous β -cell transplantation requiring immunosuppressive agents are at high

risk during a public health emergency. Having multiple risk factors for a severe course of COVID-19, these patients and are highly impacted by the pandemic and subsequent lockdown. They experience high rates of fear, social isolation, worsening of glycemic control and weight gain which requires continuous awareness amongst healthcare professionals.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by the Medical Ethical Committee of Leiden—Den Haag—Delft, the Netherlands. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CL, MR, HR, SH, and EdK designed the study. CL, MR, and HR acquired the data, CL analysed and interpreted the data. CL was

responsible for writing the article, under supervision of EdK. All authors (CL, MR, HR, MN, BB, PvdB, AdV, SH, and EdK) thoroughly revised the article and approved the final version to be published. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2024.12278/full#supplementary-material>

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GLOSSARY

Abbreviation	Full term
ACE	Angiotensin converting enzyme
ARB	Angiotensin receptor blocker
BMI	Body mass index
CGM	Continuous glucose monitoring
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
CV	Coefficient of variation
CVD	Cardiovascular disease
(e)GFR	(estimated) Glomerular filtration rate
FGM	Flash glucose monitoring
FSL	FreeStyle Libre
Hb	Hemoglobin
HbA1c	Hemoglobin type A1c (glycosylated hemoglobin)
IQR	Interquartile range
IS	Immunosuppression
ITx	Islet transplantation
IU	International units
IV	Intravenously
LDL	Low-density lipoprotein
LUMC	Leiden University Medical Center
MMF	Mycophenolate mofetil
mRNA	Messenger ribonucleic acid
NA	Not applicable
OR	Odds ratio
Pre-L	Pre-lockdown
Pred	Prednisolone
PSS	Perceived Stress Scale
PTx	Pancreas transplantation
Q1	First quartile
Q3	Third quartile
Ref.	Reference
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SD	Standard deviation
SPK	Simultaneous pancreas-kidney transplantation
T1D	Type 1 diabetes mellitus
Tac	Tacrolimus
TAR	Time above range
TBR	Time below range
TIR	Time in range
Tx	Transplantation
VAS	Visual analogue scale



Adaptative Strategy of Immunosuppressive Drugs Dosage Adjustments When Combined With Nirmatrelvir/Ritonavir in Solid Organ Transplant Recipients With COVID-19

Lidvine Boland^{1,2*}, Arnaud Devresse³, Caroline Monchaud^{4,5,6}, Sébastien Briol³, Stéphanie Belaïche^{7,8,9}, Baptiste Giguët¹⁰, Lionel Couzi¹¹, Olivier Thauinat¹², Laure Esposito¹³, Magdalena Meszaros¹⁴, Ana Roussoulières¹⁵, Vincent Haufroid^{1,2}, Yannick Le Meur^{16,17} and Florian Lemaitre^{18,19}

¹Department of Clinical Chemistry, Cliniques Universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium, ²Louvain Centre for Toxicology and Applied Pharmacology (LTAP), Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Louvain-la-Neuve, Belgium, ³Department of Nephrology, Cliniques Universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium, ⁴Service de Pharmacologie, Toxicologie et Pharmacovigilance, Centre Hospitalier Universitaire de Limoges, Limoges, France, ⁵INSERM U1248 Pharmacology and Transplantation, Limoges, France, ⁶FHU SUPORT, Limoges, France, ⁷Department of Pharmacy, Lille University Medical Center, Lille, France, ⁸Department of Hepatogastroenterology, Lille University Medical Center, Lille, France, ⁹ULR2694-METRICS, Université de Lille, Lille, France, ¹⁰Liver Disease Department, Centre Hospitalo-Universitaire Pontchaillou, Rennes, France, ¹¹Department of Nephrology, Transplantation, Dialysis and Apheresis, Centre Hospitalier Universitaire de Bordeaux, Bordeaux, France, ¹²Department of Transplantation, Nephrology and Clinical Immunology, Hospices Civils de Lyon, Lyon, France, ¹³Department of Nephrology, Dialysis and Organ Transplantation, Centre Hospitalier Universitaire de Toulouse, Toulouse, France, ¹⁴Hepatogastroenterology and Liver Transplant Unit, Saint Eloi Hospital, Centre Hospitalier Universitaire de Montpellier, Montpellier, France, ¹⁵Department of Cardiology, Pulmonary Vascular Diseases and Heart Failure Clinic, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium, ¹⁶Department of Nephrology, Centre Hospitalier Régional Universitaire (CHU) de Brest, Brest, France, ¹⁷INSERM UMR1227 Lymphocytes B et Autoimmunité, Université de Bretagne Occidentale, Brest, France, ¹⁸INSERM UMR1085, Centre Hospitalier Universitaire (CHU) de Rennes, Rennes, France, ¹⁹FHU SUPORT, Rennes, France

OPEN ACCESS

*Correspondence

Lidvine Boland,
✉ lidvine.boland@
saintluc.uclouvain.be

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Nirmatrelvir/ritonavir is a promising option for preventing severe COVID-19 in solid organ transplant recipients with SARS-CoV-2 infection. However, concerns have arisen regarding potential drug interactions with calcineurin inhibitors (CNI). This two-phase multicentre retrospective study, involving 113 patients on tacrolimus and 13 on cyclosporine A, aimed to assess the feasibility and outcomes of recommendations issued by The French societies of transplantation (SFT) and pharmacology (SFPT) for CNI management in this context. The study first evaluated adherence to recommendations, CNI exposure, and clinical outcomes. Notably, 96.5% of patients on tacrolimus adhered to the recommendations, maintaining stable tacrolimus trough concentrations (C_0) during nirmatrelvir/ritonavir treatment. After reintroduction, most patients experienced increased C_0 , with 42.9% surpassing 15 ng/mL, including three

Abbreviations: AUC_{0–120h}, area under the concentration-time curve over 5 days; C_0 , trough concentration; CNI, calcineurin inhibitors; COVID-19, coronavirus infectious disease 19; CsA, cyclosporine A; eGFR, estimated glomerular filtration rate; IQR, interquartile range; ISD, immunosuppressive drugs; PK, pharmacokinetic; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SFPT, Société Française de Pharmacologie et Thérapeutique; SFT, Société Francophone de Transplantation; TDM, therapeutic drug monitoring.

