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Impact of delayed graft function

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DOI: 10.3389/ti.2022.10910

Oshini Shivakumar, Rebekah Judge, Andrew Smith and Andreas Kousios

An interesting case with several teaching points for the clinical nephrologist.

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Leading the way in transplantation

Calendar of Events

12th EPITA Symposium & 31st AIDPIT Workshop	**22-24 January** Innsbruck-Igls, Austria	
ELITA 30th Anniversary & Monothematic Conference on ACLF	**09-11 March** Madrid, Spain	
ELPAT Working Group Meeting	**24-26 March** Oxford, United Kingdom	
HESPERIS Course	**20-22 April** Budapest, Hungary	
ITS Meeting	**30 April – 03 May** Outside of Europe	
EDTCO Congress	**16 September** Athens, Greece	
ESOT Congress	**17-20 September** Athens, Greece	
└ Post-Graduate Course Pre-Congress Activity	**16 September** Athens, Greece	
└ Science Day Pre-Congress Activity	**16 September** Athens, Greece	
Machine Perfusion Hands-on Course	**17-20 September** Athens, Greece	
ELITA Consensus on Liver Graft Assessment & Discard	**November**	

ESOT Transplant Live Online
ESOT Mentorship Programme Online
ESOT Grants Programme
Quarterly Transplant International Webinars
Bi-weekly Webinars
Bi-monthly Online Live Events
Quarterly Newsletter Education, ETPO
Monthly Newsletter Members, Community

Legend

Area

- ORGAN SPECIFIC
- TRANSPLANT SCIENCE
- TRANSPLANT PROFESSIONS
- EDUCATION
- PATIENT INCLUSION
- MACHINE PERFUSION

Audience

- SENIOR PROFESSIONALS
- YOUNG PROFESSIONALS
- PATIENT ADVOCATES

ACLF - Acute-on-Chronic Liver Failure
AIDPIT - Artificial Insulin Delivery, Pancreas and Islet Transplantation
EDTCO - The European Donation and Transplant Coordination Organisation
ELITA - The European Liver and Intestine Transplant Associationi
ELPAT - The European Platform on Ethical, Legal and Psychosocial Aspects of Organ Transplantation
EPITA - The European Pancreas and Islet Transplant Association
ESOT - European Society for Organ Transplantation
ITS - International Transplant Science

ABSTRACT SUBMISSION

ELITA
ESOT

The European
Liver and Intestine
Transplant Association

EF CLIF
EUROPEAN FOUNDATION
FOR THE STUDY OF
CHRONIC LIVER FAILURE

ELITA 30th Anniversary Meeting & ELITA-EF CLIF Monothematic Conference about ACLF, Alcohol and Liver Transplantation

9–11 March 2023, Madrid, Spain

#ESOT_ELITA

30th
ANNIVERSARY

ABSTRACT SUBMISSION

EDTCO
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EDTCO ORGAN DONATION CONGRESS 2023

Towards a new era in donor coordination

16 September 2023
Athens, Greece

#ESOT_EDTCO

TRANSPLANT TRIAL WATCH
published: 01 December 2022
doi: 10.3389/ti.2022.10970

Transplant Trial Watch

Simon R. Knight[1,2]*

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Keywords: donor management, liver transplantation outcome, patient preferences, kidney transplantation, randomised controlled trial

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

RANDOMISED CONTROLLED TRIAL 1

Donor Simvastatin Treatment is Safe and Might Improve Outcomes After Liver Transplantation: A Randomized Clinical Trial. *by Pagano, D., et al. Transplantation 2022 [record in progress].*

Aims

The aim of this study was to evaluate the safety and efficacy of administration of simvastatin to liver donors after brain death (DBDs) on outcomes following liver transplantation.

Interventions

Liver allograft DBDs were randomised to receive either 80 mg of simvastatin or placebo.

Participants

58 liver transplant recipients (>18 years) with DBDs over 18 years of age.

Outcomes

The primary outcome was patient survival and graft survival posttransplantation. The secondary outcomes were severe complications.

Follow-Up

180 days post-transplant.

CET Conclusion

This small single-centre study investigated the administration of a single dose of simvastatin intraoperatively in brain-dead donors on outcomes following liver transplantation. Although

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Knight SR (2022) Transplant Trial Watch. Transpl Int 35:10970. doi: 10.3389/ti.2022.10970

recruitment stopped prematurely due to the pandemic, the study demonstrated superior graft survival in the study group with some mechanistic evidence of changes in inflammatory gene expression. The study is well designed, with use of double-blinding, placebo control and intent-to-treat analysis. In reality, it is underpowered with a significant risk of type 1 error due to the low event rate in the primary endpoint. As with any donor intervention study, it would be important to understand the impact of the intervention on all retrieved organs, not just the liver, and no reference is made to this. Nonetheless, the findings certainly warrant further investigation and the large ongoing SIGNET study in the UK should provide more insight.

Jadad Score
5.

Data Analysis
Modified intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
ISRCTN27083228.

Funding Source
Non-industry funded.

RANDOMISED CONTROLLED TRIAL 2

Patient Preferences for Waiting Time and Kidney Quality.

by Mehrotra, S., et al. Clinical Journal of The American Society of Nephrology: CJASN 2022 [published ahead of print].

Aims
This study aimed to investigate patient preferences when presented with choices between a lower-quality kidney offered today or a higher-quality kidney offered in the future.

Interventions
Each participant was randomised to receive one of 24 sets of questions, with each set including six questions.

Participants
605 patients who were waiting for or had received a kidney transplant.

Outcomes
To quantify patients' assessment of the trade-off between kidney quality and waiting time.

Follow-Up
Not applicable.

CET Conclusion
This is a very interesting study from the US that posed kidney transplant offer scenarios to 605 wait-list patients. Respondents adapted their assessment of a kidney offer today in light of the potential offer that may be received in later months or years. As potential waiting time for a second offer increased, the relative importance of the graft survival for the offer on the table decreased. The average respondent was willing to forgo 4–5 years of normal transplant function to prevent waiting an additional 2 years. Younger patients, and pre-dialysis patients were prepared to wait for later, better kidney offers with longer predicted graft survival. The study was conducted in the United States and it is possible that the discard rate is higher than other countries; the authors compare to France where more marginal kidneys are used for transplantation. The implication is that the results are not necessarily translatable to countries outside of the United States. Given the variability of patient preferences, it is worth having an individualised approach to kidney offer assessment adapted to each patients' priorities. A key limitation of the study is that future kidney offers were described in terms of certainty to avoid heuristics. It is possible therefore that in real world situations patients may be even more likely to accept a marginal offer, as any future offer is not guaranteed to give better graft survival.

Funding Source
Non-industry funded.

CLINICAL IMPACT SUMMARY

Decline rates for kidneys offered for transplantation vary widely between countries, transplant centres and clinicians—a reflection of uncertainty as to quality and likely outcome. These disparities usually arise from a genuine desire to do the best thing for our patients, but patients are rarely involved in depth in these decisions and it is likely that their priorities sometimes differ from those of the clinicians treating them.

In a recent paper from the US, Mehrotra et al. use a discrete choice experiment to explore patient preferences over organ offers and the impact of age, demographics and dialysis status on these preferences (1). They presented 605 patients with putative organ offers, with information about likely graft survival time and subsequent waiting list time if they were to

decline the offer. They found that the average patient would accept a kidney with predicted graft survival of 6.5 years to avoid 2 years of additional waiting time for a better quality kidney with 11 years of predicted graft survival. However, younger patients and those still pre-dialysis were more likely to prefer to wait for a better kidney, and older, black or less educated patients were less willing to wait longer for a better-quality kidney.

These findings suggest that in many cases, patients would much rather go ahead with a transplant now than wait longer for something slightly better. Of course, the real world is not quite as black and white as this—there is no guarantee that a subsequent kidney offer would be better than the one currently presented. Predictions of graft survival and waiting time are not exact—the current paper uses predicted survival based upon KDPI, which has a c-statistic of 0.62 indicating only moderate predictive ability for graft survival (2). The authors recognise some limitations—particularly that patients making hypothetical decisions may act differently to a real decision, and that some included patients were post-transplant and may feel differently about risk compared to their own pre-transplant status.

If nothing else, this study demonstrates the importance of involving patients in the organ decision process. For example, the authors advocate recording patients' risk preferences on the wait list so that these can be taken into account either during allocation or upon consideration of an offer. For this to work in real clinical practice, we need improved predictive models for transplant and wait-list outcomes, and tools to present these in a patient-friendly manner.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

SK has received consultancy fees from OrganOx Ltd. for research design in the past.

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ESOT | Transplant International

EDITORIAL
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Reflecting on an Intense Year for Transplant International

Thierry Berney[1]*, *Maria Irene Bellini*[2,3], *Nuria Montserrat*[2], *Maarten Naesens*[2], *Thomas Neyens*[4] *and Stefan Schneeberger*[2]

[1]Editor-in-Chief, Transplant International, [2]Deputy Editor-in-Chief, Transplant International, [3]Social Media Editor, Transplant International, [4]Statistical Editor, Transplant International

Editorial

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The spirit of *Transplant International* is characterized by anticipation of the developments lying ahead and attempt to grow through generated opportunities. In an increasingly digital world and amid growing concerns for the environment, we adopted a paperless format years before our competitors. We have taken advantage of the potential offered by digitalization and designed a journal with a completely novel appearance, with the animated covers as a hallmark (1). Open science, embodied in the FAIR acronym (Findability, Accessibility, Interoperability, Reusability), is not only a self serving principle but has become a requirement from academic institutions to their scholars. Also, and more compellingly, most funding agencies increasingly demand that scientific outputs resulting from public financial support be made freely available (2). We strongly believe that these changes are here to stay and that all scientific research will be published open access in the near future. In line with all these important changes, and to further adapt to these challenging times, we have made our selection of a new publisher and opted to move to gold open access in 2022 (3). This change was very well adopted by the community and resulted in close to 250, carefully peer reviewed, high-quality articles published in regular or special issues in 2022. We would like to thank the authors who have chosen *Transplant International* to publish their cutting-edge research. In this issue, we also wish to highlight the articles that have received the most attention in 2022 and congratulate their authors. Our thanks also go to our executive and associate editors and our reviewers, whose work and expertise are the bedrock of *Transplant International*. Happy New Year and may 2023 bring exciting achievements to all!

TOP 10 2022 MOST CITED ARTICLES

Bio-Engineering of Pre-Vascularized Islet Organoids for the Treatment of Type 1 Diabetes	Wassmer et al.
Immune Response to Third Dose BNT162b2 COVID-19 Vaccine Among Kidney Transplant Recipients—A Prospective Study	Yahav et al.
Machine Perfusion for Human Heart Preservation: A Systematic Review	Qin et al.
BNT162b2 Third Booster Dose Significantly Increases the Humoral Response Assessed by Both RBD IgG and Neutralizing Antibodies in Renal Transplant Recipients	Hod et al.
Sense and Sensibilities of Organ Perfusion as a Kidney and Liver Viability Assessment Platform	Verstraeten et al.
Comparison of mRNA-1273 and BNT162b2 SARS-CoV-2 mRNA Vaccine Immunogenicity in Kidney Transplant Recipients	Haller et al.
Perfusate Composition and Duration of *Ex-Vivo* Normothermic Perfusion in Kidney Transplantation: A Systematic Review	Fard et al.
Surrogate Endpoints for Late Kidney Transplantation Failure	Naesens et al.
Kidney Transplants From Donors on Extracorporeal Membrane Oxygenation Prior to Death Are Associated With Better Long-Term Renal Function Compared to Donors After Circulatory Death	Gregorini et al.
Kidneys for Sale: Empirical Evidence From Iran	Moeindarbari et al.

TOP 10 2022 HIGHEST ATTENTION ARTICLES (ALTMETRIC SCORES)

European Guideline for the Management of Kidney Transplant Patients With HLA Antibodies: By the European Society for Organ Transplantation Working Group	Mamode et al.
Insights From Transplant Professionals on the Use of Social Media: Implications and Responsibilities	Sandal et al.
Kidneys for Sale: Empirical Evidence From Iran	Moeindarbari et al.
Gender and Racial Disparity Among Liver Transplantation Professionals: Report of a Global Survey	Aguilera et al.
Donor Autonomy and Self-Sacrifice in Living Organ Donation: An Ethical Legal and Psychological Aspects of Transplantation (ELPAT) View	Mamode et al.
From Haphazard to a Sustainable Normothermic Regional Perfusion Service: A Blueprint for the Introduction of Novel Perfusion Technologies	Hunt et al.
Criminal, Legal, and Ethical Kidney Donation and Transplantation: A Conceptual Framework to Enable Innovation	Roth et al.
Kidneys for Sale: Are We There Yet?	Jackson et al.
Kidneys for Sale? A Commentary on Moeindarbari's and Feizi's Study on the Iranian Model	Ambagtsheer et al.
An Analysis by the European Committee on Organ Transplantation of the Council of Europe Outlining the International Landscape of Donors and Recipients Sex in Solid Organ Transplantation	Cozzi et al.

TOP 10 2022 MOST DOWNLOADED ARTICLES

European Guideline for the Management of Kidney Transplant Patients With HLA Antibodies: By the European Society for Organ Transplantation Working Group	Mamode et al.
Bio-Engineering of Pre-Vascularized Islet Organoids for the Treatment of Type 1 Diabetes	Wassmer et al.
Proposed Definitions of T Cell-Mediated Rejection and Tubulointerstitial Inflammation as Clinical Trial Endpoints in Kidney Transplantation	Seron et al.
Evolution of the Definition of Rejection in Kidney Transplantation and Its Use as an Endpoint in Clinical Trials	Becker et al.
Plasma Biomarkers for Clinical Assessment of Bone Mineral Density in Heart Transplanted Patients—A Single-Center Study at Skåne University Hospital in Lund	Löfdahl et al.
Sense and Sensibilities of Organ Perfusion as a Kidney and Liver Viability Assessment Platform	Verstraeten et al.
Metagenomic Next-Generation Sequencing for Diagnosing Infections in Lung Transplant Recipients: A Retrospective Study	Ju et al.
Prolonged-Release Once-Daily Formulation of Tacrolimus Versus Standard-of-Care Tacrolimus in *de novo* Kidney Transplant Patients Across Europe	Budde et al.
Demonstrating Benefit-Risk Profiles of Novel Therapeutic Strategies in Kidney Transplantation: Opportunities and Challenges of Real-World Evidence	Helanterä et al.
Perfusate Composition and Duration of *Ex-Vivo* Normothermic Perfusion in Kidney Transplantation: A Systematic Review	Fard et al.

TOP 10 2022 MOST VIEWED ARTICLES

Kidneys for Sale: Empirical Evidence from Iran	Moeindarbari et al.
Comparison of mRNA-1273 and BNT162b2 SARS-CoV-2 mRNA Vaccine Immunogenicity in Kidney Transplant Recipients	Haller et al.
European Guideline for the Management of Kidney Transplant Patients With HLA Antibodies: By the European Society for Organ Transplantation Working Group	Mamode et al.
Bio-Engineering of Pre-Vascularized Islet Organoids for the Treatment of Type 1 Diabetes	Wassmer et al.
Kidneys for Sale: Are We There Yet?	Jackson et al.
Cardiac Xenotransplantation: Progress in Preclinical Models and Prospects for Clinical Translation	Singh et al.
From Haphazard to a Sustainable Normothermic Regional Perfusion Service: A Blueprint for the Introduction of Novel Perfusion Technologies	Hunt et al.
Patient Selection for Downstaging of Hepatocellular Carcinoma Prior to Liver Transplantation—Adjusting the Odds?	Seehofer et al.
Mortality and Causes of Death After Liver Transplantation: Analysis of Sex Differences in a Large Nationwide Cohort	Serrano et al.
Donor Autonomy and Self-Sacrifice in Living Organ Donation: An Ethical Legal and Psychological Aspects of Transplantation (ELPAT) View	Mamode et al.

EDITORS' PICKS: 2022 COVER ARTICLES

January	Bio-Engineering of Pre-Vascularized Islet Organoids for the Treatment of Type 1 Diabetes	Wassmer et al.
February	Prolonged Organ Extraction Time Negatively Impacts Kidney Transplantation Outcome	Maassen et al
March	Cardiac Xenotransplantation: Progress in Preclinical Models and Prospects for Clinical Translation	Singh et al.
April	Patient Selection for Downstaging of Hepatocellular Carcinoma Prior to Liver Transplantation—Adjusting the Odds?	Seehofer et al.
May	A National Survey Comparing Patients' and Transplant Professionals' Research Priorities in the Swiss Transplant Cohort Study	Beckmann et al.
June	Kidneys for Sale: Empirical Evidence From Iran	Moeindarbari et al.
July	Artificial Intelligence: Present and Future Potential for Solid Organ Transplantation	Peloso et al.
August	Equity in Transplantation: A Commitment for Progress in Troubled Times	Berney et al.
September	Physical Effects, Safety and Feasibility of Prehabilitation in Patients Awaiting Orthotopic Liver Transplantation, a Systematic Review	Jetten et al.
October	Outcome of COVID-19 in Kidney Transplant Recipients Through the SARS-CoV-2 Variants Eras: Role of Anti-SARS-CoV-2 Monoclonal Antibodies	Papadimitriou-Olivgeris et al.
November	Outcomes After Liver Transplantation With Incidental Cholangiocarcinoma	Safdar et al.
December	Association of DGF and Early Readmissions on Outcomes Following Kidney Transplantation	Jadlowiec et al.

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Transplant International thanks the following colleagues for their time and effort in reviewing manuscripts during 2022. Their astute assessments along with their productive and often critical comments ensure that only high-quality papers are published in Transplant International.

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Twenty Years, and More to Come: Learning What Makes Some Transplants Ultra-Long Survivors

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Keywords: kidney transplantation, graft function, long-term survival, DSA, late graft loss

A Forum discussing:

Entering the Third Decade After Kidney Transplantation: Excellent Graft Function Refers to Superior Graft but Not Patient Survival
by Reimann AV, Nilsson J, Wuethrich RP, Mueller TF, Schachtner T (2022). Transpl Int 35:10675. doi: 10.3389/ti.2022.10675

Lifetime survival of the kidney graft and normal life expectancy of the recipient have always been the ultimate goals of kidney transplantation. To survive for several decades, the kidney graft must overcome multiple potential threats, including immune-mediated cell damage, ischemia/reperfusion injury-induced cell senescence and fibrosis, suboptimal nephron mass of kidneys obtained from marginal donors, primary kidney disease recurrence; chronic or recurrent graft infections, and drug nephrotoxicity. The antirejection treatment, which protects against alloimmune-mediated injury, may do so at the expense of the patient's life expectancy. By inhibiting the immune response, anti-rejection drugs increase the risk and severity of infections and cancer. Moreover, some anti-rejection drugs can cause hypertension and severe metabolic complications such as diabetes mellitus, obesity, and hyperlipemia that augment the cardiovascular risk. Cardiovascular abnormalities developed during long exposure to dialysis (such as vascular calcification), patient frailty at time of transplantation, and the effect of long-standing previous immunosuppressive regimens used for the treatment of the primary kidney disease, may also impair long term patient survival, despite a successful transplant procedure.

Regardless of these threats, there exists a subset of kidney transplant recipients whose grafts survived for numerous years, formerly represented by young patients who recieved an azathioprine-based immunesuppressive regimen alongside kidneys from donors who died as a result of traffic accidents. Over time this population has been enriched by older patients who received standard calcineurin-based immunosuppressive regimes alongside kidneys from marginal and after-circulatory-death donors (DCD). Understanding the unique characteristics of this population may provide critical information for the management of this population.

The determinants and complications that may adversely affect ultra-long term clinical outcomes are highly heterogeneous. Some of those patients might have survived for decades without developing any relevant risk factor or complication. In contrast, others have developed relevant risk factors or complications (e.g., donor-specific antibodies), but did so too recently with respect to the start of follow-up, therefore the effect on transplant loss could not be yet detected. Some of the risk factors and complications may be still amenable to intervention even decades after transplantation. The benefit to risk ratio of therapeutic intervention at these later stages, such as immunosuppressive treatment for chronic antibody mediated rejection, should consider that decades of exposure to immunosuppression increase the susceptibility to infection and cancer,

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and that substantial nephron loss unavoidably takes place over decades after transplantation, irrespective of the ongoing alloimmune mediate injury, which leaves little margin for graft function recovery.

In this issue of the journal, Reimann et al. (1) report the determinants of clinical outcomes in kidney transplant recipients who entered the third decade of follow-up and who had received a transplant over a period that spanned from 1981 to 1999 which, compared to previous literature on the topic (2,3,4,5), encompasses the most recent transplantation years reported to date. This population included more recipients of suboptimal donors who were also on modern immunosuppression. After analysing 248 survivors 20 years or more post-transplant, the authors identified 96 patients (39%) who had superior graft function (defined by the joint presence of eGFR ≥45 ml/min, proteinuria ≤300 mg/day, and eGFR-slope ≤2 ml/min/1.73 m^2/year) and who were then compared with the remaining patients. As expected, superior graft function was associated with less exposure to pre-transplant dialysis, better graft quality (younger donor age, less DCD donors), lower rates of T cell mediated rejection, and primary kidney disease not encompassing glomerulonephritis, which included diseases that can recur very late post-transplantation, such as IgA nephropathy (6). After 10 years of further follow-up, having a superior graft function was associated with a twenty-fold reduction in the rate of death censored graft failure (DC-GF). Group membership (i.e., either superior graft function, or other patients) did not apparently affect mortality rate, although the data may not have sufficient statistical power and enough length of follow-up to detect differences in mortality. Even after controlling for group membership, the presence of donor-specific antibodies was still associated with a three-fold increase in the DC-GF rate. Such findings imply that donor-specific antibodies (DSA) remain a powerful biomarker of ongoing chronic antibody-mediated rejection even several decades after transplantation. The incidence of DSA was low in this population, being approximately 20% at 20 years, which is apparently in contrast with the 20% cumulative incidence previously documented as early as 5 years post-transplantation (7). Surprisingly, the prevalence and type of DSA, and the graft immunogenicity as estimated by the PIRCHE II score (8), did not differ between the two groups. These paradoxical findings may be related to the fact that most patients were not typed for HLA-DP and -DQ and that all the assessment was based on low resolution HLA typing. An alternative, not mutually exclusive explanation, is that differences in graft function between the two groups were related to non-alloimmune mediated graft injury and that most cases of chronic rejection started only late after transplantation. Interestingly, the type of immunosuppression apparently affected clinical outcomes, steroid-free regimens being associated with approximately one-third (hazard ratio vs. steroid-based: 1/2.844 = 0.35) the DC-GF rate, and cyclosporine-based regimens being associated with approximately one-third (hazard ration vs. cyclosporine-free: 0.30) the mortality rate. However, because the hazard ratio estimates were not fully adjusted for potential confounding factors, they can hardly be interpreted as expressing a causal relationship between immunosuppressive regimen and clinical outcomes. Rather, they may be regarded as markers of a better prognostic profile. It is interesting to note that even beyond 2 decades post-transplantation, older donor age remains associated with increased DC-GF rate. In fact, the hazard ratio of DC-GF was 1.032 per 1 year age increase. This may seem trivial, but it corresponds to almost twice the DC-GF rate when comparing donor age differences of 20 years (1.032 to the power of 20 = 1.88) and it is especially relevant as the donor age distribution varied widely, ranging between 3 and 72 years (median 32). Although older donor age is associated with inferior outcomes, the survival advantage of transplantation over remaining on the wating list still holds true for kidneys from elderly donors (9,10). Likely, this benefit (although not formally tested) was particularly high in ultra-long survivor transplant recipients.

Given the growth of the ultra-long survivor population and its heterogeneous profile, the study from Reimann et al. (1) provides novel information that may help to guide decision making in their clinical management.

AUTHOR CONTRIBUTIONS

UM wrote the manuscript.

CONFLICT OF INTEREST

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

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ORIGINAL RESEARCH
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Association of DGF and Early Readmissions on Outcomes Following Kidney Transplantation

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Concerns regarding outcomes and early resource utilization are potential deterrents to broader use of kidneys at risk for delayed graft function (DGF). We assessed outcomes specific to kidneys with DGF that required early readmission following transplant. Three groups were identified: 1) recipients with DGF not requiring readmission, 2) recipients with DGF having an isolated readmission, and 3) recipients with DGF requiring ≥2 readmissions. Most recipients either required a single readmission (26.8%, n = 247) or no readmission (56.1%, n = 517); 17.1% (n = 158), had ≥2 readmissions. Recipients requiring ≥2 readmissions were likely to be diabetic (53.8%, p = 0.04) and have longer dialysis vintage (p = 0.01). Duration of DGF was longer with increasing number of readmissions (p < 0.001). There were no differences in patient survival for those with DGF and 0, 1 and ≥2 readmissions (p = 0.13). Graft survival, however, was lower for those with ≥2 readmissions (p < 0.0001). This remained true when accounting for death-censored graft loss (p = 0.0012). Additional subgroup analysis was performed on mate kidneys with and without DGF and mate kidneys, both with DGF, with and without readmissions. For these subgroups, there were no differences in patient or graft survival. As a whole, patients with DGF have excellent outcomes, however, patients with DGF requiring ≥2 readmissions have lower graft survival. A better understanding of recipient variables contributing to multiple readmissions may allow for improvements in the utilization of DGF at-risk kidneys.

Keywords: outcomes, kidney transplant, delayed graft function, graft type, readmission

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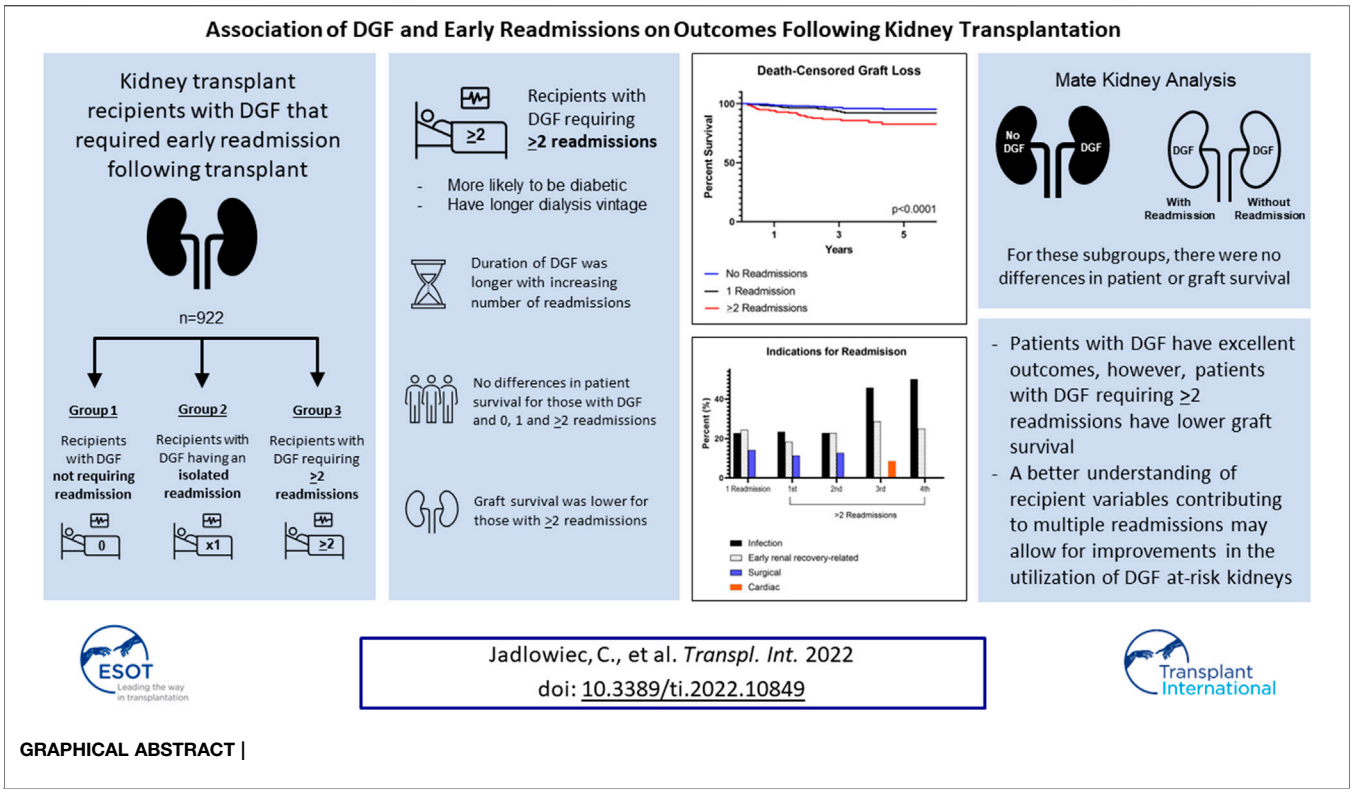

GRAPHICAL ABSTRACT |

INTRODUCTION

Delayed graft function (DGF) is a common post-transplant event. Although the incidence varies between transplant centers, it is known to occur at a higher rate with certain types of kidney allografts, such as those coming from high kidney donor profile index (KDPI) donors, acute kidney injury (AKI) donors and donation after circulatory death (DCD) donors (1-3). The clinical significance of DGF and its impact on outcomes remains debated, however outcomes-related concerns, in combination with increased need for early resource utilization, are perceived as deterrents to the broader use of kidneys at risk for DGF (1-8). These factors unfortunately predispose certain kidney allografts to underutilization and place them at a high risk for discard (8). Although donor-related factors contributing to DGF are well established, recipient-specific variables likely also play an important role in DGF, resource utilization, and transplant outcomes (9-10). Our center has gained experience in using DGF at-risk kidneys and managing post-transplant events in the outpatient setting (1-3). Given this background, we sought to assess variables and outcomes specific to kidneys with DGF that required early readmission following transplant.

METHODS

This is a retrospective review of patients with DGF who received deceased donor kidney transplants at Mayo Clinic Arizona from 2015 through 2020. Recipients with DGF were assessed based on their need for readmission. Three groups were identified: 1) recipients with DGF not requiring readmission, 2) recipients with DGF having a single isolated readmission, and 3) recipients with DGF requiring ≥2 readmissions. Living donor kidney transplants and multivisceral transplants were not included in this analysis. Recipients with early (<7 days post-transplant) technically related graft losses (n = 12) and with primary nonfunction (n = 7) were excluded as were recipients of deceased donor kidneys without DGF (n = 616) (**Figure 1**). The study was deemed exempt by the Institutional Review Board (IRB 20-000860).

DGF was defined as the need for dialysis within 7 days of kidney transplant. Acute kidney injury (AKI) donors were defined as those with Acute Kidney Injury Network (AKIN) stage 2-3 (2, increase in serum creatinine >twofold to threefold from baseline; 3, increase in serum creatinine >4.0 mg/dl or >threefold from baseline or requirement of renal replacement therapy) (1-3).

Data on readmissions was obtained using the electronic health record. The electronic health record was queried for the date of admission, date of transplant procedure, date of discharge and initial length of stay. Early readmission following kidney transplant was defined as occurring within 60 days of the index procedure. A readmission was defined as any hospital stay ≥24 h. The International Classification of Diseases (ICD) 10 codes (ICD 9 prior to October 2015) for the primary readmission discharge diagnosis were recorded. Readmissions related to early renal recovery include those attributed to volume status (overload or dehydration) and electrolyte management.

TABLE 1 | Characteristics of recipients and donors with DGF and 1, ≥2 or no readmissions.

	DGF No readmission (n = 517)	DGF Single readmission (n = 247)	DGF ≥2 readmissions (n = 158)	*p*-value
Recipient				
Age (years)	55.3 ± 12.8 (57.0)	57.1 ± 12.8 (60.0)	57.3 ± 12.7 (59.0)	0.11
Male	333 (64.4%)	160 (64.8%)	109 (69.0%)	0.56
Race				
White	214 (41.4%)	96 (38.9%)	71 (44.9%)	0.65
Black	74 (14.3%)	36 (14.6%)	20 (12.7%)	
Hispanic	126 (24.4%)	75 (30.4%)	41 (25.9%)	
American Indian/Alaska Native	58 (11.2%)	23 (9.3%)	12 (7.6%)	
Other	45 (8.7%)	17 (6.9%)	14 (8.9%)	
BMI (kg/m^2)	28.8 ± 5.6 (28.3)	28.5 ± 5.3 (28.4)	28.7 ± 5.8 (28.4)	0.78
Diabetes	222 (42.9%)	121 (49.0%)	85 (53.8%)	0.04
Ejection Fraction	61.6 ± 6.8 (62.0)	61.1 ± 7.2 (62.0)	60.7 ± 6.7 (61.0)	0.68
EF <45%	15 (2.9%)	11 (4.5%)	5 (3.2%)	0.53
6-minute walk distance (m)	375.5 ± 73.8 (366.0)	333.9 ± 67.5 (344.5)	372.8 ± 49.4 (367.5)	0.07
Midodrine pre-transplant	6 (1.2%)	3 (1.2%)	5 (3.2%)	0.18
Preemptive	45 (8.7%)	13 (5.3%)	9 (5.7%)	0.16
Length of dialysis (years)	3.6 ± 2.6 (3.2)	4.2 ± 3.3 (3.6)	4.0 ± 2.9 (3.4)	0.01
Re-Transplant	41 (7.9%)	23 (9.3%)	17 (10.8%)	0.52
Donor				
Age (years)	40.3 ± 14.8 (39.0)	41.5 ± 15.3 (41.0)	39.9 ± 15.3 (38.0)	0.46
Male	326 (63.1%)	140 (56.7%)	93 (58.9%)	0.21
Height (cm)	170.2 ± 13.8 (172.0)	168.3 ± 14.5 (168.0)	169.0 ± 16.1 (170.0)	
KDPI (%)	52.6 ± 25.1 (51.0)	56.4 ± 23.6 (53.0)	53.1 ± 24.7 (53.0)	0.13
High KDPI	64 (12.4%)	41 (16.6%)	18 (11.4%)	0.20
DCD	147 (28.4%)	82 (33.2%)	46 (29.1%)	0.40
AKI	256 (49.5%)	116 (47.0%)	74 (46.8%)	0.73
Allocation				
Local	226 (43.7%)	89 (36.0%)	60 (38.0%)	0.15
Regional	115 (21.1%)	70 (28.3%)	41 (25.9%)	
National	147 (35.2%)	88 (35.6%)	57 (36.1%)	
Induction				
Alemtuzumab	320 (61.9%)	145 (58.7%)	87 (55.1%)	0.62
Basiliximab	161 (31.1%)	82 (33.2%)	58 (36.7%)	
Thymoglobulin	36 (7.0%)	20 (8.1%)	13 (8.2%)	
CIT (hours)	20.7 ± 6.2 (21.4)	21.0 ± 6.4 (21.4)	21.4 ± 6.4 (21.7)	0.49

Percentages of missing variables are noted in **Supplementary Table S1**.

Protocolized induction and maintenance immunosuppression was used for all kidney transplant recipients. Basiliximab induction was used for patients over 65 years of age; patients less than 65 years of age received depleting induction. Those who received basiliximab were continued on maintenance corticosteroids while steroid discontinuation occurred by post-transplant day five for those receiving depleting induction. Tacrolimus and mycophenolate mofetil were used for maintenance immunosuppression. Tacrolimus was started on post-transplant day 1-2 irrespective of DGF. Tacrolimus trough levels were maintained between 8–10 ng/ml for the first month post-transplant and between 6–8 ng/ml after 1 month. All reported rejections were biopsy-proven. Early acute cellular rejection (ACR) was defined as occurring within 6 months of transplant. Estimated GFR (eGFR) was calculated using the CKD-EPI formula. Six-minute walk distance was used to assess candidate suitability for transplant as previously described (11).

Recipients are typically discharged between post-transplant days 2 and 3 regardless of DGF (12). For those with DGF, dialysis occurred as an outpatient in a community-based dialysis unit. Need to discontinue dialysis was monitored in the outpatient setting with clinic visits and laboratory studies occurring 2-3 times per week. Parameters used to guide discontinuation of dialysis included serum laboratory studies, recipient weight and urine output volume.

Outcomes

Primary outcomes were post-transplant hospital length of stay, early ACR, eGFR, and patient and allograft survival comparing recipients with DGF having 0, 1 and ≥2 post-transplant readmissions. Secondary outcomes were obtained through subgroup analyses on mate kidneys. Two subgroup analyses were completed: 1) mate kidney with and without DGF, and 2) mate kidneys both with DGF but with and without hospital readmissions. Primary outcomes were applied to the subgroup analyses.

Statistical Methods

Chi-square analysis was used for categorical variables and t-tests were used for quantitative variables. Graft and patient survival were calculated by Kaplan-Meier survival analysis. We also used a Cox proportional hazard model to adjust for baseline differences

TABLE 2 | Post-operative outcomes of recipients and donors with DGF and 1, ≥2 or no readmissions.

	DGF No readmission	DGF Single readmission	DGF ≥2 readmissions	*p*-value
LOS	3.0 (2.0, 3.0)	3.0 (2.0, 3.0)	3.0 (2.0, 4.0)	0.91
DGF days	10.5 ± 10.8 (9.0)	12.2 ± 9.8 (10.0)	16.5 ± 15.2 (13.0)	<0.001
Time to readmission (days)	—	18 (9, 30)	13 (7,22)	<0.001
ACR	21 (4.1%)	8 (3.2%)	10 (6.3%)	0.31
eGFR (ml/min)				
4 months	50.2 ± 15.9 (50.0)	49.2 ± 16.8 (50.0)	47.5 ± 18.5 (47.0)	0.26
eGFR <30 ml/min	45 (9.7%)	23 (11.0%)	26 (19.5%)	0.007
1 year	52.9 ± 17.3 (53.0)	53.7 ± 17.2 (55.3)	49.4 ± 19.7 (46.0)	0.11
eGFR <30 ml/min	36 (7.6%)	16 (8.4%)	16 (14.2%)	0.08
2 years	50.1 ± 17.7 (49.9)	51.3 ± 17.7 (51.0)	50.5 ± 19.9 (50.0)	0.86
eGFR <30 ml/min	26 (13.4%)	14 (11.7%)	8 (11.8%)	0.88

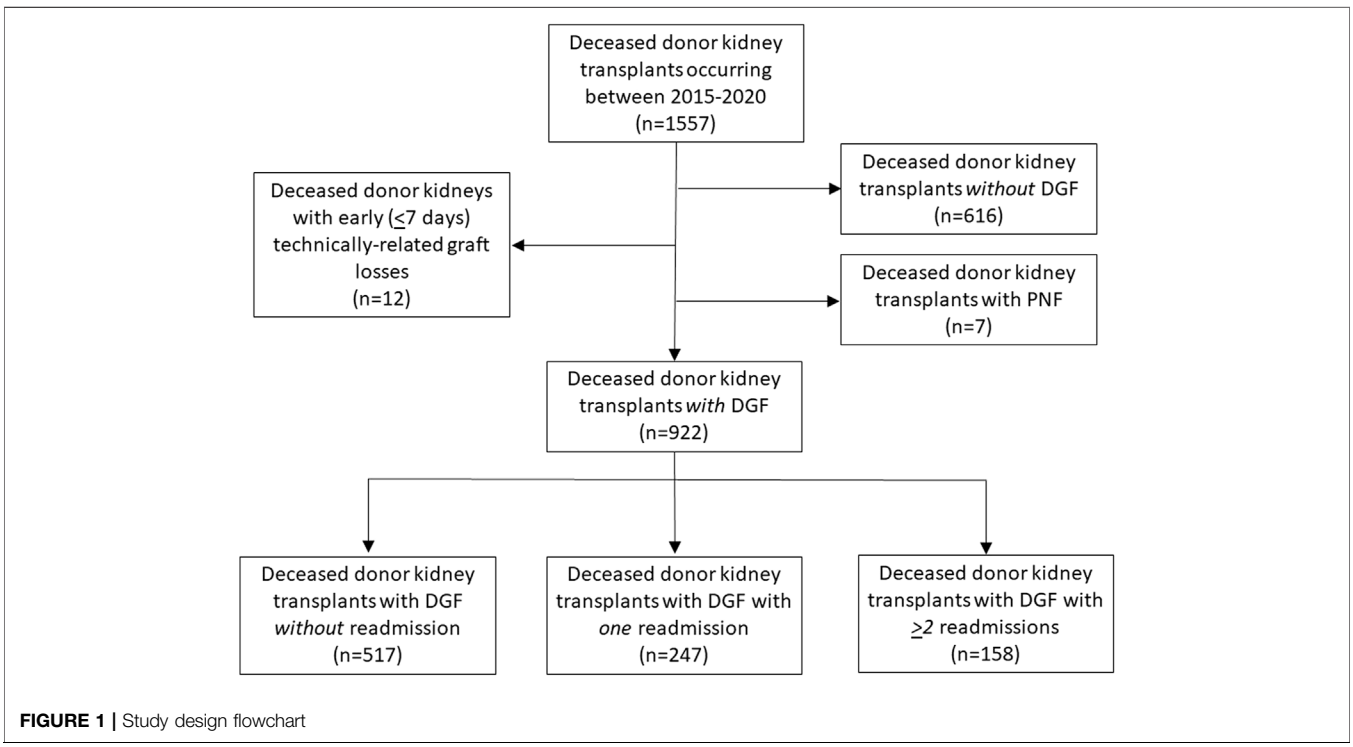

FIGURE 1 | Study design flowchart

in death-censored graft survival. A *p*-value of less than 0.05 was considered statistically significant. Descriptive statistics were reported as mean ± standard deviation, mean ± standard deviation and median, or median and interquartile range (IQR); categorical variables were reported as count and percent. Data was analyzed using GraphPad Prism 9.3.1 (2021 GraphPad Software, Inc.) and BlueSky (Version 7.40).

RESULTS

In total, there were 1557 kidney transplants during this time period. Of those, 59.2% ($n = 922$) had DGF. Of these 922 kidneys with DGF, 13.3% were high KDPI ($n = 123$), 29.7% ($n = 275$) were from DCD donors, and 48.4% ($n = 446$) were from AKI donors. Characteristics of recipients with DGF requiring 0, 1 and ≥2 readmissions are shown in **Table 1**. Most recipients either required an isolated (single) readmission (26.8%, $n = 247$) or no readmission (56.1%, $n = 517$); 17.1% ($n = 158$), had ≥2 readmissions. In general, recipients were similar in age ($p = 0.11$) and race ($p = 0.65$). Recipients for all groups were more likely to be male ($p = 0.56$) and be on dialysis at the time of transplant ($p = 0.16$). Recipients requiring ≥2 readmissions were more likely to be diabetic (53.8%, $p = 0.04$) and have longer dialysis vintage (median 3.4 years, $p = 0.01$). There were no differences observed between the three groups with regard to pre-transplant ejection fraction ($p = 0.68$), 6-minute walk distance ($p = 0.07$) and need for midodrine ($p = 0.18$).

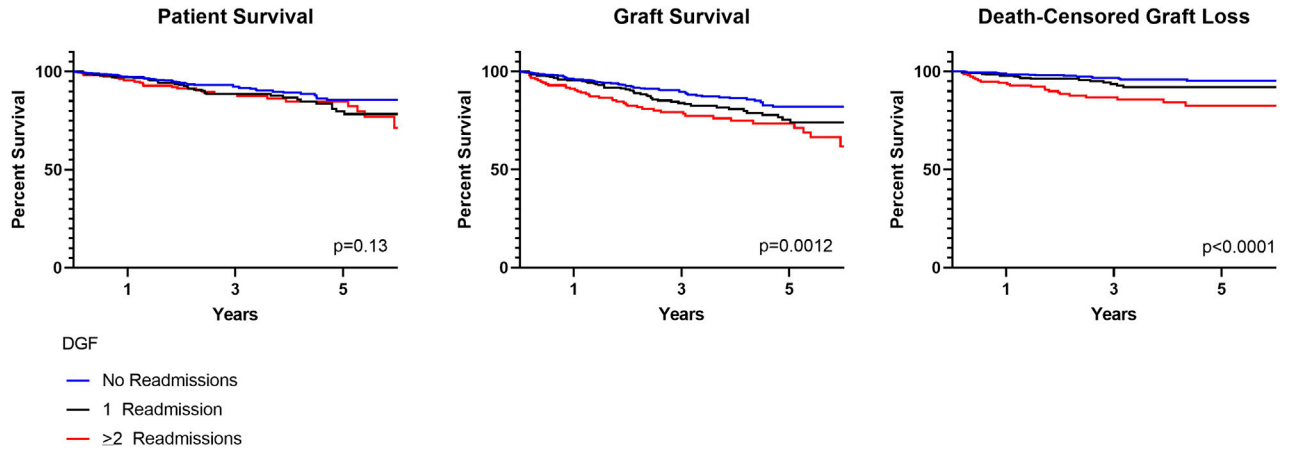

FIGURE 2 | Patient and graft survival in kidneys with DGF and 0, 1, ≥2 readmissions.

TABLE 3 | Recipient and donor characteristics of mate kidneys with and without DGF.

	Mate kidney A, with DGF (*n* = 111)	Mate kidney B, without DGF (*n* = 111)	*p*-value
Recipient			
Age (years)	56.0 ± 13.2 (58.0)	58.7 ± 12.0 (62.0)	0.11
Male	75 (67.6%)	56 (50.5%)	0.01
Race			
White	53 (47.7%)	63 (56.8%)	0.23
Black	16 (14.4%)	14 (12.%)	
Hispanic	21 (18.9%)	15 (13.5%)	
American Indian/Alaska Native	7 (6.3%)	12 (10.8%)	
Other	14 (12.6%)	7 (6.3%)	
BMI (kg/m^2)	27.9 ± 5.4 (27.5)	27.8 ± 5.6 (27.6)	0.92
Diabetes	40 (36.0%)	28 (25.2%)	0.08
Preemptive	14 (12.6%)	56 (50.5%)	<0.0001
Length of dialysis	3.4 ± 2.4 (3.0)	3.4 ± 2.6 (3.1)	0.94
Re-transplant	13 (11.7%)	8 (7.2%)	0.25
Donor			
Age (years)	39.1 ± 15.4 (37.0)		—
Male	69 (62.2%)		—
Height (cm)	169.8 ± 12.5 (170.4)		—
KDPI (%)	49.1 ± 25.7 (49.0)		—
High KDPI	14 (12.6%)		—
DCD	32 (28.8%)		—
AKI	51 (46.0%)		—
Allocation			
Local	54 (48.6%)		—
Regional	26 (23.4%)		
National	31 (27.9%)		
Induction			
Alemtuzumab	66 (59.5%)	62 (55.9%)	0.27
Basiliximab	34 (30.6%)	43 (38.7%)	
Thymoglobulin	11 (9.9%)	6 (5.4%)	
CIT (hours)	20.4 ± 6.7 (20.5)	19.8 ± 7.0 (21.0)	0.48

TABLE 4 | Post-operative outcomes for mate kidneys, with and without DGF.

