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The importance of HLA-DQ matching

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TRANSPLANT TRIAL WATCH
published: 30 September 2024
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Transplant Trial Watch

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Keywords: randomised controlled trial, kidney transplantation (KT), hypothermia, normothermia, Treg

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

Fixed Hypothermia for Expanded Criteria Organ Donors in Kidney Transplantation in France (HYPOREME): A Multicentre, Randomised Controlled Trial.

by The HYPOREME Trial Group. The Lancet Respiratory Medicine 2024 [record in progress].

Aims
This study aimed to examine the effect of donor hypothermia versus normothermia on the risk of delayed graft function.

Interventions
Donors were randomised to either hypothermia or normothermia.

Participants
365 expanded criteria kidney donors with death diagnosed based on neurological criteria and 526 graft recipients.

Outcomes
The primary endpoint was proportion of renal transplant patients with delayed graft function. Secondary endpoints in donors were the number of organs recovered and transplanted, blood pressure, body temperature, kidney function, need for vasopressors and inotropes, total volume of fluids administered, and adverse events. Secondary endpoints for recipients were graft function, length of hospital stay, vital status and adverse events.

Follow-Up
1 year after transplantation.

CET Conclusion
by Simon Knight

This multicentre, randomised trial recruited 365 extended-criteria DBD donors in 53 intensive care units in France. Donors were randomised to hypothermia (34°C–35°C) or normothermia.

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Hypothermic machine preservation was routinely used for kidneys from recruited donors. The focus of the study was the outcomes of the kidney transplant recipients. Primary endpoint was incidence of delayed graft function, which was not significantly different between groups. A small, statistically significant difference was seen in 1-year graft function, with lower serum creatinine and higher creatinine clearance. One thing that is not clear from the manuscript is how many of the donors donated organs other than kidneys, and what the outcomes were for these organs. Clearly, any donor intervention has the potential to impact all donated organs, and so this should be mandatory outcome reporting for donor intervention studies. Otherwise, the study is well designed and reported, with block randomisation, allocation concealment and blinding of outcome assessment. The clinical significance of the differences in kidney function at 1 year is small but given the simplicity of the intervention and evidence for safety, it adds to the existing evidence that donor hypothermia may be beneficial to organ recipients.

Jadad Score
3.

Data Analysis
Modified intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
ClinicalTrials.gov - NCT03098706.

Funding Source
Non-industry funded.

RANDOMISED CONTROLLED TRIAL 2

Effect Late Treatment with Autologous Expanded Regulatory T-Cell Therapy After Alemtuzumab Induction Is Safe and Facilitates Immunosuppression Minimization in Living Donor Renal Transplantation.

by Brook, M. O., et al. Transplantation 2024 [record in progress].

Aims
The aim of this study was to examine the effect of delayed infusion of autologous expanded regulatory T-cell (Treg) following alemtuzumab induction in living donor kidney transplant recipients.

Interventions
Participants were randomised to either the Treg therapy arm or to standard immunosuppression alone.

Participants
7 living donor kidney transplant patients.

Outcomes
The primary endpoints of interest were patient survival, graft survival and the incidence of biopsy-confirmed acute rejection events.

Follow-Up
18 months post-transplantation.

CET Conclusion
by Keno Mentor

The Transplantation Without Overimmunosuppression (TWO) study was originally designed to investigate the efficacy of regulatory T cells (Treg) therapy to enable the reduction of long-term immunosuppression. Treg cells were to be infused 6 months after kidney transplantation with Alemtuzumab induction therapy. However, during the COVID-19 pandemic, Alemtuzumab use was suspended because of safety concerns and the trial was re-designed based on Basiliximab induction therapy. Prior to this change, 7 patients received therapy as per the original protocol – these results are presented in this study as a proof-of-concept analysis. 3 patients were randomised to the Treg therapy group with elimination of MMF and reduction of tacrolimus, and 4 to the standard immunosuppression (alemtuzumab induction, Tacrolimus & MMF therapy) group. The patients were followed up for 18 months. MMF elimination was achieved in all patients in the treatment group, but although tacrolimus doses were decreased, average trough levels were equal between the two groups. There was 100% graft survival in both groups and no episodes of rejection in the Treg group versus one episode in the standard group. Adverse events were generally low in both groups. With such a small cohort, these results cannot be generalisable, but the study does contribute to and support existing data that demonstrates that Treg therapy is safe and potentially enables reduction of immunosuppression. Larger studies with long-term follow-up are needed to better determine the degree of benefit.

Jadad Score
3.

Data Analysis
Strict intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
International Standard Randomised Controlled Trial Number registry - 11038572.

Funding Source
Non-industry funded.

CLINICAL IMPACT SUMMARY

by John O'Callaghan

This is a thought-provoking clinical study that set out to use regulatory T-cells after renal transplantation, with alemtuzumab induction and overall reduction in immune suppression over time. The study arm received an infusion of regulatory T-cells at week 26, following which the patients would stop MMF and be maintained on tacrolimus monotherapy. In the control arm all patients would continue tacrolimus and MMF from the time of transplant. All were living donor transplants and low risk immunologically. The target inclusion was 68 patients.

The study commenced prior to the COVID pandemic, during which the use of alemtuzumab was suspended in the United Kingdom, and the study protocol had to be changed. The published study report therefore only includes data from a cohort of 7 patients, 3 of whom were in the cell therapy arm. The regimen in the study arm is interesting, with a protocol biopsy at 26 weeks, after which MMF was stopped. A second protocol biopsy was taken at 38 weeks, and if satisfactory the dose of tacrolimus was taken down to maintain reduced trough levels. An interesting feature of the regimen is the delayed infusion of regulatory T-cells at 6 months after transplant, which targets the lymphocyte repopulation phase.

Unfortunately, the direct clinical impact of the study is significantly reduced by the very much reduced patient inclusion. The 3 patients who received the regulatory T-cell infusion had no haemodynamic or inflammatory reaction. Transplant Survival at 18 months was 100%, with no acute rejection in the study arm, and one patient in the control arm with early acute rejection treated with steroids. One control patient experienced a decline in renal function due to CNI toxicity.

This paper reports on the safety of delayed infusion of regulatory T-cells, at 26 weeks after renal transplantation, and following immune induction with a leukodepleting agent. This will be a proof of concept for future studies in this field.

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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ORIGINAL RESEARCH
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High-Risk HLA-DQ Mismatches Are Associated With Adverse Outcomes After Lung Transplantation

Lisa Kleid[1†], Julia Walter[2,3,4†], Patrick Moehnle[1], Christian Wichmann[1], Julia Kovács[2,3], Andreas Humpe[1], Christian Schneider[2,3], Sebastian Michel[3,5], Nikolaus Kneidinger[3,4,6], Michael Irlbeck[7], Jan Fertmann[2,3], Andrea Dick[1†] and Teresa Kauke[2,3,8*†]*

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Human leukocyte antigen (HLA) mismatches (MM) between donor and recipient lead to eplet MM (epMM) in lung transplantation (LTX), which can induce the development of de-novo donor-specific HLA-antibodies (dnDSA), particularly HLA-DQ-dnDSA. Aim of our study was to identify risk factors for HLA-DQ-dnDSA development. We included all patients undergoing LTX between 2012 and 2020. All recipients/donors were typed for HLA 11-loci. Development of dnDSA was monitored 1-year post-LTX. EpMM were calculated using HLAMatchmaker. Differences in proportions and means were compared using Chi2-test and Students' t-test. We used Kaplan-Meier curves with LogRank test and multivariate Cox regression to compare acute cellular rejection (ACR), chronic lung allograft dysfunction (CLAD) and survival. Out of 183 patients, 22.9% patients developed HLA-DQ-dnDSA. HLA-DQ-homozygous patients were more likely to develop HLA-DQ-dnDSA than HLA-DQ-heterozygous patients (p = 0.03). Patients homozygous for HLA-DQ1 appeared to have a higher risk of developing HLA-DQ-dnDSA if they received a donor with HLA-DQB1*03:01. Several DQ-eplets were significantly associated with HLA-DQ-dnDSA development. In the multivariate analysis HLA-DQ-dnDSA was significantly associated with ACR (p = 0.03) and CLAD (p = 0.01). HLA-DQ-homozygosity, several high-risk DQ combinations and high-risk epMM result in a higher risk for HLA-DQ-dnDSA development which negatively impact clinical outcomes. Implementation in clinical practice could improve immunological compatibility and graft outcomes.

Keywords: eplet matching, *de novo* donor specific antibody, HLA-DQ antibody, risk-stratification, lung transplantation

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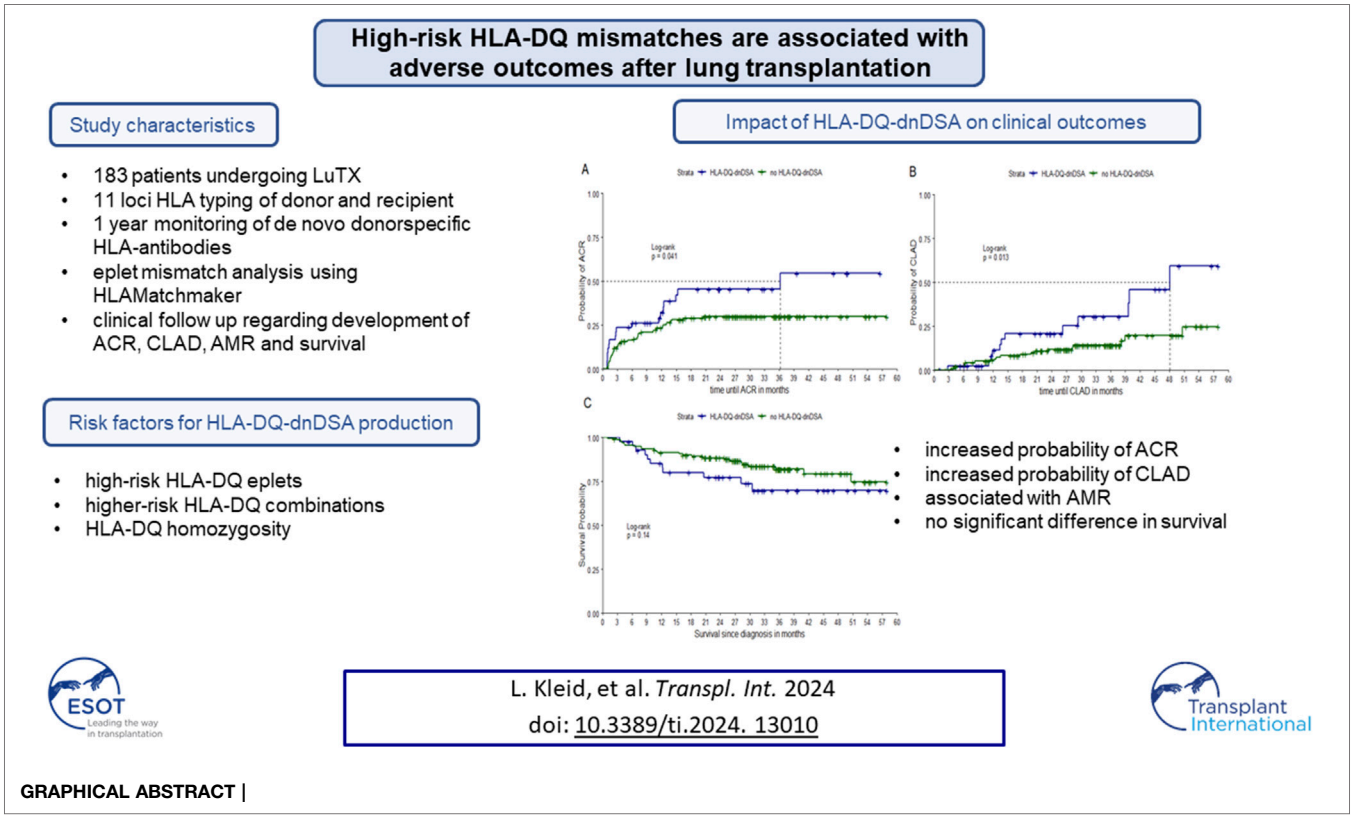

GRAPHICAL ABSTRACT |

INTRODUCTION

Lung transplantation can lead to better quality of life and prolonged survival in patients suffering from end-stage lung disease. Despite improvement of surgical techniques and advances in immunosuppression, the median survival time after lung transplantation remains at 6 years [1]. One limiting factor is the development of *de novo* donor specific HLA antibodies (dnDSA), which are part of antibody-mediated rejection (AMR) and have been associated with the development of acute and chronic rejection, primarily the bronchiolitis obliterans syndrome (BOS) [2, 3]. However, human leukocyte antigen (HLA) compatibility of donor and recipient can improve long-term graft survival of transplanted organs. Choosing histocompatible donors might reduce the risk of HLA antibody development and therefore lower the risk of rejection of the donor organ [4]. Currently, HLA-matching is not taken into account for allocation of lungs due to controversial data, urgency and organ shortage. HLA-matching is only mandatory in kidney patients and current findings show that disparities between HLA molecules are better described by epitope matching algorithms rather than matching the entire antigen [5–7]. With HLAMatchmaker, the immunogenic parts of each HLA molecule, the so-called eplets, can be calculated. Eplets are known as variable amino acid segments within a 3.0–3.5 Ångstroms radius of functional HLA epitopes, which can be directly recognized by recipients' B-lymphocytes and thus lead to the development of donor-specific antibodies (DSA) [8, 9]. Although there is consensus among experts that molecular histocompatibility is better described by eplet mismatches than antigen mismatches, the immunogenicity of the individual eplet mismatches (epMM) is still a matter of debate [10]. There is a strong need to define immunogenicity of the eplets in order to implement epitope matching into routine diagnostics. Institutions such as the International HLA & Immunogenetics Workshop Foundation work to improve patient care by facilitating collaborations between researchers. In a recent study a German research group was able to show that immunisation against HLA-class II and especially against HLA-DQ made up the largest part of *de novo* donor-specific HLA-antibodies in their lung transplant patients [11]. Both matching algorithms, PIRCHE-II and HLAMatchmaker, have proven to be helpful tools to identify patients at higher risk for the development of *de novo* DSA, especially when used together. However, immunisation seems to be determined not only by the amount of eplets present, but also by the presence of high-risk eplets resulting from certain donor-recipient constellations [12]. The aim of our present study was to identify those eplet disparities between recipient and donor that could be associated with the development of HLA-DQ-dnDSA. Beside identifying high-risk eplets, we aimed to reveal other risk factors that are associated with HLA-DQ-dnDSA development. We also aimed to confirm the association between the development of HLA-DQ-dnDSA and acute cellular rejection (ACR), antibody-

mediated rejection (AMR), chronic lung allograft dysfunction (CLAD) and survival.

Thus, we would like to present an approach to improve risk assessment in lung transplant patients to potentially improve long-term transplant outcomes.

PATIENTS AND METHODS

Lung Transplant Cohort

This retrospective study is based on data from patients who underwent lung transplantation at the Ludwig-Maximilians-University (LMU) hospital between 2012 and 2020. The main inclusion criteria was complete HLA 11-loci loci typing of both donor and recipient (HLA-A, B, C, DRB1, DRB345, DQA1, DQB1, DPA1 and DPB1). Patients with pre-transplant HLA antibodies were excluded from the study, as were patients who did not have class I or class II HLA mismatches. The majority of the patients received a standard triple immunosuppressive regimen with tacrolimus, mycophenolat-mofetil and steroids without induction therapy. All patients were followed up at the transplant centre including lung function tests, bronchoscopy and HLA antibody screening.

Our study was approved by the ethics committee of LMU (reference number 22-0166) and was carried out in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local ethical and legal requirements.

HLA Typing

All patients and donors in the study were routinely typed for 11 HLA loci. Recipients were typed by means of sequence specific oligonucleotide technique (LABType™ SSO Typing Kits, One Lambda, Inc., Canoga Park, CA, United States). Organ donors were typed using the sequence specific oligonucleotide technique (LABType™ SSO Typing Kits, One Lambda, Inc., Canoga Park, CA, United States) or real-time PCR genotyping with sequence-specific primers (LinkSēq™ HLA-ABCDRDQB1 384 Kit, One Lambda, Inc., Canoga Park, CA, United States).

HLA-Antibody Detection

According to the local transplant protocol, patients' sera are regularly tested for the presence of HLA antibodies using Luminex screening and single antigen bead technology prior to lung transplantation as well as 1, 3, 6 and 12 months after transplantation (LABScreen™ Mixed Class I and II and LABScreen™ Single-antigen HLA Class I - Combi and Class II – Group 1, One Lambda, Inc., Canoga Park, CA, United States). Patients' sera have been heat-inactivated to avoid prozone effect and were measured undiluted. All donor specificities reported could be explained by one or more of the mismatched eplets. Specificities with a mean fluorescence intensity of approximately 1.000 were considered positive. The majority of dnDSA have been detected more than once and have been classified as persistent. They have been defined as transient if they disappeared spontaneously or after treatment. All of our analysis were performed with persistent and transient dnDSA.

Antigen and Eplet Matching

HLA antigen matching was performed by comparing HLA of donor and recipient on the antigen level. In case of ambiguities, the most common alleles and their resulting serological equivalents were used. Due to the small number of patients in each group, patients and donors, homozygous either for HLA-DQ5 and/or -DQ6, were combined as HLA-DQ1 homozygous, patients homozygous for either HLA-DQ7, -DQ8 and/or DQ9 were termed as HLA-DQ3 homozygous.

Number and type of epMMs was calculated with R. Duquesnoy's HLAMatchmaker algorithm (HLAMatchmaker algorithm integrated in One Lambda Fusion software, One Lambda, Inc., Canoga Park, CA, United States) based on donors' and recipients' HLA 11-locityping results. Due to intermediate resolution results, most common alleles were used for molecular matching (eplet matching). All eplet mismatches were accepted equally regardless of whether they were verified by antibodies or not. For the calculation of the number of eplets, interlocus class II eplets have been removed. All eplet information was concordant with the Epitope Registry [HLA Epitope Registry (HLA Epitope Registry.com.br, version 3.0)]. Eplets with a high ElliPro score, according to the Epitope registry, were counted as highly immunogenic eplets.

Clinical Outcomes

The primary clinical parameters for the study were ACR, AMR, CLAD and survival.

ACR was diagnosed by graft biopsy and graded according to the ISHLT classification system [13]. A transplant biopsy was performed routinely after 4 weeks, after 3 months, and after 6 months, as part of the follow up examinations, and on demand in case of clinical suspicion. All grades were treated with steroid pulse therapy starting from A1.

AMR was defined according to the ISHLT consensus report and staged into clinical- and subclinical, and possible and probable AMR [14]. Diagnosis was based on allograft function, conspicuous features in histology such as infiltration with neutrophile granulocytes, positive immunohistochemical C4d staining, development of dnDSA and after exclusion of secondary causes.

CLAD was diagnosed and staged according to the CLAD consensus definition of the ISHLT Guidelines of 2019 [15]. CLAD was characterized by a persistent decline of FEV1 to 80% of baseline or below after exclusion and adequate treatment of secondary causes such as infection, acute cellular or antibody-mediated rejection, or airway stenosis according to current definitions.

Statistical Analysis

We reported categorical variables as absolute and relative frequencies and numerical variables as means with standard deviation (sd). We compared differences in frequencies and mean values between groups using Chi^2 or Fisher's exact test (cell-numbers < 6), and Student's t-tests, respectively. In the univariate analysis, we used Kaplan-Meier curves with LogRank-test to compare time to ACR, CLAD, and death between patients with and without HLA-DQ-dnDSA. In the multivariate analysis we Cox regression models to analyse time to event data concerning development of ACR, CLAD, and

TABLE 1 | Characteristics of study population stratified by HLA-DQ-dnDSA.

	all patients (n = 183)		HLA-DQ-dnDSA (n = 42)		no HLA-DQ-dnDSA (n = 141)		*p*-value
	mean	sd	mean	sd	Mean	sd	
Age in years	51.8	13.0	50.6	12.9	52.1	13.0	0.51
BMI	23.1	4.5	23.7	4.7	22.9	4.4	0.30
	n	%	n	%	n	%	
Sex							
Female	69	37.7%	17	40.5%	52	36.9%	0.81
Male	114	62.3%	25	59.5%	89	63.1%	
Underlying condition							
COPD	45	24.6%	9	21.4%	36	25.5%	0.22
CF	35	19.1%	7	16.7%	28	19.9%	
ILF	26	14.2%	3	7.1%	23	16.3%	
Other (PPH, LAL, EAA, bronchiectasis, sarcoidosis)	77	42.1%	23	54.8%	54	38.3%	
Type of surgery							
Single lung	28	15.3%	4	9.5%	24	17.0%	0.35
Double lung	155	84.7%	38	90.5%	117	83.0%	
Blood type							
O	73	39.9%	21	50.0%	52	36.9%	0.45
A	77	42.1%	15	35.7%	62	44.0%	
B	29	15.8%	5	11.9%	24	17.0%	
AB	4	2.2%	1	2.4%	3	2.1%	
CMV							
R-D-	42	23.0%	7	16.7%	35	24.8%	0.51
R-D+	59	32.2%	15	35.7%	44	31.2%	
R+D-	33	18.0%	10	23.8%	23	16.3%	
R+D+	49	26.8%	10	23.8%	39	27.7%	

Notes: Baseline characteristics of our study collective of lung transplanted patients, stratified by development of de novo *donor specific antibodies against HLA-DQ during the first year after transplantation. CMV serostatus was determined by ELISA. Categorical variables are reported as absolute and relative frequencies and numerical variables as means with standard deviation.* P*-values between frequencies and mean values between patients with HLA-DQ-dnDSA and patients without are from Chi² and Fisher's exact test (cell-numbers <6), and Students' t-test.*

BMI, body mass index; CF, cystic fibrosis; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; D, donor; dnDSA, de-novo *donor-specific antibody; ELISA, enzyme-linked immunosorbent assay; EAA, exogenous allergic alveolitis; HLA, human leukocyte antigen; HLA-DQ-dnDSA, donor specific antibodies against HLA-DQ; ILF, idiopathic lung fibrosis; PPH, primary pulmonary hypertension; sd, standard deviation; R, recipient.*

survival. All regression models were adjusted for age, sex, blood type, and CMV risk combination status. Results from regression analysis are reported as Hazard ratios (HR). Statistical significance in all analysis was determined using two-sided *p*-values with alpha errors of <0.05. Data analysis was performed using R Version 4.0.0 and RStudio Version 1.4. Tables and figures were created in RStudio and Microsoft Excel.

RESULTS

Study Population

A total of 608 patients underwent lung transplantation between 2012 and 2020. For 220 of these patients, complete HLA 11-locityping of donor and recipient were available. Of these, one patient was excluded due to missing HLA antibody follow up data, 32 patients due to positive HLA antibody status before transplantation, and four patients due to no HLA-class I or HLA-class II mismatches. Finally, we were able to include 183 patients in our study. Recipients and donor characteristics are shown in **Table 1**. Of the 183 patients included in the study, 62.3% of recipients were male with mean age of 51.8 (sd = 13.0) years. The most common diagnosis was chronic obstructive pulmonary disease (24.6%). Eighty-five percent of patients underwent a double lung transplantation. The majority of lung donors (78.1%) revealed two HLA-DQ mismatches. Only in 1.6% of cases there was no HLA-DQ mismatch between donor and recipient.

HLA-Antibody Development

Of the 183 patients, 52 (28.4%) developed dnDSA during 1 year after transplantation. Of all patients with dnDSA, 22/52 (42.3%) patients developed dnDSA against HLA-class I, and 45/52 patients (86.5%) against HLA class II. As previously described by Kleid L. et al [9] we evaluated molecular matching algorithms regarding the development of class I and class II antibodies. However, the main analysis in this paper focuses on HLA-DQ antibodies as the detected class II antibodies were predominantly directed against HLA-DQ (n = 42/45). Among the patients with HLA-DQ-dnDSA, the majority developed antibodies against HLA-DQ3 (59.5%). Most of the patients were immunised against more than one HLA-DQ antigen. The dnDSA characteristics of each patient is listed in the **Supplementary Material 1**.

TABLE 2 | Comparison between number of antigen- and epletMM.

	All patients (n = 183)		HLA-DQ-dnDSA (n = 42)		no HLA-DQ-dnDSA (n = 141)		*p*-value
	mean	sd	mean	sd	mean	sd	
# HLA-DQ-epMM	14.5	6.8	18.0	6.6	13.5	6.6	0.0001
# highly immunogenic HLA-DQ-epMM	11.1	5.5	14.1	4.7	10.2	5.4	<0.0001
	n	%	n	%	n	%	
HLA-DQ antigenMM							
0	3	1.6%	0	0.0%	3	2.1%	0.71
1	37	20.2%	7	16.7%	30	21.3%	
2	143	78.1%	35	83.3%	108	76.6%	

Notes: Antigen mismatch between donor and recipient was calculated by comparing their HLA-typing results. The amount of epMM was calculated with HLAMatchmaker algorithm (OneLambda Fusion software). Eplets with high ElliPro Scores according to the HLA Epregistry 3.0 were taken into account for the number of highly immunogenic eplet mismatches. The results were stratified by development of HLA-DQ-dnDSA. Mean values were compared using two sided p-values from Students' t-test.
EpMM, eplet mismatches; HLA, human leukocyte antigen; HLA-DQ-dnDSA, donor specific antibodies against HLA-DQ; sd, standard deviation; # = number.

TABLE 3 | Association between recipient/donor HLA-DQ alleles and development of HLA-DQ-dnDSA.

		HLA-DQ-dnDSA (n = 42)		no HLA-DQ-dnDSA (n = 141)		*p*-value
		n	%	n	%	
Recipient alleles						
homozygous HLA-DQ		22	52.4	45	31.9	
heterozygous HLA-DQ		20	47.6	96	68.1	0.03
Allele combination of HLA-DQ-homozygous patients						
rec: DQ1, DQ1	**do:** DQ1, DQ1	1	2.4	10	7.1	0.46
rec: DQ1, DQ1	**do**: DQ2, DQ1	0	0.0	3	2.1	1.00
rec: DQ1, DQ1	**do:** DQ2, DQ2	0	0.0	1	0.7	1.00
rec: DQ1, DQ1	**do:** DQ2, DQ3	1	2.4	2	1.4	0.54
rec: DQ1, DQ1	**do:** DQ3, DQ1	9	21.4	2	1.4	<0.0001
rec: DQ1, DQ1	**do:** DQ3, DQ3	2	4.8	4	2.8	0.62
rec: DQ1, DQ1	**do:** DQ4, DQ1	0	0.0	1	0.7	1.00
rec: DQ2, DQ2	**do**: DQ1, DQ1	-0	0.0	1	0.7	1.00
rec: DQ2, DQ2	**do**: DQ2, DQ3	1	2.4	1	0.7	0.41
rec: DQ2, DQ2	**do:** DQ3, DQ1	0	0.0	3	2.1	1.00
rec: DQ3, DQ3	**do:** DQ1, DQ1	1	2.4	5	3.5	1.00
rec: DQ3, DQ3	**do:** DQ2, DQ1	2	4.8	1	0.7	0.13
rec: DQ3, DQ3	**do:** DQ2, DQ2	0	0.0	1	0.7	1.00
rec: DQ3, DQ3	**do:** DQ2, DQ3	2	4.8	1	0.7	0.13
rec: DQ3, DQ3	**do**: DQ3, DQ1	2	4.8	7	5.0	1.00
rec: DQ3, DQ3	**do:** DQ3, DQ3	1	2.4	1	0.7	0.41
rec: DQ3, DQ3	**do:** DQ3, DQ4	0	0.0	1	0.7	1.00

Notes: The cohort was analysed according to patients and donors HLA-DQ typing. Certain cross-reactive allele groups were combined into one group as follows: HLA-DQ1 = HLA-DQ5/DQ6; HLA-DQ3 = HLA-DQ7/DQ8/DQ9. The results were stratified by development of HLA-DQ-dnDSA. P-values were derived from Chi^2 test or Fisher's exact test (cell numbers < 6).
rec, recipient; do, donor; HLA-DQ-dnDSA, donor specific antibodies against HLA-DQ.

