Transplant International

Treating split livers with HOPE

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Original Research

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EPITA Symposium, 26-29 January
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Transplant Trial Watch

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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

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RANDOMISED CONTROLLED TRIAL 1

Randomized Trial of Routine Versus On-Demand Intraoperative Extracorporeal Membrane Oxygenation in Lung Transplantation: a Feasibility Study.

by Nasir, B., et al. Journal of Heart and Lung Transplantation 2024 [record in progress].

OPEN ACCESS

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Aims
Assess the feasibility of undertaking a multicentre RCT to compare two strategies of intraoperative mechanical circulatory support (routine ECMO versus on-demand ECMO) during lung transplantation.

Interventions
Standard of care being routine ECMO versus the intervention of on-demand ECMO utilised when required during transplantation.

Participants
28 adult, lung only, primary transplant recipients where cardiopulmonary support was not mandatory were randomised.

Outcomes
The outcome measures were death, primary graft dysfunction (PGD), bleeding, cannulation site complications, and hypoperfusion-related complications (e.g., AKI, stroke, mesenteric ischemia).

Follow-Up
30 days

CET Conclusion
by John Fallon

This is a small, randomised feasibility study conducted in a single Canadian lung transplant centre with the aim of designing a large multicentre RCT to definitively assess the use of routine versus on-demand ECMO during lung only transplantation. They perform a sensible power calculation based on the Blackwelder method and discussion across all Canadian lung centres with regards historic data and possible effect sizes, giving a needed trial size of 310 patients in each arm. They apply this to collected data on local and national transplant numbers to assess are reasonable study period and recruitment window. Based on their contribution to national transplant number they go on to generate an aim within their centre during a 6-month feasibility recruitment period. They determine their trial would likely be feasible and at low risk of failure if they randomised 19 participants with fewer than 5% loss-to-follow up and less than 10% protocol violations within the 6 months. During the feasibility study period they successfully randomise 28 patients. While the numbers are insufficient to comment on the two interventions, they demonstrate that over the proposed 3-year study period with all 4 Canadian lung transplant centres it is highly likely the trial could be achieved, and a definitive answer found. This is a commendable feasibility study, with complex interventions with potentially small effect sizes, it is crucial that should one embark on the cost, effort, and patient recruitment for such trials that the risk of failure is minimised as far as possible. Strategies such as a well-thought-out simple feasibility studies are key to larger trial successes.

Jadad Score
3.

Data Analysis
Strict intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
ClinicalTrials.gov—NCT05505422.

Funding Source
No funding received.

RANDOMISED CONTROLLED TRIAL 2

Decreased Mycophenolate Mofetil Hampers Antibody Responses to a Broad Range of Vaccinations in Kidney Transplant Recipients: Results From a Randomized Controlled Study.

by Fatly, Z. A., et al. Journal of Infection 2024; 88(3): 106133.

Aims
This study aimed to investigate whether discontinuing mycophenolate mofetil (MMF) 3 months prior to vaccination would improve vaccination responses in renal transplant recipients using tacrolimus.

Interventions
Participants were randomised to either tacrolimus monotherapy (TACmono) or to tacrolimus with MMF (TAC/MMF).

Participants
79 kidney transplant recipients.

Outcomes
The main outcomes of interest were responses to pneumococcal, tetanus and influenza vaccination; relation between pneumococcal, tetanus, and influenza vaccination responses; clinical differences in vaccination responders versus non-responders; correlation between SARS-CoV-2, pneumococcal, and tetanus vaccination responses; and effect of Co-administering of influenza vaccines on pneumococcal and tetanus serological vaccination responses.

Follow-Up
21 days post-vaccination.

CET Conclusion
by John O'Callaghan
This is a very interesting paper following on from a randomised controlled trial that has been previously published. In the initial trial kidney transplant recipients were randomised to continue on tacrolimus monotherapy instead of a tacrolimus and mycophenolate combination (de Weerd et al. Transpl Int. 2022 October 24; 35:10839). In the present paper these two cohorts were monitored for their serological responses to key vaccinations: pneumococcus, tetanus, influenza. The results show a very significant difference in the vaccine responses when assessing each vaccine individually, with tacrolimus monotherapy being beneficial. In addition, only 7% responded adequately to all of pneumococcus, tetanus and influenza vaccines whilst on tacrolimus and mycophenolate monotherapy. In this group 40% responded inadequately to all 3 of these vaccinations. In contrast, 100% of those on tacrolimus monotherapy responded to at least one of the vaccines. No significant differences were seen in the clinical outcome of responders versus non-responders, but at this level of analysis the study becomes too small for the outcomes being assessed (patient survival, infection-related death and antibiotic use. In addition a small number of those in the study received the sars-cov2 vaccine when it became available. Sars-cov2 antibody levels were significantly lower following vaccination in the tacrolimus and mycophenolate group compared to the tacrolimus monotherapy group. The inhibition of both B and T-cell responses by mycophenolate hampers the body's response to vaccination and the effect is clearly shown by this study. However, in this

study, the response was only moderately dose dependent, so reducing mycophenolate dosing does not help significantly with vaccine responses, compared to stopping the drug 3 months prior to vaccinations. If reducing immune suppression is not possible then this study highlights the importance of vaccination prior to transplantation.

Trial Registration

EudraCT nr.: 2014-001372-66.

Funding Source

Non-industry funded.

CLINICAL IMPACT SUMMARY

by Simon Knight
The COVID-19 pandemic served as a reminder not just of the susceptibility of immunosuppressed patients to infection, but also to their reduced ability to show serological response to vaccination or infection. The large, UK-based Melody study included nearly 10,000 solid organ transplant recipients, and demonstrated that patients on steroid or mycophenolate mofetil (MMF) therapy were far less likely to develop an antibody response to SARS-CoV-2 [1]. Those on triple immunosuppression (antiproliferative, calcineurin inhibitor (CNI) and steroids) were significantly less likely to respond than those receiving dual or monotherapy, suggesting that it is overall immunosuppression burden that is important, rather than specific agents.

The potential benefits of immunosuppression minimisation have been well studied, largely focussing on either the metabolic benefits of steroid withdrawal, or the reduction in renal injury, infection and malignancy risk with CNI minimisation [2, 3]. Immunosuppression minimisation may have the additional benefit of improving vaccination responses in vulnerable patients.

In a recent pilot study, researchers from Erasmus Medical Centre in the Netherlands investigated the ability to withdraw MMF in low immunological-risk recipients by 9 months following renal

transplant [4]. A pre-planned sub-study investigated responses to the pneumococcal, tetanus and influenza vaccines at 12-month post-transplant [5]. Serological vaccination response was measured for all three vaccinations. Adequate serological responses were seen in 74%, 82%, and 71% of tacrolimus monotherapy patients for the pneumococcal, tetanus and influenza vaccines respectively, in comparison to 43%, 35%, and 20% patients remaining on dual therapy with MMF.

These results suggest that the ability to respond to vaccination is significantly improved within 3-months of MMF withdrawal, an effect that spans different vaccine types. It highlights the importance of vaccination prior to transplant where possible and provides more ammunition for consideration of immunosuppression minimisation in lower-risk transplant recipients. The study is too small to demonstrate whether improved vaccine response translates to measurable clinical benefit, but nonetheless provides further evidence of the importance of immunosuppressive load on vaccine responses.

Clinical Impact

3/5.

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.

ACKNOWLEDGMENTS

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HOPE Mitigates Ischemia-Reperfusion Injury in Ex-Situ Split Grafts: A Comparative Study With Living Donation in Pediatric Liver Transplantation

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Optimizing graft preservation is key for ex-situ split grafts in pediatric liver transplantation (PSLT). Hypothermic Oxygenated Perfusion (HOPE) improves ischemia-reperfusion injury (IRI) and post-operative outcomes in adult LT. This study compares the use of HOPE in ex-situ partial grafts to static cold storage ex-situ partial grafts (SCS-Split) and to the gold standard living donor liver transplantation (LDLT). All consecutive HOPE-Split, SCS-Split and LDLT performed between 2018–2023 for pediatric recipients were included. Post-reperfusion syndrome (PRS, drop ≥30% in systolic arterial pressure) and reperfusion biopsies served as early indicators of IRI. We included 47 pediatric recipients (15 HOPE-Split, 17 SCS-Split, and 15 LDLT). In comparison to SCS-Split, HOPE-Split had a significantly shorter cold ischemia time (CIT) (470min vs. 538 min; p =0.02), lower PRS rates (13.3% vs. 47.1%; p = 0.04) and a lower IRI score (3 vs. 4; p = 0.03). The overall IRI score (3 vs. 3; p = 0.28) and PRS (13.3% vs. 13.3%; p = 1) after HOPE-Split were comparable to LDLT, despite a longer CIT (470 min vs. 117 min; p < 0.001). Surgical complications, one-year graft, and recipient survival did not differ among the groups. In conclusion, HOPE-Split mitigates early IRI in pediatric recipients in comparison to SCS-Split, approaching the gold standard of LDLT.

Keywords: machine perfusion, organ preservation, split liver transplantation, pediatric liver transplantation, ischemia-reperfusion injury

GRAPHICAL ABSTRACT |

INTRODUCTION

Living donor liver transplantation (LDLT) provides the best achievable outcomes for pediatric recipients [1, 2]. In addition to optimal donor selection, LDLT grafts have a short static cold ischemia time (CIT) resulting in less ischemia-reperfusion injury (IRI) and improved post-LT outcomes [3, 4]. Nevertheless, in France, LDLT accounts for only 12% of all pediatric liver transplantations (PLT) and the majority of PLT are performed with ex-situ split grafts from deceased donors (PSLT) [5]. Pediatric prioritization and strict donor selection have enabled PSLT from deceased donors to yield excellent outcomes although they have not yet reached the benchmarks set by living donation in terms of graft and patient survival. One of the main independent risk factors for early graft loss in PSLT is CIT [6, 7]. One strategy to improve preservation is the use of hypothermic oxygenated perfusion (HOPE), especially in case of ex-situ split procedures. As shown in adult LT, application of HOPE is associated with reduced rates of post-reperfusion syndrome (PRS) [8], histological IRI [9] and improved post-LT outcomes [8, 10, 11]. PRS is also a major determinant of graft survival in PLT with a reported incidence up to 34%. Therefore, PLT may benefit from the implementation of HOPE to mitigate PRS an IRI [12–14]. While the safety and feasibility of HOPE in PSLT have already been established, there is currently no data on the impact of HOPE on early IRI indicators available [9, 15, 16]. Thus,

this study will focus on the impact of HOPE on IRI in ex-situ split grafts for pediatric recipients in comparison to the gold standard LDLT.

METHOD

Study Design

This retrospective study focuses on PSLT and aims at investigating the impact of HOPE on ex-situ split grafts from deceased donors (HOPE-Split) in comparison to the gold standard LDLT and ex-situ grafts splitted during SCS (SCS-Split).

We included all PSLT performed prospectively from 2018 to 2023 with at least 6 months follow-up, including LDLT, SCS-Split and HOPE-Split procedures (**Supplementary Figure S1**). Of note, 5 *in-situ* splits were performed at our center during the study period and were excluded due to small sample size.

Graft selection for deceased donor was based on current data [17] relying on donor age (<45 years), body mass index (<25 kg/m^2), intensive care unit stay (<7 days), cardiac arrest and donor biology.

The implementation of HOPE in the pediatric setting followed the IDEAL recommendations for surgical innovation [18]. The safety and benefit of HOPE has been established in adult LT [11, 19] allowing for its application in pediatric LT. The safety of HOPE-Split has been previously assessed in case series and the surgical technique has been refined through Stage I and IIa studies [9, 16]. To further

investigate this strategy and expand its indications (Stage IIb), this study focused on SPLT, aiming to compared HOPE-Split to the gold standard LDLT, and was approved by the local ethics committee (CSEHCL_21_202).

HOPE Split Procedure

The procedure for liver graft splitting during HOPE has been previously standardized and reported [16]. The first step of the Split procedure was performed during static cold storage. It consisted in the pedicular dissection aiming at identifying the portal vein and the hepatic artery division. The portal vein was not divided allowing for the perfusion of both partial grafts with a single cannula. A cholangiography was performed to assess biliary anatomy prior to parenchymal transection. The second step, namely parenchymal transection, was performed during HOPE. Both grafts were perfused at a pressure of a maximum of 5 mmHg with a portal flow ranging from 200 to 300 mL/min.

Since 2022, in line with the findings from *Ravaioli et al.* in adult LT [8], HOPE was initiated at the beginning of the back table preparation [20].

Endpoints

We specifically investigate the impact of HOPE on surrogate markers of early IRI in pediatric recipients, namely post-reperfusion syndrome (PRS) and histological ischemia-reperfusion injuries. PRS in pediatric recipients was defined according to *Zhang et al.* as a drop of systolic arterial pressure (SAP) of more than 30% within the first 5 min following reperfusion [13]. To refine PRS assessment, increase of norepinephrine (NE), the use of other vasoactive drugs such as adrenaline, median post-reperfusion SAP or mean arterial pressure (MAP) and acute kidney injury requiring dialysis (AKI) were also evaluated. IRI based on reperfusion biopsy were assessed as previously described [9]. A blinded reading by one experienced pathologist was performed and histological IRI was ranked as grade 0 for absence of IRI, grade 1 for minimal IRI, grade 2 for mild IRI, grade 3 for moderate IRI and grade 4 for severe IRI. A histological IRI $\geq$ grade 3 (moderate to severe) was considered as a high-grade injury. Overall IRI score based on each compartment evaluation (Neutrophilic infiltrate, necrosis, congestion) was calculated.

To assess the impact of HOPE on graft preservation we evaluated CIT and total preservation time. CIT was defined by static cold storage duration from *in situ* cold flush in the donor to either the beginning of HOPE or the implantation of the graft.

In addition, early graft function was assessed and graded according to the Olthoff criteria (Early Allograft Dysfunction [EAD]) [21] and to the LGraft7 score [22]. 1 year graft and patient survival were assessed as well as overall morbidity using the Comprehensive Complication Index (CCI©) [23] and the Clavien-Dindo classification [24].

Statistical Analysis

Categorical variables were expressed in quantities and percentages while continuous variables were expressed as median with interquartile range (IQR). Continuous variables were compared using the Kruskal-Wallis with *post hoc* Dunn's test to compare the 3 study groups or with the Mann Whitney test. Categorical variables were compared using the chi-square test or the Fisher's exact test. Kaplan Meier curves with a log rank test were used to compare graft and patient survival.

p-values <0.05 were considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics for Windows (Version 26.0. Armonk, NY: IBM Corp) and GraphPad Prism (Version 8.0.0 for Windows, GraphPad Software, San Diego, California United States).

RESULTS

Study Cohort

Donor characteristics were similar between the HOPE-Split and SCS-Split group (**Table 1**), with a median age of 20 years, a median BMI of 21.6 kg/m². The main donor cause of death was traumatic (59%), and 18.7% of donors had a cardiac arrest prior to graft procurement.

Living donors were mostly the father of the recipient (66.7%), with a median age of 36 years and a median BMI of 23.1 kg/m².

As shown in **Table 1**, recipient characteristics regarding median age, weight and PELD (Pediatric End Stage Liver Disease) were comparable between groups. The main cause for PSLT was biliary atresia (53%) with significantly more retransplantations in the HOPE-Split group (28.5%; *p* = 0.03). Recipients in both HOPE-Split and SCS-Split presented with a trend toward higher rate of acute liver failure (14.2% and 17.6% vs. 0% respectively; *p* = 0.24) and high urgency listing (46.7% and 58.8% vs. 20% respectively; *p* = 0.08) compared to LDLT recipients.

Ischemia Reperfusion Injury
Graft Preservation

In the HOPE-Split group, HOPE was performed for a median time of 100 min with a significant shorter CIT in comparison to SCS-Split (470 min vs. 538 min; *p* = 0.01). Total ex-vivo preservation time was not significantly different between the two groups (568 min vs. 538 min; *p* = 0.36).

Overall, the LDLT group presented with the shortest CIT compared to both HOPE-Split and SCS-Split groups (117 min; *p* < 0.001) (**Figure 1**).

Post-Reperfusion Syndrome

The HOPE-Split group showed a significant reduction of PRS compared to the SCS-Split group (13.3% vs. 47.1%; *p* = 0.04) with significantly less additional post-reperfusion vasoactive drugs (13.3% vs. 52.9%; *p* = 0.02) (**Figure 1**). No difference was observed regarding post-LT AKI (13.3% vs. 11.8%; *p* = 0.89).

In comparison to LDLT, the PRS rate (13.3% vs. 13.3%; *p* = 1), NE increase (26.7% vs. 14.3%; *p* = 0.41) and the use of other vasoactive drugs (13.3% vs. 6.7%; *p* = 0.54) were not significantly different in the HOPE-Split group.

TABLE 1 | Donor-recipients characteristics, surgical data and post-operative outcomes.

	LDLT *n* = 15	HOPE-Split *n* = 15	SCS-Split *n* = 17	HOPE-Split vs SCS-Split p value	HOPE-Split vs LDLT p value
Donor Characteristics					
Age (y)	36 [30–38]	21 [17–28]	20 [18–30]	0.91	<0.001
Sex (M)	66.7 (10)	73.3 (11)	41.2 (7)	0.07	0.69
BMI (kg/m^2)	23.1 [20.4–26]	18.9 [17.4–23.5]	22.5 [20.1–22.9]	0.71	0.10
COD					
Traumatic	-	66.7 (10)	52.9 (9)	0.43	-
Hypoxic Brain Injury	-	6.7 (1)	29.4 (5)	0.18	-
CerebroVascular	-	26.7 (4)	17.6 (3)	0.54	-
Cardiac Arrest	-	13.3 (2)	23.5 (4)	0.46	-
Recipient Characteristics					
Age (months)	17 [9.5–56.5]	43 [19.5–51]	21 [13–38]	0.17	0.39
Sex (M)	33.3 (5)	26.7 (4)	41.2 (7)	0.38	0.69
Weight (kg)	10.5 [7.5–16]	15 [10–17]	10 [8.5–14]	0.14	0.46
PELD	16 [9–21]	19 [16–21]	23 [15–29]	0.35	0.15
BA	60 (9)	57.1 (8)	47 (8)	0.46	0.71
Tumor	20 (3)	0 (0)	5.8 (1)	0.34	0.68
Urgency	20 (3)	46.7 (7)	58.8 (10)	0.49	0.12
ALF	0 (0)	14.2 (2)	17.6 (3)	0.74	0.14
reLT	6.7 (1)	28.5 (4)	0 (0)	0.02	0.14
Liver Transplantation					
Preservation Time (min)	117 [99–139]	568 [525–608]	538 [514–567]	0.19	<0.001
CIT (min)	117 [99–139]	470 [376–505]	538 [514–567]	<0.001	<0.001
WIT (min)	32 [29–36]	36 [34–39]	33 [31–37]	0.04	0.03
Transfusion (mL/kg)	24 [18–37]	35 [23–47]	47 [27–69]	0.24	0.17
GRWR (%)	2.2 [1.5–3.2]	2.4 [1.8–2.6]	2.8 [2.4–3.2]	0.11	0.95
Post-operative Outcomes					
EAD	20 (3)	66.7 (10)	70.6 (12)	0.81	0.01
LGraft7[a]	−3.31 [-3.69;-2.02]	−3.05 [-4.17;-2.25]	−3.25 [-4.26;-2.13]	1	0.74
Risk LGraft7[b]	3.5 [2.4–11.7]	6.6 [1.5–10.3]	3.7 [1.4–10.6]	0.78	0.98
PNF	0 (0)	6.6 (1)	5.8 (1)	0.93	0.31
PRS	13.3 (2)	13.3 (2)	47 (8)	0.04	1
AKI	0 (0)	13.3 (2)	11.8 (2)	0.89	0.14
Early Laparotomy	46.7 (7)	40 (6)	35.3 (6)	0.78	0.71
Biliary Complications	40 (6)	33.3 (5)	35.3 (6)	0.91	0.71
Anastomotic stricture	40 (6)	20 (3)	17.6 (3)	0.86	0.23
Non anastomotic stricture	0 (0)	13.3 (2)	17.6 (3)	0.73	0.14
Arterial Complications	0 (0)	20 (3)	11.8 (2)	0.52	0.07
Stenosis	0 (0)	13.3 (2)	0 (0)	0.12	0.14
Thrombosis	0 (0)	0 (0)	11.8 (2)	0.17	-
Pseudoaneurysm	0 (0)	6.7 (1)	0 (0)	0.28	0.31
CCI 3 months	53 [39–79]	68 [41–99]	71 [46–90]	0.71	0.37
CCI 12 months	71 [44–86]	94 [44–99]	92 [63–99]	1	0.27
Graft Survival (3 m)	100 (15)	86.7 (13)	94.1 (16)	0.47	0.14
Patient survival (3 m)	100 (15)	86.7 (13)	94.1 (16)	0.47	0.14
Graft survival (1 year)	100 (15)	86.7 (13)	88.2 (15)	0.89	0.14
Patient survival (1 year)	100 (15)	86.7 (13)	94.1 (16)	0.47	0.14

Values are expressed as % (n) or median [interquartile range].

(BMI: body mass index, COD: cause of death, BA: biliary atresia; ALF: acute liver failure, reLT: retransplantation, PELD: Pediatric End-Stage Liver Disease, CIT: cold ischemia time, WIT: warm ischemia time, GRWR: graft over recipient weight ratio, EAD: early allograft dysfunction, PNF: primary non function, PRS: post reperfusion syndrome, AKI: acute kindey injury requiring dialysis, CCI: Comprehensive Complication Index).

[a]*Continuous variables were compared using the Mann Whitney test. Categorical variables were compared using the Fisher's exact test.*

[b]*LGRAFT, score was presented (negative value) as well as the risk of graft loss (percentage).*

Reperfusion Biopsy

The HOPE-Split group exhibited a trend toward less high-grade IRI (moderate to severe, grade ≥3; 26.7% vs. 47.1%; *p* = 0.23) and a significantly lower neutrophilic infiltrate (6.7% vs. 41.2%; *p* = 0.02) with a significantly lower overall IRI score (3 [2–5] vs. 4 [4–7]; *p* = 0.03) compared to SCS-Split (**Figure 1**).

In comparison to LDLT, HOPE-Split exhibited a trend toward more histological high-grade IRI (26.7% vs. 13.3%; *p* = 0.36)
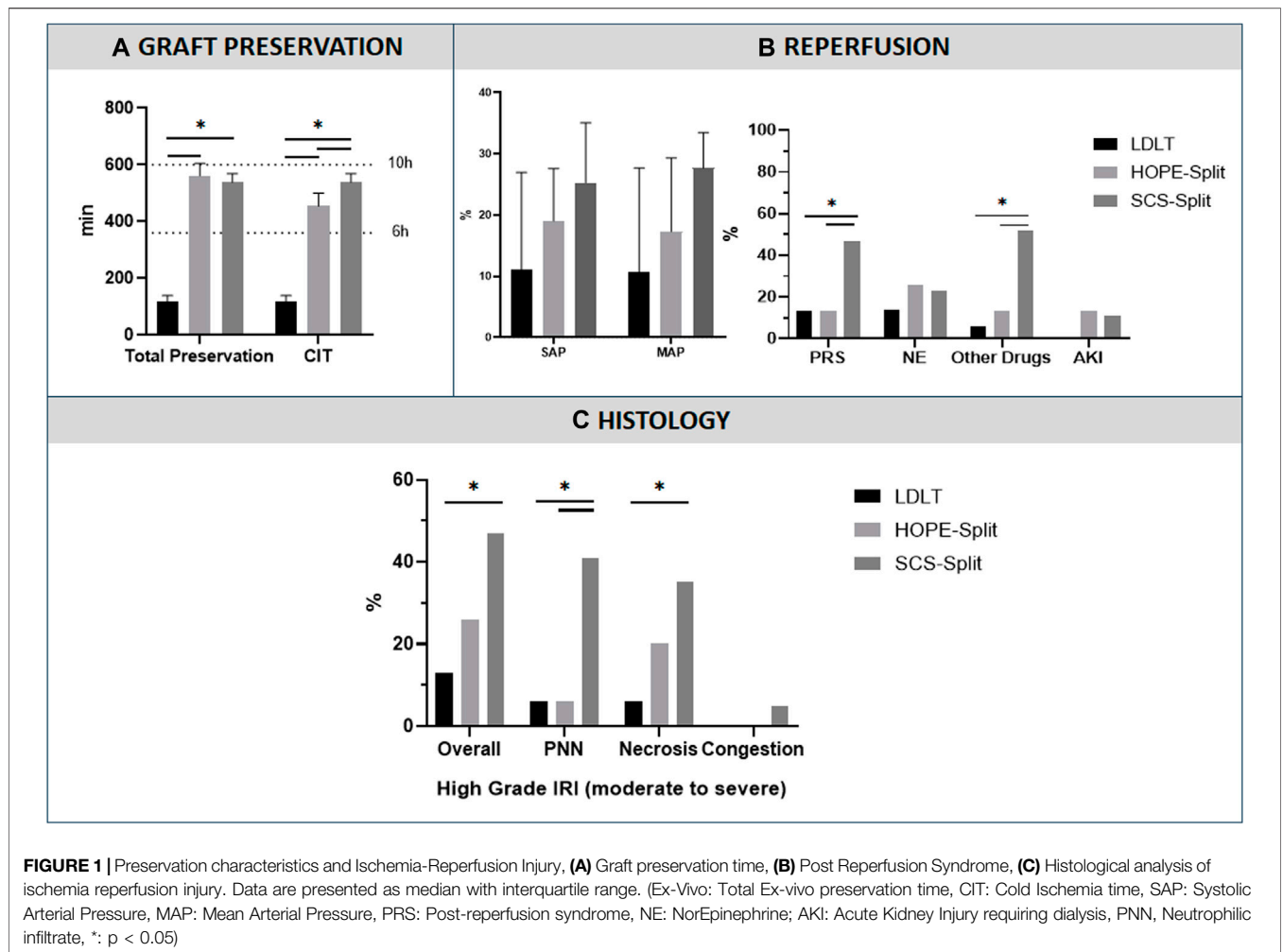
FIGURE 1 | Preservation characteristics and Ischemia-Reperfusion Injury, **(A)** Graft preservation time, **(B)** Post Reperfusion Syndrome, **(C)** Histological analysis of ischemia reperfusion injury. Data are presented as median with interquartile range. (Ex-Vivo: Total Ex-vivo preservation time, CIT: Cold Ischemia time, SAP: Systolic Arterial Pressure, MAP: Mean Arterial Pressure, PRS: Post-reperfusion syndrome, NE: NorEpinephrine; AKI: Acute Kidney Injury requiring dialysis, PNN, Neutrophilic infiltrate, *: p < 0.05)

without significant difference regarding the overall injury score (3 [2–5] vs. 3 [2-3]; $p = 0.28$).

Early Post-Operative Outcomes

The HOPE-Split group exhibited significantly less ALT release during the first four post-operative days (**Figure 2**) with a trend toward lower AST and ALT peak (523 UI/L/100 g vs. 909 UI/L/100 g; $p = 0.30$ and 303 UI/L/100 g vs. 440 UI/L/100 g; $p = 0.19$) compared to SCS-Split.

In comparison to LDLT, the HOPE-Split group exhibited a significant higher AST peak (523 UI/L/100 g vs. 244 UI/L/100 g; $p = 0.01$) and ALT peak (303 UI/L/100 g vs. 205 UI/L/100 g; $p = 0.25$) resulting in a significant higher rate of EAD (66.7% vs. 20%; $p = 0.007$).

Factor V normalization was similar between HOPE, SCS and LD (**Figure 2**).

The HOPE-Split group exhibited similar rates of early laparotomy (40%; $p = 0.81$), biliary complications (33.3%; $p = 0.92$) and arterial complications (20%; $p = 0.21$) compared to both LDLT and SCS-Split (**Table 1**).

One year graft and patient survival were 86.7% ($n = 13/15$) in the HOPE-Split group without statistically significant differences

compared to both LDLT and SCS-Split (**Table 1**; **Supplementary Figure S1**).

DISCUSSION

This is the first study to investigate the impact of HOPE on early IRI in PSLT by a direct comparison with the gold standard LDLT and standard ex-situ split grafts. We were able to show that HOPE-Split significantly reduced PRS and IRI on reperfusion biopsy in comparison to SCS-Split, resulting in comparable IRI profiles to LDLT.

Graft preservation remains a key challenge in PSLT as CIT has been shown to be an independent risk factor for graft loss [6]. In addition, *Lauterio et al.* [7] recently showed that CIT >6 h and >10 h were associated with graft failure in a cohort of in-situ PSLT. Besides, CIT is related to PRS [12] and IRI which are known risk factors for graft loss [13]. HOPE has been shown to improve graft preservation by actively oxygenating the graft associated with shorter CIT [8], translating into decreased PRS [10, 25, 26], decreased EAD, ischemic type biliary complications

FIGURE 2 | Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Factor V during the first post-operative days. Data are expressed as median and interquartile range. POD, Post-operative days; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
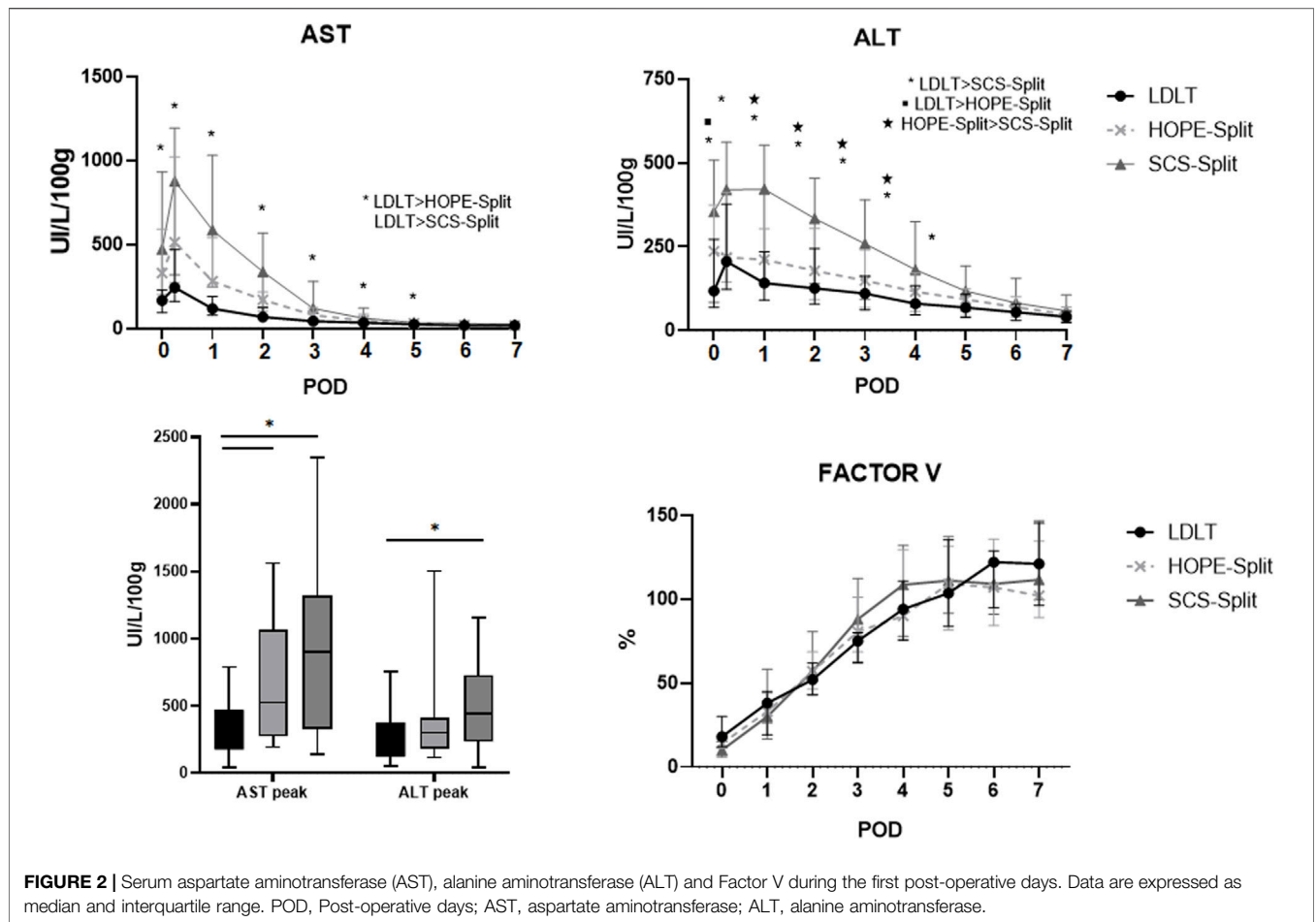
and graft loss [11] in adult LT. In our institution, we therefore implemented HOPE for ex-situ split liver grafts since 2020 aiming at improving graft preservation in PSLT. In the present study we compared for the first time HOPE-Split to LDLT gold standard and to SCS-Split to evaluate the impact of HOPE on early ischemia-reperfusion events, namely, PRS and IRI injury on reperfusion biopsy.

First, the HOPE-Split group exhibited lower rates of PRS, as well as improved hemodynamic stability upon reperfusion compared to SCS-Split. This observation is in line with previous data from adult split transplantation using HOPE [10, 26]. In addition, we observed a lower grade of histological IRI and less neutrophilic infiltrate in the HOPE-Split group. This allowed the HOPE-Split grafts to approach outcomes with LDLT regarding early IRI without statistically significant differences in PRS and IRI on reperfusion biopsy. These clinical observations are supported by experimental data showing a reduction of mitochondrial damage with HOPE which translates into a reduction of the hepatic inflammasome [27, 28]. Indeed, HOPE replaces cold ischemia by an active oxygenation of the graft during preservation thus improving mitochondrial function, uploading the ATP cellular pool [28, 29] and mitigating IRI [10, 25, 26]. Applying HOPE during ex-situ liver splitting thus

combines the benefit of shorter CIT, inherent to this strategy, to mitochondrial metabolism recovery.

Second, all PSLT groups showed a 1-year graft survival rate of >85% which is comparable to the data from the ELTR registry and the UNOS data base [3, 4]. Improved preservation characteristics did not result in a decrease in overall morbidity or mortality in our study. Additionally, meaningful statistical adjustments for recipient risk factors were not possible due to the small sample size. Nevertheless, early IRI events such as PRS [12, 13] and IRI on reperfusion biopsy [30] have been shown to significantly impact long-term post-LT outcomes in larger cohorts, including LDLT [12] and serve as early surrogates of graft quality.

Altogether, these data suggest that HOPE-Split could mitigate left partial liver graft IRI similar to the impact of HOPE in whole liver transplantation [8, 31]. The presented results demonstrate that HOPE, by replacing CIT during ex-situ liver splitting, may be a promising strategy to expand donor selection criteria especially for split liver grafts [14]. Besides, performing back-table preparation during active perfusion can further improve graft preservation allowing for a CIT <6 h, similar to in-situ split grafts [8, 20], which may facilitate logistics. Graft evaluation [32] and specific scenarios that might benefit the most from HOPE still need to be explored to

safely increase the donor pool for pediatric recipients through tailored preservation strategies [14]. In addition to PLT, HOPE may also facilitate the access to partial grafts for adult recipients with oncological indications in the context of the RAPID procedure (Resection And Partial Liver Segment 2/3 Transplantation with Delayed Total Hepatectomy) [33].

Our study has some limitations inherent to its retrospective design. A small sample size and a focus on short-term follow-up do not allow to draw robust conclusion regarding the potential benefit of HOPE on long-term clinical outcomes. According to the IDEAL framework for surgical innovation [18], larger scale prospective trials (Stage III) are mandatory to provide robust data on the independent effect of HOPE in PLT. This will soon be assessed in a multicenter national prospective randomized trial (HOPE-Split) supported from the French Ministry of Health through a grant from the National Hospital Clinical Research Program. Regarding PRS, there exist several definitions in the literature and preoperative management may differ from center to center [13]. However, in this single center study, there was a protocolized standard of care for PRS management in all recipients included.

In conclusion, HOPE-Split allows to reduce PRS rates and histological IRI in comparison to SCS-Split, resulting in early IRI profiles comparable to LDLT. Improving early IRI with HOPE in PSLT could benefit high-risk donor-recipient scenarios and allow expanding selection criteria for ex-situ split grafts. Future multicenter trials should now evaluate long-term outcomes of HOPE-Split in larger cohorts and identify specific situation that might benefit the most from dynamic preservation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article are not publicly available but will be made available by the corresponding author upon reasonable request.

ETHICS STATEMENT

The studies involving humans were approved by the Comité Scientifique et Ethique des Hospices Civils de Lyon. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for

participation in this study was provided by the participants' legal guardians/next of kin.

AUTHOR CONTRIBUTIONS

GR and XM: contributed equally as first authors, designed the study, acquired the data, performed the statistical analysis, interpreted the data and wrote the manuscript. SC-F performed the histological analysis and critically reviewed the data. J-YM, KM, MR, TA, CD, NB, and RD: designed the study, interpreted the data, critically reviewed the data and drafted a final version of the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.12686/full#supplementary-material

SUPPLEMENTARY FIGURE S1 | Study flow chart.

SUPPLEMENTARY FIGURE S2 | One year graft and patient survival.

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Prevention and Rehabilitation After Heart Transplantation: A Clinical Consensus Statement of the European Association of Preventive Cardiology, Heart Failure Association of the ESC, and the European Cardio Thoracic Transplant Association, a Section of ESOT

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Little is known either about either physical activity patterns, or other lifestyle-related prevention measures in heart transplantation (HTx) recipients. The history of HTx started more than 50 years ago but there are still no guidelines or position papers highlighting the features of prevention and rehabilitation after HTx. The aims of this scientific statement are (i) to explain the importance of prevention and rehabilitation after HTx, and (ii) to promote the factors (modifiable/non-modifiable) that should be addressed after HTx to improve patients' physical capacity, quality of life and survival. All HTx team members have their role to play in the care of these patients and multidisciplinary prevention and rehabilitation programmes designed for transplant recipients. HTx recipients are clearly not healthy disease-free subjects yet they also significantly differ from heart failure patients or those who are supported with mechanical circulatory support. Therefore, prevention and rehabilitation after HTx both need to be specifically tailored to this patient population and be multidisciplinary in nature. Prevention and rehabilitation programmes should be initiated early after HTx and continued during the entire post-transplant journey. This clinical consensus statement focuses on the importance and the characteristics of prevention and rehabilitation designed for HTx recipients.

Keywords: diabetes, dyslipidaemia, exercise training, heart failure, heart transplantation

INTRODUCTION

Heart transplantation (HTx) continues to be the most optimal therapeutic option for selected patients with end-stage heart disease of varying aetiologies, with more than 6,000 heart transplants performed annually worldwide [1].

The history of HTx started more than 50 years ago but only recently European guidelines and position papers have recognized the importance of prevention (including medication, nutrition, and exercise prescription) in cardiovascular disease. However, optimal prevention in HTx recipients remains to be formulated [2, 3].

The aims of this clinical consensus statement are (i) to point out the importance and content of prevention and rehabilitation after HTx, and (ii) to promote the (non-)modifiable risk factors that should be addressed after HTx to improve patients' capabilities and functional capacity, quality of life and survival.

FEATURES OF THE HEART TRANSPLANT POPULATION

Little is known about the physical activity (PA) patterns, among other prevention measures, among patients after HTx. Firstly, HTx recipients face total denervation of the transplanted heart, with a significant impact on their response to exercise and daily-life activities [1]. Secondly, life-long immunosuppression leads to a recip-ients' immunocompromised status and atypical clinical symptoms and is also associated with numerous post-transplantation compli-cations. Hence, it would be incorrect to consider HTx recipients as individuals without any chronic heart disease anymore, and they are clinically absolutely different from chronic heart failure (CHF) patients or those who are supported by mechanical circulatory support (e.g., left ventricular assist device). Therefore, prevention and rehabilitation after HTx is very specific and requires a multidis-ciplinary approach, and such programmes should be initiated early after HTx and continued during the whole post-transplant journey.

RISK CONDITIONS AFTER HEART TRANSPLANTATION: THE NEED FOR MULTIDISCIPLINARY PREVENTION AND REHABILITATION

With the improved survival over time of patients with heart disease due the availability of better medical, surgical, and device-

based therapies, the clinical characteristics of the HTx recipient popu-lation have also evolved. Recent changes in donated organ alloca-tion policies in the United States in 2018 [4–6] and in many other countries around the world [7, 8] have led to organ allocations going to sicker, device-dependent and more frail end-stage CHF patients. In the effort to maximize the positive impact of HTx on post-transplant functional capacity, exactly these patients are expected to experi-ence greater benefits from intensive rehabilitation programmes.

Actually, HTx is not a complete cure for CHF. Long-term survival of HTx patients remains limited and exercise capac-ity and health-related quality of life (HRQoL) of HTx recipients remains inferior to age-matched healthy people [9]. To improve the exercise/functional capacity and HRQoL of HTx recipients, mul-tidisciplinary cardiac rehabilitation (CR) is an integral component in most clinical HTx programmes [10]. Hence, multidisciplinary pre-vention and rehabilitation will benefit from including the different healthcare practitioners, such as the heart transplant cardiologist, endocrinologist, lipidologist, nutritionist, physiotherapist, physia-trist, psychiatrist/psychologist, and social workers. Exercise training (ET) leads to an improved exercise/functional capacity, which in turn facilitates many PAs of everyday life. Tailored programmes of rehabilitation and prevention may better avoid complications that otherwise would negatively impact the patients' HRQoL. Therefore, preoperative risk factors such as frailty, sarcopenia, overweight and physical deconditioning should be assessed and addressed accordingly.

The holistic multidimensional prevention and treatment of dia-betes, overweight, dyslipidaemia, and psychological wellness should not be restricted to the immediate postoperative recovery period but should be reassessed and readdressed periodically to counter-act their potential negative clinical impact after HTx.

Exercise training might play an important role in counteract-ing some of the side-effects associated with immunosuppression, such as diabetes, dyslipidaemia and hypertension, as well as skele-tal muscle dysfunction and an increased risk for infections. ET has the potential to alleviate these side-effects to some degree [11], albeit systematic studies are lacking. A structured collection of patient-reported outcome measures using modern devices and software should be implemented to measure and tailor the man-agement and prevention of complications after HTx. Indeed, once a significant survival benefit has been reached, HTx profession-als and patients face a mindset from improving survival towards improvement in HRQoL.

Areas for intervention should include lifestyle changes, psy-chosocial support, in addition to targeting improved control of hypertension, dyslipidaemia, atherosclerosis, and cardiac allograft vasculopathy (CAV) [12].

PREVENTION AFTER HEART TRANSPLANTATION

Lifestyle Changes and Sex

Metabolic derangements such as dyslipidaemia and post-transplant diabetes mellitus (PTDM) are important risk factors for graft fail-ure, coronary events and mortality after organ transplantation [13]. Other lifestyle-related factors contributing to an increased cardio-vascular risk and functional disability include a reduced exercise capacity [14], and significant psychosocial issues (i.e., clinical depres-sion, mood disorders with negative affect, demoralization, and coping problems) [15]. Therefore, lifestyle modification should be an important part of the management of HTx patients. Key elements of such lifestyle management programmes include PA counselling and exercise prescription, nutritional counselling and healthy nutri-tion intervention, smoking cessation, psychosocial management, education and strategies to improve self-efficacy, self-care, and self-confidence. Other specific factors that should be taken into account include education and management of the side-effects of medical therapy (e.g., anti-rejection and antihypertensive drugs, pre-vention and management of infections), interventions and services to help promote a return to work and social rehabilitation [16], as well as counselling on sexual activities. In general, patients can resume their sexual activities after HTx, if they can perform mild to mod-erate levels of PA without symptoms (i.e., empirically 1 W/kg body weight, which is the required tolerance of the physical demand of sexual activity) [17]. Therefore, transplant patients may benefit from education, counselling and CR [17–19]. Around 50% of HTx recipients report a decreased sexual frequency and/or libido [17]. HTx specific concerns include having difficulty integrating the new organ into their "sense of self" (i.e., avoiding sexual activity out of concern to protect their heart, anxieties about assuming the sexual identity of the donor, and perceptions of sexual unattractiveness). CR can improve the exercise capacity of patients after HTx, which may also enhance sexual functioning [18–20].

Psychosocial Support and Return to Work

The psychosocial impact of referral, waiting for, and receiving a HTx is profound and variable, with many patients feeling fearful, anxious, guilty and inadequate, with concerns about the impact on their family [21, 22]. Despite the benefits of receiving a HTx, recipients need continual psychosocial as well as medical support, based on the understanding of the many complex challenges that can confront them. Moreover, patients often feel restricted in their ability to return to a normal life, due to the need for regular medical check-ups and possible hospital admissions.