	Mate kidney A, with DGF	Mate kidney B, without DGF	*p*-value
Length of stay (days)	3.0 (2.0, 3.0)	2.0 (2.0, 3.0)	0.002
DGF duration (days)	12.8 ± 11.7 (11.0)	—	—
ACR	4 (3.6%)	5 (4.5%)	0.73
Readmission	48 (43.2%)	33 (29.7%)	0.04
Number readmissions/patient	1.0 (1.0, 2.0)	1.0 (1.0, 1.0)	0.08
Number of readmissions			0.02
None	63 (56.8%)	78 (70.3%)	
One	28 (25.2%)	26 (23.4%)	
≥ Two	20 (18.0%)	7 (6.3%)	
eGFR (ml/min)			
4 months	47.4 ± 17.1 (47.2)	47.9 ± 13.8 (50.0)	0.80
eGFR <30 ml/min	17 (17.2%)	3 (3.0%)	0.0008
1 year	52.2 ± 18.5 (51.2)	52.4 ± 17.1 (53.0)	0.94
eGFR <30 ml/min	9 (9.7%)	5 (5.6%)	0.29
2 years	49.7 ± 18.3 (49.0)	51.0 ± 17.8 (51.0)	0.69
eGFR <30 ml/min	9 (16.7%)	2 (3.4%)	0.01

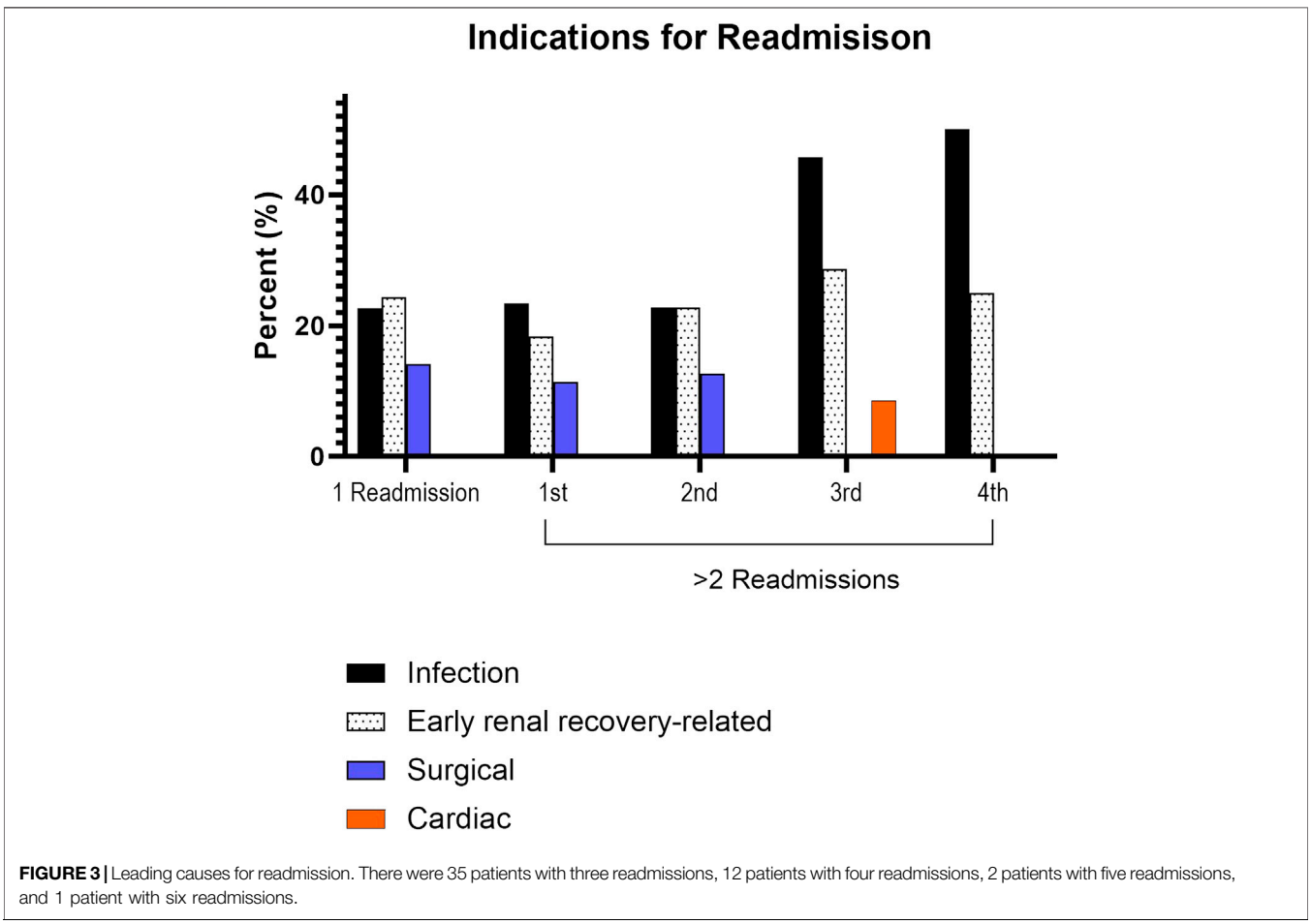

FIGURE 3 | Leading causes for readmission. There were 35 patients with three readmissions, 12 patients with four readmissions, 2 patients with five readmissions, and 1 patient with six readmissions.

TABLE 5 | Recipient and donor characteristics of mate kidneys, both with DGF, with and without readmission.

	DGF, mate kidney A, readmission (*n* = 89)	DGF, mate kidney B, no readmission (*n* = 89)	*p*-value
Recipient			
Age (years)	55.8 ± 12.2 (55.0)	57.8 ± 11.6 (59.0)	0.26
Male	67 (75.3%)	51 (57.3%)	0.01
Race			
White	31 (34.8%)	38 (42.7%)	0.75
Black	15 (16.9%)	14 (15.7%)	
Hispanic	26 (29.2%)	22 (24.7%)	
Other	17 (19.1%)	15 (16.9%)	
BMI (kg/m^2)	29.1 ± 5.5 (28.8)	29.2 ± 5.5 (29.2)	0.89
Diabetes	45 (50.6%)	38 (42.7%)	0.29
Preemptive	2 (2.3%)	6 (6.7%)	0.15
Length of dialysis	4.0 ± 2.7 (3.6)	3.7 ± 1.8 (3.6)	0.53
Re-transplant	6 (6.7%)	4 (4.5%)	0.52
Donor			
Age (years)	39.7 ± 14.0 (38.0)		—
Male	134 (64.1%)		—
Height (cm)	170.4 ± 13.2 (171.5)		—
KDPI (%)	53.0 ± 23.7 (51.0)		—
High KDPI	27 (12.9%)		—
DCD	62 (29.7%)		—
AKI	122 (58.4%)		—
Allocation			
Local	86 (41.1%)		—
Regional	47 (22.5%)		
National	76 (36.4%)		
Induction			
Alemtuzumab	56 (62.9%)	50 (56.2%)	0.27
Basiliximab	27 (83.1%)	36 (40.4%)	
Thymoglobulin	6 (6.7%)	3 (3.4%)	
CIT (hours)	21.3 ± 5.8 (21.3)	21.1 ± 6.0 (21.6)	0.88

Donor characteristics for the three groups are shown in **Table 1**. Overall, there were no differences noted. Donors were similar in age ($p = 0.46$) and more likely to be male ($p = 0.21$). The median KDPI score was 52.0% ($p = 0.13$); high KPDI (KDPI ≥85%) kidneys were equally distributed between the three groups (12.4% vs. 16.6% vs. 11.4%, $p = 0.20$). A similar distribution of AKI (49.5% vs. 47.0% vs. 46.8%, $p = 0.73$) and DCD (28.4% vs. 33.2% vs. 29.1%, $p = 0.40$) kidneys allografts was observed between the groups. Distribution of locally, regionally, and nationally allocated kidneys ($p = 0.15$) and cold ischemia time (CIT, median 21.4 h, $p = 0.49$) were also similar and did not vary between the three groups. Alemtuzumab was the most commonly used induction agent for all groups ($p = 0.62$).

Post-operative outcomes for recipients with DGF requiring 0, 1 and ≥2 readmissions are shown in **Table 2**. Duration of DGF increased along with number of readmissions. Median DGF duration was 9 days for those not requiring readmission, 10 days for those with an isolated readmission and 13 days for those requiring ≥2 readmissions ($p < 0.001$). Readmissions occurred later post-transplant for those recipients with one readmission compared to those with ≥2 readmissions (median 18 vs. 13 days, $p < 0.001$). Recipients with one readmission were also more likely to have had resolution of DGF prior to readmission compared to those with ≥2 readmissions (median 6 vs. 1 day(s), $p < 0.001$). Despite differences in duration of DGF, there were no differences in initial hospital length of stay (LOS) (median 3.0 days, $p = 0.91$) or early ACR events ($p = 0.31$). At 4 months post-transplant, there were no differences between the groups with regard to overall eGFR ($p = 0.26$), although the percentage of individuals with an eGFR <30 ml/min was higher for those requiring ≥2 readmissions (9.7% vs. 11.0% vs. 19.5%, $p = 0.007$). At 1- and 2-years post-transplant, there were no differences in overall eGFR (1-year, $p = 0.11$; 2-year, $p = 0.86$) or eGFR <30 ml/min (1-year, $p = 0.08$; 2-year, $p = 0.88$).

There were no differences in patient survival for those with DGF and 0, 1 and >2 readmissions ($p = 0.13$). Graft survival, however, was lower for those with ≥2 readmissions ($p = 0.0012$). This remained true when accounting for death-censored graft loss ($p < 0.0001$) (**Figure 2**). At 1 year, patient survival was 97.3%, 97.2% and 95.6% for those with 0, 1 and ≥2 readmissions; kidney graft survival was 96.1%, 95.5%, and 91.1%. Median follow-up was 2.7 years (IQR 2.1–6.6) for recipients with 0 readmissions, 3.1 years (IQR 2.1–4.9) for recipients with one readmission and 3.1 years (IQR 2.0–5.0) for recipients with ≥2 readmissions. Cardiopulmonary events accounted for the most common cause of patient death occurring less than 1-year post-transplant in all groups. Death with function followed by infection accounted for the most common causes of graft loss occurring less than 1-year post-transplant. In a cox proportional hazards regression model accounting for presence of pre-transplant diabetes and dialysis duration, ≥2 readmissions was associated with an increased risk for death-censored graft loss (HR 3.1, 95% CI 1.8–5.3) (**Supplementary Table S1**).

Causes for Post-Transplant Readmission

In assessing the initial index readmission for those with 1 and ≥2 readmissions, infection, early renal recovery related factors, and surgical complications were the most common indications observed (**Figure 3**). Subsequent readmissions for those with ≥2 readmissions are shown in **Figure 3**. Infection and factors related to early renal recovery remained the most common causes for readmission in subsequent readmissions.

Subgroup Analysis on Mate Kidneys With and Without DGF

Of the 922 kidney transplants with DGF, 111 had mate kidneys without DGF. Patient characteristics for mate kidneys with and without DGF are shown in **Table 3**. Recipients of mate kidneys with DGF were more likely to be male (67.6% vs. 50.5%, $p = 0.01$) and less likely to be preemptive (12.6% vs. 50.5%, $p < 0.0001$). Other characteristics such as age ($p = 0.11$), race ($p = 0.23$), presence of diabetes ($p = 0.08$), and dialysis vintage ($p = 0.94$) were similar between the two groups.

Median donor age was 37 years and 62.2% of donors were male (**Table 4**). The median KDPI score was 49.0%; 12.6% of allografts were high KDPI, 28.8% came from DCD donors, and 46.0% came from AKI donors. Alemtuzumab remained the most

TABLE 6 | Post-operative outcomes of mate kidneys, both with DGF, with and without readmission.

	DGF, mate kidney A, readmission	DGF, mate kidney B, no readmission	*p*-value
Length of stay (days)	3.0 (2.0, 4.0)	3.0 (2.0, 4.0)	0.96
DGF duration (days)	12.8 ± 7.6 (12.0)	12.9 ± 12.9 (11.0)	0.98
ACR	6 (6.7%)	6 (6.7%)	>0.99
Number of readmissions			—
None	—	89 (100%)	
One	64 (71.9%)		
≥2 Two	25 (28.1%)		
eGFR (ml/min)			
4 months	50.2 ± 17.2 (50.0)	51.1 ± 15.9 (53.0)	0.73
eGFR <30 ml/min	7 (7.9%)	8 (9.0%)	0.79
1 year	52.6 ± 19.6 (56.4)	53.6 ± 16.7 (58.0)	0.76
eGFR <30 ml/min	8 (9.0%)	6 (6.7%)	0.58
2 years	52.7 ± 22.7 (50.8)	45.7 ± 16.6 (46.8)	0.16
eGFR <30 ml/min	6 (6.7%)	7 (7.9%)	0.77

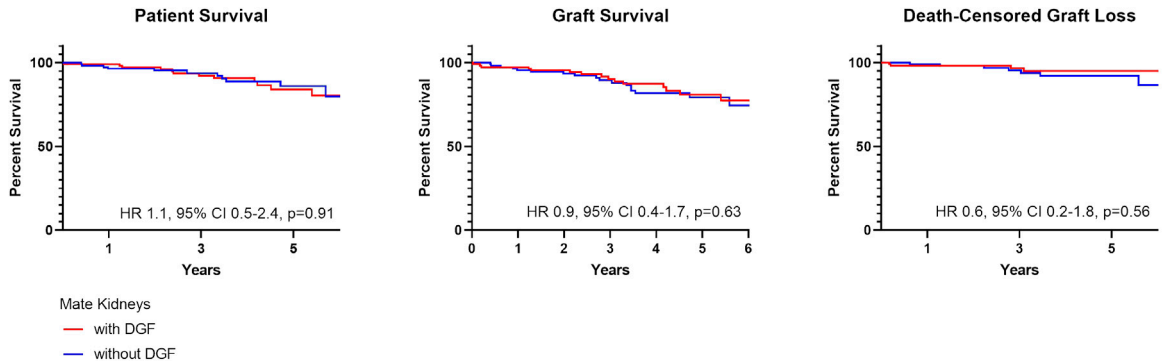

FIGURE 4 | Patient and graft survival in mate kidneys, with and without DGF.

common induction agent used for both groups ($p = 0.27$). There were no differences in CIT ($p = 0.48$).

Post-operative outcomes are shown in **Table 4**. Hospital length of stay was longer in mate kidneys with DGF (median 3.0 vs. 2.0 days, $p = 0.002$) and the median duration of DGF was 11.0 days. Readmissions were more common for mate kidneys with DGF (43.2% vs. 29.7%, $p = 0.04$). Although the overall number of readmissions per recipient did not vary between those with and without DGF (median 1.0, $p = 0.08$), mate kidneys mate kidneys with DGF were more likely to have >2 readmissions (18.0% vs. 6.3%, $p = 0.02$). Early ACR events were uncommon and did not vary between the two groups (3.6% vs. 4.5%, $p = 0.75$). There were no differences in overall eGFR at 4-months ($p = 0.80$), 1-year ($p = 0.94$) and 2-year ($p = 0.69$) although eGFR <30 ml/min was more commonly observed in mate kidneys with DGF at 4 months (17.2% vs. 3.0%, $p = 0.0008$) and 2 years (16.7% vs. 3.4%, $p = 0.01$).

In comparing mate kidneys with and without DGF, there were no differences in patient (HR 1.1, 95% CI 0.5–2.4, $p = 0.91$) or graft survival (HR 0.9, 95% CI 0.4–1.7, $p = 0.63$) (**Figure 4**). This remained true when accounting for death-censored graft loss (HR 0.6, 95% CI 0.2–1.8, $p = 0.56$).

Subgroup Analysis for Mate Kidneys With DGF With and Without Readmission

Of the 922 kidney transplants with DGF, there were 89 mate kidneys with and without readmission. Recipient characteristics of DGF mate kidneys with and without readmission are shown in **Table 5**. Recipient characteristics were overall similar between the two groups with no differences noted in age ($p = 0.26$), body mass index (BMI) ($p = 0.89$), and presence of diabetes ($p = 0.29$). Recipients requiring readmission were more likely to be male (75.3% vs. 57.3%, $p = 0.01$). A similar distribution of preemptive recipients was noted in both groups (2.3% vs. 6.7%, $p = 0.15$). There were no differences in dialysis vintage ($p = 0.53$).

The median donor age was 38 years and 64.1% of donors were male (**Table 5**). Median KDPI was 51.0%; 12.9% of kidneys were high KDPI, 29.7% were from DCD donors,

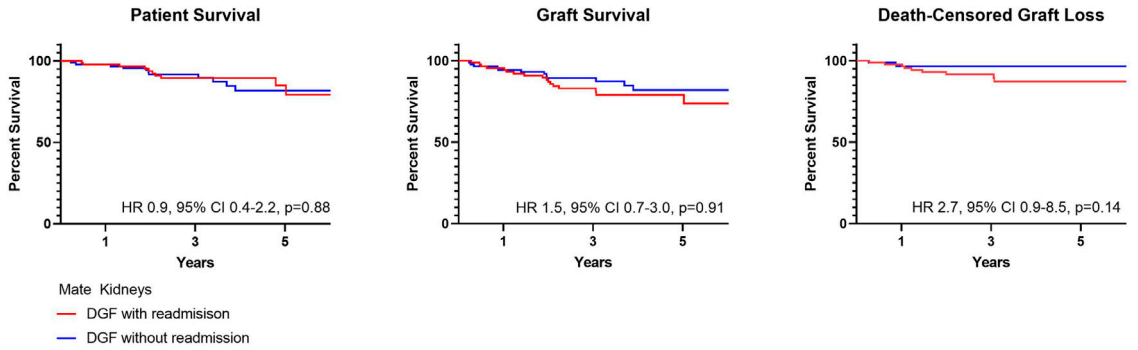

FIGURE 5 | Patient and graft survival in mate kidneys, both with DGF, with and without readmissions.

and 58.4% came from AKI donors. There were no differences in induction with alemtuzumab being the most commonly used induction agent (p = 0.27). CIT was similar for both groups (p = 0.88).

Post-Transplant outcomes from DGF mate kidneys with and without readmission are shown in **Table 6**. There were no differences in DGF duration (p = 0.98), hospital length of stay (p = 0.96) and early ACR events (p > 0.99). There likewise were no differences in patient (HR 0.9, 95% CI 0.4–2.2, p = 0.88) or graft survival (HR 1.5, 95% CI 0.7–3.0, p = 0.91) (**Figure 5**).

DISCUSSION

Concerns related to outcomes, along with increased need for early resource utilization, such as dialysis and hospital readmissions, are believed to be deterrents to the broader use of kidneys at risk for DGF (1-8). Although certain types of kidney allografts are at increased risk for DGF, recipient-specific variables likely play an equally important role in DGF, resource utilization, and transplant outcomes (9-10). As such, the aim of this study was to assess variables and outcomes specific to kidneys with DGF that did and did not require readmission following transplant.

In this study, 59.2% of recipients experienced DGF following transplant. Despite 13.3% of recipients receiving high KDPI kidneys, 29.7% receiving DCD kidneys and 48.4% receiving AKI kidneys, the overall median hospital length of stay was 3 days and the majority of recipients either did not have any readmissions (56.1%) or had an isolated single admission (26.8%). These findings are consistent with our center's experience in using DGF at-risk allografts (2-3,13). Only a small percentage (17.1%) of recipients with DGF required multiple readmissions. Those recipients were more likely to be diabetic and have longer dialysis vintage. In comparing graft characteristics between those with 0, 1, and ≥2 readmissions high KPDI, DCD and AKI kidneys remained equally represented suggesting that use of DGF at-risk allografts does not necessarily result in increased length of hospital stay and readmissions.

DGF continues to be viewed as an adverse event within the transplant community. This negative connotation associated with DGF is largely driven by studies suggesting a correlation between hospital readmissions, increased resource utilization and other inferior outcomes possibly as a result of surgical complications, infection and rejection (4-5,14-15). These concerns likely limit broader utilization of kidneys at risk for DGF, such as those coming from AKI, high KDPI and DCD donors (8,16). More recent studies have suggested that there is in fact significant heterogeneity within DGF (1-3,17-18). In this cohort, we found that the majority of recipients with DGF had excellent patient and graft survival and lower graft survival was noted only for those with ≥2 readmissions; this finding remained true when accounting for death-censored graft loss (**Figure 2**) (14-15). This finding was further supported by data coming from mate kidney comparisons. Differences in patient or graft survival were not observed in mate kidneys with and without DGF (**Figure 4**) or mate kidneys with DGF with and without early readmission (**Figure 5**). These findings suggest that other factors, independent of DGF, are responsible for kidney transplant outcomes. Despite broad representation of kidney allografts coming from high KDPI, DCD and AKI donor, only a small subset of recipients, those requiring ≥2 admissions, demonstrated inferior survival. For that subset of recipients, comorbidities related to diabetes and dialysis vintage likely played a significant contributing role in outcomes (19-20). Based on this experience, one can conclude that transplant outcome determinants are influenced by the presence and severity of recipient comorbidities, rather than DGF (19-21). Given this risk, additional attention should be given to recipients with early frequent readmissions to try to mitigate longer-term inferior outcomes (20).

Competing variables contribute to transplant outcomes (1,19). As such, active risk reduction strategies should be undertaken for factors that are able to be controlled. In this study, the majority of recipients received depleting induction and had calcineurin inhibitors (CNIs) started early post-transplant despite the presence of DGF. As a result, the overall prevalence of early ACR was notably low. Delay in CNI initiation, along with use of non-depleting induction, in the setting of DGF, has resulted in a body of literature linking DGF, ACR and early allograft fibrosis (22-24). Early initiation of CNIs, with achievement of therapeutic trough levels is, in fact, an important risk modifier that should be undertaken in the

setting of DGF (2-3). Similarly, infection, renal recovery related factors and surgical complications accounted for many early readmissions although outcomes were not affected when these events were self-limited (21). For our center, there may be an opportunity to use less depleting induction while still minimizing risk for early rejection through early aggressive initiation of CNIs. Other potential strategies might include improvements in post-transplant diabetes management thereby reducing hyperglycemia and infection risk, as well as a modified outpatient protocol for those presenting with their first hospital readmission. Closer monitoring of patients presenting with their first readmission with a dedicated outpatient care team would perhaps reduce risk for subsequent admissions and adverse outcomes. As such, strategies to minimize recurring readmission events, particularly in the context of recipient comorbidities such as diabetes, warrants further consideration (1,19).

To our knowledge, this is the first study assessing both variables and outcomes specific to DGF kidneys as well as differences in readmission outcomes. It is, however, important to note that, as a single center study, there are limitations as a result of biases introduced through center-specific protocolized practices. As a center with experience in DGF at-risk kidney allograft utilization, the outcomes described here are reflective of carefully selected organs. Donor-recipient pairing remains a crucial component influencing outcomes. Nonetheless, we feel that this data is meaningful. DGF is common event that continues to be associated with a negative connotation throughout the transplant community. More broadly, this association has been linked to kidney allografts that have good outcomes, such as those from DCD and AKI donors, and continues to be a deterrent to broader utilization. As such, we hope to improve utilization of these discard at-risk organs by sharing our experience.

DGF is a common occurrence in high KDPI, DCD and AKI kidneys. Patients with DGF have excellent outcomes as a whole, however, patients with DGF requiring ≥2 readmissions have lower graft survival compared to those with DGF and 0 or 1 readmissions. Independent of DGF, the presence and severity of recipient comorbidities affect transplant outcomes. A better understanding of recipient variables contributing to multiple readmissions may allow for better utilization of DGF at-risk kidneys.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

CJ—Study conception and design, acquisition of data, analysis and interpretation of data, drafting of manuscript, critical revision of manuscript; PF—Acquisition of data, analysis and interpretation of data, drafting of manuscript, critical revision of manuscript; EM—Acquisition of data, analysis and interpretation of data, drafting of manuscript, critical revision of manuscript; JW—Acquisition of data, analysis and interpretation of data, drafting of manuscript, critical revision of manuscript; DD—Analysis and interpretation of data, drafting of manuscript, critical revision of manuscript; PB—Analysis and interpretation of data, drafting of manuscript, critical revision of manuscript; AM—Analysis and interpretation of data, drafting of manuscript, critical revision of manuscript; NK—Analysis and interpretation of data, drafting of manuscript, critical revision of manuscript; KR—Analysis and interpretation of data, drafting of manuscript, critical revision of manuscript; HK—Analysis and interpretation of data, drafting of manuscript, critical revision of manuscript; RH—Study conception and design, analysis and interpretation of data, drafting of manuscript, critical revision of manuscript.

CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10849/full#supplementary-material

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Use of Machine Learning Consensus Clustering to Identify Distinct Subtypes of Kidney Transplant Recipients With DGF and Associated Outcomes

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Data and transplant community opinion on delayed graft function (DGF), and its impact on outcomes, remains varied. An unsupervised machine learning consensus clustering approach was applied to categorize the clinical phenotypes of kidney transplant (KT) recipients with DGF using OPTN/UNOS data. DGF was observed in 20.9% (n = 17,073) of KT and most kidneys had a KDPI score <85%. Four distinct clusters were identified. Cluster 1 recipients were young, high PRA re-transplants. Cluster 2 recipients were older diabetics and more likely to receive higher KDPI kidneys. Cluster 3 recipients were young, black, and non-diabetic; they received lower KDPI kidneys. Cluster 4 recipients were middle-aged, had diabetes or hypertension and received well-matched standard KDPI kidneys. By cluster, one-year patient survival was 95.7%, 92.5%, 97.2% and 94.3% (p < 0.001); one-year graft survival was 89.7%, 87.1%, 91.6%, and 88.7% (p < 0.001). There were no differences between clusters after accounting for death-censored graft loss (p = 0.08). Clinically meaningful differences in recipient characteristics were noted between clusters, however, after accounting for death and return to dialysis, there were no differences in death-censored graft loss. Greater emphasis on recipient comorbidities as contributors to DGF and outcomes may help improve utilization of DGF at-risk kidneys.

Keywords: kidney transplant, delayed graft function, clustering, machine learning, artificial intelligence

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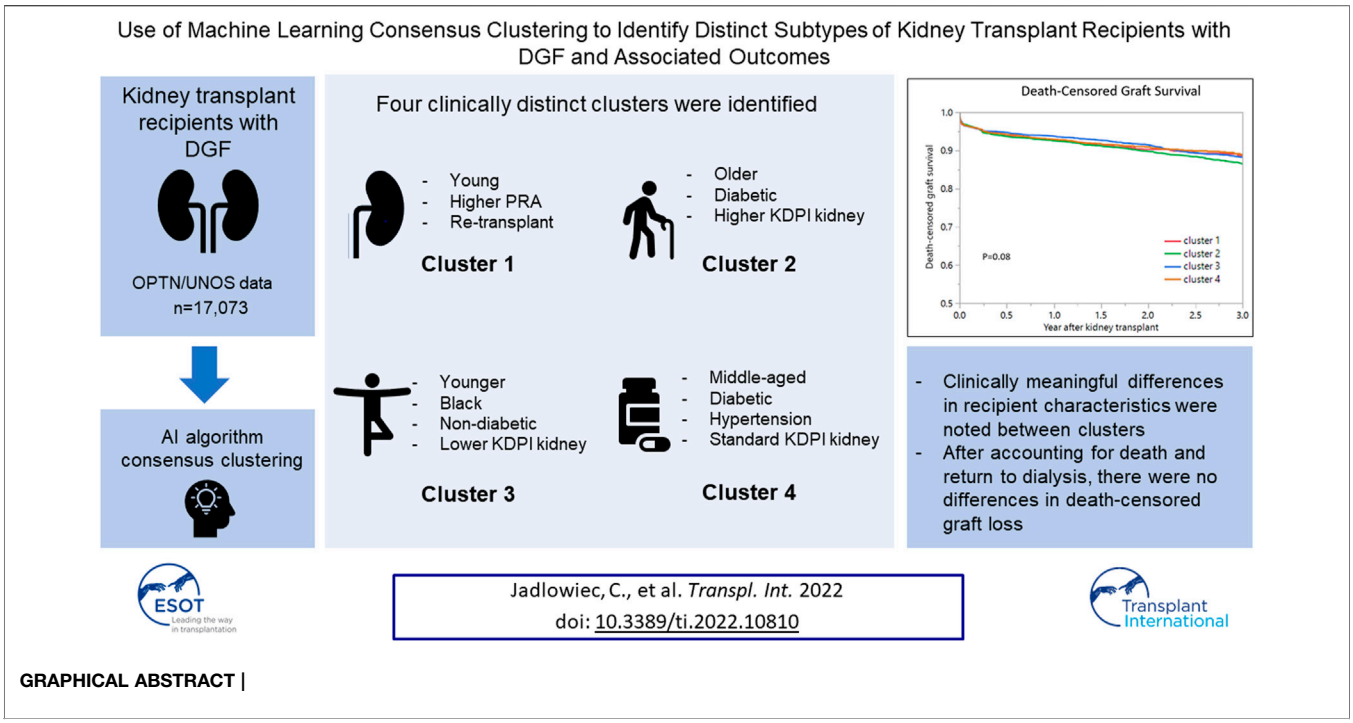
Abbreviations: ANOVA, analysis of variance; BMI, body mass index; CIT, cold ischemia time; DGF, delayed graft function; ECD, extended criterion donor; HLA, human leukocyte antigen; HTN, hypertension; KDPI, kidney donor profile index; KT, kidney transplant; MICE, multiple imputation by chained equation; ML, machine learning; mTOR, mammalian target of rapamycin; OPTN, Organ Procurement and Transplantation Network; PRA, panel reactive antibody; PVD, peripheral vascular disease; UNOS, United Network for Organ Sharing.



GRAPHICAL ABSTRACT |

INTRODUCTION

Delayed graft function (DGF) is common following kidney transplantation (KT) and its incidence varies anywhere from ≤30% in standard kidney donor profile index (KDPI) kidneys to upwards of 60% in kidneys allografts coming from donation after circulatory death (DCD), severe acute kidney injury (AKI), and high KDPI (KDPI ≥85%) donor (1–4). Although the definition of DGF, need for dialysis within 7 days of KT, is simplistic and allows for consistency, the reporting of DGF as a binary outcome in data analyses fails to capture complex clinical nuances that contribute to outcomes. Donor-related characteristics, such as DCD status and acute kidney injury, are commonly identified risk-factors for DGF, although recipient-specific characteristics and transplant events also play significant roles and influence outcomes (1–3, 5, 6). Published data and transplant community opinion on DGF, and its impact on outcomes, remains varied. Many studies have shown an association between DGF and inferior survival (7–9). While other studies have shown that select DGF subgroups have equivocal outcomes compared to those with primary function (1–4). The observed inconsistencies in DGF outcomes are possibly related to how DGF data is analyzed, with many studies focusing on predetermined individual donor-, recipient-, or transplant characteristics rather than a balanced interpretation of competing variables (1–9).

Artificial intelligence and machine learning (ML) function as clinical decision support tools have been used to help individualize patient care, including organ transplantation (10–15). Unsupervised consensus clustering, a type of ML, can be applied to clinical data and its application has allowed for the discovery of novel data patterns and distinct subtypes (16–18). It has facilitated the discovery of similarities and heterogeneities among data variables and has also distinguished data into clinically meaningful clusters independent of predefined risk-variables (16, 17). Recent studies have demonstrated that distinct subtypes identified by ML consensus clustering approach can forecast different clinical outcomes (19–21). To better understand differing DGF outcomes, we used an unsupervised ML consensus clustering approach to categorize clinical phenotypes of KT recipients with DGF and their paired donors.

MATERIALS AND METHODS

Adult patients who received a kidney-only transplant in the United States from 2015 to 2019 were identified using the Organ Procurement and Transplantation Network (OPTN)/United Network for Organ Sharing (UNOS) database. All KT patients with DGF were included. DGF was defined as the need for dialysis within 7 days after KT. Multivisceral transplant recipients were not included in this dataset. After accounting for all adult kidney-only transplant recipients (n = 81,548), adult kidney-only transplant recipients without DGF (n = 64,475) were excluded. The Mayo Clinic Institutional Review Board approved this study (IRB 21-007698).

Recipient-, donor-, and transplant-related variables shown in **Table 1**, in addition to recipient ABO, positive hepatitis C serostatus, hepatitis B surface antigen, human immunodeficiency virus serostatus, working income, public insurance, United States resident, undergraduate education or higher, serum albumin, ABO incompatibility, Ebstein-Barr and cytomegalovirus status, were abstracted from the OPTN/UNOS database. All variables had ≤5% missing data (**Supplementary**

TABLE 1 | Clinical characteristics, according to clusters, of kidney transplant recipients with DGF.

	All (*n* = 17,073)	Cluster 1 (*n* = 1,891)	Cluster 2 (*n* = 6,918)	Cluster 3 (*n* = 5,442)	Cluster 4 (*n* = 2,822)	*p*-value
Recipient Characteristics						
Age (year)	54.1 ± 12.6 (56)	47.2 ± 12.6 (48)	61.5 ± 8.3 (62)	45.9 ± 11.6 (46)	56.3 ± 11.5 (58)	<0.001
Male sex	11475 (67%)	1199 (63%)	4854 (70%)	3746 (69%)	1676 (59)	<0.001
Race						<0.001
White	5208 (30%)	753 (40%)	2022 (29%)	1167 (21%)	1266 (45%)	
Black	6645 (39%)	681 (36%)	2627 (38%)	2692 (49%)	645 (23%)	
Hispanic	3506 (21%)	324 (17%)	1464 (21%)	1059 (20%)	659 (23%)	
Other	1714 (10%)	133 (7%)	805 (12%)	524 (10%)	252 (9%)	
Body mass index (kg/m^2)	29.3 ± 5.5 (29.0)	27.5 ± 5.6 (27.0)	30.1 ± 5.2 (29.9)	28.8 ± 5.8 (28.2)	29.7 ± 5.3 (29.4)	<0.001
No. of kidney transplant(s)	1.1 ± 0.4	2.1 ± 0.4	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	<0.001
PRA, median (IQR)	0 (0, 39)	98 (83, 100)	0 (0, 3)	0 (0, 16)	0 (0, 57)	<0.001
Dialysis duration						<0.001
Preemptive	610 (4%)	74 (4%)	225 (3%)	183 (3%)	128 (5%)	
<1 year	1054 (6%)	126 (7%)	406 (6%)	302 (6%)	220 (8%)	
1–3 years	3120 (18%)	445 (23%)	1199 (17%)	734 (13%)	742 (26%)	
>3 years	12289 (72%)	1246 (66%)	5088 (74%)	4223 (78%)	1732 (61%)	
Cause of kidney disease						<0.001
Diabetes mellitus	5998 (35%)	74 (4%)	4163 (60%)	600 (11%)	1161 (41%)	
Hypertension	4151 (24%)	171 (9%)	1300 (19%)	2101 (39%)	579 (21%)	
Glomerular disease	2780 (16%)	313 (16%)	595 (9%)	1443 (27%)	429 (15%)	
PKD	976 (6%)	35 (2%)	302 (4%)	406 (7%)	233 (8%)	
Other	3168 (19%)	1298 (69%)	558 (8%)	892 (16%)	420 (15%)	
Comorbidities						
Diabetes mellitus	7404 (43%)	349 (18%)	4788 (69%)	901 (17%)	1366 (48%)	<0.001
Malignancy	1584 (9%)	213 (11%)	766 (11%)	316 (6%)	289 (10%)	<0.001
PVD	1941 (11%)	144 (8%)	1159 (17%)	304 (6%)	334 (12%)	<0.001
Functional status						<0.001
10–30%	53 (0%)	2 (0%)	30 (1%)	13 (0%)	8 (0%)	
40–70%	8789 (52%)	872 (46%)	3829 (55%)	2609 (48%)	1479 (53%)	
80–100%	8231 (48%)	1017 (54%)	3059 (44%)	2820 (52%)	1335 (47%)	
Donor Characteristics						
Kidney donor status						<0.001
Non-ECD	13528 (79%)	1697 (90%)	4530 (65%)	5161 (95%)	2140 (76%)	
ECD	2778 (16%)	145 (8%)	2160 (31%)	61 (1%)	412 (15%)	
Living donor	767 (5%)	49 (3%)	228 (3%)	220 (4%)	270 (10%)	
Age	41.4 ± 14.6 (43)	37.9 ± 13.5 (39)	49.5 ± 11.0 (51)	31.3 ± 13.1 (31)	43.2 ± 12.9 (45)	<0.001
Male sex	10571 (62%)	1223 (65%)	4057 (59%)	3565 (65%)	1726 (61%)	<0.001
Race						<0.001
White	11691 (68%)	1258 (66%)	4804 (69%)	3575 (66%)	2054 (73%)	
Black	2247 (13%)	258 (14%)	924 (13%)	836 (15%)	229 (8%)	
Hispanic	2350 (14%)	290 (15%)	841 (12%)	810 (15%)	409 (14%)	
Other	785 (5%)	85 (4%)	349 (5%)	221 (4%)	130 (5%)	
Hypertension	5678 (33%)	516 (27%)	3401 (49%)	829 (15%)	932 (33%)	<0.001
KDPI						<0.001
Living donor	767 (4%)	49 (3%)	228 (3%)	220 (4%)	270 (9%)	
KDPI<85	14611 (86%)	1795 (95%)	5265 (76%)	5160 (95%)	2391 (85%)	
KDPI≥85	1695 (10%)	47 (2%)	1425 (21%)	62 (1%)	161 (6%)	
Transplant-Related Characteristics						
HLA mismatch ABDR	4 (4, 5)	3 (2, 4)	5 (4, 5)	5 (4, 5)	3 (2, 3)	<0.001
CIT (hours)	19.0 ± 9.3 (18.4)	19.6 ± 8.5 (19.3)	20.3 ± 9.6 (19.4)	17.3 ± 8.8 (16.4)	18.5 ± 9.8 (18.6)	<0.001
Kidney on pump	8280 (48%)	701 (37%)	3961 (57%)	2396 (44%)	1222 (43%)	<0.001
Allocation type						<0.001
Local	10996 (64%)	752 (40%)	4347 (63%)	4208 (77%)	1689 (60%)	
Regional	2748 (16%)	325 (17%)	1437 (21%)	574 (11%)	412 (15%)	
National	3329 (20%)	814 (43%)	1134 (16%)	660 (12%)	721 (25%)	
Induction Immunosuppression						
Thymoglobulin	10777 (63%)	1425 (75%)	4136 (60%)	3478 (64%)	1738 (62%)	<0.001
Alemtuzumab	2651 (15%)	270 (14%)	973 (14%)	965 (18%)	443 (16%)	<0.001
Basiliximab	3308 (19%)	122 (6%)	1744 (25%)	877 (16%)	565 (20%)	<0.001
Other	240 (1%)	27 (1%)	105 (1%)	65 (1%)	43 (1%)	0.44
No induction	965 (6%)	87 (5%)	404 (6%)	310 (6%)	164 (6%)	0.21

(Continued on following page)

TABLE 1 | (*Continued*) Clinical characteristics, according to clusters, of kidney transplant recipients with DGF.

	All (*n* = 17,073)	Cluster 1 (*n* = 1,891)	Cluster 2 (*n* = 6,918)	Cluster 3 (*n* = 5,442)	Cluster 4 (*n* = 2,822)	*p*-value
Maintenance Immunosuppression						
Tacrolimus	15513 (91%)	1742 (92%)	6250 (90%)	4958 (91%)	2563 (91%)	0.10
Cyclosporine	152 (1%)	25 (1%)	56 (1%)	47 (1%)	24 (1%)	0.20
Mycophenolate	15678 (92%)	1746 (92%)	6329 (92%)	5020 (92%)	2583 (92%)	0.35
Azathioprine	61 (0%)	10 (1%)	21 (0%)	19 (0%)	11 (0%)	0.53
mTOR inhibitors	46 (0%)	4 (0%)	24 (0%)	9 (0%)	9 (0%)	0.24
Steroid	12337 (72%)	1523 (81%)	4875 (70%)	3930 (72%)	2009 (71%)	<0.001

Table S1). We imputed missing data using multiple imputation by chained equation (MICE) method (12). One-year acute rejection was defined as clinical acute rejection, independent of chronic rejection, occurring within the first-year post-transplantation as reported to UNOS.

Clustering Analysis

An unsupervised ML was applied by conducting a consensus clustering approach to categorize clinical phenotypes of KT recipients with DGF (13). A pre-specified subsampling parameter of 80% with 100 iterations and the number of potential clusters (k) ranging from 2 to 10 were used to avoid producing an excessive number of clusters that would not be clinically useful. The optimal number of clusters was determined by examining the consensus matrix (CM) heat map, cumulative distribution function (CDF), cluster-consensus plots with the within-cluster consensus scores, and the proportion of ambiguously clustered pairs (PAC). The within-cluster consensus score, ranging between 0 and 1, was defined as the average consensus value for all pairs of individuals belonging to the same cluster (14). A value closer to one indicates better cluster stability. PAC, ranging between 0 and 1, was calculated as the proportion of all sample pairs with consensus values falling within the predetermined boundaries (15). A value closer to zero indicates better cluster stability (16). To avoid cherry picking results, we used validated clustering approaches including examination of the consensus matrix (CM) heat map, cumulative distribution function (CDF), cluster-consensus plots with the within-cluster consensus scores, and the proportion of ambiguously clustered pairs (19, 21–23). The detailed consensus cluster algorithms used in this study for reproducibility are provided in **Online Supplementary**.

Outcomes

Outcomes identified included acute rejection within the first post-transplant year and 1- and 3-year patient, kidney allograft and death-censored graft survival.

Statistical Analysis

After each KT recipient with DGF was assigned a cluster using the consensus clustering approach, we performed a comparison of clinical characteristics and posttransplant outcomes among the assigned clusters. Clinical characteristics among the assigned clusters were compared using Chi-squared analysis for categorical variables and analysis of variance (ANOVA) for continuous variables. The key characteristics of each cluster were identified using the standardized mean difference between each cluster and the overall cohort with the pre-specified cut-off of >0.3. The cumulative risks of death-censored graft failure and death after KT were estimated using Kaplan-Meier analysis, and the risks among the assigned cluster were compared using Cox proportional hazard analysis. As OPTN/UNOS only reported whether allograft rejection occurred within 1 year after KT but did not specify the occurrence date, we compared the risk of 1-year acute allograft rejection among the assigned clusters using logistic regression analysis. We did not adjust the association of the assigned cluster and posttransplant outcomes in multivariable analysis for difference in baseline characteristics because unsupervised consensus clustering approach purposefully generated clinically distinct clusters. R, version 4.0.3 (RStudio, Inc., Boston, MA; http://www.rstudio.com/) was used for statistical analyses; ConsensusClusterPlus package (version 1.46.0) for consensus clustering analysis, and the MICE command in R for multivariable imputation by chained equation (24).

RESULTS

During this study period, a total of 81,548 adult patients received a KT, and of those, 20.9% (n = 17,073) had DGF. Consensus clustering analysis was performed on the 17,073 KT recipients with DGF.

Figure 1A shows the CDF plot consensus distributions for each cluster of KT recipients with DGF; the delta area plot shows the relative change in the area under the CDF curve (**Figure 1B**). The largest changes in area occurred between k = 2 and k = 4, at which point the relative increase in area became noticeably smaller. As shown in the CM heat map (**Figure 1C**), the ML algorithm identified cluster 2 and cluster 4 with clear boundaries, indicating good cluster stability over repeated iterations. The mean cluster consensus score was comparable between k = 2 and k = 4 (p > 0.05) (**Figure 2A**). Favorable low PAC was demonstrated for 4 clusters than 2 clusters (**Figure 2B**). Thus, using baseline variables at the time of transplant, the consensus clustering analysis identified 4 clusters that best represented the data pattern of our KT recipients with DGF.

Clinical Characteristics of DGF Clusters

Table 1 shows recipient-, donor-, and transplant-related characteristics of included patients. DGF was observed in

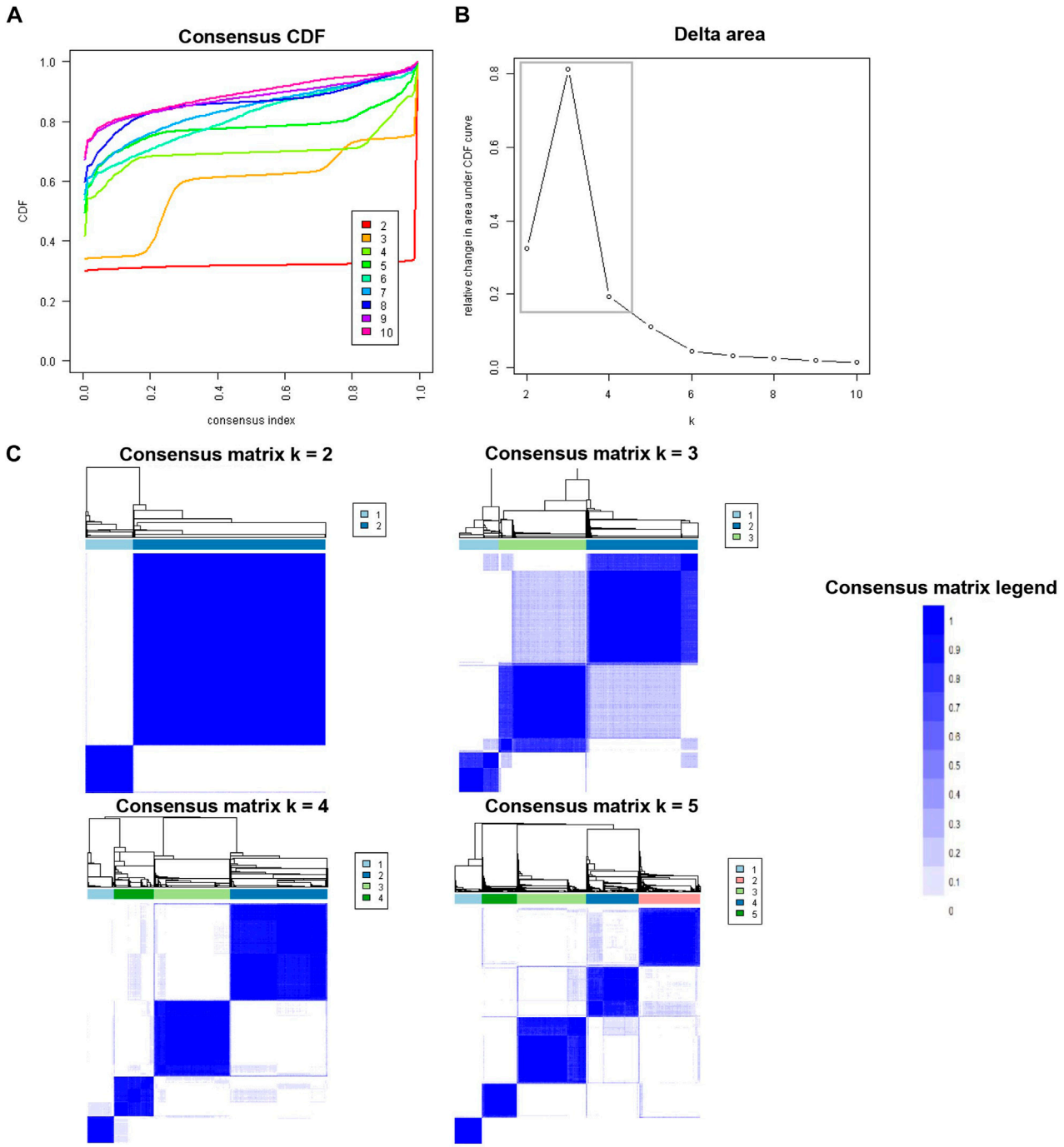


FIGURE 1 | (A) CDF plot displaying consensus distributions for each k; **(B)** Delta area plot reflecting the relative changes in the area under the CDF curve. **(C)** Consensus matrix heat map depicting consensus values on a white to blue color scale of each cluster.

20.9% of kidney transplants (n = 17,073) that occurred during this study period. The majority of recipients with DGF were male (67%, n = 11,475) and had more than 3 years of time on dialysis (72%, n = 12,289). Most kidneys with DGF were non-extended criterion donor (ECD) (79%, n = 13,528) standard KDPI kidneys (86%, n = 14,611). Donors of kidneys with DGF had a median age of 43 years, were likely to be male (62%, n = 10,571), white (68%, n = 11,691), be transplanted by local centers (64%, n = 10,996), and have a median CIT of 18.4 h.