Risk Factors for HLA-DQ-dnDSA Development

There was no significant difference between the number of HLA DQ-antigen mismatches regarding HLA-DQ-dnDSA development. The number of HLA-DQ epMM, as well as the number of highly immunogenic HLA-DQ epMM, was significantly higher in patients who developed HLA-DQ-dnDSA (**Table 2**).

According to our data, recipients who were homozygous for HLA-DQ were significantly more likely to develop HLA-DQ-dnDSA compared to HLA-DQ heterozygous recipients (52.4% vs. 47.6%, *p*-value = 0.03). HLA-DQ1 homozygous recipients transplanted with HLA-DQ3/DQ1 donors were at a higher risk to develop HLA-DQ-dnDSA than patients transplanted with donors of other genotypes (21.4%, *p*-value <0.0001). Absolute and relative frequencies and *p*-values of allele combinations in homozygous recipients stratified by HLA-DQ-dnDSA are summarized in **Table 3**. If both, recipient and donor, had the allele combination DQ3/DQ1 this was significantly associated with not having HLA-DQ-dnDSA (0.0% vs. 12.1%, *p*-value = 0.01).

The following HLA-DQ eplets were significantly more prevalent in the HLA-DQ-dnDSA group: 55PP (50.0% vs. 22.7%, *p*-value = 0.001), 55PPD (47.6% vs. 23.4%, *p*-value =

TABLE 4 | Description of "high-risk" eplets.

eplet	Polymorphic AA residues	Main alleles (most common)	ElliPro Score	HLA-DQ-dnDSA (n = 42)		no HLA-DQ-dnDSA (n = 141)		*p*-value
				n	%	n	%	
55PP	55P56P	DQ3 (DQB1*03:01, DQB1*03:02, DQB1*03:03)	High	21	50.0	32	22.7	0.001
55PPD	55P56P57P	DQ3 (DQB1*03:01, DQB1*03:03)	High	20	47.6	33	23.4	0.004
66ER	66E67V70R71T	DQ3 (DQB1*03:01, DQB1*03:02, DQB1*03:03) scattered on DQ1 (DQB1*06:04 DQB1*06:05, DQB1*06:06)	High	20	47.6	32	22.7	0.003
182N	182N	DQ3 (DQB1*03:01, DQB1*03:02, DQB1*03:03), DQ4	High	19	45.2	32	22.7	0.01
70RT	70R71T	DQ3 (DQB1*03:01, DQB1*03:02, DQB1*03:03) scattered on DQ1 (DQB1*06:01 DQB1*06:04 DQB1*06:05, DQB1*06:06)	High	19	45.2	30	21.3	0.004
45EV	45E46V47Y	DQ3 (DQB1*03:01)	High	20	47.6	28	19.9	0.001
167H	167H	DQ3 (DQB1*03:01), DQ1 (DQB1*06:01)	High	19	45.2	29	20.6	0.003
66IL	66I69L	DQA1*02,03,05	Intermediate	11	26.2	17	12.1	0.05
61FT	61F64T55R	several DQA-alleles (except DQA1*01)	High	12	28.6	14	9.9	0.005
84QL	84Q86E87L89T90T125A	DQ3 (DQB1*03:01, DQB1*03:02, DQB1*03:03), DQ2, DQ4	High	12	28.6	13	9.2	0.003

Notes: List of eplets that were significantly associated with the development of de novo *HLA-DQ-dnDSA, including their properties such as polymorphic amino acid residues, representing alleles and ElliPro scores according to the HLA Epitope Registry (HLA Epitope Registry.com.br). P-values were derived from Chi² test or Fisher's exact test (cell numbers < 6). AA, amino acid; HLA-DQ-dnDSA,* de-novo *donor-specific antibodies against HLA-DQ; … = also represented on other rare alleles.*

0.004), 66ER (47.6% vs. 22.7%, *p*-value = 0.003), 182N (45.2% vs. 22.7%, *p*-value = 0.01), 70RT (45.2% vs. 21.3%, *p*-value = 0.004), 45EV (47.6% vs. 19.9%, *p*-value = 0.001), 167H (45.2% vs. 20.6%, *p*-value = 0.003), 66IL (26.2% vs. 12.1%, *p*-value = 0.05), 61FT (28.6% vs. 9.9%, *p*-value = 0.005), 84QL (28.6% vs. 9.2%, *p*-value = 0.003). The absolute and relative frequencies and *p*-values for these "high-risk eplets" are shown in **Table 4**. The eplet 130Q was significantly more prevalent in patients without HLA-DQ-dnDSA (**Supplementary Material 2**).

HLA-DQ-dnDSA and Clinical Outcomes

Among the 183 patients included in our study, 58 patients suffered from ACR. Of these, 18 patients were positive for HLA-DQ-dnDSA. A total of 10 patients with diagnosed ACR died, three of them within the first year after transplantation.

Within our cohort, 52 patients showed signs of a possible or probable AMR. Of these, 47 were staged as subclinical and five patients as possible clinical AMR. Among the five patients with clinical AMR, there were 3 patients with severe outcome who died within the first year; all had HLA-DQ-dnDSA. Patients with dnDSA (class I, class II, HLA-DQ) showed significantly more clinical and subclinical AMR (**Table 5**).

Concerning long term outcome, 35 patients were diagnosed with CLAD of which 12 were positive for HLA-DQ-dnDSA. A total of 7 patients died, all of them after more than 1 year after transplantation.

Kaplan-Meier Curves

Figure 1 shows Kaplan-Meier curves of time until first detection of HLA-DQ-dnDSA, by being homozygous for HLA-DQ, by having a high-risk allele combination (homozygous for HLA-DQ1 in combination with HLA-DQ3/DQ1 donors), and by having at least one high-risk eplet mismatch. Patients with these risk-factors had a significantly higher risk to develop HLA-DQ-dnDSA compared to patients without.

Figure 2 shows Kaplan-Meier curves of time until ACR, CLAD, and death stratified by development of HLA-DQ-dnDSA. We found that having HLA-DQ-dnDSA was significantly associated with time to ACR (*p*-value = 0.04) and time to CLAD (*p*-value = 0.01). However, we did not find a significant association between HLA-DQ-dnDSA and overall survival (*p*-value = 0.14).

Even though our study focused on HLA-DQ-dnDSA we additionally analysed HLA class I and class II antibodies with clinical outcome data. The results of this analysis displayed as Kaplan-Meier curves can be found in the **Supplementary Material 3, 4**. HLA class I dnDSA were not significantly associated with clinical outcomes, HLA class II dnDSA were significantly associated with time to CLAD.

Multivariate Regression Analysis

The multivariate Cox regression models of time to HLA-DQ-dnDSA confirmed the significant association between homozygosity of HLA-DQ (model 1), and the high-risk allele combination (model 2) and the development of HLA-DQ-dnDSA from the univariate analysis. In contrast to the presence of at least one high-risk eplet (model 3) the number of high-risk eplets was still significantly associated with time to HLA-DQ-dnDSA (model 4).

In the multivariate Cox regression, no direct correlation was found between the above-mentioned risk factors and survival or time to ACR. However, the high-risk allele combination was significantly associated with time to CLAD.

The association of HLA-DQ-dnDSA with time to ACR (HR = 1.85, *p*-value = 0.04) and time to CLAD (HR = 2.61, *p*-value = 0.01) revealed significant results. Survival time and HLA-DQ-dnDSA were not significantly associated. **Table 6** shows results of all regression models.

TABLE 5 | Association of dnDSA with antibody-mediated rejection.

	Class-I-DSA (n = 22)		No class-I-DSA (n = 161)		*p*-value
	%		n	%	
AMR					
Yes	18	81.8%	34	21.1%	<0.0001
No	4	18.2%	127	78.9%	
AMR subtype					
None	4	18.2%	127	78.9%	<0.0001
1a	3	13.6%	2	1.2%	
2a	15	68.2%	30	18.6%	
2b	0	0.0%	2	1.2%	
	Class-II-DSA (n = 45)		**No class-II-DSA (n = 138)**		***p*-value**
	%		n	%	
AMR					
Yes	32	71.1%	20	14.5%	<0.0001
No	13	28.9%	118	85.5%	
AMR subtype					
None	13	28.9%	118	85.5%	<0.0001
1a	5	11.1%	0	0.0%	
2a	25	55.6%	20	14.5%	
2b	2	4.4%	0	0.0%	
	DQ-DSA (n = 42)		**No DQ-DSA (n = 141)**		***p*-value**
	%		n	%	
AMR					
Yes	29	69.0%	23	16.3%	<0.0001
No	13	31.0%	118	83.7%	
AMR subtype					
None	13	31.0%	118	83.7%	<0.0001
1a	4	9.5%	1	0.7%	
2a	23	54.8%	22	15.6%	
2b	2	4.8%	0	0.0%	

Notes: Overview of patients with diagnosed with antibody-mediated rejection classified according to the ISHLT consensus guidelines and stratified by development of HLA class I, class II or HLA-DQ-dnDSA.

AMR, antibody-mediated rejection; ISHLT, the international society of heart and lung transplantation; 1a = possible clinical antibody-mediated rejection; 2a = possible subclinical antibody-mediated rejection, 2b = probable subclinical antibody-mediated rejection; HLA, human leucocyte antigen; HLA-DQ-dnDSA, de-novo *donor-specific antibodies against HLA-DQ.*

DISCUSSION

In this study, we analysed 183 lung transplant patients for their HLA-antibody status and the impact of the presence of HLA-DQ-dnDSA on their clinical outcomes. We used antigen- and eplet-based HLA-matching as a new approach in addition to determining quantitative amount of mismatches for risk assessment. Our purpose is to add valuable information to the growing knowledge about risk factors for dnDSA and rejection after lung transplantation. In addition, our results indicate an easy applicable approach to identify high risk patient donor combinations that can be used in clinical practice.

We found that patients with HLA-DQ-dnDSA were at a significantly higher risk for developing ACR and CLAD. This finding further supports the association of HLA-DQ-dnDSA, with ACR, CLAD, and overall survival found in other studies [16–18]. Our results also strengthen the association of class I, class II and HLA-DQ-dnDSA with AMR, which is confirmatory in nature as dnDSA are in most cases one of the diagnosis criteria for AMR. We could not confirm the effects on survival, which might be due to a shorter follow-up period. However, our results are in line with Ennis et al, who investigated the impact of *de novo* HLA-DQ antibodies resulting from a DQA1*05 + DQB1*02/DQB*03:01 mismatch, and showed that these dnDSA are associated with CLAD but not survival [19].

A few studies indicate that HLA-DQ-dnDSA are the most prevalent in cardiothoracic transplant patients and there is evidence of inferior graft outcomes [16, 20]. This has also been described in renal [21] and cardiac transplantation [22]. Increased expression of HLA-class II molecules in inflamed lung tissue might be one explanation [23, 24]. The development of an ACR can affect long-term complications such as the development of CLAD and have a negative impact on patients' survival. Lowering the immunological risk for developing HLA-DQ-dnDSA and therefore the risk for ACR, AMR and CLAD will contribute to improve graft outcome.

Regarding antigen-based HLA-matching, we could show that the number of antigen mismatches does not play a major role. Rather, it is important to look a bit closer at the patient's own HLA-DQB1 typing. We were able to demonstrate that HLA-DQ-

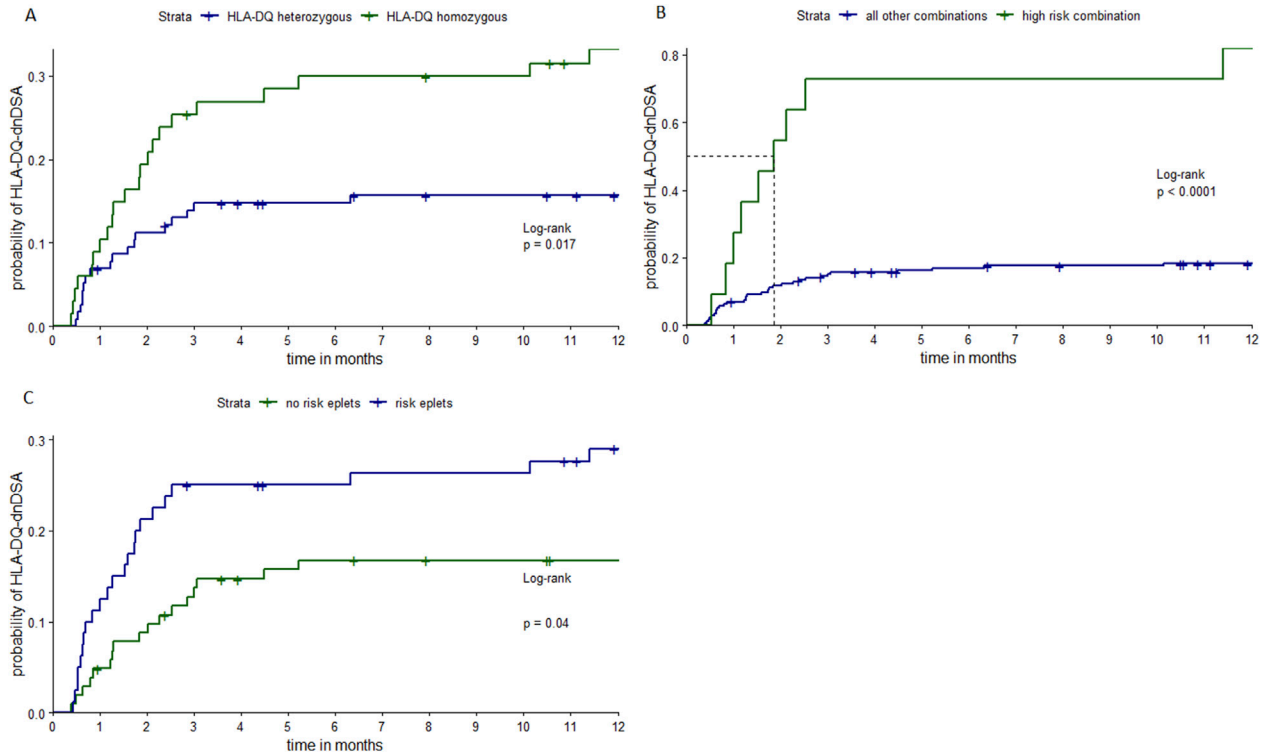

FIGURE 1 | Kaplan-Meier curves stratified by different risk-factors. Notes: Kaplan-Meier curves of time until first detection of HLA-DQ-dnDSA, stratified by being homozygous or heterozygous for HLA-DQ **(A)**, by having the high-risk allele combination (homozygous HLA-DQ1 patients, recipients transplanted with HLA-DQ3/DQ1 typed donors) or any other allele combination **(B)** and stratified by the presence of one or more high-risk eplets **(C)**. Patients and donors who were homozygous either for HLA-DQ5 and/or -DQ6 were combined as HLA-DQ1 homozygous, patients homozygous for either HLA-DQ7, -DQ8 and/or DQ9 were termed as HLA-DQ3 homozygous. The following eplets are determined as high-risk eplets 55PP, 55PPD, 66ER, 182N, 70RT, 45EV, 167H, 66IL, 61FT, 84QL. *P*-values from LogRank test. HLA, human leucocyte antigen; HLA-DQ-dnDSA, *de-novo* donor-specific antibodies against HLA-DQ locus.

homozygous patients have a significantly higher risk to develop HLA-DQ-dnDSA than HLA-DQ heterozygous patients. The risk of developing HLA-DQ-dnDSA was 2.34 times higher compared to heterozygous patients. HLA-DQ homozygosity is a risk factor as these patients are facing more structural differences than heterozygous patients. One specific recipient-donor antigen combination, recipient homozygous for HLA-DQ1 with HLA-DQ1/DQ3 donors, was significantly associated with the development of HLA-DQ-dnDSA. This high-risk donor recipient constellation was also described in the publication of McCaughan et al [20]. They showed similar findings within their patient cohort and assume electrostatic potentials as a possible explanation for the increased immunogenicity. They also described that a combination of the foreign HLA-DQA1 and HLA-DQB1 structures could be crucial for immunisation. Besides confirming the aforementioned risk-constellation, the added value of our study is reflected in the clinical outcome parameters of our patient cohort. Unfortunately, we were not able to compare the allele combinations on high resolution as the number of combinations was too high and the number of patients within each combination too small. Although one of the strengths of our study is the large patient cohort, the number of patients with HLA-DQ-dnDSA was small, especially for the analysis of high-risk donor-recipient combinations. Further research on this topic and larger cohorts might help to see whether more high risk or low risk combinations can be revealed.

Regarding HLA eplet matching, we were able to show that the number of epMM was associated with the development of HLA-DQ-dnDSA. Previously we had shown that it was associated with the development of HLA-antibodies [11]. Similar results have also been reported by Hiho et al. [25]. Both works show that comparing the number of molecular mismatches can be an approach for risk stratification in lung transplantation. One limitation of using and comparing eplet matching data are uncertainties not only in terminology but also in their application, as recently described and summarized by Tambur et al [26]. Depending on user preferences and different versions of the HLAMatchmaker algorithm, eplet matching results or eplets loads can lead to discordant results concerning number and type of mismatched eplets. In our study, eplets designated as "antibody-confirmed" and those lacking confirmation were treated equally due to the fluid nature of classification. An eplet labelled as unverified presently could potentially undergo experimental validation by a research team in the foreseeable future. Moreover, the validation process lacks clear regulation and consistency, making comparisons challenging [27]. Tambur

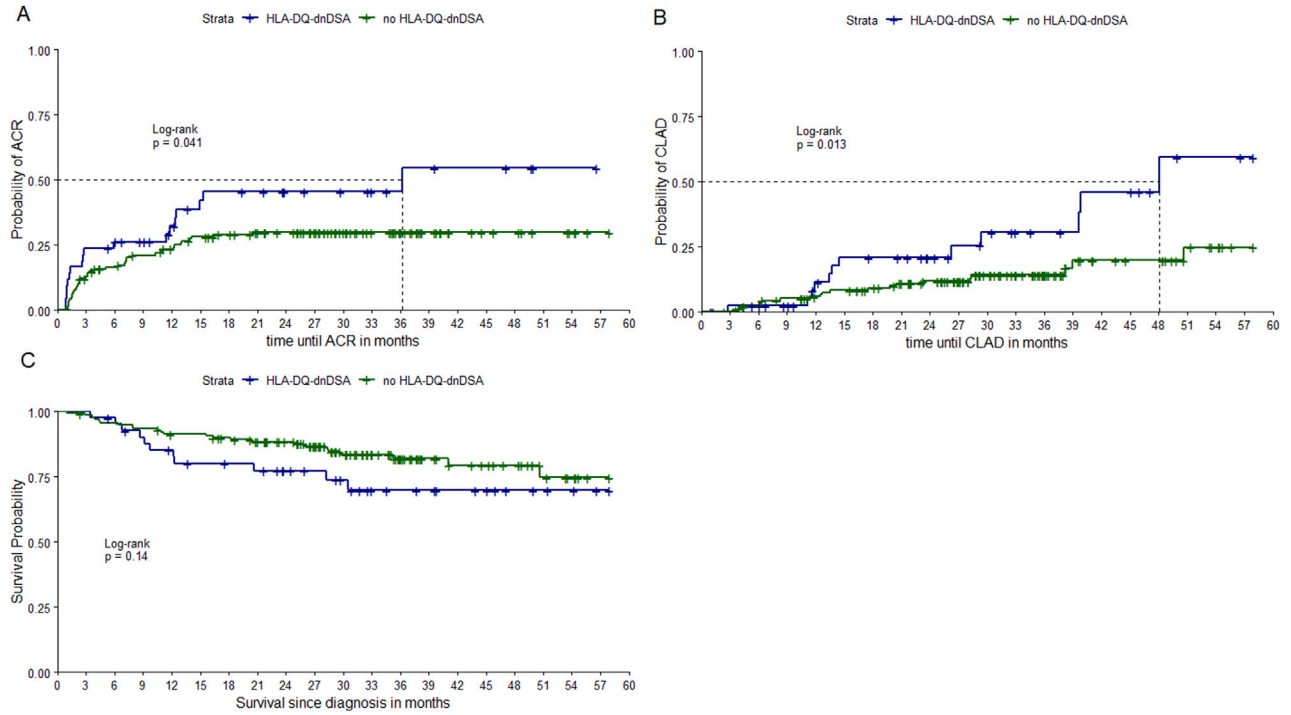

FIGURE 2 | Kaplan Meier curves stratified by HLA-DQ-dnDSA. Notes: Kaplan-Meier curves of time until ACR, time until CLAD and time until death, stratified by development of HLA-DQ-dnDSA. *P*-values from LogRank test. ACR, acute cellular rejection; CLAD, chronic allograft dysfunction; HLA-DQ-dnDSA, *de-novo* donor-specific antibodies against HLA-DQ locus. Notes: Kaplan-Meier curves of time until ACR **(A)**, time until CLAD **(B)** and time until death **(C)**, stratified by development of HLA-DQ-dnDSA.

TABLE 6 | Results of regression analyses.

		Beta	HR	se	z-value	*p*-value
Cox regression of time to HLA-DQ-dnDSA						
Model 1	Recipient allele homozygous vs. Heterozygous	0.68	1.97	0.32	2.10	0.04
Model 2	High risk allele combination	1.82	6.17	0.39	4.61	<0.0001
Model 3	High risk eplet yes vs. No	0.57	1.76	0.32	1.78	0.08
Model 4	# of high risk eplets	0.16	1.17	0.04	3.97	<0.0001
Cox regression of time to CLAD						
Model 5	Recipient allele homozygous vs. Heterozygous	–0.58	0.56	0.42	–1.40	0.16
Model 6	High risk allele combination	1.15	3.15	0.56	2.04	0.04
Model 7	High risk eplet yes vs. No	0.14	1.15	0.36	0.39	0.70
Model 8	# Of high risk eplets	0.04	1.04	0,05	0.92	0.36
cox regression of ACR, CLAD, and survival						
Model 9	HLA-DQ-dnDSA and ACR	0.62	1.85	0.29	2.14	0.03
Model 10	HLA-DQ-dnDSA and CLAD	0.96	2.61	0.38	2.55	0.01
Model 11	HLA-DQ-dnDSA and survival	0.61	1.83	0.38	1.62	0.11

Notes: Results from Cox regression analysis of development of HLA-DQ-dnDSA (models 1–4), time to CLAD recipient allele homozygous/heterozygous (model 5), high-risk allele combination (model 6), high-risk eplet yes vs. no (model 7), #of high risk eplets (model 8), and Cox regression analysis of time to ACR (model 9), CLAD (model 10) and survival (model 11). All Cox regression models are adjusted by age, sex, and CMV risk combination.

ACR, acute cellular rejection; CLAD, chronic lung allograft dysfunction; CMV, cytomegalovirus; HLA, human leucocyte antigen; HLA-DQ-dnDSA, de-novo donor-specific antibodies against HLA-DQ; OR, odds ratio; se = standard error; # = number.

et al. also point out that the number of eplet mismatches should be considered with caution. Combining recipient and donor eplets into one so called HLAMatchmaker "eplet universe" and not considering individual alleles, bears the risk of creating potential ambiguities and the immunologic validity of this concept still needs to be determined. Therefore, it is important to also look at specific eplets. In this study we identified potential high-risk eplets that have a greater potential to induce the development of dnDSA. Our findings are similar to the data of Schawalder et al, who analysed child-

specific anti-HLA DQ-antibodies after pregnancy [28]. They found that the eplets 55PP and 45EV are highly reacting eplets and we were able to confirm these findings within our lung transplant cohort, and additionally identified several more high-risk eplets. Hereby, one must bear in mind that different versions of the Matchmaker have been used (2.1 and 3.0). There were also patients with high-risk epMMs who did not develop HLA-DQ-dnDSA in our cohort. When we compared these patients to patients with high-risk epMM and HLA-DQ-dnDSA, we found that being female slightly elevated the risk for immunisation. This might be explained by prior contact to foreign HLA during pregnancies. Immunisation is a multifactorial process and adherence to immunosuppressive therapy might also be a factor. It would be interesting to monitor patients' compliance in further studies. Nevertheless, we clearly identified several high-risk eplets that were significantly more immunogenic than others which in most cases resulted from a HLA-DQB1*03:01 mismatch. Snanoud and colleagues reported similar findings in their kidney transplant cohort [29].

As described by Schawalder et al., one major limitation in defining immunogenicity of eplets is to identify the true target of the antibody. Each HLA-mismatch leads to a set of overlapping eplets, each eplet on its own or several eplets might explain the reaction pattern in the Luminex Assay [28]. Especially the HLA-DQ locus is very complex as it is composed of an α and β chain, each carrying individual immunogenic eplets. Moreover, a distinction between anti-HLA-DQA1 and -DQB1 antibodies in Luminex data interpretation is sometimes not possible. Most of our high-risk eplets were derived from donor´s HLA-DQB1, however in their cohort of lung transplant patients González-López et al showed that also HLA-DQA1 epMM could lead to inferior graft outcomes [30].

One limitation of our study is the resolution of our HLA typings. Analysis with high-resolution typing and comparing donor and recipient on the amino acid level might help to reveal the true antibody targets. Available typing information has improved over the last years and hopefully studies with more recent high-resolution typing data will help to better perform eplet analysis and make molecular mismatch methods more accurate.

Although further research on this topic is necessary, there is a clear tendency towards HLA-DQB1*03:01 as a highly immunogenic HLA mismatch, regarding both the antigen and the responsible eplets. Randomised clinical trials are needed to gain a better understanding of the clinical relevance and potentially the significance of increased immunosuppression in a high-risk constellation.

CONCLUSION

Specific HLA-DQ mismatches seem to be particularly responsible for the development of *de novo* HLA-antibodies after lung transplantation, which in return result in inferior graft outcomes. EpMM analysis might be a helpful tool for risk assessment in order to support clinicians in identifying patients at higher risk for HLA-DQ-dnDSA. Although it will take some time until molecular matching algorithms will be ready to be consistently used in clinical routine, our analysis has highlighted HLA-DQ phenotypes of high-risk recipients, recipient-donor combinations and high-risk eplets for risk-assessment. With this early information about increased humoral risk, adjustment of immunosuppression or closer follow-up could lead to improved long-term survival in lung transplant patients.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by Ethikkommission der medizinischen Fakultät, Ludwig-Maximilians-Universität München. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because retrospective non-interventional observational study.

AUTHOR CONTRIBUTIONS

Conceptualization and Design: LK, AD, TK, AH, CW, and PM. Collection and assembly of data: LK and JK. Data analysis and interpretation: LK, JW, AD, and TK. Resources: JF, CS, MI, NK, and TK. Writing of the original draft: LK, JW, AD, and TK. Editing the manuscript: AH, CW, and PM. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.13010/full#supplementary-material

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Analysis of Rejection, Infection and Surgical Outcomes in Type I Versus Type II Diabetic Recipients After Simultaneous Pancreas-Kidney Transplantation

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Given the increasing frequency of simultaneous pancreas-kidney transplants performed in recipients with Type II diabetes and CKD, we sought to evaluate possible differences in the rates of allograft rejection, infection, and surgical complications in 298 Type I (T1D) versus 47 Type II (T2D) diabetic recipients of simultaneous pancreas-kidney transplants between 2006-2017. There were no significant differences in patient or graft survival. The risk of biopsy-proven rejection of both grafts was not significantly different between T2D and T1D recipients ($HR_{pancreas}$ = 1.04, p = 0.93; HR_{kidney} = 0.96; p = 0.93). Rejection-free survival in both grafts were also not different between the two diabetes types ($p_{pancreas}$ = 0.57; p_{kidney} = 0.41). T2D had a significantly lower incidence of *de novo* DSA at 1 year (21% vs. 39%, p = 0.02). There was no difference in T2D vs. T1D recipients regarding readmissions (HR = 0.77, p = 0.25), infections (HR = 0.77, p = 0.18), major surgical complications (HR = 0.89, p = 0.79) and thrombosis (HR = 0.92, p = 0.90). In conclusion, rejection, infections, and surgical complications after simultaneous pancreas-kidney transplant are not statistically significantly different in T2D compared to T1D recipients.