The main objective of HTx is to increase survival [23, 24], as well as to improve PA and HRQoL, allowing patients to return to their daily activities [8, 25, 26] including returning to paid work. However, the rate of successful return to work after HTx is low and varies between different countries [17, 27–31]. Despite a satisfactory objective and subjective functional status, some patients choose not to return to work. Age [32], previous work [17] and time out of work before transplantation [15, 27, 31, 32] are determining factors in most studies, while the impact of functional capacity and educational level appears to be controversial [30]. The type of work previously performed may also play a role, with a return to work being more common in white-collar workers [32]. Identifying factors that affect post-transplant patient's motivation to return to work

might help health professionals to adopt the best course of treatment and psychological support, in order to fulfill this goal. Nevertheless, a return to work should not be considered as the only manifestation or measure of a patient's real psychosocial condition [33–35]. After a long period of illness and the prospect of death, post-transplant patients tend to attach great importance to factors other than work, for example, giving priority to relationships with family and friends, spirituality and free time [27].

On the other hand, HTx recipients make use of all coping strategies, with predominance on problem-focused strategies. The use of these active coping strategies encompass behaviours that may lead to greater adherence to treatment [36]. Psychologically prepared individuals use more active coping strategies, which high-lights the importance of psychological support during the referral and transplantation process [36]. Hence, life-long rehabilitation and psychosocial support should be provided to HTx patients after the surgery. PA is one beneficial factor to improve peak oxygen uptake (VO_{2peak}) in heart transplanted recipients based on the results of cardiopulmonary exercise testing (CPET) [37–40]. Although the exercise/functional capacity after HTx will improve significantly, changes in the type of work will sometimes be necessary, adapting it to the new situation and retraining for a different occupation. Obviously, the patient's financial need and the lack of public health insurance will increase the urgency to return to work.

There is also the impact of immunosuppressive (IS) drugs on the recipients' mental health, which can lead to anxiety and depres-sion (which are strong predictors for poor medication compliance or increased hospitalization rates in transplant recipients) [41]. Com-mon symptoms after cardiac surgery include fatigue or loss of energy, changes in sleep pattern, alterations to appetite, which often "may be misinterpreted by healthcare providers, researchers or patients as mood-related," precisely because they resemble the somatic symptoms of depression [42]. Studies have demonstrated that psychosocial factors, particularly coping style and social support, may be significant predictors of morbidity and mortality in patients awaiting HTx [43, 44] and in the intermediate term after successful HTx [34, 45, 46]. Furthermore, recent studies found an increasing dete-rioration of emotional wellbeing in the long-term life course after HTx [47]. Hence, the existence of depression limits the return to work [32, 48]. It has also been shown that the patient's feeling of ill-ness or the subjective assessment of his or her capacity for work, which does not always coincide with the real situation, can facilitate or limit return to work [17, 25, 32, 49]. The presence of clinical complica-tions (e.g., rejection, infections, etc.), as well as the presence of comorbidities will limit the return even after the patients recover from the transplantation [30]. While pre-transplant depression does not impact outcomes, patients with post-transplant depression are more likely to experience a complicated course, suggesting the need for increased vigilance regarding depression in such patients [50]. Moreover, patients with post-transplant depression within the first year have a significantly higher 5-year mortality [49].

All these aspects are advised during CR to improve the rate of return to work after HTx. In addition to ET, the rehabilitation process should focus on the psychological and educational aspects of the patient, self-esteem improvement and self-management of the illness, in function of the motivation to return to work.

Complications of Immunosuppression

There is a common misconception that post-HTx recipients' func-tional/exercise capacity usually returns to normal spontaneously. But the need for IS therapy actually may impair such recovery. Some of these IS-related complications, such as dyslipidaemia or osteoporosis, could be prevented, and some, such as tremor and leukopenia, can be managed by modifying the doses of the IS agents. Other adverse effects require prescription of new therapies (to address infections, endocrine abnormalities, neurological complica-tions, nephropathy and arterial hypertension [AH]) [6, 51–53]. Guide-lines for addressing the complications of IS therapy include regular screening for adverse events, minimizing drug doses, drug substi-tution, and drug withdrawal if possible, as well as initiating targeted therapies for a specific complication [54, 55]. It is important to assess for contraindications and drug interactions when medically treating complications associated with immunosuppression [56].

The available IS agents vary with respect to their risk for inducing PTDM. Mycophenolate mofetil and azathioprine have not been shown to have a large impact on insulin action or glu-cose metabolism and so do not appear to have a major role in PTDM. There is however increasing evidence that the commonly used IS agents, particularly calcineurin inhibitors (CNIs; tacrolimus, cyclosporine) and inhibitors of the mammalian target of rapamycin (mTOR; sirolimus and everolimus), may contribute to PTDM [53]. A major modifiable risk factor for the development of PTDM is IS therapy, but risk versus benefit analysis is needed to balance the risk of developing PTDM versus the risk of rejection. However, there are reports that both glucose intolerance and dyslipidaemia, that occur predominantly with mTOR inhibitors, improve as the drug dose is reduced [54]. However, increasing the risk of allograft rejection or transplant dysfunction is not justified by trying to man-age glucose intolerance and dyslipidaemia after HTx. High-dose corticosteroids, often used as part of induction protocols in the immediate post-transplant period, have a much greater negative impact on insulin sensitivity than the chronic low-dose cortios-teroids that are commonly prescribed in the maintenance of many IS protocols [55].

Reduction of mycophenolate mofetil or everolimus initiation may be beneficial in HTx patients presenting with leukopenia or neu-tropenia and lead to modest, short-term renal function improve-ments [55]. Other causes of leukopenia should be excluded such as infectious (invasive pulmonary aspergillosis, cytomegalovirus) and haematological complications [56–60].

Cardiovascular events may occur at an increased rate even independently of the HTx recipients' age, sex, and cause of CHF. After HTx, development of dyslipidaemia, atherosclero-sis and AH is also associated with the prescribed doses of

immunosuppression [60]. Pharmacological treatment of dyslipidaemia is however effective [56]. Moreover, uncontrolled severe hyperlipidaemia and severe hypertriglyceridaemia associated with everolimus treatment may occur [61, 62]. Effective blood pressure (BP) control with antihypertensives has been shown to enhance graft survival and reduce the risk of future cardiovascular events in HTx recipients [63].

Malignancy after HTx is one the leading causes of mortality in the long term after HTx. According to the International Society for Heart and Lung Transplantation (ISHLT) guidelines, annual check-ups were recommended to screen all HTx recipients for breast, colon and prostate cancer and to have a close skin cancer surveillance, including education on preventive measures [56, 59]. The oncogenic Epstein—Barr virus is common in immunocompromised patients and also a key pathogenic driver in many post-transplant lymphoproliferative disorders (PTLD) cases [59]. However, chronic immunosuppression should be minimized in heart recipients with low immunological risk as possible, particularly at high risk for malignancy [56].

Complications after HTx vary according to the time from surgery and the IS therapy used as well as progression of pre-existing conditions. Future research needs thus to focus on reaching an optimal and customized balance between efficacy and toxicities of IS strategies [64].

Arterial Hypertension

Pre-transplant AH is an important predictor of post-transplant AH [65]. According to the ISHLT registry, the prevalence of AH among HTx patients is 50%–90% and is associated with an increased cardiovascular morbidity and mortality [66]. There are several con-tributors to the development of AH after HTx such as the use of CNIs and corticosteroids for immunosuppression, the negative effect of cardiac denervation and nephropathy, which addition-ally activates the renin—angiotensin system, hereby increasing the intravascular fluid volume and peripheral resistance [56, 67, 68]. Activation of the sympathetic nervous system, vasoconstric-tion affecting endothelin-1, reduction in circulatory nitric oxide and prostaglandin concentrations, are the main mechanisms of CNI-induced AH [69]. Polymorphisms resulting in CYP3A5 loss of function may also significantly influence drug metabolism and exposure, and lead to higher incidence of CNI-related nephrotoxicity [70].

According to the 2018 European Society of Cardiol-ogy/ European Society of Hypertension (ESC/ESH) guidelines and 2023 ESH guidelines for the management of AH, a diagnosis of AH is established when office systolic BP is > 140 mmHg and/or diastolic BP is > 90 mmHg [68, 71]. According to the SPRINT clinical trial, among patients who were at increased cardiovascular risk, targeting a systolic BP < 120 mmHg resulted in lower rates of major adverse cardiovascular events and lower all-cause mortal-ity [72]. However, there are no data about specific BP levels that should be maintained in the HTx population other than referring to the recommended BP levels to non-HTx recipient population levels and taking into account patients' ethnicity and the other recipients' comorbidities

(i.e., chronic kidney disease [CKD], etc.). Throughout the management of AH, ambulatory BP monitoring is warranted, in particular when in doubt regarding the accurate diagnosis or the adequacy of BP control.

Once AH is diagnosed as per respective guidelines, interventions need to be implemented as the benefits in HTx patients are similar to those in patients with AH at large [71]. Efforts to achieve the lowest possible effective CNI serum level (tacrolimus, cyclosporine) and, if possible, to discontinue corticosteroids by the end of the first year post-transplant are warranted [56]. Finally, antihypertensive therapies should be initiated if needed. Drug selection is empiric and depends on BP responses [56]. Adequate BP control with a calcium channel blockers (CCB) or angiotensin-converting enzyme (ACE) inhibitor is warranted to avoid CKD [56]. Data show that the majority of HTx recipients required only a single antihypertensive drug, and CCB are primarily used [73]. The reason for such choice is their neutral effect on cardiac and renal function and minimal interaction with IS drugs [74], whereas ACE inhibitors and angiotensin receptor blockers (ARBs) may be beneficial among diabetic recipients, and a two-drug regimen can include both CCB and ACE inhibitor/ARB [56, 75, 76]. Another concern about using ACE inhibitors and ARBs, especially in patients with CKD, is that the serum creatinine level tends to rise when starting these drugs, although several studies have shown that an acute rise in creatinine may demonstrate that the drug is actually protecting the kidney. This phenomenon was described as "pre-renal success," proposing that the decline in glomerular filtration rate is haemodynamic, secondary to a fall in intraglomerular pressure as a result of efferent vasodilatation, and therefore should not be reversed. Caution is advised when initiating ACE inhibitor/ARB therapy in these high-risk groups as well as in patients with potassium levels >5.0 mmol/L at baseline, at high risk of pre-renal acute kidney injury, with known renal insufficiency, and with previous deterioration in renal function on these medications [77].

In conclusion, a first-line antihypertensive therapy in heart recipients without diabetes or with advanced CKD (stages IV–V) or at high risk of acute kidney injury development is CCB. Otherwise, HTx patients with diabetes or CKD (stages I–III) will benefit from ACE inhibitor/ARB prescription. If first-line antihypertensive medications (CCB or ACE inhibitors/ARBs) are not effective, then instead of its dosage up-titration either CCB or ACE inhibitors/ARBs should be added.

Dyslipidaemia

Dyslipidaemia is frequent in HTx recipients, not just because of background history and comorbidities, but also because IS therapy has a negative impact on the lipid profile [78]. Dyslipidaemia is associated with CAV and cardiovascular events [79].

Statins have been shown to reduce CAV and improve long-term outcomes and should be started during the very first weeks in all HTx patients, adults and children, regardless of cholesterol levels [56, 80].

The adagio "the lower the better" also applies to the HTx population, aiming at a minimal low-density lipoprotein

TABLE 1 | Recommendations for the doses of dyslipidaemia tablet medications in the heart transplant population[56,84].

Drug	Doses of dyslipidaemia drugs	Risks of myositis	Interactions between dyslipidaemia drugs and common medications prescribed to heart recipients
Pravastatin (preferred drug)	20–40 mg	Myositis (lower)	Major interactions with cyclosporine – is not generally recommended! Preference should be for the combination of fluvastatin and cyclosporine
Simvastatin	5–20 mg >20 not recommended	Myositis (higher)	
Atorvastatin	5–20 mg	Myositis (higher)	Moderate interactions with tacrolimus, possible use under the control of CO targets of tacrolimus
Fluvastatin (preferred drug)	40–80 mg	Myositis (lower)	
Lovastatin	20 mg	Myositis (higher)	
Rosuvastatin (limited in case of chronic kidney disease)	5–20 mg	Myositis	The combination of warfarin with rosuvastatin/lovastatin—moderate interactions, not recommended
Ezetimibe	10 mg	—	Cyclosporine, anticoagulants
Fenofibrate	145 mg	Myositis (lower)	Cyclosporine, anticoagulants

cholesterol of ≤70 mg/dL (1.8 mmol/L). Initial statin doses should be lower than those recommended for hyperlipidaemia, due to concern for pharmacological interactions with CNIs, and up-titration should be careful, especially in patients on cyclosporine [79].

Interactions between statins and CNIs are well documented, but recent data, though limited, suggest that the combination of tacrolimus and statins may be safe. Retrospective studies sup-port the safety of high-dose statin therapy in patients treated with tacrolimus-based immunosuppression, with greater statin dose to be associated with a reduction in adverse cardiovascular out-comes after HTx [81, 82]. Pravastatin was included in the guidelines due its beneficial effects on cholesterol levels and improved inci-dence of allograft rejection causing haemodynamic compromise, 1-year survival, and the incidence of CAV [56, 83]. Dosage of statins in the HTx population is lower than in general population because of the high risk of myopathy or myositis development [56]. Rec-ommendations for dyslipidaemia medications are presented in **Table 1** [56, 84].

Ezetimibe may be used as an alternative, or additionally to a maximally tolerated dose of statin in patients who are poorly tolerant to statins, or those with significant dyslipidaemia despite maximal dose statin treatment and dietary advice.

Recently, a retrospective study showed the effectiveness of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors in lowering cholesterol levels in HTx patients, hereby stabilizing coronary intimal hyperplasia [85]. For the specific management of hypertriglyceridaemia, caution is warranted when using fibrates in.

Thromboembolic Complications

Venous thromboembolism (VTE), including deep venous thrombo-sis and pulmonary embolism, is a frequent complication after HTx, being six times more common among HTx recipients than among the general population [86, 87]. The highest risk is during the first post-operative year [86].

"Classic" risk factors for VTE, such as recent hospitalization, being older, obese, previous history of VTE or having renal dysfunction, all increase the risk of VTE after HTx [86]. However, specific risks exist in HTx recipients: indeed, the use of mTOR inhibitors has been associated with a significant increased risk for VTE, even when controlling for other risk factors [88, 89]. Although strong evidence is still lacking, in order to reduce the risk of VTE after HTx [36], weight loss should be recommended in obese patients [87]. The use of mTOR inhibitors after HTx should take into account the risk of VTE over time [88, 89]. However, indications for mTOR initiation after HTx is based on patients' comorbidities and/or post-transplant complications. Developed complications, that is, CAV, CKD, malignancy, neurological complications and so forth, outweigh the risk of VTE when conversion to mTOR should be considered. A more aggressive approach to thromboprophylaxis is advised in order to minimize thromboembolic complications [86–89], taking into consideration the potential interactions with other drugs administered to HTx patients.

Osteoporosis

Osteoporosis represents a serious complication for HTx recip-ients, mainly resulting in vertebral compression fractures, with a prevalence ranging from 14% to 40% [90]. Bone mineral density.

(BMD) is rapidly reduced during the first 6–12 months after HTx [91]. The most important risk factors are pre-transplant bone disease [92] and post-transplant IS therapy, in particular glucocorticoids and CNI use [92]. Other factors such as aging, tobacco use, alcohol con-sumption, nutritional deficiencies, immobility, and hypogonadism can further enhance this risk [93]. After an initial increase of bone resorption markers associated with a decrease of bone formation markers, there is later attenuation in the rate of BMD loss after the first year post-HTx, reflecting a partial normalization of bone for-mation/degradation markers in the later post-HTx period [94]. Sup-plementation with calcium and vitamin D alone may not prevent significant BMD loss [94], while a recent meta-analysis [95] concluded that bisphosphonates effectively reduced the loss of vertebral BMD in early stage after HTx. Resistance ET can provide an additional osteogenic stimulus, and the combination of resistance ET with bisphosphonates is more efficacious than bisphosphonates alone in restoring BMD [11].

Post-Transplant Diabetes Mellitus

Post-transplant diabetes mellitus is a common complication after HTx, occurring in 20%–30% of HTx recipients [96, 97]. Pre-existing diabetes risk, IS agents and infections are major contributors to the development of PTDM [54]. However, the few available retrospective studies in HTx show that PTDM may increase the likelihood of rejection, AH, renal failure and infection rates [98–100].

Screening for PTDM should be performed once stable doses of immunosuppression are reached. Glycated haemoglobin should not be used as a sole screening measure in the first year after HTx as it could underestimate PTDM due to blood loss and greater red blood cell turnover.

Once diagnosed, PTDM will require action to achieve gen-eral treatment targets of diabetes management. On top of health lifestyle measures that include PA, oral hypoglycaemic medications alone or in combination with insulin therapy are warranted. The new oral antidiabetics such as sodium—glucose cotransporter 2 (SGLT2) inhibitors and glucagon-like peptide-1 receptor agonists (GLP1-RA) seem to be safe according to the first studies and are promising, especially in the setting of transplant vasculopa-thy (GLP1-RA) or renal failure (SGLT2 inhibitors) after HTx [101, 102]. However, after HTx there are no indications to continue SGLT2 inhibitor therapy if it was prescribed prior to surgery. Data on using SGLT2 inhibitors in HTx recipients are limited. According to recent data, SGLT2 inhibitors did not deteriorate heart transplant function and were efficient in diabetes management but during the first year after HTx its possible adverse effects (i.e., urinary tract infection, dyslipidaemia and increased urination) outweighed expected bene-fits. Larger studies of GLP1-RA and SGLT2 inhibitors are needed in patients after solid organ transplantation [102]. There is no evidence that the risk of PTDM can be decreased (e.g., by use of cyclosporine instead of tacrolimus or steroid avoidance/withdrawal), without increasing the risk of rejection.

Vaccination

The timing of vaccination appears to be critical to optimize responses. The first 6 months after transplantation are associ-ated with the poorest immune response because the patients are usually receiving the highest doses of immunosuppression [103]. No reduction or discontinuation of immunosuppression should be considered because of vaccination in transplant individuals. In the post-transplant setting, inactivated vaccines can be safely administered starting at 3–6 months post-transplantation except for influenza vaccine which can be given as early as 1 month post-transplantation [104].

COVID-19 vaccination is advised in all patients with CHF and a compromised immune system, including patients following HTx receiving IS therapy. They are however unlikely to generate a completely protective immune response after COVID-19 vaccina-tion, and therefore need additional personal measures including facemask wearing and social distancing for added protection [105]. According to the ISHLT guidance, both mRNA (Pfizer/BioNTech, Moderna) and non-replicating viral vector (Sputnik V, AstraZeneca, Johnson & Johnson)

vaccination can be used after HTx [106]. The additional dose of the vaccine beyond the standard scheme may increase the efficacy of vaccination in these patients [106].

FACTORS AFFECTING EXERCISE CAPACITY

Effect of Cardiac de- and Reinnervation on Exercise Capacity

Heart transplantation leads to total (i.e., both sympathetic and parasympathetic) denervation of the transplanted heart. Initially this results in increased resting heart rate (HR), due to the loss of vagal tone on the sinoatrial node, and a blunted HR increase during exercise [1, 11]. In denervated patients, no increase in HR is observed the first few minutes after exercise initiation. This phase is followed by a rise in HR rate presumably due to circulating catecholamines, but the increase is reduced compared to healthy subjects [11]. The decreased HR reserve (HRR) after HTx has generally been assigned an important role in the well-described exercise limitation observed in most patients; however, there is no consensus about the relative importance of HRR compared with contractile and diastolic reserve or peripheral factors [107].

Cardiac reinnervation occurs in a subgroup of HTx recipients. Both sympathetic and some degree of parasympathetic reinnerva-tion has been demonstrated, although the latter is much less well established [108]. The degree of reinnervation depends on several factors, including time from transplantation, age of recipient and donor as well frequency of rejection episodes [13].

Reinnervation after HTx is associated with a higher peak HR and HRR as well as a greater exercise tolerance [109]. High-intensity interval training (HIT) after HTx can increase peak HR and HRR, but whether this indicates an effect of exercise on reinnervation is not known. HIT clearly improves VO_{2peak} but the improvement in VO_{2peak} and HRR is not correlated, hence the improvement in VO_{2peak} is largely related to peripheral (muscular) adaptation [110].

Post-Heart Transplant Arrhythmias During Exercise

Transplantation leads to a denervation of the sympathetic and parasympathetic nerve fibers, that significantly restricts HR vari-ability [111]. Moreover, reactive tachycardia in response to underlying physiologic distress (e.g., pain, hypovolaemia, intense effort, etc.) may be blunted since the response of the grafted tissue to intrin-sic catecholamines is variable [112]. The transplanted heart shows a delayed chronotropic response to exercise due to a reliance on cir-culating catecholamines [37]. Denervation gradually leads to an emp-tying of the catecholamine stores in the myocardium, meaning that the transplanted heart is then reliant on the stimulation of circu-lating catecholamines. Overall, the catecholamine receptors display an increased sensitivity. In some cases, this can lead to an increased incidence of cardiac arrhythmia, whereby for decades therapy with beta-blockers was not considered the

preferred option because it has the capacity to significantly reduce exercise tolerance [113, 114]. However, in recent years some evidence has shown that after HTx beta-blockers are useful and effective in the treatment of cardiac arrhythmias, left ventricular systolic dysfunction and AH and in long term after HTx can lead to a HR reduction [114, 115]. Most sudden cardiac deaths occurring in HTx recipients are related to trans-plant coronary artery disease or CAV [113]. Most cases of sudden cardiac death have asystolic presentation, and ventricular fibrilla-tion occurs only in 10% of the patients with moderately depressed or preserved left ventricular ejection fraction [116]. According to current evidence, ET appears to be a safe intervention in HTx recipients and it has not been related to an increased risk of devel-oping cardiac arrhythmias [9]. However, after the first detection of post-transplantation cardiac arrhythmia, HTx recipients should be examined with transthoracic echocardiography, 24 h electrocar-diogram (ECG) monitoring, endomyocardial biopsy and coronary angiography to rule out allograft rejection and CAV [55, 113]. Transplant recipients undergoing exercise therapy should begin in a supervised setting with continuous ECG monitoring. More than one-third of subjects exhibit a partial normalization of HR response to exercise from6 months to 1 year after surgery [39].

Atherosclerosis and Cardiac Allograft Vasculopathy

Cardiac allograft vasculopathy is one of the leading causes of increased morbidity and mortality (which occurs in 32% of the patients at 5–10-year follow-up) in HTx recipients [117, 118]. CAV is multifactorial and is caused by immunologic mechanisms, and stim-ulated by non-immunologic factors leading to persistent endothe-lial injury [119, 120]. Intimal hyperplasia progresses towards coronary obstruction, which impairs perfusion up to the point of graft failure [118].

Prediction or early clinical diagnosis of CAV is difficult. Owing to the denervation of the transplanted heart, patients with CAV do not usually experience chest pain, but typically are asymptomatic until they present with sudden death or congestive heart failure [117]. Surveillance against CAV is currently performed by periodic coronary angiography. Intracoronary imaging with intravascular ultrasound or optical coherence tomography is useful in serial assessment of disease progression [121]. However, these techniques are invasive and expensive. Therefore, promising non-invasive modalities for an early detection of CAV like computed tomog-raphy angiography with coronary fractional flow reserve, car-diac magnetic resonance imaging and positron emission tomog-raphy assessment are under investigation [122]. Future studies will confirm whether these techniques may allow stratifying high-risk HTx patients for CAV development reliably and non-invasively, and may therefore substitute the current gold standard invasive intra-coronary imaging.

Conversion from a CNI- to a sirolimus-based IS regimen attenu-ates CAV progression and results in a positive remodelling effect on the coronary artery wall. Beneficial volumetric changes occur with conversion to sirolimus resulting in reduced rates of CAV-related events and improved late survival, in which the greatest benefits are achieved when patients are converted early (within 6 months to 2 years) following HTx [123]. On the other hand, total cholesterol and triglycerides seem to increase significantly in the sirolimus con-verters, although the dyslipidaemia associated with sirolimus does not translate into higher rates of cardiac events [123]. Actually, com-pared with continued CNI therapy, sirolimus attenuates plaque progression in recipients with early conversion, but contributes to increases in necrotic core and dense calcium volume in those with late conversion [124].

Post-Heart Transplant Nutrition

Optimal nutrition is an important part of the management for the heart transplanted patient. Weight control and lipid-lowering diet are recommended for all heart transplanted patients, irrespective of their sex, age, or aetiology of CHF [56, 78, 125]. Excess body weight worsens lipid profile and glycaemic control, and increases the risk of atherosclerosis and AH. CNIs (tacrolimus, cyclosporine) are well-known potent IS agents affecting electrolyte levels with known drug interactions. Grapefruits, pomelo, ginger, St. John's wort and turmeric juice should be avoided after HTx due to drug inter-actions and changes in immunosuppression blood levels [126, 127]. In addition, tacrolimus affects potassium and magnesium levels and mTOR inhibitors cause elevation in blood lipid levels. Appropri-ate nutrition considering the blood electrolyte and lipid levels should be tailored in every heart transplanted patient and those with diabetes should be counselled regarding weight control and low-glycaemic diet [55, 125].

Skeletal Muscle Abnormalities

Skeletal muscle abnormalities are a major factor limiting exercise capacity in patients with CHF that are partially reversed by ET [128], however this has been less well studied in patients post-HTx. Following HTx, patients demonstrate numerous skeletal muscle deficits that include loss of mass and function alongside evi-dence of mitochondrial abnormalities, a shift towards Type II more-fatiguable glycolytic fibres, and reduced fibre capillarity [129]. Skeletal muscle deficits following HTx can persist for months and are closely correlated to measures of exercise capacity [130], which provides a fundamental explanation for why many HTx patients are unable to retain normal exercise/functional capacity promptly. For-tunately, traditional endurance or strength ET regimes performed over 3–6 months can normalize at least some of the skeletal mus-cle deficits post-HTx, by increasing muscle mass and strength in line with greater mitochondrial function/morphology and Type I fatigue-resistant oxidative fibres, although low fibre capillarity ratio persists [129]. Collectively, therefore, skeletal muscle is a key periph-eral organ post-HTx that limits exercise/functional capacity but is modifiable by sustained ET.

The Impact of Frailty on Post-heart Transplant Follow-Up

Multidimensional frailty (including the physical, psycho-cognitive, social, nutritional domain) is highly prevalent in

TABLE 2 | The 10 items of the AGILE tool with relative scoring divided by domain of frailty (physical, mental, nutritional, and socioeconomic).

No.	Item	Score	Frailty domain
1	Feel everything is an effort	Yes – 1, No – 0	Physical
2	Help up/down stairs	Yes – 1, No – 0	
3	Grip strength[a]	Yes – 1, No – 0	
4	Temporal orientation deficit[b]	Yes – 1, No – 0	Mental
5	Delayed recall deficit[c]	Yes – 1, No – 0	
6	Feel depressed	Yes – 1, No – 0	
7	Weight loss over 4.5 kg in the last year	Yes – 1, No – 0	Nutritional
8	Help in eating	Yes – 1, No – 0	
9	Financial help from family members	Yes – 0, No – 1	Socioeconomic
10	Physical help from family members	Yes – 0, No – 1	

[a]≤30 kg in men, ≤20 kg in women at hand-held dynamometer.
[b]The subject does not refer the exact date (day/month/year).
[c]The words "bread-house-cat" are referred to the subject at the beginning of the questionnaire and then asked to the subject at this time of the questionnaire.

HTx patients [131, 132]. Among all the components of multidimensional frailty, some can be reversible (treatable) while others are irreversible (requiring sup-portive care) [132]. The identification of the major components of frailty, if present, and the role exerted by each domain are the pil-lars to prioritize interventions within a tailored plan of care after HTx [133]. Frailty which develops during the waiting list period, is often the basis to plan rehabilitative interventions. Moreover, it has been demonstrated that frailty within 6 months before HTx is associated with increased mortality and prolonged hospitaliza-tion after HTx [134, 135]. Therefore, the identification of a common language to evaluate frailty among CHF specialists is mandatory. The need for a common language to manage CHF and transplanted patients has been proposed in recommendations from the ESC, the American Heart Association (AHA), and the Society for Geri-atric Cardiology (SGC) and these emphasize the importance of awareness of the frailty syndrome in the treatment of patients with CHF [29]. Recently, a quick tool to identify multidimensional frailty was proposed to reduce the time spent in frailty evalua-tion [136–138]. AGILE is a 10-item tool evaluating mental, physical, socioeconomic and nutritional domains (**Table 2**) with the ability to predict mortality, disability and hospitalization, which is especially useful in care settings that require reliable assessment instruments with short administration time [139].

Associations Between Exercise and the Immune Landscape

Regular ET modulates the immune landscape, affecting both num-bers of immune cell subtypes as well as their activation state and responsiveness to activation [140–143]. Hence, ET might in principle support shaping the host's immunity in HTx in a way to improve graft survival as well as pathogen defence in addition to its role in aiding physical recovery. The exercise-induced effects on immunity, however, need to be viewed in context with the IS regimen used for each patient.

A better understanding of the role of individual leucocyte populations and their modulation by exercise under the various IS regimens is needed to develop effective prevention and treatment strategies against acute and chronic graft-versus-host

TABLE 3 | Phases of cardiac rehabilitation.

Phase I	In-hospital patient period
Phase II	Post-discharge pre-exercise period
Phase III	Exercise and education programme
Phase IV	Maintenance

disease [144, 145]. High-intensity interval training is more effective for enhancing aerobic fitness of sedentary males by increasing their pulmonary ventilatory and cardiac haemodynamic responses to exercise than moderate intensity-continuous ET and these experimental findings facilitate the identification of effective ET regimens to increase aerobic capacity and minimize immune death under conditions of hypoxia [146].

Endothelial cells serve as facultative antigen presenting cells and thereby take on a crucial role in graft rejection. Indeed, ET has been shown to preserve endothelial function in HTx recipients [130, 147, 148]. The maintenance or improvement of endothelial integrity and quiescence may therefore contribute to the beneficial role of endurance exercise in HTx rehabilitation.

Finally, exercise might have a role in counteracting the side-effects of immunosuppression, which include increases in plasma glucose, lipids and/or BP and skeletal muscle dysfunction in addition to an increased risk of infections. There is individual evidence that ET might alleviate these to some degree [11, 12], albeit systematic studies are lacking and relevant changes in the immune landscape in this context have not been systematically investigated.

REHABILITATION AFTER HEART TRANSPLANTATION

Early- and Long-Term Cardiac Rehabilitation

Early-term rehabilitation after HTx could be delivered in the usual phases I and II (**Table 3**) of CR. During the in-hospital phase, early mobilization—particularly in phase I but also in

TABLE 4 | Core components of cardiac rehabilitation after heart transplantation[2,75].

Component	Issue
Patient assessment and self-assessment	• Clinical stabilization • Wound healing • Risk of acute rejection • Exercise tolerance • Personal and dental hygiene, risk of communicable diseases • Avoidance of environmental risks
PA counselling	• Advice for PA as a way for return to functional lifestyle with good quality of life • Consideration of both AET and RST training • Self-monitoring of exercise intensity more relied on perceived exertion than HR range
Structured exercise training	• Phase I: respiratory physiotherapy, active and systematic mobilization of the upper and lower limbs • Phase II: AET in the second or third week after HTx, RST after 6–8 weeks • AET intensity: <50% VO_{2peak}/W_{peak} (or 10% below AT) • RST intensity: 40–70% 1-RM
Diet/nutritional counselling	• Dietary choices, particularly concerning foods to be avoided
Weight control management	• Avoidance of overweight to balance the side-effects of immunosuppressants and to limit cardiovascular risk factors for CAV • Consider weight loss for obese patients
Lipid management	• Pharmacological and non-pharmacological treatment of hyperlipidaemia as a risk factor for CAV • Choice of statin with regard to interaction with cyclosporine and other immunosuppressants
Blood pressure monitoring	• Target blood pressure: ≤140/90 mmHg • Relationship between hypertension and immunosuppression/heart denervation • Consideration of CCB and ACE inhibitors/ARB as first choice • Beta-blockers are not the first choice of therapy to manage AH due to they can delay chronotropic response but they can be added to the combination of antihypertensive drugs if CCB with/or ACE inhibitors/ARB are not efficient to reach the required blood pressure
Smoking	• Smoking cessation • Avoidance of post-HTx smoking resumption
Psychosocial management	• Clear advice on life after HTx • Structured support and intervention for psychosocial risk factors

1-RM, one repetition maximum; ACE, angiotensin-converting enzyme; AET, aerobic endurance training; AH, arterial hypertension; ARB, angiotensin receptor blocker; AT, anaerobic threshold; CAV, cardiac allograft vasculopathy; CCB, calcium channel blocker; HR, heart rate; HTx, heart transplantation; PA, physical activity; RST, resistance/strength training; VO_{2peak}, peak oxygen consumption; W_{peak}, peak workload.

phase II CR—can be initiated as soon as haemodynamic reestablishment and weaning from post-transplant intravenous drugs occurs [2]. Phase I refers to postoperative early mobilization, patient education and promotion of adherence towards following phase II activities, being the last couple also part of an eventual prehabilitation course (i.e., before HTx). Phase II CR (initiated before discharge and followed by post-discharge pre-exercise programme) in these patients usually starts with an in-patient programme (due to the complexity of intervention and related needs of strict observation), directly followed by outpatient activities [2]. The average duration of hospital stay after patients underwent HTx with uncomplicated early-term follow-up and before the discharge from the hospital is 2–3 weeks [149–151]. Considering the time difference of recipients' post-transplant stay at the hospital the duration of the phase 1 can be different, the same as when the phase II can be initiated which is more than 2–3 weeks after HTx. Time windows of residential and ambulatory CR is about 3–4 weeks and 2–3 months, respectively, varying with local policies. At discharge, heart recipients should be able to walk on a level surface for a period of 40–60 min at speeds of 80–100 m/min, 4–5 times a week [2].

Long-term multidisciplinary CR is very important after HTx [152] and includes phases III (exercise and education programme) that is followed by phase IV (maintenance) [2].

Only half of HTx recipients participate in CR programmes, and those who do have a lower 1-year readmission risk [153]. All heart transplant team healthcare specialists should participate in the post-transplant rehabilitation programmes.

Despite having a new heart, these patients may suffer from exercise limitation in relation with muscle abnormalities resulting from their previous CHF and comorbidities, anti-rejections thera-pies, corticoid myopathy and from deconditioning due to muscle pain or fatigue. HTx recipients are therefore often severely decon-ditioned [154]. Residual peripheral vasodilatory limitation may persist after surgery [9]. At least in the first months, exercise capacity is also limited by chronotropic incompetence. Therefore, improving peripheral (muscular) performance, mediated by amelioration of microvascular and/ or skeletal muscular metabolic function, is key for achieving a sustained improvement of exercise capacity. In fact, various studies have shown that regular physical ET is effective in improving exercise capacity and HRQoL in these patients [9].

Specific core components of CR after HTx—to be integrated by common components of CR in cardiovascular patients [2] —are summarized in **Table 4**.

For exercise rehabilitation, one may use any of the usual of training methods in these patients (continuous aerobic or interval training, resistance training, inspiratory muscle training). One

should, however, not rely on the HR response for train-ing prescription and assessment in case of denervation-related abnormal chronotropic response. Finally, it has been shown that these patients need long-term supervised programmes because short-term or home-based programmes without proper remote guidance may be less effective than in other patients [155].

Exercise After Heart Transplantation: Practical Implementation

After HTx, ET and PA implementation play a key role in the rehabilitation programme, and need a detailed consideration of the following steps: risk stratification assessment, shared-decision making, data monitoring, exercise modalities adjustment, and consideration of any other issues that arise.

Risk Stratification Assessment

A symptom-limited CPET prior to any exercise intervention, with monitoring of ECG, ventilatory parameters (i.e., ventilatory VO_2, CO_2 production, ventilation), workload, oxygen saturation and BP should be performed [2, 37, 156]. From this the first and second ventilatory threshold can be determined [156]. In addition, a 6-min walking test maybe used to document functional capacity where such CPET facilities are not available. One-repetition maximum (1-RM) testing is advised to assess muscle strength, or in patients who cannot tolerate completing CPET (especially in patients with a difficult postoperative course) [156]. These data can then be used for personalized exercise prescription, to maximize the safety of exercise and decide on the appropriate setting for subsequent ET (e.g., supervised, hospital, gym, home-based).

Shared Decision-Making

When prescribing exercise/PA, it is necessary to consider patient preferences to maximize adherence and to optimize the intervention goals [2].

Data Monitoring

In particular during the first few weeks of a newly started exercise/PA intervention, monitoring BP and HR ahead of exercise, HR, oxygen saturation, and Borg rating of perceived exertion dur-ing endurance ET, OMNI-RES during strength training, BP and HR after exercise are recommended [2, 156]. In some patients, continuous ECG monitoring can be beneficial (based on ischaemic, arrhythmic and clinical status).

Exercise Modality Adjustment

The HTx patient generally has—at least initially—a delayed HR response due to cardiac denervation. As a result, a warm-up period before each session and steady-state aerobic exercise are recom-mended at the beginning. Although HTx patients are expected to start with a low exercise load and muscle strength, (rapid) changes are expected to occur. Functional electrical stimulation is advised in special cases where mobilization is not possible soon after surgery. As a result, regular assessments of physical fitness (endurance capacity and muscle

strength) are needed to adjust the duration and/or intensity of the exercise sessions [154]. CPET can be advised after 6 weeks (useful in individualized ET for phases III–IV of CR) in the first 3–6 months of intervention (before the reinnervation and development of HR variability) and then at least once per year to estimate the dynamic of physical capacity, while 6-min walking tests and 1-RM tests can be executed more often (e.g., every 2 weeks) while hospital admission or check-ups in the outpatient department [2, 37, 107, 157, 158]. The end-goal should remain HIT, if tolerated by the patient, with periods of 85%–95% of maximum HR.

Other Issues

Exercise performance is also affected by nutritional status, psy-chosocial status (e.g., level of education, economic status, believes about exercise/PA) and/or motivation. Education on exercise/PA is very important, and the motivational stage should be taken into account.

High-Intensity Interval Training After Heart Transplantation

Up until a study by Haykowsky et al. [158] in 2009, exercise after HTx had, and in many institutions still has, a rather conservative approach consisting mainly of moderate intensity continuous train-ing, mostly due to uncertainty and concerns regarding denervation and consequently chronotropic incompetence and parasympa-thetic impairment. Recent studies demonstrated that HIT was safe and effective in different groups of maintenance recipients (>1 year post-HTx).

Since 2009, accumulating evidence has underscored these early findings regarding the safety and efficiency of HIT in HTx recipients. The first meta-analysis of exercise studies in solid organ transplant recipients was published in 2013 [159], which concluded that "ET is a promising but unproven intervention for improving the cardiovas-cular outcomes," has already been largely replaced by more positive recommendations. A recent European Association of Preventive Cardiology position paper stated that previous restrictions placed on HTx recipients with regard to exercise modalities, and especially HIT, do not seem to rely on evidence-based knowledge [2].

Although more research is still needed in different aspects of the field, newer studies have addressed both long-term effects of HIT [108, 160], possible mechanisms for the "HIT effect" [109], comparison of exercise modalities [110, 157], HIT in *de novo* HTx recipients [111], and demonstrated stronger evidence of reinnervation [161].

In summary, the vast majority of performed studies have proven positive effects of HIT on multiple factors as, for example, VO_{2peak}, muscle strength, chronotropic responses, CAV, body composition and HRQoL [9, 37, 38, 159, 162–165]. Although beneficial effects of HIT on HTx recipients seem to differ to some extent from patients with coronary heart disease or heart failure, with more prominent peripheral effects of exercise, rather than central adaptions such as increased stroke volume, there is no doubt that HIT is highly effective in HTx recipients and should be more frequently used.

FIGURE 1 | Benefit of exercise after heart transplantation. CAV, cardiac allograft vasculopathy.
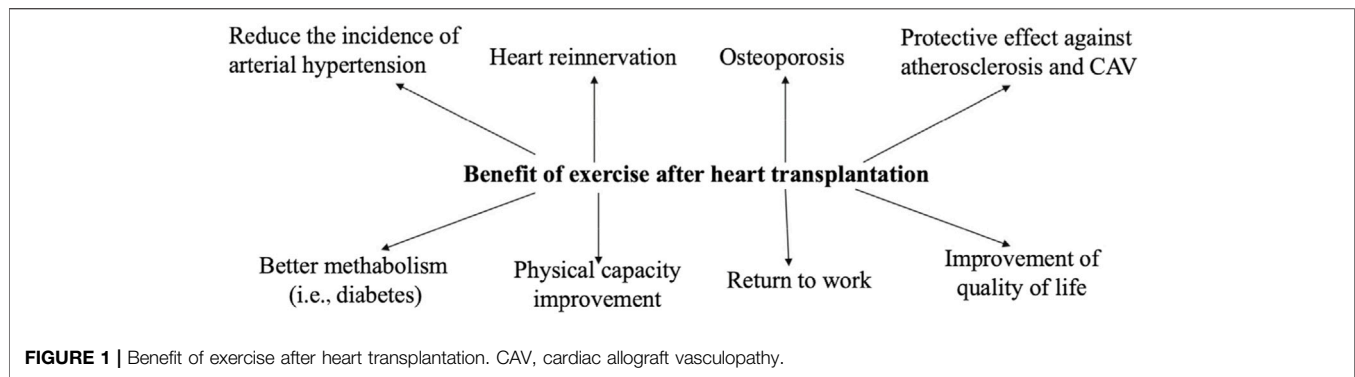
However, individual tailoring and individual considerations are still needed to determine the optimal exercise modality for each specific patient.

Benefits of Exercise Rehabilitation After Heart Transplantation

There are benefits of post-transplant exercise rehabilitation on reducing post-transplant complications as follows (**Figure 1**).

Arterial Hypertension

Exercise training is widely used for reducing BP in hypertensive subjects. Among studies investigating potential benefits of ET in HTx recipients only few have used BP as primary endpoint; therefore, most of the available data come from studies designed with different endpoints in which resting BP or ambulatory BP were secondary or exploratory variables. A 12-week training programme performed at 69% of VO_{2peak} was effective for reducing both systolic and diastolic ambulatory BP in HTx recipients [166]. Little is known about the type, frequency, or intensity of exercise that provides the greatest benefits on BP of HTx recipients. HIT proved to be more effective than no training [167], and slightly better than continued moderate exercise on reducing systolic BP [10] but it failed to reduce BP in another [40]. It is possible that denervation that occurs during transplantation surgery may reduce the hypotensive response to ET in HTx compared to what has been observed in other populations. In a recent study, a greater reduction of ambulatory BP, as well as a greater increase of maximal VO_2, were observed in patients with evidence of cardiac reinnervation compared to those without cardiac reinnervation [168].

Diabetes

The role of ET in the management of diabetes in HTx recipients has been poorly investigated. A recent meta-analysis, evaluating the effects of exercise on components of metabolic syndrome and involving patients with solid organ transplantation, showed a significant reduction of fasting blood glucose after training [169].

Osteoporosis

Strength training, alone or in association with drug therapy, has long been recognized as an effective intervention in counteract-ing bone loss in HTx recipients [170]. Six months of strength training potentiated the effects of alendronate administration on revert-ing glucocorticoid-induced osteoporosis in HTx recipients [11]. In another study strength training combined with the administration of calcitonin was more effective than calcitonin alone in restor-ing BMD in spine to within 5% of pre-transplantation levels within 8 months after HT [171].

Heart Reinnervation

The HR response to exercise is one of the most important pre-dictors of exercise capacity in transplant recipients with com-plete chronotropic competence and without relevant transplant vasculopathy or acute allograft rejection [172]. Transplant recipients with evidence of restoration of sympathetic innervation had bet-ter exercise performance compared to denervated recipients, due to a better chronotropic and inotropic response. Overall exercise time was significantly greater in reinnervated patients with a signifi-cantly greater increase of HR above baseline, and peak HR attained during exercise compared with denervated patients. Multiple stud-ies have demonstrated the benefit of ET after HTx by improving VO2peak, peak HR, and chronotropic response, and high-intensity, interval-based aerobic exercise has been documented to have supe-rior positive effect compared with moderate exercise [13]. ET was effective to reduce BP, to lead to HR variability and to increase exercise tolerance. However, it was not effective to improve arte-rial stiffness [168, 173]. The fact that the improvement in exercise capac-ity is lost after a few months without training, may suggest that the physiological mechanisms for improvement are primarily peripheral and not through cardiac remodelling [13].

Atherosclerosis

Exercise training has a protective effect against the development of CAV. In a murine model, ET reduced the onset of CAV by enhancing endothelial cell regeneration and function in the graft [33]. In humans, Nytrøen et al. [174] reported significantly reduced progress of CAV in HTx recipients undergoing HIT compared with no exercise. Among potential anti-atherosclerotic mechanisms of exercise in HTx recipients is a reduction of the inflammatory response. In a small study, exercise evoked an immediate response in several vascular, angiogenetic and platelet-derived inflammatory mediators in HTx recipients, irrespective of the training intensity [109].

Quality of Life

Studies investigating post-transplant HRQoL have clearly demon-strated that HTx recipients have significantly improved HRQoL compared to the pre-transplant stage [37]. This supports previ-ously documented evidence on the association between increased exercise capacity and better HRQoL [37]. A moderate level of exercise and intensity is insufficient to maintain the higher VO_{2peak} that were achieved after the HIT intervention [160]. It was suggested that HIT can reduce the development of anxiety symptoms in the long term, which is a frequent health issue following HTx [160]. The.