Within this group of 17,073 recipients with DGF, consensus clustering analysis identified four distinct clinical clusters as shown in **Table 1**. There were 1,891 (11%) patients in cluster 1, 6,918 (41%) patients in cluster 2, 5,442 (32%) patients in cluster 3, and 2,822 (17%) patients in cluster 4. According to

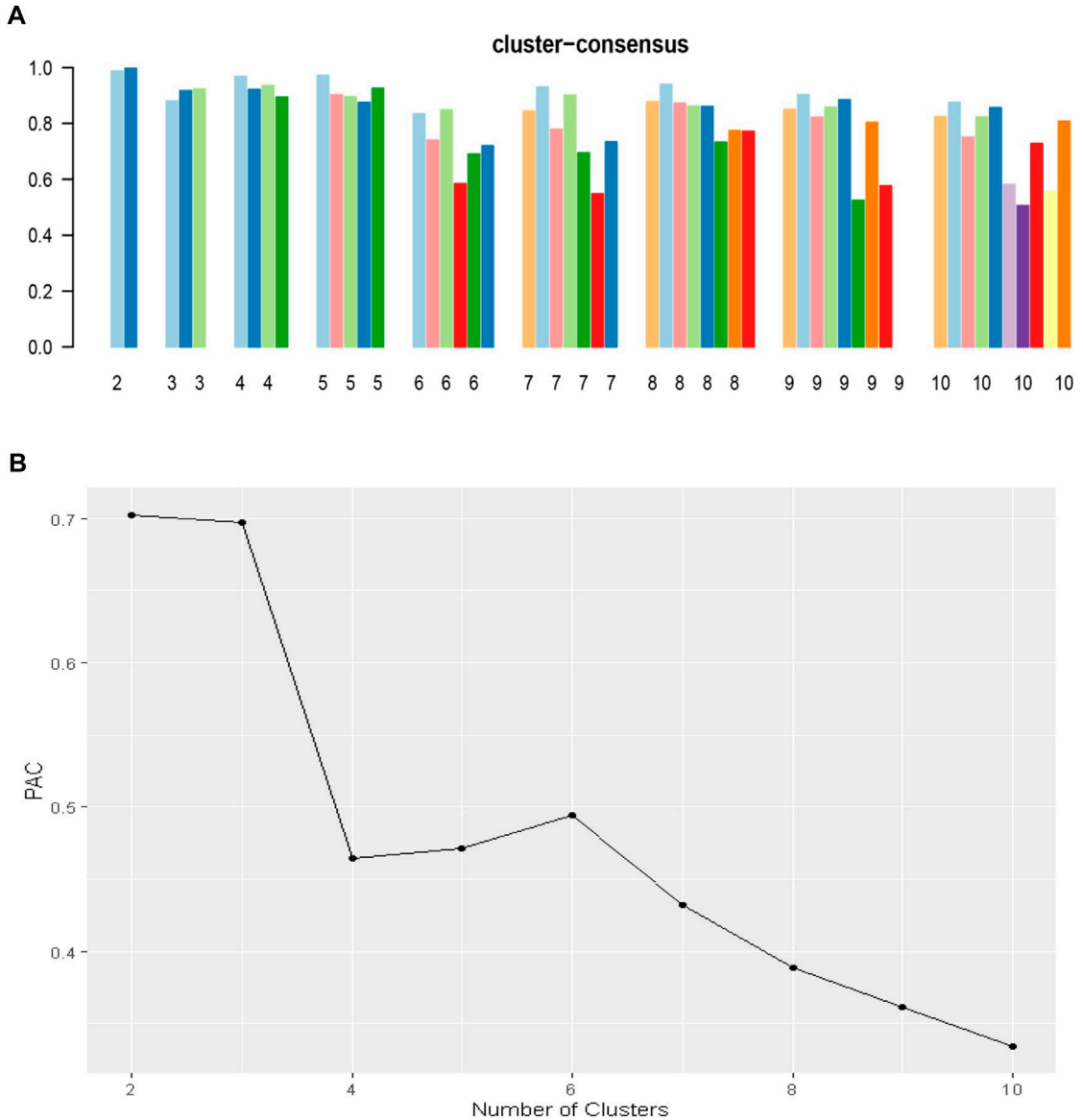


FIGURE 2 | (A) The bar plot represents the mean consensus score for different numbers of clusters (K ranges from two to ten); **(B)** The PAC values assess ambiguously clustered pairs.

standardized mean differences, shown in **Figure 3**, cluster 1 was characterized by younger (median age 48 years), low BMI, non-diabetic, kidney re-transplant recipients who had a high PRA, a low number of HLA mismatches, and received depleting induction. Cluster 1 recipients received standard KDPI kidneys (95% had a KDPI score <85%, n = 1795) and had the highest percentage of nationally allocated kidneys (43%, n = 814).

By comparison, cluster 2 recipients were the oldest (median age 62 years) of the four clusters. They had a higher BMI (30.1 ± 5.2 kg/m^2) and were likely to be diabetic (69%, n = 4,788) with the majority (74%, n = 5,088) having ≥3 years of dialysis time. Cluster 2 recipients were not sensitized. They were first-time KT recipients with a high number of HLA mismatches. Cluster 2 had more recipients with lower functional status, with 55% having a Karnofsky score between 40-70%. Out of the four clusters, cluster 2 recipients were the most likely to receive an ECD (31%, n = 2,160), high KDPI (21%, n = 1,425) kidney, although the majority (76%, n = 5,265) received standard KDPI kidneys. Peripheral vascular disease (PVD) was present in 17% of cluster 2 recipients.

Cluster 3 recipients were young in age (median age 46 years) and non-diabetic. They were more likely to be black (49%, n = 2,696) and have hypertension (39%, n = 2,101). Similar to cluster 2, they were also first-time KT recipients with a high number of HLA mismatches and a low PRA. They were unlikely to receive an ECD (1%, n = 61), high KDPI (1%, n = 62) kidney. Instead, the majority of cluster 3 recipients received standard KDPI kidneys (76%, n = 5,265), from young (median age 31 years), non-

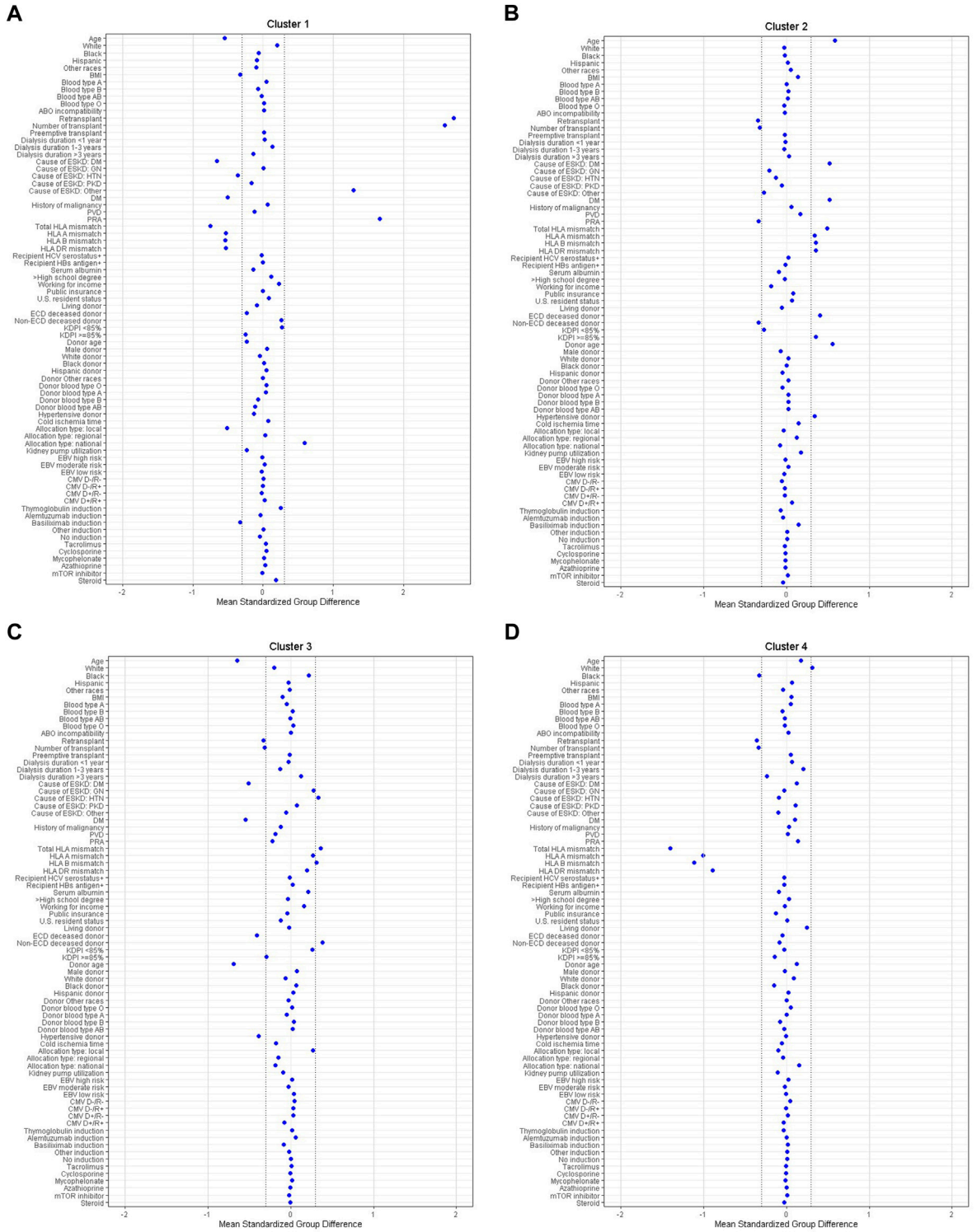


FIGURE 3 | (A–D) The standardized differences in Clusters 1–4 of DGF for each of baseline parameters. The x axis is the standardized differences value, and the y axis shows baseline parameters. The dashed vertical lines represent the standardized differences cutoffs of <−0.3 or >0.3. Abbreviations: BMI, body mass index; CMV, cytomegalovirus; D, donor; DGF, delayed graft function; DM, diabetes mellitus; EBV, Epstein-Barr virus; ECD, extended criteria donor; ESKD, end stage kidney disease; GN, glomerulonephritis; HBs, hepatitis B surface; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HLA, human leucocyte antigen; HTN, hypertension; KDPI, kidney donor profile index; mTOR, mammalian target of rapamycin; PKD, polycystic kidney disease; PRA, panel reactive antibody; PVD, peripheral vascular disease; R, recipient.

TABLE 2 | Posttransplant outcomes, according to clusters, of kidney transplant recipients with DGF.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
1-Year				
Patient survival	95.7% (1.55, 1.17–2.07)	92.5% (2.66, 2.19–3.24)	97.2% (1, ref)	94.3% (1.98, 1.56–2.52)
Graft survival	89.7% (1.22, 1.03–1.46)	87.1% (1.52, 1.35–1.71)	91.6% (1, ref)	88.7% (1.33, 1.15–1.55)
Death-censored graft survival	92.7% (1.15, 0.94–1.41)	92.5% (1.18, 1.03, 1.36)	93.7% (1, ref)	92.9% (1.12, 0.93–1.34)
1-year acute rejection	10.2% (2.86, 2.24–3.64)	5.3% (1.42, 1.14–1.76)	7.0% (1.90, 1.52–2.36)	3.8% (1, ref)
3-Year				
Patient survival	88.7% (1.63, 1.32–2.03)	81.6% (2.78, 2.39–3.24)	93.2% (1, ref)	86.7% (1.98, 1.64–2.39)
Graft survival	81.1% (1.20, 1.04–1.39)	74.6% (1.58, 1.43–1.75)	83.8% (1, ref)	80.0% (1.29, 1.14–1.47)
Death-censored graft survival	88.6% (1.05, 0.88–1.26)	86.5% (1.16, 1.03–1.32)	88.2% (1, ref)	88.9% (1.03, 0.87–1.20)

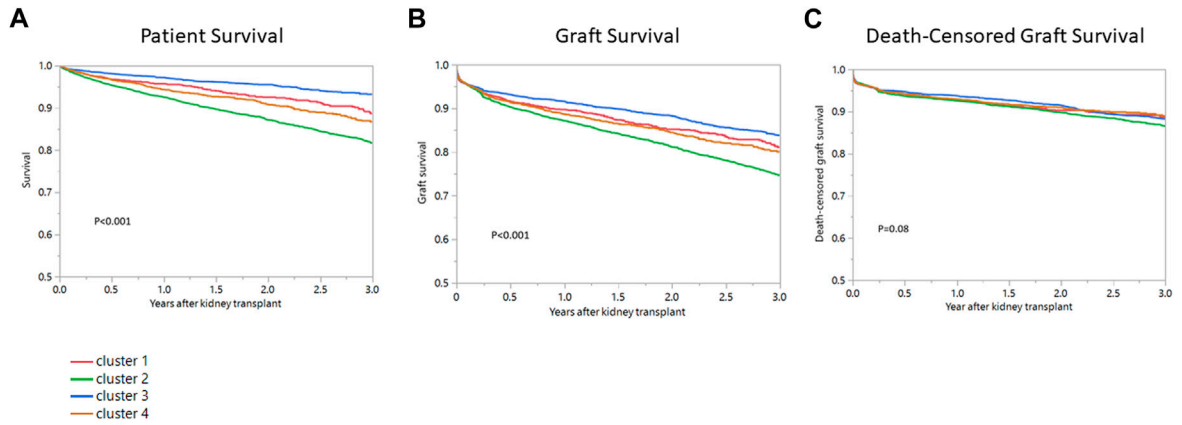


FIGURE 4 | (A) Patient survival, **(B)** Graft survival, **(C)** Death-censored graft survival after kidney transplant among four clusters of kidney transplant recipients with DGF in the United States.

hypertensive donors. These kidneys came from local donors (77%, n = 4,208). Cluster 3 kidneys had the shortest CIT (median 16.4 h).

Lastly, cluster 4 recipients were middle aged (median age 58 years), first-time KT recipients with greater than 3 years of dialysis times, a low PRA, and a lower number of HLA mismatches. Recipients in cluster 4 were likely to have kidney disease as a result of diabetes (41%) or hypertension (21%). Forty-eight percent (n = 1,366) were diabetic and 12% (n = 334) had PVD. Recipient functional status was also lower in cluster 4, with 53% of recipients having a Karnofsky score between 40%–70%. The majority received non-ECD (76%, n = 2,140), standard KDPI (85%, n = 2,391) kidneys that largely came from local donors (60%, n = 1,889).

Posttransplant Outcomes of DGF Clusters

Table 2 and **Figure 4** show cluster-based posttransplant outcomes. Median follow-up time for patient survival was 412 days (IQR 199-971). Median follow-up time for graft survival was 391 days (IQR 188-945). One-year patient survival in clusters 1, 2, 3 and 4 was 95.7%, 92.5%, 97.2% and 94.3%. Cluster 3 had the most favorable patient survival (ref) with cluster 2 (HR 2.66, 95% CI 2.19–3.24) having the worst (p < 0.001) (**Table 2**, **Figure 4A**). One-year graft survival in clusters 1, 2, 3 and 4 was 89.7%, 87.1%, 91.6%, 88.7% (**Table 2**, **Figure 4B**). Similar to patient survival, cluster 3 recipients had the best 1-year graft survival (ref) with cluster 2 (HR 1.54, 95% 1.35-1.71) recipients having the worst (p < 0.001). One-year death-censored graft survival in clusters 1, 2, 3 and 4 was 92.7%, 92.5%, 93.7%, and 92.9% (**Table 2**, **Figure 4C**) and there were no differences in death-censored graft survival when comparing clusters (p < 0.08).

One-year acute rejection in clusters 1, 2, 3 and 4 was 10.2%, 5.3%, 7.0%, 3.0% (**Table 2**). Cluster 4 had the lowest observed acute rejection within the first-year post-transplant (ref). Clusters 1 (HR 2.86, 2.24–3.64) and 3 (HR 1.90, 95% CI 1.52–2.36) had the highest number of reported acute rejection events.

DISCUSSION

The clinical significance of DGF and its impact on KT outcomes continues to be debated and some of the reported variation in outcomes is likely a reflection of how DGF data is analyzed (1–9). The interpretation of DGF data remains heavily influenced as a result of predefined study constructs based on fixed and isolated donor-, recipient-, and transplant characteristics, such as donor DCD status, CIT, or rejection (1–9). To better understand

differing DGF outcomes and viewpoints, we used an unsupervised ML consensus clustering approach to categorize the clinical phenotypes of KT recipients with DGF and their paired donors.

During this recent study period, the overall incidence of DGF in the US was 20.9%. The majority of recipients with DGF were males who were on dialysis ≥3 years and who received non-ECD, standard KDPI kidneys. Within this group of 17,073 recipients with DGF, consensus clustering analysis identified four distinct clinical clusters. Cluster 1 was characterized by younger, low BMI, non-diabetic, kidney re-transplant recipients who had a high PRA. Cluster 2 recipients were the oldest of the four clusters, had a higher BMI, were likely to have lower functional status, and be diabetic with 3+ years of dialysis vintage. They were also the most likely to receive ECD high KDPI kidneys. Cluster 3 recipients were young and non-diabetic. They were more likely to be black, have hypertension and receive higher HLA mismatched, lower KDPI kidneys. Lastly, cluster 4 recipients were middle-aged, first-time KT recipients with either diabetes or hypertension, lower functional status, dialysis duration ≥3 years, and a low PRA. Patient and graft survival varied by cluster, however, after accounting for death with a functioning graft, there were nosurvival differences between the four clusters suggesting that recipient comorbidities played an important role in graft outcomes (**Figure 4C**).

Although DGF is often attributed to donor quality and CIT, the majority of kidney allografts used during this study period came from non-ECD, standard KDPI, younger donors with a median CIT of 18.4 h (1–3, 5, 6). Only a small percentage of donors had hypertension, and the majority of kidneys were transplanted locally. Clinically significant differences in recipient comorbidities were notable between the clusters. Cluster 1 recipients were highly sensitized re-transplants, cluster 2 recipients were older diabetics, cluster 3 recipients were young non-diabetic black first-time transplants with hypertension, and cluster 4 recipients were predominantly middle-aged, recipients with diabetes or hypertension and lower functional status. As might be predicted, patient survival was best in 3 and lower in clusters 2 and 4. Despite varying cluster-specific recipient comorbidities, there were however no difference in death-censored graft survival between the four clusters.

The lack of difference in death-censored graft loss suggests that different factors contributed to survival across the four clusters. Recipient comorbidities, such as diabetes, dialysis vintage, PVD and dialysis vintage, likely played a significant role for clusters 2 and 3. Lack of difference in death-censored graft loss between clusters 2 and 4 suggests that there is increased room to increase use of ECD and high KDPI allografts for patients with these demographics. High KDPI kidneys continue to be at significant risk of discard and recipients with demographics shown in cluster 2 and 4 are well suited for these allografts (24). Although recipients in cluster 4 received more standard KDPI low HLA mismatched allografts, ultimately there were no differences in death-censored graft survival. Although cluster 1 recipients were younger in age and had less comorbidities, they were sensitized re-transplants. They carried the highest risk for rejection and likely had decreased survival as a result of risk factors such as infection due to over-immunosuppression, rejection as a result of infection or reactivation of preexisting donor specific antibodies or recurrent disease. Outcomes related to cluster 3 recipients were possibly the most surprising. Based on comorbidities, these recipients would perhaps be predicted to have the best outcomes. This finding possibly underscores that racial disparities in transplant impact outcomes and that variables, such as risk for rejection, socioeconomic barriers and access to healthcare, disproportionately affect minorities (21, 25). Although graft quality, demonstrated though use of predominantly standard KDPI allografts was observed in cluster 4, better HLA matching, need for a more personalized approach to immunosuppression or better post-transplant support, might result in improved outcomes.

DGF is often felt to be a risk factor for early acute rejection (7, 26). The overall incidence of acute rejection post-transplant has been reported to range between 10% and 29% with the inclusion of subclinical rejection (27, 28). In this study, the reported incidence of acute rejection was low ranging from 3.8% to 10.2% with the majority of recipients, regardless of PRA or age, received depleting induction. Although historically rejection data as reported in UNOS has had limitations due to underreporting, the use of depleting induction remains a widespread practice preference in the United States and these lower rejection rates may be reflective of several factors (21, 30). Increasingly, many centers are moving towards earlier initiation of CNIs in combination with use of depleting therapy in the setting of DGF to minimize this early rejection occurrences (1–4). The highest incidence of acute rejection was observed in cluster 1 recipients who were highly sensitized re-transplants. Despite this being an *at-risk* group for rejection, the reported incidence was only 10.2%. Cluster 3 recipients had the second highest reported incidence of acute rejection at 7.0%. Although this group was not sensitized, risk factors such as young recipient age, black race, and high HLA mismatches may have played a role in the higher number of rejection events (3, 30–33). Cluster 2 recipients were the oldest and the most likely to receive ECD high KDPI kidneys and also receive non-depleting induction. While cluster 2 was possibly at higher risk for a longer duration of DGF due to recipient and donor characteristics, there was not an increase in acute rejection episodes noted. The results from this analysis suggest that the overall incidence of acute rejection for kidneys with DGF is low (25–29).

In using the OPTN/UNOS national registry data, there are several limitations. This clustering analysis included only recipients with DGF. As such, there is not a comparison group for similarly matched recipients and donors without DGF. Because of the registry nature of this study, there is lack of detail regarding exact causes for DGF, mortality and graft loss. We also do not know the outcomes for mate kidneys from the same donor. Missing data remains an inherent limitation of the UNOS dataset. Although we acknowledge this as a limitation, all variables in our study had missing data <5%, and it is unlikely that missing data imputation substantially altered the results of our analysis. Additionally, we acknowledge that the current

working definition of DGF has inherent limitations such that it is simplistic and does not account for additional complexities, such as DGF duration and oliguria. Forthcoming updated guidelines in terminology specific to DGF will be helpful in addressing these current limitations. These limitations highlight the need for better reporting practices specific to DGF. Lastly, although unsupervised ML clustering applied in this study provided detailed information on distinct phenotypes and outcomes pertaining to kidney transplant recipients with DGF, the clinical characteristics attributed to the clusters were not necessarily novel and unsupervised ML clustering approaches have limitations in that they do not directly generate risk prediction for each individual. Future studies using supervised ML prediction models to predict outcomes of kidney transplant recipients with DGF are needed for validation.

Despite these limitations, the interpretation of DGF data to date remains heavily influenced as a result of predefined study constructs. Unsupervised clustering machine learning algorithms help us understand the characteristics of different clusters of kidney transplant patients with DGF within the current transplant practice in the U.S., and the algorithms do not use labeled outcomes. Unlike supervised machine learning models, unsupervised machine learning models do not have issues with overfitting and do not have limitations of variables in the clustering algorithms. To our knowledge however, this is the first ML clustering approach to look at the impact of DGF on KT outcomes. Outcomes specific to DGF have been varied and have often been reported as isolated analyses focusing on individual donor-, recipient-, or transplant characteristics rather than isolated interpretation of competing variables. By applying a ML clustering approach, this study has allowed for an unbiased assessment of KT outcomes for those with DGF.

Clinical outcomes specific to DGF are currently described in a binary fashion, however factors contributing to DGF are complex, nonbinary and varied. Significant variation exists between different studies reporting on DGF and much of this variation can be accounted for by differences in analyses. In this study, unsupervised ML was applied to KT recipients with DGF and their paired donors and this resulted in the identification of four clinically distinct clusters with differing post-transplant outcomes. The majority of kidneys utilized in the United States continue to come from standard KDPI non-ECD donors and more obvious clinical heterogeneity is notable in cluster-specific recipient comorbidities. The majority of kidneys with DGF in the United States come from standard KDPI donors. Clinically meaningful differences in recipient characteristics were noted between clusters, and, after accounting for death and return to dialysis, there were no differences in death-censored graft loss. Immunologic, cardiac, metabolic, and socioeconomic contributors likely play significant roles in varying outcomes and, although DGF is a predefined clinical endpoint, recipient comorbidities assume an important role in survival outcomes.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: The Organ Procurement and Transplantation Network (OPTN)/United Network for Organ Sharing (UNOS) database (accession number: DATA0006605).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Mayo Clinic Institutional Review Board (IRB 21-007698). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

CJ, NL, WK, PP, and MC: participated in research design, writing of manuscript, data analysis, and performance of the research. CT and WC: participated in research design, writing of manuscript, data analysis, performance of the research, and analytic tools.

AUTHOR DISCLAIMER

The interpretation and reporting of this data are the responsibility of the authors and in no way should be seen as an official policy of or interpretation by the OPTN or the United States government.

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.2022.10810/full#supplementary-material

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ORIGINAL RESEARCH
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Poor Outcomes of Patients With NAFLD and Moderate Renal Dysfunction or Short-Term Dialysis Receiving a Liver Transplant Alone

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The outcomes of patients with moderate renal impairment and the impact of liver disease etiology on renal function recovery after liver transplant alone (LTA) are largely unknown. We explored whether NAFLD patients with pre-LTA moderate renal dysfunction (GFR 25–45 ml/min/1.73 m^2) may be more susceptible to develop post-LTA severe renal dysfunction (GFR<15 ml/min/1.73 m^2) than ALD patients, as well as other overall outcomes. Using the UNOS/OPTN database, we selected patients undergoing liver transplant for NAFLD or ALD (2006–2016), 15,103 of whom received LTA. NAFLD patients with moderate renal dysfunction were more likely to develop subsequent GFR<15 ml/min/1.73 m^2 than ALD patients (11.1% vs. 7.38%, $p < 0.001$). Patients on short-term dialysis pre-LTA (≤12 weeks) were more likely to develop severe renal dysfunction (31.7% vs. 18.1%), especially in NAFLD patients, and were more likely to receive a further kidney transplant (15.3% vs. 3.7%) and had lower survival (48.6% vs. 50.4%) after LTA ($p < 0.001$ for all). NAFLD was an independent risk factor for post-LTA severe renal dysfunction (HR = 1.2, $p = 0.02$). NAFLD patients with moderate renal dysfunction and those receiving short-term dialysis prior to LTA are at a higher risk of developing subsequent severe renal dysfunction. Underlying etiology of liver disease may play a role in predicting development and progression of renal failure in patients receiving LTA.

Keywords: liver transplantation, non-alcoholic steatohepatitis, acute kidney injury, alcohol-related liver disease, chronic kidney disease

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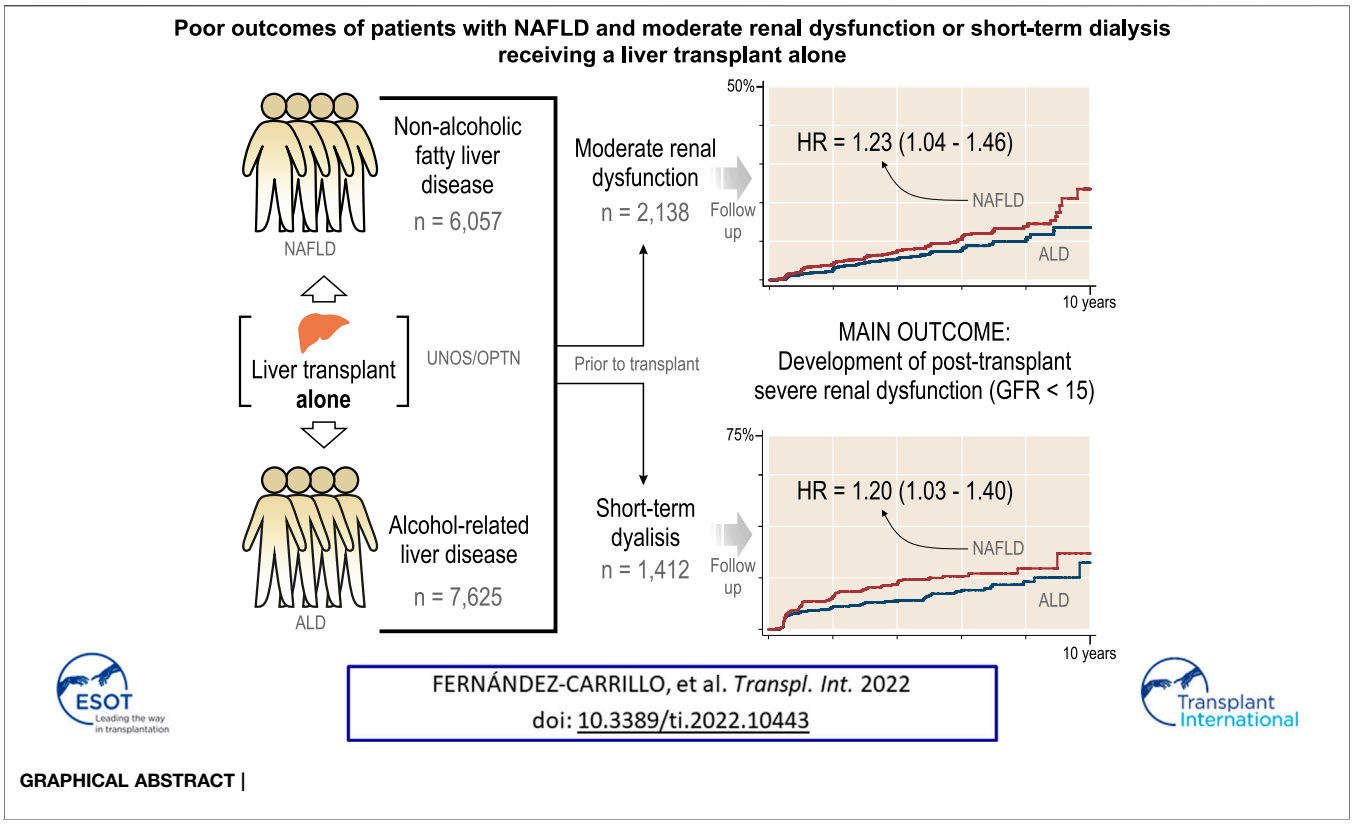
Abbreviations: AKI, acute kidney injury; ALD, alcohol-related liver disease; BMI, body mass index; CKD, chronic kidney disease; CI, confidence interval; Cr, serum creatinine; GFR, glomerular filtration rate; HR, hazard ratio; INR, international normalized ratio; KDIGO, Kidney Disease Improving Global Outcomes; KT, kidney transplant; MELD, Model for End-Stage Liver Disease; NAFLD, non-alcoholic fatty liver disease; LT, liver transplantation; LTA, liver transplant alone; OPTN, Organ Procurement and Transplantation Network; SLKT, simultaneous liver-kidney transplantation; T2DM, type 2 diabetes mellitus; UNOS, United Network for Organ Sharing.



GRAPHICAL ABSTRACT |

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a major health problem which has recently become the second leading indication for liver transplantation (LT) in the United States (1-4). NAFLD is also the most rapidly increasing indication for simultaneous liver-kidney transplant (SLKT) (5). In addition to a high prevalence of cardiovascular risk factors in NAFLD patients, there is an association between NAFLD and chronic kidney disease (CKD), which is independent of metabolic syndrome or cirrhosis (6-8). Moreover, a recent study has shown an independent association between pre-LT renal dysfunction and a worse graft and overall survival after transplant in NAFLD patients (9). In previous research we found that, compared with those with NAFLD, patients with alcohol-related liver disease (ALD) and renal dysfunction prior to LT have better outcomes after LT (10). This suggests that NAFLD may be more frequently associated with causes of renal dysfunction that have less reversion potential and that the etiology of liver disease may impact the recovery of renal function after LT. Previous studies are focused on patients with the most impaired renal function, such as those with creatinine (Cr) $\geq$ 2.5 mg/dl or with a need for dialysis (10,11). There is scarce information regarding outcomes of patients with moderate renal impairment after LT, and the impact of liver disease etiology on renal function recovery has not been fully addressed (12). Presumably, a higher incidence of structural kidney injury in the NAFLD population and overestimation of renal function when using serum Cr, may lead to overlook a significant and irreversible renal impairment in this vulnerable group of patients (13).

Beyond NAFLD-related indication, overall SLKT has been growing since 2002, when the Model for End-stage Liver Disease (MELD) score was adopted to guide graft allocation (14). The MELD score includes Cr. Renal dysfunction, which occurs in up to 30% of listed patients for LT, strongly influences the outcomes of patients with end-stage liver disease (15-21). The increase in SLKT has potentially resulted in important inequalities since kidney grafts may have been diverted from highly-prioritized kidney transplant (KT) candidates toward certain subsets of cirrhotic patients whose native kidneys might have recovered after liver transplant alone (LTA) (22-24). In view of this, certain proposals have been made by the Organ Procurement and Transplantation Network (OPTN) to offer some guidance on SLKT allocation, resulting in the inclusion of the latest consensus in OPTN official policies of 2017 (24-30). These are valuable criteria, but they still lack solid demonstration of a benefit in survival, and other studies show that glomerular filtration rate (GFR) alone may not guide SLKT indication (12,31,32). New predictive factors are needed in order to better support the decision making process. In this regard, it is remarkable that, with some exceptions, published studies overlooked a potential role of the etiology of liver disease for the indication of SLKT (5,9,10,12).

Based on these considerations, we hypothesize that NAFLD patients with renal dysfunction who receive LTA have worse kidney-related outcomes and reduced survival. Therefore, we aimed at exploring these variables in NAFLD patients with pre-LTA moderate renal dysfunction, compared to ALD patients with similar renal function impairment, who represent the other leading indication for LT. To better address the issue of SLKT indication, we assessed the same outcomes for those NAFLD patients on short-term dialysis vs. ALD patients.

PATIENTS AND METHODS

Study Population

Using the United Network for Organ Sharing/Organ Procurement and Transplantation Network (UNOS/OPTN) database, we selected adult patients undergoing LT between January 1st, 2006 and January 1st, 2016 and with at least 1 year of available follow up data. This timeframe predates the UNOS SLKT policy (implemented in 2017) aimed at standardizing kidney allocation criteria in transplant candidates with acute or chronic kidney injury. Patients with only NAFLD or ALD as a single diagnosis were selected using codes 4214 and 4215 respectively, excluding any concomitant diagnoses. As previously described, we also considered NAFLD as the most likely underlying etiology of liver disease in those patients classified as cryptogenic or idiopathic cirrhosis (codes 4208 and 4213) and a body mass index (BMI) > 30 (3,5) In addition, diagnoses were manually reviewed where the code was 999 ("Other specify"), and patients matching the above criteria were included in the analysis. Patients with hepatocellular carcinoma or any other malignancy were excluded. Patients receiving both kidney and liver grafts on the same day or with a date mismatch of up to 24 h were classified as SLKT, whereas the rest of the patients were classified as recipients of LTA. Other multi-organ transplants were excluded. This study was approved by the University of Pittsburgh Institutional Revision Board as a consent-waived study with the number PRO18020615, and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the 2000 Declaration of Helsinki as well as the Declaration of Istanbul 2008.

Variable and Outcome Definitions

Glomerular filtration rate (GFR) at the time of transplant is the standard parameter to assess kidney function endorsed by UNOS guidelines. GFR was estimated at that single time point by the formula $141 \times \min(Cr/\kappa, 1)^{\alpha} \times \max(Cr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] $\times 1.159$ [if black] (28,33). Clinically meaningful cutoffs for pre-LTA GFR were used to define three categories (>45, 45–25 and <25 ml/min/1.73 m^2), of which the intermediate category (45–25 ml/min/1.73 m^2) was defined as moderate renal dysfunction. An upper threshold of 45 ml/min/1.73 m^2 is widely accepted as mildly to moderately decreased renal function (34). Although a lower cut-off of 30 ml/min/1.73 m^2 is used in many studies for this category, 25 ml/min/1.73 m^2 was used to cover a wider scope of clinical situations and follows UNOS/OPTN's recommendations to define sustained acute kidney injury (AKI). The UNOS/OPTN database does not allow accurate distinction between acute or chronic kidney disease, while the OPTN policy recommends 25 instead of 30 for sustained acute kidney injury (AKI) (28,34). Given that Cr levels alone are commonly used in clinical practice, Cr at the time of transplant was also included in the analysis. Clinically meaningful cutoffs for pre-LTA Cr were used to define three categories of Cr elevation (<1.5 mg/dl, low; 1.5–2.5 mg/dl, moderate; > 2.5 mg/dl, high). Dialysis during the last week prior to LT is recorded in the UNOS/OPTN database and was used to define the group of patients on dialysis prior to LTA. Such patients were not included in the groups with pre-LTA GFR <25 or Cr > 2.5 mg/dl. Dialysis length was unavailable for LTA patients, for whom short-term dialysis (≤12 weeks) was assumed, since they did not receive a KT. Post-LTA severe renal dysfunction was an outcome defined as GFR <15 ml/min/1.73 m^2 that persisted at least 6 months after LTA. This cut-off corresponds with the KDIGO G5 category and a Cr ≥ 4 mg/dl in patients within the age range of the study population. KT after LTA was matched with the LTA patients using patient code.

Statistical Analysis

Summary statistics are reported as means (standard deviation) or n (%) for continuous or categorical variables, respectively. Wherever dispersion is high, median (interquartile range) is shown. The Chi-square test was used to analyze differences between categorical variables. A comparison of continuous variables between groups was performed using the Student t test. Survival rates were estimated using Kaplan-Meier curves of death-free, kidney transplant-free, and kidney failure-free survival and compared with the log-rank test. Cox proportional hazards and competing risk logistic models adjusted for age, gender, race, diabetes, and BMI (>40 vs. < 40) were developed to investigate which variables were independently associated with severe renal dysfunction and further kidney transplant after liver transplant alone. All reported p-values were two-tailed. The level of statistical significance was set at $p < 0.05$. Statistical analyses were performed with STATA software version 15.1.

RESULTS

Between January 1st' 2006 and January 1st' 2016, we identified 59,363 patients that had received a LT across the United States. A total of 15,103 fulfilled the inclusion and exclusion criteria of the study and underwent LT because of NAFLD or ALD as the only indication (**Figure 1**). Of them, 13,682 (90.6%) underwent LTA and 1,421 (9.4%) underwent SLKT.

Characteristics of Patients with NAFLD or ALD Without Pre-LTA Dialysis

A total of 12,088 patients out of 13,682 who underwent LTA (88.3%), did not receive dialysis treatment and had computable GFR. NAFLD was the indication for LTA in 5,427 (44.9%) of

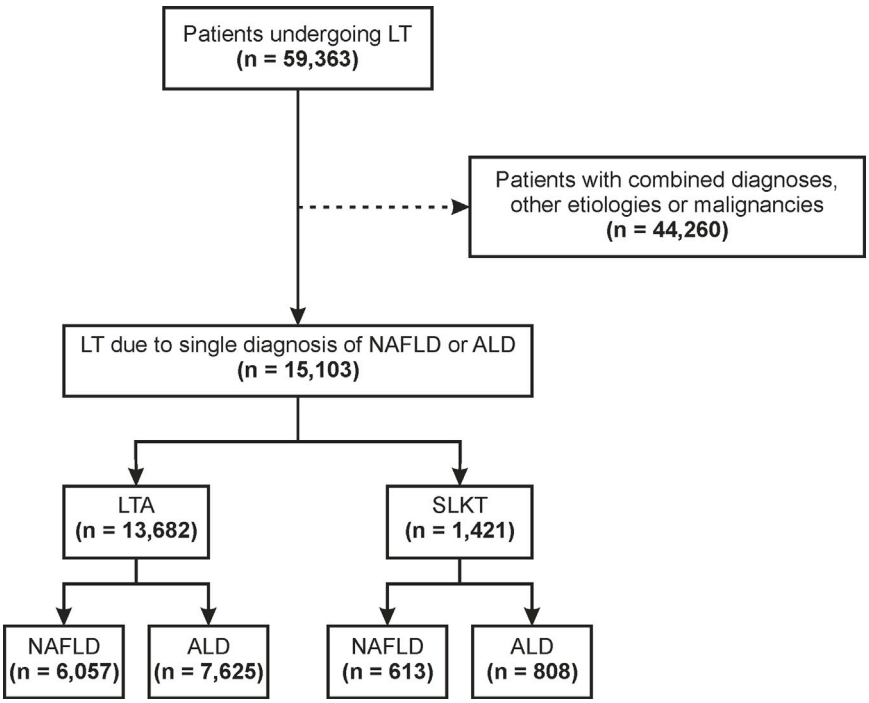


FIGURE 1 | Flowchart for patient selection ALD, alcohol-related liver disease; LT, liver transplant; LTA, liver transplant alone; NAFLD, non-alcoholic fatty liver disease; SLKT, simultaneous liver-kidney transplant.

them while 6,661 (55.1%) underwent LTA for ALD. Within the group of NAFLD patients, there was a smaller predominance of male gender and a lower proportion of Hispanic and Black ethnicities as compared with ALD ones (male gender, 56.8% vs. 78.6%; Hispanic, 11.4% vs. 13.9%; Black, 1.9% vs. 3.7%; $p < 0.001$ for all) (**Table 1**). Additionally, NAFLD patients were older and had a higher BMI, as well as a higher proportion of type 2 diabetes mellitus (T2DM) (mean age, 59 vs. 55 years; mean BMI, 33 vs. 29; T2DM, 45.7% vs. 17.3%; $p < 0.001$ for all). Mean GFR was lower in NAFLD patients than in ALD patients (62.87 vs. 70.54 ml/min/1.73 m^2, $p < 0.001$). ALD patients showed a slightly more impaired liver function with higher MELD scores (21 vs. 22; $p < 0.001$), due to higher bilirubin levels and INR.

Impact of Moderate Renal Dysfunction Before LTA

First, we assessed the three pre-transplant GFR categories (>45, 45–25, and <25 ml/min1.73 m^2) and their impact after LTA. Stratification of NAFLD or ALD patients by these categories showed three clearly differentiated curves for survival, development of post-LTA severe renal dysfunction (GFR <15 ml/min/1.73 m^2) and further KT indication ($p \leq 0.01$ for all) (**Figure 2**). Second, we focused on those patients with pre-transplant moderate renal impairment and compared them by liver-disease etiology, over a median time of 4.92 years (95% CI 4.80–4.99). Either according to predefined categories of GFR (45–25 ml/min1.73 m^2) or Cr levels (1.5–2.5 mg/dl), NAFLD patients developed post-LTA severe renal dysfunction more frequently than ALD patients (GFR: 11.1% vs. 7.38%, $p < 0.001$; Cr: 10.5% vs. 7.1%, $p = 0.045$) (**Figure 3**; **Supplementary Figure S1**, respectively). In addition, NAFLD patients developed post-LTA severe renal dysfunction earlier than ALD patients, for whom this mainly happened after 2 years (**Figure 2**). There was no difference in overall post-transplant survival or need for KT between both etiologies in patients with moderate renal dysfunction, either using GFR or Cr levels (**Figure 3**; **Supplementary Figure S1**, respectively). However, of the patients with best renal function prior to LT (GFR >45 ml/min1.73 m^2 or Cr levels <1.5 mg/dl), those with NAFLD still showed a higher cumulative incidence of post-LTA severe renal dysfunction vs. those with ALD (GFR: 5.22% vs. 3.23%, $p = 0.006$; Cr: 17.3% vs. 9.5%, $p < 0.001$) (**Supplementary Figure S2**).

Guided by the above unadjusted analysis, we built Cox proportional hazard models for incidence of severe renal dysfunction and for KT indication after LTA, in which the etiology of liver disease was included as an explanatory variable (**Tables 2A**,**B**). Both moderate or more severely impaired GFR prior to transplant were independent predictors of post-LTA severe renal dysfunction (GFR 45–25: HR 2.18, 95% CI 1.83–2.61; GFR <25: HR 3.61, 95% CI 2.99–4.36; $p < 0.001$ for both). These two categories were found to be as well the strongest risk factors impacting on further need of KT (GFR 45–25: HR

TABLE 1 | Baseline characteristics of LTA recipients, not receiving pre-transplant dialysis, according to the etiology of liver disease.

Characteristics	NAFLD	ALD	*p* value
	n = 5,427	*n* = 6,661	
Age (years)	59 ± 8	55 ± 9	<**0.001**
Gender (n, %)			<**0.001**
Male	3,080 (56.8)	5,237 (78.6)	
Female	2,347 (43.2)	1,424 (21.4)	
Race (n, %)			<**0.001**
White	4,560 (84)	5,319 (79.9)	
Hispanic	620 (11.4)	928 (13.9)	
Black	103 (1.9)	244 (3.7)	
Others	144 (2.7)	170 (2.5)	
BMI	33 ± 6	29 ± 5	<**0.001**
BMI > 40 (n, %)	549 (10.1)	191 (2.9)	<**0.001**
T2DM (n, %)	2,458 (45.7)	1,141 (17.3)	<**0.001**
GFR levels (ml/min/1.73 m^2)	62.87 ± 29.5	70.54 ± 31.5	<**0.001**
GFR (n, %)			<**0.001**
GFR > 45 (n, %)	3,666 (68)	5,028 (76)	
GFR (25 – 45) (n, %)	1,216 (22)	1,064 (16)	
GFR < 25 (n, %)	545 (10)	569 (8.5)	
Creatinine levels (mg/dl)	1.41 ± 0.90	1.38 ± 0.92	0.085
Cr < 1.5 (n, %)	3,628 (66.9)	4,714 (70.7)	<**0.001**
Cr (1.5–2.5) (n, %)	1,330 (24.5)	1,337 (20.1)	<**0.001**
Cr > 2.5 (n, %)	469 (8.6)	610 (9.2)	<**0.001**
Albumin levels (g/dl)	3.01 ± 0.67	3.03 ± 0.68	0.13
Total Bilirubin levels (mg/dl)	3.6 (2–7.2)	4.8 (2.4–10.8)	<**0.001**
INR	1.87 ± 0.80	2.04 ± 1.59	<**0.001**
MELD score	21 ± 8	22 ± 9	<**0.001**
Ascites (n, %)	4,434 (82.2)	5,645 (85.3)	<**0.001**
SBP (n, %)	302 (5.7)	656 (10.0)	<**0.001**
On ventilator (n, %)	107 (2.0)	158 (2.4)	0.15
Portal vein thrombosis (n, %)	791 (14.7)	656 (9.9)	<**0.001**

**Others includes Asian, American Indian/Alaska Native, Hawaiian/other Pacific Islander, and Multiracial.*

Values are shown as mean ± standard deviation, excepting bilirubin levels, which are shown as median (interquartile range) due to non-normal distribution.

ALD, alcohol-related liver disease; BMI, body mass index; Cr, serum creatinine; GFR, glomerular filtration rate; INR, international normalized ratio; LTA, liver transplant alone; NAFLD, non-alcoholic fatty liver disease; MELD, model for end-stage liver disease; SBP, spontaneous bacterial peritonitis; T2DM, type 2 diabetes mellitus.

2.72, 95% CI 1.88–3.94; GFR <25: HR 4.77, 95% CI 3.26–7.00; $p < 0.001$ for both). Interestingly, NAFLD was an independent risk factor for development of post-LTA severe renal dysfunction (HR 1.23, 95% CI 1.04–1.46; $p = 0.017$), although it did not predict KT indication. In addition, Black race and T2DM, two well-known risk factors of CKD were also associated with severe renal dysfunction after LTA (Black race: HR 1.89, 95% CI 1.31–2.72, $p = 0.001$; T2DM: HR 1.74, 95% CI 1.47–2.07, $p < 0.001$). Likewise, T2DM was associated with KT indication after LTA (HR 1.71, 95% CI 1.20–2.44; $p = 0.003$). Given the high prevalence of T2DM within NAFLD patients, we assessed a potential interaction between etiology and T2DM, which was found not significant, suggesting that their impact may be independent (HR: 1.15, 95% CI 0.81–1.63). Age was independently associated with the need for KT only (HR 0.98, 95% CI 0.96–0.99), while gender or BMI >40 were not. Similar results were obtained using Cr levels categories instead of GFR (**Supplementary Tables S1A, B**). Finally, we performed a competing risk analysis for severe renal dysfunction, considering KT as the competing factor, which strongly supported Cox regression results (**Supplementary Table S3**).

Analysis of Patients With Re-Transplantation After LTA

One hundred and sixty three patients out of 13,682 that underwent LTA (1.2%), had already received a previous liver transplant. Serum creatinine at the time of the second transplant was available in 130 patients. We performed a dedicated analysis to assess if this especial population showed similar outcomes to the ones of the overall LTA population. NAFLD was the indication in 59 (45.4%) of them while 71 (54.6%) underwent re-LTA for ALD (**Supplementary Table S2**). NAFLD patients were older, had a higher BMI and were more frequently affected by T2DM than ALD patients (mean age, 56 vs. 53, $p = 0.024$; mean BMI 31 vs. 27, $p < 0.001$; T2DM, 43% vs. 22%, $p = 0.043$). Baseline GFR and Cr levels did not differ between the two groups. However, among those with baseline moderate renal dysfunction, a total of 26.7% patients with NAFLD developed post-LTA severe renal dysfunction while such event was not observed in the ALD group (26.7% vs. 0%, $p = 0.053$) (**Supplementary Figure S3A**). Similar results were obtained when using the predefined moderate cutoff for Cr (moderate, 33.3% vs. 0%, $p = 0.045$) (**Supplementary Figure S3B**). Survival did not differ between both groups according to the etiology and no further KT indication did occur in this subgroup of patients.

Impact of Pre-LTA Short-Term Dialysis According to the Etiology of Liver Disease

Short-term dialysis was performed in 1,576 patients (11.5%) out of 13,682 undergoing LTA prior to surgery. Within this population, 622 patients (39.5%) had NAFLD and 954 patients (60.5%) had ALD. MELD scores were significantly higher for ALD patients than for NAFLD patients (39 vs. 38, $p < 0.001$) (**Table 3**). Compared to LTA recipients that did not receive dialysis, these patients were younger and had a higher MELD score, mainly accounting for bilirubin levels (age, 54 vs. 57 years; MELD score, 38 vs. 22; bilirubin, 14.4 vs. 4.2 mg/dl; $p < 0.001$ for all), and exhibited ascites more frequently (93.9% vs. 83.9%, $p < 0.001$). Thus, the short-term dialysis group appeared to have a more severe clinical condition overall, related to either acute-on-chronic liver failure or advanced chronic liver disease.