Keywords: infection, rejection, complication, pancreas-kidney transplantation, type 2 diabetes

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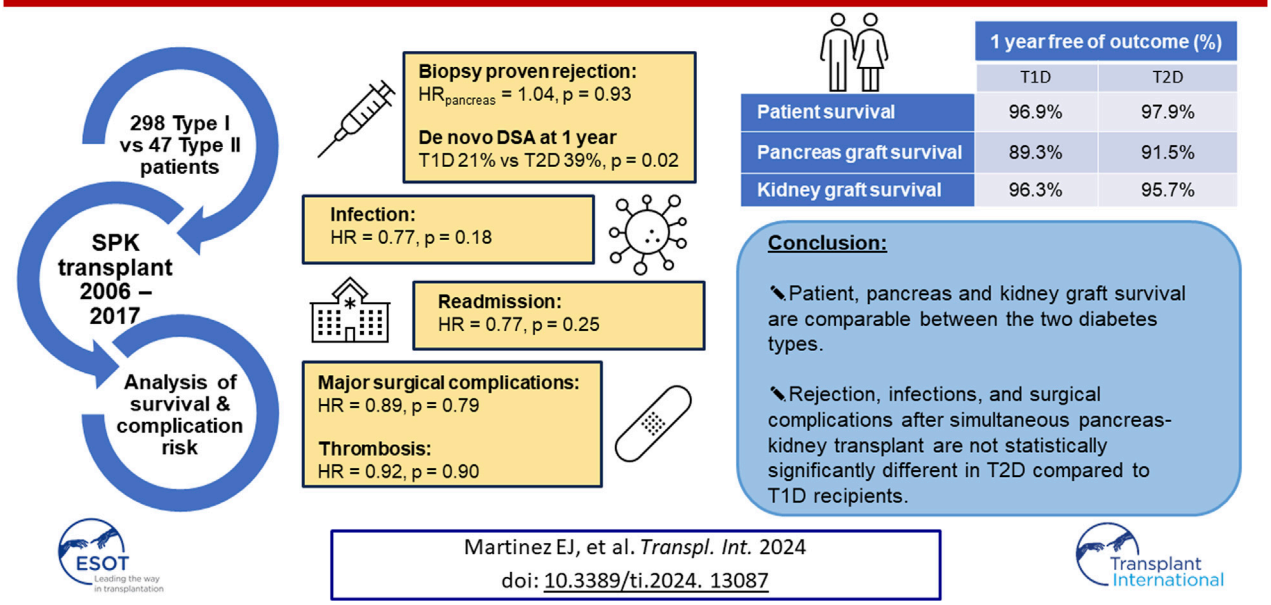
Abbreviations: ALEM, Alemtuzumab; ATG, Antithymocyte globulin; BAS, Basiliximab; BMI, body mass index; BPR, biopsy proven rejection; CIT, cold ischemia time; CMV, cytomegalovirus; dnDSA, *de novo* anti-HLA donor specific antibody; DCD, donation after circulatory death; ESRD, end stage renal disease; KDPI, kidney donor profile index; OPTN/UNOS, Organ Procurement and Transplant Network/United Network for Organ Sharing; PDRI, pancreas donor risk index; SPKT, simultaneous pancreas kidney transplant; STAR, Standard Transplant Analysis and Research; T1D, Type I diabetes mellitus; T2D, Type II diabetes mellitus; UW, University of Wisconsin.



GRAPHICAL ABSTRACT |

INTRODUCTION

Simultaneous pancreas-kidney transplantation (SPKT) in Type I diabetes (T1D)with end-stage renal disease (ESRD) has produced significant improvement in prolongation and quality of life. Patient survival approaches 97% and 92% at 1 year and 3 years, respectively [1]. The half-life of pancreas allografts has increased to 15.5 years [2] secondary to advances in immunosuppressive therapy, surgical techniques, and immune monitoring [1, 3–5]. SPKT is also associated with improved kidney graft survival [6, 7] and improved preservation of kidney graft ultrastructure and function [8] compared to deceased donor kidney transplant alone.

Concerning SPKT in Type II diabetes mellitus (T2D) patients with ESRD, many studies have addressed the outcomes of pancreas transplantation for such patients [9]. Such studies have found comparable results between the two types of recipients regarding various endpoints including insulin resistance and β-cell function [3], kidney and pancreas graft survival [9–16], post-transplant glycemic control, BMI control [9, 17], and patient survival [6, 11, 18].

However, the effect of diabetes type on graft rejection after pancreas transplantation is less well understood. Differing rates of allograft rejection are observed in other abdominal solid organ transplants based on the primary etiology of the organ failure, especially with autoimmune components [19–30]. Several studies have evaluated the effects of donor-specific anti-HLA antibodies (DSA) on graft outcomes [31–33] and noted significantly decreased kidney and pancreas allograft survival [33–36]. None of these studies, however, account for the type of diabetes as a distinguishing factor.

In addition, T2D patients may be obese and consequently may have an increased risk of surgical site infections [37, 38] and worse graft outcomes [39]. The inflammatory milieu of T2D may impact the risk of surgical infections, thrombosis, etc., [40–42]. While these theoretical risks may exist, the outcomes of T1D and T2D SPKT recipients with respect to important specific surgical and infection-related outcomes have not been thoroughly evaluated.

Thus, in this study, we sought to comprehensively examine whether the type of diabetes impacts the rates of acute biopsy-proven rejection and DSA development as well as other key surgical and infectious complications. Additionally, we globally analyze factors contributing to these outcomes in the T1D and T2D SPKT populations.

MATERIAL AND METHODS

A single center retrospective review of prospectively collected data from a comprehensive in-house Transplant Database, electronic medical records, and the UNOS/OPTN STAR file was approved by the local Institutional Review Board. Analysis included primary SPKT recipients from 2006–2017 with 1-year minimum post-transplant follow-up. Diabetes mellitus types were determined by a holistic assessment with a grading system that included factors of patients' age at diabetes onset, need for immediate use of insulin, pre-transplant fasting

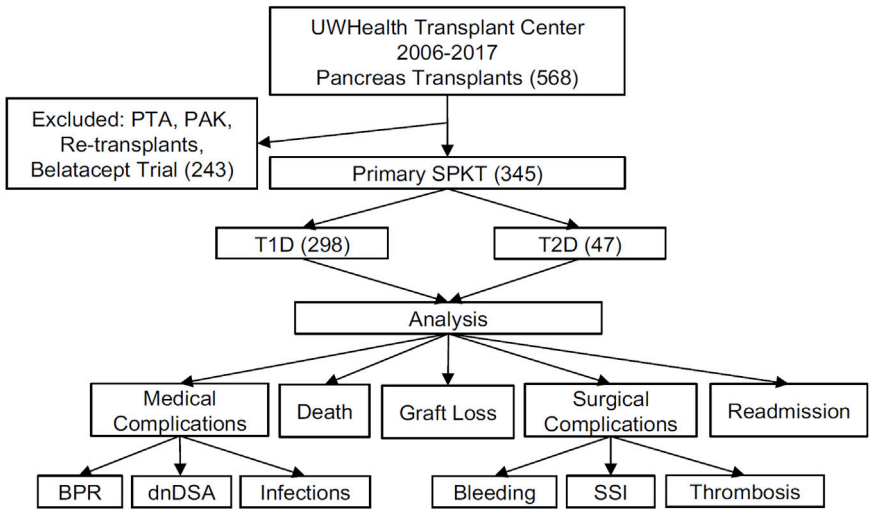


FIGURE 1 | Schematic diagram of study design and data analysis. UWHC, University of Wisconsin Hospital and Clinics; PTA, Pancreas Transplant Alone; PAK, Pancreas After Kidney (transplant); SPKT, Simultaneous Pancreas Kidney Transplant; PTA, Pancreas Transplant Alone; PAK, Pancreas After Kidney (transplant); T1D, Type 1 Diabetes Mellitus; T2D, Type 2 Diabetes Mellitus; BPR, Biopsy Proven Rejection; dnDSA, *de novo* Donor Specific Antibody; SSI, Surgical Site Infection.

C-peptide, family history of diabetes, and the presence of autoantibodies (GAD65, Insulin- and Islet-antibodies) [43]. Primary outcomes included patient and graft survival, incidence of biopsy proven pancreas and kidney rejection and dnDSA, readmissions, infections, and surgical complications, including bleeding, pancreatic graft thromboses and other surgical site complications (**Figure 1**).

Clinical Management

Systemic venous drainage and enteric exocrine drainage were performed in all SPKTs. Most patients were transferred to the transplant floor post-operatively with aspirin as the sole anticoagulation and without NG tube placement. Each patient's immunosuppressive therapy was protocolized based on pre-transplant immunologic risk assessment. Either Alemtuzumab (ALEM) (30 mg, 1 dose), anti-thymocyte globulin (ATG) (1.5 mg/kg, 3-4 doses), or basiliximab (BAS) (20 mg, 2 doses) were used for induction therapy. Oral tacrolimus (initial target levels 8–10 ng/mL in the first year and 6–8 ng/mL thereafter) and oral mycophenolic acid (720 mg twice daily) were used as maintenance therapy in all patients. Dexamethasone 100 mg IV was administered intraoperatively and tapered to prednisone thereafter per protocol. Post-induction, selected patients underwent either early steroid withdrawal protocol or a rapid steroid taper to prednisone 5 mg daily by 1 month. All recipients receiving BAS induction received a more delayed steroid taper to prednisone 5–10 mg daily by 6 months. Nystatin and trimethoprim-sulfamethoxazole were given for 3 months and 1 year respectively. CMV prophylaxis with valganciclovir or acyclovir was given for 6 and 3 months depending on the recipients' risk. Virtual crossmatching has been our standard minimal compatibility testing for the entire study period.

Outcome Definitions

Graft Failure

Per UNOS definitions, pancreas graft failure was defined by graft pancreatectomy, reregistration for pancreas transplant, registration for islet transplantation, use of insulin >0.5 unit/kg/day for 90 consecutive days, or recipient death. Kidney graft failure was defined by graft nephrectomy, return to maintenance dialysis, or recipient death [44].

Graft Rejection

Pancreas allograf biopsy indications included post-transplant elevation of amylase or lipase, DSA increase or dnDSA, and hyperglycemia. Pancreas and kidney biopsies were evaluated by light microscopy with assignment of a grade (indeterminate/borderline, I, II, and III) and degree of immunohistochemical staining for C4D (none, <5%, or >5%) according to the Banff grading schema [45]. Acute rejection outcome represents cellular rejection or antibody mediated rejection or both.

De Novo DSA

Donor-specific anti-HLA Class I and II antibodies were detected pre- and post-transplant using Luminex single antigen beads (One Lambda, Canoga Park, CA). Antibodies were identified using multiple criteria including patterns of epitope reactivity, mean fluorescence intensity (MFI) value, specific bead behaviors, and assay background [46]. Since 2014, routine post-transplant monitoring of DSA has been performed on all transplant recipients at 6 and 12 months, and annually thereafter. Patients with a pretransplant calculated panel reactive antibody greater than zero were tested at an additional 6-week time point, and patients with pre-transplant DSA were tested at additional 3-week, 6-week, and 3-month time points. All patients undergoing kidney or pancreas transplant biopsy for any reason had DSA testing as a part of the biopsy visit [35, 47]. The strength

of *de novo* DSA (dnDSA) was represented as the sum of the MFI of all DSA. Patients were diagnosed with dnDSA if any one of the following occurred: i) no detectable pre-transplant DSA followed by the development of new antibodies post-transplant, ii) the sum MFI increased by at least 2 fold, or iii) new alleles were detected post-transplant.

Infections

Post-transplant infections were categorized as bacterial or opportunistic infections (including virus, fungus, *listeria*, *nocardia*, and CMV viremia) and surgical site related. Surgical site infections were defined as any wound or intraabdominal infection within 90 days post-transplantation. Urinary tract infections (UTI) within the first-year post-transplantation were also assessed.

Surgical Complications

Surgical complications were categorized as either bleeding, non-bleeding or thrombotic complications (see **Table 2** footnote for specific complications). Pancreatic graft thrombotic events were defined as either partial thrombosis resulting in continued graft function or complete thrombosis requiring transplant pancreatectomy or causing early graft failure within 90 days post-transplantation.

Statistical Analysis

Differences in recipient and donor demographic factors between T1D and T2D recipients were analyzed using t-tests and Chi-square tests or Fisher's exact tests. Multivariable Cox Proportional Hazards models, or multiple logistic regression, when appropriate, were used to investigate the association of all outcomes with diabetes types, while adjusting for recipient's BMI, age at time of transplant, PDRI, KDPI, and induction immunosuppression. Death-censored-, rejection-free-, readmission free-, infection-free-, major surgical complication-free-, *de novo* DSA free-, thrombosis free-survival and thrombosis related to graft failure free-survival were compared between T1D and T2D using Kaplan Meier curves and log-rank tests. Post-transplant outcomes relating to the average number of episodes within the first year were analyzed using t-tests. Analyses were conducted using SAS software (version 9.4, SAS Institute Inc., Cary, NC) and p-values less than 0.05 were considered to be statistically significant.

RESULTS

Study Population

A total of 345 SPKTs were categorized as 298 T1Ds and 47 T2Ds. The average post-transplant follow-up was 6.7 ± 3.6 years. Donor demographic factors were not significantly different between T1D and T2D recipients (**Table 1**). Several recipient demographic factors, not surprisingly, were significantly different between the cohorts. Besides the expected differences in several recipient factors such as age, BMI, ethnicity and duration of diabetes, T2D patients has lower positivity for GAD65 autoantibody and was more frequently treated with ATG and ALEM induction and early steroid withdrawal compared to T1D patients ($p < .001$). Lastly, there was no significant difference in the presence of pre-transplant DSA, or degree of pre-transplant DSA between the two groups.

Patient and Graft Survival

Patient survival (97.9% in T2D vs. 96.9% in T1D at 1 year) and pancreas graft survival (91.5% in T2D vs. 89.3% in T1D at 1 year) were not statistically significantly different between T1D and T2D SPKT recipients (**Figures 2A, B**; **Table 2**). Kidney graft survival was also not different between the two types of diabetes recipients (95.7% in T2D vs. 96.3% in T1D at 1 year) (**Figure 2D**; **Table 2**).

Pancreas Rejection

Pancreas biopsy-proven rejection (BPR)-free survival and 1-year BPR-free survival were similar between the two types of diabetic recipients (89.0% for T2D and 87.3% for T1D) (**Figure 2C**; **Table 2**). Further stratification of rejection endpoints by grade of rejection, C4d positivity, and assessing average episodes per patient (**Table 3**) also failed to elucidate statistically significantly different rejection outcomes in the T1D vs. T2D recipients. Multivariable analysis (**Table 4**) showed that diabetes type has little association with overall pancreas BPR or other rejection subcategories. Interestingly, increasing BMI was a significant protective factor against pancreas BPR with and without Indeterminate/borderline pathology included, Grade 1 BPR, and C4d > 5% staining on biopsy (HR = 0.90, 0.89, 0.86, 0.88 respectively, all $p < 0.05$). Increasing PDRI was not significantly associated with any pancreas rejection endpoints. Meanwhile, increasing KDPI was significantly associated with a higher risk of pancreas BPR with Indeterminate/borderline pathology included (HR = 1.02, $p = 0.03$) but was not significant when excluding Indeterminate/borderline pathology. Increasing age at transplant was protective against C4d > 5% staining on biopsy (HR = 0.95, $p = 0.01$). Compared to BAS, both ALEM and ATG showed a trend, though not significant, to being protective toward overall pancreas BPR and BPR subcategories. Univariate analysis by induction type failed to demonstrate significant differences in index outcomes (**Table 5**).

Kidney Rejection

Overall kidney rejection-free survival between T2D and T1D was not significantly different ($p = 0.41$) (**Figure 2E**; **Table 2**). In univariate analysis, the rate of kidney BPR within the first year was 8.8% in T2D recipients vs. 12.4% in T1D recipients ($p = 0.47$) (**Table 3**). The lack of association between diabetes type and kidney graft rejection was confirmed in multivariable analysis (**Table 4**). Unlike for pancreas graft rejection, neither BMI nor KDPI were significantly associated with an increased risk of kidney rejection. Older age at transplant was marginally protective against rejection (HR = 0.97, $p = 0.05$) (**Table 4**). Compared to BAS, ATG was significantly associated with decreased kidney BPR (HR = 0.40, $p = 0.04$).

De Novo DSA

Overall dnDSA-free survival between T2D and T1D was significantly different ($p = 0.03$) (**Figure 2F**; **Table 2**). A significantly lower incidence of dnDSA was observed in T2D

TABLE 1 | SPKT donor and recipient demographics.

	T1D (n = 298)	T2D (n = 47)	P-value
Donor – pre-transplant			
Age, years (mean ± sd)	29.1 ± 12.6	27 ± 12	0.28
Males	176 (59.1%)	27 (57%)	0.83
BMI, kg/m^2 (mean ± sd)	24.0 ± 4.4	23.7 ± 4.2	0.61
Type of transplant (%DBD)	81.9%	72.3%	0.12
PDRI (mean ± sd)	1.31 ± 0.5	1.3 ± 0.4	0.59
KDPI (mean ± sd)	22.7% ± 18.7%	23.1% ± 15.2%	0.91
Pancreas cold ischemic time, hours (mean ± sd)	12.6 ± 4.1	12.8 ± 3.8	0.82
Kidney cold ischemic time, hours (mean ± sd)	13.9 ± 4.3	14.8 ± 3.8	0.23
CMV (% positive)	49%	55%	0.74
EBV (% positive)	88.4%	80%	0.27
Donor HLA Mismatch			0.67
0	2 (0.7%)	0 (0%)	
1	3 (1%)	0 (0%)	
2	12 (4%)	3 (6.4%)	
3	45 (15.1%)	4 (8.5%)	
4	86 (28.9%)	13 (27.7%)	
5	95 (31.9%)	20 (42.6%)	
6	55 (18.5%)	7 (15%)	
Recipient – pre-transplant			
Males (%)	179 (60.1%)	40(85.1%)	<.001
Recipient Race			<.001
American Indian or Alaska Native (%)	2 (0.7%)	2 (4.3%)	
Asian (%)	3 (1.0%)	4 (8.5%)	
Black or African American (%)	23 (7.7%)	10 (21%)	
White (%)	270 (90.6%)	31 (66%)	
Age at the time of diabetes mellitus diagnosis, years (mean ± sd)	13.7 ± 7.6	28.3 ± 9.1	<.001
25%–75% quartile range	8.0–18.0	21.0–35.0	
Median	12.0	27.0	
Age at the time of transplant, years (mean ± sd)	42.5 ± 9.1	47.9 ± 9.1	<.001
25%–75% quartile range	35.3–49.4	39.5–55.4	
Median	42.3	51.8	
Recipient Onset of Diabetes Greater than 30 Years			<.001
No (%)	290 (97.3%)	25 (53.2%)	
Yes (%)	8 (2.7%)	22 (46.8%)	
BMI, kg/m^2 (mean ± sd)	25.6 ± 3.7	27.3 ± 3.4	0.004
25%–75% quartile range	23.0–27.8	24.9–29.6	
Median	25.2	27.4	
C-peptide, ng/mL (mean ± sd)	0.19 ± 0.39	3.67 ± 3.24	<.001
25%–75% quartile range	0.10–0.10	1.33–4.90	
Median	0.10	3.20	
HbA1c, % (mean ± sd)	8.38 ± 1.62	7.71 ± 1.46	0.01
25%–75% quartile range	7.20–9.30	6.65–8.80	
Median	8.30	7.70	
Family history of diabetes (% yes)	55%	85.1%	<.001
Insulin requirements pre-transplant, unit/day (mean ± sd)	39.1 ± 16.1	44.5 ± 28.4	0.21
25%–75% quartile range	27.0–50.0	20.0–60.0	
Median	37.0	40.5	
CMV (% positive)	39.4%	55.3%	0.04
EBV (% positive)	94.5%	97.9%	0.03
PRA (% mean ± sd)	7 ± 20.0	6.3 ± 16.3	0.83
Pre-transplant DSA			0.12
Negative (%)	283 (95.3%)	42 (89.4%)	
<1000 MFI (%)	8 (2.69%)	4 (8.5%)	
>1000 MFI (%)	6 (2.02%)	1 (2.13%)	
NA (%)	1 (0.003%)	0 (0%)	
Auto antibody status			
Number of tested patients	42	28	
Any auto-antibody (% positive)	73.8%	28.6%	<.001
GAD65 (% positive)	55.9%	11.1%	<.001
Insulin Ab (% positive)	63.2%	17.9%	<.001
Islet IgG (% positive)	0.0%	8%	0.14
Steroid immunosuppression			0.01
Early steroid withdrawal (%)	6 (2%)	6 (12.8%)	

(Continued on following page)

TABLE 1 | (*Continued*) SPKT donor and recipient demographics.

	T1D (n = 298)	T2D (n = 47)	P-value
Induction and maintenance (%)	266 (89.26%)	41 (87.23%)	
Induction immunosuppression			<.001
Anti-thymocyte globulin (ATG) (%)	46 (15.4%)	22 (46.8%)	
Alemtuzumab (ALEM) (%)	79 (26.5%)	10 (21.3%)	
Basiliximab (BAS) (%)	173 (58%)	15 (31.9%)	

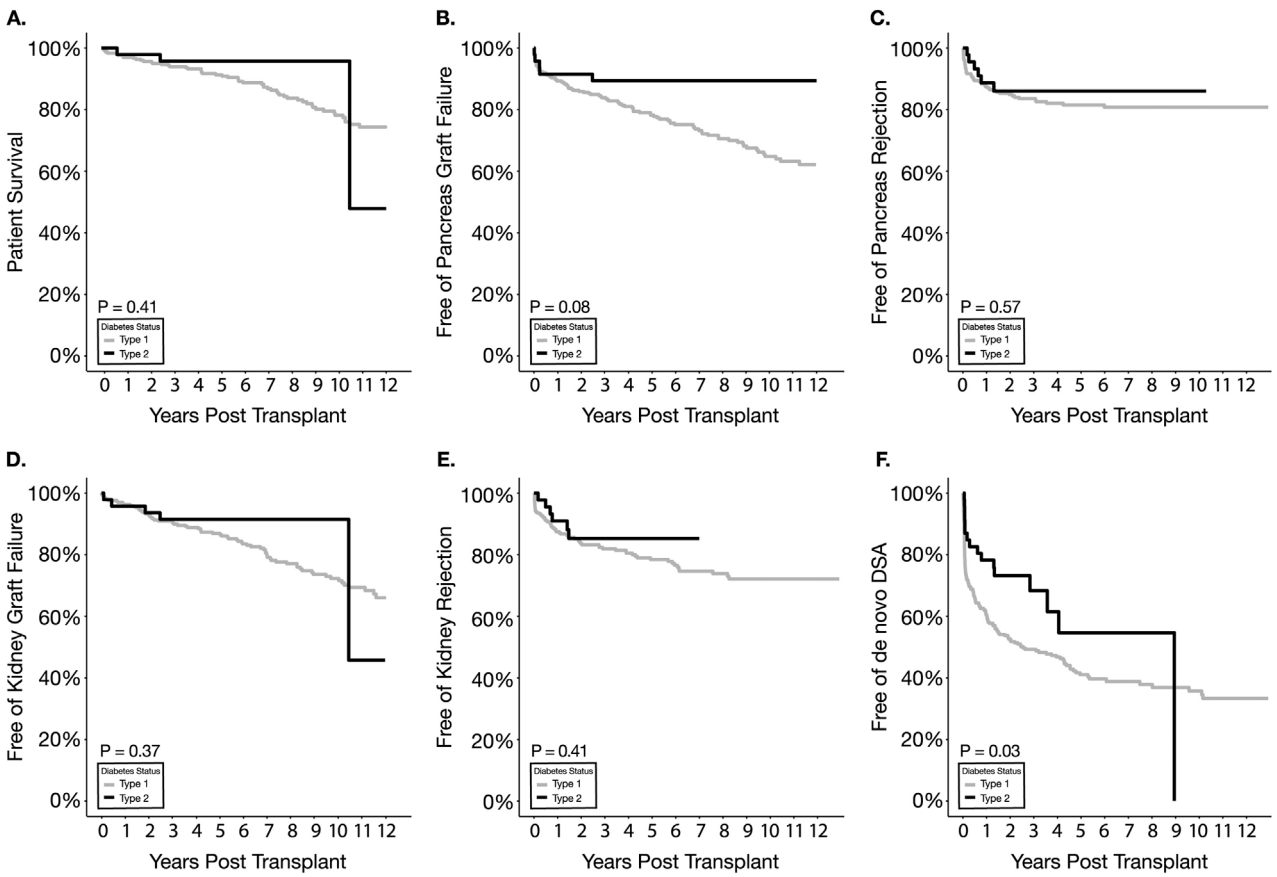


FIGURE 2 | Kaplan Meier survival estimates for patient survival **(A)**, pancreas graft failure **(B)**, pancreas graft rejection **(C)**, kidney graft failure **(D)**, kidney graft rejection **(E)**, and de novo DSA **(F)**.

(21%) compared to T1D (39%) within the first year (p = 0.02) (**Table 3**). Multivariable analysis, however, showed that type of diabetes has no association with developing *de novo* DSA while suggesting that increasing BMI was protective against such an outcome (HR = 0.95, p = 0.02) (**Table 4**). Regarding peri-operative induction agent use, compared to BAS, ALEM was significantly associated with decreased development of dnDSA (HR = 0.38, p < .001).

Readmission

Kaplan-Meier analysis of freedom from readmission showed no difference between the two types of diabetes (p = 0.07) (**Figure 3A**; **Table 2**). The percentage of readmissions within the first-year post-transplantation was not significantly different when comparing T2D with T1D recipients (47% vs. 60.7%, p = 0.11) though there was a trend to fewer readmissions in T2D recipients (**Table 3**). Positive trends in favor of T2D were also identified in the average number of readmission episodes per patient within the first year as well as overall readmissions within the first 90 days, though the results did not reach statistical significance. In the multivariable analysis (**Table 4**), neither type of diabetes nor other factors were associated with overall readmission risk.

TABLE 2 | Summary of major Rejection, Infection and Surgical Complication Endpoints. Kaplan-Meier Survival Estimates.