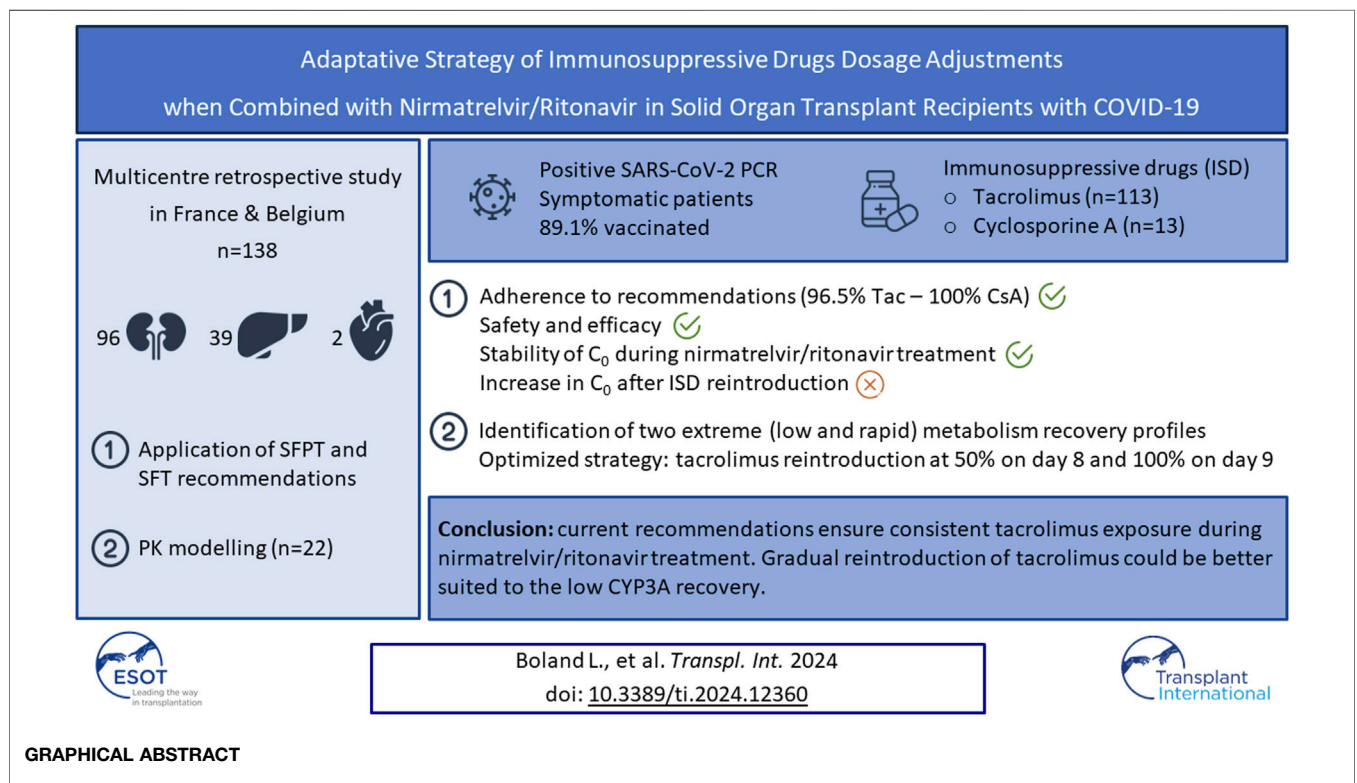
patients exceeding 40 ng/mL. Similar trends were observed in cyclosporine A patients, with no COVID-19-related hospitalizations. Moreover, data from 22 patients were used to refine the reintroduction strategy. Modelling analyses suggested reintroducing tacrolimus at 50% of the initial dose on day 8, and then at 100% from day 9 as the optimal approach. In conclusion, the current strategy effectively maintains consistent tacrolimus exposure during nirmatrelvir/ritonavir treatment, and a stepwise reintroduction of tacrolimus may be better suited to the low CYP3A recovery.

Keywords: drug-drug interactions, drug monitoring, nirmatrelvir/ritonavir, pharmacokinetic modelling, tacrolimus

INTRODUCTION

Nirmatrelvir/ritonavir (Paxlovid[®]) is the current first-line treatment to prevent hospitalization and death related to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, also known as coronavirus infectious disease 19 (COVID-19) [1]. In the phase III trial EPIC-HR, the drug has been shown to decrease hospitalization and death from severe COVID-19 by 89% for high-risk patients [2]. However, due to the high potency of drug metabolism inhibition of ritonavir, the combination of nirmatrelvir/ritonavir with calcineurin inhibitors (tacrolimus and cyclosporine) and m-TOR inhibitors (everolimus and sirolimus) can lead to their accumulation and subsequent adverse drug reactions, the most worrisome being acute renal failure [3–5]. Despite this potential safety issue, and because the immunosuppressed patients are a high-risk group for severe COVID-19, nirmatrelvir/ritonavir has been prescribed to

patients under immunosuppressive treatment with various risk mitigation approaches [6, 7]. In this context, the French societies of Transplantation (Société Francophone de Transplantation—SFT) and Pharmacology (Société Française de Pharmacologie et Thérapeutique—SFPT) have published recommendations to manage immunosuppressants dose adjustment, with the aim of decreasing the risk of accumulation during the nirmatrelvir/ritonavir treatment course in solid organ transplant recipients. In short, these recommendations are: to discontinue tacrolimus 12 h before nirmatrelvir/ritonavir initiation; or to decrease the cyclosporine (CsA) dose to 20% of the initial daily dose and administer it once a day; or to decrease everolimus and sirolimus dose to 12.5% of the initial dose and administer it every other day. For tacrolimus, everolimus, and sirolimus, reintroduction of the dose prior to the course of nirmatrelvir/ritonavir can be considered on day 7, while CsA can be resumed at full dose



on day 8 [8]. Specific therapeutic drug monitoring (TDM) of immunosuppressive drugs (ISD) has also been suggested.