After LTA, patients on prior short-term dialysis had a lower survival, developed severe renal dysfunction more frequently and were more likely to receive a further KT during a median follow-up of 3.98 years (95% CI 3.89–4.02) (survival, 48.64% vs. 50.4%; GFR <15 ml/min1.73 m^2, 31.7% vs. 18.1%; KT, 15.3% vs. 3.7%; $p < 0.001$ for the three outcomes) (**Figures 4A–C**). When stratifying by etiology, patients with NAFLD on prior short-term dialysis showed a trend towards a greater frequency of post-LTA severe renal dysfunction (27.85% vs. 21.42%, $p = 0.055$) (**Figure 4D**). Cox proportional hazards models were constructed to explore the risk factors for severe renal dysfunction

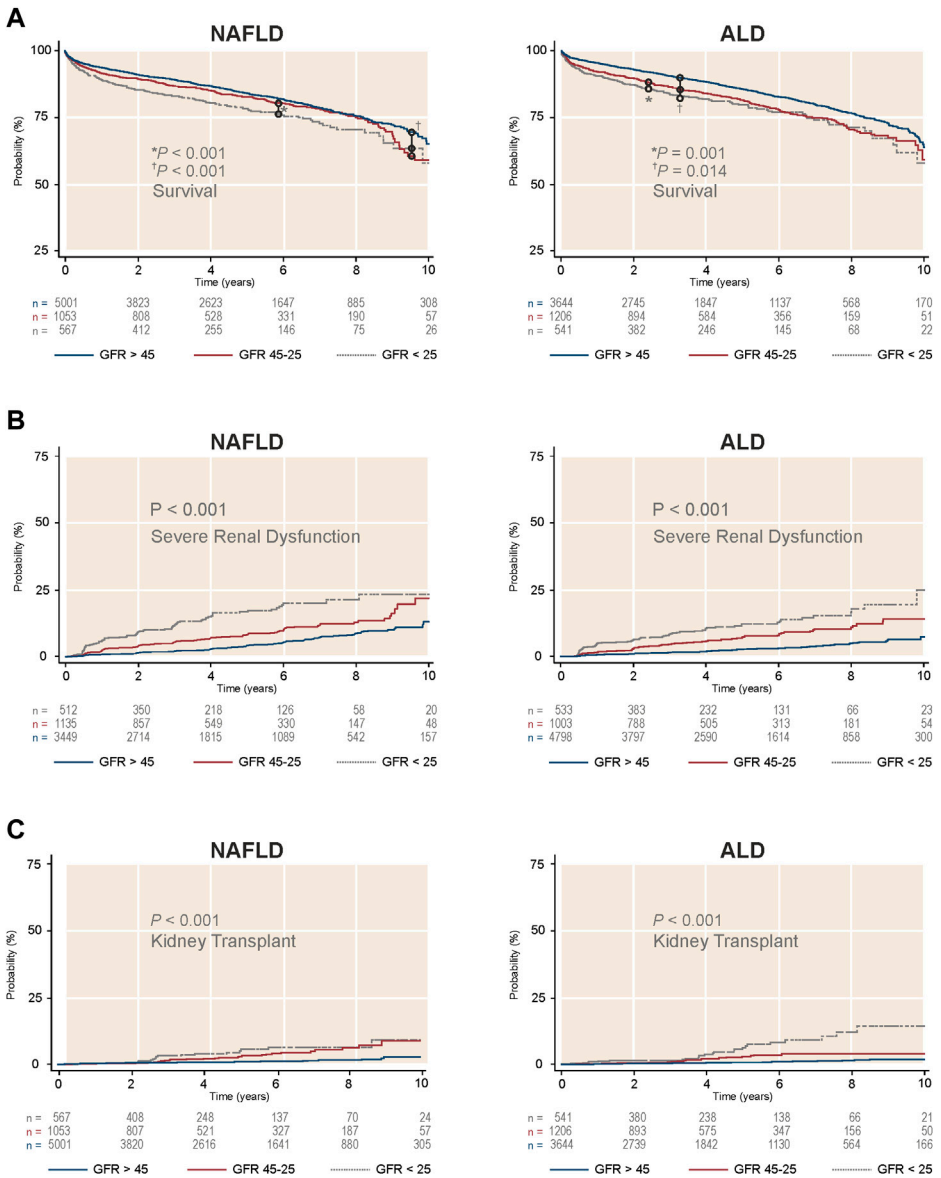


FIGURE 2 | Survival and cumulative incidence of severe renal dysfunction and further kidney transplant in LTA recipients without prior dialysis according to GFR categories and stratified by etiology of liver disease. **(A)** Survival by liver disease etiology. **(B)** Cumulative incidence of severe renal dysfunction by liver disease etiology. **(C)** Cumulative incidence of kidney transplant indication by liver disease etiology. ALD, alcohol-related liver disease; GFR, glomerular, filtration rate; NAFLD, non-alcoholic liver disease.

development after LTA in this population. Therefore, pre-LTA GFR was substituted by the binary covariate prior short-term dialysis. Interestingly, NAFLD etiology was an independent risk factor for post-LTA severe renal dysfunction (HR 1.20, 95% CI 1.03–1.40; $p = 0.020$), yet prior dialysis was the risk factor that showed the strongest impact (HR 3.29, 95% CI 2.79–3.89; $p < 0.001$) (**Table 4**). Age, male gender, Black race, and T2DM were other factors independently associated with this outcome (Age: HR 1.01, 95% CI 1.001–1.02; male gender: HR 1.33, 95% CI 1.14–1.56; T2DM: HR 1.71, 95% CI 1.46–2.00; $p < 0.05$ for all). Again, we did not find significant interaction between etiology and T2DM (HR: 1.08, 95% CI 0.79–1.47).

DISCUSSION

NAFLD is a major cause of advanced liver disease in the United States and worldwide, and is an increasing indication for LT and SLKT (3,4,35). The number of SLKT has been rising during the MELD era due to frequent kidney

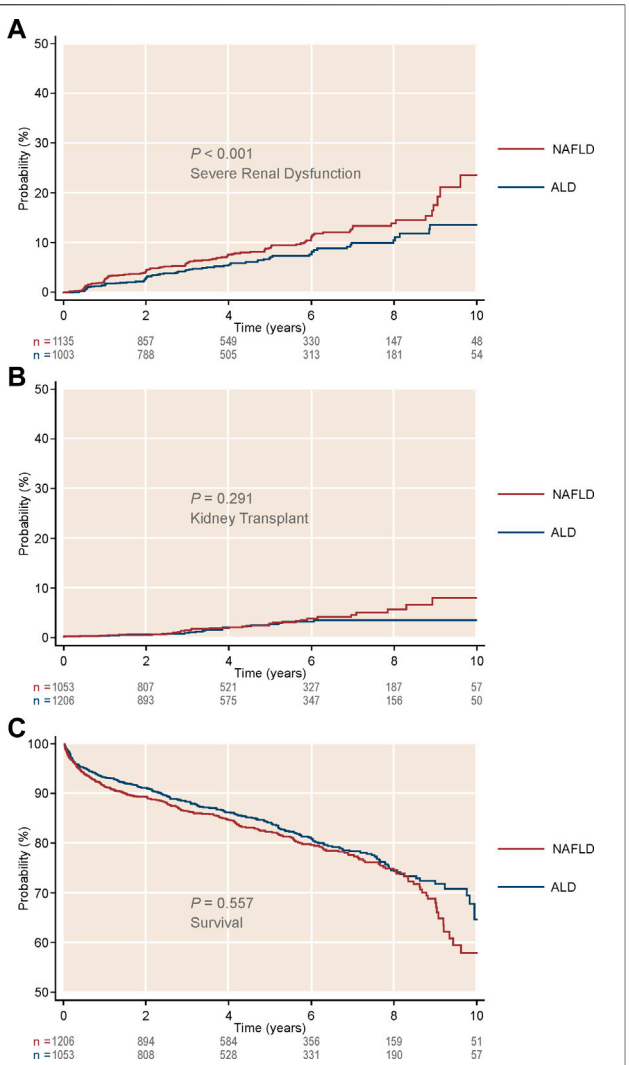


FIGURE 3 | Cumulative incidence of kidney-related outcomes, as well as survival, in patients with intermediate glomerular filtration rate (45–25 ml/min/1.73 m^2) after receiving a liver transplant alone. Stratification was done by liver disease etiology. **(A)** Cumulative incidence of severe renal dysfunction by etiology. **(B)** Cumulative incidence of kidney transplant by etiology. **(C)** Survival by etiology. ALD, alcohol-related liver disease; NAFLD, non-alcoholic liver disease.

dysfunction related to chronic liver disease. On the other hand, NAFLD has been independently associated with CKD (6-8). However, the impact of the underlying etiology of the liver disease has been largely disregarded in previous studies on LTA and SLKT. A recent study showed suboptimal post-LT outcomes in patients with NAFLD and renal dysfunction (GFR <30 ml/min1.73 m^2) including LTA and SLKT (9). Whether the underlying etiology influences the outcome of renal dysfunction after LT remains elusive. Therefore, we aimed at addressing this knowledge gap. In the current study, we show that the impact of mild or moderate renal dysfunction was more pronounced in patients with NAFLD than in ALD patients.

TABLE 2A | Cox proportional hazards model for severe renal dysfunction development.

	HR	95% confidence interval	*p value*
NAFLD	1.231	1.037–1.462	**0.017**
Age	1.008	0.998–1.017	0.118
Gender (male)	0.975	0.830–1.46	0.763
Hispanic	1.075	0.863–1.340	0.517
Black	1.888	1.311–2.719	**0.001**
T2DM	1.744	1.471–2.067	< **0.001**
BMI >40	0.968	0.718–1.304	0.829
GFR 45–25	2.184	1.829–2.609	< **0.001**
GFR <25	3.608	2.989–4.356	< **0.001**

BMI, body mass index; GFR, glomerular filtration rate; HR, hazard ratio; NAFLD, non-alcoholic fatty liver disease; T2DM, type 2 diabetes mellitus.

TABLE 2B | Cox proportional hazards model for kidney transplant after liver transplant alone in patients without pre-transplant dialysis.

	HR	95% confidence interval	*p* value
NAFLD	1.076	0.756–1.531	0.684
Age	0.980	0.962–0.998	**0.032**
Gender (male)	1.260	0.890–1.783	0.193
Hispanic	1.045	0.669–1.634	0.846
Black	1.123	0.458–2.756	0.800
T2DM	1.711	1.202–2.436	**0.003**
BMI >40	1.186	0.677–2.078	0.551
GFR 45–25	2.719	1.879–3.937	< **0.001**
GFR <25	4.774	3.258–6.997	< **0.001**

BMI, body mass index; GFR, glomerular filtration rate; HR, hazard ratio; NAFLD, non-alcoholic fatty liver disease; T2DM, type 2 diabetes mellitus.

After stratification of patients receiving LTA into three clinically relevant categories based on GFR or Cr, we identified three respective groups who had different rates of survival, development of severe renal dysfunction (GFR <15 ml/min1.73 m^2), and need for KT. When focusing on moderate renal dysfunction before transplantation (45–25 ml/min/1.73 m^2), NAFLD patients showed increased incidence of post-LTA severe renal dysfunction compared to patients with ALD. This is clinically relevant since mild to moderate Cr elevation is commonly found in NAFLD patients listed for liver transplantation. The ability to predict renal function recovery after LT in patients with chronic liver disease is quite limited, and may potentially be more difficult in patients with some degree of structural kidney injury, which is common in NAFLD (6-8,36,37). Even among those patients with good pre-LTA renal function, NAFLD patients developed post-LTA severe renal dysfunction more frequently, which strongly suggests the existence of underlying structural kidney disease with poor functional recovery potential. The lack of kidney function recovery was also observed in NAFLD patients undergoing liver re-transplantation, which reinforces this notion. Prospective studies looking for serum biomarkers predictive of renal function recovery in patients with moderate renal dysfunction listed for LTA are warranted.

TABLE 3 | Baseline characteristics of the patients on short-term dialysis receiving a liver transplant alone, according to the etiology of liver disease.

Characteristic	NAFLD	ALD	*p* value
	n = 622	*n* = 954	
Age (years)	57 ± 9	52 ± 10	<**0.001**
Gender (n, %)			<**0.001**
Female	316 (50.8)	269 (28.2)	
Male	306 (49.2)	685 (71.8)	
Race			0.64
White	449 (72.2)	676 (70.9)	
Hispanic	136 (21.9)	212 (22.2)	
Black	16 (2.6)	38 (4.0)	
Others	21 (3.3)	28 (2.9)	
BMI	34 ± 6	29 ± 6	<**0.001**
BMI >40	94 (15.1)	58 (6.1)	<**0.001**
T2DM (n, %)	258 (42.0)	154 (16.4)	<**0.001**
Albumin levels (g/dl)	3.28 ± 0.78	3.28 ± 0.82	0.94
Total Bilirubin levels (mg/dl)	13.7 (5.7–29.1)	14.9 (6.9–28.8)	0.37
INR	2.42 ± 1	2.27 ± 1	**0.001**
MELD score	38 ± 6	39 ± 6	<**0.001**
Ascites (n, %)	581 (93.7)	893 (94.1)	0.15
SBP (n, %)	70 (11.4)	121 (13.0)	0.39
On ventilator (n, %)	133 (21.4)	249 (26.1)	**0.035**
Portal vein thrombosis (n, %)	99 (16.1)	82 (8.7)	<**0.001**

**Others includes Asian, American Indian/Alaska Native and Multiracial.*
Values are shown as mean ± standard deviation, excepting bilirubin levels, which are shown as median (interquartile range) due to non-normal distribution.
ALD, alcohol-related liver disease; BMI, body mass index; INR, international normalized ratio; NAFLD, non-alcoholic fatty liver disease; MELD, model for end-stage liver disease; SBP, spontaneous bacterial peritonitis; T2DM, type 2 diabetes mellitus.

Our multivariable models confirmed that liver disease etiology is an independent risk factor for developing severe renal dysfunction after LTA, which was 23% more likely in patients with NAFLD. Renal function prior to LTA estimated by GFR or Cr levels was also found to be an independent risk factor in determining development of severe renal dysfunction and need for KT after LTA. Other independent risk factors for marked renal dysfunction after LTA were T2DM and Black race. T2DM, which was also a risk factor for receiving a kidney transplant during follow-up, is a well-known cardiovascular risk factor involved in metabolic syndrome and CKD. Particularly, NAFLD patients have a high incidence of T2DM (38), which in our cohort accounted for 45.7% compared to 17.3% in ALD patients. Black patients are particularly predisposed to developing CKD (39). Although this association may be mediated through a higher prevalence of arterial hypertension, we could not assess this factor in the UNOS/OPTN database. Disregarding race, arterial hypertension may be a potential confounder that could not be controlled. The fact that Black race was more frequent within ALD patients points at T2DM and potentially NAFLD itself, as main factors for the development of severe renal dysfunction. Even though metabolic syndrome is intrinsically associated with CKD, BMI >40 was not found to have an independent association in our models. All these findings suggest that there may be some subclinical underlying kidney damage in patients with NAFLD (6-8). A convoluted crosstalk among liver, visceral adipose tissue inflammation and kidneys, in addition to cardiovascular risk factors, may account for this structural renal injury (40,41).

Regarding patients who received dialysis before LT, it is important to conceptually differentiate CKD with long-term dialysis from short-term dialysis due to AKI mainly attributed to liver disease (e.g., hepatorenal syndrome or acute tubular necrosis). Concerning the latter, the required duration of dialysis to consider SLKT has been a matter of debate. The existing evidence is based on retrospective single-center experiences, spanning from 4 to 12 weeks, with significant variations among centers (22,25,26,42). Moreover, the precise indications and timing for dialysis in liver patients is not well defined, with significant heterogeneity in clinical practice (27). In our study, patients with NAFLD on short-term dialysis showed a clear trend to develop more frequently severe renal dysfunction after LTA. The multivariable analysis again showed NAFLD etiology as an independent risk factor for this outcome, along with other known risk factors such as age, male gender, Black race, and T2DM. The latest OPTN proposals and policies, issued after our study period, are fairly conservative and recommend 6 weeks of dialysis length in order to consider SLKT (24,27,28). Although this recommendation is expected to improve outcomes, new studies are needed to address whether the etiology of liver disease may be incorporated in the decision-making process.

The retrospective nature of our study limited our ability to adjust for confounding factors. While UNOS/OPTN database offers a large and representative sample over the US, some specific data were lacking, and the influence of potential changes in clinical practice over a ten-year span may not be properly reflected. Particularly, detailed history on calcineurin inhibitor use is lacking, which may influence kidney-related outcomes. Moreover, Cr level, which is known to be suboptimal for renal risk stratification in this setting, was the only marker available to estimate renal function. To mitigate this issue, we estimated GFR, which is the OPTN standard, by using the most accurate equation to date. Cr-based GFR may still be suboptimal since GFR equations were developed in non-cirrhotic patients and overestimate renal function in this population, yet this is an issue in real clinical practice rather than a study limitation (43). In addition, we could not discern between CKD and AKI, or the type of AKI, both critical conditions to guide clinical management and potential indication for SLKT (24,28,44). In this regard, AKI and CKD are closely related and AKI precedes transition to CKD in approximately 20% of patients. In the opposite direction, CKD is also a strong predictor of AKI (45,46). Given the increasing evidence that NAFLD patients have some degree of CKD, the previous considerations may be applied to this specific population. Finally, the lack of data on the precise duration of dialysis in patients receiving LTA is a limitation of the study. Assuming that most centers followed the well-accepted UNOS criteria, it is plausible that patients with indication for dialysis who underwent subsequent LTA were on renal replacement therapy for a short period. Prospective studies are needed

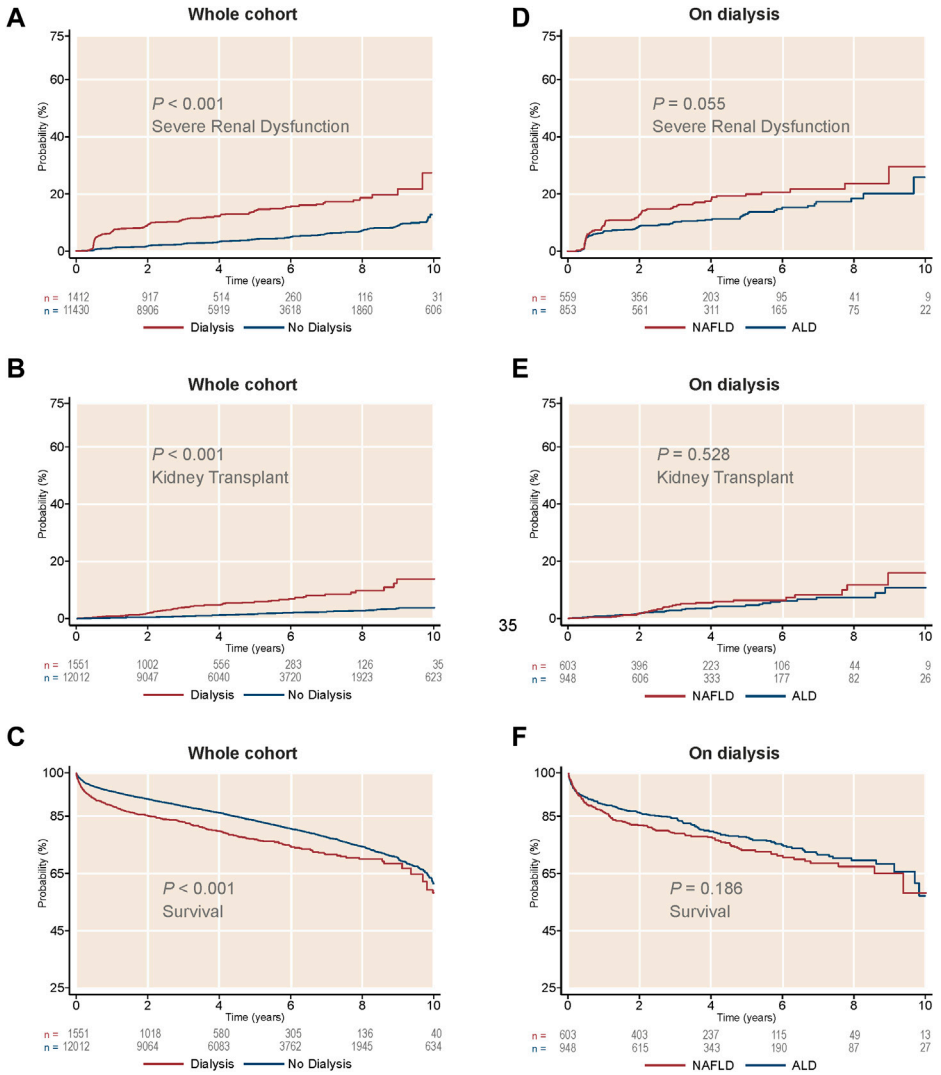


FIGURE 4 | Cumulative incidence of kidney-related outcomes, as well as survival, in patients on short-term dialysis receiving a liver transplant alone. **(A)** Cumulative incidence of severe renal dysfunction by dialysis treatment. **(B)** Cumulative incidence of kidney transplant by dialysis treatment. **(C)** Survival by dialysis treatment. **(D)** Cumulative incidence of severe renal dysfunction in patients on dialysis, by liver disease etiology. **(E)** Cumulative incidence of kidney transplant in patients on dialysis, by liver disease etiology. **(F)** Survival in patients on dialysis, by liver disease etiology. ALD, alcohol-related liver disease; NAFLD, non-alcoholic liver disease.

TABLE 4 | Cox proportional hazards model for severe renal dysfunction after liver transplant alone, including those receiving short-term dialysis prior to transplant.

	HR	95% confidence interval	*p* value
NAFLD	1.201	1.029–1.402	0.020
Age	1.009	1.001–1.018	0.033
Gender (male)	1.335	1.140–1.562	<0.001
Hispanic	1.037	0.854–1.259	0.713
Black	2.092	1.521–2.877	<0.001
T2DM	1.709	1.462–1.998	<0.001
BMI >40	1.163	0.903–1.496	0.242
Dialysis	3.290	2.786–3.886	<0.001

BMI, body mass index; HR, hazard ratio; NAFLD, non-alcoholic fatty liver disease; T2DM, type 2 diabetes.

including the precise indication and duration of dialysis prior to transplant.

In conclusion, our study shows that the underlying etiology of liver disease (NAFLD vs. ALD, the two leading LT indications) may play a role in predicting the development and progression of renal failure in patients receiving LTA. In addition, even if short-term dialysis before LTA has a strong impact on kidney-related outcomes regardless of the etiology of liver disease, it seems to be more pronounced in patients with NAFLD. Our results support the hypothesis that NAFLD patients have some degree of structural kidney disease, which could negatively impact the renal function recovery after LTA. Prospective studies are required to identify predictors and biomarkers of renal function recovery after LTA.

DATA AVAILABILITY STATEMENT

Publicly available datasets for all OPTN Member transplant centers were analyzed in this study. Formal export requests can be made here: https://optn.transplant.hrsa.gov/data/view-data-reports/request-data/ United Network for Organ Sharing/Organ Procurement and Transplantation Network (UNOS/OPTN) database.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of Pittsburgh Institutional Revision Board as a consent-waived study with the number PRO18020615. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

CF-C contributed to data analysis and writing of the main body of this article. YL contributed to statistical analysis and writing of the article. MV-C, JA, DD, AC-S, AD-R, JB, SG, NJ, AT, CH, AH, and MM contributed to critical review of this article and final approval. DL and RB contributed to study design, data analysis, writing and final approval of the article.

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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.2022.10443/full#supplementary-material

Supplementary Figure S1 | Cumulative incidence of kidney-related outcomes, as well as survival, in patients with moderate renal dysfunction (creatinine 1.5–2.5 mg/dl) after receiving a liver transplant alone. Stratification was done by liver disease etiology. **(A)** Cumulative incidence of severe renal dysfunction by etiology. **(B)** Cumulative incidence of kidney transplant by etiology. **(C)** Survival by etiology. ALD, alcohol-related liver disease; NAFLD, non-alcoholic liver disease.

Supplementary Figure S2 | Cumulative incidence of severe renal dysfunction in patients with glomerular filtration rate >45 ml/min/1.73 m^2 or low serum creatinine levels (<1.5 mg/dl) after receiving a liver transplant alone. Stratification was done by liver disease etiology. **(A)** Cumulative incidence of severe renal dysfunction in patients with glomerular filtration rate >45 ml/min/1.73 m^2. **(B)** Cumulative incidence of severe renal dysfunction in patients with creatinine <1.5 mg/dl. ALD, alcohol-related liver disease; GFR, glomerular filtration rate; NAFLD, non-alcoholic fatty liver disease.

Supplementary Figure S3 | Cumulative incidence of severe renal dysfunction after receiving a second liver transplant alone, in patients with moderate renal dysfunction. Stratification was done by liver disease etiology. **(A)** Cumulative incidence of severe renal dysfunction after receiving a second liver transplant alone in patients with glomerular filtration rate 45–25 ml/min/1.73 m^2. **(B)** Cumulative incidence of severe renal dysfunction after receiving a second liver transplant alone in patients with serum creatinine levels 1.5–2.5 mg/dl. ALD, alcohol-related liver disease; GFR, glomerular filtration rate; NAFLD, non-alcoholic liver disease.

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ORIGINAL RESEARCH
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Renal Function, Adherence and Quality of Life Improvement After Conversion From Immediate to Prolonged-Release Tacrolimus in Liver Transplantation: Prospective Ten-Year Follow-Up Study

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Immunosuppression non-adherence is a major cause of graft failure after liver transplantation. The aim of this study was to evaluate practice surrounding conversion from immediate-release to prolonged-release Tacrolimus formulation and to assess patient adherence and quality of life (QoL). One hundred and seven adult liver transplant recipients, receiving immediate-release Tacrolimus for a minimum of 6 months, were converted to prolonged-release formulation, based on a dose ratio of one (1:1). The median follow-up was 120 [IQR, 120–123] months. Tacrolimus dosage and blood level, liver and renal function, lipid and glucose profiles were recorded. In addition, questionnaires were submitted to evaluate adherence and QoL following conversion. No rejection was recorded. The median serum Tacrolimus blood level decreased over 1 month (5.80, [IQR, 2.0–10.8] vs. 3.8 [IQR, 1.4–8.7]; $p < 0.0005$). Significant improvement in renal function was noted (median GFR was 81.7 [IQR, 43.4–128.6] vs. 73.9 [IQR, 27.1–130.2]; $p = 0.0002$). At the end of the follow-up, conversion resulted in an overall decrease in non-adherence of 53.3% ($p = 0.0001$) and an improvement in QoL was reported by 76.2% of patients. Thus, 1:1 conversion from immediate to prolonged-release Tacrolimus is safe, feasible and efficient, avoiding under-therapeutic and toxic peak concentrations, improving renal function, adherence to immunosuppression and overall patient QoL.

Keywords: liver transplantation, immunosuppression, quality of life, Tacrolimus, adherence

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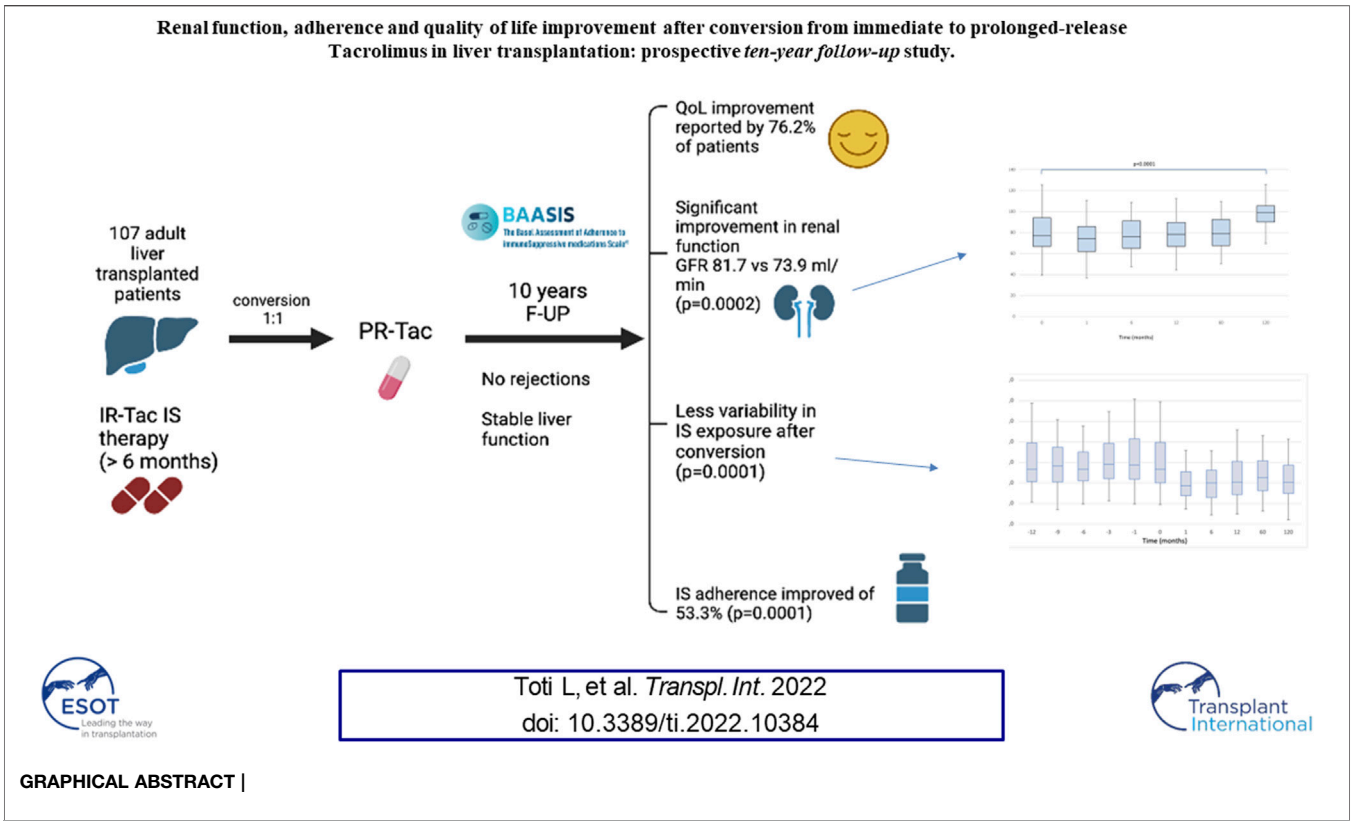
Abbreviations: BAASIS, Basel Assessment of Adherence Scale to Immunosuppressives; C/D, concentration/dose ratio; IR-Tac, immediate-release Tacrolimus formulation; PR-Tac, prolonged-release Tacrolimus formulation; VAS, Visual Analog Scale.



GRAPHICAL ABSTRACT |

INTRODUCTION

In recent decades, the introduction of new immunosuppressive drugs has contributed to graft survival in solid organ transplantation, decreasing the incidence of acute rejection and thus improving patient survival and quality of life (QoL). However, immunosuppression (IS) has several side-effects including renal failure, infections, cardiovascular diseases, metabolic disorders and *de novo* malignancies (1-4). In addition, patients are required to follow a complex IS regimen, which includes multiple drugs and personalized daily dose schedules. This therapeutic complexity is often poorly tolerated by patients and is the main cause of non-adherence after solid organ transplantation (5-7), which is estimated at between 15 and 55% (8-10). Therapeutic complexity is also the leading cause of preventable graft loss (4, 11-13). Therefore, simpler treatment regimens, such as once-daily dosing, have been suggested to help improve adherence in transplant recipients (14, 15). Furthermore, prolonged-release formulations may increase safety profiles avoiding toxic peaks and under therapeutic concentrations, which are observed in narrow therapeutic index drugs, including Tacrolimus (Tac) (16, 17).

Tac is frequently used in liver transplantation (LT). In addition to the immediate-release formulation (IR-Tac, Prograf®; Astellas Pharma US, Inc., Deerfileld, IL, USA), administered twice daily to maintain stable blood levels, a prolonged-release (PR-Tac, Advagraf®, Astellas Pharma Europe BV, Netherlands) formulation was licensed in Europe in 2007 for the prevention and treatment of graft rejection. Conversion from IR to PR-Tac has been studied in maintenance LT recipients (18-21), and the pharmacokinetic of IR-Tac and PR-Tac has been shown to be significantly different.

The main aim of this study was to explore tolerability and safety after conversion from IR to PR-Tac in adult LT patients. Secondary endpoints were patient adherence and QoL. Third endpoints were to evaluate the changes in concentration/dose ratio (C/D), C/D intra-patient variability following conversion from IR to PR-Tac, based on a dose ratio of 1 (1:1).

MATERIALS AND METHODS

This is a prospective, single arm study and patients were followed up at one, six, 12, 60 and 120 months between December 2010 and March 2021 in our hospital.

Inclusion Criteria

All adult patients, who underwent LT, who were on IR-Tac-based IS regimen for at least 6 months, with stable liver function test (LFT) and serum creatinine levels <2.0 mg/dl were enrolled in this study.

Exclusion Criteria

Patients were excluded in case of pregnancy, breastfeeding, malignancy, severe systemic infection requiring any therapy that could modify Tac pharmacokinetics, or the use of any other investigational drugs.

Standard Immunosuppression Management

In general, the IS therapeutic protocol of our centre requires that corticosteroids are not used unless the patient has autoimmune pathologies. In patients with stable liver function, Tac monotherapy is usually achieved 1 year after the transplantation (22).

Conversion Protocol to PR-Tac

The conversion from IR-Tac to PR-Tac started as soon as the new formulation was available in our hospital. All patients enrolled in the study were switched to PR-Tac, individually, during a 2 months period, and they were followed-up for at least 10 years. The starting dose of PR-Tac was exactly the same as the dose of IR-Tac taken by the patient at the time of conversion (1: 1).

Tac levels were measured in our central laboratory using a high-performance liquid chromatography-mass-spectrometry procedure (23). Patients were closely monitored during the study and Tac doses were adjusted to maintain adequate blood levels to maintain normal liver function and preventing rejection.

Clinical and Biomedical Parameters

At follow-up, physical examination and measurement of vital signs were performed, contingent adverse events were noted, and laboratory test results were checked. Arterial hypertension was defined as systolic blood pressure >140 and/or diastolic >90 mm Hg at two subsequent visits or when antihypertensive treatment was prescribed. Diabetes mellitus was defined as fasting glucose >126 mg/dl at two subsequent visits or when hypoglycemic treatment was used. Dyslipidemia was defined as cholesterolemia >220 mg/dl and/or triglyceridemia >200 mg/dl at two subsequent visits or when using hypolipidemic treatment.

LFTs including aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl-transferase and bilirubin were performed at each clinic. Elevated transaminases, defined as twice the upper limit of our laboratory cut-off (AST >68U/L; ALT >110U/L), triggered closer surveillance and a liver biopsy when LFT abnormalities persisted (24). Graft loss was defined as retransplantation or death. Renal function was assessed using the glomerular filtration rate (eGFR, Modification of Diet in Renal Disease formula, MDRD) formula.

Adherence and Quality of Life

Adherence was assessed using the "Basel Assessment of Adherence Scale to Immunosuppressives" (25) (BAASIS) questionnaire. This tool consists of a four-item validated questionnaire and a Visual Analog Scale (VAS). The first part addresses adherence including timing, missed dose and a "drug holiday" defined as >24 h interval between two consecutive doses. The VAS is a 100-point score, where patients report adherence in the previous 4 weeks from 0 to 100 (drug therapy never/always taken as prescribed) thereby assessing adherence as a continuous variable. The BAASIS form was completed by patients once pre-conversion to assess adherence to IR-Tac formulation and again at one- and ten-years following conversion to assess adherence to PR-Tac formulation and tolerability over time.

A *de novo* questionnaire (unpublished data) was developed to address those aspects of the IS regimen that influence patient care and the general perception of good or poor QoL in patients taking PR-Tac versus IR-Tac. The questionnaire was designed to be short, simple and easy to understand, to ensure a high completion rate with minimal missing data. The questionnaire, filled in anonymously by patients, collected demographic information (age, gender, and marital and employment status), and included three additional questions. Demographics were collected to facilitate the interpretation of the data at the end of the study. The first question was completed in the pre-conversion phase and queried the possible difficulty of taking multiple daily doses of drugs using a binary response option (YES/NO). The response was followed by a four-point Likert scale measuring the degree of difficulty, with two positive (very, quite) and two negative (little, very little) quantitative responses. At 12 and 120 months, questions two and three were administered. The second question assessed the possible satisfaction of the new drug regimen using a binary response option (YES/NO). The third question evaluated the perception of an improvement in QoL following the intake of the single-dose drug with a four-point Likert scale measuring the degree of improvement with two positive (very, quite) and two negative (little, very little) quantitative responses.

A positive response to the first question assumed dissatisfaction with taking multiple daily drugs, which was confirmed by positive responses on the Likert scale. Positive responses to questions 2 and 3 indicated satisfaction with the new therapeutic regimen and increased perception of QoL.

Statistical Analysis

Data were presented as means (standard deviation), medians (interquartile range; IQR), or frequencies (percentage) as appropriate. For adherence data, categorical variables collected during follow-up were compared to baseline values using Fisher's exact-test, while continuous data were compared to baseline values using the paired Student's t-test. To simultaneously subject-wise as well as time-related changes, and possible interactions between them, all other variables were analyzed using multivariate linear mixed models modelling timepoints as a repeated within-subject factor and employing an unstructured estimate of the covariance matrix. As opposed to general linear models, mixed models have the advantage of being able to account for heterogeneous distances between timepoints, missing data as well as unequal variances and covariances. To account for possible confounding due to inter-patient variability, all models included gender, categorized disease etiology, time

TABLE 1 | Patient baseline characteristics.

Characteristic (N = 107 patients)	Median/[IQR] or no. (%)
Sex, Males	69 (64.5%)
Age at conversion (years)	55 [48–61.5]
Time from IR-Tac to conversion (months)	55 [31–81]
Indication for LT	
Hepatitis C virus	27 (25.2%)
Hepatitis B virus	24 (22.4%)
Alcohol	12 (11.2%)
HCC	29 (27.1%)
Other	15 (14.1%)
Weight at baseline, kg	69 [62–75]
Comorbidity	
Diabetes mellitus	14 (13.1%)
Hypertension	21 (19.6%)
Hyperlipidemia	26 (24.3%)
Renal impairment (eGFR <60 ml/min/1.73 m^2)	14 (13.1%)

between therapy inception and conversion, and Tac blood levels (primary endpoint only) as covariates of interest.

Whenever a statistically significant ($p < 0.05$) overall effect of time was found, pairwise comparisons between timepoints were performed and corrected for multiple comparisons across pairs of timepoints using the Dunn–Šidák procedure.

Written informed consent was obtained from each patient prior to enrolment, without any patient refusing to participate in the study.

This study was conducted in accordance with the Declaration of Helsinki and approved by an Independent Ethics Committee prior to implementation.

RESULTS

One hundred and seven Caucasian adult LT recipients with a median age of 55 (IQR, 48–61.5) years were enrolled into the study. The median time from LT to study enrolment was 55 (IQR, 31–81) months. Patient characteristics are summarized in **Table 1**.

Primary Endpoint (Tacrolimus Tolerability and Safety)

At enrolment, 74 patients (69%) were on IR-Tac monotherapy. Median Tac daily dose was 2.0 (IQR, 1.5–3.0) mg and similar values were reported within the first 12-month after conversion. Eight-six out of 107 (81.9%) patients continued with the same Tac dosage after 1 year and the dosage was decreased in six patients (5.7%), increased in 11 patients (10.5%) and two (1.9%) patients were withdrawn from PR-Tac: the first patient due to frequent episodes of hypertension, diarrhea and vertigo and reconverted to IR-Tac; the second one due to *de novo* intestinal adenocarcinoma and subsequently switched to mTOR-inhibitor monotherapy.

TABLE 2 | Tacrolimus dosage modifications at 12 and 120 months.

Overall to IR-Tac	12 months	120 months
No modification	86 (80.4%)	56 (52.3%)
Decreased	6 (5.7%)	24 (22.4%)
Increased	11 (10.5%)	11 (10.3%)
Converted to another drug	2 (1.9%)	8 (7.5%)
Deaths	—	8 (7.5%)

By the end of the follow-up, 91 (85%) patients were still on the PR-Tac IS regimen: 56 patients (52.3%) were maintained on the same dosage as baseline; 24 patients (22.4%) had their dosage decreased and 11 patients (10.3%) increased. Six (5.6%) were converted to a different IS drug between 12th and 120th month. Three patients (2.8%) initiated single drug treatment with mycophenolate mofetil due to blood hypertension at 28, 56 and 68 months respectively. Three patients (2.8%) were converted to mTOR-inhibitor due to HCC recurrence, breast cancer and colon adenocarcinoma at 58, 89 and 112 months, respectively. A total of eight (7.4%) patients died due to a cardiovascular accident ($n = 4$) or malignancy ($n = 4$; lung cancer ($n = 2$) and esophagus cancer ($n = 2$)) (**Table 2**).

The median serum Tac blood levels were 5.80 (IQR, 4.1–7.1) ng/ml and 3.80 (IQR, 3.1–4.5) ng/ml respectively ($p < 0.0001$) at baseline and 1 month after conversion, respectively. When the LFTs remained stable no dose adjustments were considered. In patients without dose adjustments, the median Tac blood levels remained 2.0 mg [IQR, 1.5–3.0] We found a significant effect of the timepoint factor ($p < 0.0001$) on Tac blood levels, which in post-hoc comparison appeared to be associated with the following differences: baseline vs. 1, 6, 60 and 120 months (1 m < baseline: $p < 0.0001$; 6 m < baseline: $p = 0.023$; 60 m < baseline: $p = 0.002$, 120 m < baseline: $p = 0.001$); 1-month vs. 6, 60 and 120 months (1 m < 6 m: $p = 0.023$; 1 m < 60 m: $p = 0.003$; 1 m > 120 m: $p = 0.001$); 6 months vs. 12, 60 and 120 months (6 m < 12 m: $p = 0.01$, 6 m > 60: $p = 0.001$, 6 m > 120 m: $p = 0.001$) (**Figure 1**).

No patient experienced clinical or biopsy-proven acute rejection (BPAR) after conversion. The 10-year survival was 92.6%.

There was no statistically significant effect for the timepoint factor on liver function. Even the comparison of glucose levels and cholesterol and triglycerides values between the pre and post conversion periods was not statistically significant. We found a significant effect of the timepoint factor ($p < 0.0001$) on eGFR (**Figure 2**), which in post-hoc comparison appeared to be associated with the following differences: baseline<120 months ($p < 0.0001$); baseline>1 month ($p < 0.0001$); baseline>6 months ($p = 0.049$); month 1<6, 12, 60 and 120 (all $p < 0.001$).

Secondary End Points (Adherence and QoL)

The BAASIS questionnaire addressed different aspects of adherence, with the aim of identifying those areas where adherence has significantly increased. At baseline, 84 (78.5%)

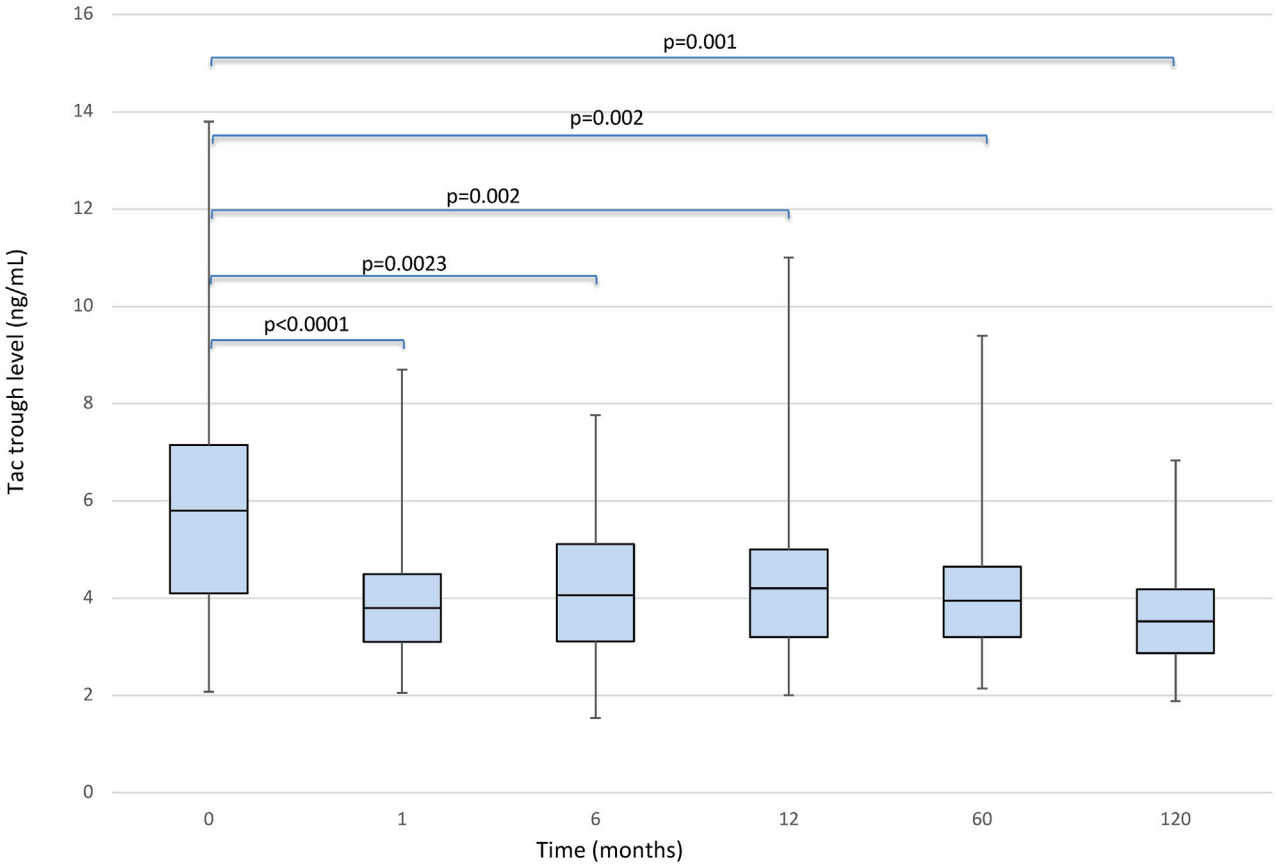


FIGURE 1 | Boxplots showing median, interquartile range and 5th/95th percentile of Tacrolimus trough level as a function of timepoint.

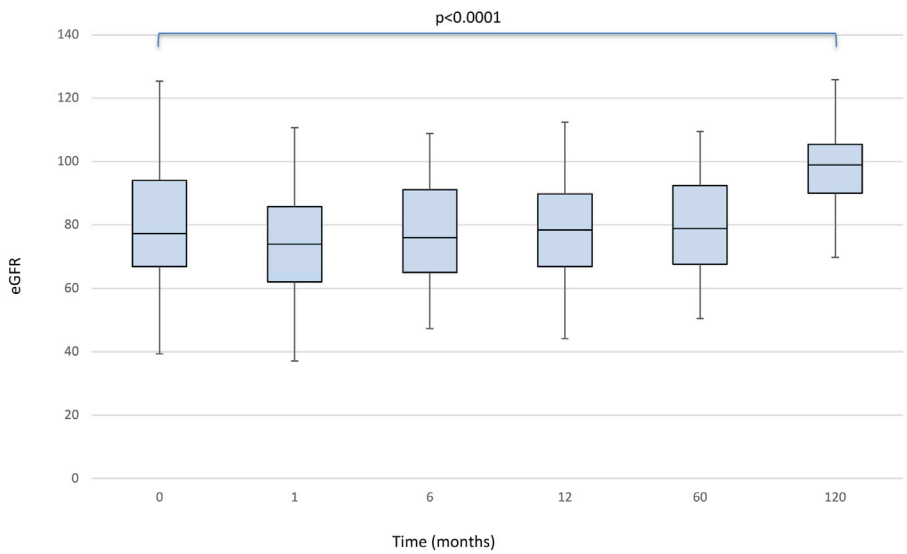


FIGURE 2 | Boxplots showing median, interquartile range and 5th/95th percentile of eGFR as a function of timepoint.

TABLE 3 | Adherence evaluation at Baseline, 12 and 120 months by BAASIS and VAS.