Outcomes	p-value (overall event-free survival)	1 year free of outcome (%)	
		T1D	T2D
Survival			
Patient survival	0.41	96.9%	97.9%
Pancreas graft survival	0.08	89.3%	91.5%
Kidney graft survival	0.37	96.3%	95.7%
Rejection			
Pancreas Rejection	0.57	87.3%	89.0%
Kidney Rejection	0.41	87.6%	91.2%
Post-transplant complications			
De novo DSA	0.03	60.6%	78.5%
Readmission	0.07	39.2%	52.7%
Infection[a]	0.12	27.6%	33.3%
Infection (UTI)	0.27	63.9%	75.8%
Major surgical complication[b]	0.84	84.5%	84.6%
Thrombosis[c]	0.46	90.7%	93.5%

[a]*Infection, unless otherwise specified, includes both bacterial and opportunistic infections.*

[b]*Major surgical complication includes both bleeding and non-bleeding complication but exclude thrombosis events. Bleeding complication is defined as any of the following: intraperitoneal (intra-abdominal) bleeding, bleeding from Jackson Pratt drain site, gastrointestinal or enteric anastomotic bleeding, pancreas arterial or venous anastomotic bleeding, renal arterial or venous anastomotic bleeding, and intravesicular hematoma. Non-bleeding complications include: chylous ascites, duodenojejunostomy leak, pancreatic enzyme leak without enteric leak (capsular or retrograde via common bile duct or pancreatic duct), pancreatic pseudocyst, ureteroneocystostomy leak, ureteral stricture, and lymphocele.*

[c]*Included both partial and complete thrombosis events. Specific diagnoses included partial thrombosis of the pancreatic allograft arterial or venous systems (e.g., portions of iliac Y graft, superior mesenteric artery or vein, splenic artery or vein), or complete occlusive thrombus of the pancreatic arterial or venous systems leading to pancreatectomy and early graft loss.*

Post-Transplant Infections

No statistical difference was observed between T2D and T1D recipients with respect to overall infection-free survival (p = 0.12) and UTI-free survival (p = 0.27) (**Figure 3B**; **Table 2**). There was no significant difference between the two types of diabetic recipients regarding the sub-categories of infection (**Table 3**). Multivariable analysis also supported the similarity between the two types in overall infection, UTI, surgical- and non-surgical site infection (**Table 4**). Increasing BMI was significantly associated with decreased risk of UTI (HR = 0.95, p = 0.04), whereas using ALEM was significantly associated with an increased risk of non-surgical site infection (HR = 1.49, p = 0.03).

Major Surgical Complications

Overall surgical complication-free survival was not significantly different between the two groups (**Figure 3C**; **Table 2**). A significant difference was not observed in the frequency or distribution of major surgical complications or subtypes (i.e., bleeding and non-bleeding) within the first -year post-transplantation between T1D and T2D recipients (**Table 3**). Multivariable analysis also showed that none of the variables tested, including diabetes types, were significantly associated with an increased risk of major surgical complication (**Table 4**).

Thrombosis Events

No difference in thrombosis-free survival was detected between T1D and T2D recipients with 1-year survivals of 90.3% and 94.8% in T1D and T2D respectively (**Figure 3D**; **Table 2**). Within the first 90 days post-SPKT, partial pancreatic thrombotic events and pancreas graft failures secondary to thrombosis were also not different between T1D and T2D in both univariate and multivariable analyses (**Table 3**, **4**). Interestingly, on multivariable analysis, increasing BMI was significantly associated with a lower risk of thrombosis (HR = 0.88, p = 0.02).

DISCUSSION

Whereas the majority of studies focus on patient and graft survival outcomes between T1D and T2D recipients, few address key infectious, surgical, and immunological outcomes. The current study addresses this gap and demonstrates that similar post-transplant outcomes, such as the incidence of acute BPR, readmissions, infections, UTIs, thrombosis, and other major surgical complications can be achieved between T1D and T2D SPKT recipients. Also, consistent with findings from previous studies demonstrating improvement in patient survival with advancing eras [9, 10, 43], the present study demonstrates acceptable and comparable patient-, pancreas allograft- and kidney allograft-survival in T2D versus T1D SPKT recipients.

Organ transplant recipients whose primary etiology of organ failure is autoimmune in nature may have higher rates of rejection and recurrence, especially in kidney transplantation [19–23] and liver transplantation [24–30]. However, a UNOS registry review did not find a significant association of rejection between T2D and T1D when combining kidney and pancreas rejection outcomes [10]. This study has the caveat however that kidney and pancreas rejection were not analyzed separately, and the majority of centers did not perform routine pancreas allograft biopsies in SPKT recipients, thereby potentially leading to underreporting of pancreas rejection. Thus, we posited that T1D SPKT recipients may experience higher rates of pancreas rejection than T2D SPKT recipients given the autoimmune nature of diabetes in the former. However, we did not observe

TABLE 3 | Univariate analysis for post-transplant outcomes.

	T1D (n = 298)	T2D (n = 47)	P-value
Pancreas graft rejection (Biopsy proven)			
Number patients with at least 1 rejection episode within 1st year			
BPR without Indeterminate/borderline	36 (12.6%)	5 (11%)	0.71
BPR with Indeterminate/borderline	39 (13.7%)	5 (11%)	0.57
Grade 1	22 (7.77%)	5 (11%)	0.50
Grade 2	11 (3.89%)	0 (0%)	0.18
Grade 3	6 (2.12%)	0 (0%)	0.33
Indeterminate/borderline	7 (2.48%)	0 (0%)	0.29
C4d > 5% on biopsy	20 (7.07%)	1 (2.2%)	0.22
Average episodes per patient within 1st year			
BPR without Indeterminate/borderline (mean ± sd)	0.13 ± 0.40	0.09 ± 0.29	0.41
BPR with Indeterminate/borderline (mean ± sd)	0.16 ± 0.46	0.09 ± 0.29	0.22
Grade 1 (mean ± sd)	0.08 ± 0.31	0.09 ± 0.29	0.84
Grade 2 (mean ± sd)	0.03 ± 0.18	0 ± 0	0.002
Grade 3 (mean ± sd)	0.02 ± 0.17	0 ± 0	0.03
Indeterminate/borderline (mean ± sd)	0.02 ± 0.18	0 ± 0	0.02
C4d > 5% on biopsy (mean ± sd)	0.07 ± 0.34	0 ± 0	<0.001
Kidney graft rejection (Biopsy proven)			
Number of patients with at least 1 rejection episode at 1 year	36 (12.4%)	4 (8.8%)	0.47
Average episodes per patient within 1st year (mean ± sd)	0.10 ± 0.30	0.07 ± 0.26	0.51
De Novo DSA within 1st year(%)	116 (39.4%)	10 (21.3%)	0.02
Readmission			
Number of patients with at least 1 readmission episode at 1 year	179 (60.7%)	22 (47%)	0.11
Average episodes per patient within 1st year (mean ± sd)	1.11 ± 1.29	0.88 ± 1.18	0.28
Number of patients at 90 days			
Any readmission	137 (46.8%)	19 (41%)	0.42
Wound-related	7 (2.43%)	2 (4.4%)	0.45
Infection-related	56 (19.2%)	10 (22%)	0.70
Rejection-related	22 (7.67%)	1 (2.2%)	0.17
Other-related	93 (31.8%)	15 (32%)	0.89
Infection (number and % of patient who have at least 1 episode)			
Bacterial infection within the 1st year	139 (47.1%)	21 (45%)	0.64
Opportunistic infection within the 1st year	141 (47.9%)	20 (43%)	0.73
Surgical site infection (within 90 days)	47 (16.0%)	6 (13%)	0.55
Non surgical site infection (within 90 days)	143 (48.2%)	18 (39%)	0.27
UTI within the 1st year	88 (30.3%)	11 (24%)	0.36
Major surgical complication (number and % of patient who have at least 1 episode)			
Any complication within 1st year	45 (15.5%)	7 (15%)	0.96
Non-bleeding fluid collection within 1st year	41 (14.2%)	7 (15%)	0.84
Bleeding complications within 1st year	7 (2.46%)	0 (0%)	0.29
Pancreas graft thrombosis event (number and % of patient who have at least 1 episode)			
Thrombosis (partial and complete) within 90 days	25 (8.52%)	3 (6.5%)	0.62
Graft failures due to thrombosis within 90 days	9 (3.09%)	1 (2.2%)	0.73

a significantly different 1-year pancreas, or kidney, BPR rate in T2D vs. T1D patients. The overall rates of rejection in our population are consistent with those previously reported in the literature (4%–38%) [48–53]. The current study provides greater granularity particular to rejection type and severity compared to prior studies [6, 9, 10, 14, 15, 52]. These prior studies, additionally, did not meticulously categorize T1D and T2D recipients [14, 15, 54], assess pancreas BPR separately from kidney BPR [10, 14, 15, 55, 56] or specifically look at pancreas BPR [6, 9, 52, 57]. Thus, the current study adds a more comprehensive assessment of the rejection risk confronting T2D SPKT recipients. It also suggests a very low overall incidence of pancreas antibody-mediated rejection (ABMR) based on the ~6% overall incidence of C4d>5% staining on biopsies in both T1D and T2D recipients, which is consistent with previously reported data [50].

While not definitive, our data does suggest a possible signal with regard to more rejection in T1D recipients. For example, we observed a greater number of episodes of Grade 2-3 ACR, indeterminate ACR, and C4d+ rejection, and numerically more patients with these rejection diagnoses within the first year in T1D patients. Moreover, we observed a higher incidence of dnDSA in T1D patients compared to T2D patients. Thus, this type of diabetes may be associated with an increased risk of pancreas rejection endpoints. Though we observed a higher rate of pancreas rejection signals by univariate analysis, we failed to detect a significant difference in multivariable analyses. Given the lack of major differences between T1D and T2D SPKT recipients, the current study suggests that a primary autoimmune pathology does not pose a substantially increased risk of BPR, nor does it suggest T2D confers higher rates of pancreas or kidney BPR. Thus, the type of

TABLE 4 | Multivariable analysis for post-transplant outcomes.

Outcomes	Type II vs. type I		BMI		PDRI		KDPI		Age at transplant		ALEM vs. BAS		ATG vs. BAS	
	HR (95% CI) or OR (95% CI)	P-value	HR (95% CI) or OR (95% CI)	P-value	HR (95% CI) or OR (95% CI)	P-value	HR (95% CI) or OR (95% CI)	P-value	HR (95% CI) or OR (95% CI)	P-value	HR (95% CI) or OR (95% CI)	P-value	HR (95% CI) or OR (95% CI)	P-value
Biopsy proven rejection (BPR) of pancreas graft without Indeterminate/ borderline	1.04 (0.42–2.55)	0.93	0.90 (0.83–0.98)	0.01	0.65 (0.25–1.66)	0.37	1.02 (0.99–1.04)	0.08	0.98 (0.95–1.01)	0.19	0.48 (0.22–1.03)	0.06	0.81 (0.38–1.72)	0.81
BPR with Indeterminate/ borderline	0.88 (0.36–2.14)	0.77	0.89 (0.83–0.96)	0.003	0.57 (0.24–1.39)	0.22	1.02 (1.00–1.05)	0.03	0.98 (0.96–1.01)	0.30	0.55 (0.27–1.11)	0.09	0.89 (0.44–1.83)	0.76
Grade 1 BPR	1.40 (0.51–3.85)	0.52	0.86 (0.78–0.95)	0.003	0.89 (0.28–2.78)	0.84	1.01 (0.98–1.04)	0.48	0.98 (0.94–1.01)	0.21	0.69 (0.28–1.71)	0.42	1.28 (0.55–2.96)	0.56
C4d > 5% on biopsy	0.46 (0.06–3.52)	0.45	0.88 (0.79–0.99)	0.03	0.74 (0.21–2.65)	0.65	1.01 (0.98–1.04)	0.46	0.95 (0.91–0.99)	0.01	0.69 (0.28–1.74)	0.44	0.40 (0.09–1.71)	0.21
Kidney graft rejection (Biopsy proven)	0.96 (0.40–2.30)	0.93	1.02 (0.95–1.10)	0.54	1.11 (0.54–2.24)	0.78	1.02 (0.99–1.04)	0.07	0.97 (0.94–1.00)	0.05	0.72 (0.41–1.27)	0.26	0.40 (0.16–0.97)	0.04
De Novo DSA	0.70 (0.41–1.21)	0.20	0.95 (0.91–0.99)	0.02	1.38 (0.86–2.23)	0.18	0.99 (0.98–1.01)	0.80	0.99 (0.98–1.01)	0.59	0.38 (0.26–0.57)	<.001	0.63 (0.40–1.00)	0.05
Readmission	0.77 (0.50–1.20)	0.25	0.97 (0.93–1.00)	0.08	1.00 (0.68–1.47)	0.99	1.01 (0.99–1.02)	0.08	0.99 (0.98–1.01)	0.20	1.02 (0.76–1.39)	0.87	1.06 (0.74–1.53)	0.74
Infection (Any)	0.77 (0.52–1.13)	0.18	0.97 (0.94–1.00)	0.08	0.97 (0.67–1.40)	0.86	1.01 (0.99–1.02)	0.11	0.99 (0.98–1.01)	0.58	1.21 (0.92–1.61)	0.17	1.28 (0.93–1.77)	0.13
Surgical site infection[a]	0.74 (0.30–1.84)	0.52	1.02 (0.94–1.10)	0.62	1.14 (0.52–2.52)	0.73	1.01 (0.98–1.03)	0.59	0.98 (0.95–1.02)	0.31	1.44 (0.76–2.77)	0.27	1.54 (0.74–3.23)	0.24
Non-surgical site infection[a]	0.87 (0.51–1.46)	0.60	0.96 (0.92–1.01)	0.09	1.03 (0.63–1.67)	0.91	1.01 (0.99–71.02)	0.28	0.99 (0.97–1.01)	0.32	1.49 (1.03–2.14)	0.03	1.08 (0.69–1.69)	0.73
UTI	0.91 (0.51–1.62)	0.74	0.95 (0.91–0.99)	0.04	0.98 (0.57–1.67)	0.94	1.01 (0.99–1.03)	0.10	0.97 (0.95–0.99)	0.05	0.76 (0.49–1.16)	0.20	1.06 (0.66–1.71)	0.79
Major surgical complication	0.89 (0.38–2.10)	0.79	1.01 (0.94–1.08)	0.80	1.93 (0.99–3.77)	0.05	0.99 (0.97–1.02)	0.85	1.00 (0.97–1.03)	0.93	0.83 (0.43–1.60)	0.57	1.09 (0.54–2.22)	0.80
Thrombosis	0.92 (0.26–3.22)	0.90	0.88 (0.79–0.98)	0.02	2.13 (0.86–5.28)	0.10	1.00 (0.97–1.03)	0.96	1.02 (0.98–1.06)	0.41	1.22 (0.54–2.76)	0.64	0.47 (0.13–1.62)	0.23

[a]*Logistic Regression was used instead of Cox Hazard Model.*

TABLE 5 | Univariate analysis for post-transplant outcomes.

	T1D (fail/Total) (%)	T2D (fail/Total) (%)	P-value
Induction			
Anti-Thymoglobulin			
Outcomes within 1 year post-transplant			
Pancreas - BPR without Indeterminate/borderline	2/46 (4.4%)	3/22 (14%)	0.20
Pancreas - BPR with Indeterminate/borderline	3/46 (7.5%)	3/22 (14%)	0.38
Death-censored pancreas graft failure	4/46 (8.7%)	1/22 (4.5%)	0.55
Kidney rejection	1/46 (2.2%)	1/22 (4.6%)	0.58
Death-censored kidney graft failure	1/46 (2.2%)	1/22 (4.6%)	0.58
Basiliximab			
Outcomes within 1 year post-transplant			
Pancreas - BPR without Indeterminate/borderline	29/173 (16.7%)	0/15 (0.0%)	0.10
Pancreas - BPR with Indeterminate/borderline	31/173 (17.9%)	0/15 (0.0%)	0.09
Death-censored pancreas graft failure	13/173 (7.51%)	1/15 (6.7%)	0.92
Kidney rejection	26/173 (15.1%)	1/15 (6.7%)	0.39
Death-censored kidney graft failure	5/173 (2.89%)	0/15 (0.0%)	0.51
Alemtuzumab			
Outcomes within 1 year post-transplant			
Pancreas - BPR without Indeterminate/borderline	5/79 (6.4%)	2/10 (20%)	0.14
Pancreas - BPR with Indeterminate/borderline	5/79 (6.4%)	2/10 (20%)	0.14
Death-censored pancreas graft failure	10/79 (13%)	2/10 (20%)	0.55
Kidney rejection	9/79 (11%)	2/10 (20%)	0.40
Death-censored kidney graft failure	0/79 (0%)	1/10 (10%)	0.005

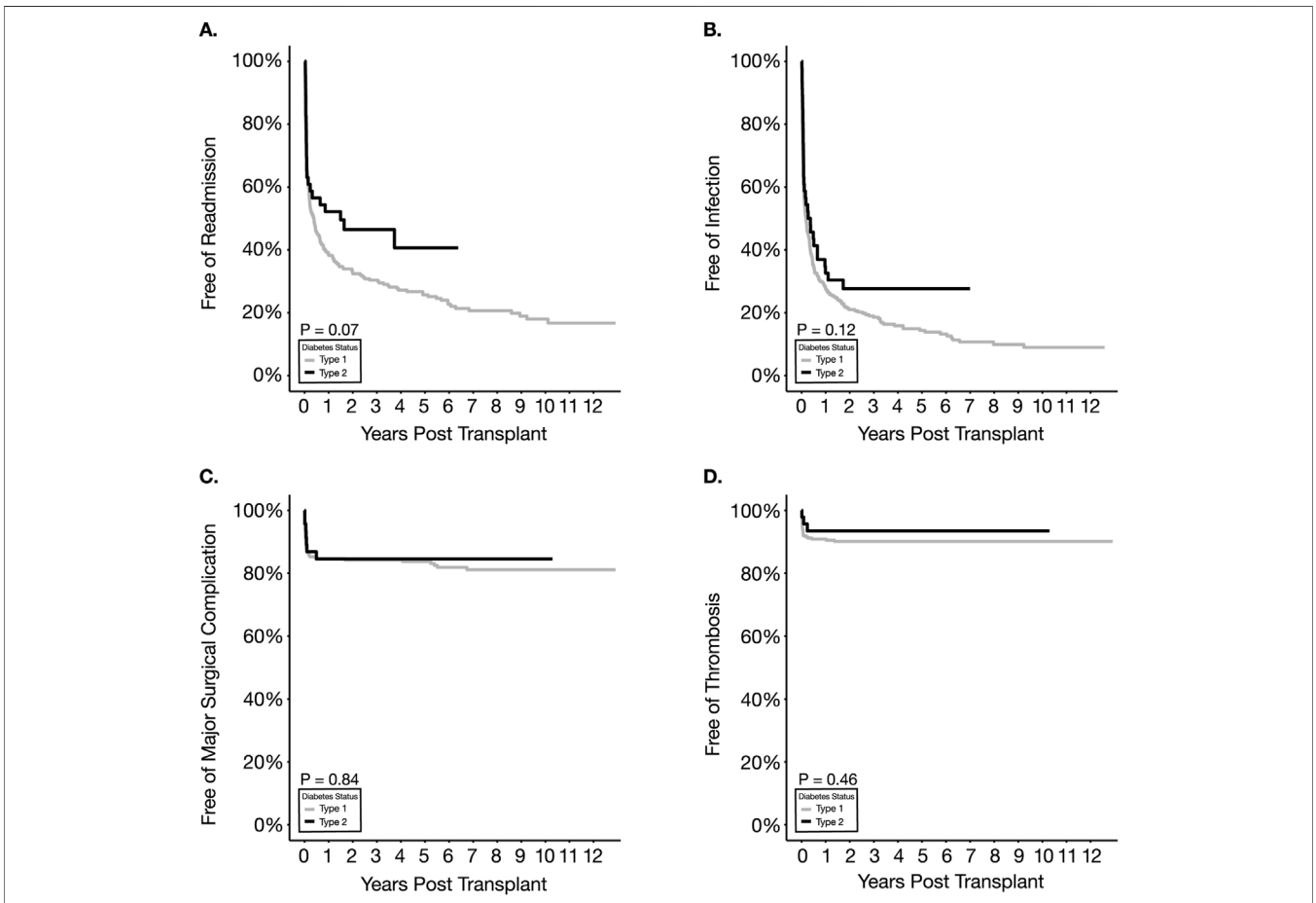


FIGURE 3 | Kaplan Meier survival estimates for readmission **(A)**, infection **(B)**, major surgical complications **(C)** and thrombosis **(D)**.

diabetes thus should not affect candidacy for SPKT from the rejection perspective.

Similar patient and graft survival outcomes have been described with both T-cell depleting and non-depleting agents for SPKT [53, 58–60]. Overall, lower rates of early acute rejection have been described in SPKT with T-cell-depleting agents versus non-depleting agents [53]. Comparing types of T-cell-depleting therapies, ALEM versus ATG has been associated with comparable surgical complications, readmissions, thromboses, and bleeding [61]. These studies however involve very few T2D recipients. The results of our study are congruent with these findings and indicate that, compared to BAS, ALEM induction might be beneficial for pancreas graft rejection and was associated with lower risk of dnDSA development, while ATG induction was associated with reduced kidney graft rejection. We also did not find an association between ALEM and kidney graft rejection, consistent with Sampaio et al [10]. Induction trends in our cohort are also consistent with those reported in T2D recipients represented in registry data, with an increasing trend toward use of T-cell-depleting antibodies in more recent eras [9]. Larger cohorts of T2D SPKT recipients are needed to make definitive conclusions regarding any differences in the rejection rate between induction regimens based on diabetes type.

The development of dnDSA after pancreas and SPK transplantation has been identified as a significant risk factor for pancreas and kidney rejection, and for graft failure [33–35]. We demonstrated a significantly lower incidence of dnDSA within the first year in T2D versus T1D SPKT recipients. This result may be explained by differences in induction immunosuppression mentioned earlier (i.e., more BAS induction in T1D vs. T2D recipients), and therefore should not necessarily be construed as definitively indicating T2D SPKT recipients would require less intensive immunosuppression or less vigorous postoperative-immune monitoring, though these benefits remain a possibility.

Previous analysis of SPKT registry data from over a decade ago [10] and more recent UK registry data [55] has suggested that the type of diabetes did not significantly impact the rate of surgical complications including abscess formation, anastomotic leak, pancreatitis, and primary non-function. Obesity, frequently associated with T2D, on the other hand, has been associated with increased risk of postoperative infections, a need for postoperative invasive procedures [62, 63], increased risk of patient death, pancreas graft loss, and kidney graft loss [39]. Our findings demonstrate no difference in risks of major surgical complications (bleeding and non-bleeding), surgical site infections, incidental image-identified pancreatic graft thrombotic lesions, and pancreatic graft losses secondary to thrombosis in T2D vs. T1D recipients. In the absence of significantly worse infectious and surgical complications and similar rejection rates between T2D and T1D SPKT recipients, it seems very reasonable to continue to offer selected IDDM/CKD patients an SPKT regardless of their diabetes labels. Prospective trials would also be valuable to definitively compare efficacy and safety outcome endpoints, but await a significant multi-center effort to accrue a sufficient number of patients. In the meantime, we recommend a careful and systematic center-specific approach to offering SPKT to T2D/CKD patients.

Given the rising rates of T2D-associated CKD and obesity, safe criteria for SPKT in the T2D/CKD population should be established [64, 65]. Though we found some marginal protective effect associated with older age with regard to pancreas and kidney rejection, elderly patients tend to preform poorly due to having more comorbidities. UNOS/OPTN policy still requires patients to be insulin-dependent, though weight or BMI restrictions were recently eliminated [44]. Consequently, the indication for SPKT for T2D and CKD at most centers in the US is quite narrow and the majority of T2D/CKD patients presenting to centers are not considered candidates for SPKT but are generally offered a kidney transplant alone. Morbidly obese patients with CKD who do not require insulin most likely have residual beta cell mass, and their diabetes could be reversed by bariatric surgery [66–70]. However, if they have undetectable or minimal C-peptide, their diabetes is unlikely reversed by bariatric surgery alone. CKD patients whose diabetes is controlled by non-insulin oral or injectable agents, diet or exercise are not eligible for pancreas transplantation currently in the US based on allocation policy. However, it is well understood by the transplant community that once they receive a kidney transplant and the requisite immunosuppression, the patient's diabetes will worsen and ultimately require long-term insulin for control. In this situation, they may benefit from a pancreas-after-kidney transplant, but would it be reasonable to offer a "preemptive" SPKT to this population, preempting their requirement for insulin, just as we offer kidneys preemptively in patients with CKD prior to dialysis? Understanding the relative mortality risk of T2D/CKD waiting list patients who are controlled without insulin to those who are on insulin may support future policy decisions.

We recognize potential limitations to the broader applicability of the results presented here given the non-randomized, single-center, and retrospective nature of our study. Despite using an objective multiparametric approach to classify diabetes type, mis-categorization is possible as not all patients fit neatly into the classically defined T1D and T2D categories, though we believe that this approach is more holistic and objective. We also acknowledge that dnDSA and rejection may still develop after our 1 year minimum follow-up period. Therefore, to ensure valid conclusions can be made, we limited our incidence analysis of immunological, surgical, and infectious complications to the first year or less so that every patient had an equal chance to realize these complications. Consequently, we cannot describe medium or longer-term outcomes relative to these complications. Lastly, our T2D population is relatively small compared to registry data, albeit one of the larger single-center experiences presented to date. However, we feel that the granularity of our data, the recent cohort, and the greater homogeneity of candidate selection, surgical technique, immunosuppression, and post-operative practices at a single center than exists in registry data are benefits to teasing out differences between these populations and to provide updated information. Nonetheless, we believe these data provide useful guidance by comprehensively

examining immunological, infectious, and surgical complications after SPKT in T2D recipients.

In conclusion, with the increasing prevalence of T2D related ESRD and an increasing trend of SPKT performed in T2D(9) this study found similar outcomes regarding rejection, major surgical complications, infections, and readmissions between SPKT T1D and T2D recipients. It further demonstrates the success that SPKT can achieve in carefully selected T2D recipients, and provides valuable reassurance to the transplant community for continued careful protocolized application of SPKT to low cardiovascular risk T2D/CKD patients.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by the UW School of Medicine and Public Health IRB. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because some patients have lost their grafts or died, if informed consent would be mandated then this would bias this study; therefore, consent was waived by our IRB. This is a retrospective study and because this is a retrospective study and for the above reasons our IRB consistently allows studies of this nature to have waived consent.

AUTHOR CONTRIBUTIONS

All authors participated in the design of the study, interpretation of the results and review of the manuscript. EM, PP, JW, and BW collected the data; EM, PP, LS, GL, and NM analyzed the data; EM, PP, and JO wrote the manuscript; TA-Q, DM, SP, HS, DK, RR, and JO reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

The authors of this manuscript have conflicts of interest to disclose as described by the Transplant International. JO is co-founder of, has equity interest in and serves as Chair of the Scientific Advisory Board of Regenerative Medical Solutions, Inc. He receives clinical trial support from Veloxis Pharmaceuticals, CareDx Transplant Management, Inc., Natera, Inc. and Vertex Pharmaceuticals, Inc. DK reports serving as a scientific advisor for, or member of, eGenesis, and receiving research funding from Medeor Pharma and the National Institutes of Health.

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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Evolving Trends in the Management of Duodenal Leaks After Pancreas Transplantation: A Single-Centre Experience

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Duodenal leaks (DL) contribute to most graft losses following pancreas transplantation. However, there is a paucity of literature comparing graft preservation approach versus upfront graft pancreatectomy in these patients. We reviewed all pancreas transplants performed in our institution between 2000 and 2020 and identified the recipients developing DL to compare based on their management: percutaneous drainage vs. operative graft preservation vs. upfront pancreatectomy. Of the 595 patients undergoing pancreas transplantation, 74 (12.4%) developed a duodenal leak with a median follow up of 108 months. Forty-five (61%) were managed by graft preservation strategies, with the rest being treated with upfront graft pancreatectomy. DL managed by graft preservation strategies had similar graft survival rates at 1 and 5-year compared to the matched cohort of population without DL (95% and 59% vs. 91% and 62%; p = 0.78). Multivariate analysis identified male recipient (OR: OR: 6.18; CI95%: 1.26–41.09; p = 0.04) to have higher odds of undergoing an upfront graft pancreatectomy. In appropriately selected recipients with DL, graft preservation strategies utilizing either interventional radiology guided percutaneous drainage or laparotomy with/without repair of leak can achieve comparable long-term graft survival rates compared to recipients without DL.