The aims of the PAXLOV-IS study were: 1) to evaluate the application of the French recommendations and their impact on exposure to tacrolimus and on clinical outcomes in solid organ transplant patients, and 2) to present the results of simulations aimed at proposing an optimized tacrolimus dosage adjustment algorithm when combined with nirmatrelvir/ritonavir.

MATERIALS AND METHODS

This two-step retrospective study was conducted in France and Belgium on behalf of the SFT. Between January and August 2022, data on solid organ transplant patients treated with nirmatrelvir/ritonavir from seven French and two Belgian transplantation centers (Bordeaux, Brest, Brussels, Lyon, Montpellier, Rennes, and Toulouse) were collected. Paxlovid[®] was prescribed to prevent severe complications of SARS-CoV-2 infection in accordance with its summary of product characteristics. The initiation of nirmatrelvir/ritonavir occurred within 5 days after the first symptoms of SARS-CoV-2 infection, for a duration of 5 days, and the dose was adapted to renal function: 150 mg nirmatrelvir + 100 mg ritonavir twice a day if the estimated glomerular filtration rate (eGFR) was below 60 mL/min/1.73 m², or 300 mg nirmatrelvir + 100 mg ritonavir for an eGFR above 60 mL/min/1.73 m².

The following characteristics were collected from medical records and anonymized: sex, weight, age, COVID-19 vaccine status and COVID-19 symptoms, type of transplantation, post-transplantation time, plasma or serum creatinine, glomerular filtration rate estimated using the CKD-EPI formula, liver enzymes, immunosuppressive treatment (type, dose, trough concentrations from baseline to the first measurement after the end of the nirmatrelvir/ritonavir course), and adverse events. The study was authorized by the institutional review board and ethics committee of Limoges Hospital and was registered under #15-2023-03.

Study Step 1: Application of the SFPT and SFT Recommendations

The first step of this study was to evaluate the application of SFPT and SFT recommendations and their impact on tacrolimus exposure and clinical outcomes, particularly the adverse events potentially related to ISD. The SFPT and SFT recommended interrupting tacrolimus during the 5 days of nirmatrelvir/ritonavir treatment (days 1–5). Reintroduction of tacrolimus was performed at full dose 36 h after the last dose of nirmatrelvir/ritonavir (on the morning of day 7). For CsA, no interruption was recommended, but the dose had to be reduced to one-fifth of the usual dose while on nirmatrelvir/ritonavir and maintained over the 5 days of treatment. The CsA dose was then progressively increased to 50% of the dose administered prior to nirmatrelvir/ritonavir treatment on day 6, 75% on day 7, and full dose on day 8. Other concomitant medications were withdrawn or adapted according to the SFPT recommendations.

Study Step 2: Pharmacokinetic Modelling

Data were included in the pharmacokinetic (PK) modelling step if at least three trough concentrations (C_0) were available before, during, and between 8 and 16 days after nirmatrelvir/ritonavir treatment. The pharmacokinetics of tacrolimus were modelled using the MWPharm++ software, as previously described [9]. Individual pharmacokinetic parameters were estimated. Different scenarios were tested to fit the concentration data from the tacrolimus reintroduction period (i.e., day 8–16 period). Tacrolimus areas under the concentration-time curves over 5 days (AUC_{0-120h}) were estimated and compared for the 5 days before and the 5 days during nirmatrelvir/ritonavir treatment. The half-life of tacrolimus during nirmatrelvir/ritonavir treatment was also calculated using the following formula:

$$T_{1/2} = (\ln 2 \times 48) / (\ln(C_{48h}) - \ln(C_{96h}))$$

where $T_{1/2}$ is tacrolimus half-life, C_{48h} is the estimated concentration of tacrolimus on day 2, and C_{96h} is the estimated concentration on day 4.

The nadir C_0 before tacrolimus reintroduction and the maximal C_0 reached during tacrolimus reintroduction were estimated to identify patients with early drug accumulation during tacrolimus reintroduction. Plasma or serum creatinine levels were compared before and at the end of the treatment course. When available, the CYP3A5 genotype was also gathered and PK parameters were compared between CYP3A5 expressors and non-expressors.

To fit the tacrolimus concentration data measured during tacrolimus reintroduction (from the morning of day 7, 36 h after cessation of nirmatrelvir/ritonavir), different scenarios of metabolism inhibition resolution were applied. This analysis allowed for the selection of the most appropriate strategy for tacrolimus resumption, ensuring sufficient immunosuppressive exposure, while mitigating the risk of drug accumulation. Two extreme scenarios for metabolism recovery were observed in the patients of the study and subsequently tested: 1) a “low metabolism recovery profile” with a progressive metabolism recovery from day 8 to 100% on day 12 and 2) a “rapid metabolism recovery profile,” with a partial (50%) metabolism recovery on day 7 and a complete recovery on day 9.

Then, different strategies of tacrolimus reintroduction were simulated based on a dose regimen of 6 mg once a day: 1) 100% of the dose prior to treatment from day 7, 2) 100% of the dose from day 8, 3) 100% of the dose from day 9, 4) 50% of the dose prior to treatment on day 8, 100% from day 9; 5) 50% of the dose on day 9, then 100% from day 10; and 6) 50% of the dose on days 8 and 9, and then 100% from day 10. An adjudication committee composed of a nephrologist, a clinical pharmacist, and two pharmacologists selected the best scenario to ensure sufficient immunosuppressive exposure while mitigating the risk of drug accumulation.