HIT group reported significantly less anxiety symptoms, but there were no long-term differences in VO_{2peak}, muscular capacity, or CAV between the groups [160]. In addition, pediatric HTx adoles-cents do not meet their required PA recommendations. Despite this, they have low normal exercise capacity and report a normal HRQoL. Efforts to engage adolescents to increase their PA should be encouraged [175]. Young adult transplant patients are to be care-fully evaluated for psychosocial risks to avoid non-compliance and reduced HRQoL in the long term [176].

GAPS IN KNOWLEDGE

Life-long follow-up proved its benefit after HTx but it may limit recipients in their socialization and may deteriorate their men-tal health. So future research should focus on incorporating telemedicine, remote consultations and developing digital platform. In addition, CR programmes should be initiated early after HTx and then should be life-long continued. And future perspectives are to organize and to implement CR programmes in the long term as a part of outpatient follow-up. Future projects should provide the particular exercise recommendations for HTx individuals based on their condition and time after surgery. Moreover, further research is needed to establish long-term impacts of rehabilitation and ET on cardiovascular disease incidence and progression.

CONCLUSION, INCLUDING OPEN QUESTIONS AND FUTURE RESEARCH

The number of HTx patients increases and it is important to initiate prevention and multidisciplinary rehabilitation from the beginning after surgery and to continue them after discharge. All heart trans-plant team members have their role and need to participate in transplant recipients' prevention and rehabilitation programmes. After HTx prevention can be defined as a comprehensive set of measures, aiming to reduce the recurrence or development of car-diovascular disease and to improve long-term prognosis. Despite the profound benefits of receiving a heart transplant, recipients need continual psychosocial as well as medical support, based on the understanding of the many complex challenges that confront them. Life-long participation in CR programmes has been shown to improve symptoms and allograft function in the long term. There is a wide range of risk factors (modifiable/non-modifiable) that should be addressed after transplantation and taking them into account may reduce the number of cardiovascular complications and improve recipients' prognosis.

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

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Transjugular Intrahepatic Portosystemic Shunt Is Associated With Better Waitlist Management of Liver Transplant Candidates With Hepatocellular Carcinoma

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Transjugular intrahepatic portosystemic shunt (TIPS) reduces portal hypertension complications. Its impact on hepatocellular carcinoma (HCC) remains unclear. We evaluated 42,843 liver transplant candidates with HCC from the Scientific Registry of Transplant Recipients (2002–2022). 4,484 patients with and without TIPS were propensity score-matched 1:3. Analysing wait-list changes in total tumor volume, HCC count, and alpha-fetoprotein levels, and assessing survival from listing and transplantation; TIPS correlated with a decreased nodule count (−0.24 vs. 0.04, $p = 0.028$) over a median wait period of 284 days (IQR 195–493) and better overall survival from listing (95.6% vs. 91.5% at 1 year, $p < 0.0001$). It was not associated with changes in tumor volume (0.28 vs. 0.11 cm³/month, $p = 0.58$) and AFP (14.37 vs. 20.67 ng/mL, $p = 0.42$). Post-transplant survival rates (91.8% vs. 91.7% at 1 year, $p = 0.25$) and HCC recurrence (5.1% vs. 5.9% at 5 years, $p = 0.14$) were similar, with a median follow-up of 4.98 years (IQR 2.5–8.08). While TIPS was associated with a reduced nodule count and improved waitlist survival, it did not significantly impact HCC growth or aggressiveness. These findings suggest potential benefits of TIPS in HCC management, but further studies need to confirm TIPS safety.

Keywords: liver transplantation, recurrence, survival, progression, hepatocellular carcinoma

INTRODUCTION

Transjugular intrahepatic portosystemic shunt (TIPS) is a valuable interventional strategy to alleviate portal hypertension complications. It effectively diverts blood flow from the portal vein to the hepatic veins, lowering portal pressure and its subsequent clinical manifestations including ascites and variceal bleeding [1, 2]. Despite its clinical advantages in portal hypertension, the role of TIPS in the management of patients remains unclear [3, 4]. Some authors have revealed no association between TIPS and *de novo* HCC nodules, while others caution against a potentially increased risk of HCC

Abbreviations: AFP: alpha fetoprotein; BMI: body mass index; OS: overall survival; HCC: hepatocellular carcinoma; MELD: Model for End-Stage Liver Disease; SRTR: Scientific Registry of Transplant Recipients; TIPS: transjugular intrahepatic portosystemic shunt; TTV: total tumoral volume.

Transjugular Intrahepatic Portosystemic Shunt is associated with better waitlist management of liver transplant candidates with hepatocellular carcinoma

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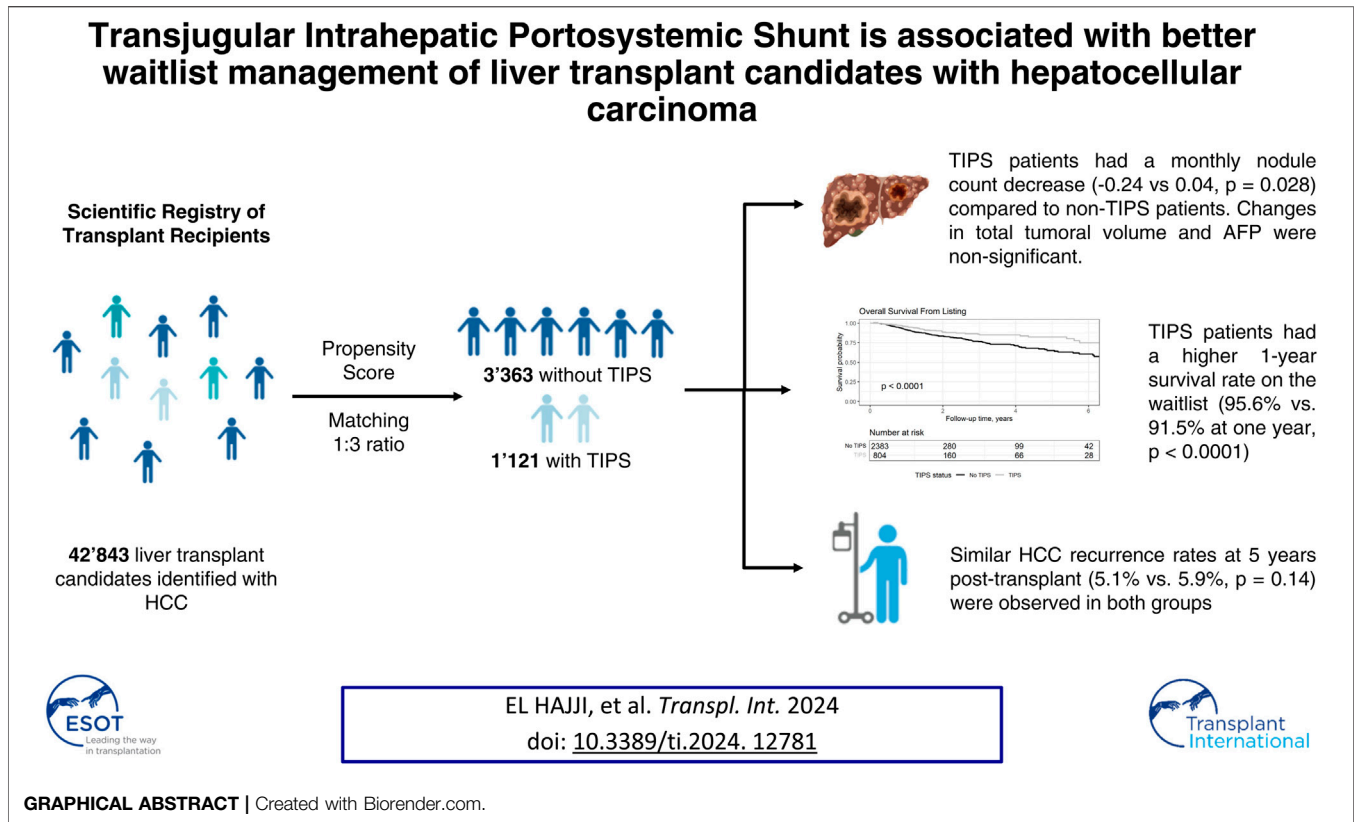
occurrence [5–8]. Concerns regarding the potential influence on tumor behavior persist, especially considering the limited sample sizes of many studies. Alterations in hepatic blood flow dynamics could theoretically promote tumor growth or metastasis through various mechanisms, including hypoxic liver injury, dissemination at insertion, or reduced response to locoregional treatment [9, 10].

We took advantage of a large prospective database from the Scientific Registry of Transplant Recipients (SRTR), which includes mandatory data from all liver transplant candidates in the United States. While on the list, HCC patients undergo periodic imaging and alpha-fetoprotein (AFP) assessments to benefit from exception Model for End-Stage Liver Disease (MELD) points. This dataset granted us access to data on HCC characteristics, such as size, number, and AFP, while patients were on the waitlist. Our study focused on comparing patients with and without TIPS at the time of listing to elucidate its impact on the progression of HCC.

MATERIALS AND METHODS

Study Population

This study utilized data from the SRTR database, a prospective registry that contains information on all donors, wait-listed candidates, and transplant recipients in the United States. The SRTR registry, submitted by members of the Organ Procurement and Transplantation Network (OPTN), encompasses a comprehensive list of patients registered from February 01, 2002, which corresponds to MELD implementation in the United States, to June 2, 2022, date of data retrieving.

Our study selected patients diagnosed with HCC as their primary or secondary diagnosis and compared them with (1,132) versus without TIPS (21,393) at the time of listing. Patients with liver tumors other than HCC were also excluded from the study. The TIPS status was determined prior to listing using the "CAN_TIPSS" label. We aimed to investigate the variations in HCC characteristics among patients on the waiting list for transplantation.

Data Collection

Data management and analysis were conducted using the R studio software (version 2022.07.2 + 576) [11]. Patient characteristics included age, sex, body mass index (BMI), underlying liver disease diagnosis, date of listing, date of transplantation, date of death, and time of follow-up. We classified the underlying liver diseases as viral, non-alcoholic steatohepatitis (NASH), and alcoholic liver disease (OH). An "other" category encompassing less prevalent etiologies like metabolic disease, cholestatic disease, drug exposure, and autoimmune disorders, each constituting less than 5% of the studied population. MELD was calculated in accordance with the 2016 revision by the United Network for Organ Sharing using a custom R function that assigned a minimum value of 1 to any log-scaled values less than 1 to prevent negative scores. Sodium levels were capped between 125 and 137 mg/dL, whereas

creatinine levels were capped at 4 mmol/L. The maximum attainable MELD score was 40.

We collected HCC characteristics at each MELD exception update from the "MPEXCEPT" list, allowing longitudinal monitoring of each patient. The characteristics included the HCC diameter, count, and AFP levels. For patients with multiple HCCs, the total tumor volume (TTV) was calculated by summing the volumes (calculated as the volume of a sphere $V = 4/3\pi r^3$) of the individual HCCs. The tumor burden was also evaluated based on the number of tumors. We assessed changes in TTV and tumor count between the first (at listing) and last (or pre-transplant) assessments, measuring changes per patient in volume in cm³ per month and count in units per year. Changes in AFP levels were expressed in ng/mL per month. In terms of therapeutic interventions, HCC treatments were categorized as: "curative" when cryotherapy, thermoablation, chemical ablation, or surgery were used; "locoregional chemotherapy" when chemoembolization was used; "mixed" when both modalities were used; or "untreated" in the absence of HCC-directed treatment.

Propensity Score Matching

Propensity score matching (PSM) was performed using the "MatchIt" package to achieve covariate balance and mitigate selection bias between groups with and without TIPS [12]. Prior to performing the matching, we ensured that only patients with complete data for the matching criteria and their outcomes were evaluated. Matching utilized nearest-neighbor matching with a 3:1 pairing ratio to optimize the analysis. Patients were matched based on age, body mass index (BMI), underlying liver disease, initial calculated TTV, nodule count, AFP levels, waitlist HCC treatment category, and the calculated MELD score. The aim of this study was to minimize differences in liver function and initial HCC characteristics between the TIPS

and non-TIPS groups to better capture the effect of TIPS on HCC, including TTV, nodule count, and AFP levels.

Statistical Analysis

Survival was first evaluated from listing by censoring transplanted patients in the matched cohort. Post-transplant survival was then studied in patients who eventually underwent transplantation from the matched cohort. We used the listing date, transplantation date, and death date to compute the survival curves. Post-transplant HCC recurrence was determined following a procedure previously used by our group and others in the same cohort [13, 14]. Notably, this procedure provides an accurate assessment of recurrence rate.

Statistical analyses were conducted using the R Studio software. The analytical results were visualized using the "gtsummary" package [15]. To compare sample distributions, we employed the Welch two-sample t-test, Wilcoxon test, and Pearson's chi-squared test. For survival analysis, we utilized both the "survival" and "survminer" packages [16, 17]. The Kaplan-Meier method was used to assess overall survival (OS), and differences between groups were assessed using the log-rank test. The cumulative incidence risk of HCC was calculated using the "tidycmprsk" package, and the differences were compared using Gray's test [18]. Statistical significance was set at a threshold of $p < 0.05$.

RESULTS

Demographics

During the study period (data dating back from February 1, 2002, until June 2, 2022), a total of 42,843 patients diagnosed with HCC were placed on the waiting list. Patient characteristics are reported in **Table 1**, and the measured outcomes of HCC

TABLE 1 | Demographics of the selected HCC patients compared between patients with (TIPS) and without (No TIPS) a history of TIPS.

Demographics	No TIPS, N = 40,691	TIPS, N = 2,152	p-value[a]
Age at listing (years), Mean (SD)	59.61 (7.9)	58.97 (7.7)	<0.001
Gender, n (%)			0.15
F	9,565 (24)	477 (22)	
M	31,126 (76)	1,675 (78)	
Body mass index (kg/m²), Mean (SD)	28.91 (5.4)	29.60 (5.6)	<0.001
Underlying liver disease, n (%)			
Hepatitis B	1,497 (3.7)	43 (2.0)	
Hepatitis C	12,958 (32)	570 (26)	
Hepatitis C and B	180 (0.4)	6 (0.3)	
Hepatitis viral other	16 (<0.1)	1 (<0.1)	
NASH	3,348 (8.2)	249 (12)	
OH	4,814 (12)	498 (23)	
Other	17,878 (44)	785 (36)	
Last calculated MELD score, Mean (SD)	14.08 (7.6)	16.60 (7.1)	<0.001
Unknown	1,140	73	
Waitlist HCC treatment, n (%)			<0.001
curative	3,166 (7.8)	164 (7.6)	
locoregional	20,390 (50)	953 (44)	
mixed	2,332 (5.7)	75 (3.5)	
untreated	14,803 (36)	960 (45)	

[a]Welch Two Sample t-test; Pearson's Chi-squared test.

TABLE 2 | Outcomes on HCC evolution measured on the whole cohort and compared between patients with and without TIPS.

Outcomes	No TIPS, N = 40,691	TIPS, N = 2,152	p-value[a]
TTV at listing (cm³), Mean (SD)	17.16 (243.1)	13.34 (16.1)	0.004
Unknown	*4,653*	*238*	
TTV change (cm³/month), Mean (SD)	−0.28 (10.9)	−0.04 (12.5)	0.51
Unknown	*17,699*	*952*	
Number of tumors at listing, Mean (SD)	1.30 (0.6)	1.27 (0.6)	0.030
Number of tumors change (unit/year), Mean (SD)	−0.04 (6.7)	−0.21 (1.7)	0.010
Unknown	*15,813*	*861*	
AFP at listing (ng/mL), Mean (SD)	140.80 (1,230.5)	119.36 (1,002.3)	0.34
Unknown	*811*	*42*	
AFP change (ng/mL per month), Mean (SD)	−14.78 (3,293.3)	13.29 (167.0)	0.18
Unknown	*14,520*	*815*	

[a]*Welch Two Sample t-test.*
Italicized data are missing values.

progression are shown in **Table 2**. Patients with TIPS were younger, had a higher BMI, and had a higher prevalence of alcohol-related liver disease. These patients also displayed more advanced liver disease, as indicated by higher Model for MELD scores, but less advanced HCC staging as shown by their TTV et number of tumors at listing.

Propensity Score Matching

Considering the disparities between the groups, we implemented propensity score matching to equilibrate the data. This approach allowed us to investigate the specific effects of TIPS on HCC volume, number, and AFP changes over a median waiting time 284 days (IQR 195–493). The matching process was performed on a 3:1 basis and accounted for the covariates described in the Methods section. The balanced data are presented in **Table 3**.

HCC-Related Data

Following propensity matching, the HCC characteristics between patients with and without TIPS did not reach

statistical significance anymore, as outlined in **Table 4**, this was done to match the patients on tumor biology as closely as possible. We then explored the waitlist changes to capture the effect of TIPS on HCC progression. A negative change in HCC volume or count indicates an effective tumor treatment or resection. Conversely, a positive monthly change was indicative of ineffective treatment (or absence of treatment) and/or more aggressive HCC.

TIPS was associated with a decrease in the number of HCC, potentially indicating more efficient treatment of these lesions. There were no significant changes in volume or AFP dynamics between the groups as presented in **Table 4**.

Of note, we also performed a sensitivity analysis, also including patients with missing data. Similar outcomes have been observed, with a decrease in the number of HCC, and no change in volume and AFP dynamics (data not shown).

Overall Survival From Listing

We compared overall survival (OS) from listing between patients with and without TIPS in the matched cohort. OS at 1, 5, and

TABLE 3 | Balanced table of the matched cohort.

Demographics	No TIPS, N = 3,363	TIPS, N = 1,121	p-value[a]
Age at listing (years), Mean (SD)	59.26 (8.1)	59.34 (7.7)	0.78
Gender, n (%)			0.65
F	839 (25)	272 (24)	
M	2,524 (75)	849 (76)	
Body mass index (kg/m²), Mean (SD)	29.96 (5.7)	29.79 (5.6)	0.38
Underlying liver disease, n (%)			
Hepatitis B	36 (1.1)	20 (1.8)	
Hepatitis C	878 (26)	289 (26)	
Hepatitis C and B	2 (<0.1)	2 (0.2)	
NASH	447 (13)	134 (12)	
OH	690 (21)	252 (22)	
Other	1,310 (39)	424 (38)	
Last calculated MELD score, Mean (SD)	16.17 (8.2)	16.23 (6.7)	0.78
Waitlist HCC treatment, n (%)			0.77
curative	260 (7.7)	84 (7.5)	
locoregional	1,836 (55)	612 (55)	
mixed	138 (4.1)	54 (4.8)	
untreated	1,129 (34)	371 (33)	

[a]*Welch Two Sample t-test; Pearson's Chi-squared test.*

TABLE 4 | Outcomes on HCC evolution after matching.

Outcomes	No TIPS, N = 3,363	TIPS, N = 1,121	p-value[a]
TTV at listing (cm³), Mean (SD)	12.23 (18.0)	12.70 (16.1)	0.41
TTV change (cm³/month), Mean (SD)	0.11 (13.2)	0.28 (6.5)	0.58
Number of tumors at listing, Mean (SD)	1.27 (0.5)	1.28 (0.6)	0.60
Number of tumors change (unit/year), Mean (SD)	0.04 (6.5)	−0.24 (1.9)	0.028
AFP at listing (ng/mL), Mean (SD)	55.38 (262.5)	56.27 (334.3)	0.94
AFP change (ng/mL per month), Mean (SD)	20.67 (328.3)	14.37 (177.9)	0.42

[a]*Welch Two Sample t-test.*
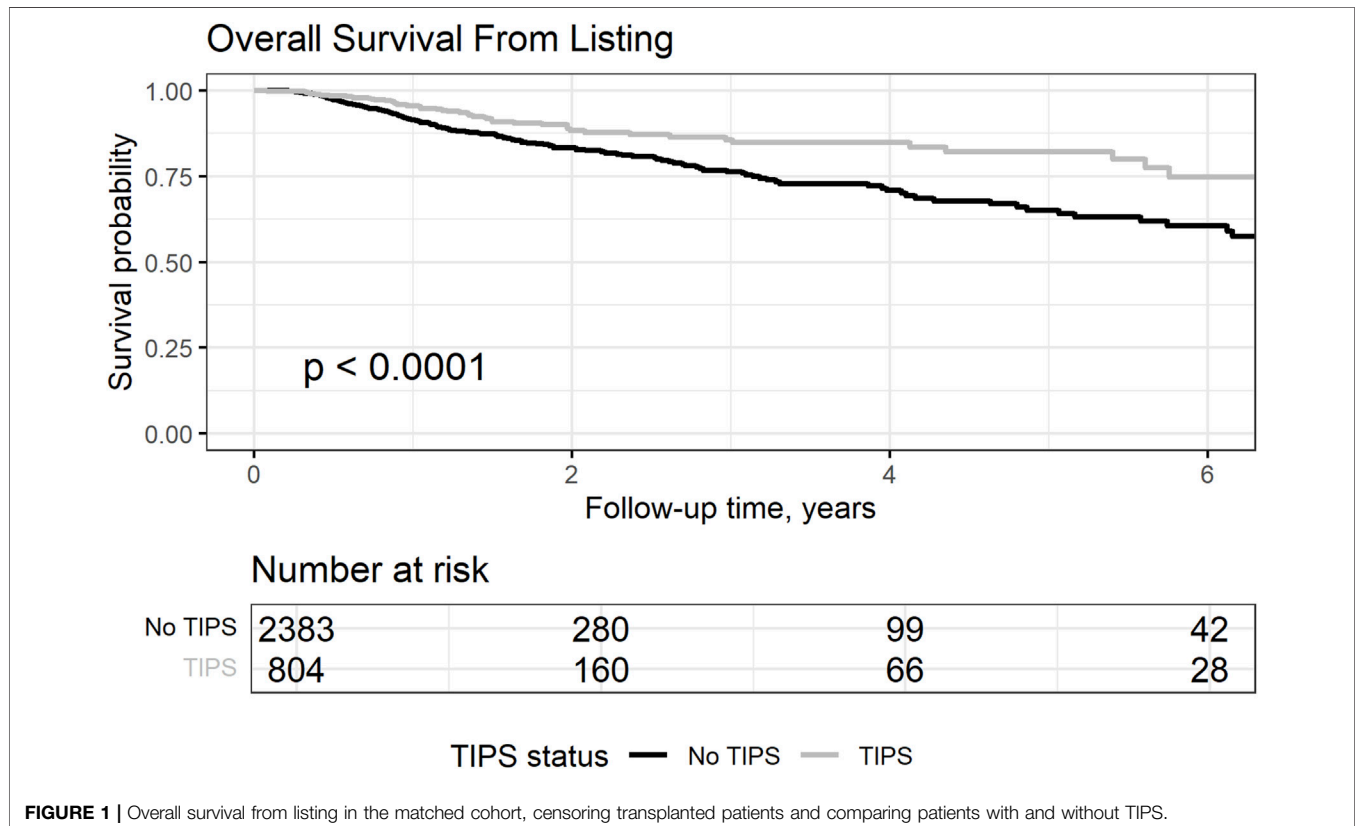
FIGURE 1 | Overall survival from listing in the matched cohort, censoring transplanted patients and comparing patients with and without TIPS.

10 years accounted for 95.6%, 82.1%, and 66%, respectively, in the TIPS and 91.5%, 65.1%, and 52%, respectively, in the no-TIPS group (log-rank test: $p < 0.0001$), as shown in **Figure 1**. Despite a longer waiting time to transplant for the TIPS group, which was 324 days (IQR 210; 607) compared to 272 days (IQR 191; 463) for the non-TIPS group (Wilcoxon test $p < 0.001$), survival rates were notably higher in the TIPS group.

We further explored the causes of the observed differences in the survival rates. Removal from the waitlist concerned 26% (N = 268) of TIPS and 24% (N = 888) of non-TIPS patients. Among them, 50% (N = 135) of TIPS vs. 46% (N = 408) of non-TIPS patients were too ill to be transplanted, and 23% (N = 56) of TIPS vs. 29% (N = 224) of non-TIPS patients died. When exploring the causes of death, hemorrhage-related death was more

frequent in the non-TIPS group (1.8%, N = 1 in TIPS and 7.6%, N = 17 in non-TIPS patients).

Post-Transplantation Outcomes

Analysis of post-transplant overall survival rates revealed no statistically significant difference between patients with and without TIPS (**Figure 2**). At 1, 5, and 10 years post-transplant, survival rates were comparable between the TIPS (92.6%, 79.6%, and 68.8%, respectively) and no-TIPS group (93.4%, 78.3%, and 67.1%, respectively, $p = 0.39$). The time of follow-up from listing was also similar (5.14 versus 4.88 years, $p = 0.14$). These results suggest that TIPS does not affect post-transplantation survival in patients with HCC, which aligns with previous data [19, 20].

Considering the observed HCC dynamics while on the waitlist, we further explored whether this could have an impact on the risk

FIGURE 2 | Overall survival from transplantation in the matched cohort comparing patient with and without TIPS.

FIGURE 3 | Competed cumulative risk incidence of HCC recurrence comparing patients with and without TIPS. Gray's test *p* = 0.14.
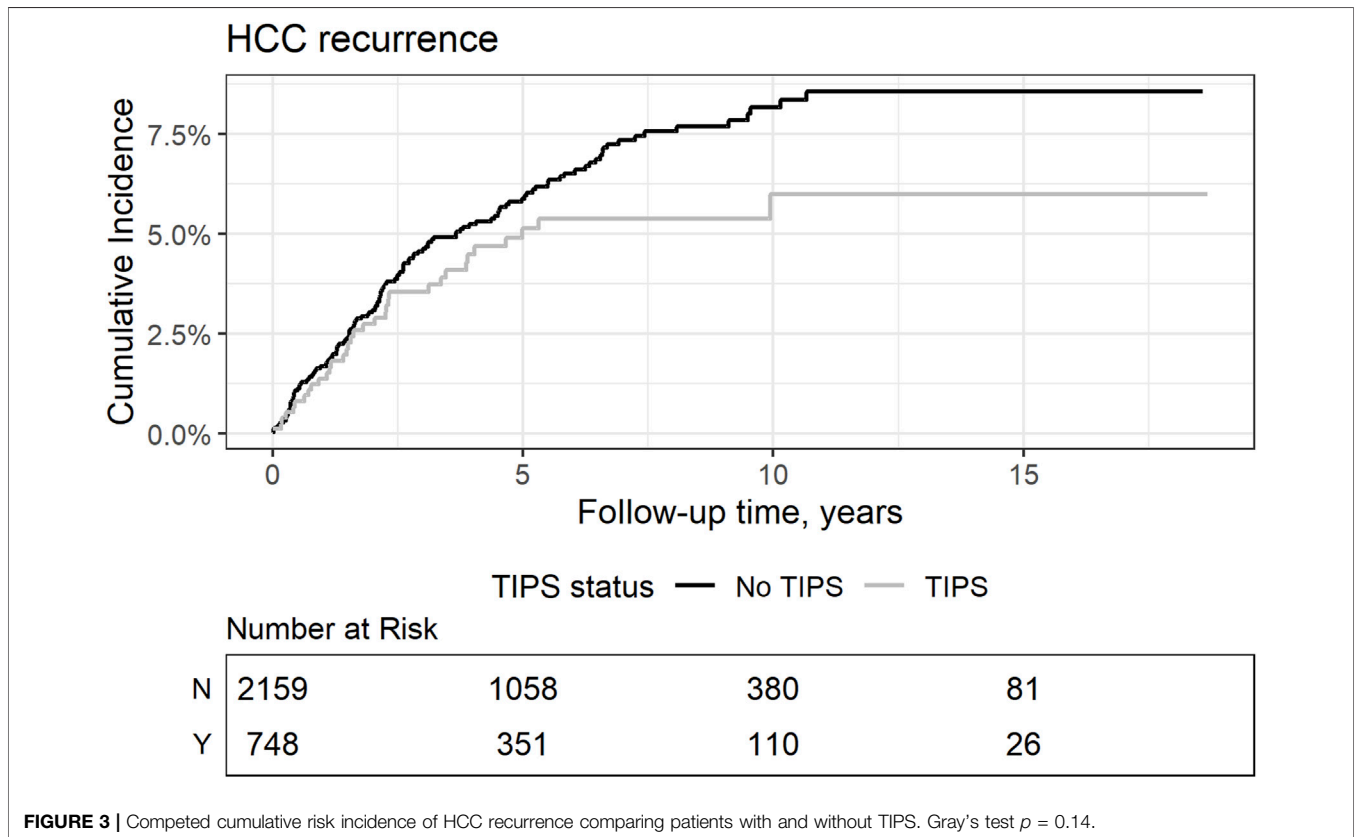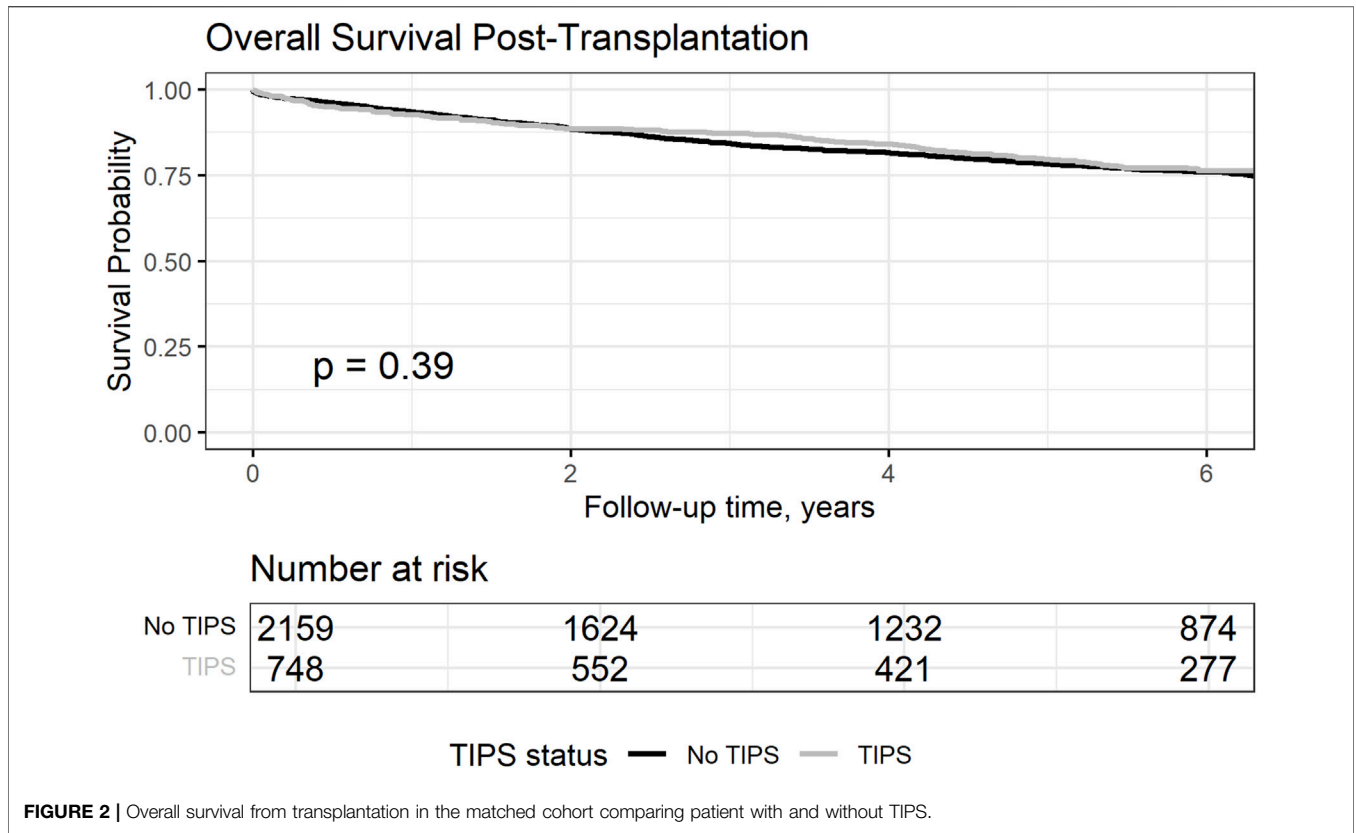
of posttransplant HCC recurrence (**Figure 3**). The cumulative risk incidence of post-transplant HCC recurrence at 5 years was similar between the groups (5.1% vs. 5.9% without TIPS, Gray's test, $p = 0.14$).

DISCUSSION

Our study contributes to the growing body of literature that explores the potential impact of TIPS on HCC. Utilizing a large patient cohort, we present novel insights into the specific advantages conferred by TIPS, especially in the context of tumor burden and survival dynamics among patients awaiting transplantation. A significant finding in our study is that patients with TIPS not only exhibited improved survival while on the waiting list, but also a reduction in the number of HCC nodules. Furthermore, TIPS was not associated with a significant impact on HCC volume or AFP changes.

In line with previous studies [19-24] our findings highlight the benefits of TIPS placement on survival outcomes. This effect may stem from its efficacy in alleviating portal hypertension, enabling concurrent treatment, or reducing bleeding events, potentially serving as a bridge to liver transplantation [22, 25]. Conversely, in cases of advanced HCC, other studies have found that TIPS significantly improved OS by reducing bleeding episodes [26]. However, when assessing TACE efficacy specifically in HCC patients with TIPS, Kuo et al. [10] observed reduced efficacy and shorter overall survival (OS) in the TIPS group. A full understanding of how TIPS influences HCC behavior and treatment response requires further cellular-level investigations that may help establish a conclusive link between TIPS placement and enhanced overall survival.

Prediction models have been developed to examine HCC recurrence after liver transplantation, focusing on factors such as nodule count, size, AFP levels, and vascular invasion, among others [27, 28]. Although the effect of TIPS on posttransplant recurrence has not been extensively explored, our study highlights that TIPS does not affect the risk of HCC recurrence.

Consistent with our results, a meta-analysis of 859 patients by Chen et al. [6] reported that TIPS placement did not increase the risk of HCC development among patients with cirrhosis. This might be due to the reduced proliferative activity of hepatocytes observed after TIPS placement, as reported by Delhaye et al. [29] In contrast, two different studies investigated the impact of TIPS on hepatic blood flow [30, 31] noted increased hepatic blood flow, particularly during the arterial phase of imaging. This observation raises concerns about potential HCC growth subsequent to arterialization of the liver. However, to our knowledge, a direct correlation between TIPS placement and HCC growth has not been established.

The significant difference in OS between the TIPS and non-TIPS groups is noteworthy. This highlights the effectiveness of TIPS as a bridging therapy to enhance life expectancy even in the presence of HCC. The decrease in hemorrhage-related deaths in the TIPS group further supports this notion, indicating the role of this procedure in mitigating the risks associated with portal hypertension.

The precise mechanisms by which TIPS modifies the liver parenchyma and HCC dynamics remain only partially understood. Further histopathological investigations should be performed to understand how TIPS modifies liver vascularization, enabling a more comprehensive treatment strategy for these patients.

Although our study employed propensity score matching, the potential for unmeasured confounders remains a limitation. Moreover, the presence of missing data in our analysis indicates the need for more comprehensive data collection in future studies, including the date of TIPS placement and its correlation with HCC appearance, which could offer insights into the immediate complications of the procedure and potential cancer dissemination in cases of misplacement. Eventually, we acknowledge the heterogenous nature of the SRTR dataset and the potential bias introduced by varying levels of experience and expertise across different centers. Experienced interventional radiology teams could indeed influence the outcomes observed in the TIPS group and future analyses should be include this confounding factor. Future prospective studies are required to validate our findings and to further elucidate the nuanced effects of TIPS on HCC behavior.

In conclusion, our findings support the general beneficial use of TIPS in HCC patients. Although the procedure may stabilize or decrease new tumor formation, it appears that it does not affect HCC growth according to our analyses. Coupled with the observed reduction in hemorrhage-related deaths and improved overall survival, TIPS has emerged as an efficient intervention, particularly for patients awaiting liver transplantation. However, establishing the definitive benefits and risks of TIPS in these patients should be accomplished in future prospective studies.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The data extracted from the SRTR research database is maintained by HHRI solely for the use of the author. The recipient of released data will abide by the terms stated in the Agreement Clauses. Requests to access these datasets should be directed to sofia.elhaijji@hcuge.ch.

ETHICS STATEMENT

The studies involving humans were approved by the Scientific Registry of Transplant Recipients under the Health Resources and Services Administration (HRSA) of the U.S. Department of Health and Human Services (HHS), through the Chronic Disease Research Group (CDRG) of the Hennepin Healthcare Research Institute (HHRI), with offices at 914 S. 8th Street, Suite S4.100, Minneapolis, Minnesota 55404. The studies were conducted in

accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

SE led the data collection, analysis, and wrote the manuscript. CT co-developed the study design and contributed to manuscript writing and revisions. SL and BM assisted in data interpretation and provided manuscript feedback. FC and PC reviewed and revised the manuscript, ensuring accuracy and clarity. Each member played a key role in their respective areas, collaboratively advancing the study. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

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Effect of Donor Cigarette Smoking in Kidney Transplantation: Re-Evaluation of Long-Term Outcomes

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Cigarette smoking is a common risk factor associated with negative long-term outcomes in kidney transplant recipients. However, whether donor smoking decreases graft longevity or negatively impacts recipient survival after kidney transplantation remains unknown. Therefore, this study aims to investigate the long-term outcome in patients who received a kidney graft from a deceased smoking or non-smoking donor. A total of 580 patients were divided into two groups: patients who received a graft from a smoking donor ($n = 276$) and those who received a graft from a non-smoking donor ($n = 304$). Analysis of demographic factors showed that the non-smoking cohort was older, had more extended criteria donors and longer warm ischemia times. The primary composite endpoint of patient and graft survival was better in the smoking donor cohort when analyzed using Kaplan-Meier method but not when controlled for covariates in multivariate analyses. These findings do not support a previously reported negative impact of deceased donor smoking on kidney transplant recipients. Thus, the underlying results should not be interpreted in favor of a positive donor smoking history, but rather remind the transplant community that donor smoking should not be considered as a deciding factor in refusing an otherwise acceptable kidney graft.

Keywords: kidney transplantation, donor criteria, smoking, graft survival, patient survival

INTRODUCTION

Critical evaluation of donor-associated characteristics in kidney transplantation (KTX) represents an ever-growing topic since the ongoing paucity of kidney grafts remains a cardinal problem in transplant medicine. This demands optimal utilization of every potentially suitable organ. Nevertheless, a high percentage of kidney grafts is still discarded, and several donor-associated characteristics have been identified that contribute to this, including donor age, diabetes, hypertension, and death from cerebrovascular accidents [1, 2]. However, for other donor-associated characteristics, one faces the dilemma of a yet not fully elucidated impact on outcomes following KTX. This eventually results in discarding suitable kidney grafts, further contributing to the ever-growing organ shortage. Nevertheless, potentially harmful donor-associated characteristics pose a risk for impaired outcomes after KTX and should, therefore, be avoided [3]. Donor smoking (DS) is a common and thus highly relevant potential donor-associated risk factor that has only been poorly studied for its impact on long-term outcomes post-KTX.

Effect of Donor Cigarette Smoking in Kidney Transplantation: Re-evaluation of Long-term Outcomes

Whether smoking in deceased kidney donors significantly decreases graft longevity or negatively impacts recipient survival post-kidney transplantation is currently unknown

Study design

Retrospective single center cohort study

580 patients

276 (47.6%) : graft from a smoking donor (DS+)

304 (52.4%): graft from a non-smoking donor (DS-)

Follow up: 36 months

The DS- cohort had a higher WIT, older age, poorer HLA matching and a higher ECD rate, which may have negatively affected patient and graft survival

No evidence of inferiority of grafts from cigarette-smoking donors. Caution in declining kidney allografts that are potentially suitable but do have a positive smoking status

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GRAPHICAL ABSTRACT | Created with BioRender.com.

The World Health Organization reports that 22.3% of the world's population used tobacco in 2020, making it the leading risk factor for death among men [4, 5]. In particular, a high prevalence of smoking (15.7% in 2018) has been observed over the past 20 years among 55–64 olds, who represent the majority of today's donor cohort [6]. There is a large body of evidence linking cigarette smoking in KTX recipients to multiple adverse events, including an increased likelihood of cardiovascular events, risk of death and graft loss [7]. A negative smoking history or smoking cessation, even after the start of renal replacement therapy, is highly beneficial, as a 5-year smoking cessation before KTX has been shown to reduce the risk of graft failure [8]. While cigarette smoking in KTX recipients impairs patient and graft survival and long-term functional outcomes [7, 9], data for kidney recipients who received a graft from a smoking donor is still limited. Only a few studies have investigated the impact of DS in KTX and have reported inconsistent results regarding graft and recipient survival [10–13]. Of interest, none of these studies were conducted within the Eurotransplant (ET) region, solely used brain-dead donors, or included patients from the last decade.

Although there is little evidence that the quality of kidney grafts from smoking donors is compromised, DS is among the factors that significantly increase the discard odds for kidney grafts [14]. One possible explanation is that smoking is associated with the development of glomerulosclerosis and the progression of pre-existing renal diseases. As recently confirmed by Ataka et al., the rate of glomerulosclerosis was increased in smoking living kidney donors [15]. Nevertheless, the significance of these pathological changes is still unclear for long-term outcomes after

KTX, especially in deceased donors [14]. Additionally, smoking is associated with the development of arteriosclerosis, which could be a crucial factor in discarding organs from smoking donors, at least from a surgical point of view [16]. A high likelihood of arteriosclerosis represents a technical challenge and thus increases the risk of prolonged warm ischemia and early graft loss due to vascular complications.

Notably, whether smoking in deceased kidney donors significantly decreases graft longevity or negatively impacts recipient survival post-KTX remains unknown. Therefore, there is an unmet need for further investigation and the practice of discarding kidneys from cigarette-smoking donors, regardless of organ quality, should be critically re-evaluated. Hence, this study aims to investigate the effects of DS on long-term patient, graft, and functional outcomes post-KTX in a contemporary cohort from the ET region.

MATERIALS AND METHODS

Study Design and Study Population

The study design was a retrospective single-center cohort study with a 36 months follow-up. The initial study population comprised patients who received a kidney graft at the University Hospital Münster, Germany, between 2006 and 2016. Patients were screened for inclusion if they met the eligibility criteria of being over 18 years of age, transplanted with a post-mortem donated kidney, and without combined organ transplantation. A total of 1,122 patients were identified, of whom 542 were excluded due to insufficient donor or recipient

FIGURE 1 | Study design and patient selection within the underlying retrospective cohort study, including a 36-month follow-up. A total of 580 patients met the following inclusion criteria: kidney transplantation after brain-dead donation, donor or recipient age above 18 years, and a complete donor and recipient dataset. Patients were stratified into two groups: (1) patients receiving a graft from a smoking donor (DS+) and (2) patients receiving a graft from a non-smoking donor (DS−).

data or not meeting the inclusion criteria (**Figure 1**). The remaining 580 patients met the eligibility criteria and were further stratified into two groups: 1) patients who received a graft from a smoking donor (DS+) and 2) patients who received a graft from a non-smoking donor (DS−). All the data used in the analysis were de-identified. Written informed consent was weaved because the study was a retrospective chart review. The study was conducted in accordance with the ethical principles in the Declaration of Helsinki. The local ethics committee approved the conduct of the study (Ethik-Kommission Westfalen-Lippe, permit number: 2021-788-f-S).

Patient Cohort and Outcome Characteristics

Only kidney grafts from brain-dead donors were included in the study. All grafts were procured on behalf of ET, and donor characteristics were obtained from the Eurotransplant Network Information System (ENIS). Recipient data were collected retrospectively from a prospective clinical database. Donor characteristics included age, sex, body mass index (BMI), cardiopulmonary resuscitation (CPR), duration of CPR (in minutes), presence of hypertension or diabetes mellitus, cold and warm ischemia time (WIT), need for vasopressors during donor evaluation, length of stay in the intensive care unit prior to donation, highest and most recent (at time of procurement) serum creatinine (sCr) levels (in μmol/L) during donor evaluation, diuresis before donation, cytomegalovirus (CMV) status, human leukocyte antigen (HLA) mismatch, and presence of more than one renal artery. Additionally, the kidney donor risk index (KDRI) and kidney donor profile index (KDPI) were calculated using the known variables [17]. Extended criteria donor (ECD) status was defined as age ≥60 years or 50–59 years with at least two of the following

conditions: a history of hypertension, a sCr level of 1.5 mg/dL, and a cerebrovascular cause of death. Recipient characteristics involved age, sex, dialysis vintage, history of hypertension, and the reason for end-stage kidney disease (ESKD).

Outcome Parameters

A composite endpoint (event-free survival) was defined as the primary endpoint and included graft loss and patient survival. Graft loss was defined as the need to reinitiate dialysis. The primary endpoint was estimated using the Kaplan-Meier method and compared using the log-rank test.