Keywords: duodenal leaks, pancreas transplantation, complications after transplantation, graft preservation, graft salvage

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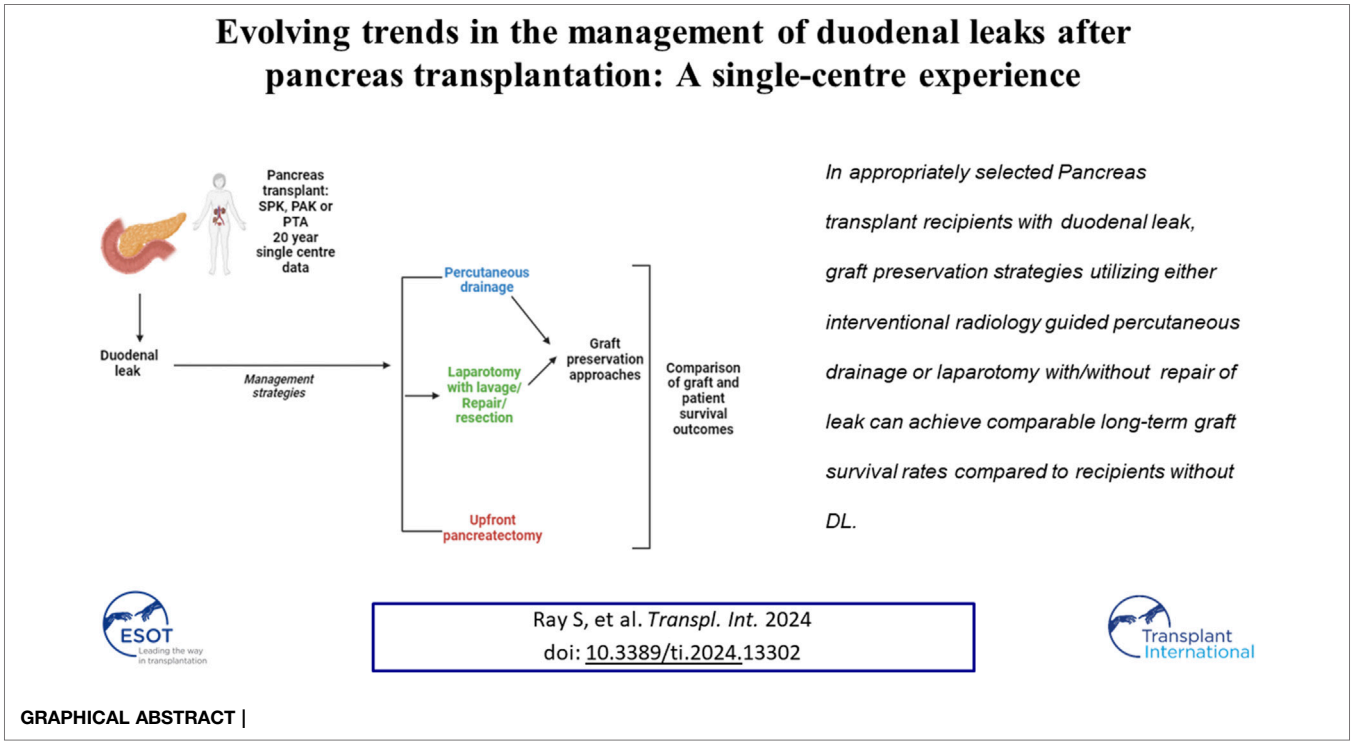
Abbreviations: DL, Duodenal leak; IR, Interventional radiology; HbA1C, Hemoglobin A1C; SPK, Simultaneous pancreas kidney; PAK, Pancreas after kidney; PTA, Pancreas transplant alone; STROBE, Strengthening the reporting of observational studies in epidemiology; IR, Interventional radiology; GIA, Gastrointestinal anastomosis; PD, Peritoneal dialysis; KP, Kidney-pancreas; HCV, Hepatitis C Virus; DSA, Donor specific antibodies; IVIg, Intravenous immunoglobulin; CMV, Cytomegalovirus; PCP, Pneumocystis carinii pneumonia; GS, Graft survival; OS, Overall survival; DLFS, Duodenal leak free survival; HR, Hazard ratio; CI95%, 95% confidence interval; OR, Odd's ratio; PSM, Propensity score matching; BMI, Body mass index; DCD, Donation after cardiac death; DBD, Donation after brain death; IQR, Interquartile range.



GRAPHICAL ABSTRACT |

INTRODUCTION

Recent studies have demonstrated pancreas transplantation to be the only effective method to restore euglycemia by normalizing HbA1c levels over a stable course of time following surgery [1–3]. In addition, it also improves the survival of patients with end-stage diabetic nephropathy, compared to kidney transplantation alone [4]. With advances in surgical techniques, immunosuppressive regimen, and donor-recipient selection criteria, there has been a significant improvement in the 5- and 10-year graft survival rates over the last 2 decades [5, 6]. Despite this, duodenal leaks (DL) continue to be an important complication in the setting of pancreas transplantation, with an incidence reported as high as 5%–10% in the literature, resulting in graft loss in more than 50% of those cases [7]. In most cases, the conventional treatment for graft duodenal leaks following pancreas transplantation has been laparotomy with upfront graft pancreatectomy, inevitably leading to graft loss. However, there is a paucity of literature on the efficacy of graft preservation techniques in managing duodenal leaks following pancreas transplantation.

In addition to the radical approach of upfront graft pancreatectomy for patients with graft duodenal leaks, more conservative graft-saving approaches have been increasingly employed in our institutional practice over the last 5–10 years. These approaches include interventional radiology (IR) guided percutaneous drain placement, as well as laparotomy with lavage or repair of the duodenal leak. We aimed to analyze the short- and long-term outcomes of these recipients in our 20-year cohort of pancreas transplantation and compare their results based on the management approach: upfront graft pancreatectomy versus graft-preserving strategies. The secondary objective of this study was to identify the peri-operative characteristics of these patients with duodenal leaks to determine the most appropriate initial management approach from the available options.

PATIENTS AND METHODS

Study Population

All consecutive patients who underwent pancreas transplantation: Simultaneous pancreas-kidney (SPK), Pancreas after kidney (PAK) and Pancreas transplant alone (PTA), between January 2000 and December 2020 at the Toronto General Hospital, University Health Network were included in this study. Patients who underwent pancreas transplantation as part of a multi-visceral transplantation were excluded. All patients included in the study had a minimum of 12 months follow-up following transplantation. The study was reviewed by the ethical board (REB) of the Toronto General Hospital and approved for the study period (CAPCR ID: 21-6151.1) and adhered to the methodologic guidance from the STROBE statement [8]. The presenting complaints, physical findings, and relevant investigations (blood work, cultures, and imaging) were analyzed retrospectively from a prospectively collected transplant database. Donor and recipient demographic and peri-operative data were collected using the institutional electronic patient records database.

TABLE 1 | Demographic, pre-operative and peri-operative characteristics of Duodenal leak (DL) group compared with the control group of patients in overall cohort of pancreas transplantation recipients.

Variables	Overall (n = 595)	DL (n = 74)	Control (n = 521)	P-value
Donor Age (years) (IQR)	25 (19–34)	23.5 (18–36)	25 (19–34)	0.74
DCD donors (%)	31 (5.2)	2 (2.7)	29 (5.5)	0.03
Donor BMI (kg/m^2) (IQR)	23.2 (20.8–26.1)	24.3 (19.9–27.5)	23.1 (21–25.9)	0.48
Donor WIT (DCD; mins) (IQR)	26 (22.5–28)	10 (10–10)	26 (24–28)	0.02
Donor CIT (mins) (IQR)	542 (442.2–644.7)	527.5 (440.5–615.5)	545 (443.5–647)	0.19
Recipient Age (years) (IQR)	43.5 (37.3–50.5)	42 (36.7–47)	43.9 (37.4–50.7)	0.16
Recipient gender (% male)	376 (63.2)	47 (63.5)	329 (63.1)	>0.99
Recipient BMI (kg/m^2) (IQR)	24.7 (21.8–27.9)	25.8 (22.6–29.1)	24.6 (21.6–27.7)	0.02
Recipient CMV status				0.82
CMV Mismatch (D+/R-) (%)	86 (14.5)	12 (16.2)	74 (14.2)	
CMV infection (R+) (%)	65 (10.9)	9 (12.2)	56 (10.7)	
Recipient EBV status				0.53
EBV Mismatch (D+/R-) (%)	27 (4.5)	2 (2.7)	25 (4.8)	
EBV infection (R+) (%)	4 (0.7)	0 (0)	4 (0.8)	
Transplant category				0.42
SPK (%)	433 (72.8)	53 (71.6)	380 (72.9)	
PAK (%)	140 (23.5)	20 (27)	120 (23)	
PTA (%)	22 (3.7)	1 (1.4)	21 (4)	
Pre-transplant IS(%)	40 (6.7)	4 (5.4)	36 (6.9)	0.81
Pre-transplant Dialysis (%)	215 (36.1)	25 (33.8)	190 (36.5)	0.75
Pre-transplant cardiac intervention (%)	215 (36.1)	27 (36.5)	188 (36.1)	>0.99
Pre-transplant infections (%)	53 (8.9)	8 (10.8)	45 (8.6)	0.69
Post-transplant dialysis (%)	25 (4.2)	4 (5.4)	21 (4)	0.81
Post-transplant DVT (%)	5 (0.8)	1 (1.4)	4 (0.8)	>0.99
Post-transplant pneumonia (%)	14 (2.4)	3 (4.1)	11 (2.1)	0.53
Post-transplant CLABSI (%)	1 (0.2)	0 (0)	1 (0.2)	>0.99
Post-transplant stay (Days) (IQR)	9.6 (8–13.2)	10.7 (8.6–18.7)	9.6 (7.8–12.7)	0.01
Graft related complications				
Arterial thrombosis (%)	3 (0.5)	1 (1.4)	2 (0.4)	0.82
Portal vein thrombosis (%)	15 (2.5)	2 (2.7)	13 (2.5)	>0.99
Hemorrhage (%)	27 (4.5)	5 (6.7)	22 (4.2)	0.61
Graft rejection (pancreas) (%)	74 (12.4)	12 (16.2)	62 (11.9)	0.41
Graft rejection (Kidney) (%)	36 (6.1)	12 (16.2)	24 (4.6)	0.06
Graft loss (pancreas) (%)	150 (25.2)	35 (47.3)	115 (22.1)	<0.001
Graft loss (Kidney) (%)	53 (8.9)	9 (12.1)	44 (8.4)	0.78
Re-transplantation (pancreas) (%)	42 (7.1)	15 (20.3)	27 (5.2)	<0.001
Overall mortality (%)	112 (18.8)	14 (18.9)	98 (18.8)	>0.99

*** All continuous variables expressed as medians, unless specified otherwise.*

Legends: DL, Duodenal leak; IQR, Interquartile range; DCD, Donation after cardiac death; BMI, Body mass index; WIT, Warm ischemia time; CIT, Cold ischemia time; CMV, Cytomegalovirus; EBV, Epstein Barr Virus; D/R, Donor/Recipient; SPK, Simultaneous pancreas kidney; PAK, Pancreas after kidney; PTA, Pancreas transplant alone; IS, Immunosuppression; DVT, Deep venous thrombosis; CLABSI, Central line associated bloodstream infections.

Duodenal Leak

Duodenal leak (DL) was suspected in recipients presenting with fever, hyperamylasemia, elevated leucocyte count, or abdominal pain along with fluid and free air adjacent to the graft duodenum on imaging (by CT scan); the diagnosis was confirmed upon surgical exploration or imaging-guided percutaneous drainage (elevated drain fluid amylase levels: more than 3 times the serum amylase at the corresponding time point). Duodenal leaks were categorized into three groups based on their management modality: IR guided percutaneous drainage, laparotomy without pancreatectomy (lavage or repair of leak), and upfront graft pancreatectomy according to the modality of their DL management. The first two constituted the graft preserving or graft salvage approaches for management of duodenal leaks. DL patients in the first group were treated using intravenous antibiotics, fluids, nutritional support, and other supportive measures alongside percutaneous drainage of collections under interventional radiology (IR) guidance. DL patients who underwent laparotomy without graft pancreatectomy had a laparotomy with lavage and drainage with or without definitive leak repair. DL patients in the third group, who underwent upfront graft pancreatectomy had a laparotomy with resection of the graft at the time of index presentation.

Surgical Procedures

All organ recoveries and transplantations were performed according to the standard institutional protocol with systemic venous drainage and enteric drainage of exocrine pancreas secretion described previously by our group [9]. Briefly, at the back-table preparation, the duodenal segment was shortened, ensuring adequate vascularity of the graft adjacent to the staple line, which was routinely inverted with a Lembert suture.

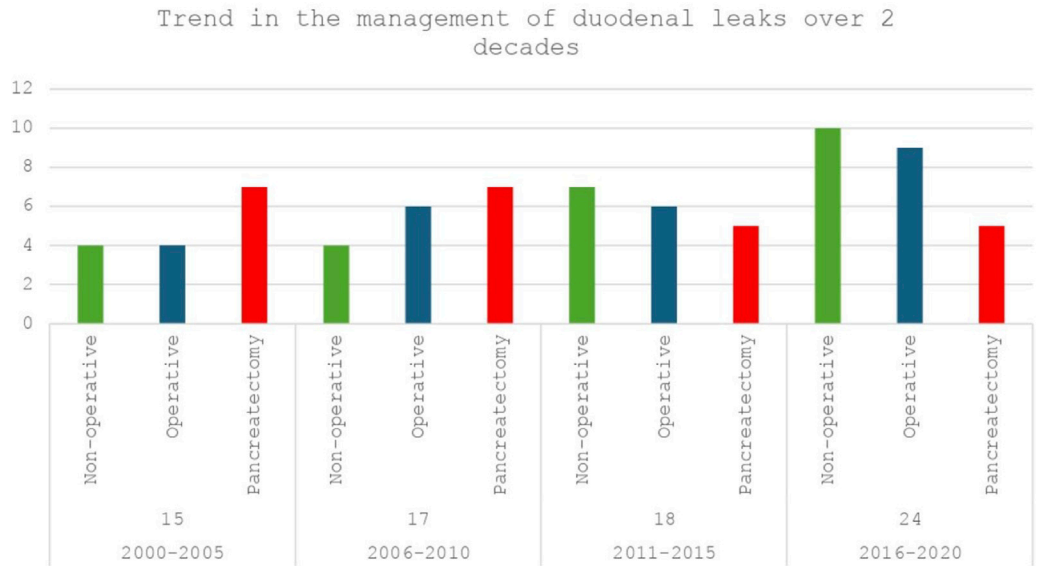


FIGURE 1 | Trend of management of duodenal leaks (DL) after pancreas transplantation at the Toronto general hospital over 2 decades (2000–2020): Non-operative (IR guided drain placement) vs. Operative (Laparotomy with lavage/repair) vs. Upfront pancreatectomy.

Systemic venous drainage to the vena cava and exocrine drainage to a Roux-en-Y limb of the jejunum was routinely performed. The duodenal-jejunal anastomosis was performed in a 2-layer hand-sewn fashion and was approximately 2-3 cm long. The final orientation of the graft was behind the right colon (retro-colic), with the head up and tail towards the pelvis. A drain was left adjacent to the graft in all patients.

Intraoperative systemic anticoagulation was employed in recipients undergoing PAK or PTA only. The kidney transplant was performed before the pancreas transplant in all cases of SPK. Prophylactic antibiotics included IV cefazolin and Metronidazole before skin incision. Pre-transplant peritoneal dialysis (PD) cell count and culture sensitivity were assessed in all Kidney-pancreas (KP) patients on peritoneal dialysis as a part of the pre-transplant sepsis screen. Oral Glecaprevir/Pibrentasvir (Maviret) was administered 2–4 h before the transplant in recipients with Hepatitis C Virus (HCV) Nucleic acid testing-positive donors. Postoperative anticoagulation and antiaggregating therapy consisted of daily prophylactic with 5000 U of unfractionated heparin, and acetylsalicylic acid, 81 mg. Protocol graft ultrasound was performed on day 1 following the transplant.

Immunosuppression

All recipients had a negative antihuman globulin complement-dependent cytotoxic T cell (before 2013) or flow cytometry crossmatch (after 2013) at the time of transplantation. Donor-specific antibodies (DSA) did not preclude transplantation, provided the crossmatch was negative. Thymoglobulin induction (3–5 mg/kg recipient body weight for SPK/PAK and up to 7 mg/kg for PTA) was administered daily over 5–7 days. Patients receiving basiliximab (Simulect) were administered an intravenous dose of 20 mg within 2 h before transplant surgery and a second dose within 12 h and on the fourth day after transplant. All patients received methylprednisolone 500 mg intraoperatively, followed by a rapid taper from 200 to 20 mg/d on day 5. The oral prednisone dosage was started at 20 mg/d, reduced to 5 mg/d at 6 months, and maintained between 2.5 and 5 mg/d thereafter. Tacrolimus (target level of 10–15 μg/L at day 7 and 5–10 μg/L at 6 months) and mycophenolate mofetil (500 mg twice a day; higher doses up to 1,000 mg BID for PTA, if tolerated) were initiated on postoperative days 2–5. Recipients with DSA also received intravenous immunoglobulin (IVIg) (1 g/kg) perioperatively.

CMV and Other Prophylaxis

CMV-negative recipients of CMV-positive organs (Mismatch) received valganciclovir for 6 months with 6 months of monitoring post cessation of therapy, and CMV-positive recipients/CMV infection (Donor positive or negative) received 3 months of therapy. In high-risk patients (i.e., CMV-positive organ to CMV-naive recipients), CMV viremia was monitored by quantitative polymerase chain reaction for 3 months after the cessation of valganciclovir; a 6-week course of valganciclovir was started in those patients who became viremic. Ganciclovir's initial dose was 5 mg/kg IV daily, followed by an oral dose of 1 g three times a day or 900 mg of oral valganiclovir per day whenever the patient could tolerate oral medications. Acyclovir 400 mg BID prophylaxis for 3 months was given in CMV-negative recipients with negative donors. Pneumocystis carinii pneumonia (PCP) prophylaxis was started in all recipients (Cotrimoxazole Single strength alternate day).

Follow-Up and Survival Endpoints

Duration of follow-up and outcomes of interest such as postoperative complications (arterial/venous/systemic), 90-day

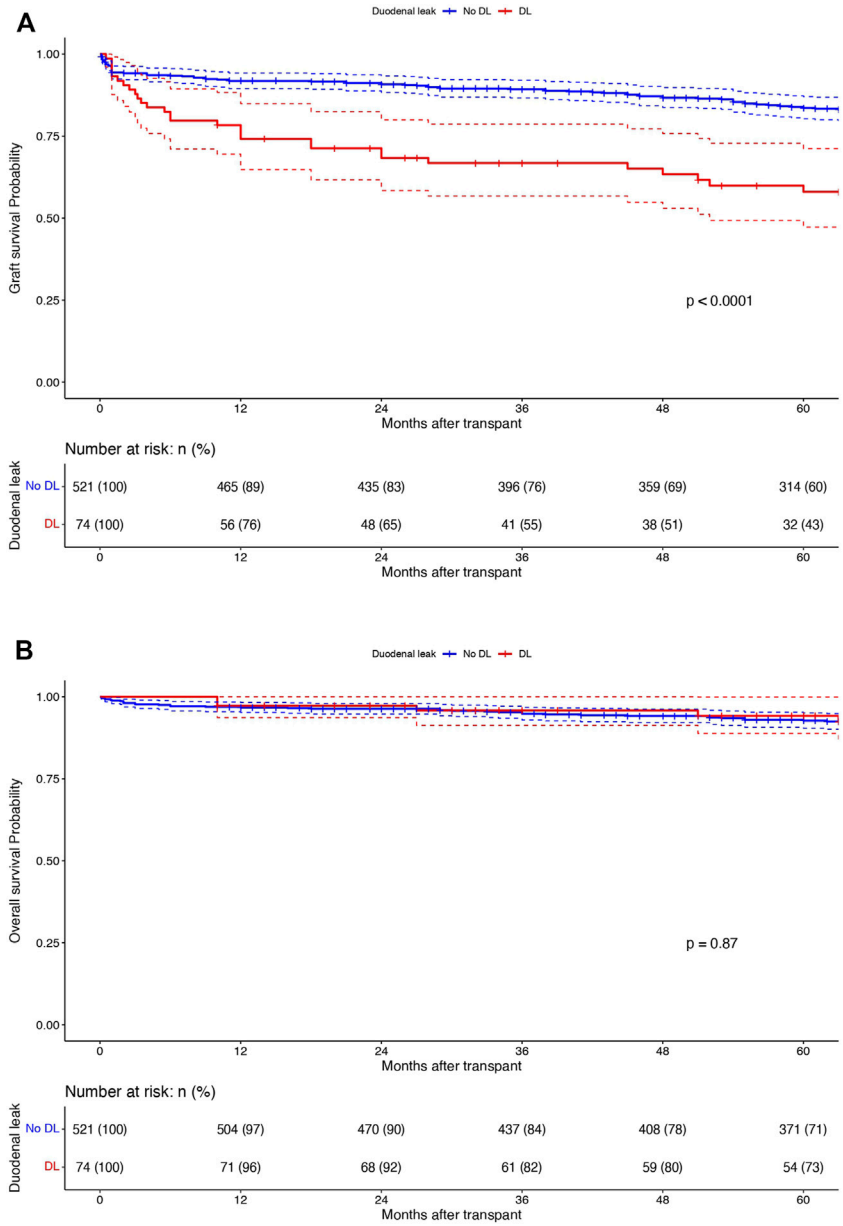


FIGURE 2 | Kaplan-Meier curves displaying **(A)** graft survivals and **(B)** overall survivals in patients with (n = 74; red) and without DL (n = 521; blue). X-axis: months after transplant. Y-axis: survival probabilities. Comparison using Log-rank test.

post-transplant mortality, infections (bacterial, viral, and fungal), rejection episodes (pancreas/kidney/both; pancreas rejections classified by the Maryland system) [10] pancreas graft failure (defined as a return to insulin dependency), cause of graft failure and loss, and death on follow-up were collected. Patients in the percutaneous drainage and laparotomy groups requiring graft pancreatectomy at any point during the study period were considered to have experienced graft loss. Similarly, re-laparotomy during the study period was recorded as a separate event in the postoperative outcomes for the three groups. Graft survival (GS) was defined as the time from transplantation to graft failure (pancreas alone or combined pancreas-kidney). Overall survival (OS) was defined as the time from transplantation to the time of death (from any cause). Duodenal leak-free survival (DLFS) was defined as the months survived without a duodenal leak after the index pancreas transplantation.

Statistical Analysis

Continuous data were expressed as median (25–75 inter-quartiles), were not categorized, and were compared using the Mann-Whitney *U* test or Kruskal-Wallis test, as appropriate.

TABLE 2 | Cox proportional hazards model in the whole population (n = 595) to identify factors associated with graft survival (GS) (Reduced model).

Variables	Graft survival		
	HR	95% CI	P-value
Recipient age (years)[a]			
Spline for age ≤37.3 years	0.66	0.42–1.08	0.06
Spline for age >37.3 and ≤50.5 years	0.68	0.40–1.08	0.39
Spline for age >50.5 years	0.78	0.43–1.43	0.35
Recipient BMI (kg/m [2])[a]			
Spline for BMI ≤21.8 kg/m [2]	0.95	0.50–1.79	0.85
Spline for BMI >21.8 and ≤27.9 kg/m [2]	1.06	0.58–1.93	0.83
Spline for BMI >27.9 kg/m [2]	1.08	0.55–2.10	0.80
Male recipient	1.27	0.87–1.85	0.15
CMV status			
Negative	ref	ref	ref
CMV infection (R+)	1.38	0.86–2.21	0.08
CMV mismatch (D+/R-)	1.16	0.73–1.85	0.42
Transplant category			
Simultaneous pancreas kidney	ref	ref	ref
Pancreas after kidney	1.48	1.01–2.16	0.01
Pancreas transplant alone	1.53	0.54–4.34	0.35
Pre-transplant infection	1.58	1.15–2.65	0.02
Donor type (DCD vs. DBD)	1.27	0.46–3.52	0.59
Donor CIT (mins)[a]			
Spline for CIT ≤442.2 min	1.09	0.61–1.93	0.73
Spline for CIT >442.2 and ≤644.7 min	1.13	0.67–1.92	0.57
Spline for CIT >644.7 min	1.12	0.64–1.97	0.63
Post transplant dialysis	1.87	1.04–3.37	0.006
Graft PV thrombosis	1.67	0.64–4.03	0.18
Duodenal leak	3.45	2.10–5.68	<0.001
Graft rejection (pancreas)	1.62	1.07–2.45	0.004

Abbreviations: GS, graft survival; HR, hazard ratio; CI, confidence interval; BMI, body mass index; CMV, cytomegalovirus; DCD, donation after cardiac death; DBD, donation after brain death; CIT, cold ischemia time; PV, portal vein.

[a]Non-linear variables transformed using cubic spline functions with 3 degrees of freedom; knots are placed at the 25th and 75th percentiles of the variable in the overall population.

TABLE 3 | Cox proportional hazards model in the whole population (n = 595) to identify factors associated with overall survival (OS) (Reduced model).

Variables	OS		
	HR	95% CI	P val
Recipient age (yrs)			
Spline for age ≤37.3 years	1.12	0.49–2.55	0.85
Spline for age>37.3 and ≤50.5 years	1.26	0.96–4.81	0.09
Spline for age >50.5 years	4.06	1.78–9.30	0.008
Recipient BMI (kg/m [2])			
Spline for BMI ≤21.8 kg/m [2]	0.49	0.26–0.93	0.04
Spline for BMI >21.8 and ≤27.9 kg/m [2]	0.51	0.28–0.93	0.03
Spline for BMI >27.9 kg/m [2]	0.82	0.43–1.56	0.56
Male recipient	0.77	0.55–1.19	0.25
CMV status			
Negative	ref	ref	ref
CMV infection (R+)	0.79	0.41–1.53	0.49
CMV mismatch (D+/R-)	0.99	0.49–2.00	0.98
Transplant category			
SPK	ref	ref	ref
PAK	0.98	0.59–1.63	0.95
PTA	0.77	0.18–3.34	0.73
Donor type (DCD vs. DBD)	0.69	0.25–1.89	0.52
Donor CIT (mins)			
Spline for CIT ≤442.2 min	0.74	0.38–1.44	0.39
Spline for CIT >442.2 and ≤644.7 min	1.14	0.63–2.05	0.67
Spline for CIT >644.7 min	1.12	0.59–2.13	0.73
Post transplant pneumonia	4.02	1.63–9.89	<0.001
Duodenal leak	0.38	0.05–2.89	0.36

Legends: OS, overall survival; HR, hazard ratio; CI, confidence interval; BMI, body mass index; CMV, cytomegalovirus; SPK, simultaneous pancreas kidney; PAK, pancreas after kidney; PTA, pancreas transplant alone; DCD, donation after cardiac death; DBD, donation after brain death; CIT, cold ischemia time.

**Non-linear variables transformed using cubic spline functions with 3 degrees of freedom; knots are placed at the 25th and 75th percentiles of the variable in the overall population.*

Categorical data are expressed as percentages and were compared using Pearson's chi-square test or Fisher's exact test, as appropriate. Statistical significance testing was 2-sided. Unless indicated otherwise, a p-value <0.05 was considered statistically significant for all tests. There were no missing values regarding the endpoints of this study, including the events of duodenal leakage, recurrence, or death, and the times to duodenal leakage, recurrence, or death, and no variable had more than 10% of data missing. Continuous variables were transformed in the regressions using natural splines with three degrees of freedom to avoid non-linear relationship misspecification due to the non-normal distribution of biological data. Survival probabilities were computed using the Kaplan-Meier estimate and compared using the log-rank test.