RESULTS

Patient Characteristics

A total of 138 patients were included (63% males), with a median age of 59 years (interquartile range: 48–66). Among

TABLE 1 | Baseline characteristics of the studied population.

	Median (IQR)	N
Age (years)	59 (48–66)	138
Sex (M/F)	87/50	137
Weight (kg)	66 (57–79.5)	99
Baseline GFR (CKD-EPI mL/min)	60.5 (45–77.6)	138
Tacrolimus daily dose (mg)	3.75 (2.875–6)	112
Cyclosporine daily dose (mg)	120 (100–150)	13

M, male; F, female.

them, 96 underwent kidney transplantation (including 3 kidney-pancreas transplants), 39 received liver transplants, and 2 received heart transplants. The majority of patients (121) had undergone transplantation for more than 12 months prior to the study. The median eGFR was 60.5 (IQR: 45.0–77.6) mL/min/1.73 m². Baseline patient characteristics are listed in **Table 1**.

COVID-19 Infection

A total of 123 patients (89.1%) received two to five doses of mRNA SARS-CoV-2 vaccine prior to COVID-19 infection. The serological response after vaccination was assessed in 82 patients. 20% were non-responders (IgG anti-S < 3 BAU), 23% presented a weak response (IgG anti-S between 3 and 250 BAU), and 57% had a good response (IgG anti-S > 250 BAU). At nirmatrelvir/ritonavir initiation, all patients showed symptoms, including cough (54%), fever (41%), rhinorrhea (38%), sore throat (32%), headache (30%), asthenia (27%), and/or gastrointestinal disorders (7%). All patients had a positive COVID-19 test (PCR).

Further genotyping of 35 patients revealed Omicron SARS CoV-2 variants.

Immunosuppressive Drug Dosing Adjustment

113 patients were on tacrolimus (82%) and 13 on cyclosporine (9%). The remaining patients were on either belatacept or mTor inhibitors and were not included in the analysis (**Figure 1**).

According to the SFPT and SFT recommendations, all but 4 patients (109/113, 96.5%) discontinued tacrolimus during the 5-day nirmatrelvir/ritonavir treatment: two had a reduced dose of tacrolimus (1.75 mg/d and 0.5 mg/d) and the other two stopped nirmatrelvir/ritonavir before the end due to side effects (digestive intolerance) and resumed tacrolimus on day 4. The SFPT and SFT dose adjustment guidelines were followed for the 13 patients on CsA.

Trough Concentrations of ISD

Tacrolimus trough concentrations were measured in 103 patients before the introduction of nirmatrelvir/ritonavir. **Figure 2** shows the evolution of tacrolimus C₀ during and after nirmatrelvir/ritonavir administration. TDM was performed in 33 patients after the completion of antiviral treatment (day 6 or 7). For those patients, the median tacrolimus C₀ remained stable: 5.2 (IQR: 4.3–6.4) ng/mL before nirmatrelvir/ritonavir introduction and 4.4 (IQR: 3.4–5.3) ng/mL before tacrolimus resumption. After tacrolimus reintroduction, C₀ was monitored in 59 patients: 35 patients between days 8 and 12 and 24 patients after day 12 (between days 13 and 73). In the early reintroduction period

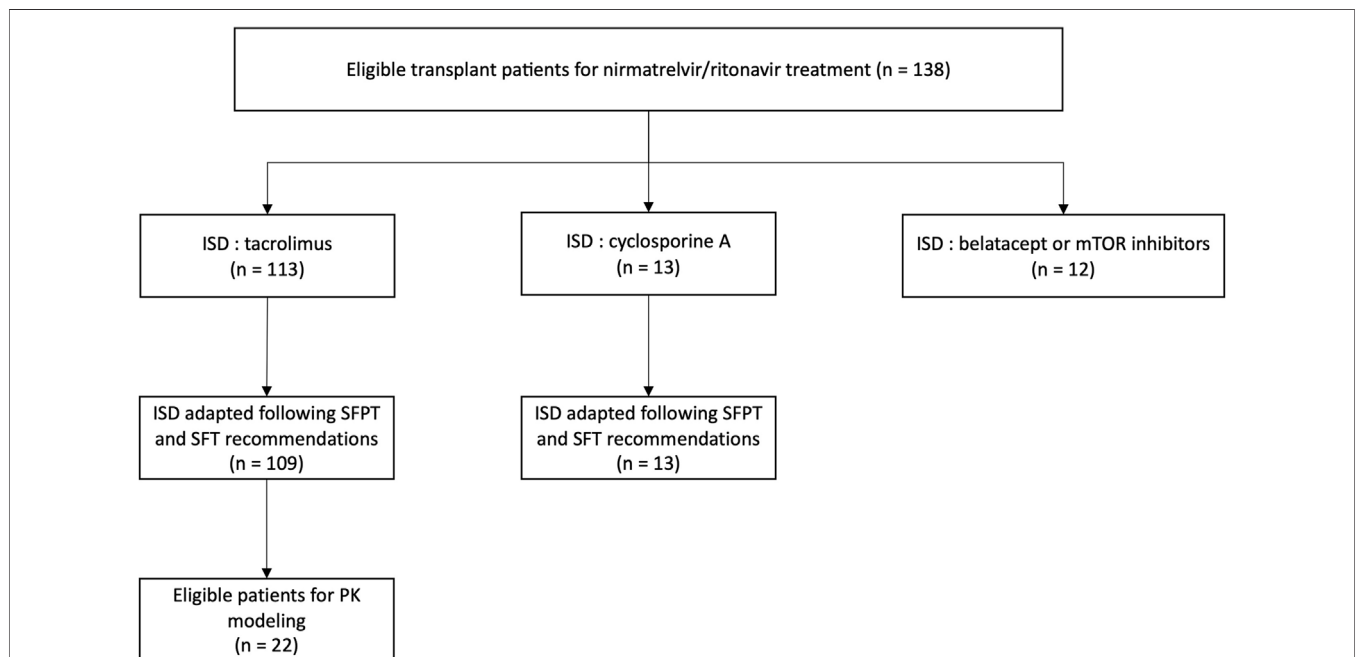


FIGURE 1 | Study flowchart and distribution of immunosuppressive treatment within the population (n = 138). ISD, immunosuppressive drug; SFPT, Société Française de Pharmacologie et Thérapeutique; SFT, Société Francophone de Transplantation.