The postoperative routine follow-up was conducted three (baseline), 6, 12, 24, and 36 months post-KTX. Blood and urine samples were collected immediately postoperatively and during routine follow-up. Renal function was defined as a secondary outcome parameter and was measured by the estimated glomerular filtration rate (eGFR; mL/h/1.73 kg^2 and estimated using the Chronic Kidney Disease Epidemiology Collaboration [CKD EPI] formula), protein excretion (PE) per day (mg/d), and urine protein/creatinine ratio (UPCR, mg/g creatinine). Other secondary outcome measures included primary non-function (PNF, defined as the need for continued dialysis within 90 days after KTX), delayed graft function (DGF, defined as any need for dialysis within the first week after KTX), biopsy-proven acute rejection, new onset of diabetes after transplantation, and the following cardiovascular events: myocardial infarction, angina pectoris, coronary artery revascularization, or congestive heart failure after transplantation.

Statistical Analysis

Group comparisons were performed using the Mann-Whitney U test for not normally distributed data, Fisher's exact test for categorical variables, and the Student's t-test for normally distributed data. Normally distributed continuous variables (tested by the Kolmogorov-Smirnov test) were shown as mean with standard deviation (SD), and not normally distributed continuous variables were presented as median with interquartile range (IQR). The probability of event-free survival, which included patient survival and the probability of graft loss, was estimated using the Kaplan-Meier method, and all three endpoints were compared using the log-rank test (for p-values ≤ 0.05). Recipient kidney function (eGFR) was analyzed using a mixed model for repeated measurements. Time points in each group were compared using a one-way analysis of variance (ANOVA). Additionally, the DS+ group was compared to the DS− group within each time point. All p-values were adjusted using the Holm-Šídák method. Results are presented as the median and a 95% confidence interval. Cox proportional hazards regression models were fitted to determine the influence of donor variables (smoking, age, cold ischemia time, warm ischemia time, CPR, sCr at procurement, hypertension, diabetes mellitus, ECD and KDPI) on event-free survival, patient survival, graft loss, as well as reduced renal function (transformed to a dichotomous endpoint of eGFR </ > 30 mL/h/1.73 kg^2). To solely focus on donor variables, recipient characteristics were omitted in the Cox proportional hazards

TABLE 1 | Donor characteristics.

	DS− *n* = 304	DS+ *n* = 276	*p*-value
Age (years, mean ± SD)	56.79 ± 16.23	51.11 ± 12.04	**<0.001**[a]
Sex (n, % males)	143 (47.0)	144 (52.2)	0.217[b]
Body mass index [kg/m^2, median (IQR)]	26.0 (24.0; 28.0)	26.0 (24.0; 29.0)	0.738[c]
Cardiopulmonary resuscitation (n, %)	58 (19.1)	69 (25.0)	0.085[b]
Duration of cardiac arrest [min, median (IQR)]	20.00 (10.00; 46.25)	20.00 (10.00; 40.00)	0.401[c]
Hypertension (n, %)	98 (32.2)	88 (31.9)	0.928[b]
Diabetes mellitus (n, %)	32 (10.5)	21 (7.6)	0.223[b]
Cold ischemia time [h, median, (IQR)]	10.03 (7.19; 13.40)	11.00 (8.09; 13.40)	0.126[c]
Kidney donor profile index [median, (IQR)]	67.00 (46.00; 87.00)	69.50 (46.00; 91.00)	0.566[c]
Kidney donor risk index [median, (IQR)]	1.20 (0.97; 1.54)	1.21 (0.98; 1.63)	0.583[c]
Extended criteria donors (n, %)	174 (57.2)	115 (41.7)	**<0.001**[b]
Perioperative vasopressors (n, %)	44 (14.5)	30 (10.9)	0.194[b]
Time at intensive care unit prior to donation [days, median, (IQR)]	3.0 (2.0; 6.0)	3.0 (2.0; 7.0)	0.821[c]
Diuresis prior to donation [m/h, median (IQR)]	160.0 (108.3; 221.6)	159.9 (100.0; 229.9)	0.752[c]
Cytomegalovirus risk status			0.518[b]
low (n, %)	98 (32.2)	100 (36.2)	
Intermediate (n, %)	79 (26.0)	63 (22.8)	
High (n, %)	127 (41.8)	112 (40.6)	
Human leukocyte antigen mismatch			
0 (n, %)	49 (16.1)	54 (19.6)	**0.017**[b]
1–3 (n, %)	151 (49.7)	157 (56.9)	
4–6 (n, %)	103 (33.9)	64 (23.2)	
Multiple renal arteries (>1)			
(n, %)	59 (19.4)	62 (22.6)	0.454[b]

Results are presented as mean ± standard deviation (SD), median with interquartile range (IQR), or relative frequency. Cytomegalovirus risk status based on donor (d) and recipient (r) status: low = d−/r−, intermediate = d−/r+ or d+/r+, high = d+/r−.
[a]*Student's t-test.*
[b]*Chi-square test.*
[c]*Mann-Whitney U test. Significant p values are highlighted in bold for clarity.*

regression models. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated. All statistical analyses and graphics were performed using IBM SPSS® Statistics 24 for Windows (IBM Corporation, Somers, NY, United States) and GraphPad Prism 10 software for Windows (GraphPad Software, CA, United States).

RESULTS

Five hundred and eighty patients were found eligible and were further stratified based on the history of smoking in the deceased donor. Within the study's cohort, 276 patients (47.6%) received a graft from a smoking donor, and 304 patients (52.4%) received a graft from a non-smoking donor (**Figure 1**).

With respect to demographic parameters, kidney donors in the DS+ and DS− cohorts were largely comparable (**Table 1**). However, the DS− group was older (56.8 vs. 51.1 years; *p* < 0.001), included more ECD donors (*n* = 174 [57.2%] vs. *n* = 115 [41.7%]; *p* < 0.001), and showed a less favorable HLA mismatch (**Table 1**). Baseline recipient demographics were also largely comparable (**Table 2**). However, recipients in the DS− cohort were significantly more often diagnosed with chronic pyelonephritis compared to the DS+ group.

When analyzing the primary endpoint, a higher probability of event-free survival (combined patient and graft survival) was

observed in the DS+ group compared to the DS− group (*p* = 0.004) (**Figure 2A**). Interestingly, long-term patient survival did not differ significantly between both groups (*p* = 0.072) (**Figure 2B**). Nevertheless, the probability of graft loss was higher in patients who received a DS− graft than in those who received a DS+ graft (*p* = 0.024) (**Figure 2C**).

The DS+ and the DS− cohorts exhibited comparable renal function at 3, 6, 12, 24, and 36 months after KTX (**Figure 3**). However, significantly higher eGFR rates were observed in the DS− cohort at 6 months after KTX compared to the 3-month baseline (*p* = 0.022). Similarly, renal function after KTX, estimated by PE and UPCR at 1, 2 and 3 years after KTX, demonstrated comparable results for the DS+ and the DS− groups (**Table 3**). Comparison of the additional secondary endpoints showed no differences between the DS+ and DS− cohorts in the incidence of DGF, PNF, biopsy-proven rejection, new-onset of diabetes after transplantation, or cardiovascular events after KTX (**Table 3**).

Since DS is thought to be associated with the development of macroscopic renal artery arteriosclerosis, implantation times were analyzed for the DS− (**Figure 4A**) and DS+ (**Figure 4B**) cohorts. **Figure 4C** illustrates that WIT was longer in the DS− cohort (35.0 min vs. 33.5 min; *p* = 0.047). Additionally, donor arteriosclerosis might pose a technical challenge when conducting arterial anastomosis, subsequently resulting in technical and thrombotic vascular complications. Therefore,

	DS– n = 304	DS+ n = 276	p-value
Age (mean ± SD)	57.78 ± 12.94	54.05 ± 11.71	0.171[a]
Sex (n, % male)	188 (61.8)	171 (62.0)	0.977[b]
Dialysis vintage [months, median, (IQR)]	58.0 (33.0; 88.0)	78.0 (48.0; 99.75)	0.199[c]
Hypertension before transplantation (n, %)	267 (87.8)	246 (89.1)	0.624[b]
Diagnosis of end-stage renal disease (n, %)			
Glomerulonephritis	101 (33.2)	99 (35.9)	0.541[d]
Diabetic nephropathy	23 (7.6)	28 (10.1)	0.306[d]
Hypertensive nephropathy	21 (6.9)	18 (6,5)	0.870[d]
Obstructive nephropathy	3 (1.0)	2 (0.7)	>0.999[d]
Fokal segmental glomeruloslerosis	14 (4.6)	13 (4.7)	>0.999[d]
Interstitial nephritis	9 (3.0)	17 (6.2)	0.072[d]
Vasculitis	6 (2.0)	4 (1.4)	0.755[d]
Chronic pyelonephritis	18 (5.9)	4 (1.4)	**0.005**[d]
Alport Syndrome	3 (1.0)	6 (2.2)	0.321[d]
Autosomal dominant polycystic kidney disease 2	38 (12.5)	35 (12.7)	>0.999[d]
Benign nephrosclerosis	5 (1.6)	6 (2.2)	0.764[d]
Other	62 (20.4)	42 (15.2)	0.129[d]

Results are presented as mean ± standard deviation (SD), median with interquartile range (IQR), or relative frequency.
[a]*Student's t-test.*
[b]*Chi-square test.*
[c]*Mann-Whitney U test.*
[d]*Fischer's exact test. Significant p values are highlighted in bold for clarity.*

the proportion of vascular complications (including postoperative bleeding and vascular occlusion) was further analyzed (**Figure 4D**). Overall, a low rate of vascular complications leading to graft loss within 90 days was present in both cohorts. Interestingly, the relative number of graft losses due to vascular complications was higher in the DS– group (64.3%) compared to the DS+ cohort (42.9%); however, the comparison was not noticeable ($p = 0.397$).

Univariate and multivariate Cox regression models were used to analyze independent donor-associated risk factors. The following endpoints were explored: event-free survival (including patient and graft survival), patient survival, graft survival, and marginal renal function (eGFR <30 mL/h/1.73 m^2, **Tables 4–7**). As presented in **Table 4**, the Cox regression model revealed that DS status was associated with event-free survival in the univariate analysis (HR [0.48; 0.29–0.80], $p = 0.005$), but did not reach statistical significance in multivariate analysis (HR [0.62; 0.35–1.09], $p = 0.095$). Similar, DS positively affected the probability of graft loss in the univariate analysis (HR [0.43; 0.21–0.86], $p = 0.017$), but did not reach statistical significance in multivariate analysis (**Table 5**). DS status was not associated with patient survival (**Table 6**). Furthermore, regarding renal function, DS+ status was associated with better (eGFR >30 mL/h/1.73 m^2) graft function in the univariate analysis (HR [0.55; 0.38–0.80], $p = 0.002$), but did not reach statistical significance in multivariate analysis (**Table 7**).

Donor age (univariate analysis (HR [1.03; 1.01–1.05], $p < 0.001$), multivariate analysis (HR [1.03; 1.01–1.05], $p = 0.003$)) and ECD status (univariate analysis (HR [1.83; 1.08–3.10], $p = 0.025$), multi-variate analysis (HR [1.96; 1.12–3.43], $p = 0.019$)) were significantly associated with worse event-free survival in the Coy regression analyses (**Table 4**). Similarly, donor age

contributed to a higher probability of graft loss in univariate (HR [1.04; 1.02–1.07], $p = 0.001$) and multivariate (HR [1.04; 1.01–1.07], $p = 0.009$) Cox regression models (**Table 5**). Patient survival (**Table 6**) was also negatively influenced by donor age (univariate analysis (HR [1.03; 1.01–1.10], $p = 0.008$), multivariate analysis (HR [1.03; 1.00–1.06], $p = 0.025$)) and ECD status (univariate analysis (HR [2.24; 1.08–4.63], $p = 0.030$), multi-variate analysis (HR [2.38; 1.10–5.13], $p = 0.027$)). Finally, donor age was associated with impaired renal function in univariate (HR [1.05; 1.03–1.06], $p < 0.001$) and multivariate (HR [1.04; 1.02–1.06], $p < 0.001$) analyses (**Table 7**).

DISCUSSION

The shortage of donor organs for kidney transplantation is undoubtedly a pressing issue for the transplant community. Additionally, demographic changes in society and increasingly poor donor quality are leading to a more and more demanding kidney allocation process in which donor-associated characteristics must be critically balanced. Therefore, the aim of this study was to investigate the role of cigarette smoking as a potential donor-associated risk factor and its long-term effects after KTX in a representative and contemporary cohort from the ET area. Over a 36-month follow-up period, this study evaluated 580 patients for survival (patient and graft) and functional outcomes after receiving a kidney allograft from a smoking or non-smoking deceased donor. Overall, this study found no evidence of inferiority of grafts from cigarette-smoking deceased donors. In addition, this study found no affirmation of an increased risk for recipients. In contrast, we observed that the primary composite endpoint of event-free
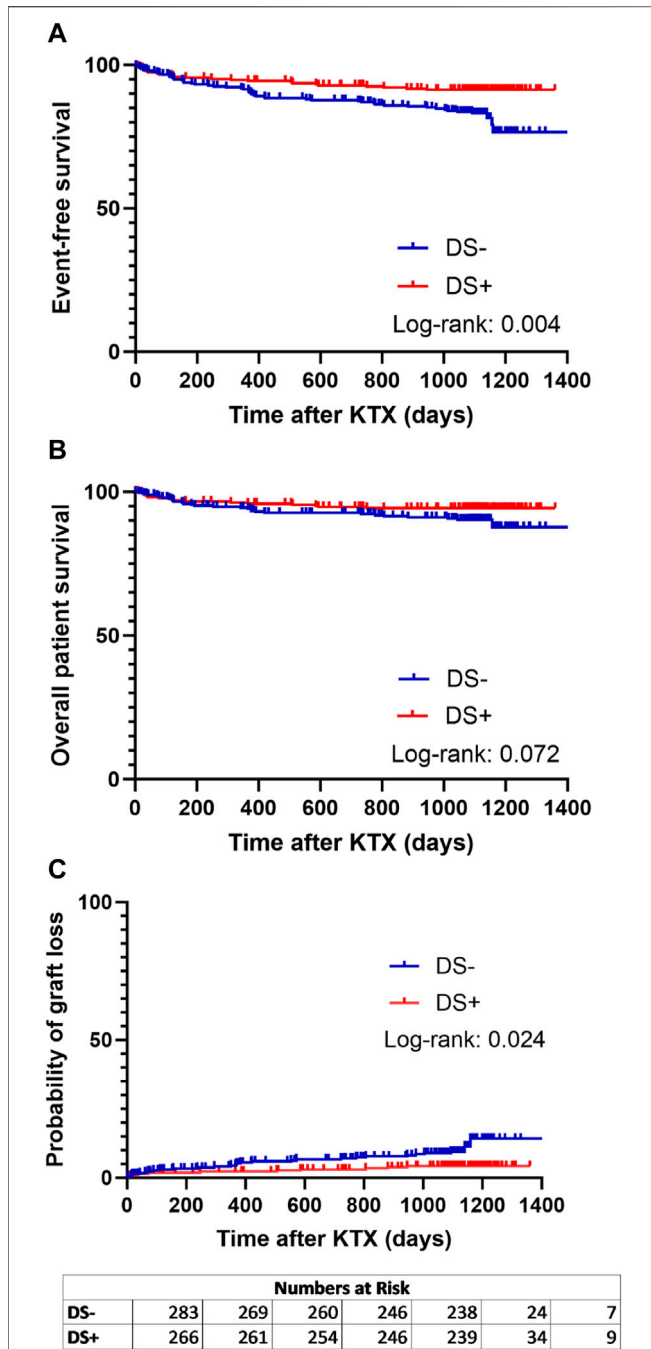
FIGURE 2 | Analysis of event-free survival **(A)** (defined as combined patient and graft survival), **(B)** overall patient survival, and **(C)** probability of graft loss separated for patients receiving a graft from a smoking donor (DS+) and patients receiving a graft from a non-smoking donor (DS−). Survival rates of DS+ (red lines) and DS− (blue lines) recipients following kidney transplantation (KTX) were estimated using Kaplan-Meier methodology and compared using the log-rank test.

FIGURE 3 | Estimated glomerular filtration rate (eGFR mL/min/1.73 m^2) for analysis of post-transplant graft function up to 36 months after KTX. Comparisons of eGFR within each group were conducted using a one-way analysis of variance (ANOVA). The DS+ group was compared to the DS− group within each time point. All p-values were adjusted using the Holm-Šídák method. A p-value less than 0.05 was considered statistically, $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$; $****p \leq 0.0001$.

survival and graft survival was better in the DS+ cohort when analyzed using the Kaplan-Meier method.

One approach to explain the findings in the DS+ group might be the analysis of the DS− baseline characteristics. The DS− cohort had a higher WIT, older age, poorer HLA matching and a higher ECD rate, which may have negatively affected patient and graft survival. In line with this, the postoperative increase in eGFR 6 months after KTX compared to baseline in DS− patients could indicate impaired kidney allograft function in the DS− cohort.
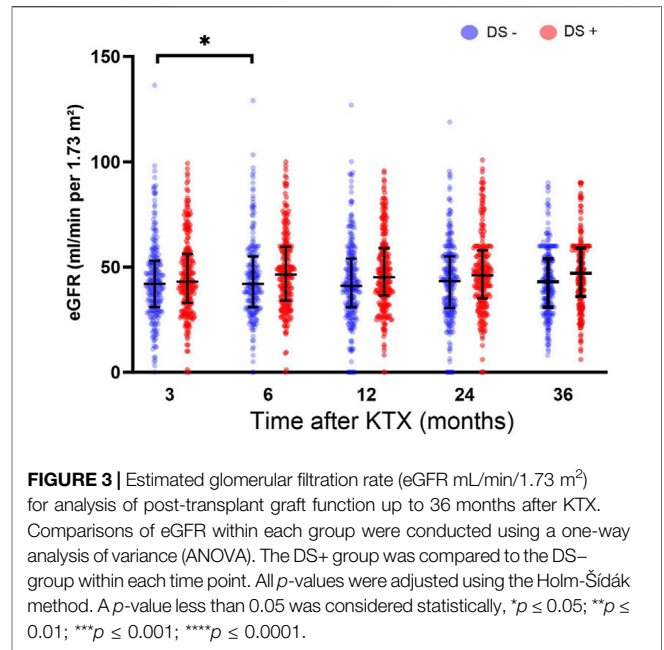
The positive results for the DS+ cohort should not be interpreted in favor of a positive donor smoking history in KTX. Moreover, this demonstrates an inherent and rather worrying bias. One could argue that DS is currently perceived as an additional risk factor, and a smoking history might encourage transplant professionals to decline an offered kidney graft, for which smoking is the tipping point. Hence, one could suggest that if transplant professionals accept a kidney graft from a donor with a history of smoking, other donor-associated factors (e.g., age or HLA matching) must be in favor of using that graft. Accordingly, the observed results of DS as a protective factor associated with improved graft survival should be interpreted with caution, not because of the misinterpretation that DS is protective (for which no logical pathophysiological explanation can be found), but rather because it reflects the direct impact of DS on allocation. We hypothesize that many suitable organs from smoking donors must have been rejected to create such a favorable outcome, as demonstrated in this analysis. Therefore, DS might represent a potentially misleading selection bias in kidney allograft allocating, which is a dilemma in today's era of donor organ shortage and decreasing organ quality, especially, since there is no substantial evidence that DS adversely affects long-term patient or allograft outcomes. Thus, cigarette smoking should not be used as a reason to accept a potentially less suitable donor. More importantly, however, DS should not be considered as a deciding factor in refusing a kidney graft.

TABLE 3 | Secondary endpoints.

	DS− *n* = 304	DS+ *n* = 276	*p*-value
Primary non-function (*n*, %)	11 (3.6)	18 (6.5)	0.089[a]
Delayed graft function (*n*, %)	65 (21.4)	69 (25.0)	0.330[a]
Biopsy-proven acute rejection (*n*, %)	152 (50.0)	129 (46.7)	0.259[a]
New onset of diabetes after transplantation (*n*, %)	40 (13.2)	33 (12.0)	0.176[a]
Cardiovascular event after transplantation (*n*, %)	30 (9.9)	27 (9.8)	0.972[a]
Parameters of kidney function (mean ± SD)			
Protein excretion per day 1 year after KTX (mg/d)	13.41 ± 23.00	14.73 ± 40.04	0.417[b]
Protein excretion per day 2 years after KTX (mg/d)	13.63 ± 26.56	10.90 ± 18.47	0.243[b]
Protein excretion per day 3 years after KTX (mg/d)	16.59 ± 41.78	15.71 ± 39.82	0.972[b]
Urine protein/creatinine ratio 1 year after KTX (mg/g creatinine)	210.6 ± 358.5	236.2 ± 704.6	0.988[b]
Urine protein/creatinine ratio 2 years after KTX (mg/g creatinine)	213.1 ± 419.1	178.7 ± 409.5	0.453[b]
Urine protein/creatinine ratio 3 years after KTX (mg/g creatinine)	224.2 ± 547.5	232.1 ± 626.2	0.928[b]

Results are presented as mean ± standard deviation (SD), median with interquartile range (IQR), or relative frequency.
[a]Chi-square test.
[b]Mixed effects model, p-values were adjusted using the Holm-Šídák method.
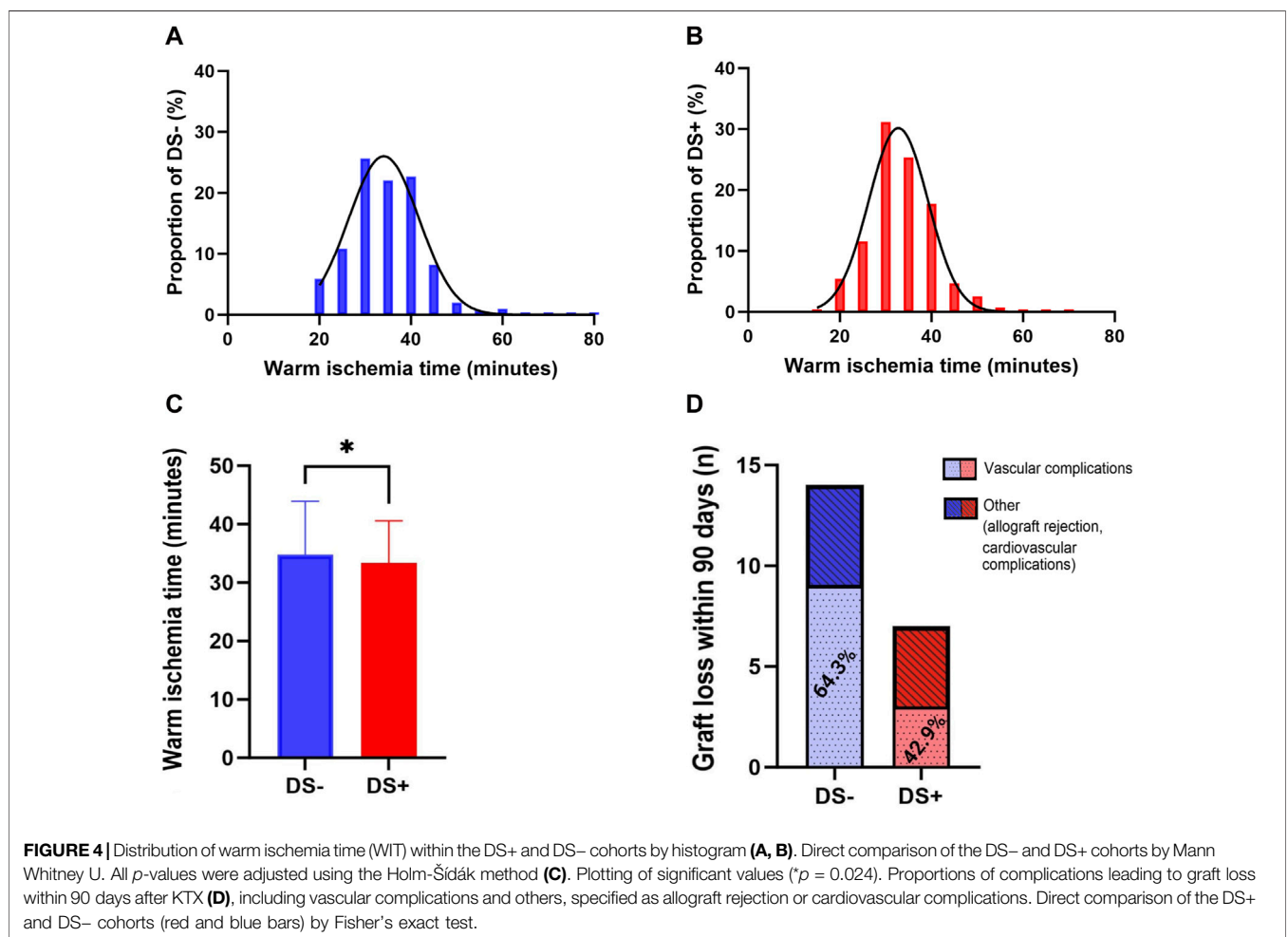
FIGURE 4 | Distribution of warm ischemia time (WIT) within the DS+ and DS− cohorts by histogram **(A, B)**. Direct comparison of the DS− and DS+ cohorts by Mann Whitney U. All *p*-values were adjusted using the Holm-Šídák method **(C)**. Plotting of significant values (*p = 0.024). Proportions of complications leading to graft loss within 90 days after KTX **(D)**, including vascular complications and others, specified as allograft rejection or cardiovascular complications. Direct comparison of the DS+ and DS− cohorts (red and blue bars) by Fisher's exact test.

TABLE 4 | Cox regression model of event-free survival.

Donor characteristics	Univariate		Multivariate	
	p-value	HR (95% CI)	p-value	HR (95% CI)
Smoking (yes/no)	**0.005**	0.48 (0.29–0.80)	0.095	0.62 (0.35–1.09)
Age (years)	**<0.001**	1.03 (1.01–1.05)	**0.003**	1.03 (1.01–1.05)
Cold ischemia time (hours)	0.925	1.00 (0.94–1.06)	0.171	1.04 (0.98–1.11)
Warm ischemia time (min)	0.236	1.02 (1.00–1.04)	0.475	1.01 (0.98–1.04)
Cardiopulmonary resuscitation (yes/no)	0.681	0.88 (0.49–1.59)	0.963	1.015 (0.54–1.90)
Serum creatinine (μmol/L)	0.989	1.00 (1.00–1.00)	0.146	1.00 (1.00–1.00)
Hypertension (yes/no)	0.083	1.52 (0.95–2.45)	0.333	1.30 (0.77–2.19)
Diabetes mellitus (yes/no)	0.782	1.12 (0.51–2.44)	0.776	0.89 (0.39–2.03)
Kidney donor risk index	0.456	1.00 (0.99–1.01)	0.140	0.99 (0.98–1.00)
Extended criteria donor (yes/no)	**0.025**	1.83 (1.08–3.10)	**0.019**	1.96 (1.12–3.43)

HR, Hazard ratios; CI, 95% confidence intervals.
Significant p values are highlighted in bold for clarity.

TABLE 5 | Cox regression model of graft loss.

Donor Characteristics	Univariate		Multivariate	
	p-value	HR (95% CI)	p-value	HR (95% CI)
Smoking (yes/no)	**0.017**	0.43 (0.21–0.86)	0.102	0.51 (0.23–1.14)
Age (years)	**0.001**	1.04 (1.02–1.07)	**0.009**	1.04 (1.01–1.07)
Cold ischemia time (hours)	0.434	0.97 (0.89–1.05)	0.927	1.00 (0.92–1.08)
Warm ischemia time (min)	0.170	1.02 (0.99–1.06)	0.424	1.02 (0.98–1.05)
Cardiopulmonary resuscitation (yes/no)	0.797	1.10 (0.52–2.33)	0.325	1.50 (0.67–3.36)
Serum creatinine (μmol/L)	0.774	1.00 (0.99–1.00)	0.917	1.00 (0.99–1.01)
Hypertension (yes/no)	**0.018**	2.16 (1.14–4.08)	0.253	1.50 (0.75–3.02)
Diabetes mellitus (yes/no)	0.381	1.52 (0.59–3.90)	0.944	1.04 (0.38–2.85)
Kidney donor risk index	0.193	0.99 (0.98–1.00)	0.078	0.99 (0.98–1.00)
Extended criteria donor (yes/no)	0.193	1.59 (0.79–3.20)	0.138	1.76 (0.83–3.69)

HR, Hazard ratios; CI, 95% confidence intervals.
Significant p values are highlighted in bold for clarity.

TABLE 6 | Cox regression model of patient survival.

Donor characteristics	Univariate		Multivariate	
	p-value	HR (95% CI)	p-value	HR (95% CI)
Smoking (yes/no)	0.093	0.57 (0.30–1.10)	0.731	0.88 (0.43–1.81)
Age (years)	**0.008**	1.03 (1.01–1.10)	**0.025**	1.03 (1.00–1.06)
Cold ischemia time (hours)	0.877	0.99 (0.92–1.07)	0.169	1.06 (0.98–1.14)
Warm ischemia time (min)	**0.024**	1.04 (1.01–1.07)	**0.032**	1.03 (1.00–1.06)
Cardiopulmonary resuscitation (yes/no)	0.480	0.75 (0.33–1.69)	0.559	0.77 (0.31–1.88)
Serum creatinine (μmol/L)	0.985	1.00 (1.00–1.00)	0.110	1.00 (1.00–1.01)
Hypertension (yes/no)	0.264	1.43 (0.76–2.70)	0.386	1.36 (0.68–2.70)
Diabetes mellitus (yes/no)	0.833	1.12 (0.34–3.14)	0.854	0.90 (0.30–2.69)
Kidney donor risk index	0.963	1.00 (0.99–1.01)	0.416	0.99 (0.98–1.01)
Extended criteria donor (yes/no)	**0.030**	2.24 (1.08–4.63)	**0.027**	2.38 (1.10–5.13)

HR, Hazard ratios; CI, 95% confidence intervals.
Significant p values are highlighted in bold for clarity.

Although it appears highly unlikely that donor smoking has a direct, causative positive effect on the outcome in our study cohort it is noteworthy that smoking has been found to be protective in other disease. A "smoker's paradox," referring to the decreased mortality in smokers after acute coronary syndrome and stroke, has been described, but the available data is limited, partially questionable and has been refuted by more recent analyses [18, 19]. Nevertheless, there is robust evidence for a protective effect of smoking on the risk of Parkinson's disease [20] and ulcerative colitis [21]. However, plausible biologic mechanisms remain scare. One possible explanation is the immunomodulatory and anti-

TABLE 7 | Cox regression model of renal function.

Donor characteristics	Univariate		Multivariate	
	p-value	HR (95% CI)	p-value	HR (95% CI)
Smoking (yes/no)	**0.002**	0.55 (0.38–0.80)	0.495	0.86 (0.56–1.33)
Age (years)	**<0.001**	1.05 (1.03–1.06)	**<0.001**	1.04 (1.02–1.06)
Cold ischemia time (hours)	**0.043**	0.95 (0.91–1.00)	0.710	1.01 (0.96–1.07)
Warm ischemia time (min)	0.102	1.02 (1.00–1.04)	0.070	1.02 (1.00–1.05)
Cardiopulmonary resuscitation (yes/no)	0.157	0.70 (0.42–1.15)	0.483	0.82 (0.47–1.44)
Serum creatinine (μmol/L)	0.624	1.00 (1.00–1.00)	0.617	1.00 (1.00–1.00)
Hypertension (yes/no)	**0.002**	1.77 (1.23–2.55)	0.077	1.45 (0.96–2.19)
Diabetes mellitus (yes/no)	**0.011**	1.89 (1.16–3.10)	0.845	0.94 (0.51–1.74)
Kidney donor risk index	0.817	1.00 (1.00–1.01)	0.308	0.99 (0.99–1.00)
Extended criteria donor (yes/no)	0.343	1.21 (0.82–1.80)	0.337	1.23 (0.81–1.88)

HR, Hazard ratios; CI, 95% confidence intervals.
Significant p values are highlighted in bold for clarity.

inflammatory effect of nicotine mediated by the activation of nicotinic acetylcholine receptor α7 in immune cells, but it remains questionable if this donor-associated protective mechanism can translate into long-term improvement in the recipient and outweigh the proven negative effects of smoking.

Only a few studies have evaluated smoking as a donor-associated risk factor in KTX, and there is an ambiguity in the current literature. Heldt et al. and Underwood et al. conducted single-center studies to investigate DS in living kidney donation and reported variable results. On the one hand, Heldt et al. showed a significantly lower graft function (GFR) in recipients from smoking donors, whereas Underwood et al. did not demonstrate an effect of DS on graft survival, but observed a negative correlation between DS and recipient survival [12, 13]. Two studies have focused on the effect of DS in deceased donation. Lin et al. demonstrated that DS was associated with an increased risk of graft loss (adjusted HR = 1.05, p = 0.028) and impaired patient survival (adjusted HR = 1.06, p = 0.021) in a retrospective registry analysis (United Network for Organ Sharing dataset) of deceased donors, including non-heart-beating donors, between 1994 and 1999 [10]. Later, Gillott et al. carried out a registry analysis (United Kingdom Transplant Registry, including patients from 2001 to 2013) and confirmed increased recipient mortality in a cohort receiving DS grafts (HR = 1.12, p = 0.044). However, no effect on graft survival was observed [11]. Thus, DS might affect patient-related outcomes more frequently than kidney allograft function and consequently graft survival. Gillott et al. revised possible approaches to explain impaired patient-related outcomes after receiving a kidney allograft from a smoking donor. The authors argue that the association between smoking and endothelial dysfunction might have a synergistic effect with other recipient comorbidities, which could increase mortality. Another possible explanation could be immune-related alterations and interactions that could be associated with increased mortality. Nevertheless, evidence of long-term pathophysiological consequences of DS leading to impaired patient survival remains scarce.

It is paramount to critically compare the above findings with our data. First, our study is the first in the field of ET. This is important because there are well-described and profound differences in demographics, allocation, and patient and graft survival outcomes between the United States, the United Kingdom, and the ET region [22]. Thus, direct comparisons remain difficult. Second, these findings may also indicate a changing role of DS in KTX over time, particularly in the face of donor shortages and increasing rates of ECD. In line with this, when only ECD from the United Network for Organ Sharing dataset was analyzed, no negative effect of DS on graft survival was found [23].

Nevertheless, our data set and analysis have several limitations. First, we do not have adequate information regarding the respective pack years for the DS+ cohort, which would have allowed us to perform a much more granular analysis, calculate a linear relationship, and conduct subgroup analysis stratified by pack years. This is of special interest since smoking-associated histological injury and graft function after KTX depend on the donor's cumulative smoking dose [13, 15]. However, lacking this information adds a more realistic and real-world aspect to our study, as it represents the actual information on which the transplant professionals involved have to base their decision on whether to decline or accept a kidney offer. In addition, there is no official data regarding the smoking prevalence among organ donors within the ET area. Therefore, one could only gauge the possible impact of discarding organs from smokers on the current organ shortage. However, the age-standardized prevalence of smoking among individuals aged 15 years and older in Western Europe is between 22.7% (female) and 28.8% (male) [6]. Since approximately 97% of all kidney donors in the ET area are 15 years and older, this further illustrates the impact of discarding otherwise suitable kidney grafts based on DS. Moreover, our findings need to be evaluated concerning the sample size, which represents an additional limitation of this retrospective cohort study. On the other hand, the recipient cohort included can be regarded as advantageous for this investigation since baseline characteristics or immunosuppression protocols show no

differences. As it has been previously argued that DS exerts its potentially negative effects via the development of glomerulosclerosis in the donor, the availability of implantation biopsies would have also strengthened the study. Unfortunately, our data set has a very low frequency of biopsies, which does not allow further analysis.

CONCLUSION

This retrospective cohort study investigated 580 patients regarding the effect of DS on graft longevity and recipient survival with a 36-month follow-up. We observed a significant improvement in the primary composite endpoint, including patient survival and the probability of graft loss, in the DS+ cohort. However, this favorable effect of DS+ was not noticeable after controlling for other donor-associated factors using multivariate analysis. Thus, this study found no evidence of inferiority of grafts from cigarette-smoking deceased donors and no evidence of an increased risk for recipients. In conclusion, we strongly suggest caution in declining kidney allografts that are potentially suitable but do have a positive cigarette smoking status.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The raw data supporting the conclusion of this article will be made available by the authors upon reasonable request. Requests to access these datasets should be directed to felix.becker@ukmuenster.de.

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SGLT2 Inhibitors Correct Fluid Overload in Adult Kidney Transplant Recipients—A Prospective Observational Study

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In this longitudinal observational study, we measured urinary glucose concentration, body composition and volume status (bioimpedance spectroscopy) and plasma renin and aldosterone concentrations in $n = 22$ kidney transplant recipients (KTRs) initiating on SGLT2I at baseline (BL), and after 1 week and 1, 3, and 6 months. Estimated glomerular filtration rate (eGFR) decreased by -2 mL/min/1.73 m^2 (IQR -10–0) after 1 week and remained stable thereafter. Urinary glucose concentration was 10 (3–24) g/g creatinine after 1 week and correlated with eGFR ($r^2 = 0.273$; $p = 0.057$). SGLT2I did not affect HbA1c, fasting blood glucose, body weight, fat or lean mass. SGLT2I decreased fluid overload dependent on baseline overhydration (OH, $r^2 = 0.54$, $p = 0.0003$) without occurrence of dehydration. Plasma aldosterone increased at day 7, while plasma renin did not change significantly. In conclusion, SGLT2I corrected fluid overload in patients with elevated overhydration at baseline, while in euvolemic KTRs fluid status remained stable without reduction of body water below the reference range, thus promoting the safety of SGLT2I therapy in patients following kidney transplantation. Glucosuria, together with effects of SGLT2I on blood glucose control and body weight, is attenuated in KTRs dependent on eGFR.

Keywords: SGLT2 inhibitor, bioimpedance spectroscopy, kidney transplantation, glucosuria, fluid overload

Abbreviations: ATM, adipose tissue mass; BCM, Body Composition Monitor (Fresenius Medical Care); BL, baseline; BMI, body mass index; CKD, chronic kidney disease; ECW, extracellular water; eGFR, estimated glomerular filtration rate, estimated with MDRD formula; FTI, fat tissue index; FU, follow up; ICW, intracellular water; KTRs, kidney transplant recipients; LTI, lean tissue index; LTM, lean tissue mass; OH, overhydration; PTDM, post-transplantation diabetes mellitus; RAAS, renin angiotensin aldosterone system; SGLT2I, SGLT2 inhibitors; TBW, total body water.

GRAPHICAL ABSTRACT

INTRODUCTION

Comprehensive care of kidney transplant recipients (KTRs) aims to maximize kidney allograft survival and on top of that seeks to reduce the patients' cardiovascular risk while balancing side effects of immunosuppressive therapy, including control of blood glucose, body weight, blood pressure, and fluid status [1]. SGLT2 inhibitors (SGLT2I) have emerged as an effective therapy to reduce proteinuria and progression in patients with chronic kidney disease (CKD) [2, 3]. Owing to their mechanism of action, SGLT2I modulate body weight, body composition and fluid status. In obese patients with type 2 diabetes mellitus and normal kidney function, we have used bioimpedance spectroscopy to investigate changes in body composition underlying the reduction of body weight with SGLT2I and observed a persistent reduction of adipose mass and a transient reduction of extracellular water after a few days [4]. This initial reduction of extracellular fluid was counter regulated by an increase of plasma renin activity and serum aldosterone concentration, and fluid status returned to initial values after 1–3 months [4]. Other studies also found a reduction of adipose mass after initiation of SGLT2I in diabetes patients, and some studies reported a reduction of lean tissue or muscle mass [5]. Latent or apparent fluid overload is frequently present in CKD, and is associated with disease progression, increase in systolic blood pressure and natriuretic peptides as parameters of cardiovascular stress [6]. Bioimpedance spectroscopy measurements of fluid status in CKD patients revealed a reduction of fluid

overload by SGLT2I without occurrence of exsiccosis, again accompanied by activation of renin-angiotensin-aldosterone-system (RAAS) [7, 8].

Despite the numerous cardio- and reno-protective effects of SGLT2I, the use of SGLT2I in the vulnerable cohort of KTRs has not yet been studied in sufficient depth and is therefore still restrictive [9, 10]. Potential concerns in this cohort include the risk of acute kidney injury due to potential interference in volume homeostasis, and urinary tract infections. Use of SGLT2I in KTRs was initially described in several observational studies with encouraging results regarding safety and glycemic control [11–18]. In a small, randomized controlled trial with $n = 49$ patients, empagliflozin was safe, and improved glycemic control in stable KTRs with median eGFR of 66 (range 41–83) mL/min/1.73 m^2 [19]. In a multicenter cohort study from South Korea, SGLT2I were shown to have beneficial effects on graft function in diabetic KTRs [20]. Although the study design was retrospective, this study was the first to demonstrate reno-protective effects in KTRs. More recently, a large observational study confirmed the benefits of SGLT2I treatment in $n = 339$ KTRs with regard to blood glucose control and reduction of proteinuria, and named urinary tract infections as the most frequent adverse event [21]. While this evidence is indicative of reno-protective effects of SGLT2I in KTRs, data on effects of SGLT2I on volume homeostasis after kidney transplantation is sparse. This study therefore investigated the effects of SGLT2I on urinary glucose excretion, body composition and fluid status in KTRs.

PATIENTS AND METHODS

Study Design

Adult kidney transplant recipients from the transplant center outpatient clinic of the University Hospital of Tuebingen who had decided to be treated with SGLT2I between March 2021 and July 2022 were requested to participate in this longitudinal observational study. The decision to embark on an SGLT2I was made solely by the attending transplant nephrologist. Patients were included independently of CKD etiology, the presence or absence of diabetes, and the SGLT2I prescribed. Time since transplantation was at least 6 months. Specified time points for the study visits were baseline (BL, the day of the prescription of the SGLT2I) and 1 week, 1 month, 3 months, and 6 months of follow up (FU) after initiation of the SGLTI.

The study was approved by the local ethics committee of the University of Tuebingen (648/2016BO1). A written informed consent was obtained from all patients. The study was registered at the German Clinical Trials Register (DRKS00028560).

Assessment of Body Composition and Fluid Status

At each study appointment, body composition and fluid status were measured using the Body Composition Monitor (BCM, Fresenius Medical Care). This device uses bioimpedance spectroscopy to detect fluid overload, and was initially developed to help to determine dry weight in patients undergoing dialysis [22]. The BCM differentiates between intra- and extracellular water by measuring bioimpedance with 50 frequencies between 5 and 1,000 kHz, whereby the low frequencies cannot pass cell membranes [23]. The BCM device calculates intracellular water (ICW) as the difference between extracellular (ECW) and total body water (TBW) on the basis of the "body volume model," and parameters of lean and adipose tissue on the basis of the "body composition model." Excess fluid, which is mainly located in the extracellular compartment, is calculated by the BCM device from normally hydrated lean and adipose tissue masses, and is defined as overhydration (OH). Reference values for OH in healthy individuals lie between −1 and +1 L [24]. Values obtained for OH, ECW, ICW, and TBW were normalized to a body surface area of 1.73 m^2.

Laboratory Measurements and Assessment of Urogenital Infections

Laboratory values were determined in the central laboratory (Institute for Clinical Chemistry and Pathobiochemistry) of the University Hospital of Tuebingen. In addition to the standard of care diagnostic parameters after kidney transplantation, plasma renin and aldosterone concentrations were measured in plasma samples and urinary glucose concentration was measured in a spot urine sample. The patients were monitored for urogenital infections by medical history and urinary dipstick.

Statistical Analysis

Parameters are reported with number and percentage (nominal parameters) or median and quartiles (continuous parameters) and illustrated as absolute values or delta values from baseline. Friedman test was performed to test for changes during the complete follow up. Wilcoxon-Mann-Whitney-Test (Wilcoxon signed rank test, referred to in short as Wilcoxon test) for paired samples was performed to test for differences between two respective follow up time points. Bonferroni correction was used to correct for multiple testing. A linear regression model was fitted for univariate correlations.

Statistical significance was defined as a significance threshold of $p < 0.05$. The statistical software packages R studio version 4.1.2 and Microsoft Office Professional Plus 2019 Excel version 1808 were used to perform data analysis.

RESULTS

Characterization of the Study Cohort

A total of 22 patients were included in the study, and follow up after 6 months was available in $n = 19$. Actual time points of all FU visits and number of patients are shown in the flowchart (**Figure 1**). N = 1 patient died during follow up due to age (85 years) and frailty. N = 1 patient terminated dapagliflozin due to an itchy rash on the forehead (temporal relation with the initiation of dapagliflozin, however causal relation uncertain, itching was not completely resolved after termination of dapagliflozin) and urinary tract infection. Other missing values are due to missed study visit of a patient due to the long distance to the transplant outpatient clinic.

Baseline patient characteristics are displayed in **Table 1**. Diabetes mellitus was present in $n = 18$ study patients ($n = 10$ with pre-existing diabetes and $n = 8$ with post-transplantation diabetes mellitus, PTDM) and $n = 2$ patients had elevated HbA1c without manifest diabetes mellitus. Blood glucose control in combination with other expected favorable effects was the main reason for initiation of SGLT2I in $n = 19$ patients. SGLT2I prescribed was dapagliflozin 10 mg in $n = 20$ patients and 5 mg in $n = 2$ patients. Empagliflozin, which was not approved for CKD at the time of the study, was not used in this cohort. N = 10 patients received medication with loop diuretics at baseline ($n = 9$ torasemide, $n = 1$ furosemide).