BAASIS	Baseline (*n* = 107)	Follow-up at 12 months (*n* = 105)	*p*-value**	Follow-up at 120 months (*n* = 91)	*p*-value**
	N (%)	N (%)		N (%)	
ITEM 1: Dose not taken	84 (78.5%)	24 (22.8%)	0.0001	23 (25.2%)	0.0001
ITEM 2: Consecutive doses not taken	66 (61.7%)	12 (11.4%)	0.0001	19 (20.9%)	0.0001
ITEM 3: Dose taken with delay	63 (58.9%)	15 (14.3%)	0.0001	21 (23.1%)	0.0001
ITEM 4: Dose auto-reduced	5 (4.7%)	3 (2.8%)	0.7214	-	0.0634
Overall Adherence*	17 (15.9%)			62 (68.1%)	0.0001

VAS	Median	IQR	Median	IQR	*p*-value***	Mean	IQR	*p*-value***
SCALE 0-100	90	75–100	97	85–100	0.0009	95	87–100	0.0008

TABLE 4 | QoL questionnaire administered at 12 and 120 months: items 1, 2 and 3.

N	Item	Answer	Baseline N = 107	12 months *n* = 105	120 months *n* = 91
1	Do you consider it difficult to take two or more doses of immunosuppressant drugs during the day?	Yes	66 (61.7%)	69 (65.7%)	61 (67%)
		No	41 (38.3%)	36 (34.3%)	30 (33%)
	• Take one or more types of drugs	Very difficult	40 (60.6%)	49 (71.0%)	45 (73.8%)
		Average	26 (39.4%)	20 (29.0%)	16 (26.2%)
		Easy	—	—	—
	• Take one or more tablets for type of drug	Very difficult	26 (39.4%)	50 (72.4%)	48 (78.7%)
		Average	40 (60.6%)	19 (27.5%)	13 (21.3%)
		Easy	3 (6.54%)	—	—
	• Take the drug at different times	Very difficult	30 (45.4%)	67 (97.1%)	60 (98.4%)
		Average	31 (47.0%)	2 (2.9%)	1 (1.6%)
		Easy	5 (7.6%)	—	—
2	Indicate the degree of satisfaction of the new regimen of taking the drug	Very satisfying	—	98 (93.3%)	79 (86.8%)
		Average	—	1 (0.9%)	10 (11.0%)
		Unsatisfactory	—	1 (0.9%)	—
		Indifferent	—	5 (4.9%)	2 (2.2%)
3	Do you feel an improvement in the quality of your life?	Yes	—	80 (76.2%)	75 (82.4%)
		No	—	25 (23.8%)	16 (17.6%)
	• Indicate how much your life has improved	Very much	—	66 (82.5%)	79 (86.8%)
		Average	—	10 (12.5%)	11 (12.1%)
		Very little	—	4 (5.0%)	1 (1.1%)

patients reported forgetting to take at least one drug dose in the previous 4 weeks, whereas this dropped to 24 (22.4%) and 27 (25.2%) patients at 12 and 120 months, respectively ($p < 0.0001$). Sixty-six patients (61.7%) declared at baseline that they had possibly missed two consecutive drug doses, which dropped to 12 (11.4%; $p < 0.0001$) and 22 (20.5%; $p < 0.0001$) patients at 12 and 120 months, respectively. Sixty-three patients (58.9%) did not respect the therapeutic intake time at baseline but this decreased to 15 (14%; $p < 0.0001$) and 25 (23.3%; $p < 0.0001$) patients at 12 and 120 months, respectively. Five patients (4.7%) admitted to taking lower drug dosages than medically prescribed, which dropped to three (2.8%; $p = 0.72$) and no (0%, $p = 0.06$) patients at 12 and 120 months, respectively.

Median VAS ratings of patient adherence were 90 (IQR,75–100) at baseline and were significantly higher at 12 months (97 [IQR, 85–100]; $p = 0.0009$) and 120 months (95 [IQR, 87–100]; $p = 0.0008$) (**Table 3**).

The three separate questions regarding QoL were completed by all participants at baseline. At baseline 66 (61.7%) patients indicated that they experienced difficulty with taking multiple doses of immunosuppressants daily, which was similar after 12 months ($n = 69$; 65.7%) and 120 months ($n = 61$; 67%). Filter questions showed that 40 (60.6%), 49 (71.0%) and 45 (73.8%) of patients found it very difficult to take more than one type of drug at baseline, 12 months and 120 months, respectively. Twenty-six (39.4%), 20 (72.4%) and 48 (78.7%) patients found it very difficult to take one or more doses of the same drug at baseline, 12 months and 120 months, respectively. Thirty (45.4%), 67 (97.1%) and 60 (98.4%) patients found it very difficult to take drugs at different times at baseline, 12 months and 120 months, respectively.

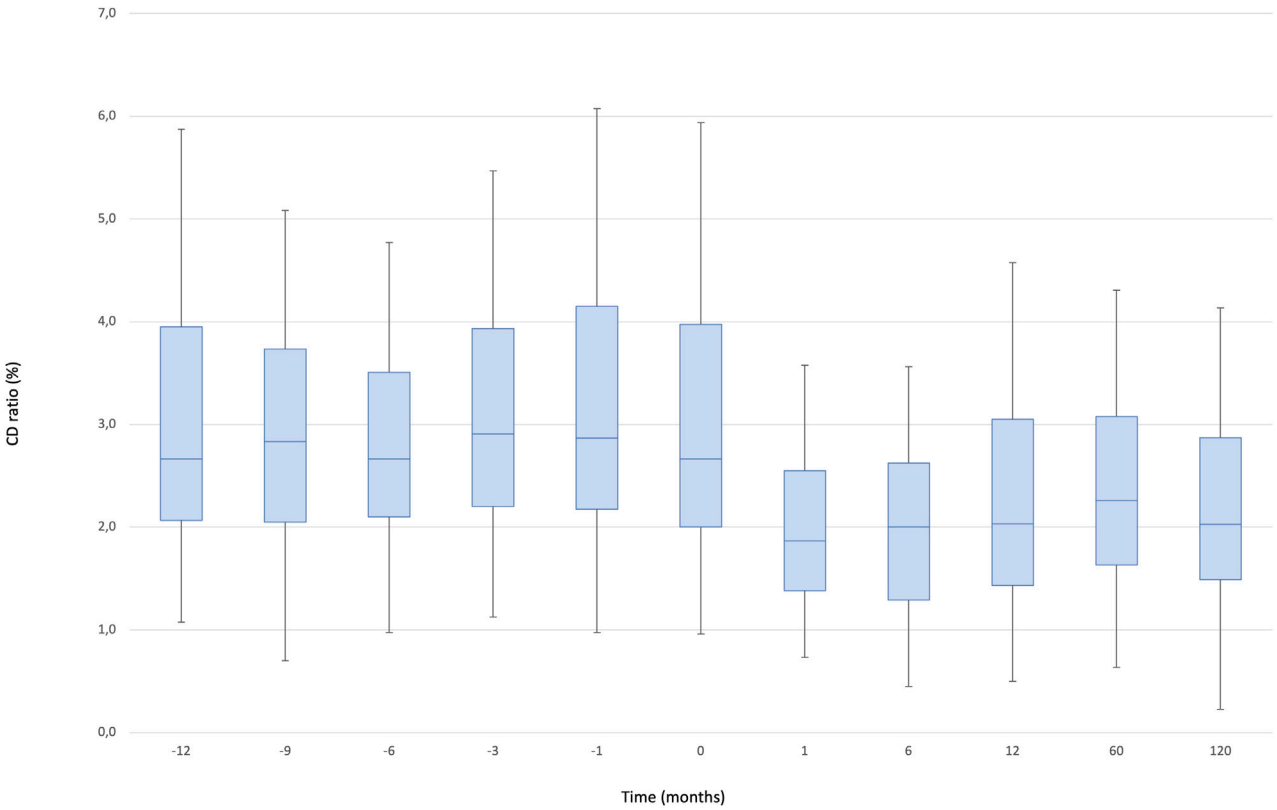


FIGURE 3 | Boxplots showing median, interquartile range and 5th/95th percentile of CD/ratio as a function of timepoint.

The second item addressed the degree of satisfaction with the PR-Tac therapeutic regimen. The data showed that 98 (93.3%) and 79 (86.8%) patients were very satisfied at 12 and 120 months, respectively. The third item asked if there had been an improvement in QoL after conversion. Eighty (76.2%) and 75 (82.4%) patients confirmed that their QoL had improved at 12 and 120 months respectively, with the filter question showing that 82.5% and 86.8% felt that QoL had very much improved at 12 and 120 months, respectively (**Table 4**).

Third Endpoint (C/D Ratio and Intra-patient Variability)

The median C/D ratio at baseline was 2.67 (IQR, 2.7–4.0). After one and 12 months, the ratio decreased to 1.87 (IQR, 1.9–2.6) and 2.03 (IQR, 2.0–2.6), respectively and remained stable during the follow up (2.03 [IQR, 2.0–2.9]: $p < 0.000001$) (**Figure 3**).

We observed a significant effect of the timepoint factor ($p < 0.0001$) associated with the following differences: 12 months vs. baseline, 1, 6, 60, 120 months (12 m < baseline: $p = 0.0001$, 12 m > 1 m: $p = 0.001$, 12 m > 6 m: $p = 0.007$, 12 m < 60 m: $p = 0.001$, 12 m > 120 m: $p = 0.053$), 6 months vs. baseline, 1, 12, 60, 120 months (6 m < baseline, 6 m > 1 m, 6 m < 12 m, 6 m < 60 m, 6 m < 120 m; all $p = 0.0001$), 1 month vs. baseline, 6, 12, 60, 120 months (1 m < baseline, 1 m < 6 m, 1 m < 12 m, 1 m < 60 m, 1 m < 120 m; all $p = 0.0001$), 120 months vs. baseline, 1, 6, 12, 60 months (120 m < baseline, 120 m > 1 m, 120 m > 6 m, 120 m < 12 m, 120 m < 60 m; all $p = 0.0001$). In addition, we compared 10 consecutive pre-conversion timepoints to 10 consecutive post-conversion measurements to evaluate the change of Tac blood levels and dose in a long-term observation: the mean C/D ratio was significantly higher pre-conversion compared to post conversion (3.29 [IQR, 2.7–4] versus 2.58[IQR, 2.3–2.9]: $p = 0.008$), while the coefficient of variation of the C/D ratio was significantly lower pre-conversion compared to post-conversion (2.12 versus 1.19: $p = 0.003$).

DISCUSSION

IR-Tac was considered a pillar of immunosuppressive therapy for solid organ transplantation for nearly 20 years, with excellent protection against organ rejection. Many studies have evaluated the effectiveness of converting from IR-Tac to PR-Tac, the latter able to facilitate adherence, to improve the QoL of transplant recipients and consequently their long-term results. (26, 27). In

2005, Florman et al. (6) reported the first conversion pharmacokinetics for stable LT recipients, concluding that the steady-state Tacrolimus exposure of PR-Tac was equivalent to IR-Tac after conversion on a milligram-for-milligram basis in stable LT recipients.

In our study the Tac blood levels decreased following conversion in 76% of cases and remained stable to the end of follow-up, which is similar to previously reported studies (19, 28).

An important aspect of this study is the side-effect profile of the anti-rejection drugs correlating to tac blood levels. The initial phase showed a decrease in Tac blood levels over time. Despite this, graft function remained stable with good function and was maintained over time with less side-effects. Time impacted significantly on serum Tac blood levels, which dropped sharply in the early post-conversion period.

With normal aging, nephron loss occurs and is detectable to some extent by the age-related decrease in eGFR (29, 30). Several studies that have analyzed the deterioration of renal function with increasing age in the healthy population show that eGFR shows a physiologic decrease between 0.3 and 1.4 ml/min/1.73 m^2/year (31, 32). In addition, calcineurin inhibitors, widely recognized as the mainstay of IS used to prevent graft rejection, have an important nephrotoxic side-effect profile. The expected gradual reduction in eGFR in LT recipients is the result of different mechanisms including immunologically mediated damage concurrent to the IS side-effects, nephrotoxicity and the development of cardiovascular risk factors (29, 33). However, a significant improvement in renal function was seen in our study: using eGFR, a significant effect of the timepoint factor ($p < 0.0001$) was seen, with a retrospective comparison showing the following differences at baseline vs. 1 month ($p < 0.0001$), baseline vs. 6 months ($p = 0.049$), month 1 vs. 6, 12, 60, and 120 (all $p < 0.001$). There were no new cases of posttransplant diabetes or glucose intolerance or any increase in adverse events associated with Tacrolimus use after conversion to PR-Tac.

It can be hypothesized that extended-release Tac may influence drug absorption and avoid drug peaks whilst maintaining adequate drug blood levels to avoid rejection.

Self-reporting adherence instruments having a tendency to overestimate adherence and under-report non-adherence due to increased awareness and pleasing the physician. However, BAASIS is considered a valid tool as it uses a rigorous definition of non-adherence, classifying a patient as non-adherent in case of positive answer to any of the four questions to be given. Non-adherence at study entry was considerably high especially regarding the evening dose, which has also been found by previous studies (6,34). At the end of the follow-up, however, these high adherence rates increased even further. The data clearly demonstrate that simply reducing the number of daily doses positively influences adherence leading to improved compliance and patient satisfaction. The important improvement in adherence following conversion to PR-Tac formulation is also evidenced in previously published studies. (35-37)

Adherence rates improved significantly between baseline and the end of the study in terms of missing one or more doses, violating drug timing and autonomous prescription modification. Patients themselves felt more adherent at the end of the study with 97% of patients defining themselves as "adherent" at 120 months compared with 90% at baseline. Patients reported having difficulty taking more than one IS drug in 61.7% of cases before conversion, not knowing about PR-Tac, subsequently, 93.3% defining the PR-Tac regimen as "very satisfactory." During medical interviews, 68% referred the evening dose as being the most difficult to self-administer for perceived interference with social life and sense of freedom. This has radically changed following conversion to PR-Tac.

Freedom of choice relating to time of drug administration directly impacted on perception of improvement in QoL for the majority of patients. QoL improvement was reported in over 82.4% of patients in our study following conversion. The reason why we decided to use a *de novo* questionnaire, drawn up and validated in collaboration with the Center for Psychology of our hospital, is due to the fact that in the literature, in our opinion, there were no validated questionnaires that had the characteristics, suitable for a complete evaluation of our patients which would allow us to obtain such complete results and which went hand in hand with the BAASIS, used for the assessment of adherence. The fact that no patient enrolled, with the sole constraint of taking IR-Tac for at least 6 months before signing the informed consent to the study, was for us surprising evidence of the excellent relationship of trust that we establish every day with all the people we follow in our post-transplant clinic and further confirmation of the patients' desire to seek "simpler" therapy regimens to follow.

Despite the absence of a control group that may evidence bias, we have considered the group itself before conversion as satisfactory for comparison.

There is significantly less intrasubject variability in exposure after conversion to PR-Tac: the mean C/D ratio was significantly ($p = 0.008$) higher pre-conversion as compared to post conversion, while the coefficient of variation of the C/D ratio was significantly ($p = 0.003$) lower pre-conversion compared to post-conversion, indicating greater stability post-conversion compared to pre-conversion.

In conclusion, our study demonstrates that 1:1 conversion from IR-TAC to PR-TAC is safe, feasible, efficient, and well-tolerated. Hepatic and renal function was closely monitored and no major dose adjustment to correct low Tac blood levels were required. Stable LT patients can be successfully switched from IR-Tac to PR-Tac formulation without risk of acute rejection even in the short term. A simplified formulation of Tac can improve patient adherence and their QoL. Improvement of renal function is probably due to lower Tac blood level exposure.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Independent Ethics Committee Fondazione PTV "Policlinico Tor Vergata," Rome. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LT and GT conceived of the presented study. LT, TM, FB, IL, and LB collected the data and revised the manuscript critically. LT and FB organized the database. NT performed the statistical analysis. LT wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

CONFLICT OF INTEREST

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

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Donor Skeletal Muscle Quality Affects Graft Mortality After Living Donor Liver Transplantation- A Single Center, Retrospective Study

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The recipient muscle status is closely associated with postoperative poor survival in recipients of living donor liver transplantation (LDLT). However, it is uncertain whether LDLT donor muscle quality and quantity affect graft quality. Hence, we analyzed the correlation between donor muscle status and graft function. We measured the skeletal muscle mass index (SMI) and intramuscular adipose tissue content (IMAC) of 380 LDLT donors. We examined the correlation between donor SMI or IMAC and graft mortality, the occurrence rates of small-for-size graft (SFSG) syndrome, and 6-month graft survival rates. The donor SMI had no effect on the occurrence of SFSG syndrome and graft survival, while a high IMAC in both male and female donors was significantly correlated with the rate of SFSG syndrome [high vs low: (male donors) 15.8% vs. 2.5%, $p = 0.0003$; (female donors) 12.8% vs. 3.1%, $p = 0.0234$] and 6-month graft survival rates [(male donors) 87.7% vs 95.9%, $p = 0.02$; (female donors) 83.0% vs. 99.0%, $p < 0.0001$]. Multivariate analysis revealed that a high donor IMAC (HR; 5.42, CI; 2.13–13.8, $p = 0.0004$) was an independent risk factor for 6-month graft survival, and the donor IMAC is useful for donor selection for high-risk recipients.

Keywords: sarcopenia, intramuscular adipose tissue content, small-for-graft size syndrome, donor muscle status, graft quality

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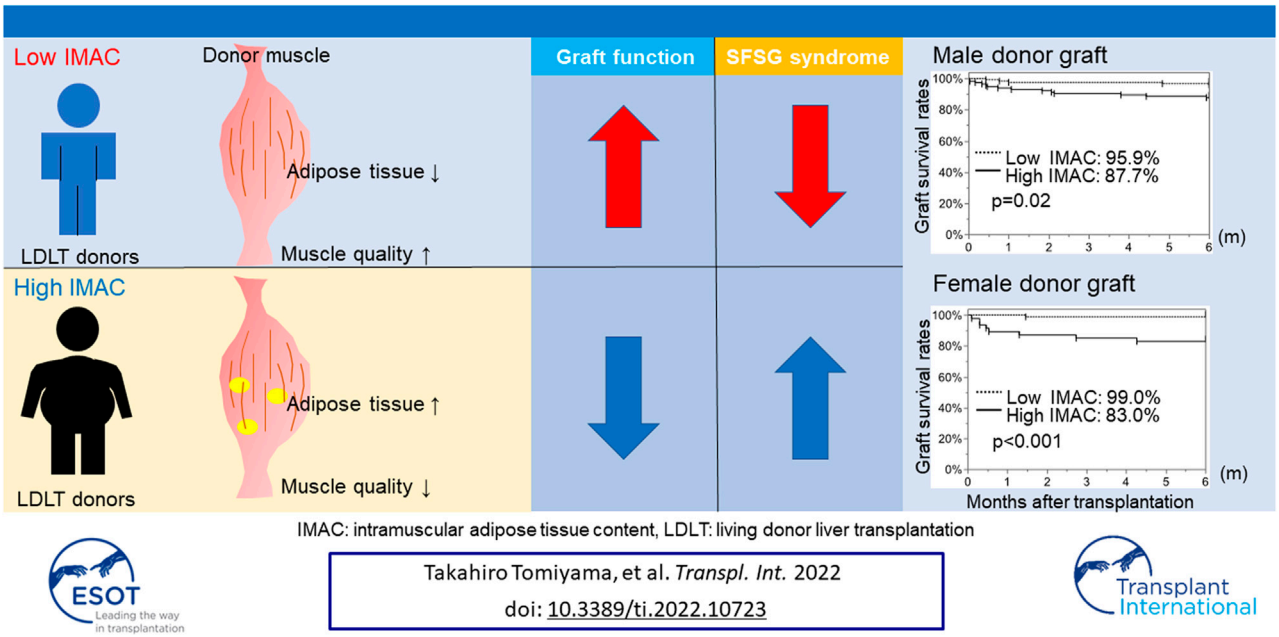
INTRODUCTION

Sarcopenia, defined as an age-dependent decrease in muscle mass and function, is reportedly an independent risk factor of poor survival in the presence of several diseases (1 –5). In the field of liver transplantation, preoperative recipient sarcopenia is reportedly correlated with increasing sepsis and mortality rates in recipients after living donor liver transplantation (LDLT) (6). In addition, the transplant recipient preoperative skeletal muscle mass-to-visceral fat area ratio, visceral adiposity, low muscularity, and high intramuscular adipose tissue content (IMAC) are closely associated with high postoperative recipient mortality following LDLT (7, 8). These findings indicate that low

Abbreviations: CT, computed tomography; DM, diabetes mellitus; GV/SLV, graft volume/recipient standard liver volume; HCC, hepatocellular carcinoma; HR, hazard ratio; ICU, intensive care unit; IL-6, interleukin-6; IMAC, high intramuscular adipose tissue content; LDLT, living donor liver transplantation; MELD, Model for End-Stage Liver Disease; POD, postoperative day; PT-INR, prothrombin-time international normalized ratio; SMI, skeletal muscle mass index; T-bil, total-bilirubin.



GRAPHICAL ABSTRACT |

quantity and quality of muscle in the recipient preoperatively closely correlate with postoperative mortality in LDLT recipients.

However, these correlations with liver transplantation and muscle quality and quantity are not surprising because the liver is strongly affected by muscle tissue (9). Skeletal muscle tissue secretes a hormone, called myokine, which regulates muscle metabolism, increases insulin sensitivity, and influences adipose tissue mass and fat deposition in the liver (10,11). On the other hand, adipose tissue can release hormones, called adipokines, which regulate lipid metabolism, decrease insulin sensitivity, and influence fibrogenesis in the liver (10, 12). Thus, skeletal muscle is closely involved in determining the liver condition.

The effect of skeletal muscle in LDLT donors has not been fully examined. LDLT donors are healthy and lack severe comorbidities. Preoperative blood tests are performed to confirm that there are no abnormalities. In addition, donor liver steatosis and cold ischemic time are reportedly graft quality markers in deceased donor liver transplantation (DDLT) (13, 14). If a donor has mild obesity or fatty liver in LDLT, dietary restriction and exercise are implemented, and LDLT is performed after complete improvement of obesity and fatty liver. In LDLT, the cold ischemic time is very short and much less likely to be affected compared to DDLT. The population of LDLT donors is quite homogeneous compared to that of DDLT donors. However, there is diversity in LDLT donor body shape and muscle mass. In LDLT, exercise and diet improve the health of the donor (15), and regular exercise reduces intrahepatic adiposity, increases β-oxidation of fatty acids, and induces hepato-protective autophagy (16). Hence, donor muscularity may reflect the health status of the liver and the condition of the graft, and it may be useful to base the decision of donor selection in LDLT on donor muscularity.

In the present study, the pretransplant donor skeletal muscle mass index (SMI) and IMAC were retrospectively evaluated, and the impact of the SMI and IMAC on graft survival was assessed.

METHODS

Patients

The study protocol was approved by the Institutional Review Board of the Kyushu University Hospital, approval number 2019–354. This study was conducted according to the Declaration of Helsinki of 1996. Written informed consent was obtained from all patients before LDLT. In total, 380 adult patients (age >17 years) underwent LDLT at Kyushu University Hospital, Japan, between January 2007 and March 2018. Recipients who could be followed for at least 6 months after LDLT were included. If an LDLT donor had mild obesity or fatty liver, dietary restriction and exercise were implemented, and LDLT was performed after complete improvement of obesity and fatty liver. In our cohort, only two donors underwent weight loss before the donation.

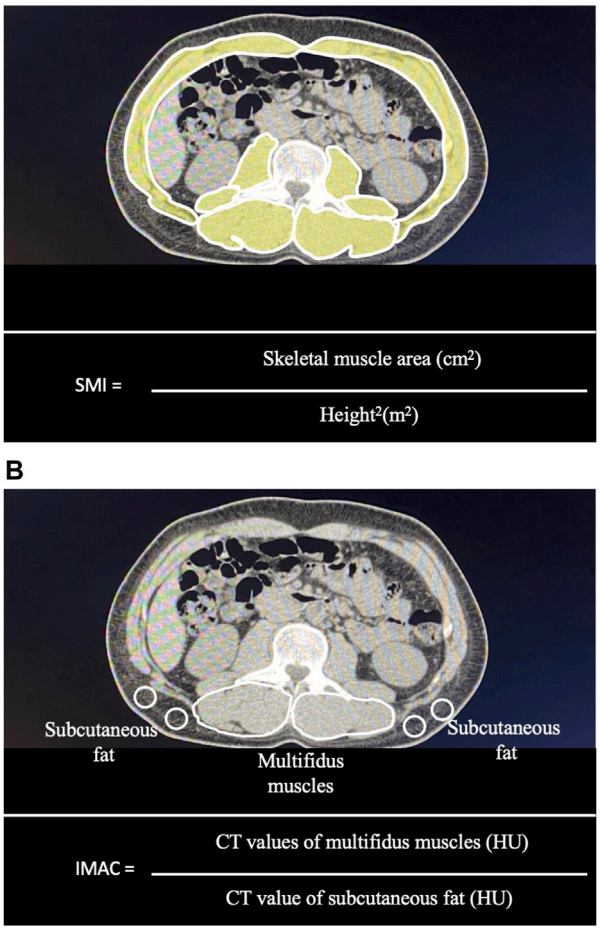


FIGURE 1 | Measurement of the skeletal muscle mass index (SMI) and intramuscular adipose tissue content (IMAC) using a cross-sectional CT image obtained at the third lumbar vertebral level: **(A)** Skeletal muscle areas were measured by manual tracing. Skeletal muscle areas were normalized with patient height in m^2 and expressed as the SMI. **(B)** The preoperative mean CT value for the right and left multifidus muscles (in Hounsfield units, HU) was divided by the mean CT value for four points of subcutaneous fat and expressed as the IMAC.

Image Analysis

Computed tomography (CT) scanning was performed within 1 month preoperatively. The SMI and IMAC were calculated as previously reported (5, 17). Briefly, the skeletal muscle area and IMAC were calculated using cross-sectional CT images obtained at the third lumbar vertebral level. Skeletal muscle areas were measured by manual tracing and normalized with patient height in m^2 and expressed as the SMI (**Figure 1A**). The preoperative mean CT value for the right and left multifidus muscles (in Hounsfield units, HU) was divided by the mean CT value for four points of subcutaneous fat and expressed as the IMAC (**Figure 1B**). A higher IMAC indicates a larger amount of adipose tissue in the skeletal muscle and, therefore, muscle that is of poorer quality.

Selection Criteria

The selection criteria for the recipients and donors have been previously described (18, 19).

The selection criteria for LDLT for patients without hepatocellular carcinoma (HCC) were as follows: 1) no other potentially curative modality available and 2) no other organ failure present. There was no limitation on recipient age. The selection criteria for LDLT for patients with HCC were as follows: 1) no other potentially curative modality available, 2) no extrahepatic metastasis, and 3) no major vascular invasion. The Model for End-Stage Liver Disease (MELD) score was calculated using a formula reported by Kamath et al. (20).

Donors were selected from among candidates who had volunteered for the procedure (18). They were required to be within three degrees of consanguinity or the spouse of the recipient and to be between 20 and 65 years of age. For donors not within three degrees of consanguinity with the recipient, individual approval was obtained from the Ethic Committee of Kyushu University Hospital. Good Samaritan donation was not used. The standard liver volume of recipients was calculated according to the formula of Urata (21). Three-dimensional CT was performed for volumetric analysis and delineation of vascular anatomy. Decisions regarding graft type were based on the preoperatively predicted graft volume/recipient standard liver volume (GV/SLV) ratio. Left lobe + caudate lobe grafts were basically used when the preoperatively predicted GV/SLV ratio was ≥35%, but relatively small grafts, such as those with a GV/SLV between 30% and 35%, were selected when the donor was younger than 30 years of age (18). When the GV/SLV ratio of the left lobe + caudate lobe graft was <35% and remnant liver volume after right lobectomy was ≥35%, a right lobe graft was used. A posterior segment graft was considered when the donor's vascular anatomy was suitable for this purpose (22).

Surgical Technique

The graft procurement technique and recipient surgery have been previously described (23). Splenectomy was performed using a vessel sealing system (Ligasure; Covidien Japan, Tokyo, Japan) and automatic suturing device (Endo GIA, Covidien Japan or Powered ECHELON, ETHICON, New Brunswick, NJ, United States) as described previously (19, 24).

Postoperative Management

The perioperative management of recipients, including the immunosuppression regimens, have been described previously (18, 19, 25). Briefly, immunosuppression was initiated using a protocol based on either tacrolimus (Prograf: Astellas Pharma, Tokyo, Japan) or cyclosporine A (Neoral; Novartis Pharma K.K, Tokyo, Japan) with mycophenolate mofetil (CellCept; Pfizer, New York, America) and steroids. The target trough concentration for tacrolimus was set at 10 ng/ml for 3 months after LDLT, followed by 5–10 ng/ml. The target trough concentration for cyclosporine A was set at 250 ng/ml for 3 months after LDLT, followed by 150–200 ng/ml. Methylprednisolone was initiated on the day of the

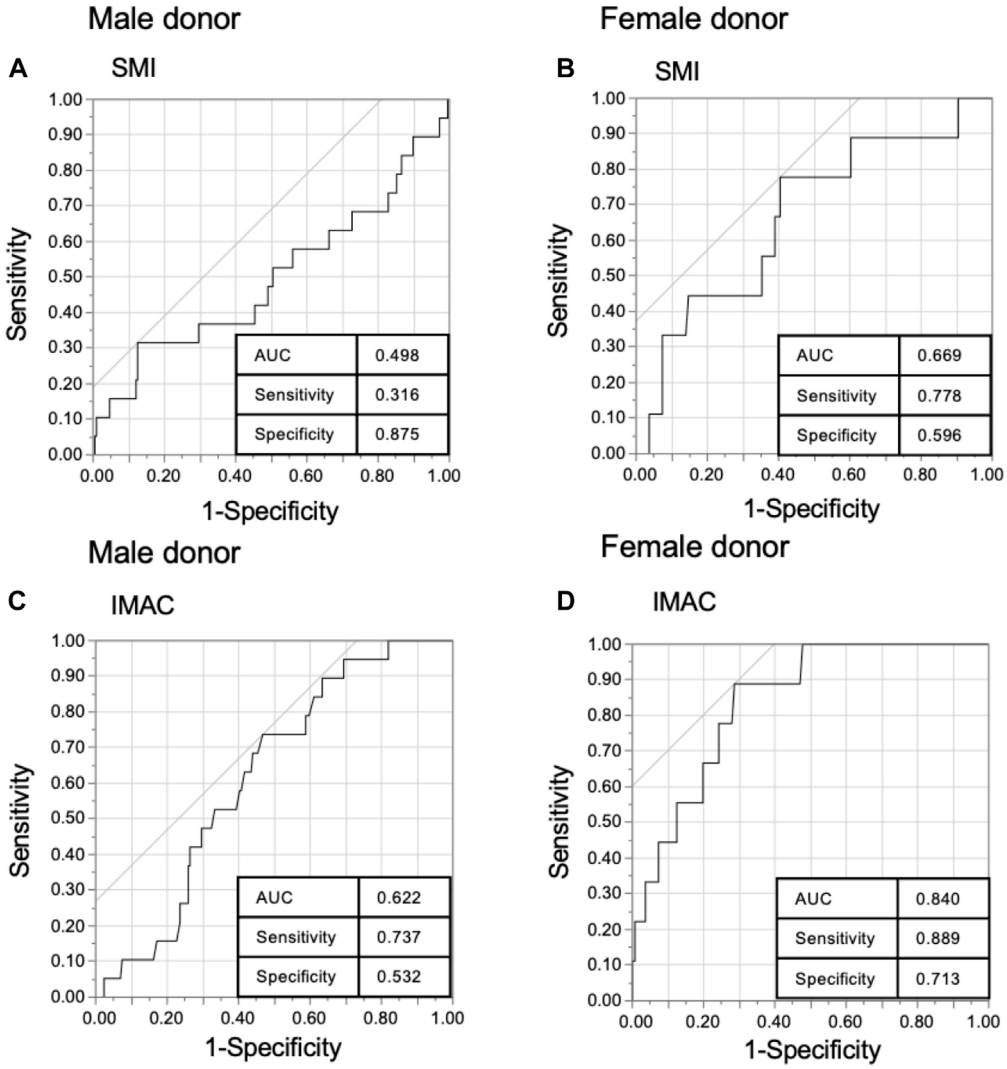


FIGURE 2 | Receiver operating characteristics (ROC) curve of the donor muscle mass index (SMI) and intramuscular adipose tissue content (IMAC). ROC of **(A)** male donor SMI, **(B)** female donor SMI, **(C)** male donor IMAC, and **(D)** female donor IMAC with 6-month graft survival in living donor liver transplantation.

LDLT, after which the dose was tapered, and prednisolone was sustained 7 days after the LDLT. Prednisolone treatment was tapered and discontinued 6 months after LDTL. Mycophenolate mofetil was used, beginning with 2 or 3 g on the day after LDLT; the dose was tapered and discontinued 6 months after LDLT. The trough concentration of mycophenolate mofetil was not measured.

Portal, hepatic arterial, and hepatic venous flows were assessed using Doppler ultrasonography twice per day until postoperative-day (POD) 7 and once per day thereafter during the first admission. For recipients with simultaneous splenectomy and LDLT, portal vein thrombosis prevention was not routinely performed. When the platelet count increased to 500,000/ml or higher during the follow-up period, 100 mg of aspirin was administered, which was discontinued when the platelet count decreased below 500,000/ml.

The abdominal drain was removed when the daily ascites volume became lower than 500 ml.

Endpoints

The primary endpoint was 6-month graft survival. If there was a significant difference in the first endpoint, we also examined laboratory data and the amount of abdominal drainage as secondary endpoints. Six-month graft loss was defined as recipient death or re-transplantation within 6 months.

Parameters Analyzed

Data Analysis

Categorical variables are presented as numbers and percentages and all patient background information was compared using the Pearson's chi-square test. Based on their distributions,

TABLE 1 | Difference in patient characteristic between male high SMI group and low SMI group.

Variables	Male donor SMI		*p*-value
	High (*n* = 43)	Low (*n* = 192)	
Preoperative donor variables			
Age (years)	33 (20–62)	36 (20–63)	0.0636
Graft (right lobe)	10 (23.3%)	80 (41.7%)	0.0248
Actual GV/SLV (%)	40.3 (23.2–73.1)	39.8 (22.6–70.1)	0.7135
Actual GRWR (%)	0.787 (0.430–1.35)	0.770 (0.397–1.42)	0.7616
ABO (Incompatible)	5 (11.6%)	32 (16.7%)	0.4122
Recipient preoperative variables			
Age (years)	55 (19–76)	57 (20–74)	0.3485
Sex (male)	15 (34.9%)	78 (40.6%)	0.4865
Primary diagnosis			
Hepatocellular disease	30 (69.8%)	130 (67.7%)	
Cholestatic disease	20 (20.9%)	32 (16.7%)	
Others	4 (9.30%)	30 (15.6%)	
HBsAb (yes)	9 (20.9%)	50 (26.0%)	0.5070
HCVAb (yes)	18 (41.9%)	70 (36.5%)	0.5082
HCC (yes)	19 (44.2%)	76 (39.6%)	0.5783
Body mass index (kg/m^2)	24.1 (15.8–32.0)	23.7 (14.9–35.6)	0.9234
ICU or hospital statement (yes)	14 (32.6%)	67 (34.9%)	0.7706
DM (yes)	10 (23.3%)	38 (19.8%)	0.6105
MELD	14 (4–29)	15 (4–54)	0.0760
Splenectomy (yes)	32 (74.4%)	163 (84.9%)	0.0985
Pre-transplant WBC count (x10^3/μL)	4.06 (1.46–15.7)	4.06 (0.39–20.6)	0.5546
Pre-transplant Platelet count (x10^4/μL)	7.40 (2.60–44.6)	6.85 (0.90–30.2)	0.0710
Intraoperative parameters			
Recipient operation time (h)	12.2 (8.33–21.6)	12.1 (7.55–24.8)	0.7576
Recipient blood loss (L)	3.80 (0.15–68.3)	3.84 (0.12–220)	0.7711
Cold ischemic time (min)	81.5 (42–210)	92 (35–376)	0.0550
Warm ischemic time (min)	38 (28–125)	41 (25–119)	0.6401
PVP before the end of operation	16 (9–25)	16 (6–30%)	0.6908
Recipient postoperative parameter			
Admission period (day)	25 (13–78)	25 (1–145)	0.4970
Sepsis	7 (16.3%)	12 (6.3%)	0.0292
SFSG syndrome	3 (7.0%)	18 (9.4%)	0.6183
Acute cellular or humoral rejection	7 (16.3%)	18 (9.38%)	0.1844
Graft failure within 6 months	5 (11.6%)	14 (7.29%)	0.3458
Cause of graft loss within 6 months			
Liver failure	2 (40.0%)	8 (57.1%)	
Sepsis	1 (20.0%)	4 (28.6%)	
Others	2 (20.0%)	2 (14.3%)	0.4805

Data are presented as median (range) or n (%).

DM, diabetes mellitus; GRWR, graft recipient weight ratio; GV/SLV, graft volume/recipient standard liver volume ratio; HBsAg, hepatitis B surface antigen, HCC, hepatocellular carcinoma; HCVAb, hepatitis C virus antibody; HCC, hepatocellular carcinoma; ICU, intensive care unit; MELD, Model for End-Stage Liver Disease; PVP, portal vein pressure; SFSG, small-for-size graft.

continuous variables are presented as the mean with 95% confidence interval, and they were compared using the *t*-test.

Graft survival data were analyzed using the Kaplan–Meier method and compared using the log-rank test. Continuous variables were compared using the *t*-test and categorical variables were compared using the chi-squared (?^{2}) test. Any variable in the univariate analysis was identified as significant at $p < 0.05$, or variables at $p < 0.2$ were considered candidates for the multivariate Cox analysis. The results are shown as hazard ratios (HRs) with 95% confidence intervals (CIs). A value of $p < 0.05$ was considered to indicate statistical significance. All statistical data were generated using JMP Pro 15 (SAS Institute, Cary, NC, United States).

RESULTS

Measurement of Donor SMI and IMAC and Examination of the Cutoff Value

There was a significant difference in donor SMI and IMAC by sex [male donor (n = 235) vs. female donor (n = 145); mean

TABLE 2 | Difference in patient characteristic between female high SMI group and low SMI group.

Variables	Female donor SMI		*p*-value
	High (*n* = 89)	Low (*n* = 56)	
Preoperative donor variables			
Age (years)	38 (21–64)	39 (21–62)	0.2019
Graft (right lobe)	61 (68.5%)	45 (80.4%)	0.1182
Actual GV/SLV (%)	42.5 (26.9–63.0)	40.6 (28.9–56.4)	0.2386
Actual GRWR (%)	0.782 (0.524–1.21)	0.755 (0.509–1.19)	0.3869
ABO (Incompatible)	17 (19.1%)	9 (16.7%)	0.6433
Recipient preoperative variables			
Age (years)	56 (17–73)	71 (21–71)	0.4245
Sex (male)	39 (43.8%)	33 (58.9%)	0.0765
Primary diagnosis			
Hepatocellular disease	62 (70.0%)	36 (64.3%)	0.3052
Cholestatic disease	20 (22.5%)	11 (19.6%)	
Others	7 (7.87%)	9 (16.1%)	
HBsAb (yes)	14 (15.7%)	10 (17.9%)	0.7372
HCVAb (yes)	28 (31.5%)	15 (26.8%)	0.5484
HCC (yes)	24 (27.0%)	17 (30.4%)	0.6589
Body mass index (kg/m^2)	23.3 (17.0–32.9)	23.3 (17.2–29.0)	0.4330
ICU or hospital statement (yes)	25 (28.4%)	21 (37.5%)	0.2540
DM (yes)	10 (11.2%)	8 (14.3%)	0.5876
MELD	16 (5–44)	17 (5–45)	0.2161
Splenectomy (yes)	78 (87.6%)	48 (85.7%)	0.7379
Pre-transplant WBC count (x10^3/μL)	3.99 (1.04–15.9)	4.20 (1.17–15.8)	0.9446
Pre-transplant Platelet count (x10^4/μL)	7.00 (1.2–36.2)	6.25 (1.7–34.8)	0.7092
Intraoperative parameters			
Recipient operation time (h)	12.6 (8.10–18.0)	12.5 (8.47–20.7)	0.2898
Recipient blood loss (L)	3.70 (0.58–26.4)	5.62 (0.20–50.4)	0.0770
Cold ischemic time (min)	113 (39–261)	157 (50–367)	0.0013
Warm ischemic time (min)	41 (25–103)	44.5 (22–83)	0.4413
PVP before the end of operation	15 (7–25)	14.5 (9–22)	0.5789
Recipient postoperative parameter			
Admission period (day)	27 (9–172)	29 (3–80)	0.2890
Sepsis	5 (5.62%)	4 (7.14%)	0.7110
SFSG syndrome	6 (6.7%)	3 (5.4%)	0.7366
Acute cellular or humoral rejection	7 (7.87%)	5 (8.93%)	0.8210
Graft failure within 6 months	7 (7.87%)	2 (3.57%)	0.2968
Cause of graft loss within 6 months			
Liver failure	3 (42.9%)	2 (100%)	0.1515
Sepsis	4 (57.1%)	0 (0%)	
Others	0 (0%)	0 (0%)	

Data are presented as median (range) or n (%).

DM, diabetes mellitus; GRWR, graft recipient weight ratio; GV/SLV, graft volume/recipient standard liver volume ratio; HBsAg, hepatitis B surface antigen, HCC, hepatocellular carcinoma; HCVAb, hepatitis C virus antibody; HCC, hepatocellular carcinoma; ICU, intensive care unit; MELD, Model for End-Stage Liver Disease; PVP, portal vein pressure; SFSG, small-for-size graft.

SMI: 50.1 vs. 39.4, $p < 0.0001$, mean IMAC: −0.557 vs. −0.507, $p < 0.0001$]. Thus, we separated data from male and female donors for further analysis. The optimal cutoff values for predicting primary 6-month graft loss were derived from receiver operating characteristic curves, with SMI cutoff values for men and women of 57.0 and 37.5 (sensitivity 31.6% and 77.8%, respectively; specificity 87.5% and 59.6%, respectively), and IMAC cut-off values for men and women of −0.553 and −0.473, respectively (sensitivity 73.7% and 88.9%, respectively; specificity 53.2% and 71.3%, respectively) (**Figures 2A–D**).

Correlation of Preoperative Donor Muscle Condition With Patient Characteristics

As shown in **Table 1**, depicting the male donor SMI analysis, there were significant differences in the rates of right lobe grafts

TABLE 3 | Difference in patient characteristic between male high IMAC group and low IMAC group.

Variables	Male donor IMAC		*p*-value
	Low (*n* = 121)	High (*n* = 114)	
Preoperative donor variables			
Age (years)	34 (20–63)	37 (20–62)	0.1697
Graft (right lobe)	54 (44.6%)	36 (31.6%)	0.0397
Actual GV/SLV (%)	40.8 (26.8–70.1)	38.2 (22.6–73.1)	0.0283
ABO (Incompatible)	0.792 (0.482–1.42)	0.737 (0.397–1.35)	0.0552
Recipient preoperative variables	17 (14.1%)	20 (17.5%)	0.4623
Age (years)	55 (22–74)	57 (19–76)	0.6764
Sex (male)	54 (44.6%)	39 (34.2%)	0.1026
Primary diagnosis			
Hepatocellular disease	86 (71.1%)	74 (64.9%)	0.5522
Cholestatic disease	20 (16.5%)	21 (18.4%)	
Others	15 (12.4%)	19 (16.7%)	
HBsAb (yes)	28 (23.1%)	31 (27.2%)	0.4740
HCVAb (yes)	47 (38.8%)	41 (36.0%)	0.6487
HCC (yes)	53 (43.8%)	42 (36.8%)	0.2773
Body mass index (kg/m^2)	23.7 (14.9–35.0)	24.1 (15.8–35.6)	0.7142
ICU or hospital statement (yes)	41 (33.9%)	40 (35.1%)	0.8462
DM (yes)	40 (15.8%)	19 (19.6%)	0.3982
MELD	15 (4–54)	15 (4–39)	0.8274
Splenectomy (yes)	102 (84.3%)	93 (81.6%)	0.5794
Pre-transplant WBC count (x10^3/μL)	4.10 (0.39–16.1)	3.96 (0.96–20.6)	0.5805
Pre-transplant Platelet count (x10^4/μL)	6.9 (1.8–41.5)	7.0 (0.9–44.6)	0.5164
Intraoperative parameters			
Recipient operation time (h)	12.2 (7.55–23.1)	12.0 (7.57–24.9)	0.3754
Recipient blood loss (L)	3.79 (0.14–29.9)	3.90 (0.12–22.0)	0.0362
Cold ischemic time (min)	88 (35–313)	89 (38–376)	0.9574
Warm ischemic time (min)	41 (26–104)	40.5 (25–125)	0.5239
PVP before the end of operation	15 (6–30)	16 (8–26)	0.1224
Recipient postoperative parameter			
Admission period (day)	24 (9–145)	29 (1–133)	0.0492
Sepsis	7 (5.79%)	12 (10.5%)	0.1827
SFSG syndrome	3 (2.5%)	18 (15.8%)	0.0004
Acute cellular or humoral rejection	13 (10.7%)	12 (10.5%)	0.9569
Graft failure within 6 months	5 (4.13%)	14 (12.3%)	0.0220
Cause of graft loss within 6 months			
Liver failure	3 (60.0%)	7 (50.0%)	
Sepsis	1 (20.0%)	4 (28.6%)	
Others	1 (20.0%)	3 (21.4%)	0.9156

Data are presented as median (range) or n (%).

DM, diabetes mellitus; GRWR, graft recipient weight ratio; GV/SLV, graft volume/recipient standard liver volume ratio; HBsAg, hepatitis B surface antigen, HCC, hepatocellular carcinoma; HCVAb, hepatitis C virus antibody; HCC, hepatocellular carcinoma; ICU, intensive care unit; MELD, Model for End-Stage Liver Disease; PVP, portal vein pressure; SFSG, small-for-size graft.

and sepsis after transplantation between the low-SMI and high-SMI groups (low-SMI group vs. high-SMI group; right lobe rate: 58.3% vs. 76.7%, $p = 0.0248$, sepsis rate: 6.3% vs. 16.3%, $p = 0.0292$). **Table 2** shows the patient characteristics in female donors. Cold ischemic time was significantly longer in the low-SMI group than in the high-SMI group (157 min vs. 113 min, $p = 0.0013$), but there was no significant difference in recipient postoperative parameters. **Table 3** shows the patient characteristics for the male donor IMAC analysis. In the high-IMAC group, the rates of right lobe graft and GV/SLV were lower (38.2% vs. 40.8%, $p = 0.0283$) and intraoperative recipient blood loss was higher (3.90L vs. 3.79L, $p = 0.0362$) than in the low-IMAC group. Regarding recipient postoperative parameters, the admission period was longer (29 vs. 24 days, $p = 0.0492$) and the rates of SFSG syndrome (15.8% vs. 2.5%, $p = 0.0004$) and graft failure (12.3% vs. 4.13%, $p = 0.0220$) were higher in the high-IMAC than in the low-IMAC group. **Table 4** shows the patient characteristics for the female donor IMAC analysis. The rate of ABO incompatibility was lower (8.51% vs. 22.5%, $p = 0.0406$) and the MELD score was higher (19 vs. 16, $p = 0.0005$) in the high-IMAC group than in the low-IMAC group. Regarding postoperative parameters, the rates of sepsis, SFSG syndrome,

TABLE 4 | Difference in patient characteristic between female high IMAC group and low IMAC group.