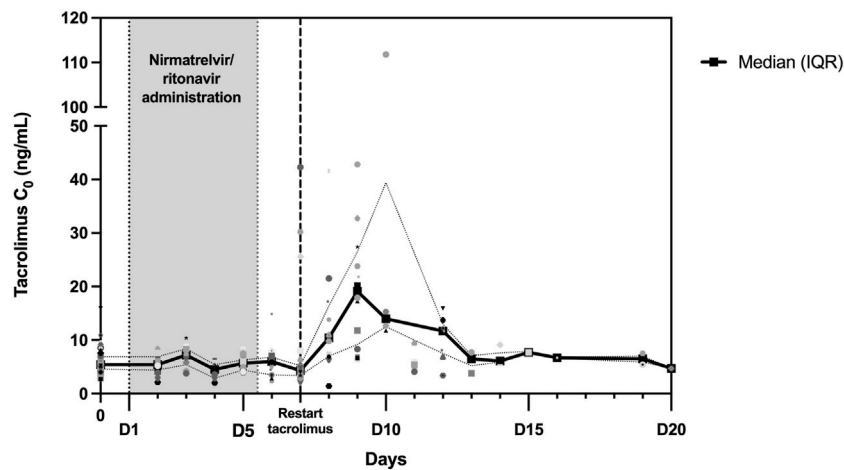


FIGURE 2 | Trough concentrations (C_0) of tacrolimus during and following nirmatrelvir/ritonavir treatment. Between day 0 and day 20, a total of 192 tacrolimus C_0 values were collected and are depicted in this figure. The black curve represents the median C_0 , while the dotted curves represent the interquartile range (25th percentile–75th percentile).

(days 8–12), C_0 increased in most patients with a median tacrolimus C_0 level of 12.7 (IQR: 6.8–20.9) ng/mL and then normalized. In fact, 15 patients (42.9%) reached concentrations above 15 ng/mL including three (8.6%) above 40 ng/mL. Notably, the highest observed C_0 exceeded 100 ng/mL; however, this was due to patient error in the tacrolimus dose.

Similar results were obtained for patients on CsA with a median C_0 of 40 (IQR: 36–70) ng/mL before nirmatrelvir/ritonavir and 111 (IQR: 42–161) ng/mL after full dose resumption.

Safety and Efficacy

49 adverse events were reported during nirmatrelvir/ritonavir treatment. Dysgeusia was the most frequent symptom (20 patients, 14.5%), followed by diarrhea (17 patients, 12.3%). Adverse events were attributed to tacrolimus toxicity in eight patients (5.8%) (three acute renal failures, two neurologic toxicities, and three gastrointestinal toxicities). One patient for whom the SFPT and SFT recommendations were not followed developed acute renal failure concomitant with a very high tacrolimus concentration. One patient who was treated with CsA experienced acute renal failure. All events were observed in patients with high trough concentrations of ISD and were reversible within a few days after dose reduction.

All patients in this cohort recovered quickly from COVID-19 and none were hospitalized for COVID-19 complications.

Pharmacokinetic Modelling

Data from 22 patients were included in the modelling step. **Table 2** summarizes the treatment and pharmacokinetic parameters of this patient subpopulation. The median tacrolimus C_0 was 5.2 (IQR: 4.6–6.7) ng/mL before the antiviral course and 4.0 (IQR: 3.4–5.0) ng/mL just before tacrolimus reintroduction (morning of day 7, $n = 12$ patients). The median estimated AUC_{0-120h} before the nirmatrelvir/ritonavir course was 900

(IQR: 684–1,213) ng.h/mL. The median AUC_{0-120h} decreased slightly to 752 (IQR: 622–895) ng.h/mL when tacrolimus was discontinued (i.e., during the antiviral treatment phase). The median decrease in AUC_{0-120h} was 11%. Among the 22 patients, 18 exhibited a decrease in the range of 0%–22%, while the remaining four patients experienced more substantial reductions in exposure, at 47%, 62%, 68%, and 82%, respectively. The median estimated half-life was 212 (IQR: 177–405) hours with some extreme values (range: 87–712 h). The predicted nadir tacrolimus C_0 in these patients was close to C_0 prior to the nirmatrelvir/ritonavir course (4.7 vs. 5.2 ng/mL).

All patients with available CYP3A4 genotypes ($n = 15$) were wild-type (CYP3A4*1/*1). Among the 19 patients with an available genotype for CYP3A5, 14 were non-expressors (CYP3A5*3/*3) and five were expressors (four CYP3A5*1/*3 and one CYP3A5*1/*1). The half-life did not differ between CYP3A5 expressors (173 h, IQR: 160–294 h) and non-expressors (212 h, IQR: 191–474 h).