Allograft Function

Median estimated GFR (eGFR, estimated with MDRD formula) at BL was 39 (IQR 33–58) mL/min/1.73 m^2 (**Table 1**) and decreased by −2 (IQR −10 – 1) mL/min/1.73 m^2 directly after initiation of SGLT2I without further decrease during 6 months of FU (**Table 2**). Median albuminuria at BL was 33 (75–174) mg/g creatinine and tended to decrease but without significant changes during FU (**Table 2**).

Glucose Metabolism and Body Composition

Glucosuria was present from the first FU visit 1 week after initiation of the SGLT2 inhibitor with a median urinary

FIGURE 1 | Flowchart of the study Abbreviations: FU, follow up; SGLT2I, SGLT2 inhibitor.
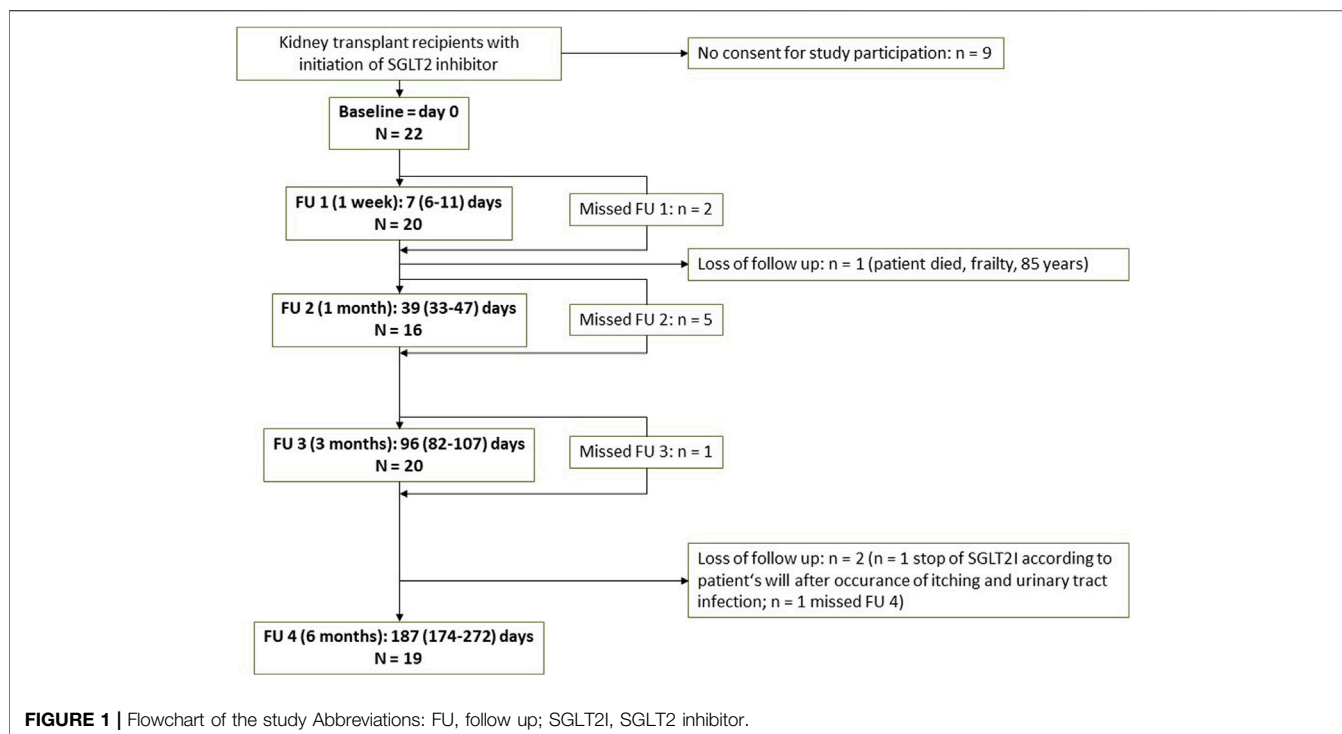
TABLE 1 | Characteristics of the study cohort.

	n	Median (quartiles) or number
Age, years	22	61 (54; 65)
Sex	22	male 16/female 6
Reason for initiation of SGLT2I	22	*n* = 17 therapy of DM + other favorable effects
		n = 2 IgAN recurrence (*n* = 1 also with DM)
		n = 1 PVAN
		n = 2 elevated HbA1c without manifest DM + other favorable effects
Dose of dapagliflozin	22	5 mg 2/10 mg 20
eGFR (MDRD), mL/min/1.73m^2	22	38.6 (33.0–57.5)
Albuminuria, mg/g Crea	21	50 (22; 145)
OH, l/1.73m^2	21	1.3 (0.3; 2.5)
Diuretic therapy	22	No diuretic therapy *n* = 10
		Furosemide *n* = 1
		Torasemide *n* = 9
		Hydrochlorothiazide (HCT) *n* = 2
RAAS inhibitor	22	Ramipril *n* = 7, Enalapril *n* = 1, Candesartan *n* = 10, no *n* = 4
Immunosuppressive therapy	22	Tacrolimus/MMF/Prednisolone 5 mg *n* = 11
		Tacrolimus/MMF *n* = 2
		CSA/MMF/Prednisolone 5 mg *n* = 3
		CSA/MMF *n* = 2
		Sirolimus/MMF/Prednisolone 5 mg *n* = 1
		Sirolimus/Prednisolone 5 mg *n* = 1
		Tacrolimus/Everolimus/Prednisolone 5 mg *n* = 1
		Tacrolimus/Azathioprine *n* = 1
Patients with DM	22	*n* = 18 (pre-existing *n* = 10, PTDM *n* = 8)
HbA1c, %	20	6.6 (6.1; 7.6)

Abbreviations: DM, diabetes mellitus; SGLT2I, SGLT2 inhibitor; GFR, glomerular filtration rate (estimated by CKD-EPI, formula); PVAN, polyoma virus associated nephropathy; OH, overhydration measured by bioimpedance spectroscopy; MMF, mycophenolate mofetil; CSA, cyclosporine A; PTDM, post-transplantation diabetes mellitus.

glucose concentration at FU 1 of 10 (3–24) g/g creatinine (**Table 1**). Glucosuria remained stable during further FU (**Figure 2A**). The degree of urinary glucose concentration correlated with eGFR and was lower in patients with lower eGFR (**Figure 2B**). There was no significant change of HbA1c or fasting plasma glucose during FU (**Table 2**).

TABLE 2 | Course of parameters during FU.

	BL value	FU 1 (1 week)	FU 2 (1 month)	FU 3 (3 months)	FU 4 (6 months)	FT
Actual FU time, days	0	7 (6; 11)	39 (33; 47)	96 (82; 107)	187 (174; 272)	
Number of patients	22	20	16	20	19	
			Delta values to BL			
GFR (MDRD), mL/min/1.73 m^2	40 (34; 58)	**−2 (-10; 1)***	−4 (−5; 4)	**−4 (-10; 1)***	−4 (−7; 0)	n.s.
Albuminuria, mg/g Crea	75 (33; 174)	−6 (−31; 19)	−1 (−21; 18)	−12 (−86; 18)	−9 (−39; 9)	n.s.
HbA1c, %	6.1 (6.6; 7.6)	−0.1 (−0.2; 0.0)	−0.1 (−0.4; 0.1)	0.0; (−0.5; 0.4)	0.0 (−0.5; 0.2)	n.s.
Fasting plasma glucose, mg/dL	92 (112; 135)	−7 (−18; 5)	−2 (−19; 6)	−1 (−21; 12)	4 (−8; 14)	n.s.
Body weight, kg	85.2 (75.6; 90.6)	−0.3 (0.8; 0.5)	−0.8 (−1.5; 1.1)	−0.6 (−3.1; 1.4)	−2.3 (−4.4; 1.0)	n.s.
BMI, kg/m^2	27.3 (25.7; 30.4)	−0.1 (0.3; 0.2)	−0.4 (−0.5; 0.4)	−0.3 (−0.9; 0.5)	−0.9 (−1.8; 0.4)	n.s.
ATM, kg	41.7 (28.9; 48.2)	0.5 (−1.2; 3.4)	−0.6 (−2.3; 1.2)	0.5 (−1.9; 2.5)	1.8 (−4.2; 4.2)	n.s.
FTI, kg/m^2	13.9 (8.9; 17.1)	0.2 (−0.4; 1.1)	0.1 (−0.6; 0.7)	0.2 (0.7; 1.1)	0.5 (−1.3; 1.4)	n.s.
LTM, kg	39.9 (32.3; 44.3)	−0.25 (−4.0; 1.4)	0.4 (−0.8; 1.4)	−0.9 (−3.3; 1.0)	−1.2 (−5.3; 0)	n.s.
LTI, kg/m^2	13.6 (11.1; 14.8)	−0.1 (−1.3; 0.6)	0.1 (−0.3; 0.3)	−0.4 (−1.1; 0.3)	−0.3 (−1.7; 0.1)	n.s.
OH, l/1.73 m^2	1.3 (0.3; 2.5)	−0.4 (−0.8; 0.1)	0.2 (−0.7; 0.4)	−0.3 (−1.0; 0.4)	0.1 (−0.8; 0.3)	n.s.
ECW, l/1.73 m^2	16.6 (15.4; 17.5)	**−0.3 (-0.7; 0.0)***	−0.2 (−0.5; 0.6	−0.5 (−1.0; 0.3)	−0.5 (−0.7; 0.2)	n.s.
Plasma renin, ng/L	21 (5; 57)	0 (0; 5)	0 (0; 4)	0 (−5; 5)	3 (0; 12)	n.s.
Plasma aldosterone, ng/L	156 (132; 211)	**45 (5; 114)***	**19 (-2; 85)***	35 (−7; 69)	**49 (6; 93)***	n.s.
			Absolute values			
Glucosuria, g/g crea		10 (3; 24)	5 (3; 22)	14 (3; 23)	11 (6; 25)	n.s.

Values are Median and interquartile range. *p < 0.05 with p-values from Wilcoxon test to baseline with Bonferroni correction for multiple testing (printed bold if significant). Friedman test (FT) was performed to test for significant changes during total FU period.
Abbreviations: BL, baseline; FU, follow up; FT, friedman test; OH, overhydration measured by bioimpedance spectroscopy; ECW, extracellular water measured by bioimpedance spectroscopy; GFR, glomerular filtration rate; BMI, body mass index; ATM, adipose tissue mass; FTI, fat tissue index; LTM, lean tissue mass; LTI, lean tissue index.

Median BL BMI was overweight with 27.3 (25.7–30.4) kg/m^2. Although BMI and body weight tended to decrease during 6 months FU, the reductions were not significant (**Table 2**). There was no significant change of adipose tissue mass (ATM), fat tissue index (FTI), lean tissue mass (LTM) or lean tissue index (LTI) during 6 months FU (**Table 2**).

Overhydration (OH) and Plasma Renin and Aldosterone Concentration

There was a wide range of OH at BL (**Figure 2C** BL) with a median OH of 1.3 (0.3–2.5) L/1.73 m^2. During follow-up, range of OH was visually narrowed down and fewer patients had values of OH above 2.0 L/1.73 m^2 (**Figure 2C**). This was reflected by a significant correlation of BL OH and delta OH after 6 months, where patients with higher BL OH had a greater decrease of OH (adjusted r^2 = 0.54, p = 0.0003, **Figure 2D**). Delta OH after 6 months did not correlate with eGFR or delta eGFR, albuminuria or delta albuminuria, or glucosuria. Extracellular water (ECW) decreased in parallel to OH (**Table 2**).

Loop diuretic therapy was increased in n = 2 patients, remained unchanged in n = 6 patients and were terminated in n = 2 patients by the end of FU (**Supplementary Figure S1**).

Plasma renin concentration did not change significantly after initiation of SGLT2I or during FU (**Table 2**; **Figure 2E**). Plasma aldosterone concentration increased from BL to the first FU visit after 7 days, but there were no further significant changes during 6 months FU (**Table 2**; **Figure 2F**).

Urogenital Infections

Urinary tract infection occurred in n = 2 male patients. In the first case, the patient had also had urinary tract infections prior to

medication with an SGLT2I, and during complete FU of 6 months, there was only one episode of a lower urinary tract infection with *Klebsiella pneumoniae* found in urinary culture and successfully treated with amoxicillin/clavulanic acid. In the second case, the patient was treated in hospital due to febrile infection and poor glucose control; there was also found *Klebsiella pneumoniae* in urinary culture and infection rapidly subsided with antibiotic therapy with amoxicillin/clavulanic acid; this patient did not wish to continue treatment with dapagliflozin due to an itchy on the forehead with timely but uncertain causal relationship to dapagliflozin.

DISCUSSION

Our study shows that SGLT2I lead to a correction of fluid overload in those patients with elevated overhydration at baseline, while in euvolemic KTRs fluid status remained stable without fluid loss or reduction of body water below the reference range. These results are in line with previous findings in non-transplant CKD patients, where SGLT2I also lead to a reduction in patients with fluid overload without causing dehydration [7, 8]. In KTRs, there is one smaller previous study also using bioimpedance analysis to investigate volume homeostasis after initiation of SGLT2I, that reported a reduction of fluid overload after 4 weeks (n = 14); in this study, mean overhydration returned to initial value after 12 months, but with a decreased sample size of n = 8 and large standard deviation, making interpretation uncertain [11]. As there is persistent glucosuria under therapy with SGLT2I, we should actually expect a lasting osmotic effect, but there was no ongoing fluid loss after SGLT2 inhibition in all these cohorts. Therefore, counter-regulation mechanisms

FIGURE 2 | Glucosuria, overhydration, plasma renin and aldosterone concentration after initiation of SGLT2 inhibitors in KTRs. Course of glucosuria [**(A)**, absolute values] and correlation of glucosuria with eGFR **(B)**; course of overhydration [**(C)**, absolute values] and correlation of baseline overhydration with delta overhydration to baseline after 6 months **(D)**; and course of plasma renin [**(E)**, delta values to baseline] and aldosterone [**(F)**, delta values to baseline] concentration. p values in **(A,E,F)** are from Wilcoxon tests, with bonferroni correction for multiple testing: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Note that the distances of x-axis in **(A,C,E,F)** are not representative of follow up time intervals. Abbreviations: KTRs, kidney transplant recipients; BL, baseline; FU, follow up; eGFR, estimated glomerular filtration rate with MDRD formula; OH, overhydration.

promoting stabilization of extracellular water seem to be active. One potential mechanism is activation of renin angiotensin aldosterone system (RAAS). In normally hydrated patients with diabetes mellitus and normal kidney function, we had observed a transient loss of extracellular water, which was counter-regulated by RAAS activation [4]. In non-transplant CKD cohorts, decrease of fluid overload was accompanied by a tendential but not significant increase of renin and aldosterone [7, 8]. In our present cohort of KTRs, SGLT2I did not lead to an increase in renin and elicited only a moderate response in aldosterone concentrations, indicating that denervation of the kidney during transplantation might have an impact on the RAAS activation. Another potential mechanism promoting stabilization of fluid status with SGLT2I is water conservation by

the antidiuretic hormone (ADH/Vasopressin). The vasopressin surrogate marker copeptin has been shown to increase after SGLT2 inhibition in non-transplant CKD patients [8]. Marton and colleagues proposed an effect called aestivation, that is known as an evolutional survival strategy in energy and water shortage, to become active with SGLT2 inhibition [25]. Aestivation-like changes of metabolism with nitrogen transfer for production of organic osmolytes with parallel water conservation via ADH might prevent the glucose-driven osmotic diuretic effect of SGLT2I and contribute to renoprotective effects of SGLT2I [25]. Most recently, this effect was examined in patients with heart failure, where, in accordance with this finding, SGLT2 inhibition lead to increased serum copeptin levels and decreased free water clearance [26].

The denervation of the transplanted kidney might also lead to a different reaction to changes in volume status than the native kidneys. Our current study shows, however, that correction of fluid overload without ongoing fluid loss with SGLT2I is also present in the cohort of KTRs, suggesting that this effect of SGLT2I is independent from innervation of the kidneys. Our observations overall confirm the safety of SGLT2I with respect to the risk of dehydration after kidney transplantation and emphasize a potential benefit of SGLT2I, particularly in patients with sub-clinic or obvious fluid overload. Of note, we investigated the use of SGLT2I in stable KTRs at least 6 months after the kidney transplantation, and so the impact on fluid status may be different early after kidney transplantation. Prospective outcome studies investigating the reno-protective effects of SGLT2I in KTRs are still pending. The Renal Lifecycle Study (NCT05374291) investigating effects of dapagliflozin on renal and cardiovascular outcomes includes KTRs and is currently recruiting.

Our study confirms that glucosuria with SGLT2I depends on the kidney function and is reduced in lower GFR after kidney transplantation. This has already been demonstrated in KTRs [19] and is in line with previous findings in non-transplant patients, where urinary glucose excretion also decreased with kidney impairment [27]. Due to this dependency of SGLT2I-mediated glucosuria from GFR, we assume that the effect of SGLT2I on blood glucose control is lower in patients with impaired kidney transplant function. This should be borne in mind when selecting a glucose-lowering therapy in these patients. Together with the limited study size, this probably explains why we did not observe significant changes of fasting plasma glucose or HbA1c in our cohort.

Although body weight tended to decrease in a range reported earlier [11–14, 16–18] there was no reduction of adipose tissue mass. Likewise, adipose tissue was not reduced by SGLT2I in a previous smaller cohort of KTRs [11]. This might be on account of lower calorie losses in the urine due to decreased eGFR. Furthermore, our cohort was overweight and therefore differed from obese cohorts, the latter showing a greater reduction of body weight and decrease in adipose tissue following initiation of SGLT2 inhibitors [4, 28]. Lean tissue was also not decreased in our cohort, which speaks against loss of muscle mass under therapy with SGLT2I in KTRs. However, effects of SGLT2I on body fat and lean tissue might become more pronounced after longer FU time and might be overseen in our study due to the small cohort size. Especially as there seem to be present, as discussed above, counter regulating effects of ongoing fluid loss like

aestivation-like metabolic changes which include consumption of amino acids from muscle tissue, further findings on the course of muscle tissue under SGLT2I must be awaited.

Our study is restricted by the small sample size and limited follow-up period. However, we present new and robust data on the impact of SGLT2I on fluid status and glucosuria after kidney transplantation. Since changes of fluid status are expected soon after the initiation of SGLT2 inhibitors, we monitored respective parameters closely at an early follow up visit after 7 days. We used bioimpedance spectroscopy as a reliable and investigator independent tool for intra-individual change of fluid status over time, producing clinically applicable parameters. Our findings promote the safety of SGLT2 inhibitors following kidney transplantation and support a broader use that will lead to further clinical experience.

In conclusion, our study demonstrates a correction of fluid overload after initiation of SGLT2I without risk of volume depletion and promotes the safety of SGLT2I therapy in patients after kidney transplantation. Glucosuria, together with effects of SGLT2I on blood glucose control and body weight, is reduced in lower kidney allograft function.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by the Ethics Committee at the Medical Faculty of the Eberhard Karls University and at the University Hospital of Tübingen, Germany. 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

Detailed author contributions are as follows: AS, MG, and FA planned the study. AS and M-LE carried out the clinical measurements. AS and MG analyzed and interpreted the data and drafted the manuscript. BB, FE, DH, and DV assisted with the clinical measurements. DV, NH, and AB contributed to analyzing and interpreting the data. 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

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Timing Considerations for Sleeve Gastrectomy in Kidney Transplant Patients: A Single Center Evaluation

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Current scientific literature is deficient in detailing the optimal timing for conducting bariatric surgery in relation to kidney transplantation. In this study, we performed a retrospective evaluation of kidney transplant recipients with BMI >35 kg/m^2. It aimed to provide data on those who received both sleeve gastrectomy (SG) and kidney transplantation (KT) simultaneously, as well as on patients who underwent SG and KT at different times, either before or after. In addition, the acceptance levels of the bariatric surgery among different scenarios were assessed. Our findings demonstrated that combined KT and SG led to successful weight loss, in contrast to undergoing kidney transplant alone, while maintaining comparable rates of graft and patient survival. Weight loss was similar between recipients who had a combined operation and those who underwent SG following the transplant. Additionally, over a median time frame of 1.7 years, patients who underwent SG before KT exhibited a statistically significant reduction in BMI at the time of the transplant. Notably, our study highlights that patients offered the combined procedure were significantly more likely to undergo SG compared to those for whom SG was presented at a different operative time than the transplant.

Keywords: kidney transplant, sleeve gastrectomy, timing, weight loss, robotic

INTRODUCTION

Obesity has emerged as a global epidemic, affecting approximately 13% of the world's adult population in 2016, a nearly threefold rise over the past four decades [1]. In the last 30 years, bariatric surgery has been established as the paramount therapeutic intervention for weight loss, specifically indicated for class III obesity and for class II obesity when accompanied by a concurrent medical condition [2, 3].

End-stage renal disease is a terminal condition characterized by a glomerular filtration rate of less than 15 mL/min. In the United States, diabetic nephropathy ranks as the most prevalent cause of ESRD, followed by hypertension [4]. Obesity contributes to the onset of non-communicable illnesses

Abbreviations: BMI, body mass index; CKD, chronic kidney disease; ESRD, end-stage renal disease; EWL, excess weight loss; HbA1c, glycated hemoglobin; KT, kidney transplantation alone; OKT, open kidney transplant; RKT, robotic kidney transplant; SG, sleeve gastrectomy.

GRAPHICAL ABSTRACT
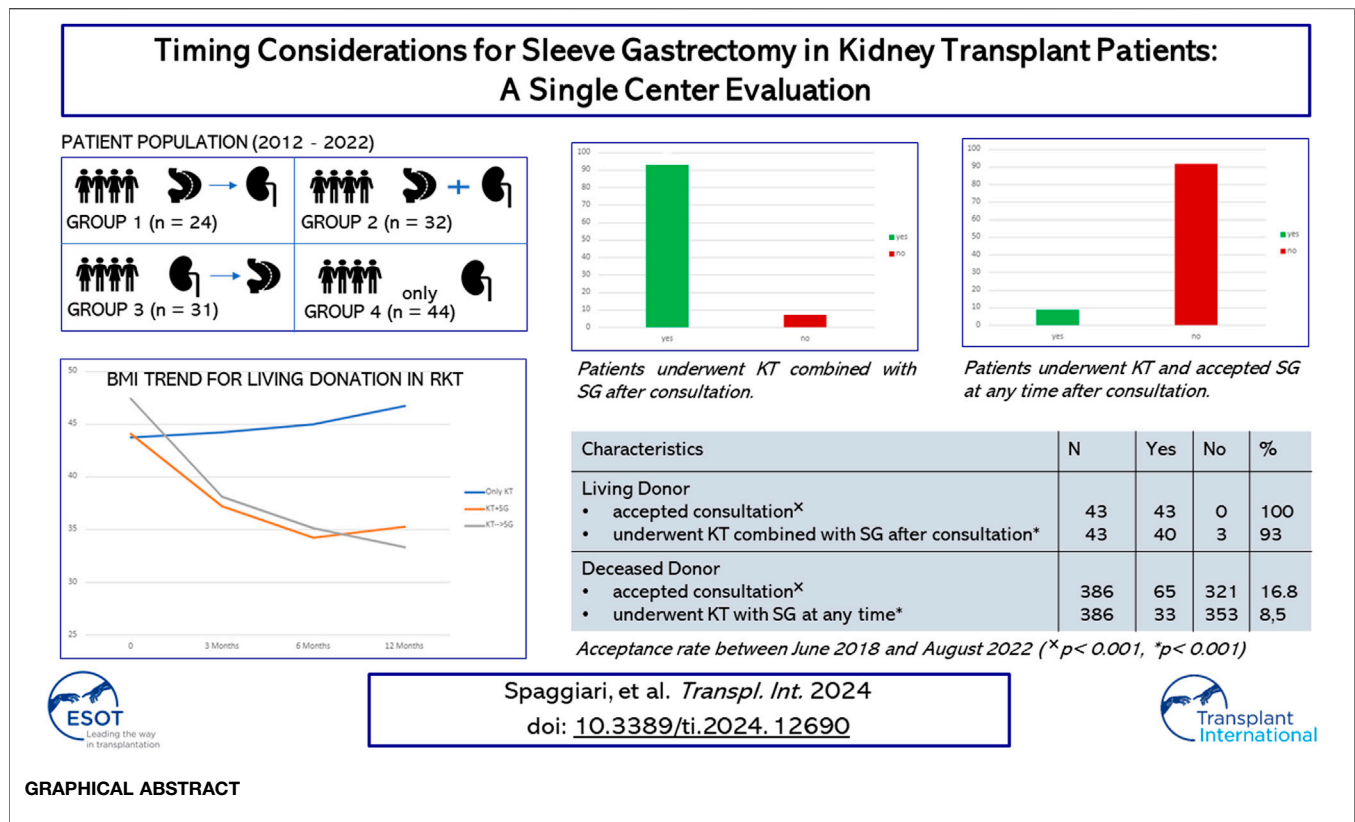
such as arterial hypertension (AHT), diabetes mellitus (DM), and atherosclerosis, all factors that also affect the development of CKD, ultimately leading to the progression to end-stage renal disease (ESRD) [5–7]. The effectiveness of kidney transplantation as the primary therapeutic approach for most ESRD patients has been extensively demonstrated, however, with the growing number and complexity of potential recipients, continuous refinement of selection criteria becomes imperative [8–10].

Prior studies have already underscored superior outcomes in patients who experience weight loss compared to those who do not [11]. In a cohort study involving 7,270 patients evaluating kidney transplant results, higher graft survival was observed in obese patients who lost more than 10% of their weight compared to obese patients who did not undergo weight loss [12]. Furthermore, weight reduction could enhance the eligibility of individuals with obesity for transplantation, potentially leading to improvements in both short-term and long-term outcomes [13, 14].

Numerous programs have incorporated robotic technology to minimize surgical risks in severely obese candidates, expanding therapeutic possibilities [15–18]. Nevertheless, the ideal timing for performing bariatric surgery in relation to kidney transplantation remains a topic of ongoing debate. This study carried out a retrospective evaluation of kidney transplant recipients with BMI >35 kg/m². It aimed to provide data on those who received both sleeve gastrectomy (SG) and kidney transplantation (KT) simultaneously, as well as on patients who underwent SG and KT at different times, either before or after. In addition, the acceptance levels of the bariatric surgery among different scenarios were assessed.

MATERIALS AND METHODS

Study Design and Patient Population

We conducted a retrospective study on patients who received kidney transplants (KT) and received bariatric surgical consultation at our center from April 2012 to August 2022. This study was approved by IRB# 2022-1122.

The multidisciplinary transplant recipient review committee at the University of Illinois Kidney Transplant Program determined the patient's eligibility for kidney transplantation. In our cohort, patients underwent both open kidney transplant (OKT) and robotic-assisted kidney transplant (RKT). Per protocol, adult patients (aged >18 years) were considered eligible for RKT if they had a body mass index (BMI) of ≥35 kg/m² at the time of listing but excluded in the presence of severe iliac atherosclerosis. Following the 1991 National Institutes of Health guidelines for bariatric procedures, all patients with a BMI exceeding 35 kg/m² were recommended to undergo consultation for bariatric surgery [19]. All patients with ESRD and a BMI greater than 35 kg/m² and a potential living donor were considered for a combined procedure. Patients on the waiting list for deceased organ transplants were offered the opportunity to participate in a weight loss program and undergo a consultation for bariatric surgery, considering

sleeve gastrectomy (SG) before or after the transplant surgical procedure. For accuracy, non-surgical weight management options were offered in patients with a BMI lower than 35 kg/m^2. However, as per our protocol, surgical management remains the primary option for candidates with a BMI over 35 kg/m^2.

In our study population, we categorized individuals into four distinct groups. *Group 1* included patients who underwent kidney transplantation after sleeve gastrectomy. *Group 2* comprised recipients who underwent a simultaneous KT and SG. *Group 3* was composed of patients who received KT before SG. Additionally, we established *Group 4*, which consisted of patients who underwent a consultation for bariatric surgery but declined to proceed with the surgical procedure.

Only recipients with at least 1-year of follow-up from the date of the KT and SG were included in the analysis. Patients who had undergone a bariatric surgical procedure other than sleeve gastrectomy and recipients who underwent simultaneous kidney-pancreas transplantation were excluded from the analysis.

As a result of constraints in the electronic health records data, we limited the sub-group analysis to examine the acceptance rate of the bariatric surgical consultation only for patients between June 2018 and August 2022. Moreover, In the calculation of the acceptance rate for the combined KT and SG, it's noteworthy that nine patients from a prior randomized clinical trial initially agreed to undergo the combined procedure but were subsequently randomized into the control group. These patients were classified as acceptors, irrespective of whether they ultimately underwent the procedure.

Data Collection and Statistical Analysis

Pre-transplant and post-transplant characteristics were collected through electronic health records. These included recipient characteristics as age, sex, ethnicity, height, weight, BMI (Body Mass Index) at the time of the KT and SG, comorbidities, dialysis information, donation type (living or deceased), type of surgery performed for the transplantation (OKT or RKT), length of surgery, length of stay, readmission rate, glomerular filtration rate (GFR) at 6 and 12 months post-transplant, serum creatinine (SCr) at 3, 6 and 12 months post-transplant, BMI post-SG at 3, 6 and 12 months, and 1-year organ and patient survival.

Excess weight loss (%) was calculated as follows: excess weight loss (%) = [(initial excess weight – postoperative excess weight)/initial excess weight] × 100, where excess weight (kg) = initial weight – ideal weight, and ideal weight (kg) = 23 × height^2.

Comprehensive descriptive analyses of all variables were performed. Qualitative variables were presented as counts and percentages. Normally, distributed quantitative variables were computed as mean ± standard deviation, and nonnormally distributed data were presented as median (range). Analysis was exclusively conducted among specific combinations (limitations section for more in-depth information). A p-values $<.05$ was considered statistically significant. The software used was IBM SPSS Statistics for Windows [20–22].

Immunosuppressive Regimen

Induction therapy, alongside a methylprednisone bolus of 500 mg, was administered to all patients. The treatment for the majority included rabbit antithymocyte globulin at a dosage of 1.5 mg/kg daily from postoperative day (POD) 0–4. African American patients, ABO incompatible, or with a positive cross-match received thymoglobulin induction. Basiliximab at 20 mg on POD 0 and 4, or alemtuzumab at 30 mg on POD 0, was administered to the remaining patients. Following this, maintenance immunosuppression was provided using either tacrolimus or cyclosporine, with tacrolimus levels targeted at 7–10 ng/mL for the initial month post-transplantation, adjusting to 3–7 ng/mL afterwards. Cyclosporine levels were aimed at 200–250 ng/mL for the first month, reducing to 150–200 ng/mL subsequently. Cyclosporine, in particular, was primarily utilized for patients considered at risk for diabetes following transplantation, in combination with mycophenolic acid and a brief 5-day steroid taper. During the induction phase, antimicrobial prophylaxis was applied. For patients or donors with positive cytomegalovirus serologies, treatment with valganciclovir at 450 mg/day was prescribed for 6 months, while those without positive serologies received a one-month course of acyclovir to prevent herpes simplex virus. Desensitization, involving a mix of plasmapheresis and intravenous immunoglobulin, was necessary for patients who were ABO incompatible, cross-match positive, or had a high panel reactive antibody count.

RESULTS

Cohort Characteristics

After a retrospective analysis of our database, we identified a total of four groups. *Group 1* included a total of 3 patients with living donors and 21 patients with deceased donors; *Group 2*–31 patients with living donors and 1 patient with deceased donor; *Group 3*–19 patients with living donors and 12 patients with deceased donors; *Group 4*–12 patients with living donors and 32 patients with deceased donors. Additional details can be found in **Table 1**; **Supplementary Tables S1, S2**.

Table 1 illustrates only patients in *Group 2* (KT + SG), *Group 3* (KT before SG), and *Group 4* (Only KT) with living donation with robotic-assisted approach. A total of 7 patients who underwent the open surgical approach is detailed alongside patients who underwent the robotic-assisted approach in **Supplementary Table S1**. Indeed, as per protocol they were not considered for the robotic approach due to presence of severe iliac atherosclerosis. **Supplementary Table S2** presents cases involving deceased donation, where both the robotic-assisted and open approaches are listed across the four distinct groups.

BMI and Excess Weight Loss

BMI values and EWL percentages at different time points (3 months, 6 months, and 12 months) post-surgery were compared across the groups (*Group 2* (KT + SG), *Group 3* (KT before SG), *Group 4* (Only KT)) in **Table 1**. Significant differences were observed in BMI at 3 months ($p = 0.023$), 6 months ($p < 0.001$), and 12 months ($p < 0.001$). Similarly, EWL percentages differed significantly at 12 months ($p < 0.001$), with smaller variations at 3 and 6 months. These differences suggest changing body weight trends among the groups over time. Nonetheless, upon comparing

TABLE 1 | Living donation with robotic-assisted approach - *Group 2* (KT + SG), *Group 3* (KT before SG), *Group 4* (Only KT).

Characteristics	Only KT (N = 12)	KT + SG (N = 24)	KT before SG (N = 17)	p
Age* (years), mean ± SD	52.9 (0.5)	43.3 (10.2)	53.7 (2.4)	0.376
Male gender, n (%)	9 (75)	10 (41.7)	7 (41.2)	0.124
Ethnicity and race, n (%)				
• Caucasian	4 (33.3)	8 (33.3)	2 (11.8)	0.412
• African-American	5 (41.7)	11 (45.8)	11 (64.7)	
• Hispanic	2 (16.7)	5 (20.8)	2 (11.8)	
• Asian	0	0	0	
• Other	1 (8.3)	0	2 (11.8)	
BMI* (kg/m2), mean ± SD	43.7 (4.1)	44.1 (5.3)	40.7 (0.4)	0.964
Co-morbidities, n (%)				
• Hypertension	11 (91.7)	24 (100)	17 (100)	0.207
• Hyperlipidemia	10 (83.3)	14 (58.3)	4 (23.5)	**0.029**
• Diabetes mellitus	6 (50)	15 (62.5)	3 (17.6)	0.072
• High cardiac risk (EF < 45%)	6 (50)	9 (37.5)	2 (11.8)	0.177
Pretransplant dialysis (months), median (range)	14 (28)	11.5 (96)	1 (97)	0.119
Time frame KT – SG (years), median (range)	NA	NA	2.24 (10.7)	NA
BMI[✱] (kg/m2), median (range)				
• 3 months	44.2 (6.2)	37.2 (24.7)	38.1 (19.2)	**0.023**
• 6 months	45 (6.4)	34.2 (25)	35.1 (20)	**< 0.001**
• 12 months	46.7 (5.2)	35.3 (26)	33.3 (20)	**< 0.001**
EWL[✱] (%), median (range)				
• 3 months	4 (8)	26.2 (36.8)	34.6 (28.6)	0.213
• 6 months	1.6 (7.2)	31.7 (41.5)	43.1 (37.3)	0.113
• 12 months	–1.4 (3.6)	27.1 (67.1)	54.3 (61.1)	**< 0.001**

*Abbreviations: EWL, excess weight loss; GFR, glomerular filtration rate; KT, kidney transplant; NA, not available. *at the time of transplantation. [✱]delta between weight at the follow-up and weight at the time of sleeve gastrectomy (or KT, for the control group). The bold values represents the statistical significance.*

TABLE 2 | Living donation with robotic-assisted approach - Group 2 (KT + SG) and Group 3 (KT before SG).

Characteristics	KT + SG (N = 24)	KT before SG (N = 17)	p
Age* (years), mean ± SD	43.3 (10.2)	53.7 (2.4)	0.516
Male gender, n (%)	10 (41.7)	7 (41.2)	0.615
Ethnicity and race, n (%)			
• Caucasian	8 (33.3)	2 (11.8)	0.119
• African-American	11 (45.8)	11 (64.7)	
• Hispanic	5 (20.8)	2 (11.8)	
• Asian	0	0	
• Other	0	2 (11.8)	
BMI* (kg/m2), mean ± SD	44.1 (5.3)	40.7 (0.4)	0.782
BMI at Sleeve Gastrectomy (kg/m2), mean ± SD	44.1 (5.3)	47.4 (6.7)	0.088
Co-morbidities, n (%)			
• Hypertension	24 (100)	17 (100)	1
• Hyperlipidemia	14 (58.3)	4 (23.5)	0.109
• Diabetes mellitus	15 (62.5)	3 (17.6)	**0.022**
• High cardiac risk (EF < 45%)	9 (37.5)	2 (11.8)	0.160
Pretransplant dialysis (months), median (range)	11.5 (96)	1 (97)	0.386
BMI[✱] (kg/m2), median (range)			
• 3 months	37.2 (24.7)	38.1 (19.2)	0.170
• 6 months	34.2 (25)	35.1 (20)	0.280
• 12 months	35.3 (26)	33.3 (20)	0.467
EWL[✱] (%), median (range)			
• 3 months	26.2 (36.8)	34.6 (28.6)	0.318
• 6 months	31.7 (41.5)	43.1 (37.3)	0.406
• 12 months	27.1 (67.1)	54.3 (61.1)	0.925

*Abbreviations: EWL, excess weight loss; GFR, glomerular filtration rate; KT, kidney transplant; NA, not available. *at the time of transplantation. [✱]delta between weight at the follow-up and weight at the time of sleeve gastrectomy (or KT, for the control group). The bold values represents the statistical significance.*
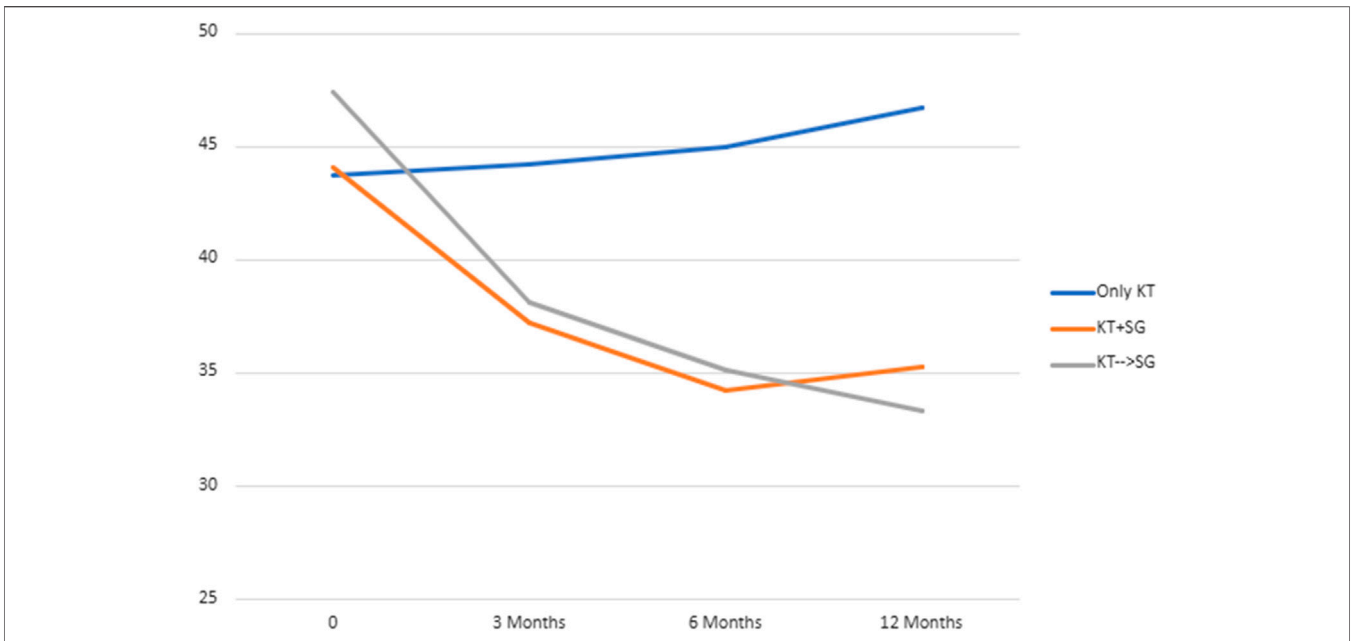
FIGURE 1 | BMI trend for living donation with robotic-assisted approach - *Group 2* (KT + SG), *Group 3* (KT before SG), *Group 4* (Only KT).
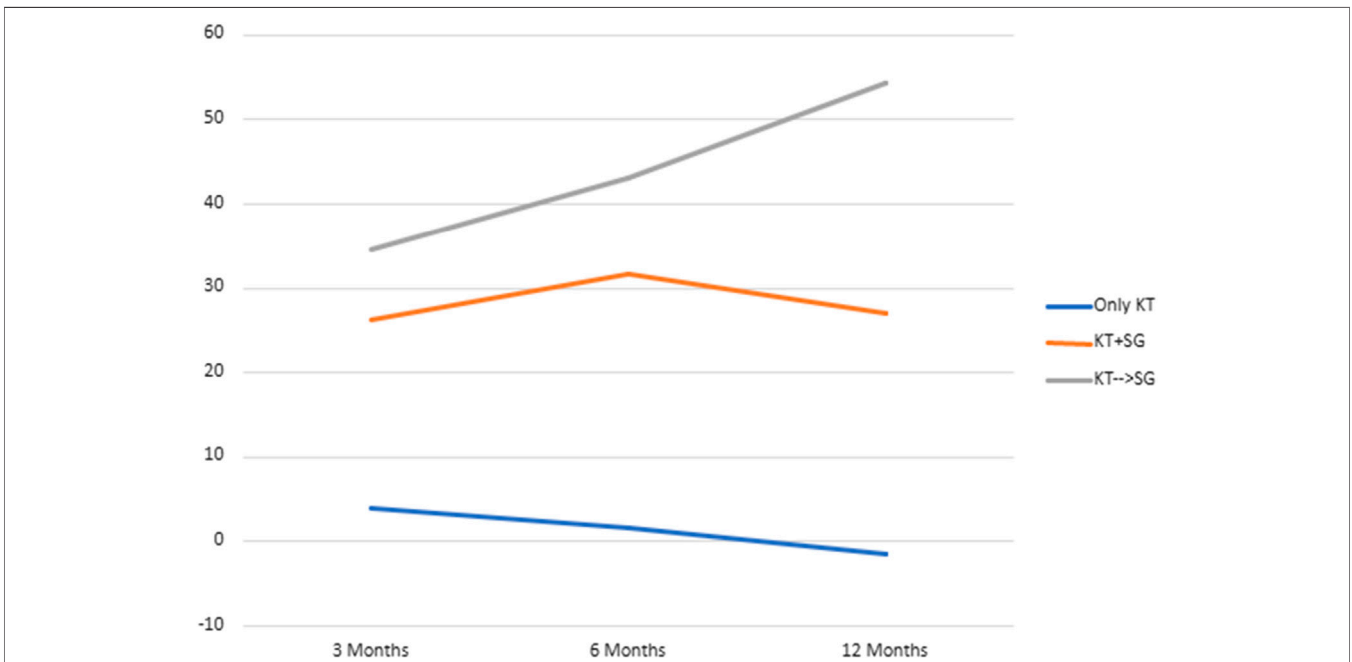
FIGURE 2 | Estimated weight loss percentage trend for living donation with robotic-assisted approach - *Group 2* (KT + SG), *Group 3* (KT before SG), *Group 4* (Only KT).

only *Group 2* and *Group 3* (**Table 2**), no statistical significance was observed at the same time points for BMI and EWL. **Figure 1** and **Figure 2** illustrate respectively the BMI and the estimated weight loss percentage trends for living donation with robotic-assisted approach between Group 2 (KT + SG), Group 3 (KT before SG), Group 4 (Only KT).

In **Supplementary Table S3**, the internal group statistics for *Group 1* (KT after SG) are detailed. The time frame from Sleeve Gastrectomy (SG) to Kidney Transplant (KT) is reported as 1.7 years (median range: 6.1). The mean BMI at Sleeve Gastrectomy is 43.8 kg/m2 (SD: 5.6), and a paired sample test reveals a significant BMI decrease ($p < 0.001$) at the time of

TABLE 3 | Living donation with robotic-assisted approach - Group 2 (KT + SG) and Group 4 (Only KT).