Variables	Female donor IMAC		*p*-value
	Low (*n* = 98)	High (*n* = 47)	
Preoperative donor variables			
Age (years)	37 (21–60)	40 (20–64)	0.2281
Graft (right lobe)	72 (73.5%)	34 (72.3%)	0.8859
Actual GV/SLV (%)	40.7 (26.9–63.0)	42.5 (28.0–54.8)	0.5307
Actual GRWR (%)	0.760 (0.509–1.22)	0.783 (0.563–1.16)	0.6636
ABO (Incompatible)	22 (22.5%)	4 (8.51%)	0.0406
Recipient preoperative variables			
Age (years)	57 (23–71)	58 (17–73)	0.9950
Sex (male)	48 (49.0%)	24 (51.1%)	0.8143
Primary diagnosis			
Hepatocellular disease	63 (64.3%)	35 (74.5%)	
Cholestatic disease	27 (27.6%)	4 (8.51%)	
Others	8 (8.16%)	8 (17.0%)	0.0171
HBsAb (yes)	16 (16.3%)	8 (17.2%)	0.9161
HCVAb (yes)	28 (28.6%)	15 (31.9%)	0.6799
HCC (yes)	25 (25.5%)	16 (34.0%)	0.2856
Body mass index (kg/m^2)	23.5 (17.0–30.4)	23.0 (17.2–32.9)	0.8357
ICU or hospital statement (yes)	30 (30.9%)	16 (30.0%)	0.7070
DM (yes)	12 (12.2%)	6 (12.7%)	0.9290
MELD	16 (5–36)	19 (9–45)	0.0005
Splenectomy (yes)	84 (85.7%)	42 (89.4%)	0.5424
Pre-transplant WBC count (x10^3/μL)	4.03 (1.06–15.8)	4.05 (1.04–15.9)	0.0799
Pre-transplant Platelet count (x10^4/μL)	7.05 (1.2–36.2)	5.70 (1.2–24.3)	0.2080
Intraoperative parameters			
Recipient operation time (h)	12.5 (8.10–20.7)	12.6 (9.33–19.3)	0.2366
Recipient blood loss (L)	3.93 (0.45–50.4)	4.42 (0.20–34.7)	0.7987
Cold ischemic time (min)	129 (39–367)	151 (50–255)	0.5455
Warm ischemic time (min)	43 (23–103)	40 (22–83)	0.2999
PVP before the end of operation	15 (7–25)	15 (7–24)	0.6381
Recipient postoperative parameter			
Admission period (day)	27 (9–172)	30 (3–128)	0.2483
Sepsis	2 (2.04%)	7 (14.9%)	0.0027
SFSG syndrome	3 (3.1%)	6 (12.8%)	0.0234
Acute cellular or humoral rejection	10 (10.2%)	2 (4.26%)	0.2236
Graft failure within 6 months	1 (1.02%)	8 (17.0%)	0.0002
Cause of graft loss within 6 months			
Liver failure	1 (100%)	4 (50%)	
Sepsis	0 (0%)	4 (50%)	
Others	0 (0%)	0 (0%)	0.3428

Data are presented as median (range) or n (%).

DM, diabetes mellitus; GRWR, graft recipient weight ratio; GV/SLV, graft volume/recipient standard liver volume ratio; HBsAg, hepatitis B surface antigen, HCC, hepatocellular carcinoma; HCVAb, hepatitis C virus antibody; HCC, hepatocellular carcinoma; ICU, intensive care unit; MELD, Model for End-Stage Liver Disease; PVP, portal vein pressure; SFSG, small-for-size graft.

and graft failure were higher in the high donor IMAC group than in the low donor IMAC group (sepsis; 14.9% vs. 2.41%, p = 0.0027; SFSG syndrome; 12.8% vs. 3.1%, p = 0.0234; graft failure within 6-month; 17.0% vs. 1.02%). In both the male and female analyses, there was no significant difference in the cause of graft loss.

In male donors, there was a significant negative correlation between preoperative donor SMI and donor age (**Figure 3A**, r = −0.1289, p = 0.0484), while for female donors, there was no correlation between the SMI and age (**Figure 3B**, r = −0.0392, p = 0.6400). In all donors, there were significant positive correlations between the preoperative donor IMAC and donor age (**Figures 3C,D**; male; r = 0.1340, p = 0.0401; female; r = 0.1792, p = 0.0310).

Comparison of Graft Function

The male and female SMI analyses revealed no significant difference in the overall graft survival rates within

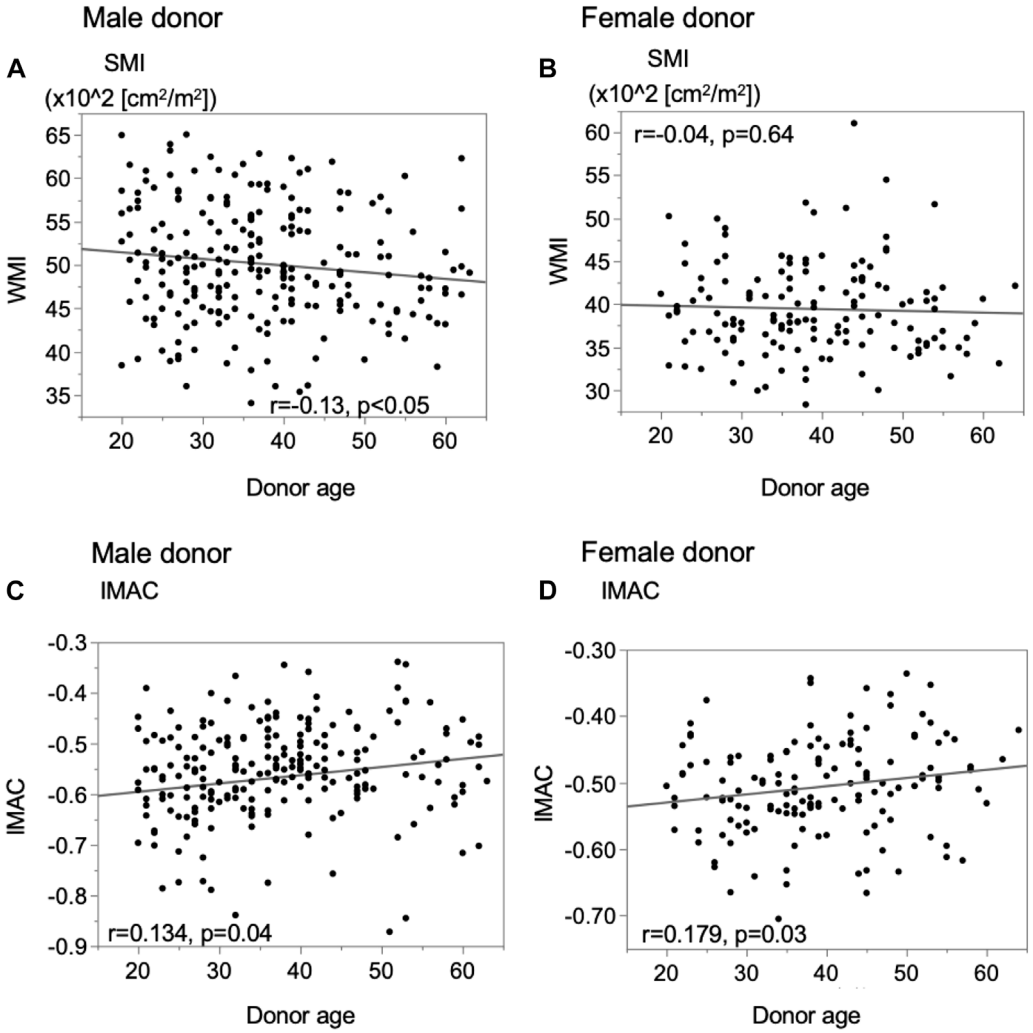


FIGURE 3 | Analysis of the correlation between donor muscle status and donor age. **(A)** Male donor SMI, **(B)** female donor SMI, **(C)** male donor IMAC, and **(D)** female donor IMAC. SMI; muscle mass index, IMAC; intramuscular adipose tissue content.

6 months after LDLT between the low-SMI group and the high-SMI group (**Figures 4A,B**; male; 92.3% vs. 88.4%, $p = 0.3570$; female; 96.4% vs. 91.1%, $p = 0.3128$). The male and female IMAC analyses showed that the overall graft survival rates in the high-IMAC group were lower than those in the low-IMAC group (**Figures 4C,D**; male; 87.7% vs. 95.9%, $p = 0.0210$; female; 83.0% vs. 99.0%, $p < 0.0001$).

The differences in graft function after LDLT between the high- and low-IMAC groups were examined. The serum total-bilirubin (T-bil) level on POD 14, prothrombin-time international normalized ratio (PT-INR) on POD 14, and drained ascites on PODs 14 and 30 were significantly higher in recipients with grafts from high-IMAC donors than from low-IMAC donors (**Figures 5A–D**; T-bil; 6.2 mg/dl vs. 4.5 mg/dl, $p = 0.0042$; PT-INR: 1.15 vs. 1.10, $p = 0.0043$; ascites on POD 14: 425 ml vs. 228 ml, $p = 0.0030$; ascites on POD 30: 95 ml vs. 41 ml, $p = 0.0355$). We examined liver steatosis in 186 LDLT donors with preserved liver biopsy tissue using hematoxylin and eosin staining to assess the correlation between liver steatosis and IMAC. There were 88 high-IMAC patients and 98 low-IMAC patients. No patient had 5% or higher steatosis in either group. There was no significant difference between the groups in the rate of donors with microvascular steatosis (1%–4%) (high IMAC vs. low IMAC: 13.6% vs. 11.2%, $p = 0.6179$).

Risk Factors for Poor Graft Survival in Patients Undergoing LDLT

We performed univariate and multivariate cox regression analyses to examine the predictive factors for graft survival within 6 months after LDLT. **Table 5** shows the results of the multivariate analysis; high donor IMAC (HR; 5.42, CI; 2.13–13.8,

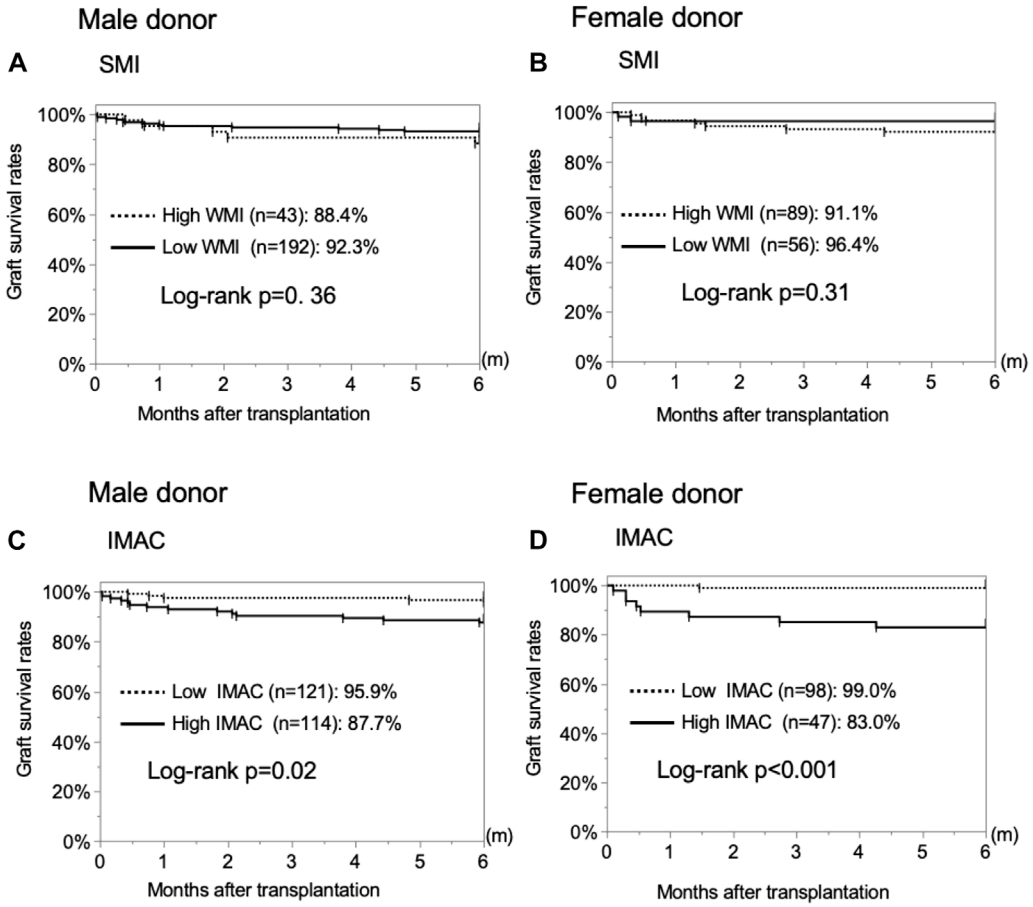


FIGURE 4 | Analysis of the correlation between donor muscle status and 6-month graft survival rates. **(A)** Male donor SMI, **(B)** female donor SMI, **(C)** male donor IMAC, and **(D)** female donor IMAC. SMI, muscle mass index; IMAC, intramuscular adipose tissue content.

p = 0.0004), high MELD score (HR; 2.24, CI; 1.04–4.82, p = 0.0384), and absence of splenectomy (HR; 4.94, CI; 2.24–10.9, p = 0.0001) were independent risk factors for graft failure within 6 months.

Stratification With Predictive Factors for Graft Survival Within 6 months

Next, we examined the significance of donor IMAC for predicting graft survival. We used two risk factors excluding IMAC to stratify the patients into three groups. In the low-risk group, including patients without risk factors, and moderate-risk group, including patients with one risk factor, the graft survival rates in the high-IMAC group were significantly lower than those in the low-IMAC group (low-risk group: 94.1% vs. 98.7%, p = 0.0381; moderate-risk group: 73.1% vs. 96.2%, p = 0.0010) (**Figures 6A,B**). However, there was no significant difference between the high- and low-IMAC groups for the high-risk group (84.6% vs. 71.4%, p = 0.4995) (**Figure 6C**). We divided the patients into four groups according to the presence or absence of the three risk factors, and the graft survival rates were stratified according to the number of risk factors (0 risk factors, 98.7%; 1 risk factor, 94.8%; 2 risk factors, 75.4%; 3 risk factors, 71.4%; p < 0.0001) (**Figure 6D**).

DISCUSSION

In this study, we examined the correlation of the donor SMI and IMAC with graft survival and function in LDLT. A high donor IMAC was correlated with poor graft prognosis and graft function deterioration. We stratified LDLT patients by three risk factors, including high IMAC, high MELD score, and absence of splenectomy, and found that the presence of two or more risk factors significantly reduced graft survival.

The usefulness of the IMAC for predicting the prognosis of patients with various diseases, such as cirrhosis and pancreatic cancer, has been reported, and a high IMAC was shown to correlate with poor prognosis (26, 27). In LDLT, Hamaguchi et al. firstly reported a significant association between the recipient IMAC and recipient early mortality (8). Miyachi et al. reported that combined high SMI and IMAC in male

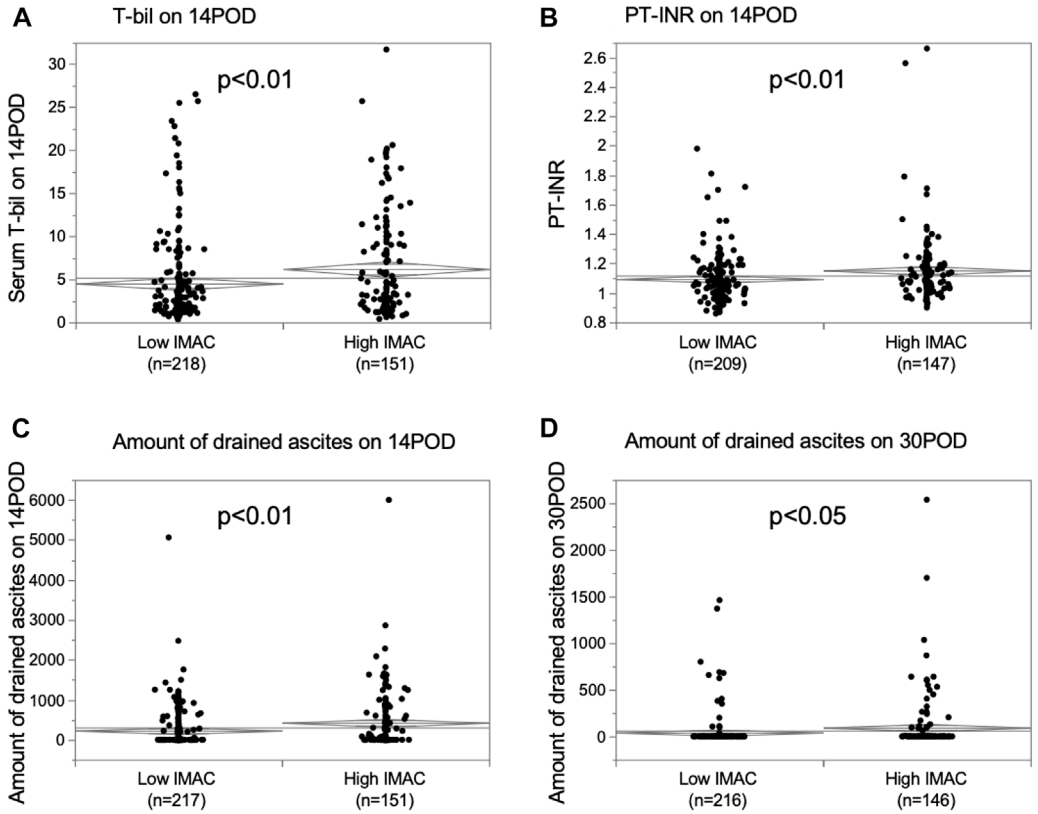


FIGURE 5 | Comparison of graft function in recipients with high IMAC graft and low IMAC graft. **(A)** Serum T-bil on POD 14, **(B)** PT-INR on POD 14, **(C)** amount of ascites on POD 14, **(D)** amount of ascites on POD 30. IMAC, intramuscular adipose tissue content; T-bil, total bilirubin; POD, postoperative day; PT-INR, prothrombin-time international normalized ratio.

donors was an independent protective factor against graft loss after LDLT (28). To our knowledge, this is the only report showing a correlation between donor muscle quality and quantity and graft mortality. Previous studies adjusted for SMI and IMAC with donor age because there was a strong correlation between donor age and donor SMI and IMAC. In our case, there was a significant, but not strong, correlation between the donor IMAC and age. The donor selection criteria varied among institutions, and we also used donor grafts from relatively elderly donors up to 65 years of age. Donors in their 50s and 60s are expected to be relatively healthy with good muscle quality and quantity. These differences in donor selection across facilities may have affected the relationship between donor age, IMAC, and SMI, and further investigations are needed in a larger cohort. Hence, we did not adjust the IMAC for donor age, and both the male and female donor IMAC showed a strong correlation with graft survival within 6 months. In our institution, donors whose body mass index is greater than 25 or who have fatty liver in the preoperative evaluation are placed on a diet before surgery. This may have influenced the relationship between donor age and preoperative IMAC and SMI.

In LDLT, there are rarely ideal conditions in terms of recipient status and donor selection. Some compromises are often necessary in donor selection because of organ shortages. Hence, we examined under which conditions IMAC assessment is useful. In low-risk recipients, whose MELD score was 20 or lower and who could not undergo splenectomy, there was a significant difference in 6-month graft survival rates between the high-IMAC group and low-IMAC group, albeit not by a large margin. Surprisingly, in the moderate-risk group, which included patients with one risk, i.e., high MELD score or absence of splenectomy, the difference was large and significant. This may indicate that donor selection considering the IMAC may be important for moderate-risk recipients scheduled to undergo LDLT. Splenectomy is a risk factor for SFSG syndrome in LDLT, and splenectomy is recommended for recipients with small grafts or portal hypertension (19). However, splenectomy is often difficult after partial splenic embolization or spontaneous bacterial peritonitis before transplantation. Our study showed that donor selection based on the IMAC may improve graft prognosis for recipients who cannot undergo simultaneous splenectomy. Although there have been several reports on graft quality assessment in LDLT, there has been no report on the effectiveness of quality assessment markers. In the future, qualitative markers that consider other background factors should be examined. Donor age has been used as a

TABLE 5 | Predictors of graft loss within 6-month.

Variables	Univariate analysis			Multivariate analysis		
	OR	95%CI	*p*-value	OR	95%CI	*p*-value
Donor variables						
WMI						
High (*n* = 132)	1.00	(References)				
Low (*n* = 248)	0.71	0.668–2.99	0.3656			
IMAC						
Low (*n* = 219)	1.00	(References)		1.00	(References)	
High (*n* = 161)	5.13	2.16–13.1	0.0003	5.42	2.13–13.8	0.0004
Sex						
Male (*n* = 235)	1.31	0.592–2.89 (References)	0.5008			
Female (*n* = 145)	1.00					
Age (year)						
<50 (*n* = 318)	1.00	(References)	0.1985	1.00	(References)	0.0514
≥50 (*n* = 62)	1.75	0.745–4.12		2.46	0.994–6.13	
Graft						
Right (*n* = 196)	1.00	(References)	0.1885	1.00	(References)	0.8211
Others (*n* = 184)	1.66	0.779–3.55		1.10	0.383–2.14	
Actual GV/SLV (%) or GRWR (%)						
≥35 and ≥0.7 (*n* = 235)	1.00	(References)	0.1781	1.00	(References)	0.2189
<35 or <0.7 (*n* = 145)	1.66	0.793–3.49		1.66	0.741–3.70	
ABO incompatible						
No (*n* = 317)	1.00	(References)	0.3941			
Yes (*n* = 63)	0.59	0.179–1.97				
Recipient variables						
Sex						
Male (*n* = 165)	1.15	0.546–2.41 (References)	0.7162			
Female (*n* = 215)	1.00					
Age (years)						
<65 (*n* = 309)	1.00	(References)	0.9003			
≥65 (*n* = 71)	0.94	0.357–2.47				
Preoperative DM						
No (*n* = 314)	1.00	(References)	0.5752			
Yes (*n* = 66)	1.29	0.525–3.19				
Hepatocellular disease						
No (*n* = 122)	1.00	(References)	0.3976			
Yes (*n* = 258)	0.72	0.338–1.54				
HCC						
Without HCC (*n* = 244)	1.00	(References)	0.2387			
With HCC (*n* = 136)	0.60	0.254–1.41				
Preoperative hospital treatment						
No (*n* = 252)	1.00	(References)	0.1662	1.00	(References)	
Yes (*n* = 127)	0.53	0.214–1.30		0.59	0.236–1.46	0.2505
MELD score						
≤21 (*n* = 295)	1.00	(References)	0.0114	1.00	(References)	0.0384
>21 (*n* = 85)	2.74	1.30–5.80		2.24	1.04–4.82	
Splenectomy						
With splenectomy (*n* = 321)	1.00	(References)	<0.0001	1.00	(References)	<0.0001
Without splenectomy (*n* = 59)	4.49	2.13–9.51		4.94	2.24–10.9	
Steatosis						
With microvesicular steatosis (*n* = 23)	1.00	(References)	0.7404			
Without steatosis (*n* = 163)		0.09–5.52				

DM, diabetes mellitus; GRWR, graft recipient weight ratio; GV/SLV, graft volume/recipient standard liver volume ratio; HCC, hepatocellular carcinoma; MELD, Model for End-Stage Liver Disease.

marker for graft quality in LDLT(18). However, liver graft quality does not uniformly decline with donor age, and it is important to assess individual changes in donor grafts because there are individual differences in aging (29). In addition, the several qualitative assessment methods previously reported require liver biopsy (30, 31), while this IMAC examination is not invasive, and the IMAC can be measured with CT images obtained before surgery, with no additional burden on the donor. In our study, the IMAC was a predictive factor for graft failure, although it was correlated with donor age. This

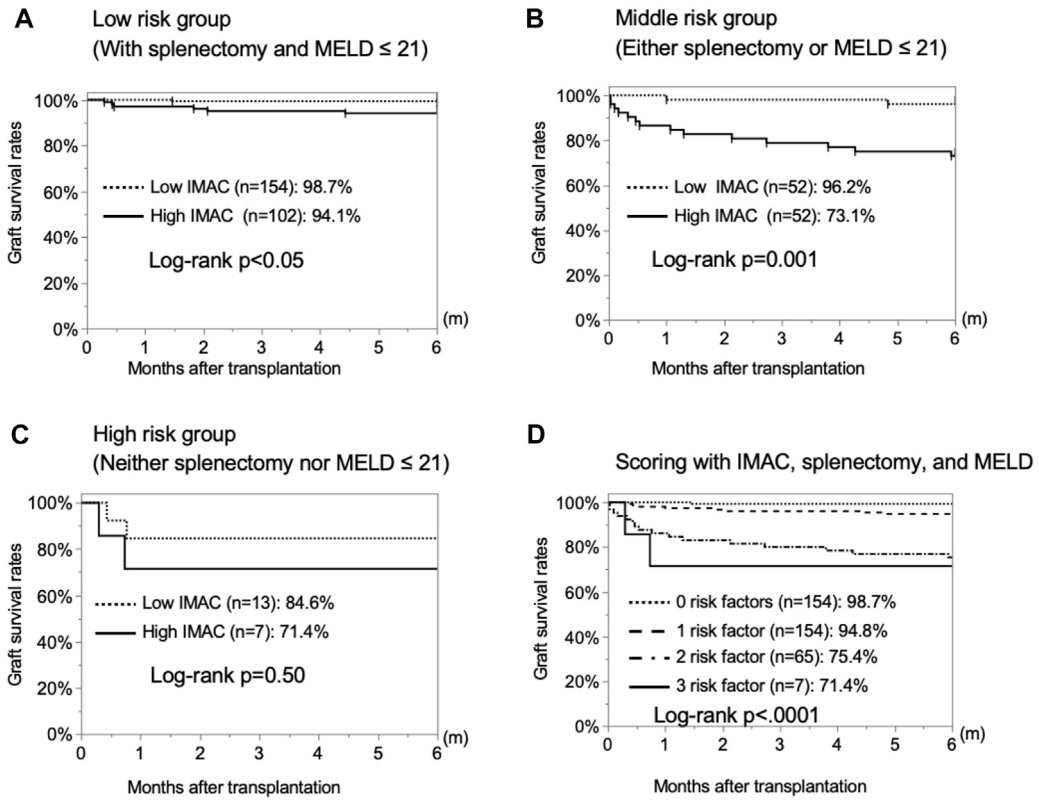


FIGURE 6 | Stratification by risk factors to predict 6-month graft survival rates. The 6-month graft survival rates of recipients with high and low IMAC grafts were examined for each of two risk factors, i.e., absence of splenectomy and high MELD score. **(A)** No risk factors, **(B)** one risk factor, **(C)** two risk factors. **(D)** Stratification for 6-month graft survival rates by the number of present risk factors among the three examined ones.

suggests that the IMAC may represent individual biological aging rather than chronological aging, and further investigations are needed.

The relationships between the musculature and liver are not well understood. Interleukin-6 (IL-6), which is implicated in both liver regeneration and metabolic functions, is secreted into the bloodstream in response to muscle contraction (32). Some epidemiological studies have reported a negative association between the amount of regular body activity and resting plasma IL-6 concentrations(33). With exercise training, IL-6 downregulation is counteracted by increased IL-6 receptor (IL-6R) expression, resulting in increased sensitivity to IL-6 (32). We hypothesized that resting plasma IL-6 concentrations are upregulated by lack of muscle use in donors with a high IMAC, and IL-6R in the liver is downregulated, thereby decreasing sensitivity and disturbing hepatocyte regeneration.

The relationship between the IMAC and graft survival was more pronounced in women. The difference may result from expression of the estrogen receptor. Estrogen is one of the most important molecular markers of liver regeneration, and it has been reported that more estrogen receptors are expressed in the male liver than in the female liver(34,35). Hence, grafts from female donors may have more directly reflected the effects of IL-6.

This study has some limitations. First, this was a retrospective and single-center study. The IMAC needs to be studied on a large scale in the future, as it is a non-invasive examination and can be evaluated using CT scans performed preoperatively. Second, there is no clear answer as to whether the IMAC should be adjusted for age. Our study did not find a strong correlation with age, while others have reported strong correlations. In any case, the IMAC is a useful prognostic marker for graft survival in LDLT, but a large-scale validation may be needed in the future to determine which IMAC or adjusted IMAC is more useful. Third, there were some significant differences in patient characteristics between the high- and low-IMAC groups. We performed univariate and multivariate analyses to more accurately examine the correlation between the IMAC and patient characteristics (**Supplementary Tables S1, S2**) because there were several significant differences in patient characteristics between the high- and low-IMAC groups. A high MELD score was significantly correlated with a high IMAC in female donors. Conversely, there no parameters were significantly correlated with a high IMAC in male donors. In the future, we need to assess the patient characteristics of a different, larger cohort and re-examine the usefulness of the IMAC.

In conclusion, the donor IMAC correlates with graft survival. Thus, the donor IMAC may be useful for predicting graft function and, by extension, graft mortality.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by 2019-354, Kyushu University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conception and design: TTomiy, KTa, and TY; development of methodology: TTomiy, KTa, TK, and TTos; acquisition of data: TTomiy, TY, NH, SI, AM, KTo, YK-F, TTomin, TK, YoN, YuN, TTos, and KM; analysis and interpretation of data: TTomiy, NH, TTos, and TY; writing review and/or revision of manuscript: TTomiy, NH, and TY; study supervision: TY.

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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.2022.10723/full#supplementary-material

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Comparison of Biliary Complications Rates After Brain Death, Donation After Circulatory Death, and Living-Donor Liver Transplantation: A Single-Center Cohort Study

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Donation-after-circulatory-death (DCD), donation-after-brain-death (DBD), and living-donation (LD) are the three possible options for liver transplantation (LT), each with unique benefits and complication rates. We aimed to compare DCD-, DBD-, and LD-LT-specific graft survival and biliary complications (BC). We collected data on 138 DCD-, 3,027 DBD- and 318 LD-LTs adult recipients from a single center and analyzed patient/graft survival. BC (leak and anastomotic/non-anastomotic stricture (AS/NAS)) were analyzed in a subset of 414 patients. One-/five-year graft survival were 88.6%/70.0% for DCD-LT, 92.6%/79.9% for DBD-LT, and, 91.7%/82.9% for LD-LT. DCD-LTs had a 1.7-/1.3-fold adjusted risk of losing their graft compared to DBD-LT and LD-LT, respectively ($p < 0.010/0.403$). Bile leaks were present in 10.1% (DCD-LTs), 7.2% (DBD-LTs), and 36.2% (LD-LTs) (ORs, DBD/LD vs. DCD: 0.7/4.2, $p = 0.402/<0.001$). AS developed in 28.3% DCD-LTs, 18.1% DBD-LTs, and 43.5% LD-LTs (ORs, DBD/LD vs. DCD: 0.5/1.8, $p = 0.018/0.006$). NAS was present in 15.2% DCD-LTs, 1.4% DBDs-LT, and 4.3% LD-LTs (ORs, DBD/LD vs. DCD: 0.1/0.3, $p = 0.001/0.005$). LTs w/o BC had better liver graft survival compared to any other groups with BC. DCD-LT and LD-LT had excellent graft survival despite significantly higher BC rates compared to DBD-LT. DCD-LT represents a valid alternative whose importance should increase further with machine/perfusion systems.

Keywords: liver transplantation, living donors, donation after brain death, donation after circulatory death, biliary anastomotic stricture, ischemic cholangiopathy, bile leak

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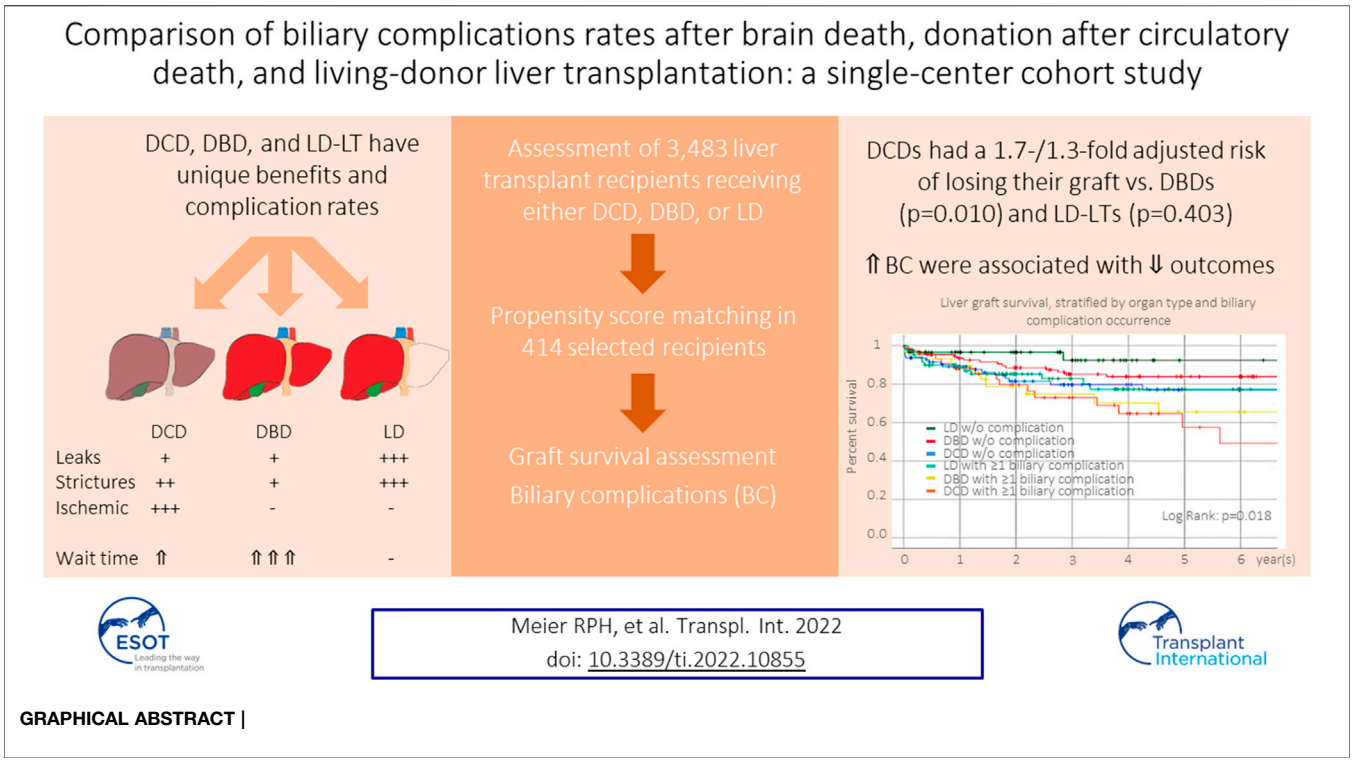

GRAPHICAL ABSTRACT |

INTRODUCTION

In regions with a high average Model for End-stage Liver Disease (MELD) score at transplant, organs from donation after brain death (DBD) donors often go to the sicker patients with high MELD scores, and so for patients with liver cancer and/or a low MELD score, organs from donation-after-circulatory-death (DCD) donors and living donors (LD) [1, 2] represent alternatives for liver transplantation (LT). DCD donors are increasingly used for LT in an effort to address organ scarcity and to decrease waiting-list mortality [3]. It is well recognized that DCD livers expose the recipient to increased risk from the inevitably longer donor warm ischemia time (dWIT). Aside from primary nonfunction [4], the most feared complication, and one of the main reasons for graft loss, is ischemic cholangiopathy (IC), defined as the appearance of intrahepatic non-anastomotic biliary strictures (NAS), which occurs in 10%–50% of cases [5–9]. The increasing use of normothermic preservation machines (NMP) might significantly modify these complication rates [10]. However, to date, NMP is not broadly available, and many US centers still avoid DCDs or apply very strict donor selection criteria [9]. In this regard, we and others have developed scores to select donors/recipients in order to optimize outcomes with a special emphasis on minimizing biliary complications [11–14]. Known risk factors for IC are donor age (>40 years) [6, 15], prolonged cold ischemic time (CIT) (>8 h) [6], prolonged dWIT (>20 min), low venous oxygen saturation ($SvO_2 \leq 60$) [15], and donor liver extraction time [8, 13]. Besides IC, other relevant ischemic complications include anastomotic biliary strictures (AS) and bile leaks which were previously shown to range between 10% and 15% in DCD cases, and not be significantly different from DBD rates [6]. Just as the use of DCD grafts has increased in recent years, so has the use of LD-LT in order to further increase organ availability [16]. The outcomes are overall excellent [17], however, a higher risk of biliary complication is present as well with anastomotic biliary stenosis and leak ranging from 10% to 35% in different series [16, 18–21]. The difficulties encountered by patients experiencing recurrent biliary issues added to the minimal, but a non-null, risk to the living donor [22] and variable access to LD, warrants a thorough assessment and selection of both donor and recipient by the transplant team.

For a given patient with all three options, the choice might be difficult to make since each modality has unique benefits, risks, and potential complications. We sought to compare biliary complications and graft survival between DCD-, DBD-, and LD-LT at a single center, with the intention to provide more data for guiding the decision between these three possible options for transplantation.

METHODS

Study Design and Patients

Approval was obtained by the Institutional Review Board of the University. Donor and recipient data were extracted from the UNOS database and included all consecutive adult liver transplants performed at the University Medical Center between 1989 and 2019 (n = 3,483), which included 138 DCD, 318 LD, and 3,027 DBD (**Table 1**). 138 DCD-LTs were compared to 138 DBD-LTs (selected using a propensity score matching

TABLE 1 | Recipient and donor baseline characteristics of donation after cardiac death, donation after brainstem death, and living donor liver transplantation.

Characteristics	DCD LT (*n* = 138)	DBD LT (*n* = 3,027)	LD LT (*n* = 318)	P-value[a]	P-value[b]
Recipient					
Age at transplant, years	57.5 ± 9.0	53.3 ± 10.7	53.9 ± 11.1	<0.001	<0.001
Gender (%)					
Male	103 (74.6)	1,942 (64.2)	158 (49.7)	0.012	<0.001
Female	35 (25.4)	1,085 (35.8)	160 (50.3)		
Pretransplant BMI, kg/m^2	27.9 ± 5.9	27.3 ± 5.9	26.2 ± 4.6	0.236	0.003
Ethnicity (%)					
American Indian	2 (1.4)	33 (1.1)	2 (0.6)	0.428	0.652
Asian	18 (13.0)	491 (16.2)	27 (8.5)		
Black	4 (2.9)	180 (5.9)	11 (3.5)		
Native Hawaiian	1 (0.7)	30 (1.0)	2 (0.6)		
Hispanic	38 (27.5)	658 (21.7)	81 (25.5)		
Multiracial	0 (0.0)	16 (0.5)	1 (0.3)		
White	75 (54.3)	1,619 (53.5)	194 (61.0)		
Etiology					
A1AT	1 (0.7)	13 (0.4)	2 (0.6)	<0.001	<0.001
Auto-immune	4 (2.9)	81 (2.7)	13 (4.1)		
Amyloidosis	1 (0.7)	0 (0.0)	0 (0.0)		
Biliary atresia	1 (0.7)	2 (0.1)	3 (0.9)		
Cholangiocarcinoma	2 (1.4)	4 (0.1)	0 (0.0)		
Cryptogenic	3 (2.2)	205 (6.8)	23 (7.2)		
EtOH	29 (21.0)	363 (12.0)	41 (12.9)		
HBV	12 (8.7)	324 (10.7)	24 (7.5)		
HCV	68 (49.3)	874 (28.9)	84 (26.4)		
NASH	9 (6.5)	132 (4.4)	25 (7.9)		
Other	0 (0.0)	780 (25.8)	39 (12.3)		
PBC	2 (1.4)	112 (3.7)	28 (8.8)		
PSC	3 (2.2)	121 (4.0)	36 (11.3)		
Wilson	3 (2.2)	16 (0.5)	0 (0.0)		
HCC					
Presence	40 (29.0)	586 (19.4)	50 (15.7)	0.005	0.001
Absence	98 (71.0)	2,441 (80.6)	268 (84.3)		
Median MELD, IRQ	23 (12–32)	38 (31–40)	18 (13–26)	<0.001	0.008
Era					
1989–2000	0 (0.0)	912 (30.1)	10 (3.1)	<0.001	<0.001
2001–2010	26 (18.8)	1,038 (34.3)	128 (40.3)		
2011–2018	112 (81.2)	1,077 (35.6)	180 (56.6)		
Donor factors					
Age, years	31.7 ± 10.3	39.5 ± 16.7	36.5 ± 10.8	<0.001	<0.001
Gender (%)					
Male	92 (66.7)	1,803 (59.6)	163 (51.3)	0.096	0.002
Female	46 (33.3)	1,224 (40.4)	155 (48.7)		
BMI, kg/m^2	25.5 ± 5.2	26.5 ± 6.1	25.8 ± 4.4	0.041	0.557
Ethnicity (%)					
American Indian	0 (0.0)	19 (0.6)	0 (0.0)	0.132	0.070
Asian	4 (2.9)	226 (7.5)	28 (8.8)		
Black	11 (8.0)	234 (7.7)	11 (3.5)		
Hispanic	34 (24.6)	677 (22.4)	69 (21.7)		
Multiracial	4 (2.9)	30 (1.0)	6 (1.9)		
Native Hawaiian	0 (0.0)	27 (0.9)	1 (0.3)		
Unknown	0 (0.0)	9 (0.3)	0 (0.0)		
White	85 (61.6)	1,805 (59.6)	203 (63.8)		
Cause of death					
Anoxia	73 (52.9)	579 (19.1)	NA	<0.001	NA
Cerebrovascular	17 (12.3)	1,213 (40.1)			
CNS tumor	0 (0.0)	8 (0.3)			
Head trauma	41 (29.7)	1,061 (35.1)			
Not reported	0 (0.0)	9 (0.3)			
Other	7 (5.1)	157 (5.2)			

(Continued on following page)

TABLE 1 | (*Continued*) Recipient and donor baseline characteristics of donation after cardiac death, donation after brainstem death, and living donor liver transplantation.

Characteristics	DCD LT (*n* = 138)	DBD LT (*n* = 3,027)	LD LT (*n* = 318)	P-value[a]	P-value[b]
Cold ischemic time, hours	7.7 ± 2.6	9.0 ± 3.9	2.4 ± 2.6	<0.001	<0.001
Donor warm ischemia time, minutes	20 ± 6	NA	NA	NA	NA
Donor hepatectomy time, minutes	41 ± 16	NA	NA	NA	NA

Data are presented as mean ± standard deviation or n (%), unless specified otherwise.

DCD, donation after cardiac death; DBD, donation after brainstem death; LD, living donor; LT, liver transplantation; BMI, body mass index, EtOH, ethanol use; HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; A1AT, alpha-1 antitrypsin; MELD, Model For End-Stage Liver Disease; CNS, central nervous system; IRQ, interquartile range.

[a]*DCD versus DBD.*

[b]*DCD versus LD. Student t-test for continuous variables, X^2 test for binary or categorical variables (global p-value).*

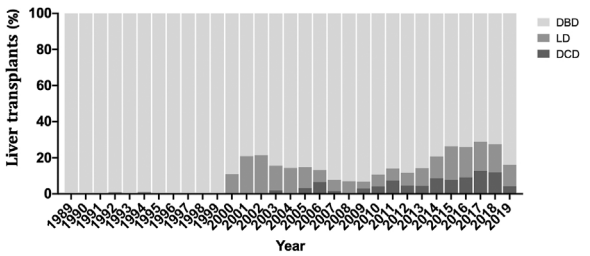

FIGURE 1 | Percentage of recipient receiving a donation after cardiac death (DCD), donation after brainstem death (DBD), or living donor liver transplantation (LD) over the study period.

technique), and 138 randomly selected LD-LTs. Ischemia times were defined as previously described [13]. Donor and recipient selection and procedures were performed as previously described [13, 23, 24]. DCD grafts were procured using the super-rapid technique with local modifications [25]. Ischemic cholangiopathy was defined by the presence of intrahepatic, non-anastomotic biliary strictures (NAS) and dilatations occurring in the absence of ductopenic rejection or recurrent primary sclerosing cholangitis. When suspected (increased alkaline phosphatase and bilirubin), NAS was diagnosed on endoscopic retrograde cholangiopancreatography (ERCP) and/or Magnetic Resonance Imaging (MRI). One DBD recipient developed secondary NAS after a hepatic artery thrombosis. The occurrence of AS and biliary leaks were collected from patients' chart reviews. The median follow-up was 6 years (min-max, 0–29 years) for the entire cohort (n = 3,483) and 3 years (min-max, 0–27 years) for the 1:1 control cohort (n = 414). In the entire cohort (n = 3,483), MELD had 1% missing data, recipient BMI and CIT had 6% missing data, and all the other variables had no missing data. In the 1:1 matched control cohort (n = 414), CIT and dWIT had 1% missing data, and all the other variables had no missing data.

Patient Selection, Organ Allocation, and Operation

Patients diagnosed with end-stage liver disease were evaluated for candidacy by a multidisciplinary team and placed on the transplant waiting list [24]. Before 2002, the United Network for Organ Sharing (UNOS) criteria were used to determine priority (no DCD-LT was performed during this time). From 2002 to present, the MELD allocation system has been used [26]. Organ selection and LT were performed as previously described [24]. All liver grafts were perfused with University of Wisconsin solution (hepatic artery and portal vein). LT was performed as previously described [24], typically utilizing the piggyback technique and duct-duct biliary anastomosis.

Statistical Analysis

Continuous variables were expressed as means, and standard deviations (SD) and categorical variables were expressed as counts and percentages. Comparison between groups was performed using the Student's t-test for continuous variables and the chi-squared test for binary or categorical variables. Propensity score matching for each patient was generated using a multivariable binary logistic regression model. DCD patients were matched 1:1 with DBD patients using recipient age, sex, and pretransplant BMI as well as donor age, sex, BMI, and cold ischemia time as a covariate with a caliper of 0.01. Due to the lower number of LD cases and the limited value of selecting one specific matching variable over another, 138 LD-LT recipients were randomly selected for comparison. To ensure that random matching was appropriate, we performed a sensitivity analysis using optimal full propensity score matching restricted to observations that had propensity scores in the extended common support region (0.06–0.75). Acceptable balance was defined by a maximum of 0.1 for the absolute value of standardized difference and by values within the 0.5–2 range for variance ratio. Survival analyses were performed using the Kaplan–Meier method and the log-rank test. Uni-/multivariate Cox proportional-hazard regression was used to compute hazard ratios (HR). We used IBM SPSS Statistics version 26 and SAS version 9.4 for all computations (IBM Corp. Armonk, NY). Ninety-five percent confidence intervals (95%CI) were reported, and an exact two-sided p-value <0.05 was considered statistically significant.

RESULTS

Patient Characteristics

During the study period, 3,483 liver transplants were performed, including 138 DCD, 3,027 DBD and 318 LD (**Figure 1**; **Table 1**). Compared to DBD, DCD recipients were significantly older and

TABLE 2 | Estimated hazard ratios for liver graft survival using a uni-/multivariate Cox proportional hazard model.