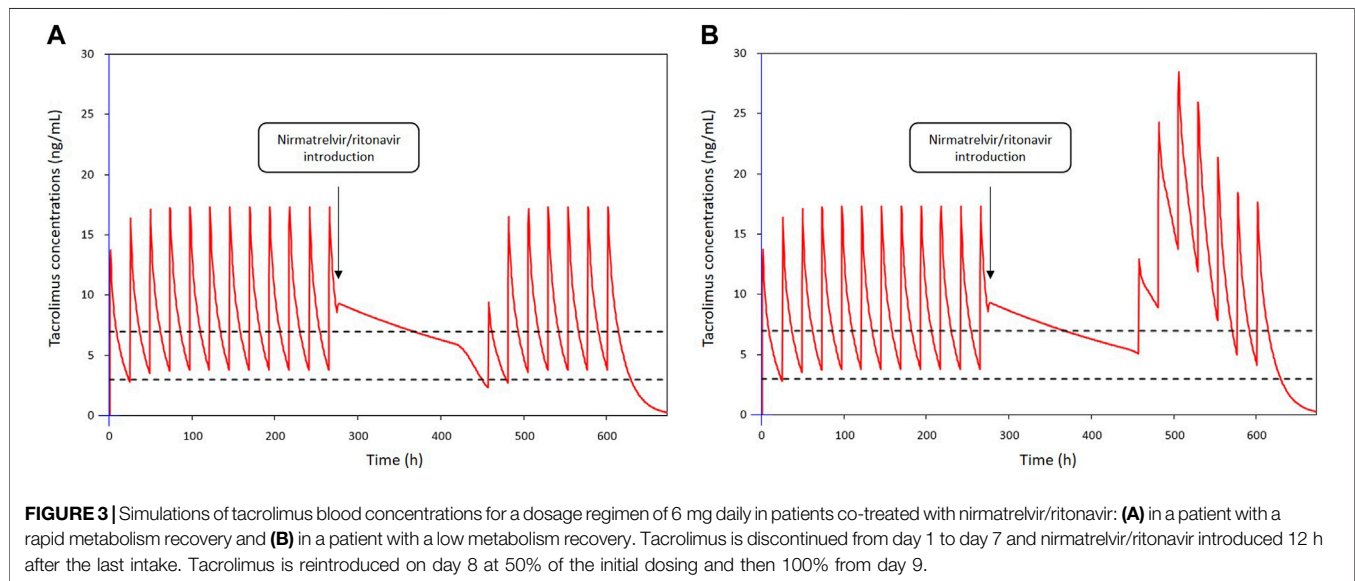
PK modelling estimated a median maximal tacrolimus C_0 of 11.2 (IQR: 8.7–19.2) ng/mL. A maximal $C_0 >10$ ng/mL, >15 ng/mL, and >20 ng/mL was estimated in respectively 13 (59%), 8 (36%), and 5 (23%) patients, respectively. However, there was only a slight difference between creatinine measured between days 9 and 16 and creatinine before the antiviral course (median variation: +2.1%, IQR: –3.4–+6.8%), with only three patients reporting an increase in creatinine above +25% of the baseline value (+27%, +31%, and +59%, respectively). None of the 22 patients included in the modelling part of the study was hospitalized for severe COVID-19 or acute renal failure.

Subsequently, the two low and rapid metabolism recovery scenarios and different tacrolimus reintroduction strategies described earlier were tested. The simulated patient receiving a once-daily dose of 6 mg tacrolimus exhibited pre-nirmatrelvir/ritonavir initiation C_0 of 3.8 ng/mL in the scenario of a rapid metabolism recovery profile, and 5.1 ng/mL in the context of a

TABLE 2 | Treatment and pharmacokinetic parameters of the 22 patients receiving tacrolimus and included in the modelling part of the study.

	Median	25th percentile	75th percentile
Tacrolimus daily dose (mg)	5	3.375	8.25
Concentration over dose ratio (ng/mL/mg)	0.96	0.75	1.49
AUC _{0-120 h} before N/R (ng.h/mL)	900.1	684.3	1213.3
AUC _{0-120 h} during N/R (ng.h/mL)	752.2	622.2	895.6
Difference in AUCs (before-during) (%)	11%	5%	20%
Half-life during antiviral treatment (h)	212	177	405
Nadir concentration (ng/mL)	4.7	3.8	5.6
Maximum post-treatment concentration (ng/mL)	11.2	8.7	19.2

N/R, nirmatrelvir/ritonavir; AUC_{0-120 h}, area under the concentration time curve between 0 and 120 h.



low metabolism recovery profile. The optimal balance was achieved by reintroducing tacrolimus at 50% of the initial dose on day 8 (60 h after nirmatrelvir/ritonavir last dose) and then 100% from day 9 (84 h after nirmatrelvir/ritonavir last dose). Using this strategy, the estimated nadir of tacrolimus C_0 after reintroduction was 2.3 ng/mL on the morning of day 8 in the case of a rapid metabolism recovery profile (**Figure 3A**) and 5.1 ng/mL in the case of a low metabolism recovery profile (**Figure 3B**). The maximum tacrolimus C_0 during the reintroduction phase was 3.8 ng/mL on the morning of day 11 in the case of a rapid metabolism recovery profile and 13.8 ng/mL on the morning of day 10 in the case of a low metabolism recovery profile.

DISCUSSION

We present a collaborative French and Belgian experience, focusing on adherence to the French national recommendations for managing drug-drug interactions between ISD and nirmatrelvir/ritonavir, along with their PK and clinical impact in 138 solid organ transplant patients. Notably, our findings highlight a high adherence rate to the

guidelines (96.5% for tacrolimus and 100% for cyclosporine A), revealing sustained tacrolimus exposure but also indicating potential accumulation after early ISD reintroduction.