Duodenal leakage was encoded as a time-dependent variable (i.e., right censored outcome). Right-censored outcomes (i.e., DLFS, OS and DFS) regressions were analyzed using Cox proportional-hazards regression models [estimated effect sizes were expressed as Hazard Ratio (HR) with 95% confidence interval (CI95%)]. To include time-dependent covariates in the Cox model, the dataset was transformed into a long format (i.e., multiple observations at each time point for one individual), and each observation was correlated to each individual using a cluster term in Cox models. Binary outcomes regressions were logistic regressions (estimated effect sizes were expressed as Odd's Ratio (OR) with CI95%). Dimensional reduction of models was performed using a semi-automated stepwise backward-forward selection of variables based on Akaike information criteria [11]. To enhance the clinical consistency of our reduced model, a set of preoperative variables clinically relevant for capturing patient profiles in pancreatic transplantation was considered as follows: recipient age, BMI and sex as well as the type of donor, cold ischemia time, and type of transplant. The reduced models included these variables regardless of whether they were selected. Additionally, the variable of interest for this study (i.e., DL) was also forced in models when appropriate (i.e., in the survival analyses).

A model of exposure was estimated using a propensity score matching (PSM), which was performed using a 5:1 (5 controls matched to 1 case) nearest neighbor matching without replacement. A propensity score was estimated with logistic regression, including clinically relevant variables or variables associated with the exposure/outcome in the exploratory

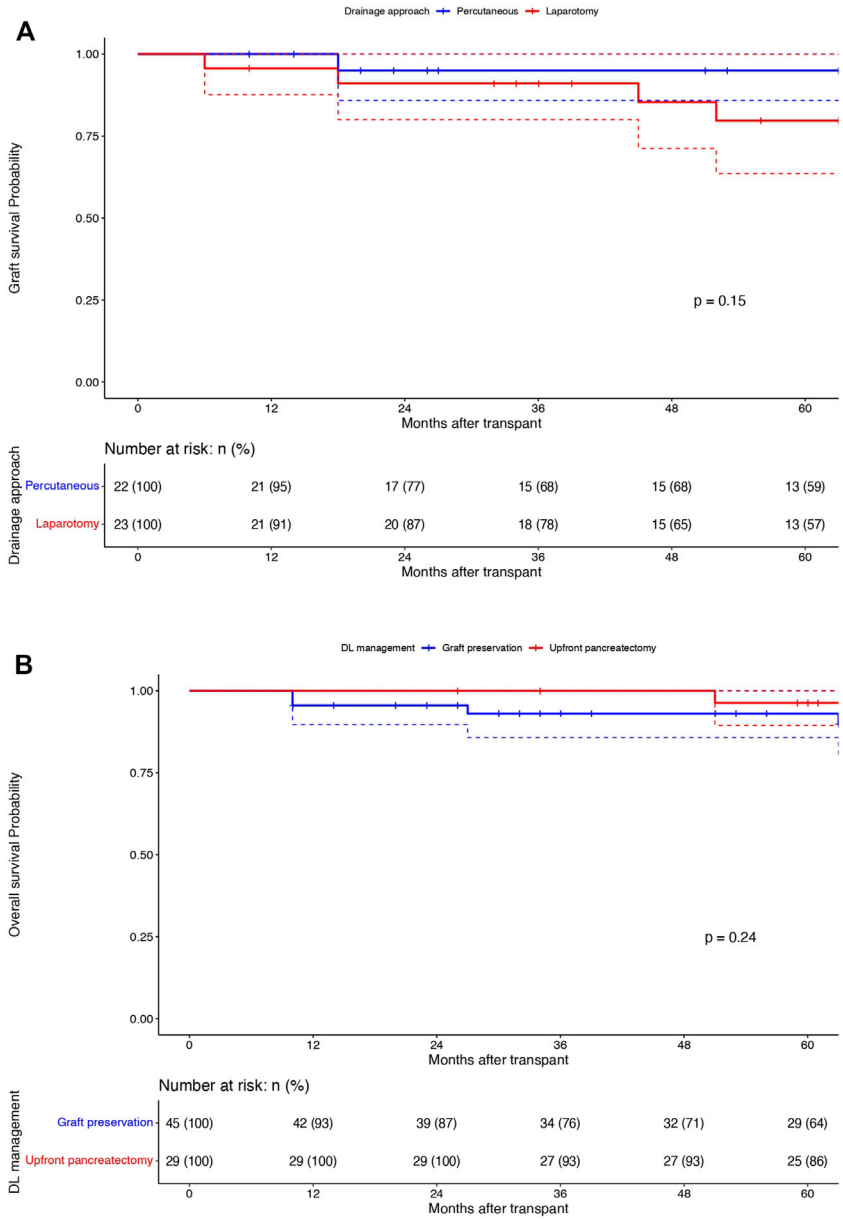


FIGURE 3 | Graft survival (1/3/5 years): Percutaneous drainage (Blue) vs. Laparotomy (Red) without pancreatectomy groups: X-axis: Months after transplant and Y-axis: Graft survival probability; comparison using Log rank test **(A)**. Overall survival (1/3/5 years): Graft preservation (Blue) vs. pancreatectomy (Red) groups: X-axis: Months after transplant and Y-axis: overall survival probability; comparison using Log rank test **(B)**.

analysis as follows: age, BMI, CMV status of the donor and the recipient, modality pancreatic transplantation (PTA, SPK, PAK), the type of donor (DCD vs. DBD), cold ischemia time, postoperative pulmonary or septic related complication, requirement for dialysis after transplant, graft portal vein thrombosis and rejection. Covariate balance before and after matching were estimated using standardized mean differences (see **Supplementary Figure S1**). The marginal effect of DL on survivals in the matched population was estimated using a weighted (incorporating the matching weights) Cox model without covariates (i.e., non-collapsible HR) with clustered variance on matching pair membership (cluster-robust standard errors).

All statistical analyses were performed using R statistical software version R version 4.2.0.

RESULTS

Study Population

A total of 595 patients underwent pancreas transplantation during study period, the majority being SPK (72.8%; n = 433),

TABLE 4 | Comparison of the demographic, pre-operative and peri-operative characteristics between the matched population of duodenal leak cohort without upfront pancreatectomy (graft preservation group) (n = 44) with the control population (n = 220); Propensity score matching (DL: Control = 1:5).

Variables	Matched control (n = 220)	DL without pancreatectomy (n = 44)	P val
Donor age (yrs) (IQR)	25.5 (20–35)	26 (19–36)	0.95
Donor BMI (kg/m^2) (IQR)	23.2 (20.9–26.1)	24.5 (20–27.1)	0.08
DCD donors (%)	10 (4.5)	2 (4.5)	>0.99
Donor CIT (mins) (IQR)	517 (414.5–601)	504 (405.2–600)	0.87
Recipient age (yrs) (IQR)	42.5 (37.4–48.4)	42.2 (38.4–46.8)	0.89
Recipient BMI (kg/m^2) (IQR)	25.8 (22.5–28.9)	25.9 (23.4–29.6)	0.67
Recipient gender (% males)	147 (66.8)	25 (56.8)	0.27
CMV mismatch (%)	20 (9.1)	4 (9.1)	0.24
EBV mismatch (%)	12 (5.5)	0 (0)	0.23
Transplant category			
SPK (%)	170 (77.3)	34 (77.3)	>0.99
PAK (%)	45 (20.5)	9 (20.5)	
PTA (%)	5 (2.3)	1 (2.3)	
Pre-transplant IS (%)	14 (6.4)	0 (0)	0.17
Pre-transplant Dialysis (%)	90 (40.9)	14 (31.8)	0.34
Pre-transplant cardiac intervention (%)	86 (39.1)	14 (31.8)	0.46
Pre-transplant infections (%)	23 (10.5)	5 (11.4)	>0.99
Post-transplant dialysis (%)	11 (5)	2 (4.5)	>0.99
Post-transplant DVT (%)	0 (0)	1 (2.3)	0.24
Post-transplant pneumonia (%)	6 (2.7)	1 (2.3)	>0.99
Post-transplant CLABSI (%)	1 (0.5)	0 (0)	>0.99
Post-transplant stay (days) (IQR)	9.7 (8.5–12.7)	10.1 (8.6–20.9)	0.31
Graft related complications			
Arterial thrombosis (%)	1 (0.5)	1 (2.3)	0.75
Portal vein thrombosis (%)	9 (4.1)	2 (4.5)	>0.99
Hemorrhage (%)	10 (4.5)	3 (6.8)	0.45
Graft rejection (pancreas) (%)	31 (14.1)	7 (15.9)	0.94
Graft loss (pancreas) (%)	45 (20.5)	6 (13.6)	0.40
Re-transplantation (pancreas) (%)	9 (4.1)	3 (6.8)	0.69
Overall mortality (%)	35 (15.9)	5 (11.4)	0.59
Median DLFS (months) (IQR)	84.5 (39.5–142.2)	12 (2.0–24.0)	**<0.001**
Median OS (months) (IQR)	99.5 (51.7–162)	83.5 (38.2–162.7)	0.55

*** All continuous variables expressed as medians, unless specified otherwise.*

Legends: DL, duodenal leak; IQR, interquartile range; BMI, body mass index; DCD, donation after cardiac death; CIT, cold ischemia time; CMV, cytomegalovirus; EBV, epstein barr virus; D/R: Donor/Recipient; SPK, simultaneous pancreas kidney; PAK, pancreas after kidney; PTA, pancreas transplant alone; IS, immunosuppression; DLFS, duodenal leak free survival; OS, overall survival.

The bold values indicate statistical significance.

followed by PAK (23.5%; n = 140) and PTA (3.7%; n = 22). Among them, 63.2% (n = 376) were males, and the median age (IQR) and BMI (IQR) were 43.5 years (37.2–50.5) and 24.7 kg/m^2 (21.8–27.9), respectively. The rate of 90-day mortality was 5% (n = 30). The descriptive analysis of the whole population and comparison between patients with and without DL is shown in **Table 1**.

DL Occurrence After Pancreatic Transplantation

After a median follow-up of 108 months, 74 patients (12.4%) were found to develop DL, with 42% (n = 31) developing leaks within 90 days following transplantation. DL patients underwent management with IR guided percutaneous drain insertion, laparotomy without graft pancreatectomy (lavage/repair), and upfront graft pancreatectomy in 29.7% (n = 22), 31.1% (n = 23), and 39.2% (n = 29) of the cases. A comparison of the demographic and peri-operative characteristics between the three groups is summarised in **Supplementary Table S1**. **Figure 1** depicts a 5-yearly comparative trend of the 3 management modalities for DL during the study period (2000-20).

Influence of DL on Overall and Graft Survivals

In the whole population (n = 595), 1-, 3- and 5-year overall and graft survivals were 96.8%, 95% and 92.9% and 89.6%, 86.5%, and 80.4%, respectively. One-, 3- and 5 years DLFS were 91%, 88.8%, and 86.8%, respectively. DL patients were associated with a decreased 1-/3-/5-year graft survival (74.2%/66.8%/58% vs. 91.9%/89.3%/83.6% respectively; $p < 0.0001$) while they had similar 1-/3-/5-year OS (96.5%/93.8%/92.4% vs. 96.7%/94.2%/92.7% respectively; $p = 0.87$) when compared to the patients without DL (**Figures 2A, B**). On multivariable analysis by Cox proportional hazards model, DL was independently associated with decreased graft survival (HR: 3.45; CI95%: 2.10–5.68; $p < 0.001$) but not with OS (HR: 0.38; CI95%: 0.05–2.89; $p = 0.36$). **Tables 2**, **3**; **Supplementary Table S2** report reduced and full Cox models to assess graft survival and overall survival risk factors in the population (n = 595).

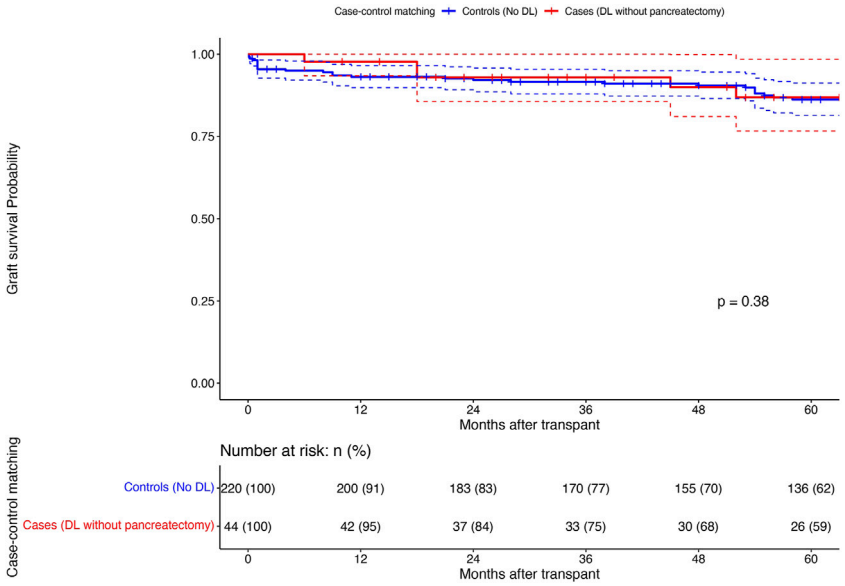


FIGURE 4 | Comparison of 1/3/5 years pancreas graft survival (Kaplan-Meier curves; Log rank test) between the DL patients without upfront pancreatectomy (n = 44; Cases; Red) and a matched (1:5) population of patients without DL (n = 220; Controls; Blue): X-axis: Months after transplant and Y-axis: Graft survival probability; comparison using Log rank test.

Management of DL (Comparison of the 3 Groups of Management of DL)

Among the DLs managed by graft salvage strategies, there was no difference in the graft survival between the percutaneous drainage arm (n = 22) and the laparotomy without pancreatectomy arm (n = 23) (95%/68%/59% vs. 91%/78%/57%; p = 0.15) (**Figure 3A**). The comparison of overall patient survival curves is demonstrated in **Figure 3B**. The overall survival rates were comparable between the graft salvage and graft pancreatectomy groups. Besides this, in the cohort of DLs managed by percutaneous drainage alone, 2 out of 22 required a laparotomy, in view of persistent undrainable collections and hemodynamic worsening. The mean interval between drain placement to removal was 17.5 days (12–53) and 8 out of 22 patients (36.3%) required repeat drain placement during the study period. In the laparotomy without pancreatectomy group, 3 out of 23 patients required re-laparotomy, with 2 culminating into graft pancreatectomy eventually. Although we observed a higher rate of renal rejection in DLs managed by graft preservation approaches compared to upfront pancreatectomy (22.7% and 17.4% vs. 10.3%), this was not significant and was managed by appropriate immunosuppressive therapy in all cases.

Matching Comparison of DL Patients (Without Upfront Graft Pancreatectomy)

Among the DL group, 44 patients who did not undergo upfront graft pancreatectomy (cases) were matched (1:5) to patients without DL (controls) (Propensity score matching; see **Supplementary Figure S1** for details). **Table 4** shows the matched population's comparative analysis between cases and controls. The marginal effect of DL on graft survival was null (HR:0.00, 95%CI:0.00–0.00; p < 0.001), with no difference in the 1/3/5-year graft survival between the cases and the matched controls (95%/75%/59% vs. 91%/77%/62%; p = 0.87) (**Figure 4**).

Identifying the High-Risk Factors in DL Cohort (Predictors of Upfront Graft Pancreatectomy)

The multivariable analysis (reduced model) identified presence of male recipient (OR:6.18; CI95%: 1.26–41.09; p = 0.04) to be associated with higher odds of requiring an upfront graft pancreatectomy on reduced model. Presence of CMV infection in the recipient (OR: 17.8; 95% CI:4.10–5,720; p = 0.02) and pre-transplant cardiovascular intervention (OR:10.8; 95%CI: 1.57–108; p = 0.03) were other variables associated with relatively higher odds of upfront pancreatectomy, although not found significant on reduced model (**Table 5**).

DISCUSSION

The present study demonstrated a 1-year graft loss of 25% in patients with DL, with 42% being diagnosed within the first 90 days after pancreas transplantation. Even though there was a demonstrable difference in the graft survival rate between the DL cohort and the patients without DL, we observed no difference in the overall survival rate at the corresponding time points. Further comparison between the patients with DL managed with graft

TABLE 5 | Logistic regression model to identify high-risk predictors in duodenal leak cohort (associated with upfront pancreatectomy) (Full and reduced regression model).

Variables	Full model			Reduced model		
	OR	95% CI	P val	OR	95% CI	P val
Donor age (yrs)						
Spline for age ≤19 years	0.61	0.00–181	0.87	0.62	0.06–5.32	0.66
Spline for age >19 and ≤34 years	0.02	0.00–1.47	0.13	0.07	0.00–0.81	0.04
Spline for age >34 years	4.20	0.01–3,607	0.63	1.64	0.15–19.41	0.68
Donor BMI (kg/m [2])						
Spline for BMI ≤20.8 kg/m [2]	0.02	0.00–1.58	0.15			
Spline for BMI >20.8 and ≤26.1 kg/m [2]	0.32	0.00–18.2	0.57			
Spline for BMI >26.1 kg/m [2]	0.32	0.00–113	0.72			
DBD vs. DCD donor	76.2	0.00–189	0.99			
Donor CIT (mins)						
Spline for CIT ≤442.2 min	31.1	0.12–840	0.33	4.80	0.36–109.68	0.26
Spline for CIT >442.2 and ≤644.7 min	6.29	0.04–120	0.57	4.17	0.44–73.75	0.25
Spline for CIT >644.7 min	0.79	0.00–87	0.95	7.50	0.69–156.43	0.13
Recipient age (yrs)						
Spline for age ≤37.3 years	1.89	0.00–2,976	0.84	0.94	0.12–7.70	0.95
Spline for age>37.3 and ≤50.5 years	0.01	0.00–2.42	0.13	0.24	0.03–1.78	0.16
Spline for age >50.5 years	1.50	0.01–859	0.88	1.60	0.14–23.46	0.71
Recipient BMI (kg/m [2])						
Spline for BMI ≤21.8 kg/m [2]	1.54	0.01–598	0.87	0.55	0.06–6.64	0.62
Spline for BMI >21.8 and ≤27.9 kg/m [2]	0.32	0.00–55.6	0.62	0.30	0.04–2.56	0.25
Spline for BMI >27.9 kg/m [2]	0.01	0.00–6.04	0.21	0.25	0.02–2.69	0.26
Male recipient	23.8	0.57–5,777	0.14	6.18	1.26–41.09	**0.04**
CMV status						
Negative	ref	ref	ref	ref	ref	ref
CMV mismatch (D+/R-)	24.8	0.66–5,347	0.12	4.28	0.83–25.69	0.09
CMV infection (R+)	17.8	4.10–5,720	**0.02**	2.01	0.32–12.52	0.44
EBV mismatch (D+/R-)	4.24	0.00-NA	>0.99			
Transplant category						
SPK	ref	ref	ref	ref	ref	ref
PAK	0.18	0.00–8.93	0.41	3.91	0.80–23.23	0.10
PTA	0.00	NA	>0.99	4.73e-06	--,8.04e+117	0.99
Pre-transplant IS	190	0.00–9,700	0.91	8.9	0.00–97	0.89
Pre-transplant dialysis	0.34	0.00–7.95	0.57			
Pre-transplant cardiac intervention	10.5	1.57–108	**0.03**			
Pre-transplant infection	0.15	0.00–21.9	0.51			

Legends: OR: Odd's ratio, CI: confidence interval, DBD: donation after brain death, DCD: donation after cardiac death, BMI: body mass index, CIT: cold ischemia time, CMV: cytomegalovirus, EBV: epstein barr virus, D/R: Donor/Recipient, SPK: simultaneous pancreas kidney, PAK: pancreas after kidney, PTA: pancreas transplant alone, IS: immunosuppression.

Non-linear variables transformed using cubic spline functions with 3 degrees of freedom; knots are placed at the 25th and 75th percentiles of the variable in the overall population. The bold values indicate statistical significance.

preservation strategies (percutaneous drainage and laparotomy without pancreatectomy) and a matched group of patients without DL demonstrated a marginal effect of DL *per se*, on short and long-term graft survival, with comparable graft survival rates at 1-/3-/5-year. Pre-transplant sepsis, graft rejection episodes, post-transplant dialysis, graft PV thrombosis were identified as the independent predictors of poor graft survival after pancreas transplantation, besides duodenal leak. In patients with duodenal leak after pancreas transplantation, development of an early leak (within 90 days), presence of CMV mismatch (D+/R−), male recipient and pre-transplant cardiovascular intervention were found to predict a worse outcome, necessitating an upfront pancreatectomy approach in most of these patients.

Management of DLs after pancreas transplantation have traditionally leaned towards an aggressive approach involving early detection and surgical intervention, with upfront graft pancreatectomy in most cases [12, 13]. The choice of graft preserving approach versus upfront graft pancreatectomy remains a matter of debate. Graft-preserving approaches, while aiming to maintain insulin independence, are associated with high rates of readmissions, relaparotomy, kidney graft rejection and failure, and sepsis, according to the limited available literature, which mostly consists of case reports and small series [14, 15]. Al-Adra et al from our centre investigated the outcome of DLs after pancreas transplantation, comparing the graft salvage approaches to upfront graft pancreatectomy in a series of 33 recipients with DL [16]. The authors reported favourable outcomes for laparotomy with definitive repair in carefully selected patients with limited peritoneal contamination and localised source of leak (13 of 14 patients with a median graft survival of 2.9 years). However, more conservative measures such as percutaneous drainage and operative drainage by lavage failed to control the leak, necessitating graft pancreatectomy in 7 out of the 8 cases. Findings of this study

were further substantiated by Fleetwood et al. in 2022 [17]. The authors compared the outcomes of graft salvage approach (repair and resection) with immediate graft pancreatectomy in a series of 33 patients with DL out of 1,153 undergoing pancreas transplantation. They found DL to be an independent predictor of 6-month graft loss (HR: 13.9; CI95%: 8.5–22.9; $p < 0.001$). However, they reported no difference in 5-year graft survival (82.5% vs. 81.5%) and overall survival (90.5% vs. 93.5%) between the graft salvage and non-leak groups beyond 6 months. This group did not attempt percutaneous drainage in any of their patients. Similar to the findings by the Wisconsin group [17], we observed no difference in the 5-year graft survival rates between DL patients who underwent graft salvage (conservative or operative) and recipients without DL. This was further substantiated by comparing the graft survival and other peri-operative variables between the DL with graft salvage cohort and a matched population of recipients without DL. We observed no difference in the 1- and 5-year graft survival rates (95% and 59% vs. 91% and 62%, respectively). Additionally, we noted a favorable outcome in terms of graft survival in the group treated by percutaneous drainage alone, with a median graft survival of 65 months.

A concern with the percutaneous drainage-alone management was a higher rate of re-admissions (10 out of 22 patients; 45.4%). These readmissions were primarily due to fever, elevated total leukocyte counts, ileus, or undrained collections. These complications were managed by upsizing the percutaneous drains or placing multiple drains in new collections under IR guidance. Previous studies have also discussed a theoretically higher risk of 90-day mortality with graft salvage approaches, potentially due to the development of generalized peritonitis and abdominal sepsis from persistent undrained collections, leading to severe systemic inflammation and septic shock. In our study, we found no significant difference in the 90-day mortality rates between the three groups, with a slightly higher incidence in the upfront pancreatectomy group compared to the graft preservation groups (27.6% vs. 13.6% and 13%; $p = 0.76$) [18, 19].

Benefits of graft salvage approaches include maintaining insulin independence and avoiding the need for re-transplantation, which is significant considering the high waitlist times and organ shortage. These benefits must be weighed against the potential risks mentioned above [20]. Therefore, triaging patients with DL into graft salvage vs. upfront graft pancreatectomy groups is important for an appropriate decision making for a multiorgan transplantation team. Clinical determinants such as hemodynamic worsening, persistently elevated blood counts, persistent undrained collections on serial imaging, and definitive evidence of unresolving sepsis or multiple undrained collections indicate the need for upfront pancreatectomy. Additionally, identification of other demographic and peri-operative factors in the donor and recipient may be helpful in decision making in these patients. We found that recipient factors such as male gender and presence of CMV infection in the recipient and pre-transplant cardiovascular intervention associated with higher odds of undergoing upfront pancreatectomy in the DL cohort, the latter two not being statistically significant. Male recipient has been shown to be associated with a higher incidence of portal vein thrombosis (higher risk of DL) in a few studies although the more recent literature has not shown any significant impact of recipient gender on the incidence of the same [21, 22]. CMV infection and pre-transplant cardiovascular interventions have been shown to be associated with delayed and very early leaks respectively, by a previous study from our centre, both of which might be presumably challenging to manage by more conservative measures due to the presence of other risk factors of healing like higher dose of immunosuppression in the early post-operative phase (in PAK and SPK) or risk of renal rejection (especially in delayed leaks) [23]. This could possibly explain the higher odds of upfront pancreatectomy (albeit not significant) in these groups of recipients in our patient population.

There were certain limitations of this study as well, foremost being the number of patients with DL. Contrary to the available literature, we observed a slightly higher incidence of DL in our cohort [23, 24]. The criteria for diagnosing DLs at our centre was mainly based on imaging using CT scan and/or drain amylase levels (in patients with percutaneous drain placement under CT guidance), the latter having a low specificity for DL. Additionally, the threshold for placing percutaneous drains in these patients has been low with a trend towards a more of a "drain first" approach over the last decade. This shift is mainly attributed to improvements in the precision of IR guidance and clinicians' preferences for managing DLs in this manner. A preference for earlier intervention in these patients has also been observed, anticipating better outcomes in terms of graft preservation. These factors may explain the higher reporting of DLs during the 20-year study period. Another important limitation was the retrospective nature of the study. Although our results with graft salvage approaches are promising, larger multicentre analysis is needed in future to validate these findings more conclusively to account for variations in operative techniques and pre- and post-transplant management protocol of pancreas transplant recipients from centre to centre.

In conclusion, despite the seemingly devastating effects of duodenal leaks on graft survival, an early diagnosis and timely intervention can decrease the short- and long-term morbidity and mortality in these patients. Graft salvage strategies have shown promising long-term results in selected patients. Triaging patients into graft salvage versus upfront graft pancreatectomy approaches, based on a combination of peri-operative donor and recipient characteristics, clinical presentation of DL, radiological severity of peritoneal contamination, and the expertise of the IR team to target undrained collections, remains the cornerstone of management for achieving favorable outcomes in pancreas transplant recipients with duodenal leaks.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by the Institutional review board Toronto general hospital (CAPCR ID: 21-6151.1).

The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because it is a retrospective observational study.