Variables	Univariate analysis[a]			Multivariate analysis[b]		
	HR	95% CI	P-value	HR	95% CI	P-value
Recipient factors						
Age at transplant, years	1.0	1.0–1.0	0.029	1.0	1.0–1.0	0.002
Gender, male	1.0	0.9–1.2	0.323	NA	NA	NA
Pretransplant BMI, kg/m^2	1.0	1.0–1.0	0.109	NA	NA	NA
Race/Ethnicity						
American Indian	1.3	0.8–2.2	0.368	NA	NA	NA
Asian	0.8	0.7–1.0	0.014	0.8	0.7–1.0	0.013
African American	1.2	0.9–1.5	0.126	NA	NA	NA
Native Hawaiian	0.5	0.2–1.2	0.123	NA	NA	NA
Hispanic	0.9	0.8–1.0	0.073	0.9	0.7–1.0	0.128
Multiracial	0.6	0.2–1.6	0.288	NA	NA	NA
Etiology						
Auto-immune	0.8	0.5–1.1	0.099	0.8	0.6–1.1	0.217
Amyloidosis	0.1	0.0 - NR	0.797	NA	NA	NA
Biliary atresia	1.8	0.4–7.2	0.412	NA	NA	NA
Cholangiocarcinoma	3.8	1.4–10.2	0.008	4.4	1.6–11.8	0.004
Cryptogenic	1.1	0.9–1.4	0.217	NA	NA	NA
EtOH	1.1	0.9–1.3	0.499	NA	NA	NA
HBV	0.9	0.7–1.1	0.272	NA	NA	NA
HCV	1.2	1.0–1.3	0.026	1.1	1.0–1.3	0.071
NASH	0.8	0.5–1.1	0.178	NA	NA	NA
PBC	1.0	0.8–1.3	0.926	NA	NA	NA
PSC	1.0	0.8–1.3	0.873	NA	NA	NA
Wilson	0.1	0.2–1.0	0.049	0.0	0.0 - NA	0.862
A1AT	0.4	0.1–1.5	0.173	NA	NA	NA
Other/unknown	0.9	0.8–1.1	0.253	NA	NA	NA
HCC	1.1	0.9–1.3	0.331	NA	NA	NA
MELD	1.0	1.0–1.0	0.037	1.0	1.0–1.0	0.945
Era						
1990–2000	NA	1 [Reference]	NA	NA	NA	NA
2001–2010	0.7	0.6–0.8	<0.001	0.7	0.6–0.8	<0.001
2011–2018	0.6	0.5–0.8	<0.001	0.6	0.5–0.7	<0.001
Donor factors						
Donor type						
DCD	NA	1 [Reference]	NA	NA	NA	NA
DBD	0.8	0.6–1.2	0.250	0.6	0.4–0.9	0.010
LD	0.7	0.5–1.1	0.153	0.8	0.5–1.4	0.403
Age, years	1.0	1.0–1.0	<0.001	1.0	1.0–1.0	<0.001
Gender, male	0.9	0.8–1.1	0.304	NA	NA	NA
BMI, kg/m^2	1.0	1.0–1.0	0.737	NA	NA	NA
Race/Ethnicity						
American Indian	0.7	0.3–2.0	0.537	NA	NA	NA
Asian	1.0	0.8–1.2	0.794	NA	NA	NA
African American	1.2	0.9–1.4	0.185	NA	NA	NA
Hispanic	1.0	0.8–1.1	0.571	NA	NA	NA
Multiracial	0.6	0.3–1.4	0.217	NA	NA	NA
Native Hawaiian	1.6	1.0–2.7	0.073	2.2	1.3–3.6	0.004
Unknown	0.8	0.3–2.1	0.631	NA	NA	NA
White	1.0	0.9–1.1	0.991	NA	NA	NA
Cause of death						
Anoxia	0.8	0.8–0.9	0.010	0.9	0.7–1.1	0.332
Cerebrovascular	1.2	1.1–1.3	0.004	1.0	0.8–1.1	0.580
Head trauma	1.0	0.8–1.1	0.434	NA	NA	NA
CNS tumor	1.2	0.4–3.1	0.758	NA	NA	NA
Other	1.1	0.9–1.3	0.566	NA	NA	NA
Not reported	0.9	0.7–1.1	0.291	NA	NA	NA
Cold ischemic time, hours	1.0	1.0–1.0	0.015	1.0	1.0–1.0	0.026

DCD, Donation after cardiac death; DBD, donation after brainstem death; LD, living donor; LT, liver transplantation; BMI, body mass index, EtOH, ethanol use; HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis, PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; A1AT, alpha-1 antitrypsin; MELD, Model For End-Stage Liver Disease; CNS, central nervous system; BMI, body mass index; CI, confidence interval; HR, hazard ratio; NR, not reported (values superior to 10^6); DCD, donation after cardiac death; DBD, donation after brainstem death (DBD); LD, living donor.

[a]*Univariate Cox proportional-hazards regression model.*

[b]*Multivariate Cox regression model. Only those variables with* $p < 0.1$ *or of key clinical interest (graft type) in the univariate analysis were entered in the multivariate analysis.*

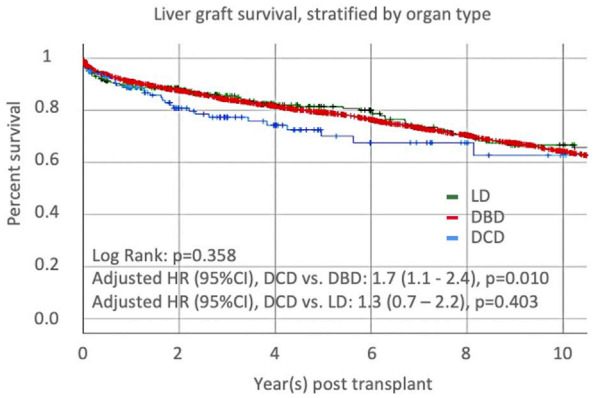

FIGURE 2 | Liver graft survival, stratified by organ type: donation after cardiac death (DCD), donation after brainstem death (DBD), or living donor liver transplantation (LD).

more likely to be males. The top two indications in DCD-LTs were cirrhosis from alcohol (EtOH) use and hepatitis C virus (HCV), and more recipients had hepatocellular carcinoma (HCC) compared to DBD-LTs. The median (interquartile range (IRQ)) MELD in DCD recipients was 23 (12–32) versus 38 (31–40) in DBD recipients ($p < 0.001$). DCD donors and LD were younger compared to DBD donors. Other baseline differences are shown in **Table 1**.

Graft Survival

Univariate Cox proportional-hazards regression identified several recipient and donor factors associated with graft loss (**Table 2**). After adjustment for variables with p-value<0.1 in the univariate model or key variable of interest (graft type), the multivariate Cox regression model identified older recipient age and Asian race, the presence of cholangiocarcinoma, era, the use of a DCD graft (compared to a DBD graft), a graft from a Native Hawaiian donor, older donor age, and increased CIT as independent risk factors for graft loss. CIT was not different between Native Hawaiian donors and non-Native Hawaiian donors, 9.7 h vs. 8.6 h, $p = 0.132$. Recipients receiving DCD grafts were 1.7 times more likely to lose their graft compared to DBD grafts, $p = 0.010$, and 1.3- times more compared LD grafts, $p = 0.410$. Protective factors against graft loss included Asian recipient ethnicity and recent transplantation era. We represented the distribution of groups within the different era (**Supplementary Figure S2A**) and confirmed the improvement of outcomes, overall and for DCD-LT, DBD-LT, and LD-LT independently (**Supplementary Figures S2B,C**). We confirmed that Graft survival at 1- and 5-year were 88.6% and 70.0% for DCD-LT, 92.6% and 79.9% for DBD-LT, and, 91.7% and 82.9% for LD-LT. Kaplan-Meier graft survival curves are shown in **Figure 2**.

Outcomes of donor after cardiac death liver transplant recipients compared to paired donation after brain death and living donor recipients.

Three groups of 138 LT recipients were constituted based on graft donation type (**Table 3**). Propensity matching allowed correction for most of the baseline variables between DCD and DBD donor/recipient characteristics. The etiology of liver disease, MELD score, HCC status, and era remained significantly different between groups. Out of 318 LD-LT recipients, 138 were randomly selected to be compared to DCDs. The sensitivity analysis included 265 LD-LT recipients. The baseline differences between the whole dataset and either the randomly matched or the propensity score-matched group remained unchanged. The differences in organ survival curves between the three donor types were mostly unchanged compared to the whole dataset (**Supplementary Figure S1**). Overall, eighteen LT recipients (4.3%, 18/414) had arterial complications (thrombosis and stenosis; no difference between groups). Eighteen patients (4.3%) were retransplanted, and there was no significant difference in retransplant rate between groups.

Biliary Complications

We studied the occurrence of anastomotic and non-anastomotic stricture and bile leak in the 414 adult recipients selected, as noted above. Anastomotic biliary strictures occurred in 28.3% of DCD recipients. Compared to DCD-LT, DBD recipients had fewer anastomotic strictures (18.1%), and LD recipients had more anastomotic strictures (43.5%) (**Table 4**; **Figure 3A**). Non-anastomotic biliary strictures developed in 15.2% of DCD recipients versus 1.4% of DBD recipients and 4.3% of LD recipients (**Table 4**; **Figure 3B**). NASs were observed much sooner after transplant in DCDs (median: 59 days) compared to DBDs (median: 409 days) or LDs (median: 172 days). Bile leak was observed in 10.1% of DCD recipients versus 7.2% of DBD recipients and 36.2% of LD recipients (**Table 4**; **Figure 3C**). Bile leaks usually occur between two to four weeks post-transplant. Patients who had leaks (regardless of the donor group) had more than a 4-time risk of developing AS and a three-time risk of developing NAS [HR (95%CI), 4.4 (3.1–6.4), $p < 0.001$ and HR (95%CI), 3.5 (1.7–7.4), $p = 0.001$), respectively]. Graft survival in the three groups ($n = 414$) was further stratified by organ type and biliary complication occurrence (none versus any) (**Figure 3D**). LD-LTs free of any biliary complications had the best graft survival, whereas DCD-LTs with ≥1 biliary complication (presence of a bile leak and/or AS and/or NAS) had the worst graft survival (global $p = 0.018$) (**Figure 3D**). Among the 21 DCD-LT patients with non-anastomotic strictures, six died (contraindication to retransplantation), two were retransplanted, three remain stent dependent, and notably, half ($n = 10$) are ultimately stent-free. LT recipients with NAS had worse graft survival compared to the NAS-free patients ($p < 0.05$). Patients with NAS had a median (IRQ) of 7 (5–10) ERCPs. We searched potential risk factors for any biliary complications in the matched/paired cohort ($n = 414$) (**Table 5**). After multivariate adjustment, the use of DBD grafts/donors with head trauma were found to be a protective factor against the occurrence of biliary complication(s). Higher donor BMI was associated with more biliary complications.

TABLE 3 | Recipient and donor baseline characteristics of donation after cardiac death donation, and matched/paired control recipients receiving a graft after brainstem death and living donor.

Characteristics	DCD LT (*n* = 138)	DBD LT (*n* = 138)	LD LT (*n* = 138)	P-value[a]	P-value[b]
Recipient					
Age at tx. years	57.5 ± 9.0	57.8 ± 10.6	55.0 ± 11.2	0.797	0.041
(min–max)	(22–75)	(18–72)	(18–75)		
Gender (%)					
Male	103 (74.6)	98 (71.0)	67 (48.6)	0.499	<0.001
Female	35 (25.4)	40 (29.0)	71 (51.4)		
Pretransplant BMI. kg/m^2	27.9 ± 5.9	27.4 ± 5.6	25.8 ± 4.3	0.478	0.001
Race/Ethnicity (%)					
White	75 (54.3)	64 (46.4)	78 (56.5)	0.053	0.583
African American	4 (2.9)	15 (10.9)	6 (4.3)		
Hispanic	38 (27.5)	29 (21.0)	41 (29.7)		
Asian	18 (13.0)	27 (19.6)	12 (8.7)		
Hawaii	1 (0.7)	2 (1.4)	1 (0.7)		
American Indian	2 (1.4)	1 (0.7)	0 (0.0)		
Etiology					
A1AT	1 (0.7)	1 (0.7)	2 (1.4)	<0.001	<0.001
Auto-immune	4 (2.9)	0 (0.0)	7 (5.1)		
Amyloidosis	1 (0.7)	0 (0.0)	0 (0.0)		
Biliary atresia	1 (0.7)	0 (0.0)	2 (1.4)		
Cholangiocarcinoma	2 (1.4)	2 (1.4)	0 (0.0)		
Cryptogenic	3 (2.2)	8 (5.8)	6 (4.3)		
EtOH	29 (21.0)	4 (2.9)	23 (16.7)		
HBV	12 (8.7)	18 (13.0)	11 (8.0)		
HCV	68 (49.3)	76 (55.1)	31 (22.5)		
NASH	9 (6.5)	5 (3.6)	19 (13.8)		
Other	0 (0.0)	3 (2.2)	12 (8.7)		
PBC	2 (1.4)	0 (0.0)	11 (8.0)		
PSC	3 (2.2)	17 (12.3)	14 (10.1)		
Wilson	3 (2.2)	4 (2.9)	0 (0.0)		
HCC					
Presence	40 (29.0)	60 (43.5)	27 (19.6)	0.012	0.068
Absence	98 (71.0)	78 (56.5)	111 (80.4)		
MELD	22.8 ± 11.1	34.2 ± 5.9	18.6 ± 7.5	<0.001	<0.001
Era					
1990–2000	0 (0.0)	19 (13.8)	0 (0.0)	<0.001	<0.001
2001–2010	26 (18.8)	31 (22.5)	1 (0.7)		
2011–2018	112 (81.2)	88 (63.8)	137 (99.3)		
Donor factors					
Age, years	31.7 ± 10.3	31.5 ± 13.5	35.7 ± 10.5	0.912	0.001
Gender (%)					
Male	92 (66.7)	87 (63.0)	64 (46.4)	0.528	0.001
Female	46 (33.3)	51 (37.0)	74 (53.6)		
BMI, kg/m^2	25.5 ± 5.2	25.9 ± 7.1	25.6 ± 4.0	0.533	0.849
Race/Ethnicity (%)					
American Indian	0 (0.0)	2 (1.4)	0 (0.0)	0.293	0.157
Asian	4 (2.9)	8 (5.8)	12 (8.7)		
African American	11 (8.0)	11 (8.0)	5 (3.6)		
Hispanic	34 (24.6)	43 (31.2)	39 (28.3)		
Multiracial	4 (2.9)	2 (1.4)	4 (2.9)		
Hawaii	0 (0.0)	1 (0.7)	1 (0.7)		
White	85 (61.6)	71 (51.4)	77 (55.8)		
Cause of death					
Anoxia	73 (52.9)	40 (29.0)	NA	<0.001	NA
Cerebrovascular	17 (12.3)	35 (25.4)			
Head trauma	41 (29.7)	62 (44.9)			
Not reported	7 (5.1)	1 (0.7)			
Cold ischemic time, hours	7.7 ± 2.6	7.6 ± 3.5	2.1 ± 1.5	0.680	<0.001

Data are presented as mean ± standard deviation or n (%).

DCD, donation after cardiac death; DBD, donation after brainstem death; LD, living donor; LT, liver transplantation; BMI, body mass index, EtOH, ethanol use; HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; A1AT, alpha-1 antitrypsin; MELD, Model For End-Stage Liver Disease; CNS, central nervous system.

[a]*DCD versus DBD.*

[b]*DCD versus LD. Student t-test for continuous variables, X^2 test for binary or categorical variables (global p-value).*

TABLE 4 | Biliary complications in liver transplant recipients after receiving a liver from a cardiac death donor, a brainstem death donor, or living donor.

Characteristics	DCD LT (*n* = 138)	DBD LT (*n* = 138)	LD LT (*n* = 138)	Odds ratio P-value[a]	Odds ratio P-value[b]
Anastomotic biliary stricture	39 (28.3)	25 (18.1)	60 (43.5)	0.5 (0.3–0.9)	1.8 (1.2–2.6)
Time to stricture, d (min–max)	87.7 (0.0–2,191.5)	98.6 (0.0–5,113.5)	54.8 (0.0–1,826.3)	0.018	0.006
Non-anastomotic biliary stricture	21 (15.2)	2 (1.4)	6 (4.3)	0.1 (0.0–0.4)	0.3 (0.1–0.7)
Time to stricture, d (min–max)	59.0 (24.0–551.0)	409.0 (53.0–765.0)	172.0 (18.0–722.0)	0.001	0.005
Bile leak (%)	14 (10.1)	10 (7.2)	50 (36.2)	0.7 (0.3–1.6)	4.2 (2.3–7.7)
Time to bile leak, d (min–max)	27.5 (1.0–334.0)	16.5 (6.0–199.0)	17.5 (1.0–169.0)	0.402	<0.001

Data are presented as median (minimum–maximum) or n (%).
DCD, donation after cardiac death; DBD, donation after brainstem death; LD, living donor; LT, liver transplantation.
[a]*DBD versus DCD.*
[b]*LD versus DCD. Student t-test for continuous variables, X^2 test for binary variables.*

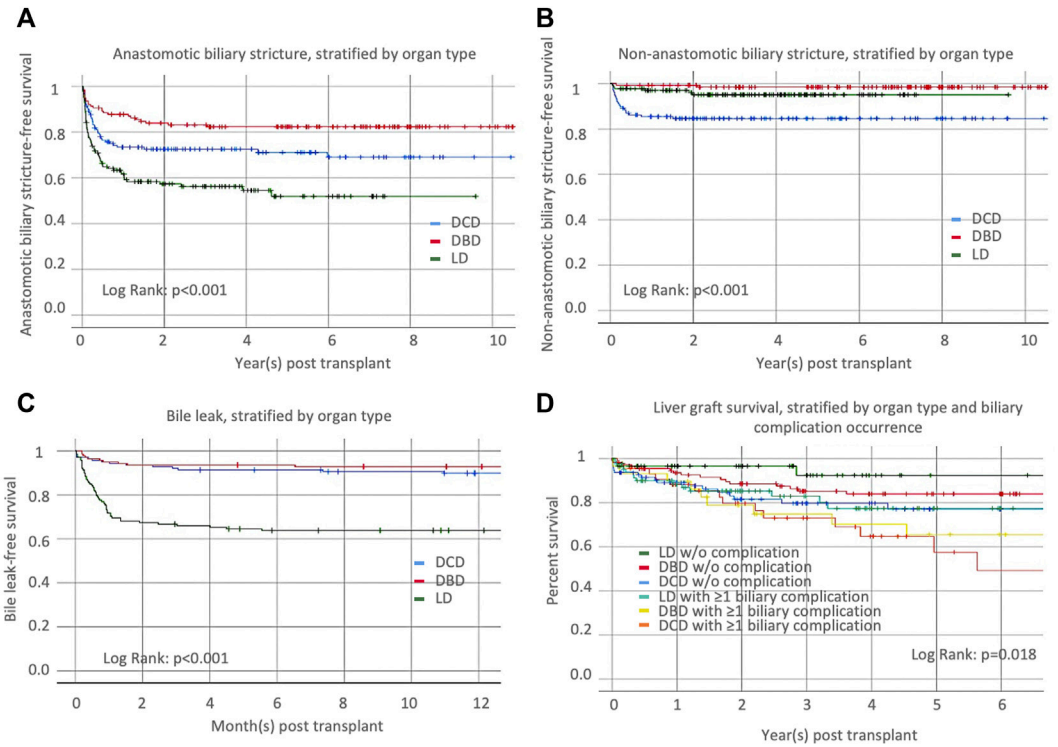

FIGURE 3 | Occurrence of **(A)** anastomotic biliary stricture, **(B)** non-anastomotic biliary stricture, and **(C)** bile leaks, stratified by organ type [donation after cardiac death (DCD), donation after brainstem death (DBD), or living donor liver transplantation (LD)]. **(D)** Liver graft survival, stratified by organ type and biliary complication occurrence.

DISCUSSION

Liver transplantation using DCD or LD donors is limited to a minority of centers because of the higher rates of ischemic cholangiopathy (DCD-LTs) or biliary complications (DCD and LD-LTs) compared with grafts from DBD donors [7, 11, 14, 23, 27]. Nevertheless, DCD- and LD-LTs often represent the only life-saving option for specific liver recipient candidates in a MELD-based allocation system. For these patients, the benefits of receiving a DCD or LD graft have the potential to increase survival and quality of life compared to staying on the transplant waiting list. Over the last few years, refinement in donor and recipient selection has allowed a significant improvement in outcomes for DCD-LT [11, 12, 14]. However, for many patients, waiting for a DBD, involving an LD, or taking a DCD offer remains a common dilemma. We thus sought to analyze and compare the outcomes and biliary complications of DCDs to DBDs and LDs in a single-center LT recipient population.

We first observed and confirmed the known increased risk of graft loss (HR of 1.7) in recipients receiving DCD livers compared to those receiving DBD grafts, which matches with previously

TABLE 5 | Estimated hazard ratios for biliary complication (any versus none) using a uni-/multivariate Cox proportional hazard model.

Variables	Univariate analysis[a]			Multivariate analysis[b]		
	HR	95% CI	P-value	HR	95% CI	P-value
Recipient factors						
Age at transplant, years	1.0	1.0–1.0	0.427	NA	NA	NA
Gender, male	0.9	0.6–1.2	0.398	NA	NA	NA
Pretransplant BMI, kg/m^2	1.0	1.0–1.0	0.960	NA	NA	NA
Race/Ethnicity						
American Indian	1.5	0.4–5.9	0.602	NA	NA	NA
Asian	0.6	0.4–1.0	0.069	1.2	0.6–2.2	0.637
African American	1.0	0.5–1.9	0.944	NA	NA	NA
Native Hawaiian	0.6	0.1–4.5	0.639	NA	NA	NA
Hispanic	1.3	0.9–1.9	0.115	NA	NA	NA
Etiology						
Auto-immune	2.2	1.1–4.6	0.026	1.3	0.6–2.9	0.437
Amyloidosis	0.1	0.0–NA	0.650	NA	NA	NA
Biliary atresia	1.1	0.2–7.8	0.934	NA	NA	NA
Cholangiocarcinoma	1.5	0.4–6.0	0.581	NA	NA	NA
Cryptogenic	1.1	0.5–2.4	0.781	NA	NA	NA
EtOH	1.2	0.8–1.8	0.486	NA	NA	NA
HBV	0.4	0.2–0.9	0.020	0.4	0.2–1.1	0.067
HCV	0.7	0.5–1.0	0.079	0.9	0.6–1.4	0.768
NASH	1.1	0.6–1.9	0.804	NA	NA	NA
PBC	2.4	1.1–5.0	0.027	1.4	0.6–3.2	0.396
PSC	1.1	0.6–1.9	0.713	NA	NA	NA
Wilson	0.7	0.2–2.8	0.623	NA	NA	NA
A1AT	2.8	0.9–8.7	0.082	2.7	0.8–8.8	0.108
Other/unknown	2.1	1.1–3.9	0.027	1.3	0.6–2.6	0.477
HCC	0.7	0.5–1.0	0.033	0.9	0.6–1.4	0.693
MELD	1.0	1.0–1.0	<0.001	1.0	1.0–1.0	0.785
Era						
1990–2000	NA	1 [Reference]	NA	NA	NA	NA
2001–2010	2.7	0.8–9.7	0.115	1.3	0.3–5.0	0.737
2011–2018	4.0	1.2–13.2	0.024	1.0	0.3–3.8	0.993
Donor factors						
Donor type						
DCD	NA	1 [Reference]	NA	NA	NA	NA
DBD	0.6	0.4–0.9	0.022	0.6	0.3–1.0	0.049
LD	2.4	1.7–3.5	<0.001	1.7	0.9–3.1	0.094
Age, years	1.0	1.0–1.0	0.002	1.0	1.0–1.0	0.260
Gender, male	0.7	0.5–1.0	0.030	0.9	0.7–1.3	0.729
BMI, kg/m^2	1.0	1.0–1.1	0.028	1.0	1.0–1.1	0.029
Race/Ethnicity						
American Indian	0.0	0.0–388.7	0.511	NA	NA	NA
Asian	1.1	0.6–2.1	0.731	NA	NA	NA
African American	1.3	0.7–2.3	0.437	NA	NA	NA
Hispanic	1.0	0.7–1.4	0.994	NA	NA	NA
Multiracial	0.5	0.1–1.9	0.299	NA	NA	NA
Native Hawaiian	1.5	0.2–11.1	0.664	NA	NA	NA
White	1.0	0.7–1.4	0.916	NA	NA	NA
Cause of death						
Anoxia	0.6	0.4–0.8	0.005	0.6	0.3–1.1	0.094
Cerebrovascular	1.1	0.7–1.7	0.659	NA	NA	NA
Head trauma	0.4	0.2–0.6	<0.001	0.5	0.3–0.9	0.028
Other	0.6	0.1–2.4	0.462	NA	NA	NA
Cold ischemic time, hours	0.9	0.9–0.9	0.000	1.0	1.0–1.1	0.249

DCD, donation after cardiac death; DBD, donation after brainstem death; LD, living donor; LT, liver transplantation; BMI, body mass index, EtOH, ethanol use; HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; A1AT, alpha-1 antitrypsin; MELD, Model For End-Stage Liver Disease; CNS, central nervous system; BMI, body mass index; CI, confidence interval; HR, hazard ratio; NR, not reported (values superior to 105); DCD, donation after cardiac death; DBD, donation after brainstem death (DBD); LD, living donor.

[a]*Univariate Cox proportional-hazards regression model.*

[b]*Multivariate Cox regression model. Only those variables with* $p < 0.1$ *in the univariate analysis were entered in the multivariate analysis.*

reported risk [28, 29], although the most recent cohort studies suggest this HR was further lowered [30, 31]. The comparison between DCD-LTs and LD-LTs was not significant, possibly due to the lower number of patients in the latter group. Nevertheless, graft survival curves showed that all three categories converged over time, suggesting that DCD is an acceptable alternative when no other organ is available. The graft survival rates for the three categories matches those observed and reported by the Toronto group in a similar analysis [32]. We identified other important predictors of graft loss and patient death in our multivariate analysis, including donor and recipient age, transplantation era, presence of cholangiocarcinoma, and cold ischemia time, considering previously described risk factors [28]. We also highlighted a detrimental effect of donor Hawaiian ethnicity and a protective effect of recipient Asian ethnicity.

The nature and frequency of biliary complications are what differentiate most long-term outcomes in DCD-LT versus DBD-LT or LD-LT. A focused analysis led us to study biliary complications in 414 recipients, including one-third of each donor type. Non-anastomotic biliary stricture developed in 15.2% of DCD recipients, which aligns with what is reported in the literature [7, 8, 11]. This complication was exceptional in DBD-LT or LD-LT in the absence of an arterial supply problem. There was a slight increase in anastomotic biliary strictures and bile leaks in DCD-LT recipients compared to DBD-LT recipients, but the increase was not prohibitive. Living donor recipients had a higher number and completely different pattern of biliary complications compared to the two other groups, as previously reported [32]. They were much more affected with anastomotic biliary strictures (43.5%) and bile leaks (36.2%) compared to DCD-/DBD-LT recipients. It is worth noting that recipients with bile leaks are the group that typically get strictures. Taken together, the type of transplant and the presence of biliary complications had an impact on organ survival. The best 1- and 5-year graft survival were achieved in LD recipients without biliary complication and the worst in DCD recipients with any type of biliary complication. This was further confirmed in a multivariate analysis where DBD grafts/donors with head trauma were the only protective factors against the occurrence of biliary complication(s). It is unclear why higher donor BMI was associated with more biliary complications. This could be a marker of graft quality (steatosis) which could have an impact on the magnitude of ischemia-reperfusion injury and biliary microcirculation damage. However, it is important to note that the magnitude of this association was limited.

The development of NAS negatively affected graft survival; however, 50% of the patients with NAS ultimately kept their graft and remained stent free in the long term.

Overall, given the reported 1- and 5-year graft survival rates and biliary complication rates, it seems that both DCD-LT and LD-LT are viable options when DBD grafts are limited or unavailable. Successful LD selection is well codified, and biliary complication rates vary between different centers [23]. Similarly, DCD donor and recipient selection criteria are center-dependent and may affect survival outcomes and the rate of biliary complications [6–9]. Large discrepancies exist in DCD utilization, the most striking one being the difference between the United States and the United Kingdom: DCD LT currently accounts for about 8% of all deceased donor LTs in the US versus 19% in the United Kingdom [9]. In our center, general DCD selection criteria included donor age younger than 60, an estimated CIT lower than 8 h, dWIT<30 min, and a recipient with a MELD score lower than the average. Several DCD scores [11, 12, 14], including ours [13], have been published to further standardize practices and ensure the best outcomes; however, local constraints (travel distance, local MELD, etc.) and practices can make these scores hard to follow in a global and protocoled manner.

Our study has limitations. We report on a retrospective cohort; thus, information bias and selection bias cannot be totally avoided. It is noteworthy that the number of missing data was low, therefore limiting information bias. Another point is that our study extends over a large period (especially for DBDs and LDs); therefore, we cannot totally exclude bias related to the evolution of surgical technique, donor/recipient selection practices, and recipient management policies. To account for this, we used "era" as an independent study variable in our multivariate analysis. Interestingly, era was significantly associated with graft survival but not with biliary complications. Moreover, the low number of DCDs and LDs and the fact that the evolution of techniques is a continuum prompted us to consider a larger study period. However, to limit its impact, we restricted the matched/paired analysis to the 2003–2019 period. Another limitation is that our conclusions are based on a single-center data analysis and should be confirmed in a multicenter cohort. From a broader perspective, it is also worth noting that the increasing use of machine perfusion devices for DCDs may change the rate and nature of complications in the future [10, 33–36]. Normothermic regional perfusion [37–39] and hypothermic oxygenated perfusion [40] might indeed have an impact on the prevention of biliary complications. To date, it remains to be demonstrated whether the *ex situ* perfusion technologies will lead to a significant risk modification that is proportional to the costs and logistic difficulties of their use and/or if this risk modification can be achieved through better organ selection. Nevertheless, the choice of proceeding with an LD versus waiting for a DCD or a DBD graft to become available will remain a point of discussion globally and at the individual patient level.

In conclusion, we exposed the differential incidence and effect of biliary complications on the outcomes after liver transplantation using brain-dead donors, donors after circulatory death, and living donors. We demonstrated that LD-LT achieved the best 1-and 5-year graft survival, and DCD-LT achieved excellent graft survival in the absence of biliary complications. DCD-LT is expected to become an equivalent alternative to DBD- and LD-LT given the further reduction of ischemia-reperfusion injury and biliary microcirculation damage offered by machine and regional perfusion systems.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of the University of California, San Francisco. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

RM and GR designed the study. RM, YK, CF, NA, JR, and GR collected the data. RM analyzed the data. RM performed the statistical analysis. RM, YK, DM, CF, NA, JR, and GR interpreted the data and wrote the manuscript. RM and GR had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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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.2022.10855/full#supplementary-material

Supplementary Figure S1 | Liver graft survival, stratified by organ type: donation after cardiac death (DCD), donation after brainstem death (DBD), or living donor liver transplantation (LD) in the matched/paired cohort.

Supplementary Figure S2 | (A) Relative numbers of liver transplant (%) in the unmatched and matched cohorts. **(B)** Liver graft survival stratified by era (all liver transplant types). **(C)** Liver graft survival, stratified by organ type: donation after cardiac death (DCD), donation after brainstem death (DBD), or living donor liver transplantation (LD), and within the different era.

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ORIGINAL RESEARCH
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Characteristics and Outcome of Post-Transplant Lymphoproliferative Disorders After Solid Organ Transplantation: A Single Center Experience of 196 Patients Over 30 Years

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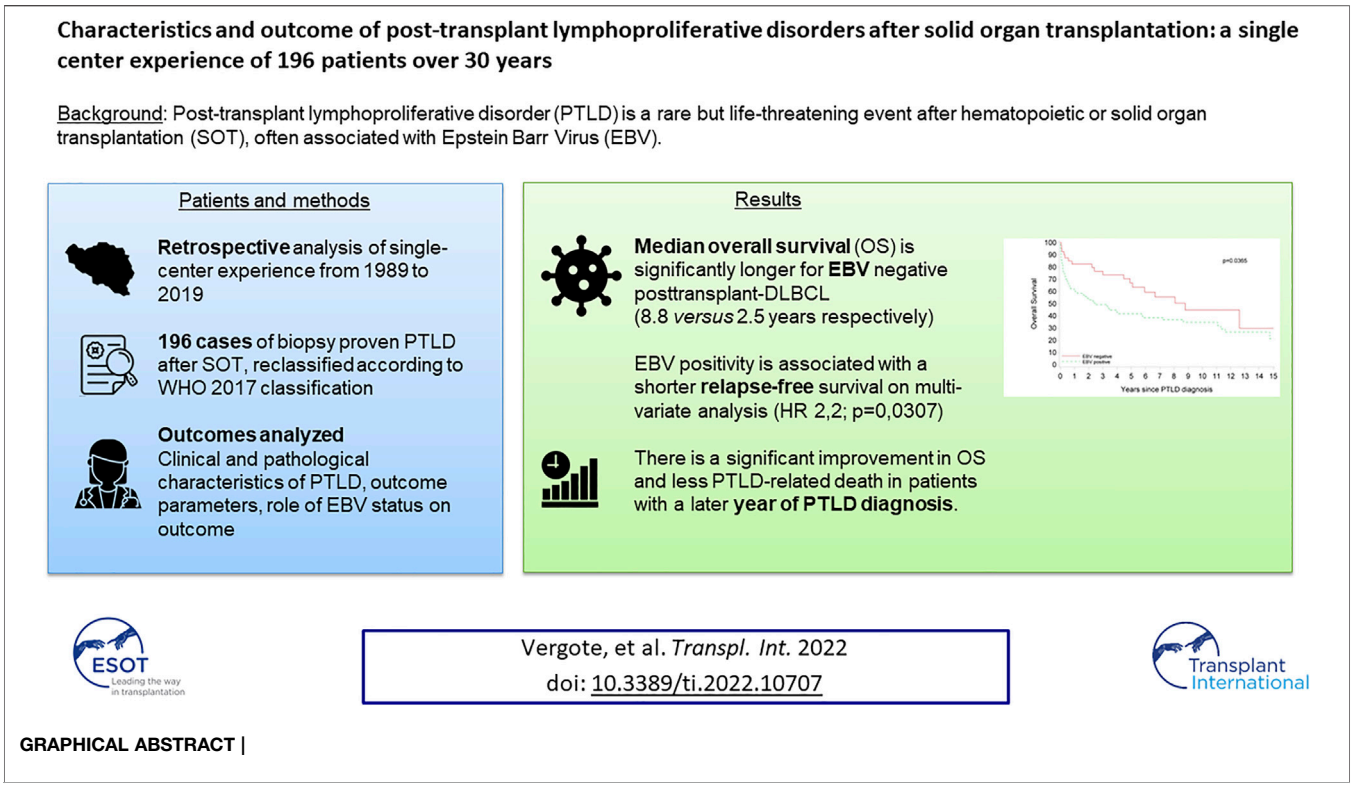
Post-transplant lymphoproliferative disorder (PTLD) is a rare but life-threatening complication after transplantation. In this retrospective, monocentric study we aimed to collect real life data regarding PTLD and determine the role of Epstein Barr Virus (EBV) status and year of diagnosis on prognosis. We identified 196 biopsy-proven PTLD after solid organ transplantation (SOT) diagnosed at the University Hospitals Leuven (Belgium) from 1989 to 2019. EBV status was positive in 61% of PTLD. The median overall survival (OS) was 5.7 years (95% CI: 2.99–11.1). Although EBV positivity was not significantly correlated with OS in multivariate analyses (HR: 1.44 (95% CI: 0.93–2.24); $p = 0.10$), subgroup analysis showed a significantly better median OS for EBV negative post-transplant diffuse large B-cell lymphoma (DLBCL) compared to EBV positive post-transplant DLBCL (8.8 *versus* 2.5 years respectively; $p = 0.0365$). There was a significant relation between year of PTLD diagnosis and OS: the more recent the PTLD diagnosis, the lower the risk for death (adjusted HR: 0.962 (95% CI: 0.931–0.933); $p = 0.017$). In conclusion, the prognosis of PTLD after SOT has improved in the past decades. Our analysis shows a significant relation between EBV status and OS in post-transplant DLBCL.

Keywords: epidemiology, transplantation, outcome, prognosis, post-transplant lymphoproliferative disorder, Epstein Barr Virus



GRAPHICAL ABSTRACT |

INTRODUCTION

Post-transplant lymphoproliferative disorders (PTLD) are a heterogeneous group of lymphoid neoplasms following solid organ transplantation (SOT) and hematopoietic stem cell transplantation (HSCT)(1,2). The cumulative incidence of PTLD is estimated at 1% after 5 years and 2.1% after 10 years in adult kidney (-pancreas) transplant recipients (3). The risk of developing PTLD depends on the type of organ transplanted and incidence density (i.e. incidence adjusted for time under immunosuppression) ranges from 1.58 per 1000 person-years (kidney), up to 2.24 (heart), 2.44 (liver) and 5.72 (lung) (4-6). The pathogenesis is complex, but two major contributing factors are recognized. Firstly, most cases (60-70%) are associated with infection with the oncogenic Epstein-Barr Virus (EBV) (7-9). Secondly, there is a diminished T-cell immune surveillance due to the iatrogenic immunosuppression in transplant recipients (4,5). The pathogenesis of EBV negative (EBV(-)) PTLD remains the subject of debate. Several hypotheses have been suggested such as the "hit-and-run" hypothesis (where EBV initiates lymphomagenesis, but is then cleared), the role of other viruses (Cytomegalovirus, Human Herpes Virus 8...), chronic antigenic stimulation and long-term immunosuppression(4,10).

The World Health Organization (WHO) 2017 classification recognizes four types of PTLD (1): Non-destructive lesions (2); Polymorphic PTLD (3); Monomorphic PTLD (including B-, T- and natural killer (NK)-cell types) (4); classic Hodgkin lymphoma PTLD (2). Historically, PTLD represents a serious and potentially life-threatening complication of transplantation, with a reported survival rate of 60% after 5 years in kidney transplant recipients (3,5).

Several single- and multicenter reports have previously been published (11-14). However, they are often hampered by their heterogenous population and limited numbers of patients. Large reports from national registries often contain many more cases, but lack detailed information (3,15). Furthermore, significant progress has been made in the past 30 years including a new WHO 2017 classification and improvement of treatment by the introduction of monoclonal antibodies against CD20. Although genomic and transcriptional studies have recently demonstrated that EBV positive (EBV(+)) and EBV(−) PTLD carry different genomic signatures, the role of EBV status on prognosis remains unclear and patients are essentially treated the same (16,17).

Here, we describe one of the largest retrospective single-center series of PTLD after SOT, comprising 196 patients with histologically proven PTLD over a 30 year period. We previously reported our experience in PTLD, including 122 cases after SOT and 18 after HSCT (18). The goal of this report was to analyze a larger group of PTLD after SOT with longer follow-up. We aimed to investigate the role of EBV status on prognosis on a large real life cohort of PTLD and to find out whether prognosis has improved in the past decades.

MATERIALS AND METHODS

Data Collection

This study was performed at the University Hospitals Leuven (Belgium), a tertiary hospital where all categories of SOT are

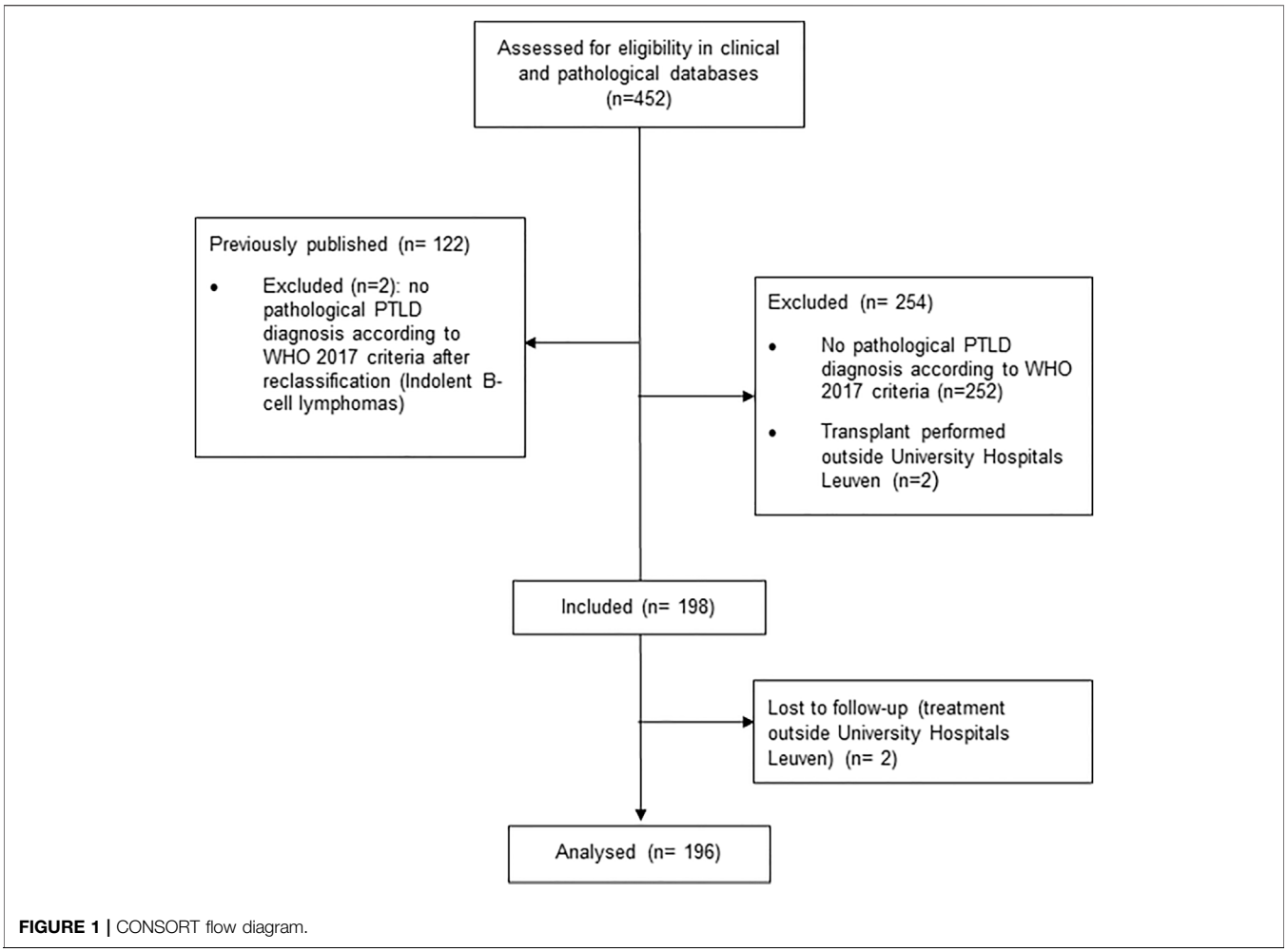


FIGURE 1 | CONSORT flow diagram.

performed. We reviewed all cases of histologically confirmed untreated PTLD after SOT, diagnosed in our hospital between January 1st, 1989 to December 31st, 2019 (**Figure 1**). Cases of indolent non-Hodgkin lymphoma (NHL) histology ($n = 2$), with the exception of EBV(+) marginal zone lymphoma, were excluded from analysis, since they are not included in the current WHO 2017 PTLD classification (2). All cases were reviewed by one expert hematopathologist (TT). Patient-related clinical characteristics included gender, age at PTLD diagnosis, Eastern Cooperative Oncology Group Performance status (ECOG PS) and pretransplant EBV serology. Transplant-related characteristics included type of organ transplanted, time from transplantation to PTLD diagnosis and type of immunosuppression. PTLD-related characteristics included: Ann Arbor Stage (19) at diagnosis, presence of B-symptoms, biochemical data (hemoglobin, creatinine clearance, albumin, lactate dehydrogenase (LDH)), number of extranodal sites involved, graft involvement and involvement of different organ systems, (sub)type of PTLD according to the WHO 2017 classification (2), presence of CD20 expression and EBV in the biopsy, year of PTLD diagnosis and data on treatment and outcome variables. If available, data on EBV polymerase chain reaction (PCR) in peripheral blood were collected. This study was approved by the Ethics Committee of University Hospitals/Catholic University Leuven (Ref: S62704 and S55498) and was conducted according to the ethical principles of the World Medical Association Declaration of Helsinki (20).

Definitions

All PTLD cases required histopathological confirmation to be included. EBV in the biopsy was determined by Epstein-Barr-encoded RNA (EBER) *in situ* hybridization (ISH). Post-transplantation EBV surveillance was not performed systematically in our hospital. International Prognostic Index (IPI) was calculated as previously described (21).

For statistical reasons, patients with combined SOT were pooled according to the transplantation requiring the highest degree of immunosuppression. Patients with combined kidney-pancreas ($n = 6$) and kidney-liver ($n = 3$) were classified as kidney transplantation. Patients with combined heart-lung ($n = 3$) and liver-lung ($n = 1$) transplant were classified as lung transplantation. Lastly patients with combined heart-kidney ($n = 1$) and combined liver-pancreas ($n = 1$) were classified as heart and liver transplantation, respectively.

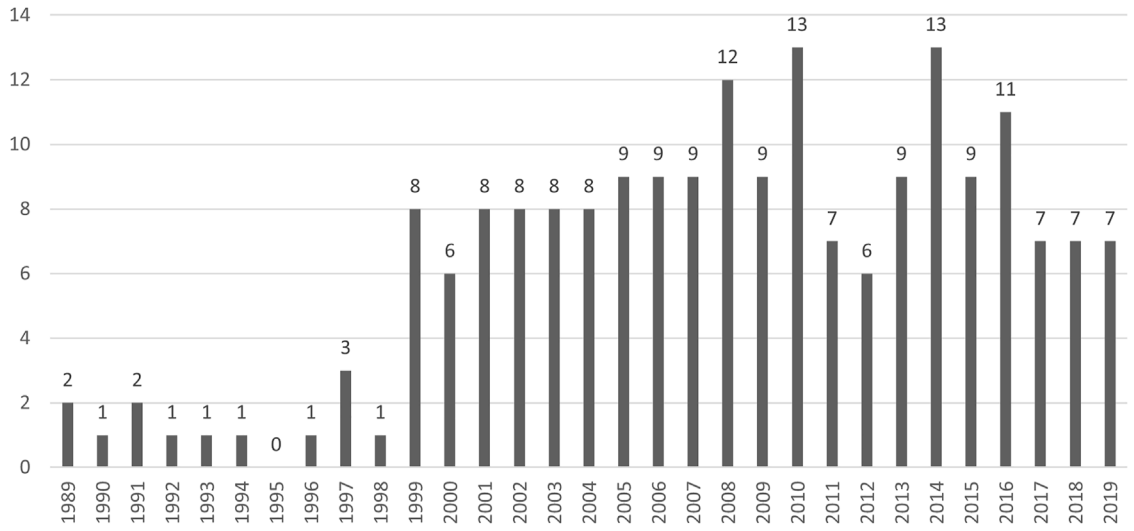


FIGURE 2 | Absolute number of PTLD diagnosis by year.

Response assessment after treatment was performed according to the Lugano criteria (22) and was based upon chart review of the available imaging protocols of computed tomography (CT) or positron emission tomography with ^{18}F-fluorodeoxyglucose combined with CT ([^{18}F]FDG-PET/CT), if possible including Deauville criteria (23). Timing of response assessment depended on the predefined initial treatment, e.g., after four cycles of rituximab for risk-stratified sequential treatment (24) and after four cycles of rituximab and four cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicine, vincristine and prednisolone) for sequential treatment(25). OS was calculated as time from biopsy-proven diagnosis till the date of death. Death was considered to be PTLD-related in any case where death was caused by either disease progression or a treatment-related complication. Relapse-free survival (RFS) was defined as time from biopsy-proven diagnosis till the date of relapse or death.

Statistical Methods

A description of the statistical methodology can be found in the **Supplementary Material**.

RESULTS

Epidemiology

Between January 1st, 1989 and December 31st, 2019, 7497 patients received a SOT at our center. We identified 196 histologically confirmed cases of PTLD after SOT in the same period. Seventeen patients were pediatric (<18 years) and 179 were adults at time of PTLD diagnosis. There was a male predominance in the adult transplant recipients (58.3%), as well in the PTLD cohort (65.3%). We observed 19 (first decade: 1990–1999), 86 (second decade: 2000–2009) and 89 cases (third decade: 2010–2019), showing a significant increase from the first to the second decade (p < 0.0001) and stable number from the second to the third decade (p = 0.97) (**Figure 2**).

Patient-, Transplant- and PTLD- Related Characteristics

Baseline patient characteristics are summarized in **Table 1**. The most common transplanted organs were kidney (n = 76; 38.8%), lung (n = 46; 23.5%), heart (n = 30; 15.3%) and liver (n = 29; 14.8%). EBV serology before transplantation was negative in 39/96 (40.6%) and positive in 57/96 patients (59.4%) with available data.

The most frequent histological type was monomorphic PTLD (n = 162, 82.7%), with DLBCL being the most frequent subtype (n = 121; 74.7%). The cell of origin according to the Hans algorithm (28) was germinal center B-cell like (GCB) in 19/56 (33.9%) and non-germinal center B-cell like (non-GCB) in 37/56 (66.1%) in the posttransplant DLBCL-type (PT-DLBCL). These data were missing in 65 patients. The majority of GCB DLBCL were EBV(-) (94.7%), whereas the majority of non-GCB DLBCL were EBV(+) (78.4%).

Other subtypes of monomorphic PTLD included plasmablastic lymphoma (n = 14; 8.6%), plasma cell malignancies (n = 3; 1.9%), T-cell NHL (T-NHL) (n = 8; 4.9%), Burkitt lymphoma (n = 8; 4.9%), Burkitt-like lymphoma with 11q aberration (n = 4; 2.5%), EBV(+) marginal zone lymphoma (n = 1; 0.6%) and B-NHL, undefined (n = 3; 1.9%).

Median time from transplant to PTLD diagnosis was 4.3 years (IQR: 1.0-10.6), with many cases occurring late (>1 year after transplantation) (n = 147; 75.0%) or very late (>10 years after transplantation) (30) (n = 46; 23.6%).

Treatment and Outcome

Treatment at first line consisted of reduction of immune suppression (RIS) (n = 178; 90.8%), rituximab (n = 120; 61.2%),

TABLE 1 | Baseline patient characteristics of 196 patients with biopsy-proven PTLD after SOT.