Characteristics	Only KT (N = 12)	KT + SG (N = 24)	p
Age* (years), mean ± SD	52.9 (0.5)	43.3 (10.2)	0.368
Male gender, n (%)	9 (75)	10 (41.7)	0.059
Ethnicity and race, n (%)			
• Caucasian	4 (33.3)	8 (33.3)	0.551
• African-American	5 (41.7)	11 (45.8)	
• Hispanic	2 (16.7)	5 (20.8)	
• Asian	**0**	0	
• Other	1 (8.3)	0	
BMI* (kg/m2), mean ± SD	43.7 (4.1)	44.1 (5.3)	0.964
Co-morbidities, n (%)			
• Hypertension	11 (91.7)	24 (100)	0.151
• Hyperlipidemia	10 (83.3)	14 (58.3)	0.134
• Diabetes mellitus	6 (50)	15 (62.5)	0.473
• High cardiac risk (EF < 45%)	6 (50)	9 (37.5)	0.473
Pretransplant dialysis (months), median (range)	14 (28)	11.5 (96)	**0.033**
Length of surgery (minutes), mean ± SD	275 (7.1)	355.7 (125.3)	**0.034**
Length of stay (days), mean ± SD	5 (1.4)	7.4 (3.4)	0.181
Readmission rate post KT, n (%)	6 (50)	15 (62.5)	0.358
GFR (mL/min), mean ± SD			
• 6 months	43 (9.1)	61.5 (17.7)	0.135
• 12 months	49.3 (4.2)	62 (14.5)	**0.020**
SCr (mg/dL), mean ± SD			
• 6 months	1.8 (0.9)	1.2 (0.2)	0.078
• 12 months	1.5 (0.4)	1.2 (0.3)	0.251
BMI✱ (kg/m2), median (range)			
• 3 months	44.2 (6.2)	37.2 (24.7)	**0.004**
• 6 months	45 (6.4)	34.2 (25)	**< 0.001**
• 12 months	46.7 (5.2)	35.3 (26)	**< 0.001**
EWL✱ (%), median (range)			
• 3 months	4 (8)	26.2 (36.8)	0.202
• 6 months	1.6 (7.2)	31.7 (41.5)	0.107
• 12 months	−1.4 (3.6)	27.1 (67.1)	**0.003**
1-year graft survival, n (%)	11 (91.7)	23 (95.8)	0.562
1-year patient survival, n (%)	12 (100)	23 (95.8)	0.667

*Abbreviations: EWL, excess weight loss; GFR, glomerular filtration rate; KT, kidney transplant; NA, not available. *at the time of transplantation. ✱delta between weight at the follow-up and weight at the time of sleeve gastrectomy (or KT, for the control group). The bold values represents the statistical significance.*

Kidney Transplant, where the mean BMI is 34.8 kg/m2 (SD: 5.1). The Pearson correlation coefficient for Delta BMI at SG and the time frame SG to KT is −0.181, with a p-value of 0.40, suggesting no significant correlation.

Supplementary Table S4 provides the internal group statistics for *Group 3* (KT before SG). The time frame from KT to SG is reported as 2.2 years (median range: 10.9). The mean BMI at KT is 44.5 kg/m2 (SD: 6.6), and the paired sample test yields a p-value of 0.38, indicating no statistically significant change in BMI at the time of SG, where the mean BMI is 45.3 kg/m2 (SD: 5.6). The Pearson correlation coefficient for Delta BMI at SG and the time frame KT to SG is 0.159, with a p-value of 0.39, suggesting no significant correlation. Both **Supplementary Tables S3, S4** include patients regardless of the type of surgical approach and the type of donor.

Graft Function and Survival, and Patient Survival

Table 3 presents data comparing *Group 2* (KT + SG) and *Group 4* (Only KT) in living donation with a robotic-assisted approach.

GFR and serum creatinine measurements are provided at 6- and 12-months post-surgery. These values did not show statistical significance between the two groups, except for GFR at 12 months (*Group 2* VS *Group 4*, 62 (SD: 14.5) VS 49.3 (SD: 4.2), p = 0.020). Additionally, **Table 3** includes 1-year graft survival percentages, with 95.8% for *Group 2*% and 91.7% for *Group 4*, and 1-year patient survival percentages of 95.8% for *Group 2*% and 100% for *Group 4*, with no statistically significant differences observed. **Supplementary Tables S1, S2** offer this information for all four groups within the context of living and deceased donation, incorporating both robotic-assisted and open approaches.

Acceptance Rate

**Table 4** describes the acceptance rate of SG consultations and the subsequent procedures in the setting of living and deceased donor kidney transplant. Among patients with living donor, all 43 individuals accepted consultation, while 93% of them underwent kidney transplant combined with sleeve gastrectomy (SG) after consultation. For patients with deceased donor, 386 patients accepted consultation, but only

TABLE 4 | Acceptance rate between June 2018 and August 2022.

Characteristics	N	Yes	No	%
Living Donor				
- accepted consultation✱	43	43	0	100
- underwent KT combined with SG after consultation*	43	40	3	93
Deceased Donor				
- accepted consultation✱	386	65	321	16.8
- underwent KT with SG at any time*	386	33	353	8.5

✱*p < 0.001,* **p < 0.001.*
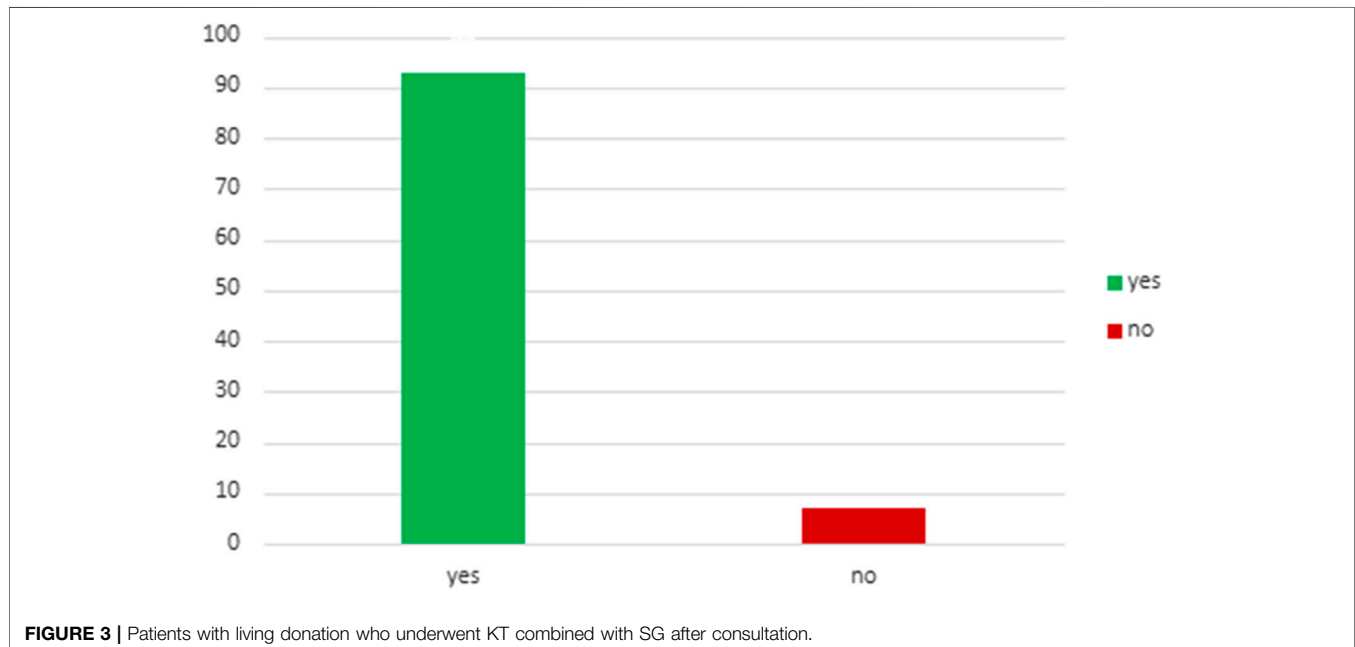
FIGURE 3 | Patients with living donation who underwent KT combined with SG after consultation.

8.5% of them proceeded with a SG at some point (before or after KT). This data underscores a notable contrast ($p < 0.001$) in the acceptance of consultations and the actual performance of the SG between living and deceased donor scenarios, highlighting the higher likelihood of proceeding with the combined procedure in the former group. **Figures 3**, **4** illustrates these findings.

DISCUSSION

In this retrospective study, we described four patient groups who underwent kidney transplantation and received bariatric surgical consultation at the University of Illinois at Chicago from April 2012 to August 2022. *Group 1* included patients who underwent kidney transplantation after sleeve gastrectomy, *Group 2* comprised recipients who underwent a simultaneous KT and SG, *Group 3* was composed of patients who received KT before SG, and *Group 4* consisted of patients who underwent a consultation for bariatric surgery but declined to proceed with the surgical procedure.

Obesity impacted 670 million adults worldwide in 2016. In the United States, the obesity rate has been steadily increasing since the 1980s, with a projected prevalence of 48.9% among American adults by 2030 [23, 24]. An increasing number of studies find obesity as a driver of chronic kidney disease progression, and the mechanisms are complex and include hemodynamic changes, inflammation, oxidative stress, and activation of the renin-angiotensin-aldosterone system [25].

Despite increased risk for early surgical complications and delayed graft function in patients with obesity, experience from multiple centers demonstrate a clear survival benefit of transplantation over dialysis, and comparable graft and patient survival rates to nonobese recipients. However, to date, obesity is associated with a lower rate of referral and waitlisting, and lower likelihood of kidney transplantation [26]. Between January 2009 and December 2018, we conducted a retrospective analysis of our cohort of patients undergoing RKT. This analysis comprised 239 patients, with a median BMI of 41.4 kg/m^2. The robotic approach has led to a statistically significant decrease in surgical site infections within this population of obese recipients, while maintaining graft and patient survival rates comparable to those of the nonobese population [18]. Based of this experience, in our current clinical protocol, we abstain from employing a definitive BMI

FIGURE 4 | Patients with deceased donation who underwent KT with SG at any time after consultation.
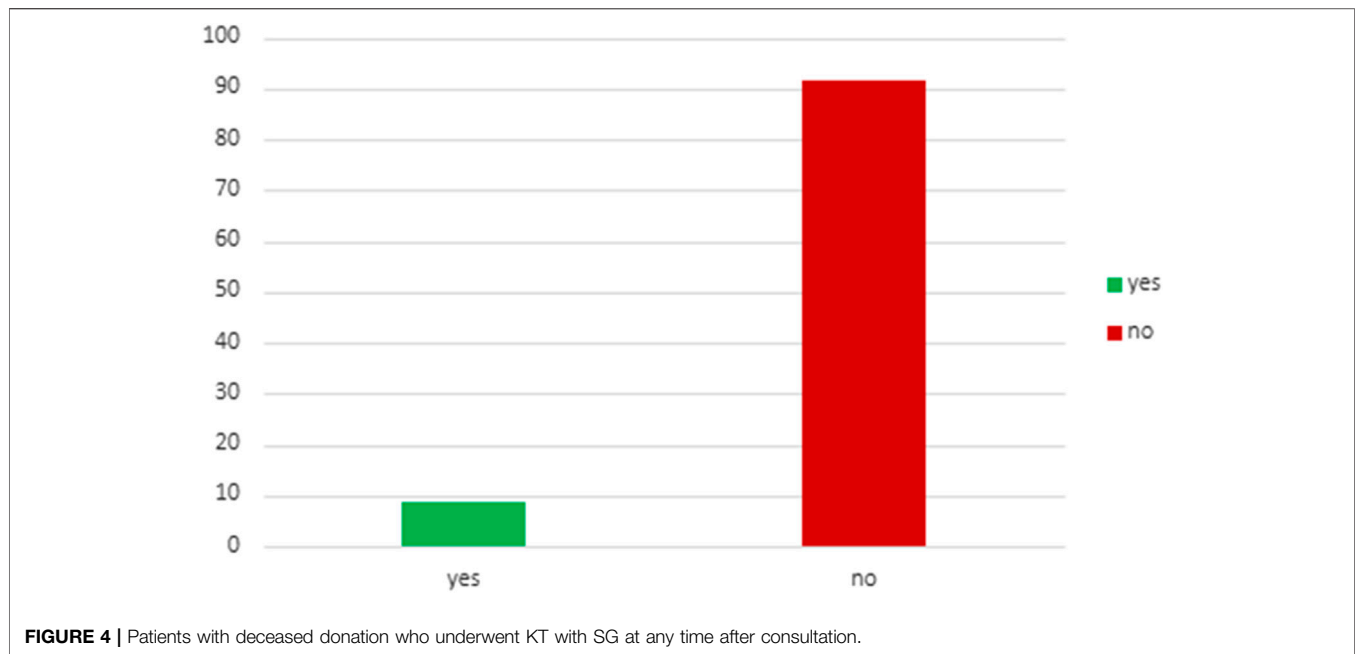
threshold for kidney recipients. Our intermediate and extended-term findings corroborate the conjecture that BMI, in isolation, is not an optimal metric for precluding transplant eligibility [27].

The optimal strategy for managing obesity in the context of ESRD patients remains uncertain. Implementing lifestyle modifications for substantial and effective weight loss poses a challenge and is frequently unsuccessful in individuals with obesity [28]. Introducing bariatric surgery before kidney transplant has become increasingly popular, with studies have shown acceptable morbidity and mortality rates [29–31]. However, a drawback to this strategy is the prolonged wait for a kidney transplant, coupled with elevated risks during dialysis [32]. Additionally, in the context of living organ donation, it's crucial to recognize that the availability of the organ is temporary. Thus, any factors contributing to a prolonged kidney transplant process may risk the feasibility of the living donor. This emphasizes the need to streamline the transplant procedure for both its success and to preserve the readiness of the living donor. One potential resolution to these issues involves combining sleeve gastrectomy and kidney transplant in the same operative time. This approach facilitates a more rapid transplantation process, requiring only a single administration of general anesthesia. As per our current protocol, all patients with ESRD and a BMI greater than 35 kg/m^2 and a potential living donor are considered for a combined procedure. Patients on the waiting list for deceased organ transplants are offered the opportunity to participate in a weight loss program and undergo a consultation for bariatric surgery, considering sleeve gastrectomy before or after the transplant surgical procedure.

In our previously randomized study, we demonstrated the efficacy and safety of the combined approach (11 patients with robotic sleeve gastrectomy and robotic-assisted kidney transplant VS 9 patients with robotic-assisted kidney transplant only) [33].

In this study, we examine a broader cohort within the combined group, incorporating details about two additional patient populations (KT after SG and KT before SG) and reporting the acceptance rate of bariatric surgery in our cohort.

Earlier articles have already addressed the outcomes of bariatric surgery both pre and post kidney transplantation [34, 35]. In their meta-analysis, Fernando et al. demonstrated that bariatric surgery is both safe and efficacious in patients with ESRD prior to KT and in those post KT, suggesting that SG should be strongly considered as part of the workup of the high BMI kidney recipient. In our study, we introduce a novel variable into the equation, illustrating that individuals undergoing simultaneous SG and KT exhibit comparable BMI and EWL trends to those of patients undergoing sleeve gastrectomy following kidney transplant. Consistent with earlier studies, we also observed a noteworthy reduction in mean BMI among patients undergoing Sleeve Gastrectomy (SG) before Kidney Transplant (KT) within a median of 1.7-year timeframe. Conversely, for patients who underwent SG after KT within a median of 2.2-year timeframe, there was no statistically significant change in mean BMI.

Graft and patient survivals, in robotic-assisted living kidney donation, were similar with no statistically significant differences noted between Group 2 (KT + SG) and Group 4 (Only KT). At the 12-month, the combined group exhibited a superior GFR compared to the KT alone group. Although long-term graft survival data is currently unavailable, we hypothesize that addressing obesity could play a pivotal role in enhancing extended graft survival. The observed improvement in GFR within the initial year suggests a positive trajectory for renal function in the combined approach, prompting the expectation that early management of obesity may contribute to sustained graft health over the long term. Also, our larger cohort did not

exhibit a statistically significant increase in the readmission rate between the two groups, a contrast to our previous randomized study findings [33]. In that earlier study, the KT + SG group had a higher readmission rate attributed to nausea and vomiting leading to dehydration and acute kidney injury (AKI). To address this issue, we implemented a strategy involving the placement of a peripherally inserted central catheter on the day of discharge and prescribed home intravenous fluid repletion with 2 L/day of crystalloid solution for the initial postoperative month. Our recent findings indicate the success of this strategy.

Discussing the management of immunosuppression in the group undergoing combined procedures is essential. The most prevalent bariatric surgeries are sleeve gastrectomy and Roux-en-Y gastric bypass (RYGB) [36, 37]. Sleeve gastrectomy is mainly a restrictive surgery that involves the removal of a large section of the stomach, while RYGB is both restrictive and malabsorptive, requiring the creation of a small stomach pouch and a Roux-en-Y gastrojejunostomy. Differing from sleeve gastrectomy, RYGB impacts the absorption processes and is specifically known to alter the pharmacokinetic dynamics of immunosuppressive drugs [38]. This specific characteristic of sleeve gastrectomy did not present any obstacles in adhering to the standard of care immunosuppression regimens set by the University of Illinois at Chicago Kidney Transplant Program.

While bariatric surgery has proven effective in this patient cohort, it's crucial to consider patients' perspectives on undergoing an additional procedure alongside the transplant. Initially, we observed significant differences in acceptance rates when proposing a combined procedure for living donor recipients versus two separate procedures for deceased donor recipients. Consequently, we conducted a more thorough investigation into the consultation rate and acceptance of the procedure, revealing substantial discrepancies in results (93% vs. 8.5%). Our interpretation of this trend is that the idea of addressing two issues in a single hospitalization is appealing to patients. It effectively minimizes logistical challenges and lessens the burden on families. However, it is crucial to bear in mind that both procedures entail intricate post-surgery care, ranging from managing immunosuppressive regimens to adapting to the lifestyle changes post-bariatric surgery. Given this, a thorough psychological assessment (evaluating psychological issues/comorbidities, social support, motivation, and capacity to manage the demands post-surgeries) is essential for the success of a combined approach, where the psychological burden may be even greater than usual [39, 40]. Indeed, a weight regain 6 months post-operation in the combined group, as opposed to the sleeve gastrectomy group following kidney transplant, could stem from the demanding nature of post-transplant care, possibly overshadowing patients' ongoing commitment to their sleeve gastrectomy education. While a more in-depth qualitative study is essential for a comprehensive understanding of this trend, the practicality of achieving comparable clinical outcomes with combined kidney transplant and sleeve gastrectomy proves beneficial in addressing both ESRD and obesity, thereby expanding the reach to more patients.

While a similar study comparing bariatric surgery before, combined, and after liver transplant has been previously published, our paper is, to the best of our knowledge, the first to present data for these groups in the context of kidney transplant and to explore acceptance rates for bariatric surgery [41].

Limitations

Our study holds considerable strength as the inaugural exploration of acceptance rates and timing for sleeve gastrectomy in kidney transplant recipients. However, our study does have certain limitations. Firstly, it is essential to acknowledge the inherent limitations associated with its retrospective design. Secondly, the comparative analysis across groups posed challenges due different type of donor (living VS deceased) and surgical approach (open VS robotic), consequently, p-values were selectively considered in specified contexts. Moreover, the creatinine-based GFR might be affected in patients experiencing substantial muscle mass loss.

Despite these constraints, our study serves as a foundational step in understanding the complex dynamics related to the surgical management of obesity in this specific patient population, paving the way for future prospective investigations to further elucidate these considerations.

Conclusion

In summary, our retrospective investigation indicates that the simultaneous kidney transplant and sleeve gastrectomy resulted in successful weight loss compared to kidney transplant alone, while maintaining similar rates of graft and patient survival. We observed a consistent trend in 1-year BMI and excess weight loss among patients who underwent simultaneous SG and KT compared to those who had KT before SG. Additionally, in a median time frame of 1.7 years, patients who underwent SG prior to KT showed a statistically significant reduction in BMI at the time of the transplant. Notably, our study highlights that patients offered the combined procedure were significantly more likely to undergo surgery compared to those for whom sleeve gastrectomy was presented at a different operative time than the transplant. Further prospective studies are necessary to obtain additional insights from the combined group.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: None. Requests to access these datasets should be directed to alessandro.martinino@uic.edu.

ETHICS STATEMENT

The studies involving humans were approved by the University of Illinois at Chicago (IRB# 2022-1122). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

MS and AM had major contributions in writing 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.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.12690/full#supplementary-material

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Ten Years of Quality Monitoring of Abdominal Organ Procurement in the Netherlands and Its Impact on Transplant Outcome

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In this study, 10 years of procurement quality monitoring data were analyzed to identify potential risk factors associated with procurement-related injury and their association with long-term graft survival. All deceased kidney, liver, and pancreas donors from 2012 to 2022 and their corresponding recipients in the Netherlands were retrospectively included. The incidence of procurement-related injuries and potential risk factors were analyzed. Of all abdominal organs procured, 23% exhibited procurement-related injuries, with a discard rate of 4.0%. In kidneys and livers, 23% of the grafts had procurement-related injury, with 2.5% and 4% of organs with procurement-related injury being discarded, respectively. In pancreas procurement, this was 27%, with a discard rate of 24%. Male donor gender and donor BMI >25 were significant risk factors for procurement-related injury in all three abdominal organs, whereas aberrant vascularization was significant only for the kidney and liver. In the multivariable Cox regression analyses, procurement-related injury was not a significant predictor for graft failure (kidney; HR 0.99, 95% CI 0.75–1.33, $p = 0.99$, liver; HR 0.92, 95% CI 0.66–1.28, $p = 0.61$, pancreas: HR 1.16; 95% CI 0.16–8.68, $p = 0.88$). The findings of this study suggest that transplant surgeons exhibited good decision-making skills in determining the acceptability and repairability of procurement-related injuries.

Keywords: organ donation, organ procurement, surgery, quality and safety, transplant outcomes

INTRODUCTION

The scarcity of donor organs has created an imbalance between their availability and the growing number of patients on the waiting list. Preventing organ loss due to complications during procurement is paramount, emphasizing the importance of evaluating procurement quality.

In the Netherlands, procurement and transplantation procedures are performed by a dedicated team of surgeons. Over the past decade, the Netherlands has implemented

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Abbreviations: BMI, Body mass index; CT, Computed Tomography; CI, Confidence interval; DGF, Delayed graft function; DBD, Donation after brain death; DCD, Donation after circulatory death; NTS, Dutch Transplantation Foundation (Nederlandse Transplantatie Stichting); eGFR, Estimated glomerular filtration rate; HR, Hazard ratio; KDRI, Kidney Donor Risk Index; MDRD, Modification of Diet in Renal Disease; NOTR, Netherlands Organ Transplant Registry; OR, Odds Ratio; PNF, Primary non function; SD, Standard deviation.

The quality of abdominal organ procurement in the Netherlands and its impact on graft survival

Aim

- Quality monitoring of organ procurement in the Netherlands
- To identify potential risk factors associated with procurement injury

Method

- Retrospective study
- Procurement data of 2012-2022

Risk factors/ impact graft survival

- Kidney:
  - Risk factors: BMI > 25, aberrant anatomy, graft side (left)
  - No impact on 5-year graft survival

- Liver
  - Risk factors: BMI >25, aberrant anatomy, DCD donor, donor male gender
  - No impact on 5 year graft survival

- Pancreas
  - Risk factors: BMI >25, Donor male gender
  - No impact on 5 year graft survival

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GRAPHICAL ABSTRACT

several initiatives to improve procurement quality. In 2010, a national training, certification, and accreditation program was introduced to educate surgeons on abdominal organ procurement procedures [1]. Before this initiative, a data analysis of livers procured in one center in the period 1996–2004 in the Netherlands showed an injury rate of 34% [2]. Subsequently, in 2012, the Quality Form System, a digital scoring system, was implemented to monitor and improve procurement quality and continues to be utilized in the Netherlands. The system involves the completion of a Quality Form for each accepted organ by both the procuring and accepting surgeons after inspection of the organ with data collection by the Dutch Transplantation Foundation. An assessment of procurement-related injuries based on the responses to these Quality Forms was conducted in 2013 [3]. This analysis showed that procurement-related injuries occurred in 25% of procured organs, with a 2% discard rate of organs with procurement related injury. The discard rate due to procurement-related injury was 13% for the pancreas, whereas it was 1% for both kidney and liver. In 23% of cases, there was a discrepancy between the evaluation of the procuring surgeon and transplanting surgeon. As the monitoring system was new, the study only included 1 year of data, resulting in a relatively small sample size of procured organs (270 kidneys, 70 livers, and 28 pancreases) [3].

Monitoring procurement-related injuries is important because of the associated risk of organ discarding. In addition, donor organ procurement-related injuries can be challenging to

manage, potentially irreversible, and may lead to diminished graft function post-transplantation. Despite its significance, there is limited literature available on long-term outcomes following procurement-related injuries [2–8]. Ausania et al. conducted a study categorizing surgical injuries in pancreas procurement and found that arterial and parenchymal injuries significantly negatively affect graft survival [5]. This finding underlines the importance of separately evaluating different categories of procurement-related injuries on graft survival. Notably, in some studies, the scoring of injuries by transplanting surgeons was not consistently available. Relying solely on the procuring surgeon to score surgical injuries might introduce a degree of variability and compromise the reliability of the scoring process.

This study aimed to assess the incidence of procurement-related injuries of abdominal organs procured between the period 2012–2022, including more data on procurement quality, and to investigate the effect of procurement-related injury on 5-year graft survival.

PATIENTS AND METHODS

Study Design and Population

This study was a retrospective analysis liver and kidney procured in the Netherlands from March 2012 to December 2022, and all pancreases procured with the intent of whole organ transplantation, between January 2014 and December 2022. The inclusion of pancreases started from 2014 because from

that year, information on pancreas acceptance for whole-organ transplantation or islet transplantation was registered. The procurement technique used is described in a National Protocol called *Postmortem* donor organ procurement, made by the Organ advisory committee on organ procurement of the Dutch Transplantation Society [9]. Information regarding the surgical technique is included in the **Supplementary Appendix**.

Data Source

The baseline characteristics of the donors were retrieved from the Eurotransplant database. Follow-up data for transplant recipients were sourced from the NOTR (Netherlands Organ Transplant Registry). Consequently, only grafts transplanted in the Netherlands were included in the follow-up analyses. The study protocol was approved by the review board of the NOTR of the Dutch Transplantation Foundation (registration no. 56765) and adhered to the principles outlined in the WMA Declaration of Helsinki and Declaration of Istanbul.

Quality Form

The Quality Form application is a mandatory system administered by the Dutch Transplantation Foundation. Procuring surgeons were required to complete a form after each procurement procedure. If an organ is transplanted in the Netherlands, the transplanting surgeon reviews the form and confirms agreement or disagreement. The Quality Form encompasses the assessment of organ quality (good, acceptable, and poor), organ injury (yes/no), arterial and venous anatomy (normal/abnormal), and the evaluation of organ injury. In accordance with the classification proposed by de Boer et al., the C1-classification denotes a preventable procurement-related injury with the organ still being transplanted [3]. The C2-classification indicates preventable procurement-related injury resulting in the organ not being transplanted (**Table 1**). If there was a disagreement of between the form completed by the procuring surgeon and the transplanting surgeon, the responses of the transplanting surgeon were used. In this study, also forms only filled out by the procuring surgeon were used.

Definitions and Study End Points

The primary outcome measure was the incidence of procurement-related injury. The secondary outcome measures included (death-censored) graft survival, incidence of primary nonfunction (PNF), and delayed graft function (DGF) in kidney transplantation.

For kidney transplant outcomes, DGF was defined as the need for dialysis within the first week after transplantation, while PNF was defined as a non-functioning graft 3 months after transplantation.

For liver transplant outcomes, PNF was defined as the need for re-transplantation or death <7 days after transplantation.

Extraction time of the organ is defined as the time duration between the start cold perfusion of the aorta and the organ's removal from the donor's body. The first warm ischemic time

TABLE 1 | Composition of the procurement-related injury classification, C1: organ transplanted, C2: organ not transplanted. Quality Form scoring system according to the system developed by de Boer et al. [2].

Type on procurement-related injury (C)	Example
Arterial	Intima dissection, partial/complete transection, no aortic patch
Venous	Tear, partial/complete transection, no caval patch
Parenchymal	Tear in capsule, parenchymal rupture

was defined as the duration from asystole in the DCD donor until the start of cold perfusion, which is applicable only to DCD donors. Cold ischemic time was defined as the duration from the start of cold perfusion until removal from cold storage or cold machine perfusion at the (receiving) transplant center. The second warm ischemic time (graft anastomosis time) was defined as the time from organ removal from static cold storage or (hypothermic) machine perfusion until reperfusion in the recipient [10]. The Modification of Diet in Renal Disease (MDRD) equation was used to calculate the eGFR in mL/min/1.73 m^2 [11]. The exclusion of ethnicity was due to its unavailability in the Eurotransplant database.

Aberrant vascular anatomy of the kidney is defined as a kidney graft with multiple renal arteries of renal veins. Aberrant vascular anatomy of the liver and pancreas is defined according to Hiatt's classification [12].

Statistical Analysis

Continuous data were presented as mean ± standard deviation (SD). Categorical data were presented as percentages (%) and absolute numbers. The Kolmogorov-Smirnoff test was used to assess whether continuous variables followed a normal distribution. Parametric tests were used to assess the differences between continuous variables. The Chi-square test was used to assess differences between categorical data. Statistical significance was set at $p < 0.05$.

To assess the potential associations between procurement-related injury of an organ and other variables, a binary logistic regression analysis (procurement-related injury versus no injury) was performed. Initially, each variable was analyzed using a univariable logistic regression model, followed by a multivariate model.

For follow up analysis only the 'C1' category organs (procurement-related injury, organ transplanted) were used. Univariate and multivariable (stepwise) binary logistic regression analyses were employed to determine associations between donor, recipient, and procedural characteristics and DGF in kidney transplant recipients. The results are presented as odds ratios (OR) with corresponding confidence intervals (CI) and *p*-values; Kaplan-Meier survival curves were used to assess death-censored graft survival, and the log-rank test was used to determine differences between the no procurement-related injury and procurement-related injury groups. Recipients who died with a functioning graft were censored, whereas recipients who died
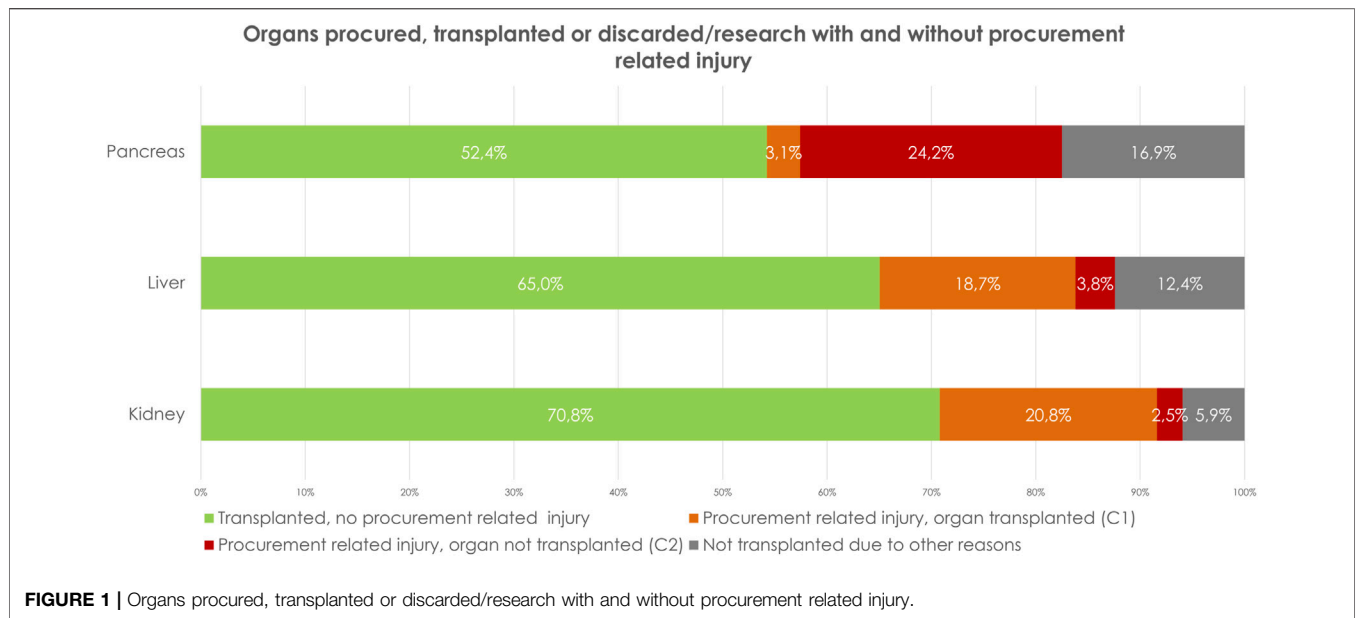
FIGURE 1 | Organs procured, transplanted or discarded/research with and without procurement related injury.

TABLE 2 | A: Number of reported, procured, and transplanted organs. **B:** Procurement related injury per organ as percentage of the total number of organ type procured.

	Kidney	Liver	Pancreas	Total
A				
Total number of organs procured with intend of transplantation	5,495 (100%)	2093 (100%)	456 (100%)	8,044
Total number of transplanted organs	5,034 (91.5%)	1753 (83.8%)	253 (55%)	7,040
B				
Procurement related injury, organ transplanted (C1)	20.8% (n = 1,144/5,495)	18.7% (n = 392/2093)	3.1% (n = 14/456)	19.3% (1,550/8,044)
Procurement related injury, organ not transplanted (C2)	2.5% (n = 135/5,495)	3.8% (n = 79/2093)	24.2% (n = 110/456)	4.0% (n = 324/8,044)
Total percentage of injury	23.3% (n = 1,279/5,495)	22.5% (n = 471/2093)	27.2% (n = 124/456)	23.3% (n = 1874/8,044)

due to graft failure were not censored. Univariable and multivariable (stepwise) Cox regression analyses were performed to identify associations between donor, recipient, procedural characteristics and death-censored kidney- and liver graft survival. Results were presented as hazard ratios (HR) with corresponding confidence intervals (CI) and p-values. Linear mixed models were used to evaluate the mean change in kidney function (expressed as eGFR) over the first 6 years post-transplantation. To assess the longitudinal effect of kidneys with no procurement-related injury versus kidneys with procurement-related injury on eGFR, we defined procurement-related injury, post-transplant time in years, and the interaction between procurement-related injury and post-transplant time as fixed effects.

For statistical analyses, IBM SPSS Statistics for Windows was used (IBM Corp. Released 2022. Version 29.0).

RESULTS

Kidney

Between March 1st' 2012, and December 31st' 2022, 5,495 kidneys were procured, and 5,034 kidneys were

transplanted. In total, 461 (8.5%) of the procured kidney grafts were not transplanted (**Figure 1**; **Table 2**). Of the procured kidneys, 73% (n = 4,003) had one renal artery, 21% (n = 1,176) had two renal arteries and 3% (n = 171) had three renal arteries and in 2% (n = 118) this was not reported. Almost 91% (n = 4,987) had one renal vein, 7% (n = 382) had two renal veins and 1% (n = 31) three renal veins, in 2% (n = 92) the number of veins was not reported.

In 1,279 grafts (23.3%) there was procurement related injury (C1+C2), of which 1,144 grafts were classified as C1 (repaired and transplanted) and 135 (2.5%) as C2 (not transplanted) (**Figure 1**; **Table 2**). Parenchymal injury was the most frequent injury type (**Table 3**). Stratifying by donor type, DCD donors had a significantly higher percentage of procurement-related injuries (C1: 20.7% vs. 20.9%, C2: 1.6% vs. 3.1%, $p < 0.01$) (**Table 4**). Additionally, a higher incidence of procurement-related injury was observed in left kidney grafts (left grafts; C1: 27%, C2: 3%, right grafts; C1: 15%, C2: 2%, $p < 0.01$). Venous injury was more frequent in left kidney grafts (58% vs. 42%,$p < 0.01$), whereas arterial injury was more frequent in right kidney grafts (43% vs. 57%, $p < 0.01$).

Comparing extraction time between C1-, C2- and no procurement related damage-grafts, showed no significant

TABLE 3 | Type of procurement related injury, kidney (percentages as total of the procured kidneys with procurement related injury) and liver (percentages as total of the procured livers with procurement related injury).

	C1	C2	Total
Kidney			
Arterial	n = 110 (8.6%)	n = 18 (1.4%)	128 (10.0%)
Venous	n = 50 (3.9%)	n = 9 (0.7%)	59 (4.6%)
Parenchymal related	n = 216 (17.0%)	n = 26 (2.0%)	242 (19%)
Not classified	n = 768 (60%)	n = 82 (6.4%)	850 (66.4%)
Total	1,144 (89%)	135 (11%)	1,279 (100%)
Liver			
Arterial	n = 125 (26.5%)	n = 26 (5.5%)	n = 151 (32%)
Venous	n = 38 (8.1%)	n = 0 (0%)	n = 38 (8.1%)
Parenchymal related	n = 199 (42.2%)	n = 42 (8.9%)	n = 241 (51.1%)
Not classified	n = 30 (6.4%)	n = 11 (2.3%)	n = 41 (8.7%)
Total	n = 392 (83.2%)	n = 79 (16.8%)	n = 471 (100%)

differences in DBD donors. In DCD donors, the extraction time was significantly longer in procurement related damaged grafts compared to grafts with no procurement related damage (C1 0: 56 ± 0:32, C2 0:55 ± 0:34, no procurement related damage 0:52 ± 0:27, $p = 0.02$) (**Table 5**).

Risk Factors Associated With Injury

In univariable logistic regression analysis, donor male gender, left kidney graft, graft with multiple arteries, and donor BMI >25 were all found to be significantly associated with a higher risk of procurement-related injury (C1+C2) The risk of procurement related injury increased when the number of renal arteries increased (**Table 6**). In multivariable logistic regression analysis (including donor -gender, donor type, age, BMI, left or right kidney, number of arteries, and number of veins), donor BMI >25, left kidney graft, and a graft with multiple arteries remained significantly associated with a higher risk of procurement-related injury (C1+C2) (**Table 6**).

Follow up of Kidneys Transplant Recipients With Procurement-Related Injury (C1)

A total of 5,034 kidneys were transplanted, of which 4,094 were transplanted in the Netherlands. The follow-up data for 4% was missing (n = 160), resulting in the inclusion of 3,934 kidney recipients in the follow up analyses. In 83% the Quality Form was completed by both the procurement surgeon and the transplant surgeons. In 16% the transplant surgeon disagreed with the procuring surgeon on at least one subject.

The characteristics of the kidney donors, recipients and the procedure are summarized in **Supplementary Table S1**, stratified by the absence (C0) or presence of procurement-related injury (C1). A significant difference was observed in donor BMI and donor gender. In total, 23% of the recipients (n = 909) received a kidney with (repaired) procurement-related injury. Most baseline characteristics were not significantly different, except that there were significantly more left kidney grafts in the C1 group (65% vs. 46%, $p < 0.01$). Additionally, more grafts in the C1 group had multiple arteries (23% vs. 33%, $p < 0.01$).

Short Term Transplant Outcome

DGF was observed in 35% (n = 1,376) of recipients, while PNF occurred in three percent (n = 120). Eight percent of the information on graft function in the first week after transplantation was missing. When comparing the incidence of immediate graft function, DGF, and PNF separately for recipients of DBD and DCD donors, no significant differences were observed between the C0 and C1 groups (**Table 7**). Comparing the incidence of immediate graft function, DGF and PNF separate per type of damage group, arterial, venous, and parenchymal related damage versus no procurement related damage, the incidence of PNF was higher in grafts from DBD donors with venous damage compared to grafts from DBD with no procurement related damage (14% versus 2.5%, $p < 0.01$). The incidence of DGF was significantly higher in grafts from DBD and DCD donors with parenchymal damage (39% versus 21% in kidney grafts from DBD donors, 56% versus 46% in kidney grafts from DCD donors) (**Table 7**).

Univariate logistic regression demonstrated that procurement-related injury did not increase the risk of developing DGF (OR, 1.14; 95% CI 0.98–1.34, $p = 0.10$) (**Table 8**). In multivariable logistic regression analyses, this was confirmed after adjustment for potential confounding factors (**Table 8**, models 1–3). Donor age, body mass index, male gender, history of hypertension, cause of death, type of donor (DCD), recipient age, history of diabetes and cardiac disease, cold ischemic time, and preservation method (cold storage) were associated with a higher risk of developing DGF based on multivariate analyses (**Supplementary Table S2**).

TABLE 4 | Procurement related damage per organ type as percentage of the total number of procured organ type, stratified by type of donor.

	DBD			**DCD**			
	C0	C1	C2	C0	C1	C2	
Kidney	77.7% (1805/2,324)	20.7% (n = 482/2,324)	1.6% (n = 37/2,324)	76.0% 2,411/3,171	20.9% (n = 662/3,171)	3.1% (n = 98/3,171)	**p=<0.01**[a]
Liver	79.7% (952/1,194)	18.6% (n = 222/1,194)	1.7% (n = 20/1,194)	74.5% (n = 670/899)	18.9% (n = 170/899)	6.6% (n = 59/899)	**p=<0.01**[a]
Pancreas	72.4% (n = 192/265)	3.8% (n = 10/265)	23.8% (n = 63/265)	73.3% (n = 140/191)	2.1% (n = 4/191)	24.6% (n = 47/191)	$p = 0.58$[a]

DBD, donation after brain death; DCD, donation after circulatory death.
[a]A Chi-square test (and Fisher extract for the pancreases) was used to investigate whether the incidence of C1 and C2 was different between donor type. Significant differences in bold.
Bold values indicate statistical siginificance of P values.

TABLE 5 | Extraction time, stratified per organ, type of donor and procurement related injury.

	C1	C2	No procurement related damage		Missing data (%)
DBD, Kidney	1:00 ± 0:30	1:04 ± 0:29	0:58 ± 0:26	p = 0.26	4
DCD, Kidney	0:56 ± 0:32	0:55 ± 0:34	0:52 ± 0:27	**p = 0.02**	
DBD, Liver	0:47 ± 0:21	0:54 ± 0:27	0:45 ± 0:20	p = 0.13	5
DCD, Liver	0:51 ± 0:26	0:46 ± 0:16	0:49 ± 0:22	p = 0.41	
DBD, Pancreas	0:58 ± 0:22	0:57 ± 0:26	0:55 ± 0:24	p = 0.62	14
DCD, Pancreas	1:17 ± 0:43	1:03 ± 0:39	0:59 ± 0:30	p = 0.09	

Bold values indicate statistical siginificance of P values.

Long Term Transplant Outcome

In a linear mixed model using eGFR as the dependent variable, there was no significant difference in the mean eGFR over time between the C0 and C1 groups at 3 months and 1–6 years post transplantation ($p = 0.77$) (**Figure 2**).

Kaplan-Meier survival analysis showed no significant differences in death-censored graft survival 5 years post transplantation between the C0 and C1 groups (log-rank test, $p = 0.44$) (**Figure 3**). A separate Kaplan-Meier survival analysis was performed for parenchymal and arterial injuries, which also showed no significant differences.

Univariable death-censored Cox regression analyses demonstrated that procurement-related injury did not increase the hazard rate of graft failure (HR 0.94; 95% CI 0.77–1.14, $p = 0.54$) (**Table 8**). This finding was further confirmed by multivariable Cox regression analysis adjusted for potential confounding factors (**Table 8**, model 1–3). Donor age, recipient age, history of cardiac disease, and cold ischemic time were identified as the significant factors associated with graft failure (**Supplementary Table S2**).

Liver

Between March 1st, 2012, and December 31st, 2022, 2093, livers were procured and 1753 were transplanted. In total, 340 (16.2%) of the procured liver grafts were not transplanted. 112 grafts were procured *en-bloc* with the pancreas.

Of the procured organs, 69.5% (n = 1,455) had a normal vascular anatomy. 14% (n = 292) had replaced or accessory left hepatic artery (Type II), 7% (n = 145) a replaced or accessory right hepatic artery (Type III), 3% (n = 61) a replaced or accessory right hepatic artery + replaced or accessory left hepatic artery (Type IV), 1% (n = 13) had the common hepatic artery arise from the superior mesenteric artery (Type V) and 0.1% (n = 2) had the common hepatic artery arise from the aorta (Type VI). In 6% (n = 125) no further classification of the aberrant anatomy was available. In 471 grafts (22.5%) there was procurement related injury (C1+C2), of which 392 grafts were classified as C1 (repaired and transplanted) and 79 (3.8%) as C2 (not transplanted) (**Figure 1**; **Table 2**). Stratifying for the

TABLE 6 | Odds ratios of risk factors for procurement related injury (C1+C2), kidney.