Nirmatrelvir/ritonavir is a valuable treatment for solid organ transplant recipients with COVID-19 who display a high risk of morbidity and mortality due to SARS-CoV-2 infection. Oral therapy is particularly interesting in outpatient settings. Nevertheless, drug-drug interactions between the antiviral and the immunosuppressive therapy remain a source of concern. The interaction between ritonavir and CYP3A4-dependent drugs can lead to significant increases in drug exposure, up to 50-fold for tacrolimus [10]. Because CNI are highly dependent on CYP3A4 metabolism, their blood concentration will increase substantially and rapidly when combined with ritonavir. This effect has been previously reported in transplant patients on ritonavir as a single agent or in association [11–13]. High concentrations of tacrolimus can lead to serious side effects such as kidney injury, seizures, posterior reversible encephalopathy, and even death [11]. Several ISD adjustment strategies have recently been reviewed by Tang et al., but no consensus has yet been reached [14]. When CNI are held during nirmatrelvir/ritonavir treatment, studies differ in terms of both timing of ISD suspension and dose

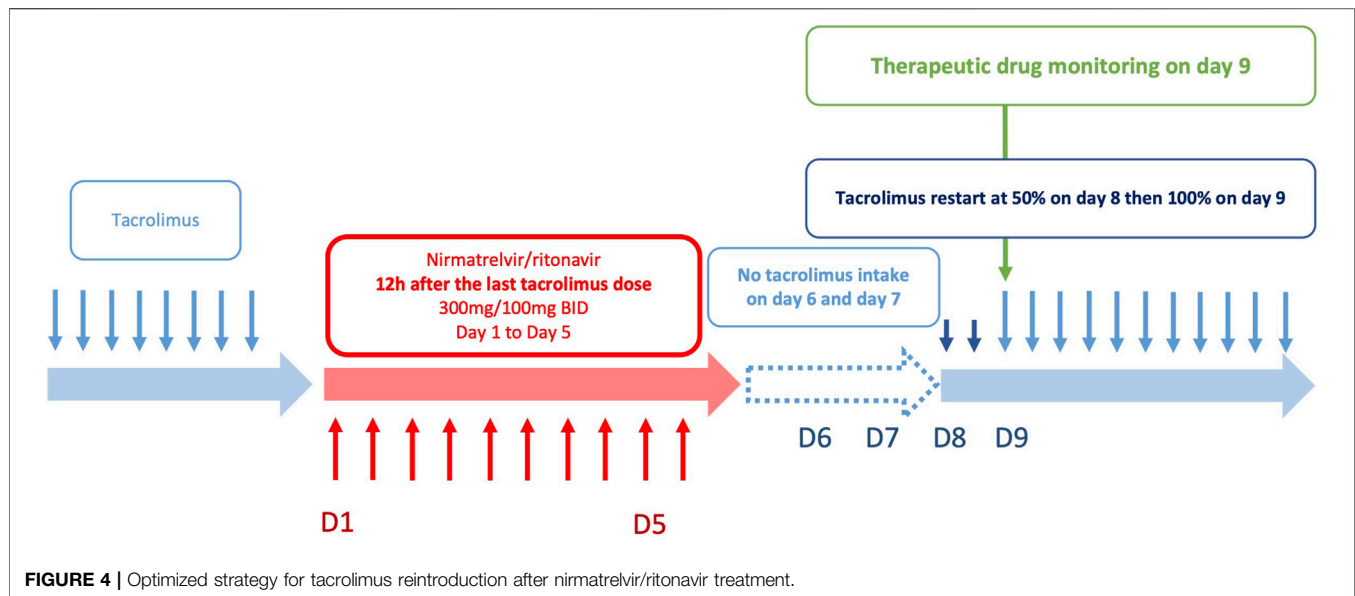


FIGURE 4 | Optimized strategy for tacrolimus reintroduction after nirmatrelvir/ritonavir treatment.

resumption. In general, tacrolimus is discontinued from the initiation of nirmatrelvir/ritonavir and resumed at partial or full dose on days 6–13 after treatment completion. Moreover, a close TDM should be considered to guide the resumption of ISD [5, 6, 15–20]. In our study, tacrolimus was discontinued 12 h before nirmatrelvir/ritonavir initiation and restarted at full dose on day 7, while CsA was decreased to 20% of the initial daily dose and resumed at full dose on day 8, according to the SFPT and SFT recommendations [8]. Tacrolimus or CsA trough concentrations were measured during and after nirmatrelvir/ritonavir treatment. This strategy was efficient in the majority of patients. Regarding safety, dysgeusia was the main reported adverse drug reaction, as expected with ritonavir. The second most frequent adverse drug reaction was diarrhea, which was probably of mixed origin (COVID-19 infection, nirmatrelvir/ritonavir, and ISD overexposure). In the whole cohort, four cases of acute renal failure (three in tacrolimus patients and one in CsA patient), two neurologic toxicities, and three gastrointestinal toxicities were reported. These events were consistent with the high exposure reported upon ISD reintroduction. Notably, a deviation from the ISD dosage adjustment was identified in one of these four cases. This is consistent with a recent pharmacovigilance study reporting that 11 out of 14 tacrolimus overexposures were linked to a lack of compliance with the French national guidelines. In two other cases, no information was reported, and only one out of 14 patients seemed to present an overexposure episode while following the guidelines [21]. Fortunately, in our study, all episodes were reversible within a few days with dose adjustments. Furthermore, none of the patients were hospitalized because of severe COVID-19.

In the second phase of the study, PK modelling was performed in a subset of patients for whom adequate data were available. We showed a sustained tacrolimus drug exposure due to metabolism inhibition during nirmatrelvir/ritonavir treatment, even after tacrolimus discontinuation. Four patients experienced a more

pronounced decrease (between 47% and 82%) without any clinical signs of acute graft rejection. However, a considerable number of patients had predicted supratherapeutic levels of tacrolimus after the cessation of nirmatrelvir/ritonavir ($C_0 > 20$ ng/mL during days 9–12 in approximately 20% of the patients). Other studies have reported supratherapeutic levels despite tacrolimus interruption during nirmatrelvir/ritonavir treatment [5, 6, 17, 19, 20]. In addition, a few case reports have illustrated the importance of tacrolimus discontinuation to avoid supratherapeutic concentrations and potentially severe adverse reactions [13, 17, 22], sometimes with rifampin [23] or phenytoin [24] treatment for toxicity reversal. Our results suggest a longer inhibition of CYP3A in some patients. Katzenmaier et al. previously reported that it may take at least 3 days after ritonavir discontinuation to restore CYP3A activity [25]. This has led us to re-evaluate the SFPT and SFT recommendations, considering patients' metabolism recovery. PK modelling of 22 patients allowed us to define two extreme (low and rapid) metabolism recovery profiles. These profiles were used to simulate different strategies for resuming tacrolimus therapy after nirmatrelvir/ritonavir treatment. The best scenario was to stop tacrolimus during nirmatrelvir/ritonavir treatment and restart tacrolimus at 50% of the initial dose from day 8 and then 100% from day 9 (Figure 4). Simulations using extensive data collected from 22 patients showed that this strategy limits the risk of tacrolimus accumulation in patients with a slow recovery metabolism while limiting the risk of low exposure in patients with a rapid metabolism recovery. This one-size-fits-all strategy provides simple and convenient management of this at-risk period following antiviral treatment and is now included in the French national recommendations. Nonetheless, TDM is essential during resumption of immunosuppressive therapy, particularly tacrolimus. This is critical for the early detection of patients who may accumulate immunosuppressants after treatment with nirmatrelvir/ritonavir (days 8–12). Tacrolimus