AUTHOR CONTRIBUTIONS

SR, acquisition of data, research design, analysis of data, drafting of the manuscript, critical analysis and revision, final approval; CH, analysis of data, research design, drafting of the manuscript, critical analysis and revision, final approval; AN, research design, drafting of the manuscript, critical revision, final approval; ZS, research design, drafting of the manuscript, critical revision, final approval; JS, drafting of the manuscript, critical revision, final approval; GS, drafting of the manuscript, critical revision, final approval; IM, drafting of the manuscript, critical revision, final approval; MS, drafting of the manuscript, critical revision, final approval; TR, co-chief supervisor of the manuscript, primary conceptualisation analytic tools, drafting of the manuscript, critical revision, final approval; CS, chief supervisor of the manuscript, primary conceptualisation, drafting of the manuscript, critical revision, final approval. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.13302/full#supplementary-material

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BRIEF RESEARCH REPORT
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The Relative Risk of COVID-19 in Solid Organ Transplant Recipients Over Waves of the Pandemic

Amanda J. Vinson[1], Alfred J. Anzalone[2], Makayla Schissel[2], Ran Dai[2], Gaurav Agarwal[3], Stephen B. Lee[4], Amy Olex[5] and Roslyn B. Mannon[6] for Membership of the National COVID Cohort Collaborative (N3C) Consortium*

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Solid organ transplant recipients (SOTR) are at increased risk from COVID-19. Over time, the absolute risk of adverse outcomes after COVID-19 has decreased in both the non-immunosuppressed/immunocompromised (non-ISC) general population, and amongst SOTR. Using the N3C, we examined the absolute risk of mortality, major adverse renal or cardiac events, and hospitalization after COVID-19 diagnosis amongst non-ISC and SOTR populations over five waves of the pandemic (Wave 1: Ancestral COVID; Wave 2: Alpha; Wave 3: Delta; Wave 4: Omicron; Wave 5: Omicron). Within each wave, we determined the relative risk of each outcome for SOTR versus the non-ISC population based on crude event rates, and then used multivariable cox proportional hazards models and logistic regression to determine the adjusted risk of each outcome based on SOT status. Throughout the pandemic, including during the Omicron wave (Wave 5), SOTR were at greater absolute risk for each outcome than non-ISC patients (p-values all <0.001). The adjusted risk of SOT status for each outcome was relatively stable over time (aHR 1.28–1.61 for mortality; aHR 1.31–1.47 for MACE; aHR 1.72–1.90 for MARCE; aHR 1.75–2.07 for AKI; and aOR 1.53–1.81 for hospitalization). Despite a reduction in the absolute risk of COVID-19 complications, the relative risk for SOTR versus the non-ISC population has not improved.

Keywords: COVID-19, pandemic, Sars-CoV-2, transplant, outcomes, variant strain, waves, relative risks

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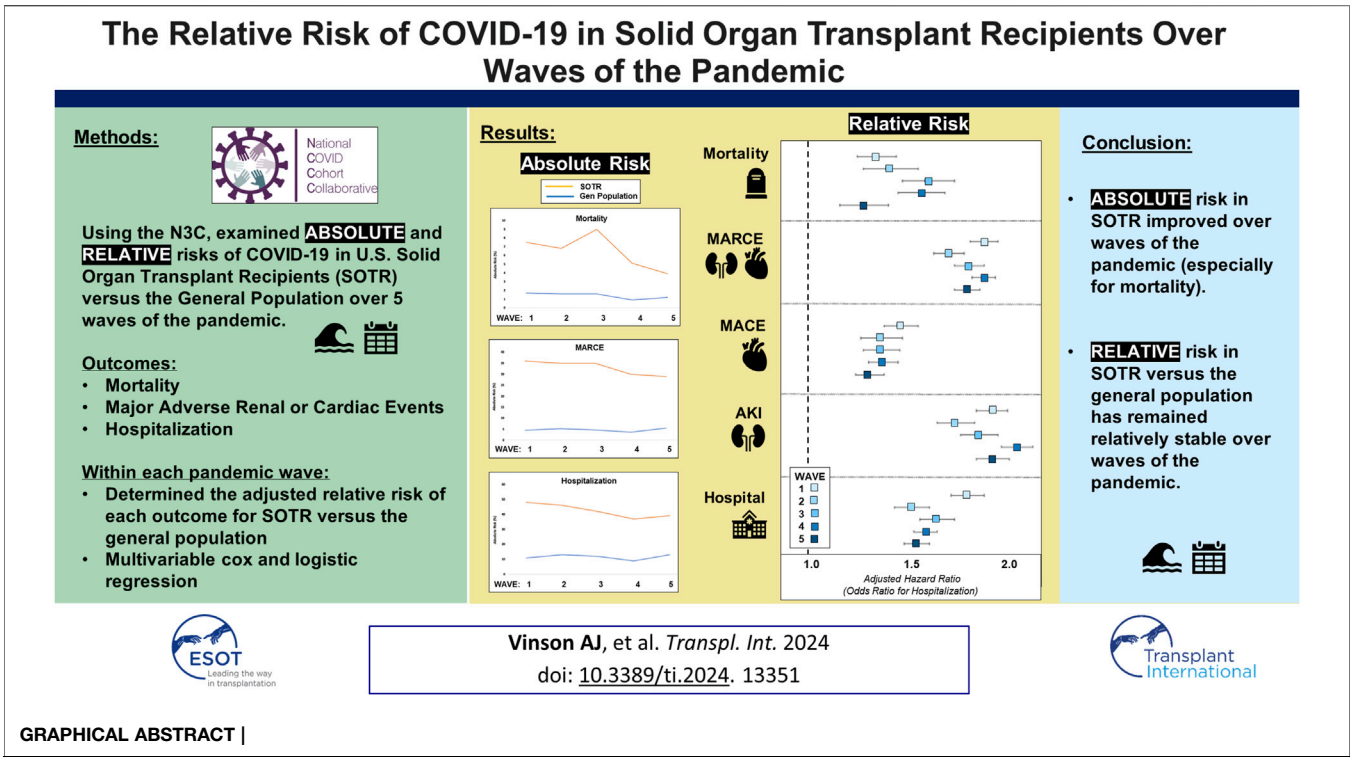
INTRODUCTION

The coronavirus 2019 (COVID-19) pandemic has had dramatic consequences for the population at large, but especially amongst solid organ transplant recipients (SOTR) who are at higher risk for severe infection and mortality [1]. The higher risk in this population is likely on account of exposure to chronic maintenance immunosuppression and greater underlying comorbidity burden [2, 3]. While SOTR benefit from COVID-19 vaccination, vaccine effectiveness among SOTR has been observed to be diminished in comparison to the general population [4]. With the development of effective vaccine programs and more efficacious therapies, COVID-19-related risk of mortality and other complications has improved over time amongst both non-immunosuppressed/immunocompromised (ISC) [5] and SOTR populations [6]. However, whether the *relative* risk associated with SOT, defined as the risk in SOTR compared to that



GRAPHICAL ABSTRACT |

observed in the general population, has improved is unknown. Therefore, in this study we aimed to examine changes in the relative risk of complications post-COVID-19 in SOTR versus non-ISC populations using the largest COVID-19 database in the United States, the National COVID Cohort Collaborative (N3C) [7].

METHODS

The N3C represents a large, national repository of 80 medical centers across the United States contributing data on nearly 9 million adult patients with COVID-19 and more than 14 million COVID-19–negative controls. This centralized and highly granular repository of electronic health record (EHR) data represents the most representative and substantive resource for studying the U.S. COVID-19 population [8]. The N3C includes patients with COVID-19 positivity or suspected positivity by lab testing or diagnostic codes for both inpatient and outpatient encounters [9]. Data is input from four primary data models—OMOP, PCORnet, TriNetX, and ACT—harmonized into the OMOP 5.3.1 data model and made available within a secure enclave for analysis at the patient- and encounter-level [7].

Using the N3C, we examined the absolute risk of 1. mortality (overall), 2. major adverse renal or cardiac events (MARCE; defined as a composite of acute kidney injury (AKI) with or without dialysis, acute myocardial infarction, angina, stent occlusion/thrombosis, stroke, transient ischemic attack, congestive heart failure or death from any cause), 3. major adverse cardiac events (MACE), 4. AKI (defined using condition codes for acute kidney injury or failure) and 5. hospitalization within 90 days of COVID-19 diagnosis amongst non-ISC and SOTR populations (kidney, liver, lung and heart recipients) over five waves of the pandemic (Wave 1: Ancestral COVID; 01/01/2020–12/31/2020; Wave 2: Alpha; 01/01/2021–06/25/2021; Wave 3: Delta; 06/26/2021–12/17/2021; Wave 4: Omicron; 12/18/2021–07/01/2022; Wave 5: Omicron; 07/02/2022–03/31/2023). Outcome events were determined based on diagnostic or procedure codes documented in the 90-day window post-COVID-19 diagnosis and were ascertained using condition codes diagnosed by a provider (e.g., SNOMED CT, ICD-10-CM), procedure codes associated with an encounter within the observation window (CPT4, ICD-10-PCS), or deaths documented within the reporting health system. Individuals without the recorded outcome were assumed to not have the outcome. Patients were considered as belonging to a given wave based on the date of their COVID-19 diagnosis. We also examined the relative risk, comparing the relative risk of each outcome within each wave of the pandemic in SOTR to non-ISC populations. Finally, multivariable Cox proportional hazard models were used to examine the adjusted relative hazard of each outcome associated with SOT status across pandemic waves (multivariable logistic regression for hospitalization at any point within 90 days of COVID diagnosis), with time 0 being date of COVID-19 diagnosis. Models were adjusted for known literature predictors of adverse outcomes after COVID-19 diagnosis, including sex, age, race/ethnicity (White, Black, Hispanic or Latino, Other),

TABLE 1 | Baseline characteristics at the time of COVID-19 diagnosis in Non-Immunosuppressed/Immunocompromised (Non-ISC) patients and Solid Organ Transplant Recipients (SOTR).

Characteristic	Overall N = 5,521,812	Non-ISC N = 5,469,182	SOTR N = 52,630	*p*-value
Age at COVID-19 Diagnosis	45 (31, 61)	45 (31, 61)	58 (46, 66)	<0.001
Age Strata				<0.001
18–44	2,682,345 (49%)	2,670,289 (49%)	12,056 (23%)	
45–65	1,828,259 (33%)	1,802,261 (33%)	25,998 (49%)	
>65	1,011,208 (18%)	996,632 (18%)	14,576 (28%)	
Sex				<0.001
Female	3,136,019 (57%)	3,113,865 (57%)	22,154 (42%)	
Male	2,385,793 (43%)	2,355,317 (43%)	30,476 (58%)	
Race/Ethnicity				<0.001
White	3,425,575 (62%)	3,397,683 (62%)	27,892 (53%)	
Black or African American	683,952 (12%)	672,713 (12%)	11,239 (21%)	
Hispanic or Latino	670,690 (12%)	662,772 (12%)	7,918 (15%)	
Other/Unknown	741,595 (13%)	736,014 (13%)	5,581 (11%)	
Comorbidities				
CKD	268,409 (4.9%)	229,872 (4.2%)	38,537 (73%)	<0.001
Hypertension	1,318,062 (24%)	1,274,263 (23%)	43,799 (83%)	<0.001
Diabetes	635,247 (12%)	609,175 (11%)	26,072 (50%)	<0.001
COPD/Asthma	549,062 (9.9%)	539,611 (9.9%)	9,451 (18%)	<0.001
Cancer	315,939 (5.7%)	305,293 (5.6%)	10,646 (20%)	<0.001
CAD	293,049 (5.3%)	278,109 (5.1%)	14,940 (28%)	<0.001
CHF	221,868 (4.0%)	206,819 (3.8%)	15,049 (29%)	<0.001
PVD	236,408 (4.3%)	224,633 (4.1%)	11,775 (22%)	<0.001
Liver Disease	213,042 (3.9%)	200,494 (3.7%)	12,548 (24%)	<0.001
Obesity	1,723,831 (31%)	1,695,716 (31%)	28,115 (53%)	<0.001
Vaccination Status				<0.001
Non-Breakthrough Infection	4,612,447 (84%)	4,571,722 (84%)	40,725 (77%)	
VAX2 Breakthrough Infection	531,876 (9.6%)	526,492 (9.6%)	5,384 (10%)	
VAX3 Breakthrough Infection	377,489 (6.8%)	370,968 (6.8%)	6,521 (12%)	
SARS-CoV-2 Variant Wave				<0.001
Ancestral COVID-19	1,492,240 (27%)	1,481,743 (27%)	10,497 (20%)	
Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1)	655,400 (12%)	649,065 (12%)	6,335 (12%)	
Delta (B.1.617.2)	926,564 (17%)	918,803 (17%)	7,761 (15%)	
Omicron (B.1.1.529, BA.2, BA.2.12.1)	1,520,782 (28%)	1,504,310 (28%)	16,472 (31%)	
Omicron (BA.5, BQ.1.1, XBB.1.5)	926,826 (17%)	915,261 (17%)	11,565 (22%)	
Transplantation Type				N/A
Non-Transplant	5,469,182 (99%)	5,469,182 (100%)	0	
Kidney	33,412 (0.6%)	N/A	33,412 (63%)	
Liver	8,545 (0.2%)	N/A	8,545 (16%)	
Lung	4,883 (<0.1%)	N/A	4,883 (9.3%)	
Heart	5,790 (0.1%)	N/A	5,790 (11%)	

comorbidities (chronic kidney disease, hypertension, diabetes, asthma/chronic obstructive pulmonary disease, cancer, peripheral vascular disease, liver disease, obesity, coronary artery disease, congestive heart failure), and vaccination status [no complete vaccination series documented, or breakthrough infection (VAX2: being ≥14 days post two doses for mRNA vaccines, one dose for Johnson & Johnson/Janssen vaccine, or two doses for other vaccines; VAX3: being ≥14 days post a booster dose of any of the above vaccine preparations following VAX2)] [4, 10].

In a secondary analysis, we examined the relative risk of each post-COVID outcome by transplanted organ type (kidney, liver, lung, or heart), rather than for SOTR collectively (based on crude event rates and multivariable modeling as above). Complete case analysis was used for all analyses.

RESULTS

Among 5.5M non-ISC and 52,630 SOTR with COVID-19, SOTR were significantly older [58 years (Q1 46, Q3 66) versus 45 years (Q1 31, Q3 61)], more likely to be male (58% versus 43%), and with greater comorbidity burden than the general, non-ISC population (73% versus 4.2% with chronic kidney disease; 83% versus 23% with hypertension; 50% versus 11% with diabetes; and 29% versus 3.8% with congestive heart failure), **Table 1**. SOTR were at significantly higher risk for all outcomes during all waves of the pandemic, **Supplementary Figures 1A–E**; generally, the absolute risk of each outcome decreased over time for both non-ISC and SOTR. Crude event rates are shown in **Supplementary Table 1**.

The relative risk (SOTR versus non-ISC) based on crude event rates for each outcome over waves of the pandemic is

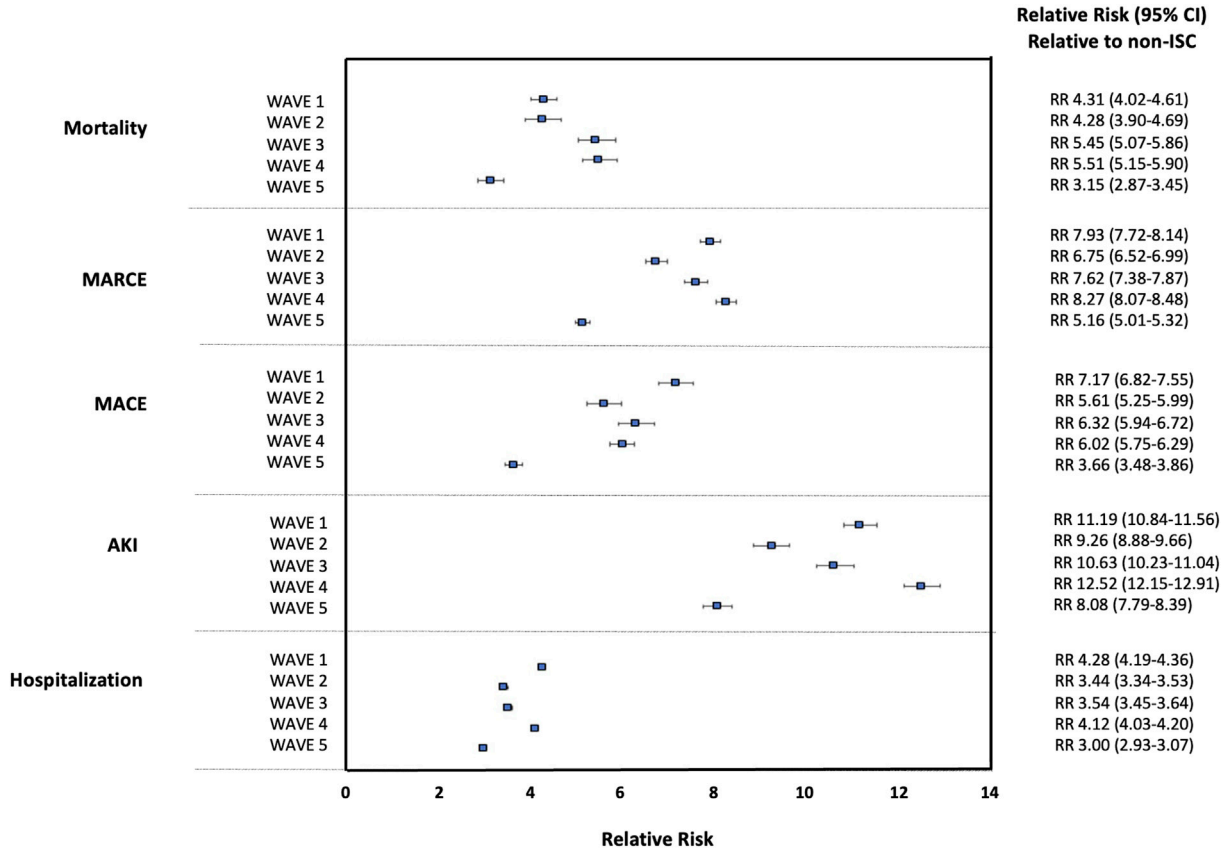


FIGURE 1 | Relative Risk for 90 days Outcomes Post-COVID-19 for Solid Organ Transplant Recipients (SOTR) Versus non-Immunosuppressed/ Immunocompromised (non-ISC) Populations Over Waves of the Pandemic.

shown in **Figure 1**. Throughout the pandemic, including during the Omicron wave (Wave 5), SOTR were at ~3-8x greater risk for each outcome than non-ISC patients and ~8-12x greater risk for AKI. Compared with the general population, the relative risk for SOTR was greatest during Wave 1 (for the outcomes of MACE and hospitalization) and Wave 4 (for the outcomes of mortality, MARCE, and AKI). The relative risk in SOTR versus the general population was significantly lower in Wave 5 for each outcome.

In multivariable models, SOT status was significantly associated with increased risk for each outcome during all waves of the pandemic (p-values all <0.001). The adjusted risk of SOT status for each outcome was relatively stable over time (aHR 1.28–1.61 for mortality; aHR 1.31–1.47 for MACE; aHR 1.72–1.90 for MARCE; aHR 1.75–2.07 for AKI; and aOR 1.53–1.81 for hospitalization), **Figure 2**. The adjusted risk of each outcome associated with SOT status (relative to the general, non-ISC population) over the five waves of the pandemic is shown in **Supplementary Table 2**. The adjusted risk for SOTR versus non-ISC was highest in Wave 1 (for the outcomes of MARCE, MACE and hospitalization) and Wave 4 (for the outcomes of mortality and AKI), though there was substantial overlap in confidence intervals with no significant differences noted in SOT-associated risk across the waves as a whole. In adjusted models, SOTR were not at significantly lower risk for any outcome during Wave 5 compared with other waves.

Finally, the relative risk for each outcome by organ type based on crude event rates is shown in **Supplementary Table 3**, and the adjusted relative risk by organ type based on multivariable modeling is shown in **Supplementary Table 4**. Relative to non-ISC, the highest mortality risk was for lung transplant recipients [aHR 1.93, 95% CI 1.53–2.43 in Wave 3 (minimum); aHR 2.27, 95% CI 1.86–2.76 in Wave 4 (maximum)], followed by kidney transplant recipients [aHR 1.46, 95% CI 1.28–1.66 in Wave 5 (minimum); aHR 1.84, 95% CI 1.68–2.01 in Wave 4 (maximum)].

DISCUSSION

Although the absolute risk associated with SARS-CoV-2 infection has decreased over time in both SOTR and the general population, SOTR remain at significantly higher risk for complications and serious adverse events post-COVID-19 than the general, non-ISC population; this has not improved over time after adjusting for potential confounders. In keeping with earlier literature [11–13],

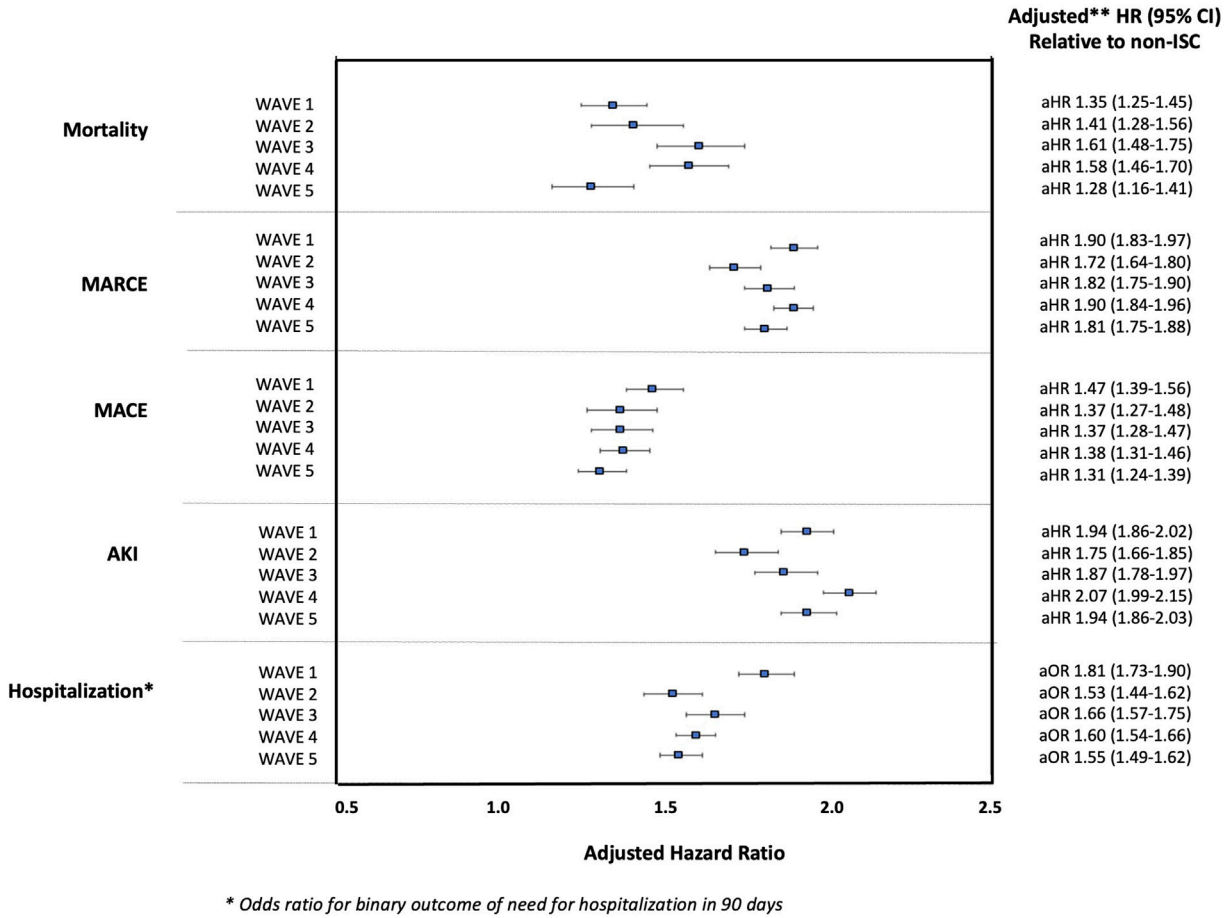


FIGURE 2 | Adjusted Hazard Ratios for 90-day Outcomes Post-COVID-19 (and Odds Ratio for Hospitalization) in SOTR Versus non-ISC Populations Over Waves of the Pandemic.

relative to the general population, the risk of death was highest amongst lung, followed by kidney transplant recipients, though the organ-specific risk across all waves of the pandemic was overall stable.

Since the onset of the pandemic, SOTR have experienced disproportionately higher rates of COVID-19 complications (including greater case-fatality ratios) than the general population, which has been attributed largely to their state of chronic immunosuppression and increased baseline comorbidity burden [1]. Relatively widespread uptake of efficacious vaccination programs and improved anti-SARS-CoV-2 therapeutic strategies have reduced the overall risk of serious adverse events post-COVID-19 [5]. The slight downtrend in relative risk for SOTR during Wave 2 may reflect prioritized access to vaccination for immunosuppressed patients during this period.

Importantly, although COVID-19 risk has diminished over time, there remains a substantial burden of illness attributable to SARS-CoV-2 infection with hospitalization rates for SOTR and non-ISC of 39.3% and 13.1% amongst those with a positive COVID-19 result recorded in the N3C during Wave 5. While a limitation of the data is that home COVID-19 testing results are not captured, these patients would typically be more likely to have asymptomatic or mild disease, and the absolute number (not percentage) of patients with each complication post-COVID would be unlikely to change (n = 4,548 and n = 120,010 hospitalizations amongst SOTR and non-ISC during Wave 5). Notably however, if asymptomatic and mild cases were completely captured, the proportion of patients requiring hospitalization after a positive test (not the absolute number) would likely be smaller. It is also important to note that the time at risk for COVID infection (duration of an individual wave) was not consistent across waves, as the dates were chosen to reflect a dominant circulating variant strain rather than a given period of time [e.g., Wave 1 (Ancestral) lasted 12 months whereas Wave 3 (Delta) lasted less than 6 months]. Therefore, comparison of isolated absolute event rates (rather than relative rates) across pandemic periods is not possible. Hence, the multivariable models we conducted (displayed in **Figure 2**), are the most accurate representation of relative risk associated with SOT status given the above limitations. An additional limitation of the current study is that within each variant period, we cannot comment on changing trends over time, rather we present the

overall absolute and relative risks for SOTR versus non-ISC populations in a given time period, acknowledging the potential for change in risk over a given wave.

Overall, there has been a reduction in the absolute risk of COVID-19 complications amongst both SOTR and non-ISC populations over the pandemic. However, risk is not negligible, and SOTR remain at significantly higher risk than the general population; SOTR continue to be disproportionately impacted by COVID-19.

DATA AVAILABILITY STATEMENT

The N3C Enclave is available for public research use. To access data, institutions must have a signed Data Use Agreement executed with the US National Center for Advancing Translational Sciences (NCATS), and investigators must complete mandatory training and submit a Data Use Request (DUR) to N3C. To request N3C data access, follow the instructions at https://covid.cd2h.org/onboarding. All code used for analyses can be made available upon request. More than 4000 researchers currently have access to data in N3C, representing more than 300 US research institution. Details are provided in the supplement.

ETHICS STATEMENT

National Institute of Health's (NIH) National COVID Cohort Collaborative (N3C) Data Utilization Request Approval committee approved the data utilization request of this project (RP-CA3365), which is approved under the authority of the National Institutes of Health Institutional Review Board and with Johns Hopkins University School of Medicine serving as a central institutional review board. The study protocol was obtained from the University of Nebraska Medical Center (0853-21-EP). The N3C data transfer to NCATS is performed under a Johns Hopkins University Reliance Protocol # IRB00249128 or individual site agreements with NIH. The N3C Data Enclave is managed under the authority of the NIH; information can be found at https://ncats.nih.gov/n3c/resources. No informed consent was obtained because the study used a limited data set.