	Univariable		Multivariable[a]	
Donor gender				
- Female	1.00	**p<0.01**	1.00	p = 0.08
- Male	1.20 [1.05–1.39]		1.13 [0.99–1.29]	
Donor type				
- DBD	1.00	p = 0.16	1.00	p = 0.44
- DCD	1.01 [0.99–1.24]		1.05 [0.93–1.20]	
Donor age				
- 0–15 years	0.80 [0.48–1.33]	p = 0.53	0.94 [0.53–1.67]	p = 0.90
- 16–25 years	1.00		1.00	
- 26–35 years	1.10 [0.78–1.56]		1.05 [0.73–1.51]	
- 36–45 years	1.08 [0.78–1.50]		1.08 [0.77–1.51]	
- 46–55 years	1.16 [0.88–1.54]		1.16 [0.86–1.55]	
- 56–65 years	1.20 [0.91–1.58]		1.14 [0.85–1.52]	
- 66–75 years	1.20 [0.90–1.59]		1.19 [0.88–1.59]	
- >75 years	0.89 [0.50–1.58]		0.93 [0.52–1.68]	
Donor BMI (kg/m²)				
- <18.5	0.87 [0.60–1.24]	**p<0.01**	0.93 [0.62–1.41]	**p<0.01**
- 18,5–25	1.00		1.00	
- 25–30	1.32 [1.15–1.52]		1.26 [1.09–1.47]	
- 30–35	1.48 [1.20–1.84]		1.44 [1.15–1.81]	
- 35–40	1.42 [1.02–1.97]		1.38 [0.98–1.93]	
- >40	1.12 [0.64–1.97]		1.08 [0.61–1.95]	
Graft side				
- Right kidney	1.00	**p<0.01**	1.00	**p<0.01**
- Left kidney	2.13 [1.89–2.44]		2.16 [1.89–2.47]	
Number of arteries				
- One	1.00	**p<0.01**	1.00	**p<0.01**
- Two	1.41 [1.22–1.64]		1.40 [1.20–1.63]	
- Three	1.74 [1.25–2.41]		1.75 [1.25–2.45]	
- Four	5.70 [2.46–13.20]		5.26 [2.22–12.46]	
Number of veins				
- One	1.00	p = 0.20	1.00	p = 0.79
- Two	0.85 [0.66–1.09]		1.04 [0.79–1.35]	
- Three	0.62 [0.23 = 1.60]		0.90 [0.34–2.38]	

[a]*In the multivariable analysis donor-gender, -age, -type, -BMI, the graft side, number of arteries and veins of the graft are all added at once in the same model.*
Bold values indicate statistical siginificance of P values.

type of injury, parenchymal injury emerged as the most frequent type of injury (**Tables 2**, **3**). Stratifying donor-type DCD donors had a significantly higher percentage of procurement-related injury for both C1 and C2 (C1: 18.9. % vs. 18.6%, C2; 6.6% vs. 1.7%, p=<0.01) (**Table 4**). Comparing extraction time between C1-, C2- and no procurement related damage-grafts, showed no significant differences (**Table 5**).

Risk Factors Associated With Injury

In univariate logistic regression analysis, donor-male gender, BMI> 25, DCD type of donor, and aberrant vascular anatomy were all significantly associated with a higher risk of procurement-related injury (C1+C2) (**Table 9**). Especially type III and type VI of aberrant vascular anatomy were associated with a higher risk of procurement related injury. Multivariable logistic

TABLE 7 | Graft function in kidney recipients, stratified by donor type, procurement related damage (no/yes: C1), and type of damage.

		Immediate graft function	Delayed graft function	Primary non function	
DBD					
No Procurement related damage		73% (n = 799)	21% (n = 233)	2.5% (n = 27)	*p* = 0.72
Procurement related damage (C1)		71% (n = 226)	25%(n = 78)	2.5% (n = 8)	
Type of damage					
	Arterial damage (vs. no damage)	68% (n = 28)	27%(n = 11)	5% (n = 2)	*p* = 0.44
	Venous damage (vs. no damage)	64% (n = 9)	7% (n = 1)	14% (n = 2)	*p*<0.01
	Parenchymal damage (vs. no damage)	61% (n = 30)	39% (n = 19)	0% (n = 0)	**p = 0.02**
DCD					
No Procurement related damage		48% (n = 826)	46% (n = 800)	4% (n = 72)	*p* = 0.11
Procurement related damage (C1)		47% (n = 246)	50% (n = 265)	2.4% (n = 13)	
Type of damage					
	Arterial damage (vs. no damage)	46% (n = 22)	48% (n = 23)	4% (n = 2)	*p* = 0.99
	Venous damage (vs. no damage)	41% (n = 11)	59% (n = 16)	0% (n = 0)	*p* = 0.43
	Parenchymal damage (vs. no damage)	43% (n = 50)	56% (n = 65)	0% (n = 0)	**p = 0.04**

Values are presented as percentage. DBD, Donation after Brain death; DCD, donation after circulatory death.
[a]A Chi-square test was used to investigate the difference in incidence in immediate graft function, delayed graft function and primary non function between the groups.
Bold values indicate statistical siginificance of P *values.*

TABLE 8 | Uni- and multivariable logistic regression analysis and Cox regression analysis evaluating the association between procurement-related injury, correcting for donor, procedural and recipient characteristics with the risk of delayed graft function and (death censored) graft failure in the kidney recipient. Results of the full model are listed in **Supplementary Table S2**.

	DGF OR [95% CI]		Graft failure HR [95% CI]	
Univariable	1.14 [0.98–1.34]	*p* = 0.10	0.94 [0.77–1.14]	*p* = 0.54
Model 1	1.10 [0.93–1.30]	*p* = 0.27	0.94 [0.78–1.15]	*p* = 0.58
Model 2	1.41 [0.8–1.27]	*p* = 0.97	0.94 [0.71–1.25]	*p* = 0.68
Model 3	1.02 [0.81–1.31]	*p* = 0.85	0.99 [0.75–1.33]	*p* = 0.99

Model 1: Procurement-related injury + donor age + donor BMI + donor gender + donor history of diabetes + donor history of hypertension + donor type + donor cause of death.
Model 2: Model 1 + first warm ischemia time + second warm ischemia time + cold ischemia time + multiple arteries + multiple veins + kidney site + machine perfusion.
Model 3: Model 2 + recipient age + recipient BMI + recipient gender + recipient diabetes + recipient cardiac disease + primary disease.
Univariable = procurement-related injury, C1 only.

regression analysis (including donor, type, age, BMI, and aberrant vascular anatomy) confirmed these associations (**Table 9**).

Follow up of Liver Transplant Recipients With Procurement-Related Injury (C1)

In total, 1753 livers were transplanted, with 1,317 whole livers transplanted in the Netherlands, which formed the basis for the follow-up analyses. In 86% (n = 1,136) the Quality Form was completed by both the procurement surgeon and the transplant surgeon. In 30% the transplant surgeon disagreed with the procuring surgeon on at least one subject. In these cases, the response of the transplant surgeon was used.

The characteristics of liver donors and their recipients are outlined in **Supplementary Table S3**, stratified by the presence or absence of procurement-related injury (C0 vs. C1). There was a significant difference in the BMI between the groups (*p*=<0.01). In total, 23% of the recipients (n = 306) received a liver with (repaired) procurement-related injury (C1). No significant

differences were observed in the baseline characteristics between the two groups.

Transplant Outcome

Twenty-five recipients (1.9%) had PNF. The incidence of PNF was not significantly different between the C0 and C1 groups of recipients (C0: 6%, n = 18 versus C1: 9%, n = 7, *p* = 0.15). In addition, the incidence of graft related injuries, (anastomotic biliary complications, hepatic vein thrombosis, and arterial thrombosis taken together), other reasons for graft failure (i.e., recurrence of disease, malignancy *de novo*, rejection, bacterial infection) and no graft failure were compared between grafts with no injury and C1-injury. This showed no significant difference in incidence.

Kaplan-Meier survival analysis showed no significant differences in death-censored graft survival 5 years post-transplantation between the C0 and C1 groups (log-rank test *p* = 0.74) (**Figure 4**). Further Kaplan-Meier survival analysis, specifically for parenchymal and arterial injuries, also demonstrated no significant differences.

Univariable death-censored Cox regression analyses indicated that procurement-related injury did not increase the hazard rate for graft failure (0.91; 95% CI [0.67–1.21], *p* = 0.51). This finding was confirmed by multivariable Cox-regression analysis adjusted for potential confounding factors (**Table 10**, model 1–3). Notably, donor age, type (DCD), recipient age, and primary disease were identified as significant factors for graft failure (**Supplementary Table S4**).
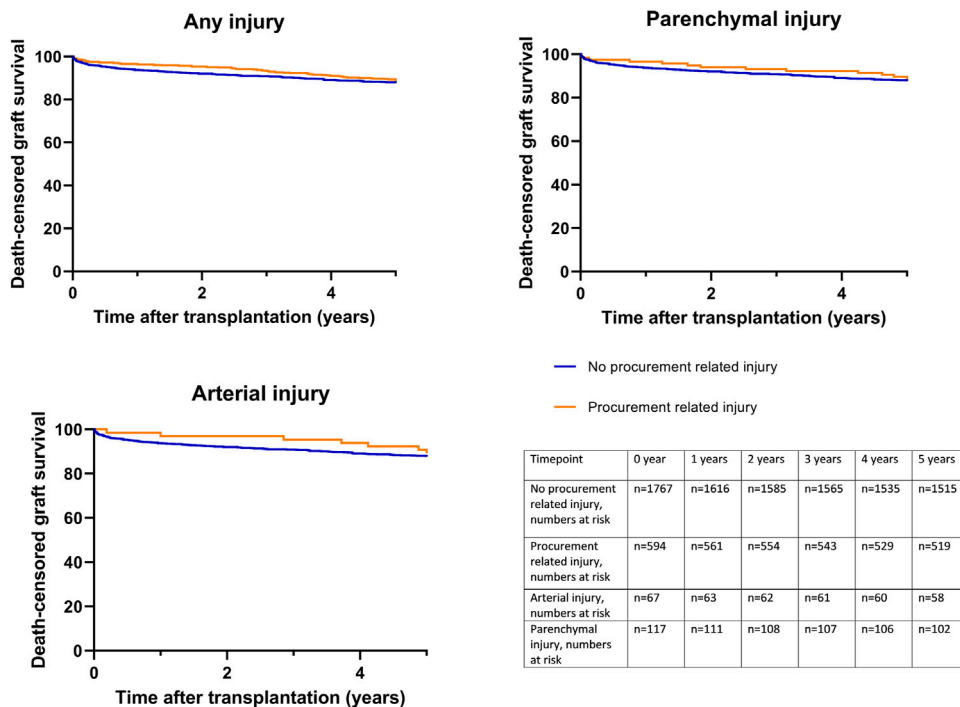
Pancreas

Between January 1st, 2014, and December 31st, 2022, 456 pancreases were procured for whole organ transplantation, 253 pancreases were transplanted as whole organs, and 16 were eventually used for islet transplantation. In total, 187 (41%) pancreases were not used of transplantation, of which 24% (n = 110) had procurement-related injury (C2) (**Figure 1**; **Table 2**). Eight of the grafts C2 grafts were used for islet transplantation and 28 were used for research.
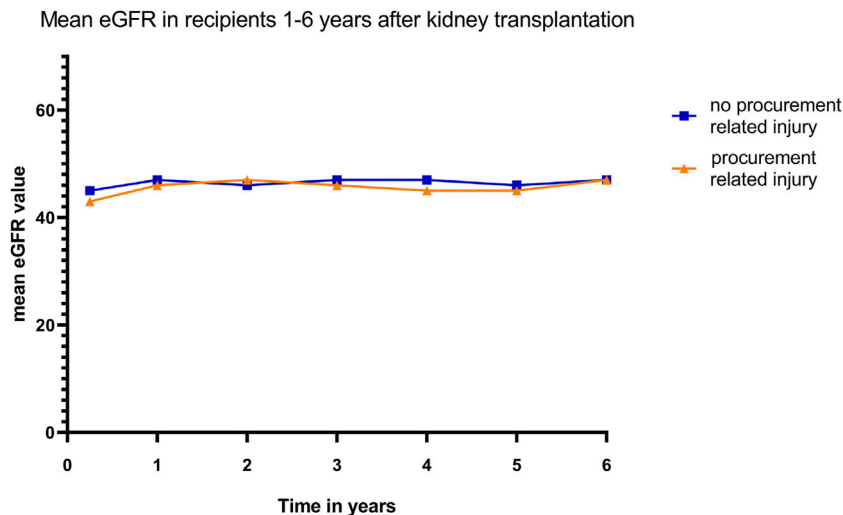
FIGURE 2 | Mean eGFR (in mL/min/1.73m^2) in kidney recipients 3 months –6 years after transplantation.

FIGURE 3 | Death-censored graft survival until 5 years post kidney transplantation, according to procurement related injury; any C1 injury, (Log rank test $p = 0.44$), parenchymal injury (Log rank test $p = 0.59$) and arterial injury (Log rank test $p = 0.78$).

FIGURE 4 | Death-censored graft survival until 5 years post liver transplantation, according to procurement related injury; any C1 injury, (Log rank test $p = 0.74$), parenchymal injury (Log rank test $p = 0.30$) and arterial injury (Log rank test $p = 0.45$).
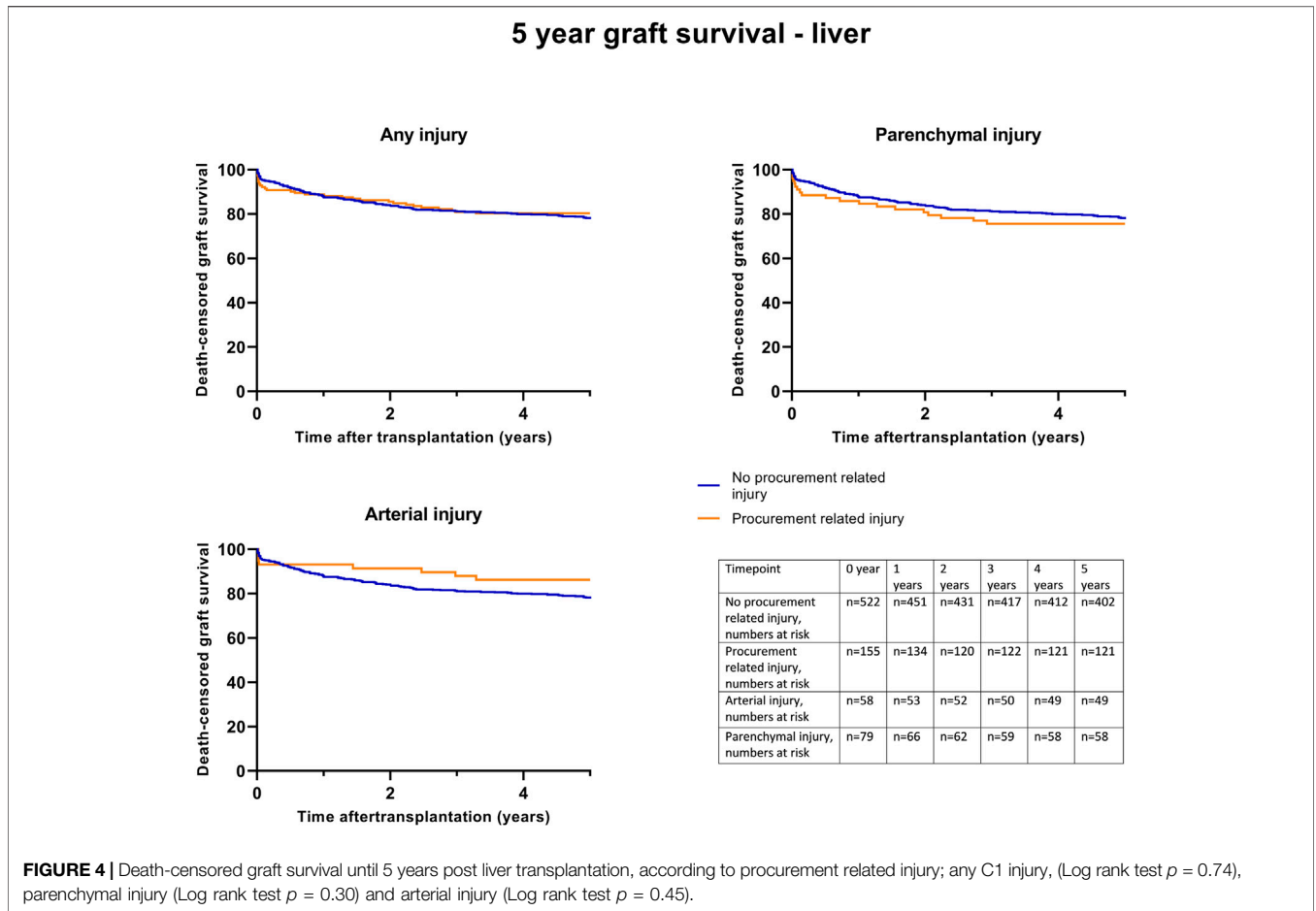
Three percent of pancreases (13/456 pancreases procured) were classified as 'C1.' After stratification by donor type, no significant differences were found in the percentages of 'C1' and 'C2' between DBD and DCD donors (**Table 4**).

Comparing extraction time between C1-C2- and no procurement related damage-grafts, showed no significant differences (**Table 4**).

Risk Factors Associated With Injury

Univariate binary logistic regression analyses showed that a donor BMI >25 was significantly associated with a higher risk of procurement-related injury (**Table 11**).

In the multivariable binary logistic regression analysis (including donor age, gender, BMI, and type), both BMI >25 and male gender emerged as significant risk factors for procurement-related injury (**Table 11**).

Follow up of Pancreas Transplant Recipients With Procurement-Related Injury (C1)

A total of 209 pancreases were transplanted into the Netherlands. In 86% of procured grafts, the Quality Form was completed, by both procuring and transplanting surgeons. In 14% disagreements arose regarding at least one subject.

Follow up data of 193 (96%) of the pancreas recipients were accessible in the database. Of the 13 pancreases transplanted with procurement-related injury, ten grafts were transplanted in the Netherlands with available follow-up data. The characteristics of pancreas donors and their recipients are outlined in **Supplementary Table S5**, stratified by the presence or absence of procurement-related injury (C0 vs. C1). There were no significant differences in baseline characteristics. Kaplan-Meier survival analysis showed no significant differences in death-censored graft survival 5 years post-transplantation between the C0 and C1 groups (log-rank test $p = 0.86$) (**Supplementary Figure S1**).

Univariable death-censored Cox regression analysis indicated that procurement-related injury did not increase the hazard rate for graft failure (HR 1.16; 95% CI 0.16–8.68, $p = 0.88$). Multivariable analysis was not performed due to small number of cases.

DISCUSSION

This national study is an extension of the study from 2017 by de Boer et al., including data from 10 years of procurement quality monitoring in the Netherlands. From all organs procured

TABLE 9 | Odds ratios of risk factors for procurement related injury (C1+C2), liver.

	Univariable		Multivariable	
Donor gender				
- Female	1.00	**p = 0.03**	1.00	**p = 0.05**
- Male	1.33 [1.09–1.64]		1.24 [1.01–1.54]	
Donor type				
- DBD	1.00	**p<0.01**	1.00	**p<0.01**
- DCD	1.34 [1.09–1.65]		1.31 [1.05–1.52]	
Donor age				
- 0–15 years	0.74 [0.34–1.62]	p = 0.88	0.78 [0.33–1.83]	p = 0.98
- 16–25 years	1.00		1.00	
- 26–35 years	1.06 [0.62–1.79]		1.02 [0.60–1.75]	
- 36–45 years	1.10 [0.68–1.79]		1.08 [0.66–1.76]	
- 46–55 years	0.98 [0.64–1.50]		0.94 [0.61–1.44]	
- 56–65 years	1.05 [0.69–1.60]		0.99 [0.64–1.52]	
- 66–75 years	0.91 [0.58–1.43]		0.93 [0.58–1.47]	
> 75 years	0.70 [0.32–1.51]		0.76 [0.34–1.68]	
Donor BMI (kg/m^2)				
- <18.5	1.02 [0.58–1.78]	**p<0.01**	1.12 [0.61–2.08]	**p = 0.03**
- 18,5–25	1.00		1.00	
- 25–30	1.45 [1.15–1.83]		1.39 [1.10–1.77]	
- 30–35	1.54 [1.08–2.20]		1.38 [0.96–1.99]	
- 35–40	1.41 [0.77–2.59]		1.42 [0.77–2.63]	
- >40	3.15 [1.25–7.97]		3.16 [1.23–8.13]	
Anatomy vascularization[b]				
- Normal	1.00	**p<0.01**	1.00	**p<0.01**
- Type II	0.99 [0.73–1.36]		1.01 [0.74–1.34]	
- Type III	2.15 [1.49–3.10]		2.13 [1.47–3.08]	
- Type IV	1.15 [0.62–2.11]		1.12 [0.60–2.07]	
- Type V	1.15 [0.32–4.22]		1.04 [0.28–3.87]	
- Type VI	3.85 [0.24–61.7]		3.98 [0.24–65.11]	
- Not classified	1.88 [1.26–2.79]		1.82 [1.22–2.71]	

[a]In the multivariable analysis donor-gender, -type, -age -BMI, and normal/abnormal anatomy regarding vascularization are all added at once in the same model.
[b]According to Hiat's classification: Type I: normal anatomy; Type II: replaced or accessory left hepatic artery; Type III: replaced or accessory right hepatic artery; Type IV: replaced or accessory right hepatic artery + replaced or accessory left hepatic artery; Type V: common hepatic artery from the superior mesenteric artery; Type VI: common hepatic artery from the aorta.
Bold values indicate statistical siginificance of P values.

TABLE 10 | Uni- and multivariable Cox regression analysis evaluating the association between procurement-related injury, correcting for donor, procedural and recipient characteristics with the risk of (death censored) graft failure in the liver recipient. Results of the full model are listed in **Supplementary Table S3**.

	Graft failure HR [95% CI]	
Univariable (procurement related injury, C1)	0.89 [0.66–1.20]	p = 0.46
Model 1	0.90 [0.65–1.25]	p = 0.90
Model 2	0.90 [0.65–1.25]	p = 0.51
Model 3	0.92 [0.66–1.28]	p = 0.61

Model 1: Procurement-related injury + donor age + donor BMI + donor gender + donor history of diabetes + donor history of hypertension + donor type + donor cause of death.
Model 2: Model 1 + first warm ischemia time + second warm ischemia time + cold ischemia time + aberrant vascular anatomy.
Model 3: Model 2 + recipient age + recipient BMI + recipient gender + primary disease.

The percentage of procurement-related injury was significantly higher in the DCD procedures than in the DBD procedures for kidney and liver grafts (**Table 4**). Potential contributing factors include the absence of circulation in DCD donation, making it more challenging to inspect vascular anatomy. In addition, time pressure to minimize warm ischemia and extraction times in DCD procedures may have been a factor, as prolonged nephrectomy and hepatectomy times are associated with worse outcomes after transplantation [13–15]. However, in multivariable analyses, DCD was found to be a significant risk factor for procurement-related injury of the liver, but not for the kidney or pancreas (**Tables 5**, **9**, **11**). In DCD liver donation, the entire liver dissection occurs after aortic cross-clamping, whereas in DBD donors, preparatory dissection is performed before the start of aortic cold flushing [16]. In kidney and pancreas procurement, there is less or no preparatory dissection, even in DBD procedures, which could explain why DCD donation was not a significant risk factor for kidney and pancreas procurement.

Higher BMI and male gender of the donor are risk factors for procurement-related injury in kidney, liver, and pancreas procurement, which is supported by other publications [3, 17]. A possible explanation for this association could be variations in fat distribution between genders. Men tend to store body fat in the abdominal (visceral) region, whereas women have a higher proportion of body fat in the gluteal-femoral region [18]. Increased visceral abdominal fat may contribute to the complexity of the procurement procedure. Potential strategies to minimize the risk of procurement related injury in high BMI patients could be to implement an upper limit for accepting donors with a BMI above 40. However, such a measure might have undesirable consequences due to the impact on donor numbers, particularly given the organs shortage.

Left kidney grafts carry a significantly higher risk of procurement-related injury (OR 2.13, 95% CI 1.89–2.44) according to our study. The percentage of left kidney grafts was higher in grafts with procurement-related injury than in those with no procurement-related injury. Venous-related injuries were more frequent in the left kidney than in the right. A possible explanation for this could be the position of

between March 2012-December 2022 (kidney + liver) and January 2014- December 2022 (pancreas); 23% (1874/8,044) had procurement-related injury (C1+C2). Of the injured organs, 4% (324/1874 organs, C2) were not transplanted. Remarkably, the rate of procurement-related injury for pancreatic grafts was notably higher at 27.2%, compared to kidney (23.3%) and liver (22.5%) grafts. Importantly, procurement-related injury did not influence death-censored 5-year graft survival.

In kidney and liver grafts, the ratios of C1-type and C2-type injuries were comparable (kidney C1: 20.8%, C2 2.5%, liver C1: 18.7%, C2: 3.8%), while in pancreatic grafts, the ratio was reversed (C1: 3.1%, C2: 24.2%), suggesting that injured pancreases are more often discarded for transplantation compared to kidney and liver grafts (**Table 2**; **Figure 1**). This tendency may stem from transplant surgeons' reluctance to use an injured pancreatic graft for whole-organ transplantation.

TABLE 11 | Odds ratios of risk factors for procurement related injury (C1+C2), pancreas.

	Univariable		Multivariable	
Donor gender				
- Female	1.00	p = 0.06	1.00	**p = 0.03**
- Male	1.55 [0.99–2.42]		1.71 [1.06–2.75]	
Donor type				
- DBD	1.00	p = 0.69	1.00	p = 0.64
- DCD	1.10 [0.70–1.72]		1.13 [0.69–1.84]	
Donor age				
- 0–15 years	0.66 [0.23–1.95]	p = 0.21	0.80 [0.27–2.41]	p = 0.13
- 16–25 years	1.00		1.00	
- 26–35 years	0.84 [0.39–1.77]		0.88 [0.41–1.87]	
- 36–45 years	1.24 [0.63–2.45]		1.36 [0.68–2.73]	
- 46–55 years	1.66 [0.89–3.10]		1.89 [0.97–3.69]	
- 56–65 years	0.46 [0.12–1.70]		0.41 [0.11–1.57]	
- 66–75 years	1.75 [0.39–7.90]		1.65 [0.35–7.78]	
Donor BMI (kg/m^2)^a				
- <25	1.00	**p = 0.02**	1.00	**p = 0.04**
- >25	1.73 [1.11–2.71]		1.65 [0.35–7.78]	

^{a}Because 55% of the donors had a BMI, of 18,5%–25% and 34% of the donors had a BMI, of 25–30, and the number of donors in the other categories (<18,5, 30–35, >40) were small, this division was chosen.

Bold values indicate statistical siginificance of P values.

the left renal vein on the ventral side of the aorta, enlarging the chance on procurement-related injury of the vein during dissection of the aorta. This result contrasts with a prior study of Taber-Hight et al., which found the right kidney to be the most likely injured organ during procurement, for which we have no clear explanation [19].

We found that kidney and liver grafts with aberrant vascular anatomy (having more than one renal artery in case of kidney procurement, and aberrant anatomy of the liver vascularization according to Hiatt's classification) were injured more frequently [12]. Knowledge of this anatomy, through the availability of a preoperative contrast-enhanced CT scan, before procurement could aid in preventing procurement-related arterial injuries [20, 21]. In the Netherlands, a contrast-enhanced (abdominal) CT scan has been performed for every DBD and DCD donor since 2023; however, the results of these policy changes on procurement-related injuries are still in progress. Specific risks related to vascular anatomy in pancreas procurement were not analyzed in this study because of the relatively small number of pancreases with this type of anatomy (the hepatic artery arising from the SMA was only reported in 13 donors). Ausenia et al., however, identified the hepatic artery arising from the SMA as a significant risk factor for procurement-related injury in pancreas procurement [5].

This study had a few limitations that need to be acknowledged. First, only grafts transplanted in the Netherlands had a quality form filled out by the transplanting surgeon. However, there is a high rate of agreement of 70%–86% between the procuring and transplanting surgeons, suggesting that forms filled out only by the procuring surgeon may be sufficient. An option could have been to only use organs with a Quality form filled out by both parties, but a part of the C2 organs are deemed not

transplantable by the procuring surgeon. For these organs, no form is available of the transplanting surgeon. Excluding these cases, would therefore cause under reporting of the C2 organs. Second, the retrospective design of this study resulted in missing data regarding information that would have been interesting to investigate further. For example, no information regarding previous abdominal surgery was available, which could be valuable information to have prior to the procurement because of possible adhesions due to prior abdominal surgery. Also further classification of the type of injury was lacking for 60% of Quality Forms of kidneys and the necessity for repairment was limited available, since this information is not consistently captured. Also investigating whether en-bloc procurement of liver and pancreas leads to less injuries would be interesting, but since the number of grafts procured en-bloc number was relatively low, we did not include this is our analyses. One of the major strengths of this study is the mandatory nature of follow-up registries for kidney and liver transplantation in the Netherlands, ensuring nearly complete follow-up data. Although since the relatively small number of pancreases with procurement-related injuries transplanted, it is difficult to draw conclusions from this analysis.

This study demonstrated that procurement-related injury in transplanted organs does not affect long-term graft survival. It is important to emphasize that this comes with a certain bias; in these organs, the procurement-related injury could be repaired, and therefore, these organs could successfully be transplanted. On the other hand, procurement-related injury contributed to the discard of 4% (324/8,044) of procured organs: 135 kidneys, 79 livers, and 110 pancreases. Every organ lost for transplantation due to preventable reasons is one too many. Therefore, further research should focus on preventive measures against procurement-related injuries. We previously demonstrated that procedures during evening/night-time have a higher incidence of procurement-related injury than day-time procedures [22]. Centralizing the organization of organ procurement could also contribute to a decrease in procurement-related injuries. Although center volume was not specifically addressed in this study, de Boer et al. showed that centers performing more procurements had significantly fewer injuries (C1+ C2) for kidney and pancreatic procurement [3]. In 2023 Lam et al. suggested that cumulative sum (CUSUM) analysis plots with data from the Quality Forms could be of value to prospectively monitor procurement-related injury in a real-time manner, which could further lead to quality improvement and bring quality monitoring to a new level [23].

In conclusion, procurement-related injuries occur in 23% of abdominal organs procured in the Netherlands, resulting in 4% of the procured grafts not being suitable for transplantation. Despite this, the majority of kidney and liver grafts with procurement-related injury are still transplanted, showing no significant differences in 5-year graft survival compared with grafts with no procurement-related injury. This suggests effective decision making by transplant surgeons in determining the acceptability and reparability of procurement-related injuries. Auditing, national training of procurement surgeons, and certification contribute to this, and are important to even lower the incidence of procurement-related injuries in the future.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by the Review board of the Netherlands Transplant registry (NOTR) of the Dutch Transplant Foundation. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

AEB and KC participated in research design. KC and AH participated in data collection. AEB, AH and KC participated in data analysis. 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.2024.12989/full#supplementary-material

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Improved Preservation of Rat Small Intestine Transplantation Graft by Introduction of Mesenchymal Stem Cell-Secreted Fractions

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Segmental grafts from living donors have advantages over grafts from deceased donors when used for small intestine transplantation. However, storage time for small intestine grafts can be extremely short and optimal graft preservation conditions for short-term storage remain undetermined. Secreted factors from mesenchymal stem cells (MSCs) that allow direct activation of preserved small intestine grafts. Freshly excised Luc-Tg LEW rat tissues were incubated in preservation solutions containing MSC-conditioned medium (MSC-CM). Preserved Luc-Tg rat-derived grafts were then transplanted to wild-type recipients, after which survival, injury score, and tight junction protein expression were examined. Luminance for each graft was determined using *in vivo* imaging. The findings indicated that 30–100 and 3–10 kDa fractions of MSC-CM have superior activating effects for small intestine preservation. Expression of the tight-junction proteins claudin-3, and zonula occludens-1 preserved for 24 h in University of Wisconsin (UW) solution containing MSC-CM with 50–100 kDa, as shown by immunostaining, also indicated effectiveness. Reflecting the improved graft preservation, MSC-CM preloading of grafts increased survival rate from 0% to 87%. This is the first report of successful transplantation of small intestine grafts preserved for more than 24 h using a rodent model to evaluate graft preservation conditions that mimic clinical conditions.

Keywords: small intestine, preservation, mesenchymal stem cells, transplantation, graft survival

INTRODUCTION

Recently, the management of intestinal failure has advanced with the establishment of the concept of an intestinal rehabilitation program. The resulting improved treatment results, especially for pediatric cases of intestinal failure, led to a reduction in number of annual intestinal transplants (ITx) worldwide to 149 in 2017 since reaching a peak of 270 per year in 2008 [1]. Nonetheless, ITx remains the ultimate alternative for patients requiring permanent parenteral nutrition. ITx using segmental grafts from living-related donors has recently been proposed to be advantageous [2].

Abbreviations: rAT-MSCs, rat adipose tissue-derived mesenchymal stem cells; CM, conditioned medium; Luc, luciferase; Tg, transgenic; LEW, Lewis; NRDFs, neonatal rat derived fibroblasts; ITx, intestinal transplants; UW, University of Wisconsin.

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GRAPHICAL ABSTRACT
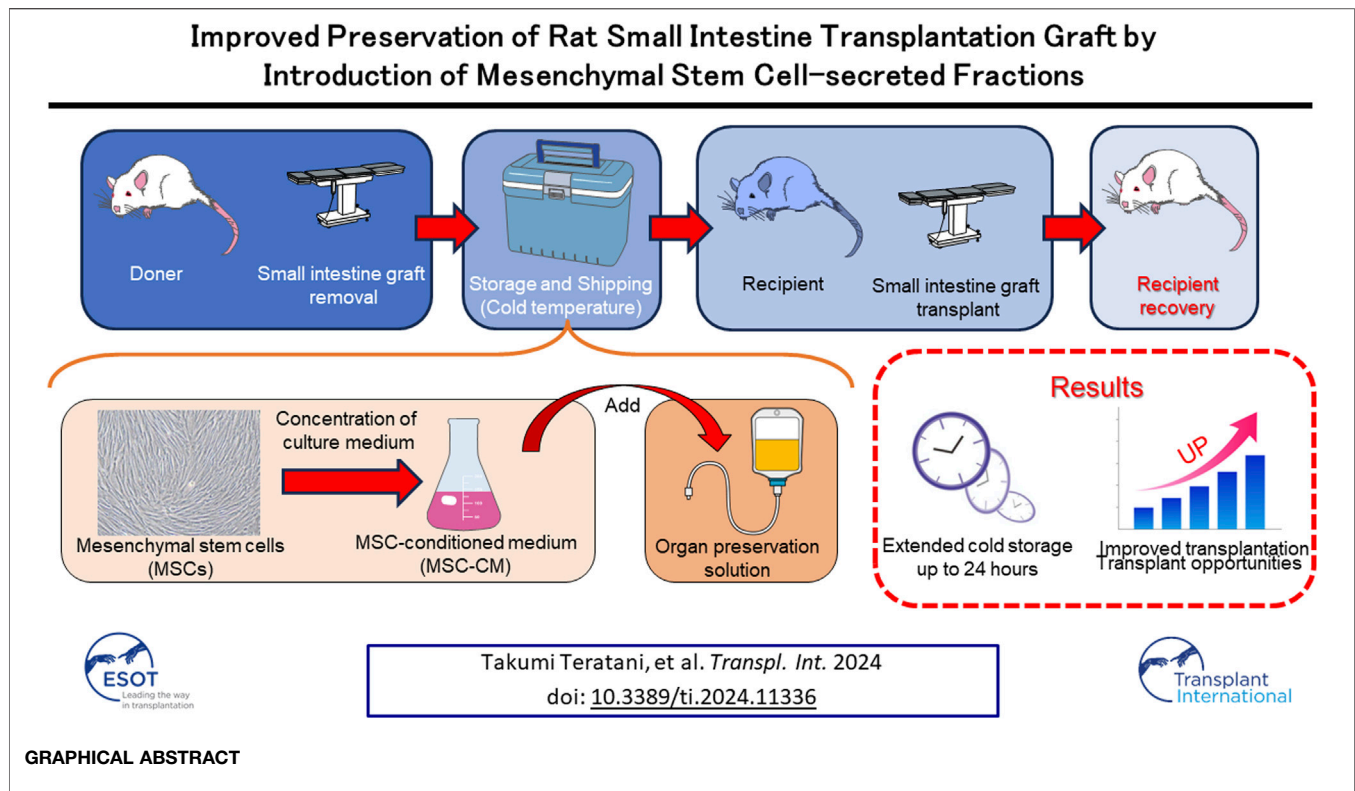
Such grafts allow tissue matching, as well as shorter cold ischemic and operating times as compared with grafts from deceased donors [3]. Graft viability prior to implantation is a key factor in organ transplantation outcomes. When using grafts from brain dead donors, ischemia reperfusion injury and preservation damage affects graft quality, especially their barrier function [4]. During preservation, mucosal injury rapidly progresses to mucosal breakdown. Tissue injury worsens with reperfusion and further impairs the mucosal barrier, favoring bacterial translocation and sepsis [5]. In this context, successful preservation of graft viability during cold-ischemic storage is critical, with prevention of hypothermia-induced cellular swelling fundamental for successful organ preservation [6, 7]. The current clinical practice for intestinal preservation (IP) is based on an *in-situ* vascular flush with cold University of Wisconsin (UW) or Histidine-Tryptophan-Ketoglutarate solution, followed by cold static storage at 4℃ [8, 9]. However, multiple studies have documented the inability of a variety of solutions, including UW solution, to maintain a clinically acceptable degree of morphological injury beyond 6–10 h of cold storage [10–12]. A breakthrough in the development of improved preservation solutions is important to address this problem.

Mesenchymal stem cells (MSCs) are multipotent and capable of differentiating into multiple lineages (osteogenic, chondrogenic, adipogenic, and neuronal) when cultured under defined *in vitro* conditions, rendering these cells useful for basic research and as a therapeutic cell source for clinical applications [13–15]. Recently, MSCs transplantation has been used to treat several human diseases as cell products authorized for commercialization by regions/countries [15–19]. These cells have broad utility with many therapeutic effects regarding organ injury and organ transplantation. Their effects on organ injury have been attributed to many secreted factors containing cytokines, which can inhibit inflammatory and immune responses [20–25]. There is also increasing evidence suggesting that the therapeutic potential of MSCs could be applied to difficulties encountered with ischemia reperfusion injury of the small intestine in experimental animal models, though application of MSCs to cold storage injury has not been adequately investigated [26–29].

In this study, we identified factors secreted from MSCs that allow direct activation of preserved small intestine grafts. This novel finding will help elucidate the precise molecular mechanisms of small intestine activation and will potentially be useful as an attractive source for preserved small intestine transplant therapy.

MATERIALS AND METHODS

Animals

All animals were housed in a specific pathogen-free animal facility at Jichi Medical University under the following conditions: 50% ± 10% relative humidity, 12/12-h light-dark cycle, and a temperature of 24℃ ± 2℃. Male wild-type Lewis (LEW) rats were purchased from Charles River (Breeding
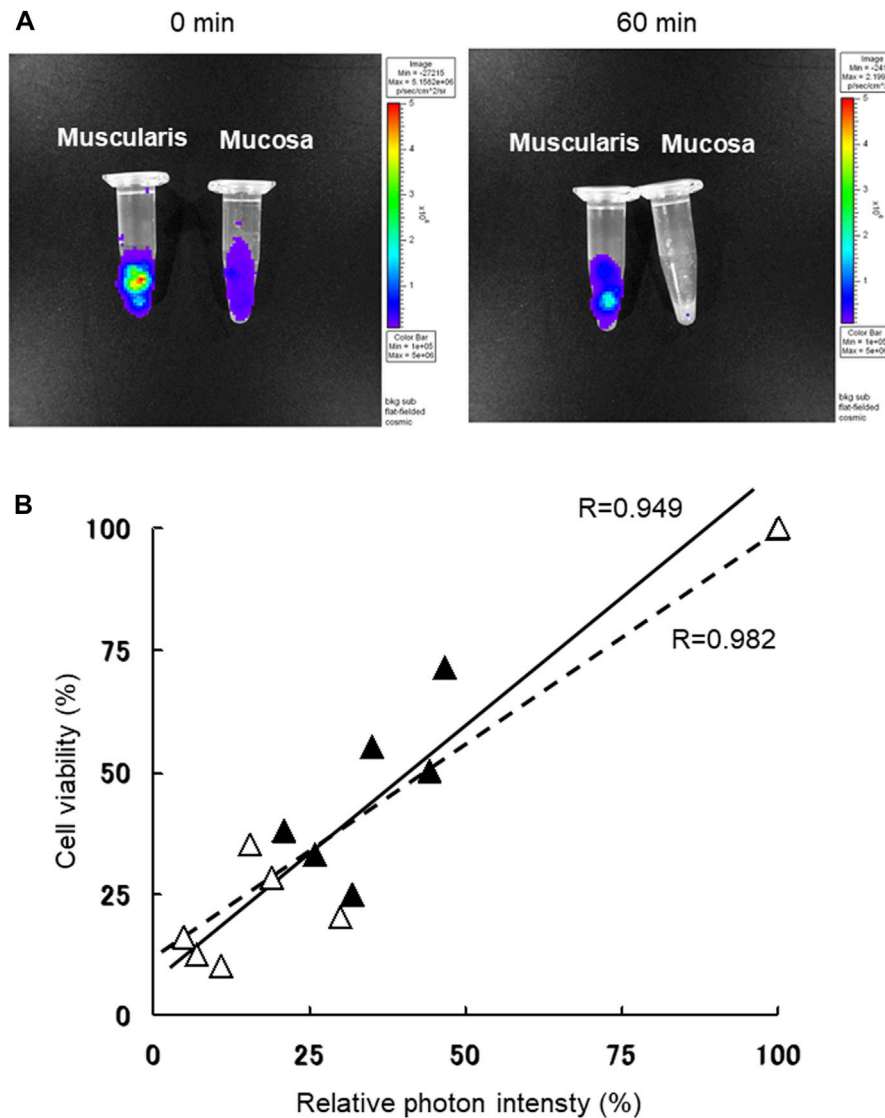
FIGURE 1 | Small intestine muscularis and mucosal viability were determined following trypan blue staining, based on bioluminescence with use of *in vivo* imaging system (IVIS). **(A)** Luciferase transgenic (Luc-Tg) LEW rat-derived small intestine tissues were separated into muscularis and mucosa before normothermic normal saline preservation. Using the IVIS, the image on the left was acquired at the starting time and on the right image after 60 min of preservation. **(B)** Photon count of Luc-Tg LEW rat trypsinized small intestine tissues was determined using the IVIS, followed by evaluation of cell viability with trypan blue staining. Black triangles and straight lines show data for muscularis, and white triangles and broken lines show data for mucosa under the same conditions shown in **(A)**.

Laboratories, Kanagawa, Japan). LEW rats used in the experiments had a body weight between 230 and 310 g.

Rat Adipose Tissue-Derived (rAT)-MSC Preparation and Culture

Wild-type LEW rat AT was sharply minced into pieces <3 mm, and rAT-MSCs isolation proceeded as described previously [30]. Isolated rAT-MSCs were seeded onto 100 mm tissue culture dishes (Nunc, Tokyo) and cultured with minimum essential medium (MEM)α supplemented with 10% fetal bovine serum.

When the cells were 70%–80% confluent, they were harvested with 0.05% trypsin-EDTA (Invitrogen, Tokyo), replated at 2.0×10^4 cells/cm [27], and cultured for 5 days. rAT-MSCs between the fifth and eighth passage were used for the experiments.

Microarray Analysis

Clariom™ D Assay for Rat (Filgen, Tokyo, Japan) was used, according to the manufacturer's instructions. Total RNA was extracted from undifferentiated rAT-MSCs and NRDFs. The process of hybridization and washing was performed using a Gene Expression Wash Pack (Agilent Technologies) and

TABLE 1 | Upregulation of cytokine genes in rAT-MSCs as compared with NRDFs ($p < 0.001$).

Name	rAT-MSCs	NRDFs	Ratio
FGF1	1,004.07	254.31	3.95
FGF2	5,011.34	633.14	7.92
FGF4	36.65	24.57	1.49
FGF5	693.31	6.31	109.96
FGF7	143.98	6.95	20.73
FGF16	45.63	7.61	6.00
FGF18	232.53	23.01	10.11
FGF22	3,462.66	630.26	5.49
FGF23	27.21	6.57	4.14
HGF	127.01	6.76	18.78
VEGF	1,157.93	845.47	1.37
VEGFC	14,993.36	7.69	1949.14
VEGFFB	18,723.8	1,126.92	16.62
VEGFB	790.95	55.6	14.23
EGF	45.78	7.79	5.87
NGF	199.14	76.37	2.61
IGF1	13.26	5.17	2.56
IGF2	1,315.06	39.85	33.00
TGFB1	1,245.00	71.27	17.47
TGFB3	37.00	19.64	1.88
HDGFL1	73.58	30.78	2.39
PGF	368.80	221.08	1.67
PDGFC	5,713.71	2,181.63	2.62
PDGFD	26.79	6.94	3.86
BCGF	19.41	7.04	2.76
TBRG1	283.64	126.22	2.25
LTBP1	2,420.02	712.98	3.34
HDGFRP3	2,686.30	14.08	190.79
CTGF	13,349.88	5,469.09	2.44

acetonitrile (Sigma, Tokyo, Japan). A DNA microarray scanner (Agilent Technologies) was used for array scanning. To ensure data reliability, weak signal spots were removed according to the manufacturer's criteria. This resulted in a data matrix of 25,721-genes with no missing data.

Preparation of Conditioned Medium

For analyses of secreted factors, rAT-MSCs were plated on 100 mm dish (using 30 dishes). Upon reaching confluence, samples were washed with phosphate-buffered saline (−) and incubated with serum-free MEMα medium. After 2 days, the supernatant was collected, centrifuged, filtered, and concentrated at 7000 × g using Amicon Ultra Centrifugal Filter Devices (Millipore, Tokyo, Japan; MW: 3 kDa, 10 kDa, 30 kDa, 50 kDa, and 100 kDa).

Procurement of Small Intestine Segments

The firefly luciferase-expressing transgenic rat was established in our laboratory as described previously [31]. Small intestine segments from Luc-Tg LEW rats were removed at 8 weeks of age, divided into 10 mm segments after heparinization (300 U/animal), and washed with Hank's Balanced Salt Solution (Invitrogen, Tokyo, Japan).