TDM based on trough concentrations measured on days 2 and 3, or even better measurement of its area under the curve, can be proposed to individualize the treatment strategy. Moreover, TDM should be performed early when ISD is restarted to rapidly detect patients with high or low ISD exposure. Volumetric absorptive microsampling (VAMS) could facilitate this process for outpatients infected with COVID-19.

This work has some limitations, including the retrospective design of the study and a limited sample size, especially for the PK modelling phase, where only 22 patients were included. The immunosuppressive treatment in the cohort predominantly consisted in tacrolimus (82%). The observations made on the 13 patients receiving cyclosporine A need confirmation in a larger population, and a similar evaluation should be considered for everolimus and sirolimus. Additionally, it would be interesting to assess the impact of CYP3A genotype on ISD exposure and potential accumulation during nirmatrelvir/ritonavir treatment and after ISD reintroduction.

CONCLUSION

This study reports the implementation of the French national recommendations for ISD drug adjustments during nirmatrelvir/ritonavir treatment in 138 solid organ recipients. These data demonstrate that discontinuing tacrolimus 12 h before the introduction of nirmatrelvir/ritonavir enables the maintenance of tacrolimus concentrations within the therapeutic range. It also ensures a tacrolimus exposure during the 5 days of treatment with nirmatrelvir/ritonavir close to the pre-treatment exposure. However, real-life data showed that some patients receiving a combination of tacrolimus-nirmatrelvir/ritonavir experienced tacrolimus accumulation when the treatment was resumed. Simulations performed on patients with repeated TDM showed that a strategy with 50% of the dose initially prescribed from day 8 (60 h after the last nirmatrelvir/ritonavir dose) and then 100% from day 9 (84 h after the last nirmatrelvir/ritonavir dose) should improve drug safety. TDM is an invaluable tool in such combination cases, allowing real-time ISD drug dosage adjustment, and should therefore be used systematically in patients receiving nirmatrelvir/ritonavir.

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.

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ETHICS STATEMENT

The studies involving humans were approved by the institutional review board and ethics committee of Limoges Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

LB, CM, StB, YL, and FL analysed the data and wrote the manuscript. AD, SéB, BG, LC, OT, LE, MM, AR, and VH were involved in the treatment of the patient and critically revised the manuscript. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

AD reports consultancy fees from Alnylam and Merck outside the submitted work. CM reports research grants (paid to institution), financial support for participation in congresses, and expertise fees from Chiesi and Astellas. StB received lecture fees from Astellas. BG reports financial support for participation in congresses from Chiesi and Gilead, speaker for Gilead, outside the submitted work. LC received lecture fees from Astellas, Chiesi, Novartis, Sandoz, Ostuka, GSK, and Biotest, and participated in advisory boards for Biotest, Hansa, and Novartis. LE received fees from Astellas, Chiesi, and Sandoz. FL received research grants (paid to institution) from Astellas, Sandoz, and Chiesi and fees to attend meetings from Viiv, MSD, Janssen-Cilag, Pfizer, and Gilead.

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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Retraction: Mesenchymal Stromal Cells for Tissue-Engineered Tissue and Organ Replacements

Transplant International Editorial Office*

A Retraction of the Review Article

Mesenchymal Stromal Cells for Tissue-Engineered Tissue and Organ Replacements

by Baiguera S, Jungebluth P, Mazzanti B, Macchiarini P. (2012). *Transpl. Int.* 2012; 25: 369-82. doi: 10.1111/j.1432-2277.2011.01426.x

Transplant International published in 2012 a review paper [1], in which another article from the same authors was cited [2]. This article had been published in the *Lancet* in 2008 and was very recently retracted for demonstrated falsification [3]. The falsification had been confirmed in a decision by the Swedish National Board for Assessment of Research Misconduct [4].

Briefly, the paper retracted by the *Lancet* described the allegedly successful transplantation of a tissue-engineered tracheal segment, reporting that “the graft immediately provided the recipient with a functional airway, improved her quality of life, and had a normal appearance and mechanical properties at 4 months” [2]. The conclusions of the investigation by the Swedish National Board for Assessment of Research Misconduct found that this statement constituted falsification [4].

The review article [1] was accepted and published in *Transplant International* based on its perceived quality and the supposed merits of the authors’ work. In this review, the authors stated that “(tissue engineering) has already provided functional tissue and organ human replacement” (sic), citing to support this point the retracted *Lancet* article [2] that they knew contained falsified data.

We are therefore retracting this review article.

Thierry Berney.

Transplant International editor-in-chief.

OPEN ACCESS

Approved by:

Thierry Berney,
University of Geneva, Switzerland

*Correspondence

Transplant International Editorial
Office,
✉ tieditorialoffice@frontierspartnerships.org

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Editorial Office

Avenue du Tribunal Fédéral 34
CH – 1005 Lausanne
Switzerland

Tel +41 (0)21 510 17 40
Fax +41 (0)21 510 17 01

tieditorialoffice@frontierspartnerships.org
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