AUTHOR CONTRIBUTIONS

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

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AUTHOR DISCLAIMER

The N3C Publication Committee confirmed that this manuscript MSID: 1936.849 is in accordance with N3C data use and attribution policies; however, this content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the N3C program.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed 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.2024.13351/full#supplementary-material

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Best oral communication of the 8th Congress
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BRIEF RESEARCH REPORT
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Management of Kidney Transplant Outpatients With COVID-19: A Single Center Experience

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Patients undergoing kidney transplant are at risk of severe COVID-19. Our single-center retrospective analysis evaluated the outcomes of kidney transplant outpatients with COVID-19 who were managed with reduced immunosuppression and treatment with molnupiravir. Between January 2022 and May 2023, we included 93 patients (62 men, average age 56 years), serum creatinine 127 (101–153) μmol/L. Molnupiravir was administered, and immunosuppressive therapy was reduced immediately following the confirmation of SARS-CoV-2 infection by PCR, which was 2 (1–3) days after the onset of symptoms. Only three (3.2%) patients required hospitalization, and one patient died. Acute kidney injury was observed in two patients. During the follow-up period of 19 (15–22) months, there was no significant increase in proteinuria, no acute or new chronic graft rejection, and kidney graft function remained stable; serum creatinine was 124 (106–159) μmol/L post-COVID-19 infection and 128 (101–161) μmol/L at the end of the follow-up period. Our results demonstrate that early initiation of molnupiravir treatment combined with a temporary reduction in immunosuppressive therapy results in favorable clinical outcomes in patients with COVID-19, with preservation of good graft function and no episodes of graft rejection.

Keywords: COVID-19, Sars-CoV-2, molnupiravir, kidney transplantation, antiviral drugs

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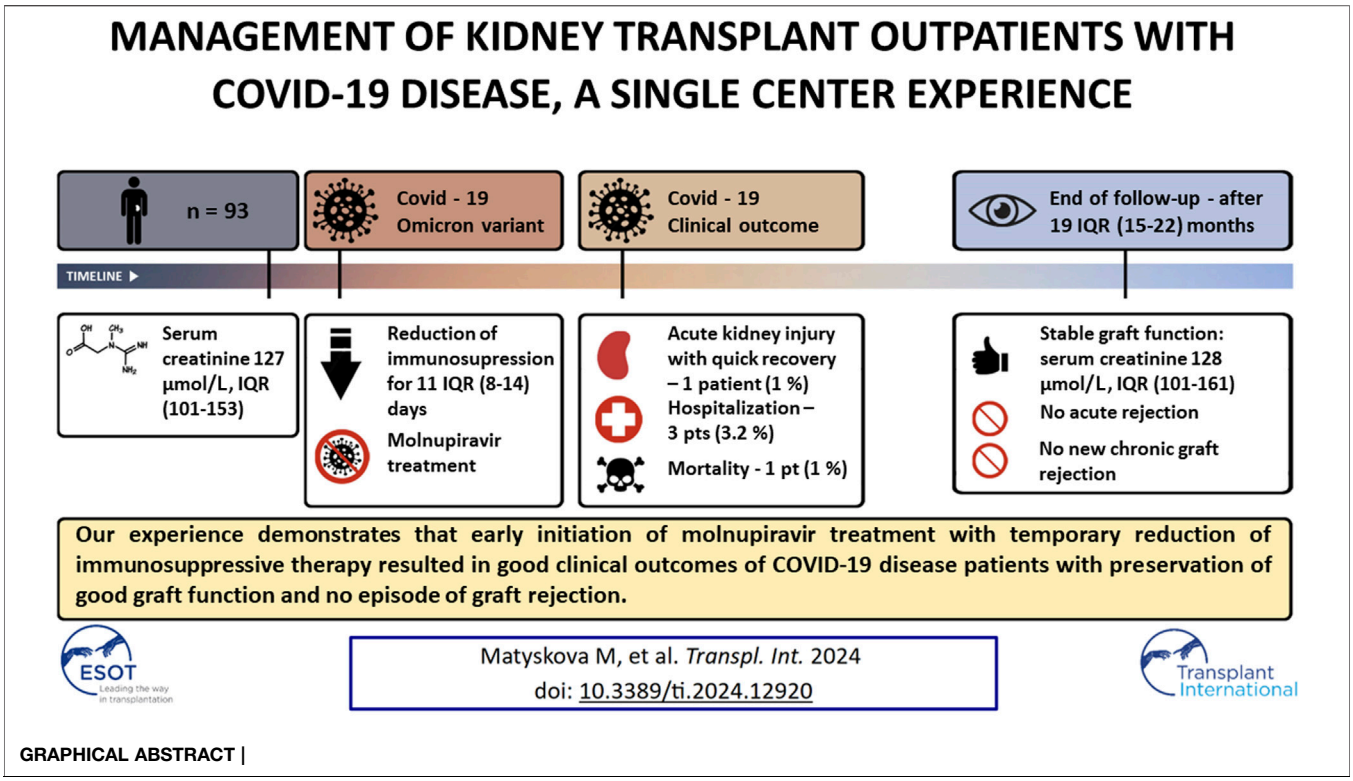
INTRODUCTION

The SARS-CoV-2 pandemic poses a serious threat, especially for vulnerable populations, including patients who are immunocompromised, and continues to remain a major burden on the healthcare system. During the initial phase of the COVID-19 pandemic, the European Renal Association Registry reported a high mortality rate among kidney transplant recipients [1]. Subsequently, with the emergence of the Omicron variant and the introduction of various vaccinations and several antiviral drugs, the severity of the disease and hospitalization rates decreased [2]. Consequently, attention is now being paid to the management of ambulatory patients. According to the ERA Descartes Working Group, antiviral treatment is a valid option for mild-to-moderate COVID-19 in patients undergoing kidney transplant [3].

Three drugs are currently available for this purpose. However, nirmatrelvir/ritonavir was not available at the beginning of the study and has notable disadvantages, mainly due to its significant interaction with immunosuppressive drugs prescribed to patients undergoing transplantation, and



GRAPHICAL ABSTRACT |

the need for dosage adjustment based on kidney function. Another drug, remdesivir, must be administered intravenously and was reserved for patients with serious COVID-19. Therefore, all patients in our study were prescribed molnupiravir, which has been approved for emergency use by both the European Medicines Agency and the Food and Drug Administration. Despite its lower efficacy, molnupiravir offers significant advantages for ambulatory management; it is administered orally, lacks drug-drug interactions, and is safe for patients with a wide range of graft functions [4]. In this study, we assessed the outcomes of kidney transplant outpatients with COVID-19 managed with molnupiravir treatment in combination with temporary immunosuppressive reduction.

METHODS

SARS-CoV-2 positive kidney transplant recipients between January 2022 and May 2023 were included in this single-center retrospective study analyzing the outcomes of kidney transplant outpatients with COVID-19. The patients were followed up until February 2024. The inclusion criteria were adult, symptoms of COVID-19, and confirmed SARS-CoV-2 infection via reverse transcriptase polymerase chain reaction testing on nasopharyngeal swab specimens. All diagnoses were confirmed in the outpatient setting. Patients diagnosed with COVID-19 during hospitalization were excluded from the study. This study did not include a control group.

All patients were educated about COVID-19 symptoms and their clinical significance. They were instructed to call our transplant center immediately if they experienced COVID symptoms. Molnupiravir was administered to all patients positive for SARS-CoV-2, with none refusing treatment. Following PCR confirmation of SARS-CoV-2, molonupiravir treatment was initiated, and immunosuppressive therapy was promptly reduced. All patients were treated with a standard dose of molnupiravir capsules (800 mg every 12 h for 5 days).

Upon COVID-19 infection, immunosuppressive therapy was temporarily reduced. Mycophenolate mofetil was discontinued, and calcineurin inhibitor treatment was maintained. For patients assessed as low rejection risk by physician and with higher target tacrolimus levels (6–8 µg/L), the doses of tacrolimus was reduced to achieve target levels of 4–6 µg/L. A similar approach was used in patients treated with cyclosporine A and an mTOR inhibitor. Immunosuppressive treatment remained unchanged for patients not treated with mycophenolate mofetil, those at a higher risk of rejection, or those already receiving minimal immunosuppressive treatment. Pre-COVID-19 doses of immunosuppressive therapy were resumed after significant clinical improvement or complete resolution of symptoms.

This study aimed to assess the safety and feasibility of the described treatment approach for COVID-19 course and outcome, kidney graft function, and incidence of rejection episodes. For this purpose, we analyzed graft function prior to COVID-19 diagnosis, 2–3 weeks after the onset of the disease, and at the end of the follow-up period (February 2024). The patients were followed-up for a median duration of 19 (15–22) months. Routine monitoring

TABLE 1 | Baseline characteristics of patients.

Characteristics	Values
Patients	93
Age (years), median (IQR)	58 (46–67)
Male (years), median (IQR)	54 (45–64)
Female (years), median (IQR)	64 (50–69)
Sex	
Male, n (%)	62 (67)
Female, n (%)	31 (33)
Time from transplantation (years), median (IQR)	6 (2–11)
Previous transplant, n (%)	10 (11)
Living donor, n (%)	5 (5.4)
Baseline serum creatinine (μmol/L), median (IQR)	127 (101–153)
Diabetes, n (%)	36 (39)
Hypertension, n (%)	89 (96)
Coronary artery disease, n (%)	15 (16)
COPD, n (%)	7 (8)
BMI (kg/m^2), median (IQR)	27.7 (25.9–31.1)
Donor specific antibodies (from 80 examined patients), n (%)	2 (3)
Vaccinated against SARS-CoV-2, n (%)	86 (93)
At least one booster dose, n (%)	61 (66)
Interval between last vaccination and COVID-19 disease (months), median (IQR)	9 (4–12)
Previous COVID-19 disease, n (%)	28 (30)
Immunosuppressive drugs in the patients	
Tacrolimus, n (%)	87 (94)
Cyclosporin A, n (%)	3 (3)
mTOR inhibitor, n (%)	3 (3)
Mycophenolate mofetil, n (%)	79 (85)
Prednisone, n (%)	92 (99)

IQR, interquartile range; COPD, chronic obstructive pulmonary disease; BMI, body mass index.

during follow-up included regular assessment of excretory kidney function and quantitative proteinuria. If a clinician suspected humoral rejection, the LUMINEX method was used to detect donor-specific antibodies, and in uncertain cases, a kidney biopsy was performed. Acute kidney injury was defined according to Kidney Disease: Improving Global Outcomes guidelines as an increase in serum creatinine to 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days [5]. The institutional ethics committee waived the requirement for informed consent from the patients because of the observational nature of the study.

RESULTS

A total of 93 patients (62 men; mean age: 56 years) were included in the study, 89% of whom had undergone their first kidney transplant. Patient characteristics are shown in **Table 1**. Continuous variables are presented as median and interquartile range (IQR). No patients were lost to follow-up.

A full course of vaccination against SARS-CoV-2 was completed by 86 patients (93%). Patients were vaccinated with the available vaccines. Among them, 53 patients received the COMIRNATY, 15 patients received the Moderna COVID-19 vaccine, and 18 patients received both vaccines. Most patients received three doses of vaccination (60 patients, 65%), 25 patients (27%) received two doses, and only one patient received a single dose. The interval between the last COVID-19 vaccine dose and disease onset was 9 (4–12) months. Only three patients did not have previous COVID-19 infection nor were vaccinated. One patient developed pneumonia and recovered, whereas the other two had favorable clinical outcomes. Immunoglobulin responses to vaccination are not routinely monitored. Details regarding vaccinations are shown in **Table 1**.

During the entire study period, the Omicron variant was the dominant type of SARS-CoV-2 virus strain in our region. From January 2022 to January 2023, subtypes BA.1, 2, and 5 were the most common. In February 2023, subtypes BQ.x, XBB.1.x, and BN.x were prevalent. From March to May 2023, XBB.1.x was the dominant subtype.

All patients completed the prescribed course of molnupiravir treatment. There was only one reported adverse event (pruritus), which was possibly related to molnupiravir treatment; however, molnupiravir was not discontinued.

Molnupiravir treatment was initiated 2 (1–3) days after the onset of COVID-19 symptoms. A runny nose and cough were the most frequent symptoms at presentation, with only three patients experiencing shortness of breath. Detailed information regarding the symptoms is presented in **Table 2**.

Immunosuppressive therapy was reduced in 87 (93.5%) patients. Mycophenolate was discontinued in 76 (82%) patients and reduced in 3 (3%) patients. The morning prednisone dose was increased to a median of 10 (10–15) mg, while 4 patients maintained a 5 mg dose. Immunosuppression was reduced for 11 (8–14) days. Immunosuppressive treatment was not reduced in 6 patients (6.5%).

The immunological risk in our patient population was generally low, with only three patients having donor-specific antibodies and two having known chronic humoral rejection prior to COVID-19 infection. A LUMINEX examination was performed on 46 (49%) patients during follow-up, and no new donor-specific antibodies were detected. Additionally, a kidney biopsy was indicated in three patients during follow-up and did not reveal any new cases of chronic or acute humoral rejection.

Overall, the clinical outcomes of patients with COVID-19 in our study have been good. We observed only one case of acute kidney injury, with recovery of kidney function occurring within 2 weeks. Hospitalization was required for three patients (3.2%), all due to pneumonia. The first patient, who was initially naive to COVID-19, recovered fully. The second patient had more severe symptoms and required artificial ventilation but eventually recovered. The third patient, who had acute kidney injury and graft failure, died. During the follow-up period, two patients died, and two patients required hemodialysis treatment due to causes not related to COVID-19. No episodes of acute rejection occurred during follow-up. By the end of the study, proteinuria and serum creatinine levels did not show a significant increase compared with the baseline values, as shown in **Table 3**.

DISCUSSION

The present study focused on the clinical outcomes of COVID-19 management using molnupiravir and a temporary reduction in

TABLE 2 | Common symptoms of COVID-19 at presentation.

Symptom	No. of patients (%)
Rhinitis	59 (63)
Cough	45 (48)
Fever	27 (29)
Sore throat	18 (19)
Subfebrile state	12 (13)
Fatigue	12 (13)
Muscle and joint pain	10 (11)
Headache	9 (10)

immunosuppressive treatment. The clinical outcomes were positive, with only 3.2% of the patients requiring hospitalization. This rate is notably lower compared with a similar small patient group with higher hospitalization rates [6–9]. A key factor that determines the severity of COVID-19 is the type of SARS-CoV-2 virus. According to the data from our hospital laboratory, which used sequencing for viral typing, Omicron was the most prevalent variant in our region during the study period.

One of the reasons for the favorable clinical outcomes in our study might be attributed to the early initiation of antiviral treatment with molnupiravir. However, a limitation of our study was the lack of a control group to establish the benefits of this treatment. Molnupiravir has been approved for emergency use because of its demonstrated efficacy in the general population. However, studies evaluating the efficacy of molnupiravir in immunosuppressed patients are limited and have produced mixed results [10, 11]. At the beginning of our study, remdesivir was reserved for severe cases of COVID-19, and molnupiravir was the only drug available for improving the outcomes of our non-hospitalized patients undergoing transplantation. In our study, owing to the effective education of our patients and timely reporting of symptoms, we were able to diagnose COVID-19 and initiate molnupiravir treatment for most patients within 2 days of symptom onset. According to the product characteristics, treatment should be initiated within 5 days, and clinical studies suggest that earlier initiation of treatment might be related to a lower hospitalization rate [12]. Consistent with other studies, molnupiravir was well tolerated and did not show evidence of nephrotoxicity [8]. Currently, the efficacy of molnupiravir remains unconvincing, its future use is questionable, and comparative studies to other available drugs, especially nirmatrelvir/ritonavir, are lacking. Moreover, there are concerns regarding the generation of potentially transmissible molnupiravir-mutated variants [13, 14].

Vaccination against COVID-19 has been established as the most effective tool for preventing severe disease, with proven efficacy in both clinical trials and real-world settings [15]. Patients undergoing kidney transplantation were prioritized for vaccination at our center, and we achieved high vaccination rates. However, these patients were at risk of having a weak response to the vaccines. Repeated vaccinations can increase protection against severe COVID-19 [16–18]. In our study, the high rate of vaccination might have contributed to the low rate of hospitalization, with only three patients without previous COVID infection and were not vaccinated. The patient who died had accumulated risk factors, including a laboratory-proven low response to the COVID-19 vaccination. A history of previous COVID-19 infection has been recognized as a protective factor against severe disease. This trend was also observed in patients undergoing kidney transplants [19]. In our study, 28 (30%) patients had a history of prior infection. The outcome in this subgroup was favorable (no hospitalization or acute kidney injury). Our study shows that despite COVID-19 vaccination and a history of previous infection, kidney transplant recipients might not be protected from SARS-CoV-2 infection; however, their symptoms are mild, and their clinical outcomes are excellent.

The reduction of immunosuppressive medication during severe infectious diseases is routine practice in kidney transplant recipients and this approach is also applied to severe COVID-19. In the case of mild COVID-19, modification to immunosuppressive therapy may not be necessary [3]. Non-adherence and self-management issues of patients undergoing kidney transplant were also described in our previous study, highlighting the need for careful management [20]. In clinical practice, to achieve optimal results, treatment should be initiated, and immunosuppression should be reduced as soon as the diagnosis of COVID-19 is confirmed, even when the severity of the disease is uncertain. At present, there are no detailed recommendations for the modification of immunosuppressive medications during COVID-19 infection, and the practice differs among transplant centers. Commonly implemented strategies include withholding mycophenolate mofetil and slightly increasing the prednisone dose, with some studies reporting a brief period of mycophenolate mofetil withholding (e.g., 5 days) [12]. In our study, the decision to resume full immunosuppressive therapy was driven by the clinical status of the patients, with therapy being reinstated only after a significant improvement in the clinical status or complete resolution of symptoms. This resulted in a period of reduced immunosuppressive therapy for a median duration of 11 days. This approach may have enhanced the ability of the patients to fight the infection, though it also increased the risk of rejection. The reported incidence of allograft rejection after COVID-19 is highly variable among studies, though generally low [9, 21]. In line with these reports, we did not observe any acute rejection, and kidney graft function was stable during follow-up. Compared with other studies, our follow-up period was considerably longer, which allowed for the detection of changes induced by immunosuppression reduction [21, 22]. We observed a low prevalence of donor-specific antibodies (only 3%) in our study, and LUMINEX examination performed in cases of suspected humoral rejection showed no *de novo* donor-specific antibodies during follow-up.

Acute kidney injury was observed in only two patients. Although published rates of acute kidney injury are typically higher, our findings are consistent with the overall mild COVID-19 symptoms observed in our cohort [23]. The first patient with acute kidney injury had mild symptoms, and regained kidney function within 2 weeks. The second patient experienced severe

TABLE 3 | Parameters of transplanted kidney function.

Parameter	At baseline	After COVID-19	End of follow-up period
Creatinine (μmol/L), median (IQR)	127 (101–153)	124 (106–159)	128 (101–161)
UPCR (g/mol), median (IQR)	12 (5–27)	12 (6–28)	9.2 (4.7–24)

UPCR, urine protein/creatinine ratio. No statistically significant differences was observed between the groups (Wilcoxon signed-rank test).

symptoms and subsequently lost graft function. Similar outcomes have been reported previously. Patients with mild disease and good graft function at baseline have a high chance of graft function recovery, while patients with severe disease symptoms and poorer baseline graft function, have a higher probability of incomplete graft function recovery [24, 25].

In conclusion, we observed favorable outcomes in our group of patients. Factors contributing to these outcomes include high rates of vaccination (including booster doses), frequent prior COVID-19 infection, early initiation of antiviral treatment with molnupiravir, and early and prolonged reduction of immunosuppressive treatment. Notably, the temporary reduction of immunosuppression did not adversely affect graft function, even during long-term follow-up. Therefore, early reduction of immunosuppression appears to be an effective strategy in the management of patients with COVID-19, including those with mild symptoms at presentation.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical approval was not required for the studies involving humans because The study was a retrospective analysis and involved already available data on human participants and followed the 1964 Declaration of Helsinki and its later amendments. 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 because The study was a retrospective analysis and involved already available data on human participants.

AUTHOR CONTRIBUTIONS

SD designed the study. SD, AP, MH, IG, PN, JP, and AM contributed to the data acquisition. PM, MM, and RS organized the database and performed the statistical analysis. RS wrote the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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LETTER TO THE EDITOR
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Preemptive Treatment of De Novo Donor Specific Anti-HLA Antibodies With IVIG Monotherapy after Lung Transplantation

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Dear Editors,

Development of *de novo* donor specific anti-HLA antibodies (dnDSA) following lung transplantation (LTX) is common and increases the risk for chronic lung allograft dysfunction (CLAD) and death [1–3]. Optimal management of asymptomatic dnDSA, in the absence of clinical antibody-mediated rejection or allograft dysfunction, is unclear. The approach varies among transplant centers. Some do not initiate specific therapy, while others pre-emptively treat with regimens that typically include high-dose intravenous immunoglobulin (IVIg) [4–6]. A recent retrospective multi-center report found improved CLAD-free survival among 30 LTX recipients with asymptomatic dnDSA who received preemptive therapy compared with 115 controls who did not receive antibody-targeted therapy or were only treated once signs of AMR developed. Various antibody reduction strategies were employed, including IVIg alone or in combination with rituximab, plasmapheresis, bortezomib, tocilizumab, and/or methylprednisolone [6].

IVIg has multiple immunomodulatory effects including neutralization of pathogenic IgG, inhibition of cytokine gene activation and activity, interaction with antigen-presenting cells to suppress T-cell activation, expansion of regulatory T-cells and inhibition of complement activity [7, 8]. We routinely administer pre-emptive high dose IVIg monotherapy for asymptomatic dnDSA in an attempt to reduce the strength of the antibody and improve long-term outcomes. The aim of this study is to review our experience with this approach.

Institutional review board approval was obtained. Data was collected from our prospective LTX registry. HLA antibody by Luminex single antigen bead assay was routinely obtained at post-op day 14, at 1-, 3-, 6-, 9-, 12- and 18-month post-transplant in conjunction with surveillance bronchoscopy with biopsy, yearly thereafter, and at any time for clinical indication. During the study period from 2/2013 and 3/2021, 230 lung transplants were performed. Sixty-three recipients (27%) developed dnDSA. We excluded 16 with a sum mean fluorescence intensity (MFI) < 3,000, 7 with clinical AMR at the time of first detection of dnDSA, 6 with non-HLA indication for IVIg and 2 re-transplants. The remaining 32 recipients with sum mean MFI ≥3,000 and no evidence of allograft dysfunction or AMR were treated with IVIg monotherapy at 2 gm/kg followed by 1 gm/kg monthly, for a minimum of 3 months or until clearance (sum MFI <1,000) up to 6 months. The cohort was predominantly

Abbreviations: ACR, acute cellular rejection; AMR, antibody-mediated rejection; CLAD, chronic lung allograft dysfunction; dnDSA, *de novo* donor specific anti-HLA antibodies; HLA, human leukocyte antigen; iDSA, immunodominant DSA; IQR, interquartile range; IVIg, intravenous immune globulin, MFI, mean fluorescence intensity.

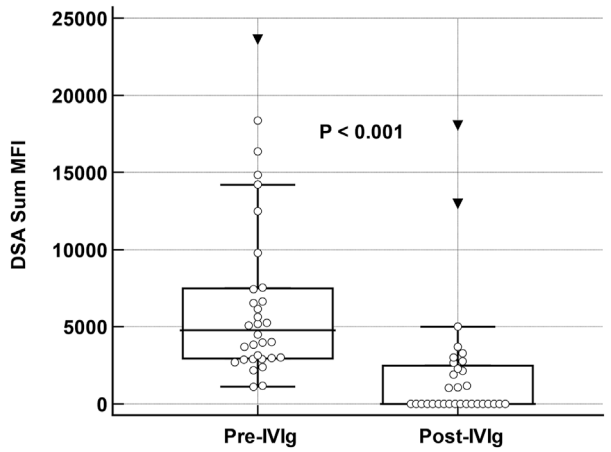

FIGURE 1 | Changes in DSA sum MFI pre and post Therapy with IVIg. Box and whisker plot demonstrating change in donor-specific antibody sum mean fluorescent intensity values at baseline and after intravenous-immune globulin monotherapy in 32 lung transplant recipients. Analysis was performed with Wilcoxon rank sum test for paired samples.

Caucasian (84%), bilateral LTX in 72%, 53% male with a median age of 62 (IQR:59, 67). Transplant indication was idiopathic pulmonary fibrosis in 15 (47%) and chronic obstructive pulmonary disease in 11 (34%). Median time to development of dnDSA was 33 days (IQR:18, 143). The immunodominant DSA was frequently class II (N = 26, 81%), most commonly DQ (N = 24, 75%). Median dnDSA sum MFI before treatment was 4782 (IQR: 2,937, 7,490) and decreased by a median of 2,993 (IQR: 2051, 6,358) after the initial course of IVIg; $P < 0.0001$. Eighteen (56%) achieved DSA clearance. (**Figure 1**) Among the 14 patients without clearing of DSA, IVIg was associated with a significant reduction in DSA strength (median sum MFI at baseline: 5,462 [IQR: 3,830, 9,770] vs. 2,714 [1899, 370] after treatment, $P = 0.017$). Additional IVIg was given to 8 patients with persistent DSA and 5 with recurrence after clearing with the first course. At last follow-up at a median of 1,330 days post-transplant (IQR: 861, 1910), clearance of DSA was present in 23 (72%).

During the observation period, CLAD had developed in 11 subjects and 10 died. CLAD-free survival at 2- and 4-year post-transplant were 79% and 62%, respectively. In comparison, CLAD-free survival in a concurrent cohort of 128 recipients with no DSA was 92% and 77% at 2- and 4-year, respectively ($P = 0.023$ by log-rank comparison of Kaplan-Meir survival curves). We observed a trend towards a lower incidence of subsequent acute cellular rejection (61% vs. 93%; $P = 0.05$) and AMR (6% vs. 36%; $P = 0.06$) in those who cleared DSA compared with those with persistent DSA after IVIg, but similar CLAD incidence and survival.

A large body of data has demonstrated a strong association between dnDSA, allograft rejection, and subsequent CLAD and mortality in lung transplant recipients. The association of stronger antibody (higher MFI) and complement fixing ability of dnDSA with worse outcomes and its detection prior to allograft dysfunction suggests that these antibodies may be involved in the pathogenesis of injury, rather than simply a marker of disease [1, 9]. In this series of asymptomatic dnDSA lung transplant recipients, we observed significant reductions in dnDSA MFI following preemptive IVIg monotherapy with clearing in over half.

In the absence of a control group, it is not possible to draw firm conclusions regarding the efficacy of this approach. Spontaneous clearing of dnDSA has been observed in up to one-third of cases [1, 10] and decreasing MFI in over half [9]. However, the definition of positive DSA in these reports was lower than our threshold MFI of 3,000. Clinical outcome, in terms of CLAD-free survival, of this cohort was comparable to other studies where additional measures were employed, such as plasma exchange and B-cell depleting therapy [4–6]. While CLAD-free survival was lower than our concurrent DSA-negative group, it was similar to the DSA-negative cohort of Keller et al (2-year CLAD-free survival of 70%) and markedly better than the no pre-emptive therapy DSA-positive group in that study (2-year CLAD-free survival of 42%) [6]. However, caution needs to be exercised in comparing these survival rates as our group was small and the populations different. Importantly, CLAD-free survival was similar among subjects who cleared DSA with pre-emptive therapy vs. those with persistent DSA in the multicenter study, similar to our observation. This could reflect a reduction in the strength of DSA and/or other immunomodulatory effects of treatment.

Taken together with the Keller study [6], our findings suggest that preemptive therapy for asymptomatic dnDSA may improve long-term outcomes in this high-risk group. The use of IVIg monotherapy appears to yield similar results to those of combination strategies that are more cumbersome and associated with greater potential for complications. However, prospective, randomized controlled trials are required to definitively assess the efficacy of preemptive therapy and the optimal regimen.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by the Corewell Health Institutional Review Board. 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

JM, SC, and RG developed the study concept and design, collected data, performed data analysis, drafted and finalized the manuscript. All authors contributed to the article and approved the submitted version.

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