Correlativity of Photon Intensity and Cell Viability

IVIS and trypan blue staining were used to confirm the correlation of photon intensity and cell viability. Muscularis

and mucosa layers from Luc-Tg LEW rat small intestine were trypsinized to the cellular level. After preservation with normal saline at normothermic conditions, photon intensity and subsequently cell viability rates were evaluated by trypan blue staining on the same specimens.

Assessment of Small Intestine Tissue Viability in Preservation Solutions

Freshly isolated Luc-Tg LEW rat small intestine segments were plated in 12 wells tissue culture plates (1 segment/well: $n = 4$/each), and stored in UW preservation solution (Astellas Pharma Inc., Tokyo, Japan) at 4°C for 24 h. Detection was performed by addition of 22 μL (2.29 mg/ml) of luciferase-based reagent (D-luciferin: Wako, Tokyo, Japan). The in vivo imaging system (IVIS; Xenogen, Allameda, CA, United States) was used for the analysis of luciferase gene expression activity. In this system, a non-invasive charged-couple device camera is used to detect bioluminescence emitted from D-luciferin, which reacts with firefly luciferase in living animals and cells.

Immunohistochemical Analysis of Preserved Small Intestine Segment

Small intestine samples were stored for 24 h at 4°C, fixed in 10% formalin, and embedded in paraffin. Histological analysis of small intestine segments was conducted on serial tissue sections stained with hematoxylin and eosin (H&E) for conventional morphological evaluation and with anti-ZO1 (Hycult Biotech, Uden, Netherlands), anti-claudin-3 (Santa Cruz Biotechnology, CA, United States), and anti-myeloperoxidase (MPO) antibodies (Hycult Biotech) for protein detection. Rhodamine- or fluorescein isothiocyanate (FITC)-conjugated secondary antibodies were applied for 30 min. Nuclei were stained using 4′,6-diamidino-2-phenylindole (DAPI).

Macroscopic and Microscopic Scoring for Small Intestine Injury

Both macroscopic and microscopic scoring systems were used for analysis of tissue damage. Subsequent to resection, the small intestine was immediately cut into 10-pieces of equal length and rapidly transferred to Petri dishes containing cold buffer on ice. Macroscopic changes in each piece were documented photographically. Histological grading of injury of formaldehyde-fixed intestine specimens, counterstained with H&E, was performed by 2-independent blinded examiners using the Park/Chiu classification [32].

Heterotopic Transplantation of Preserved Partial Small Intestine Grafts With Double Enterostomy in Syngeneic Wild-Type Rats

Luc-Tg LEW rat derived small intestine grafts (10 cm) were transplanted into wild-type LEW rats, as described previously
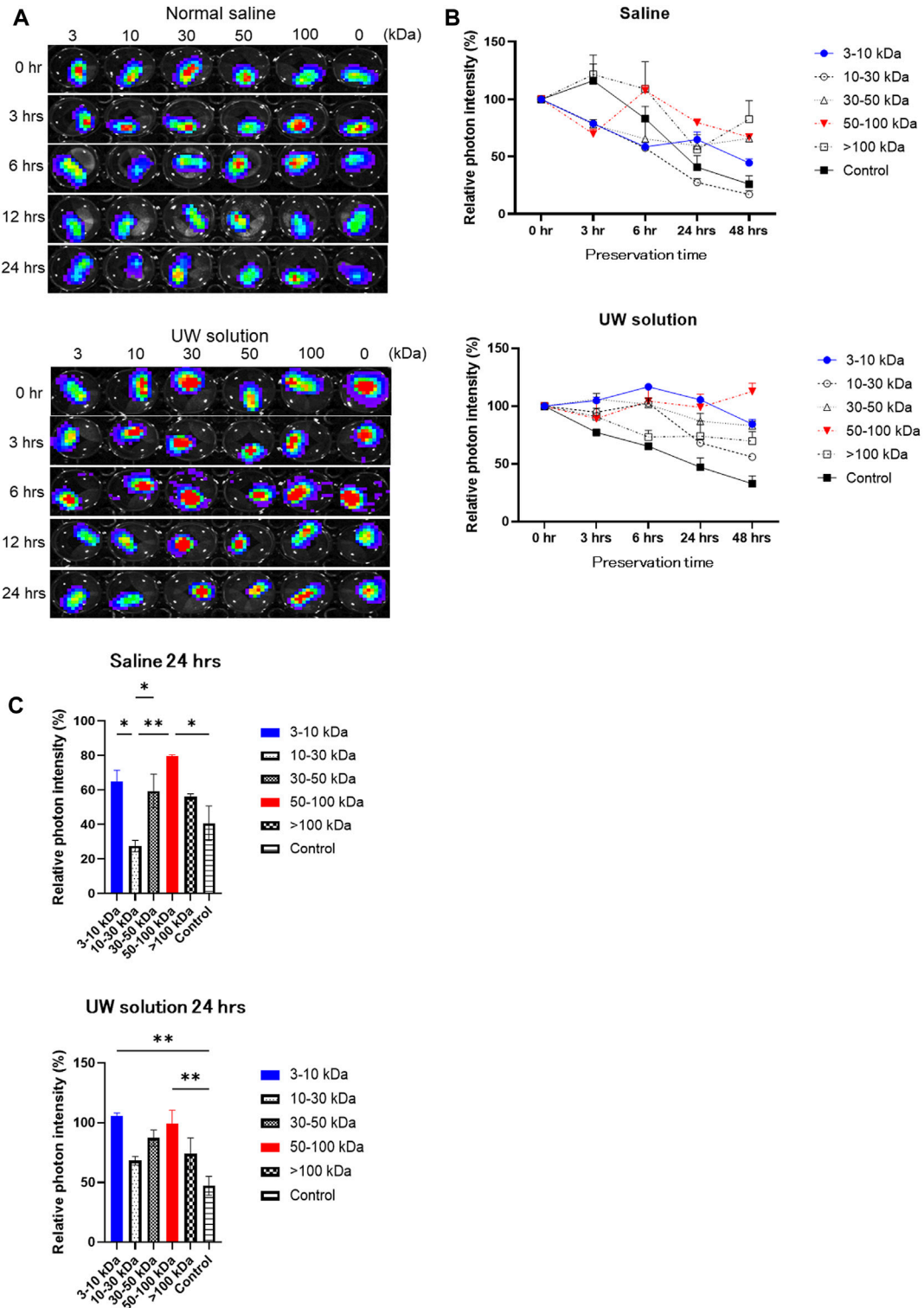
FIGURE 2 | Comparison of changes in luminescence intensity of small intestine segments in organ preservation solution following addition of each fraction of mesenchymal stem cell-conditioned (MSC) medium. **(A)** Representative time-lapse photographs of Luc-Tg LEW rat-derived small intestine segments in preservation solution treated with each fraction of MSC-conditioned medium. Shown from the left column on the plate; >100 kDa, 50–100 kDa, 30–50 kDa, 10–30 kDa, 3–10 kDa, and 0 kDa (control) fractions. **(B,C)** Relative photon intensity of small intestine segments determined using *in vivo* imaging system to assess viability. Samples were immersed in organ preservation solution at 4°C. Data shown are representative of 3 independent experiments.

[3, 13]. In brief, a transverse incision was made on the donor abdomen, and the middle and right colonic vessels were ligated and divided. The distal end of the graft intestine was cut at the ileum end and the proximal end was subsequently cut 10 cm from the distal end. After venous injection of heparin, the aorta was ligated above the origin of the superior mesenteric artery, and the intestinal lumen flushed with an adequate amount of saline.

The aorta including the superior mesenteric artery was then divided. The portal vein was divided, the graft removed *en bloc* and the vessels flushed. The graft was stored immediately in UW solution or UW + MSC-CM at 4°C. After 24 h of cold storage, the graft was placed in the recipient abdomen, the recipient's aorta was partially side-clamped, and the graft aortic conduit was anastomosed to the infrarenal abdominal aorta in an end-to-side fashion. Venous outflow was restored by end-to-side anastomosis of the portal vein to the infrarenal vena cava. Both ends of the small intestine graft were exteriorized as stomas. The incision was closed with interrupted sutures.

This rodent model was designed to investigate the status of the preserved graft and is not a sublethal model due to heterotopic partial transplantation. In a preliminary study, all recipients survived with saline transfusion at the end of the transplant procedure. Therefore, the transplant procedures were performed without transfusing saline to make this model sublethal. On postoperative days (PODs) 1, 3, and 7, D-luciferin (150 mg/kg body weight) was injected into the penile vein of each rat, and the rat anesthetized with isoflurane (Abbott Japan Co., Ltd.), to detect photons emitted from the small intestine graft. Graft luminescence was evaluated by the IVIS and quantified with the IVIS Living Image software package.

Statistical Analysis

Data are represented as means ± standard error of the mean (SEM). Pearson correlations were performed to determine the association between cell viability and relative photon intensity. To compare the mean values of relative photon intensity obtained from the preserved small intestine segment, repeated measures single-factor ANOVA followed by Tukey's multiple comparisons test was performed. For the survival study, Kaplan-Meier analysis and the log-rank test were performed. Mean values of photon intensity to compare the two groups were analyzed using a 2-tailed Student's *t*-test. Mann-Whitney's U-test was used to compare injury scores between the groups. A *p*-value < 0.05 was considered significant. All statistical analyses were performed using the GraphPad Prism software.

RESULTS

Luminescence Technology to Assess Viability of Preserved Small Intestine *In Vitro*

Using a stereomicroscope, small intestinal tissue from Luc-Tg LEW rats was strictly separated into muscularis and mucosal layers. The photon intensity from the muscularis and mucosal layers decreased as preservation time extended (**Figure 1A**). The viability of samples preserved in normal saline was lower in the mucosal layer than in the muscularis layer after 1-h. The photon count for trypsinized tissue samples preserved with normal saline under normothermic conditions was strongly correlated with cell viability, evaluated following trypan blue staining (**Figure 1B**). Both the muscularis and mucosal layers showed similar correlativity. These results demonstrate the validity of using a whole section of small intestine wall to accurately evaluate the state of preservation by measuring the photon intensity using the IVIS.

Analysis of Preserved Small Intestine Activation Factors From rAT-MSC-Secreted Fractions

For the result of DNA microarray analysis, rAT-MSC-CM contains 29 growth factors that affect the viability of cold-preserved small intestine (**Table 1**). Fractions derived from rAT-MSC-CM were evaluated to determine which were involved in activation of the preserved small intestine (**Figure 2**). During the experiment, the preservation solution was not refreshed. The photon intensity from the group receiving each fraction of conditioned medium changed over time at 4°C (**Figure 2A**). The photon intensity was quantified using color images. By comparison with controls, fractions were classified into 2 groups in terms of their effects on preserved Luc-Tg LEW rat small intestine grafts as follows: activated group (30–100 and 3–10 kDa) and less activated group (0–3 and 10–30 kDa) (**Figure 2B**). The activation of preserved small intestine grafts gradually declined in photon intensity from the peak at 24 h (**Figure 2C**), then fell below the detection limit within 4–5 days (data not shown). These results suggest that the 30–100 and 3–10 kDa fractions secreted by rAT-MSCs were superior in their activation of preserved small intestine grafts.

Histological Analysis of Preserved Small Intestine Segments

Claudin-3 and ZO-1 were found to be colocalized in epithelial villi on the surface of enterocytes in normal intestine specimens. After 3 h, the segments in UW solution alone tended to be delocalized along the intercellular membrane due to a decrease in claudin-3 expression, but colocalization was maintained in the region closer to the top of the villi (**Figure 3A**; **Table 2**). After 12 h of preservation, the expression of claudin-3 was remarkably decreased and ZO-1 expression was also decreased, in a discontinuous pattern in UW solution alone. After 24 h of cold preservation, most of the villus structure was destroyed in segments of intestine in the UW solution alone group. The expression of Claudin-3 almost disappeared, while a quantity of ZO-1 expression at the top of the villi was maintained. Only minimal change was observed in segments preserved in the UW + MSC-CM solution with respect to histological architecture, expression and colocalization of two proteins at all time points.
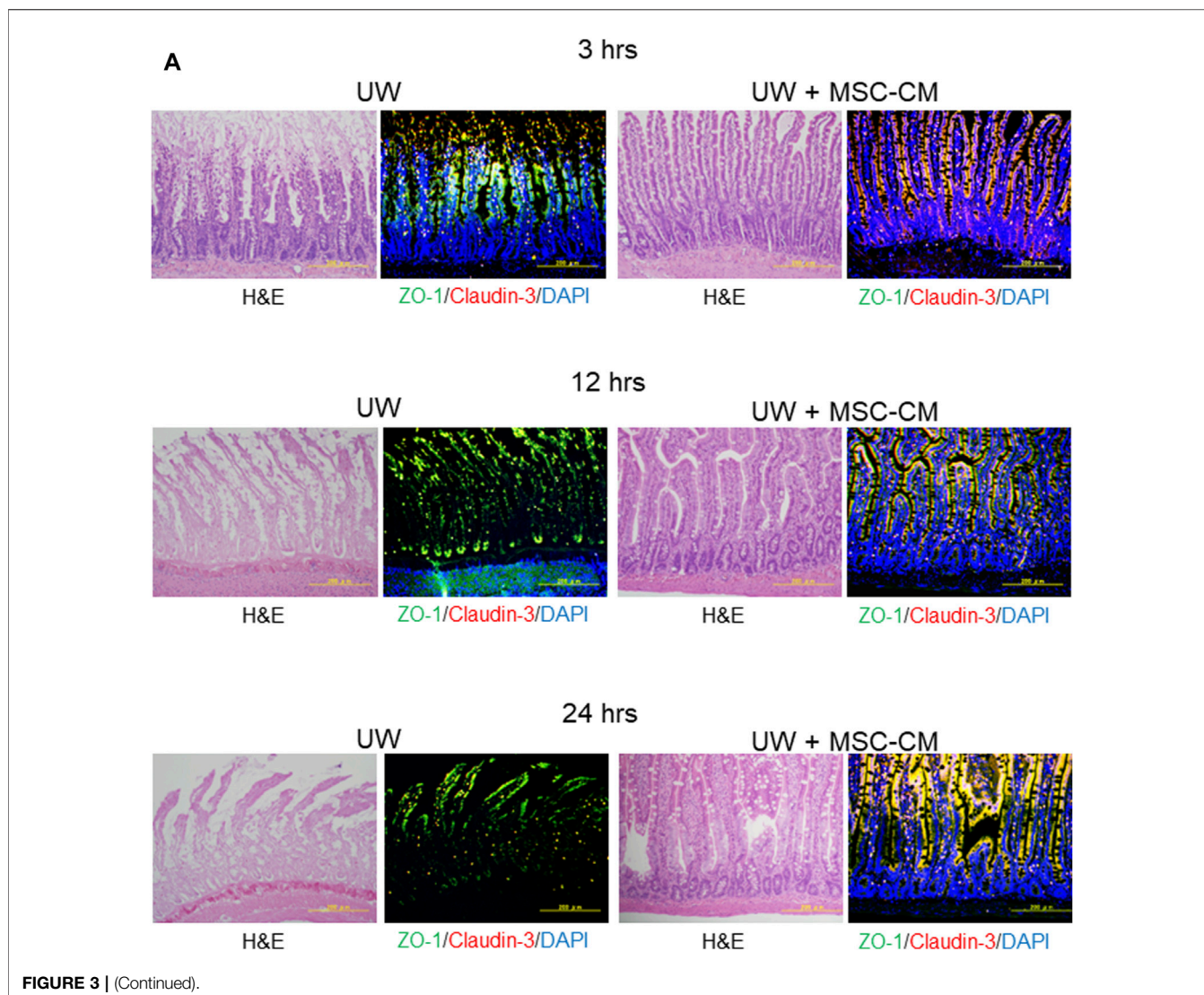
FIGURE 3 | (Continued).

Immunohistochemical staining of MPO expression was also performed to evaluate oxidative stress in preserved small intestine segments. The MPO expression was evidently reduced in the UW + MSC-CM group compared to the UW solution alone group at all time points (**Figure 3B**; **Table 2**).

Preserved Small Intestine Graft Transplantation

Luc-Tg LEW rat-derived small intestine grafts were transplanted into wild-type LEW rats after 24 h of cold preservation. The condition of the transplanted small intestine graft was evaluated daily based on the presence of relative photons using the IVIS. In the control group without MSC-CM, photon intensity showed significant attenuation over time, whereas that was increased in the group containing MSC-CM (>50 kDa fraction), similar to the increase seen in non-preserved small intestine grafts (**Figure 4A**). The small intestine

graft preserved for 24 h with either UW alone or UW + MSC-CM did not show any changes at the pre-transplant stage. Furthermore, the photon intensity rate from transplanted small intestine grafts preserved in UW solution alone decreased more rapidly than those in UW + MSC-CM [**Figure 4B**; UW: 0.6 ± 0.16 units/min (POD1), 0.009 ± 0.003 units/min (POD4); UW + MSC-CM: 1.38 ± 0.21 units/min (POD1), 2.55 ± 0.47 units/min (POD4)]. After POD14, the UW solution alone and UW + MSC-CM groups had photon intensities very similar to that of fresh small intestine transplanted grafts (**Figure 4B**).

Saline-transfused recipient rat survival was 100%; however, control rats without saline transfusion [the UW solution alone group ($n = 8$)], had 100% mortality by POD6 (**Figure 4C**). In the UW solution alone group, bleeding into the peritoneal cavity was confirmed at autopsy. In contrast, infusion of saline was unnecessary in UW + MSC-CM rats for survival.
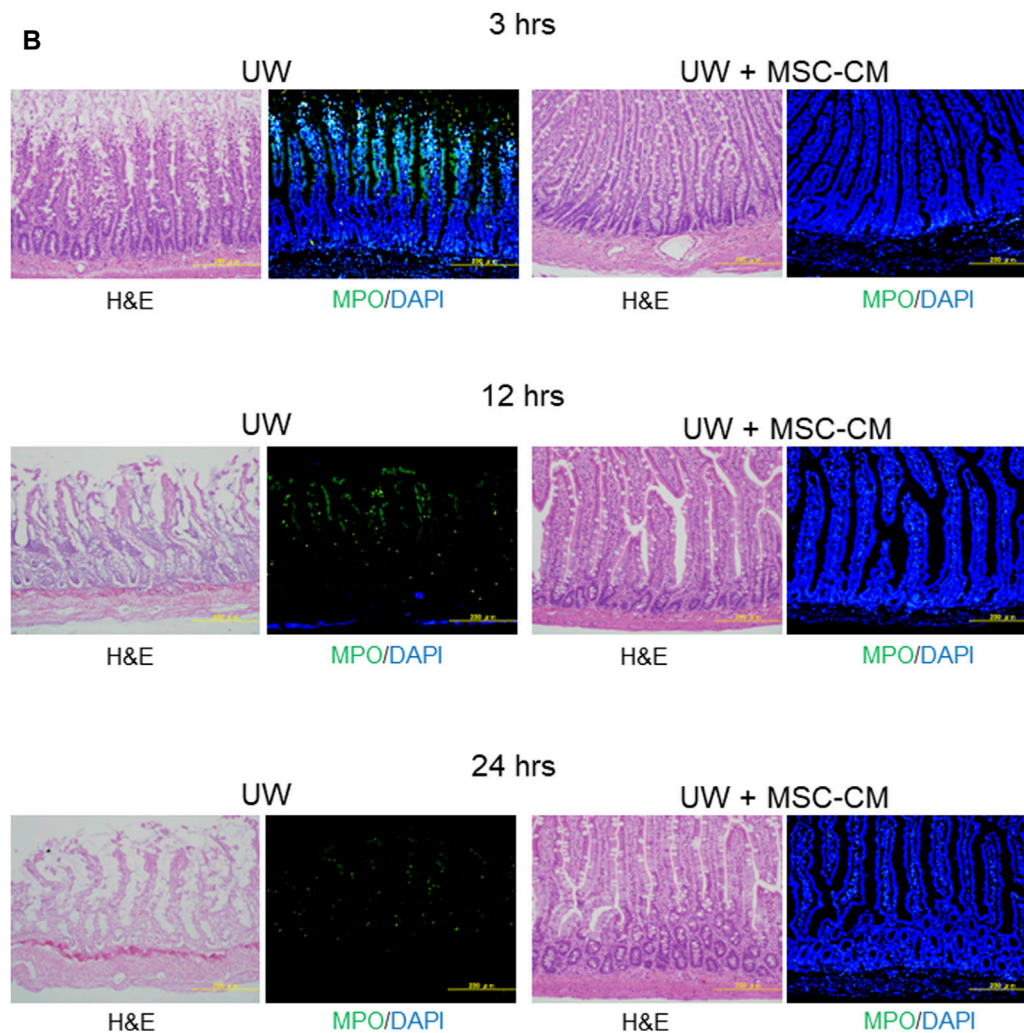
FIGURE 3 | (Continued). Assessment of tight-junction structure and oxidative stress. **(A)** Microphotographs of preserved small intestine segments. Left two columns show hematoxylin and eosin staining and immunofluorescence images of segments preserved in University of Wisconsin (UW) solution showing expression of ZO-1 (green), claudin-3 (red), and their colocalization (yellow), while right two columns show segments preserved in UW + MSC-CM at each time point. Nuclei were stained blue with DAPI. **(B)** Evaluation of oxidative stress marker MPO (green) in the same samples. The samples used were serial sections.

Gross Appearance of Transplanted Cold-Preserved Small Intestine Rat Grafts

Approximately 10 cm of ileal graft was removed under general anesthesia and kept at 4°C for 24 h in UW solution alone or UW + MSC-CM solution (**Figure 5A**). The small intestine graft preserved for 24 h with either UW alone or UW + MSC-CM did not show any changes at the pre-transplant stage. After 24 h storage, preserved grafts were transplanted into the peritoneal cavities of recipient rats and reperfused. Grafts preserved in UW solution alone were found to be edematous and congested with blood oozing from the mucosa, while those preserved in UW + MSC-CM did not have those findings (**Figures 5B, C**). Furthermore, grafts preserved in UW solution alone harvested 24 h after transplantation had marked congestion and bleeding in

microscopic sections evaluated with hematoxylin-eosin stain. Karyopyknosis and deciduation of intestinal epithelial cells were also observed as typical apoptosis (**Figure 5D**). However, grafts preserved in UW + MSC-CM solution had mild congestion and apoptosis.

After 90 min of reperfusion, small intestine graft conditions were obviously different between those preserved in UW solution alone and those in UW + MSC-CM, with the macroscopic score significantly lower in the latter (**Figures 5E, F**). Using the same samples, small intestine histopathology was examined and the microscopic score values were consistent with the macroscopic results (**Figures 5G, H**). These results suggest that UW + MSC-CM solution is beneficial for long-term storage of small intestine grafts under ischemic conditions.

TABLE 2 | Immunohistochemical results of rat small intestine grafts preserved in mesenchymal stem cell-conditioned medium.

MSC-CM (kDa)	Preservation time (hr)	Claudin-3	ZO-1	MPO	DAPI
0<Factor<3	3	+	+	+	+
	12	–	+	–	–
	24	–	+/–	–	–
3<Factor<10	3	+	+	-	+
	12	+	+	+	+
	24	+	+	+	+
10<Factor<30	3	+	+	+	+
	12	+/–	+	+	+
	24	–	+/–	+	+
30<Factor<50	3	+	+	–	+
	12	+	+	–	+
	24	+/–	+	+	+
50<Factor<100	3	+	+	–	+
	12	+	+	–	+
	24	+	+	–	+
100<Factor	3	+	+	+/–	+
	12	+	+	+	+
	24	+/–	+	+	+

DISCUSSION

The results of the present study demonstrate for the first time that isogeneic adipose derived MSC-CM supplementation of UW solution has a protective effect on intestinal grafts against 24 h extended cold static preservation in a rodent heterotopic ITx model. In addition, particular fractions of MSC-CM, including the 30–100 and 3–10 kDa fractions, were found to contain trophic factors that allow maintenance of tissue ATP content after 24 h as compared to the conditions at the initiation of storage. This method does not require complex equipment to control oxygenation or temperature, and has practical benefits, given that cold static preservation is standard practice for intestinal preservation.

Although MSCs are used for treatment of several different diseases and have recently been commercialized as therapeutic products, an urgent issue to overcome is cell supply to meet sudden demand in acute clinical situations [33, 34]. For a single treatment in clinical settings, hundreds of millions of MSCs are generally needed to attain an adequate therapeutic effect. Since several weeks are required to increase the number of cells grown in normal culture conditions, investigators have found it difficult to apply MSC-based cell therapy, especially for acute diseases. On the other hand, it has been shown that the effect of MSCs is due at least in part to paracrine factors [35]. We conducted analysis of DNA microarray and Protein-chip array to explore secretory factors of AT-MSCs (preparation of manuscript). Administration of MSC-CM in acute organ injury models has been reported to be as effective as administration of MSCs in some cases [36, 37]. MSC-CM is known to contain various cytokines, such as growth, anti-inflammatory, and anti-apoptotic factors, which regulate a large variety of physiological processes. Thus, cell-free therapy methods such as use of MSC-CM have been attracting attention due to some crucial advantages over stem-cell based applications.

Use of UW solution to flush the vasculature and store donor organs during retrieval has been the standard method employed for abdominal organ preservation since 1987 [24]. Despite the effectiveness of cold static preservation with UW solution for other intra-abdominal organs, that for small intestine preservation is somewhat limited. The maximum "safe" storage time for small intestines is brief (6–12 h) and graft quality is often compromised even with short periods of ischemia [22, 38]. With cold preservation, hypothermia elicits protective effects by delaying hypoxia-induced ATP decline and slowing down subsequent injury [39]. A previous report showed that AMP nucleosidase gene knockout in *Escherichia coli* elevates intracellular ATP levels and increases cold tolerance [40]. In addition, elevated intracellular ATP levels by deactivation of AMP deaminase was demonstrated with maintenance of decreased adenylate kinase activity in specific hibernating mammals [41]. Thus, elevation of intracellular ATP levels is critically important for cell survival at low temperatures. During cold static organ preservation, minimum cellular metabolism factors related to cell survival, including ATP synthesis and amino acid metabolism, are profoundly suppressed, which prevents consumption of essential substrates. Therefore, organs stored for a comparatively long time at low temperature cannot be revived due to irreversible changes in the energy synthesis components needed for cell survival.

Maintenance of high-energy phosphorylated compounds such as ATP, the levels of which are inversely proportional to preservation time, has been shown to be correlated with minimal changes in cell structure and function incurred during cold storage [11]. The Na^+/K^+ transporter maintains the respective gradients of such ions, relying on use of ATP as its energy source. An essential factor for successful intestinal graft transplantation under cold ischemic conditions is the ability of the preservation solution to maintain ATP levels [12]. UW preservation solution maintains ATP synthesis in the graft tissues and ameliorates the effects of cold ischemia up to 12 h. We previously presented an assay used to assess the viability of Luc-Tg LEW rat organs and tissues [42–47], and that was used in the present study to confirm that small intestine segments preserved for 24 h in 3–10 kDa and 50–100 kDa fractions of MSC-CM showed nearly equivalent
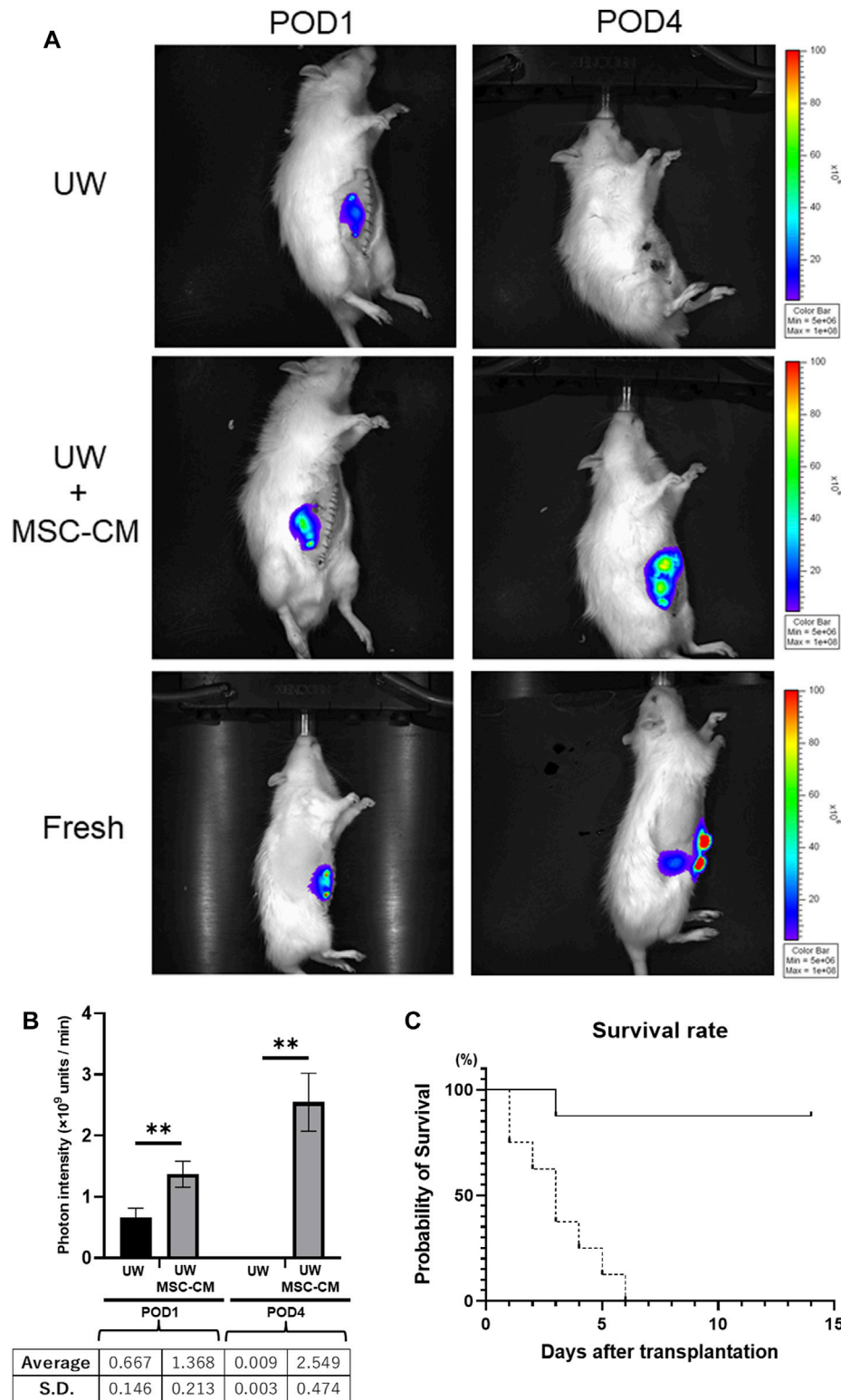
FIGURE 4 | Time course of preserved Luc-Tg derived intestine grafts in recipient. **(A)** Changes in luciferase-derived photons following transplantation of small intestine. Representative image from each group on post-operative day (POD)1 and POD4. The Fresh group included transplanted grafts without preservation. **(B)** Graph showing luminescent photon level up to 4 days following transplantation. Black bar represents University of Wisconsin (UW) alone group and white bar UW + MSC-CM group. $**p < 0.05$. **(C)** Survival rate of rats following transplantation. Solid line: UW + MSC-CM group ($n = 8$), dashed line: UW alone group ($n = 8$).

A

Preserved small intestine graft

B

UW only   UW+MSC-CM

C

UW only   UW+MSC-CM

D

UW + MSC-CM

UW
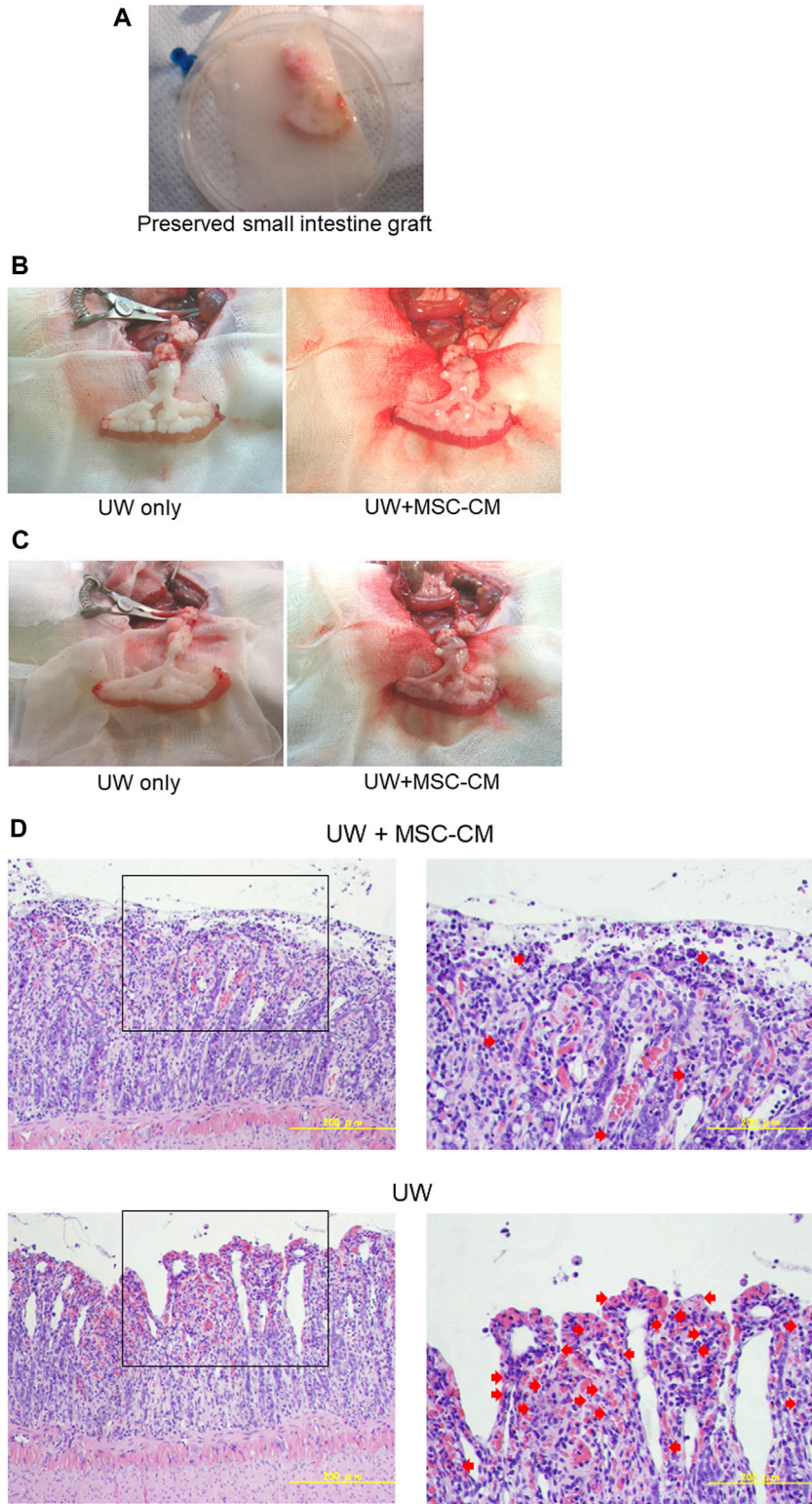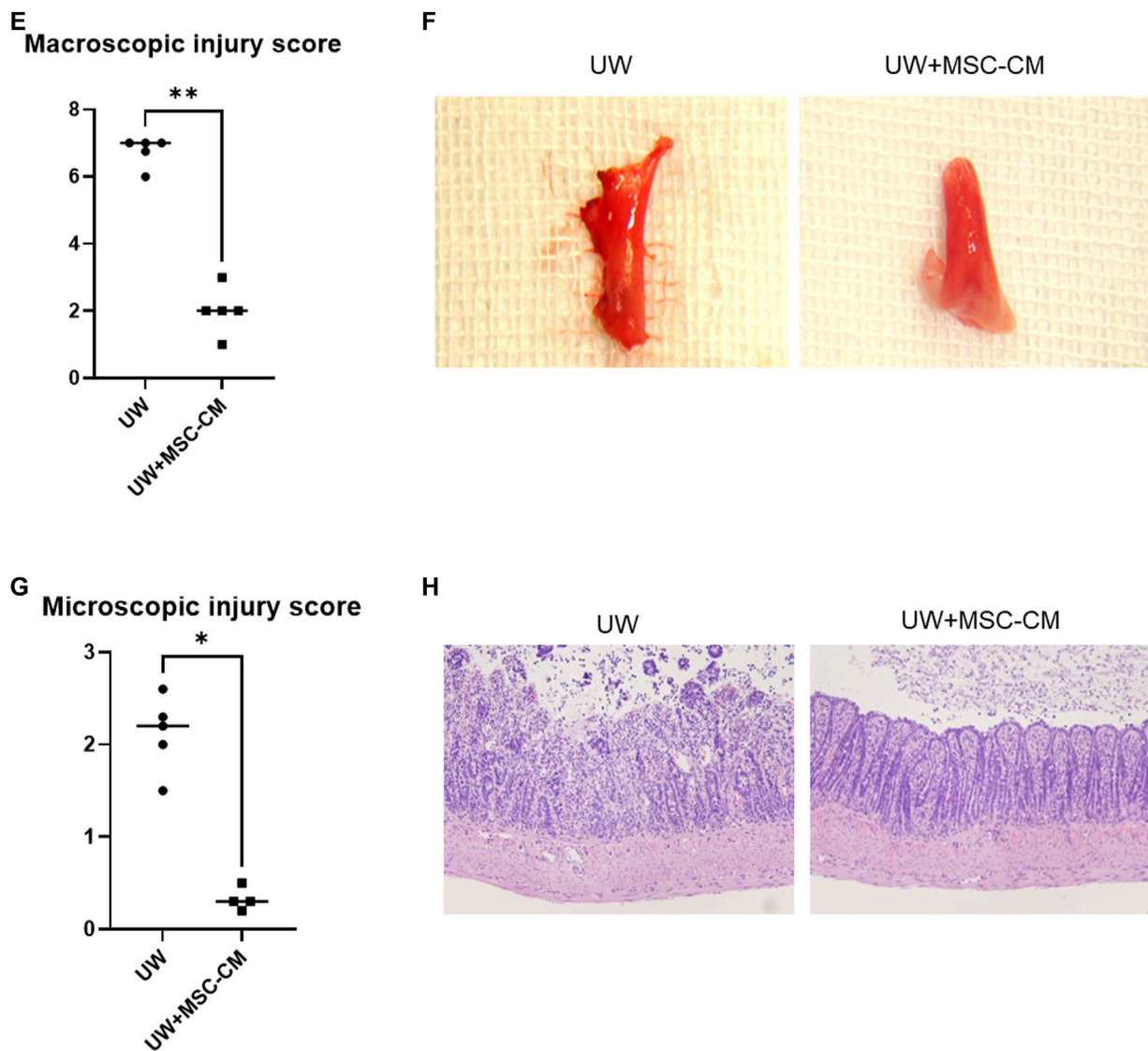
FIGURE 5 | (Continued).

FIGURE 5 | (Continued). Gross appearance of preserved small intestine grafts before and after reperfusion. **(A)** Preserved small intestine graft. **(B)** Before reperfusion (left: University of Wisconsin (UW) alone, right: UW + mesenchymal stem cell-conditioned medium (MSCX-CM). **(C)** Following reperfusion. Left: UW alone, right: UW + MSC-CM. **(D)** Representative section of small intestine graft at 24 h after transplantation. Red arrows indicate typical apoptotic cells. **(E–H)** Analysis of small intestine preservation and reperfusion injury levels in heterotopic partial transplantation model. **(E)** Macroscopic injury score. **(F)** Morphology of whole small intestine grafts. **(G)** Microscopic injury score. **(H)** Hematoxylin and eosin (H&E)-stained sections of small intestine grafts under various conditions. $*p < 0.05$ $**p < 0.01$.

photon intensity levels, which reflects tissue ATP quantity, as compared to that at the initiation of cold ischemia. The present findings also showed that grafts after preservation in UW solution alone for 24 h had blood oozing from the entire surface of the tissue upon resumption of blood flow, while grafts preserved in UW + MSC-CM solution did not. These results are consistent with histological analysis findings showing destruction of the intestinal configuration including microvasculature in the UW solution alone group. HGF is known to suppress ischemia-reperfusion injury associated with organ transplantation, particularly apoptosis [48–50]. Findings obtained with cytokine arrays have shown that AT-MSCs secrete HGF at levels approximately 10-fold greater as compared to BM-MSCs [17]. Furthermore, MSC transplantation is known to increase graft survival rates and suppress rejection reactions [51–53]. Thus, when using MSCs for this purpose, those derived from autologous tissues are best so as to evade rejection reactions. These findings directly reflect the results showing significantly improved recipient survival up to 87% using grafts preserved with MSC-CM as compared to recipients that received grafts preserved without MSC-CM, which died within 6 days following transplantation.

Major obstacles to development of reliable and safe ITx methods are largely related to bacterial infection. Cytokines, interferon-γ, and tumor necrosis factor (TNF)-α directly influence tight junction function, and also modulate both membrane microdomain localization of tight junction proteins and lipid composition of tight junctions, resulting in bacterial infection and inflammation [54]. HGF is known to suppress ischemia-reperfusion injury associated with organ transplantation, particularly apoptosis [48–50]. Through cytokine arrays, it has been found that AT-MSCs secrete approximately 10 times more HGF than BM-MSCs [17]. Furthermore, MSC transplantation is known to increase graft survival rates and suppress rejection reactions [51–53]. When using MSCs for this purpose, it is desirable that they are derived from autologous tissues to evade the MSC's own rejection reactions. Therefore, to maintain the efficiency of small intestine grafts at an optimal level, it is important to protect grafts against tight junction destruction during cold preservation and subsequent reperfusion. The present findings show that UW solution containing MSC-CM inhibits tight junction breakdown and maintains cell structure in the small intestine under cold preservation for 24 h (**Figure 3**). Furthermore, grafts preserved under these conditions were found to be transplantable into recipient rats (**Figure 5**). Mitochondrial DNA, which is released from dead cells and can induce an inflammatory response, has been shown to contribute to intestinal ischemia reperfusion injury and exacerbate the acute proinflammatory process by enhancing production of proinflammatory cytokines including TNF-α [55]. It is thus possible that reduction of tissue damage during preservation caused by addition of MSC-CM leads to a lower level of mitochondrial DNA circulation after reperfusion and suppresses the proinflammatory process, resulting in improved survival of the recipient. As noted above, it is best to use MSCs derived from autologous tissues so as to evade their rejection reactions.

Methods to expand the donor organ pool have been developed over the previous decade. Included in the marginal donor group are donated organs recovered after cardiac death, formally known as non-heart-beating donation [56]. As compared with organs transplanted after brain death, the function of organs obtained from donors after cardiac death is poor, though there is potential for significant improvement. Notably, the present results show that the 50–100 kDa fraction of MSC-CM allows grafts to attain a greater level of photon intensity after cold preservation extended to 48 h as compared to that at the beginning, which indicates maintenance of ATP production by tissue metabolism. Extrapolation of this finding suggests that use of MSC-CM as an adjunct to UW solution could contribute to improve the function of grafts obtained as donations after cardiac death that have severe ischemic injuries by *ex vivo* recovery of ATP production.

The present study has some important limitations. Since the animal model of ITx was produced using inbred syngeneic animals without rejection, assessment of the immunomodulatory effect of MSC-CM was not feasible. In addition, the present was a partial heterotopic transplantation model with a so-called Thiry-Vella loop and the native bowel

in the recipient animal remained intact. MSCs are known to secrete exosomes [57–59]. Chai et al. reported the presence of exosomes derived from MSCs in the 100 kDa–1,000 kDa fraction, which have been reported to alleviate ischemia-reperfusion injury [57]. It is suggested that our fractions above 100 kDa contain not only cytokines but also exosomes. In this study, examinations of the effects of isolated exosomes were not performed, thus it will be necessary to examine the interaction between cytokines and exosomes in a future study, while the ability to evaluate the efficacy of MSC-CM on graft intestine motility and digestive absorption function was also limited.

In conclusion, small intestine grafts maintained in cold storage with MSC-CM supplementation for 24 h were successfully transplanted in the present rat model under conditions similar to those used in clinical practice and the recipient survival rate showed dramatic improvement. Additional investigations are needed to clarify the underlying mechanisms and identify the most important factors contributing to the observed benefits when using MSC-CM so as to expand use of this approach to clinically relevant applications. As for the translational impact, it is suggested that the present results may have significant influence on discovery of pathways related to extending the preservation time of various transplant organs as well as research related to drug development.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Rats were handled and cared for according to the Institutional Guidelines for Animal Welfare. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Jichi Medical University (protocol ID: 13-116).

AUTHOR CONTRIBUTIONS

Conceptualization and design: TT and YF; *in vitro* research: TT, YF, and YS; *in vivo* research: MM and NK; data analysis: TT, YF, YS, NK, and AM; manuscript preparation: TT, NK, and AL; supervision: NS and JK. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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