		Years or number (%)
Age at diagnosis (years)	Median (IQR)	54.1 (35.2-64.5)
	Range	3.5-83
Age at diagnosis	≤60 years	122 (62.2)
	>60 years	74 (37.8)
Gender	Male	128 (65.3)
	Female	68 (34.7)
ECOG PS	0-1	138 (70.8)
	2	42 (21.5)
	3-4	15 (7.7)
	Unknown	1
Transplanted organ	Heart	30 (15.3)
	Kidney	76 (38.8)
	Lung	46 (23.5)
	Liver	29 (14.8)
	Heart-Kidney	1 (0.5)
	Kidney-Pancreas	6 (3.1)
	Kidney-Liver	3 (1.5)
	Heart-Lung	3 (1.5)
	Liver-Lung	1 (0.5)
	Liver-Pancreas	1 (0.5)
IS at diagnosis	CNI	189 (96.4)
	AM	152 (77.6)
	CS	134 (68.4)
	Sirolimus	1 (0.5)
	CNI + AM + CS	99 (55.5)
	Induction	94 (48%)
Time between transplantation and PTLD (years)	Median (IQR)	4.3 (1.0-10.6)
	Range	0.2-28
Pathology	Non-destructive	16 (8.2)
	Polymorphic	11 (5.6)
	Monomorphic	162 (82.7)
	Hodgkin	6 (3.1)
	EBV(+) mucocutaneous ulcer	1 (0.5)
EBV ISH at diagnosis	Negative	67 (26)
	Positive	119 (64)
	Unknown	10
CD 20 expression at diagnosis	Negative	31 (16.1)
	Positive	155 (80.3)
	Partially positive	7 (3.6)
	Unknown	3
Ann Arbor stage	I	31 (17.4)
	II	20 (10.3)
	III	23 (11.8)
	IV	118 (60.5)
	Unknown	1
B-symptoms	No	133 (67.9)
	Yes	63 (32.1)
Number of extranodal sites	None	38 (19.5)
	1	67 (34.4)
	>1	90 (46.2)
	Unknown	1
IPI	Low risk	61 (31.6)
	Low intermediate risk	44 (22.8)
	High intermediate risk	54 (27)
	High risk	34 (17.6)
	Unknown	3
Extranodal involvement	Graft involvement	39 (19.9)
	PCNSL	12 (6.1)
	CNS involvement, not primary	2 (1)
	Bone marrow involvement	22 (14.6)
	GI involvement	60 (30.8)
	Pulmonary involvement	51 (28)

(Continued on following page)

TABLE 1 | (*Continued*) Baseline patient characteristics of 196 patients with biopsy-proven PTLD after SOT.

		Years or number (%)
Serum levels at diagnosis	Hemoglobin <10 g/dl	70 (35.7)
	LDH elevated	87 (44.4)
	Albumin <35 g/L	87 (29)
	Creatinine ≥1.5 mg/dl	83 (42.3)

AM, antimetabolites; CNI, calcineurin inhibitors; CNS, central nervous system; CS, corticosteroids; ECOG PS, eastern cooperative oncology group performance status; EBV(+), Epstein-Barr virus positive; EBV ISH, Epstein-Barr virus in situ *hybridization; GI, gastro-intestinal; IPI, international prognostic index; IS, immunosuppressive therapy; LDH, lactate dehydrogenase; IQR, interquartile range; PCNSL, primary central nervous system lymphoma, PTLD, Post-transplant lymphoproliferative disorder.*

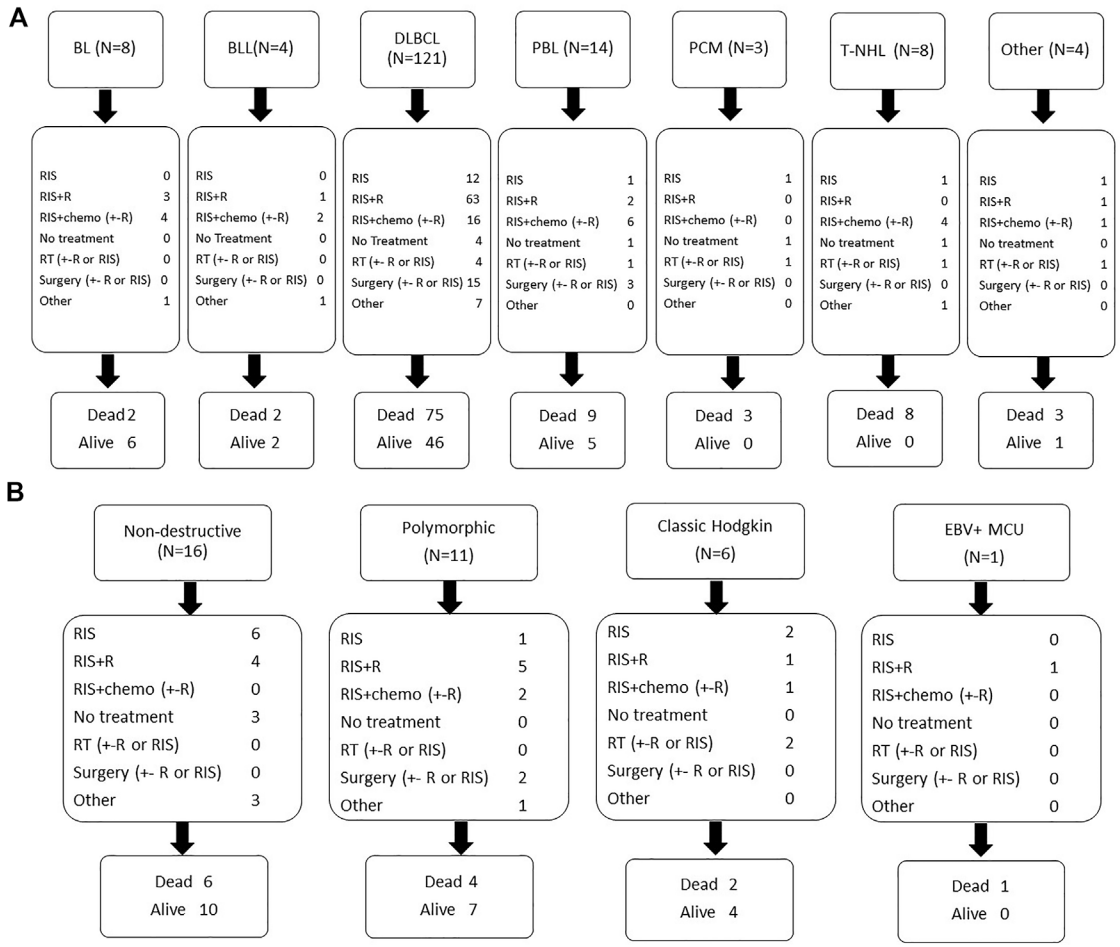


FIGURE 3 | First line treatment and outcome according to histological subtype monomorphic PTLD **(A)** and other histological subtypes **(B)**. Legend: BL: Burkitt lymphoma; BLL(11q): Burkitt-like lymphoma with 11q aberration; B-NHL,u: B-cell non-Hodgkin's lymphoma, undefined; DLBCL: diffuse large B-cell lymphoma; MCU: mucocutaneous ulcus; MZL: marginal zone lymphoma; PBL: plasmablastic lymphoma; PCM: plasma cell malignancy; PTLD: post-transplant lymphoproliferative disorder; RIS: reduction of immunosuppression; R: rituximab; RT: radiotherapy; T-NHL: T-cell non-Hodgkin's lymphoma.

chemotherapy (n = 41; 20.9%), surgery (n = 24; 12.2%), radiotherapy (n = 13; 6.6%), high-dose corticosteroids (n = 12; 6.1%) or antiviral treatment (n = 5; 2.6%). Ten patients (5.1%) received no treatment (7 supportive care, 3 spontaneous remissions of non-destructive PTLD). Eighty-three patients (42.3%) were treated with rituximab alone. Twenty-five patients were treated with RIS alone (12.8%) and 13 of these achieved a complete response (CR) (52%), of whom only 2 patients relapsed later on. Seventy-six patients (38.7%) in the cohort did not receive rituximab, mainly due to CD20 negativity (n = 26), treatment with RIS alone (n = 25), treatment in the pre-rituximab era (before 2000) (n = 19) and no treatment received (n = 10).

TABLE 2 | Reasons of death.

	Number (N = 115)	%
PTLD progression	47	40.9
Infections	21	18.3
Other malignancies	11	9.6
CVA	2	1.7
Bleeding	3	2.6
Cardiac events	7	6.1
MOF	5	4.3
Other	8	7
Unknown	11	9.6

CVA, cerebrovascular accident; MOF, multiple organ failure; PTLD, post-transplant lymphoproliferative disorder.

Response to first-line treatment was CR in 99 patients (50.5%), partial response in 25 (12.8%), stable disease in 9 (4.6%) and progressive disease in 40 patients (20.4%). Sixteen patients (8.2%) died during first line treatment and seven had received supportive care alone. Fifty-nine patients (30.1%) were refractory to first line treatment and 19 patients (9.7%) relapsed after achieving a CR. First line treatment according to histological subtypes is summarized in **Figure 3**.

After a median follow-up of 4.0 years (IQR: 0.5-8.8) after PTLD diagnosis, 115 patients (58.7%) died. Death was considered PTLD related in 46.1% (n = 53), non-PTLD related in 47% (n = 54) and unknown in 7% (n = 8). Other causes of death included mainly infections and other malignancies (**Table 2**). The cumulative incidence of PTLD-related death *versus* non-PTLD-related death is visualized in **Figure 4**.

OS rates after PTLD for the whole cohort were 67.8, 61.7 and 51.2% after 1, 2 and 5 years, respectively. The median OS was 5.7 years (95% CI 2.99–11.07). In the 99 patients achieving a CR after first line treatment, RFS was 87.9, 77.8 and 62.0% after 1, 2 and 5 years, respectively (**Figure 5**).

Uni- and Multivariate Analysis of Factors Influencing Outcome

Factors influencing CR rate in first line, PTLD-related death, OS, and RFS are summarized in **Tables 3–6**, respectively.

Higher age at transplantation, higher age at PTLD diagnosis, monomorphic histology, elevated LDH, higher IPI, poor ECOG PS (3,4) and advanced Ann Arbor stage were statistically significant adverse factors for CR rate in univariate analysis. In multivariate analysis a higher IPI score and a higher year of PTLD diagnosis were related to a lower CR rate.

Higher age at transplantation, higher age at PTLD diagnosis, monomorphic histology, extranodal disease, elevated LDH, hypoalbuminemia, higher IPI, poor ECOG PS (>1), advanced Ann Arbor stage are significantly related to PTLD-related death in univariate analysis using Cox regression models. Similar results were obtained using Fine and Gray models (results not shown). In the multivariate model hypoalbuminemia, higher IPI-score, graft organ involvement and type of transplanted organ (lung *versus* heart) were retained as factors associated with worse outcome. A

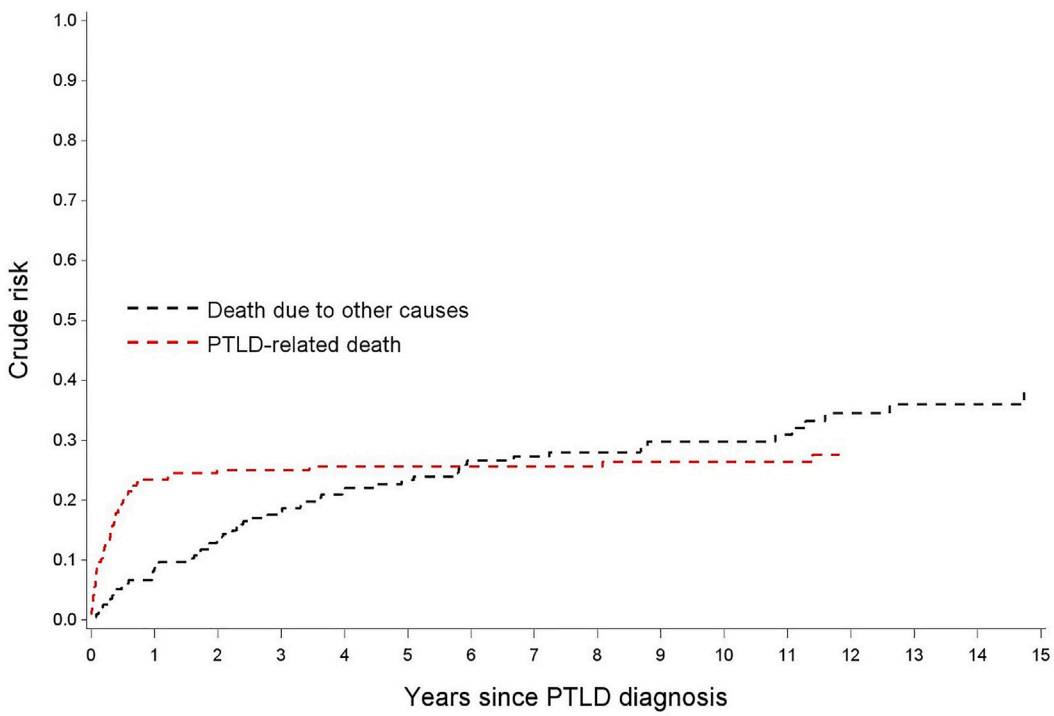


FIGURE 4 | Nelson-Aalen estimates for the cumulative incidence of PTLD-related death and for death due to other causes.

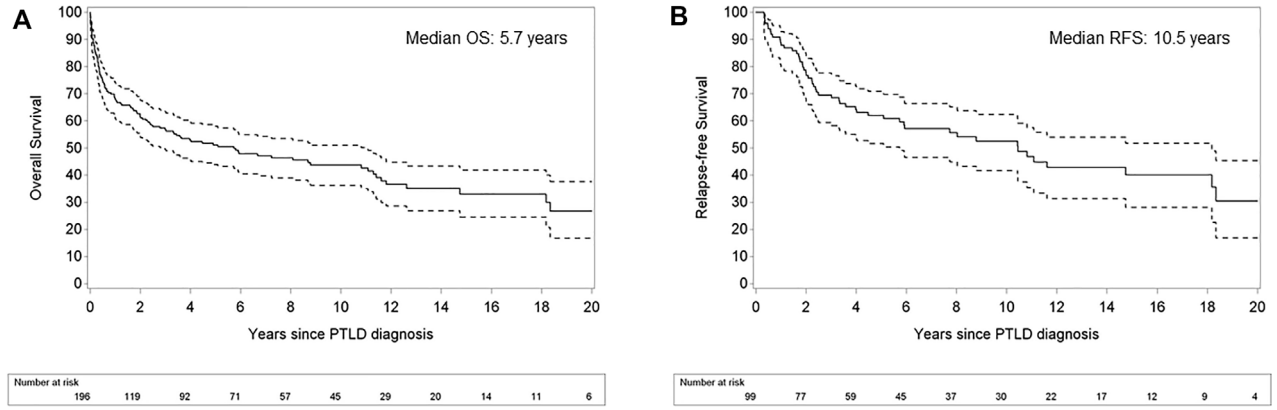


FIGURE 5 | Kaplan Meier plots for overall survival in patients with post-transplant lymphoproliferative disorder **(A)** and relapse-free survival after achievement of complete response **(B)**. Legend: Dashed lines refer to the pointwise 95% confidence interval. OS: overall survival; RFS: relapse-free survival.

TABLE 3 | Univariate and multivariate analysis (Logistic regressions) of factors influencing complete response rate.

Variable	Univariate		Multivariate[a]	*p*-value
	Odds Ratio (95% CI)	*p*-value	Odds Ratio (95% CI)	
Age at transplantation (years)	0.984 (0.970;1.000)	0.0430		
Age at PTLD diagnosis (years)	0.980 (0.966;0.995)	0.0096	0.989 (0.973;1.006)	0.2045
Age at PTLD diagnosis >60 years	0.437 (0.242;0.790)	0.0061		
EBV ISH positivity	1.351 (0.741;2.463)	0.3257	1.454 (0.758;2.788)	0.2596
Female gender	0.809 (0.449;1.459)	0.4818		
Transplanted organ		0.5318		
Kidney[b]	0.647 (0.280;1.496)	0.3082		
Liver[b]	0.483 (0.174;1.342)	0.1627		
Lung[b]	0.583 (0.234;1.450)	0.2458		
Graft organ involved	1.267 (0.622;2.581)	0.5145		
Monomorphic histology	0.423 (0.193;0.924)	0.0309		
CNS involvement		0.9992		
PCNSL	0.978 (0.304;3.147)	0.9706		
Secondary	0.978 (0.060;15.879)	0.9877		
Extranodal disease	0.534 (0.257;1.107)	0.0916		
Elevated LDH	0.305 (0.169;0.550)	<0.0001		
CD20 positivity		0.3238		
Positive	1.779 (0.808;3.913)	0.1524		
Partially positive	1.187 (0.225;6.260)	0.8394		
Hypoalbuminemia	0.672 (0.378;1.194)	0.1752		
IPI score	0.657 (0.528;0.817)	0.0002	0.659 (0.522;0.833)	0.0005
ECOG PS		0.0017		
ECOG 2[c]	0.560 (0.279;1.126)	0.1036		
ECOG 3/4[c]	0.115 (0.025;0.529)	0.0055		
Ann Arbor stage III-IV	0.451 (0.236;0.864)	0.0163		
Year of PTLD diagnosis	0.961 (0.922;1.003)	0.0661	0.955 (0.913;0.999)	0.0436

[a]*EBV status was added into the multivariate model obtained after backward selection*

[b]*Compared to heart transplant.*

[c]*Compared to ECOG PS 0-1.*

95% CI, 95% confidence interval; PTLD, post-transplant lymphoproliferative disorder; ECOG PS, eastern cooperative oncology group performance status; EBV ISH, Epstein-Barr Virus in situ *hybridization; LDH, lactate dehydrogenase; IPI, international prognostic index; PCNSL, primary central nervous system lymphoma.*

higher year of PTLD diagnosis was associated with less PTLD-related death in uni- and multivariate analysis.

Higher age at transplantation, higher age at PTLD diagnosis, monomorphic histology, extranodal disease, elevated LDH, hypoalbuminemia, a higher IPI-score, ECOG >1, advanced Ann Arbor stage were significantly adverse factors for OS in univariate analysis. In the multivariate model the IPI-score, higher age at diagnosis, hypoalbuminemia, type of transplanted organ (liver and lung transplantation compared to heart) were retained as poor prognostic factors. Higher year of PTLD diagnosis was associated with a longer OS in uni- and multivariate analysis.

TABLE 4 | Univariate and multivariate analysis (Cox regressions) of patients characteristics related to PTLD related death.

Variable	Univariate		Multivariate[a]	
	Hazard Ratio (95% CI)	*p*-value	Hazard Ratio (95% CI)	*p*-value
Age at transplantation (years)	1.029 (1.012;1.045)	0.0007		
Age at PTLD diagnosis (years)	1.030 (1.013;1.047)	0.0006		
Age at PTLD diagnosis >60 years	2.798 (1.617;4.842)	0.0002		
EBV ISH positivity	1.670 (0.884;3.157)	0.1143	1.155 (0.591;2.255)	0.6730
Female gender	0.860 (0.478;1.549)	0.6161		
Transplanted organ		0.9320		0.0162
Kidney[b]	0.855 (0.392;1.867)	0.6945	1.124 (0.498;2.534)	0.7787
Liver[b]	1.068 (0.424;2.694)	0.8883	2.477 (0.924;6.639)	0.0714
Lung[b]	1.013 (0.438;2.342)	0.9762	4.074 (1.456;11.399)	0.0075
Graft organ involved	0.834 (0.407;1.710)	0.6207	0.322 (0.135;0.772)	0.0111
Monomorphic histology	3.365 (1.211;9.352)	0.0200		
CNS involvement		0.2785		
PCNSL	2.021 (0.802;5.094)	0.1359		
Secondary	2.820 (0.388;20.494)	0.3055		
Extranodal disease	2.782 (1.105;7.003)	0.0298		
Elevated LDH	5.274 (2.799;9.937)	<0.0001		
CD20 positivity		0.3068		
Positive	0.587 (0.307;1.122)	0.1073		
Partially positive	0.708 (0.158;3.166)	0.6510		
Hypoalbuminemia	3.566 (1.939;6.561)	<0.0001	2.398 (1.256;4.577)	0.0080
IPI score	1.935 (1.562;2.399)	<0.0001	1.978 (1.554;2.519)	<0.0001
ECOG PS		<0.0001		
ECOG 2[c]	2.196 (1.163;4.148)	0.0153		
ECOG 3/4[c]	9.207 (4.581;18.504)	<0.0001		
Ann Arbor stage III-IV	4.306 (1.711;10.836)	0.0019		
Year of PTLD diagnosis	0.951 (0.916;0.988)	0.0100	0.937 (0.897;0.979)	0.0038

[a]*EBV status was added into the multivariate model obtained after backward selection.*
[b]*Compared to heart transplant.*
[c]*Compared to ECOG PS 0-1.*
Abbreviations: 95% CI: 95% confidence interval; PTLD: post-transplant lymphoproliferative disorder; ECOG PS: eastern cooperative oncology group performance status; EBV ISH: Epstein-Barr Virus in situ *hybridization; LDH: lactate dehydrogenase; IPI: international prognostic index; PCNSL: primary central nervous system lymphoma.*

Higher age at transplantation, higher age at PTLD diagnosis, elevated LDH, hypoalbuminemia, higher IPI, poor ECOG PS were significant adverse factors for RFS in univariate analysis. In the multivariate model higher age at diagnosis, EBV positivity and liver transplantation were considered prognostic factors worse RFS.

In summary, IPI was an important prognostic factor, significantly related to all four outcomes in univariate analysis and to CR rate, PTLD-related death and OS in multivariate analysis. Furthermore, hypoalbuminemia was a poor prognostic factor for PTLD-related death, OS and RFS in univariate analysis and for PTLD-related death and OS in multivariate analysis. Type of transplanted organ was significantly related to RFS, PTLD-related death and OS in multivariate analysis.

EBV

EBV status, as determined by EBV ISH at the time of diagnosis, was positive in 119 of the 186 evaluable cases (64%). The number of positive EBV was higher in early (<1 year after transplantation) PTLD cases (n = 43; 89.6%) compared to late PTLD (n = 76; 55.1%). EBV positivity was associated with type of grafted organ (highest in lung, lowest in liver transplantation) and organ-involvement in the whole PTLD cohort. There was no association between EBV status and other clinical factors (**Table 7**).

EBV status at diagnosis was not significantly related to OS in univariate (hazard ratio (HR): 1.48 (95% CI: 0.975–2.232); p = 0.066) and multivariate analysis (HR: 1.44 (95% CI: 0.928–2.239); p = 0.10). However, there was a trend towards worse OS for the EBV(+) PTLD. There was also no significant relation between EBV status and CR (odds ratio (OR): 1.35 (95% CI: 0.741–2.463); p = 0.33) and PTLD-related death (HR: 1.67 (95% CI: 0.884–3.157); p = 0.11) in univariate, nor in multivariate analysis ((OR: 1.45 (95% CI: 0.758–2.788); p = 0.26) and (HR: 1.15 (0.591–2.255); p = 0.67), respectively). However, there was a relation between EBV status and RFS in the multivariate model, where EBV positivity was a risk factor (HR: 2.29 (95% CI: 1.146–4.595); p = 0.02) (**Figure 6**).

A subgroup analysis of all cases of PT-DLBCL showed that EBV ISH was positive in 77 of the 117 evaluable cases (65.8%). Furthermore, we saw a significantly better median OS for EBV(−) PT-DLBCL compared to EBV(+) PT-DLBCL (8.8 versus 2.5 years respectively; p = 0.0365).There was no significant relation between EBV status and RFS in this group (p = 0.8852) (**Figure 7**).

EBV PCR in blood was positive in 107 of 142 evaluable cases (75.4%). However, EBV PCR was more often positive in EBV ISH positive cases (91% of 89 evaluable cases), than in EBV ISH negative cases (52% of 50 evaluable cases). This resulted in a

TABLE 5 | Univariate and multivariate analysis (Cox regressions) of patient characteristics related to overall survival.

Variable	Univariate		Multivariate[a]	
	Hazard Ratio (95% CI)	*p*-value	Hazard Ratio (95% CI)	*p*-value
Age at transplantation (years)	1.040 (1.028;1.052)	<0.0001		
Age at PTLD diagnosis (years)	1.041 (1.028;1.053)	<0.0001	1.035 (1.022;1.049)	<0.0001
Age at PTLD diagnosis >60 years	3.389 (2.321;4.948)	<0.0001		
EBV ISH positivity	1.475 (0.975;2.232)	0.0659	1.441 (0.928;2.239)	0.1037
Female gender	0.998 (0.670;1.484)	0.9903	1.290 (0.837;1.986)	0.2483
Transplanted organ		0.6780		0.0161
Kidney[b]	0.854 (0.506;1.442)	0.5553	1.197 (0.685;2.093)	0.5282
Liver[b]	1.186 (0.637;2.209)	0.5912	2.291 (1.181;4.445)	0.0142
Lung[b]	0.972 (0.546;1.729)	0.9226	2.091 (1.084;4.033)	0.0278
Graft organ involved	1.091 (0.690;1.725)	0.7088		
Monomorphic histology	2.468 (1.381;4.409)	0.0023		
CNS involvement		0.6513		
PCNSL	1.422 (0.691;2.925)	0.3393		
Secondary	1.224 (0.171;8.792)	0.8405		
Extranodal disease	1.879 (1.121;3.151)	0.0167		
Elevated LDH	2.922 (1.997;4.275)	<0.0001		
CD20 positivity		0.2877		
Positive	0.751 (0.466;1.210)	0.2393		
Partially positive	0.394 (0.092;1.683)	0.2085		
Hypoalbuminemia	2.758 (1.873;4.062)	<0.0001	1.956 (1.289;2.967)	0.0016
IPI score	1.612 (1.399;1.856)	<0.0001	1.346 (1.154;1.570)	0.0002
ECOG PS		<0.0001		
ECOG 2[c]	1.715 (1.127;2.608)	0.0117		
ECOG 3/4[c]	4.815 (2.636;8.795)	<0.0001		
Ann Arbor stage III-IV	1.902 (1.211;2.989)	0.0053		
Year of PTLD diagnosis	0.968 (0.942;0.995)	0.0196	0.962 (0.931;0.993)	0.0172

[a]*Year of PTLD diagnosis was added into the multivariate model obtained after backward selection*
[b]*Compared to heart transplant.*
[c]*Compared to ECOG PS 0-1.*
95% CI, 95% confidence interval; PTLD, post-transplant lymphoproliferative disorder; ECOG PS, eastern cooperative oncology group performance status; EBV ISH, Epstein-Barr Virus in situ *hybridization; LDH, lactate dehydrogenase; IPI, international prognostic index; PCNSL, primary central nervous system lymphoma.*

sensitivity of 91% and specificity of 48% for EBV PCR in predicting EBV ISH positivity.

Era of PTLD Diagnosis

There was a significant relation between year of PTLD diagnosis and OS, that persisted after correction for differences in patient mix in the multivariate model: the more recent the PTLD diagnosis, the lower the risk for death (HR: 0.97 (95% CI: 0.942–0.995; $p = 0.0196$) and adjusted HR: 0.962 (95%CI: 0.931–0.933; $p = 0.017$) in the Cox multivariate model.

A similar result was obtained for PTLD-related death: HR: 0.951 (95% CI: 0.916–0.988; $p = 0.01$) and adjusted HR: 0.935 (95% CI: 0.896–0.977; $p = 0.0024$) for the year of PTLD diagnosis in the multivariate Cox model. A similar conclusion was obtained in the Fine and Gray model (results not shown). However, there was no evidence of a significant relation between year of PTLD diagnosis and CR or RFS.

DISCUSSION

We investigated the baseline characteristics, outcome, role of EBV and era of PTLD diagnosis on outcome in a large cohort of biopsy-proven PTLD after SOT. We noticed a high proportion of late (>1 year after transplantation: $n = 147$; 75%) and very late PTLD (>10 years after transplantation; $n = 46$; 23.6%) in our analysis. Several reports have recently suggested that the incidence of early EBV(+) PTLD is decreasing (3, 11, 31). In our cohort the proportion of early PTLD was stable over the first, second and third decade (21.1%, 20.9% and 28.1% respectively). Other groups have suggested that a decrease in early PTLD might be a result of pre-emptive EBV viral load monitoring. However, this has not been confirmed in a recent report (11) and this strategy has not been implemented in our series. Other factors influencing the incidence of early PTLD include the changes in immunosuppressive regimens and decreased use of T-cell depleting induction therapy (32-35).

The median age at diagnosis in the current study was 54.1 years, which is comparable to previous reports (26,36-38). PTLD is typically diagnosed at an advanced stage (72.3%) with extra-nodal involvement (80.5%). Gastro-intestinal involvement (30.8%) was the most frequent extra-nodal site involved. We observed 12 cases of PCNSL (6.1%) in our cohort, less than the previously reported 10% of all PTLDs (39-41). However, it is difficult to draw definite conclusions regarding the incidence of PCNSL in PTLD due to the small group size. By far the most commonly observed histologic type of PTLD in our study was monomorphic PTLD (82.7%), with DLBCL as the most frequent subtype. Non-destructive and classic Hodgkin lymphoma PTLD were rare, as previously reported in the literature. Furthermore, we noted only

TABLE 6 | Univariate and multivariate analysis (Cox regressions) of patient characteristics related to relapse free survival.

Variable	Univariate		Multivariate	
	Hazard Ratio (95% CI)	***p*-value**	**Hazard Ratio (95% CI)**	***p*-value**
Age at transplantation (years)	1.039 (1.022;1.057)	<0.0001		
Age at PTLD diagnosis (years)	1.047 (1.029;1.066)	<0.0001	1.054 (1.034;1.074)	<0.0001
Age at PTLD diagnosis >60 years	3.576 (2.047;6.247)	<0.0001		
EBV ISH positivity	1.261 (0.678;2.346)	0.4647	2.183 (1.075;4.432)	0.0307
Female gender	0.989 (0.541;1.810)	0.9726		
Transplanted organ		0.2155		0.0103
Kidney[a]	1.000 (0.486;2.056)	0.9993	1.585 (0.734;3.424)	0.2414
Liver[a]	1.782 (0.736;4.313)	0.2003	5.244 (1.904;14.446)	0.0013
Lung[a]	0.645 (0.266;1.561)	0.3306	1.398 (0.510;3.831)	0.5153
Graft organ involved	0.903 (0.453;1.801)	0.7726		
Monomorphic histology	1.519 (0.759;3.041)	0.2376		
CNS involvement		0.5534		
PCNSL	0.862 (0.267;2.782)	0.8044		
Secondary	ND	0.9884		
Extranodal disease	1.352 (0.694;2.631)	0.3751		
Elevated LDH	2.200 (1.248;3.879)	0.0064		
CD20 positivity		0.1585		
Positive	1.317 (0.522;3.319)	0.5598		
Partially positive	ND	0.9873		
Hypoalbuminemia	2.371 (1.354;4.152)	0.0025		
IPI score	1.417 (1.146;1.751)	0.0013		
ECOG PS		0.0417		
ECOG 2[b]	1.924 (1.054;3.515)	0.0332		
ECOG 3/4[b]	ND	0.9897		
Ann Arbor stage III-IV	1.143 (0.644;2.027)	0.6479		
Year of PTLD diagnosis	0.969 (0.931;1.009)	0.1280	0.975 (0.929;1.024)	0.3078

[a]*Compared to heart transplant.*

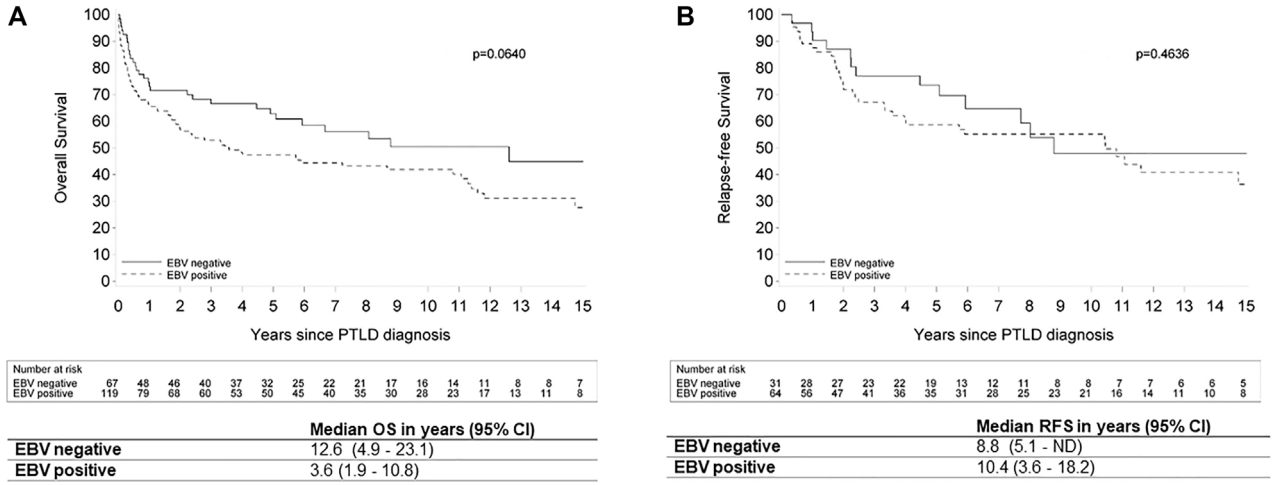
[b]*Compared to ECOG PS 0-1.*

95% CI, 95% confidence interval; PTLD, post-transplant lymphoproliferative disorder; ECOG PS, eastern cooperative oncology group performance status; EBV ISH, Epstein-Barr Virus in situ *hybridization; LDH, lactate dehydrogenase; IPI, international prognostic index; PCNSL, primary central nervous system lymphoma; ND, not determined.*

TABLE 7 | Comparison of baseline characteristics in relation to EBV status.

	EBV negative (N = 67)	**EBV positive (N = 119)**	**p**
Male Gender	45 (67.2%)	76 (63.2%)	0.75
Transplanted organ			
Heart	8 (12%)	21 (17.7%)	0.02
Liver	14 (20.1%)	13 (10.9%)	
Lung	11 (16.4%)	39 (32.8%)	
Kidney	34 (50.8%)	46 (38.7%)	
Graft organ involvement	7 (10.5%)	29 (24.4%)	0.021
Monomorphic PTLD	54 (80.6%)	98 (82.4%)	0.84
CNS involvement	2 (3%)	12 (10.1%)	0.27
CD20 positive	52 (78.8%)	96 (82.1%)	0.27
Decreased albumin	26 (38.5%)	57 (50%)	0.16
Median age at PTLD (years)	56	52.6	0.18
Median IPI	2	2	0.37
Initial therapy			0.090
RIS alone	5 (7.5%)	17 (14.3%)	
RIS + other (excluding R/chemo)	5 (7.5%)	13 (10.9%)	
RIS + R	40 (59.7%)	54 (45.4%)	
RIS + chemo	10 (14.9%)	9 (7.6%)	
RIS + R + chemo	3 (4.5%)	15 (12.6%)	
Other	4 (6.0%)	11 (9.2%)	

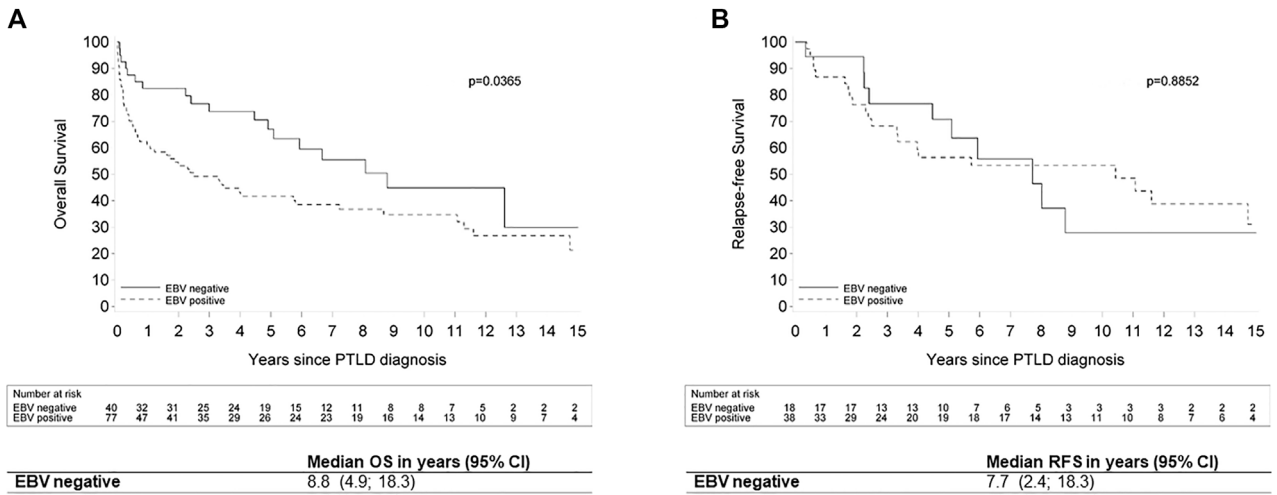
chemo, chemotherapy; EBV, Epstein-Barr Virus; IPI, international prognostic index; CNS, central nervous system; PTLD, Post-transplant lymphoproliferative disorder, R, rituximab; RIS, reduction of immunosuppression.



	Median OS in years (95% CI)
EBV negative	12.6 (4.9 - 23.1)
EBV positive	3.6 (1.9 - 10.8)

	Median RFS in years (95% CI)
EBV negative	8.8 (5.1 - ND)
EBV positive	10.4 (3.6 - 18.2)

FIGURE 6 | Kaplan Meier plots for **(A)** overall survival and **(B)** relapse-free survival by EBV status in patients with post-transplant lymphoproliferative disorder. Legend: EBV: Epstein-Barr Virus; OS: overall survival; ND: not determined; PTLD: post-transplant lymphoproliferative disorder; RFS: relapse-free survival; 95% CI: 95% confidence interval.



	Median OS in years (95% CI)
EBV negative	8.8 (4.9; 18.3)
EBV positive	2.5 (1.0; 5.8)

	Median RFS in years (95% CI)
EBV negative	7.7 (2.4; 18.3)
EBV positive	10.4 (3.3; 18.2)

FIGURE 7 | Kaplan Meier plots for **(A)** overall survival and **(B)** relapse-free survival by EBV status in patients with post-transplant diffuse large B-cell lymphoma. Legend: EBV: Epstein-Barr Virus; OS: overall survival; ND: not determined; PTLD: post-transplant lymphoproliferative disorder; RFS: relapse-free survival; 95% CI: 95% confidence interval.

11 cases (5.6%) of polymorphic PTLD, which is less than previously reported (3, 26, 37, 42). A more recent report noted a similar rate, with 5.7% polymorphic PTLD in a single center analysis of 227 adult PTLD after SOT (14). Tsai et al. also reported that PTLD morphology has changed over the past 3 decades, with a gradual increase in the number of monomorphic PTLD and a steady number of polymorphic PTLD (38). This seems to be corroborated by our results.

Burkitt lymphoma type PTLD is a rare entity, with only 8 cases over 30 years in our study. However, their prognosis is relatively good as 6 patients are currently alive and still in remission after treatment with intensified immuno/chemotherapy. We encountered 4 cases of Burkitt-like lymphoma with 11q aberration, a rare entity known to be more prevalent in immunocompromised patients (43). Furthermore, we encountered 8 T-NHLs, of which 2 were classified as hepatosplenic T-cell lymphoma and 3 cases were primary cutaneous T-NHL. Prognosis was very poor in these

patients with 6 of them dying within 1 year after the diagnosis. The poor prognosis of T-cell PTLD has previously been reported (29, 44-48). A more recent report by Barba et al showed that the outcome in 58 T/NK-cell PTLD after kidney transplantation was worse than in 148 T/NK-cell lymphomas in non-transplanted (49). They noted that transplant recipients received less anthracycline-based therapy, probably out of fear of complications in this fragile population. EBV(+) mucocutaneous ulcer has recently been described as an indolent entity occurring in patients with age-related or iatrogenic immunosuppression (2). It is currently classified as a separate entity (outside PTLD) in the WHO 2017 classification (2). However, it can occur in the post-transplant setting and needs to be considered in the differential diagnosis. We reclassified only one case of EBV(+) mucocuteanous ulcer in our cohort, which was originally classified as monomorphic PTLD, DLBCL type.

Most cases of PTLD are related to EBV. However, more recent reports suggest that up to 50% of PTLDs are EBV(−) (50). In our cohort EBV ISH was positive in 64% of all evaluable cases. Analysis of EBV DNA viremia showed a high sensitivity (91%), but low specificity (48%) in predicting EBV ISH status. Previous studies have shown that transplant recipients with PTLD have a higher viral load then recipients without PTLD. Furthermore, a higher or rapidly increasing viral load is associated with a higher risk of PTLD (4, 51-54). The low specificity of the EBV PCR in our series could possibly be attributed to the low cut-off value used (>2.7 log copies/ml or >2.18 log EBV IU/ml).

Genomic and transcriptional studies have recently demonstrated that EBV(+) and EBV(−) PTLD carry different genomic signatures(16,17). The genomic aberrations in EBV(−) PTLD are less complex and indistinguishable from those in immunocompetent DLBCL. This has led to the hypothesis that EBV(+) PT-DLBCL represent true PTLD and that EBV(−) PT-DLBCL could be considered as *de novo* lymphomas in transplant recipients (16,17). EBV(+) and EBV(-) PT-DLBCL have some different clinical characteristics. In particular, EBV(+) PT-DLBCL typically occurs early and is most often non-GCB type, whereas EBV(−) PT-DLBCL occurs later and is typically of GCB type. Furthermore, polymorphic or non-destructive lesions are usually EBV(+)(4, 16, 55). Despite these differences both groups are essentially treated with the same therapy (except EBV-specific adoptive immunotherapy). The impact of EBV status on treatment response or prognosis remains unclear (50, 56). In our cohort we found no significant relation between EBV status and CR, PTLD-related death or OS. However, we observed a significant relation between EBV status and OS in PT-DLBCL, with clinically meaningful improved survival in EBV(-) PT-DLBCL compared to EBV(+) PT-DLBCL (8.8 years versus 2.5 years, respectively). Previous reports have shown conflicting results on the relation between EBV status and OS (13, 14, 18, 25, 50, 57, 58).

As only 21 patients were treated before 2000 (when rituximab became available in Belgium), no comparison could be made regarding outcomes in the pre- and post-rituximab era. However, we investigated the impact of date of PTLD diagnosis on outcome parameters. We observed a significant improvement in OS and a diminished PTLD-related death rate with later year of PTLD diagnosis. This relation was not found with CR and RFS. It seems that the prognosis of PTLD has improved over the past decades, although the responses to first line treatment have not. Possible explanations for this finding could be achievement of deeper responses, better supportive care and risk-stratified sequential therapy (patients not achieving CR to rituximab monotherapy can still be rescued with R-CHOP chemotherapy).

RIS remains the cornerstone of PTLD treatment. Twenty-five patients were treated with RIS alone and 13 of these achieved a CR (52%). Reported response rates to RIS have been very variable, however the largest earlier reported single-center retrospective analysis of 67 PTLDs after SOT treated with RIS alone, reported an overall response rate of 45% (37% CR) (59). Responses have been known to be higher in non-destructive lesions and in EBV(+) PTLD (4). The higher rate of responses in our cohort might reflect the higher ratio of non-destructive and polymorphic lesions. Of note, RIS may be related to subsequent onset of (chronic) rejection, for instance in lung transplant recipients, which requires increased clinical surveillance (60).

The median OS in our cohort was 5.7 years. This is less than reported in the prospective phase II PTLD-1 and PTLD-2 trials, with a median OS of 6.6 years (24,25). However, only CD20-positive PTLD were included in these PTLD-1 and 2 trials. More recent real-world data showed a 3 years OS of 65.9% in CD20-positive PTLD treated with rituximab-based therapy (61). The IPI-score remained the most important poor prognostic factor in multivariate analysis for OS, CR and PTLD-related death in the current study, in concordance with earlier reports. Hypoalbuminemia and type of organ transplanted (liver and lung) were also retained in our multivariate model as poor prognostic factors for OS.

This study is limited by its retrospective design. Treatment of PTLD has obviously changed over the past decades with the incorporation of rituximab into first line treatment of CD20-positive PTLD since the early 2000s. Furthermore, some data regarding EBV serology and EBV PCR in blood were missing, since this only came into practice in the last 2 decades. Some patients reported in the current study were also reported in a previous publication (18). However, the latter study also included PLTD after HSCT and the follow-up was shorter than in the current study. In addition, we reclassified all PTLD according to the WHO 2017 classificiation (2) and added more detailed histopathological data (such as cell of origin).

In conclusion, this retrospective analysis provides real world data on 196 biopsy-proven PTLD cases, to the best of our knowledge the second largest single-institution cohort published in the literature. The OS of our patients increased in the past decade, resulting in a median OS of 5.7 years for the whole cohort. We observed a significantly improved OS for EBV(−) PT-DLBCL compared to EBV(+) PT-DLBCL.

DATA AVAILABILITY STATEMENT

Data concerns health-related information of the patients and therefore cannot be given away freely. If needed, the first author can be contacted to obtain the data.

ETHICS STATEMENT

This study was approved by the Ethics Committee of University Hospitals/Catholic University Leuven (Ref: S62704 and S55498). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

VV, TT, and DD participated in concept and design and drafting of the article. VV, TT, DD, and SF participated in data extraction. VV, CMD, SF, WL, BS, AU, JC, GV, RV, TT, and DD participated in critical revision of the article for intellectually important content.

CONFLICT OF INTEREST

VV reports consultancy fees from Beigene, BMS/Cellgene, Gilead/Kite, speaker fees from from Janssen, travel support from Amgen, Abbvie; all paid to her institution. CMD reports consultancy fees from Sirtex, PSI CRO, Terumo and Ipsen and speaker fees from Ipsen; all paid to his institution. WL reports consultancy fees from Boston-Scientific, Cook Medical, CLS Behring, Echosens, Evive Biotech, Genfit, Norgine, Abbvie, Gore and Intercept.; all paid to institution. TT reports consultancy and speaker fees from EUSApharma; all paid to his institution. TT holds a Mandate for Fundamental and Translational Research from the 'Stichting tegen Kanker' (2014-083 and 2019-091). DD reports grants/research support from Roche; personal fees/honoraria from Takeda, Novartis, Amgen, Atara Biotherapeutics, Incyte; all paid to his institution. DD holds a mandate for Clinical and Translational Research from "Kom op tegen Kanker" (2017/10908/2816). RV is a senior clinical research fellow of the Research Foundation Flanders (FWO). BS is a senior clinical investigator of the Research Foundation Flanders (1842919N) and received funding from the Foundation Against Cancer (Stichting tegen Kanker; C/2020/1380).

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10707/full#supplementary-material

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GLOSSARY

[^{18}F]FDG-PET/CT, Positron emission tomography with ^{18}F-fluorodeoxyglucose combined with computed tomography

ATG, anti-thymocyte globulin

B-NHL, B-cell non-Hodgkin lymphoma

CI, Confidence Interval

CNS, Central nervous system

CR, complete response

CT, computed tomography

DLBCL, diffuse large B-cell lymphoma

EBER, Epstein Barr-encoded RNA

EBV, Epstein Barr Virus

EBV(+), Epstein Barr Virus positive

EBV(-), Epstein Barr Virus negative

EBV ISH, Epstein Barr Virus *in situ* hybridization

ECOG PS, Eastern Cooperative Oncology Group Performance status

GCB, germinal center B-cell like

GI, gastro-intestinal

HR, hazard ratio

HSCT, hematopoietic stem cell transplantation

IPI, International Prognostic Index

IQR, interquartile range

LDH, lactate dehydrogenase

NHL, non-Hodgkin lymphoma

OR, Odds Ratio

OS, overall survival

PCNSL, primary central nervous system lymphoma

PCR, polymerase chain reaction

PTLD, Post-transplant lymphoproliferative disorder

PT-DLBCL, Post-transplant diffuse large B-cell lymphoma

R-CHOP, rituximab, cyclophosphamide, doxorubicine, vincristine and prednisolone

RFS, relapse-free survival

RIS, reduction of immune suppression

SOT, solid organ transplantation

T-NHL, T-cell non-Hodgkin lymphoma

WHO, World Health Organization

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LETTER TO THE EDITOR
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One-Year Experience With the New Kidney Allocation Policy at a Single Center and an OPO in the Midwestern United States

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Keywords: discard, kidney allocation, OPO, import kidneys, policy

Dear Editors,

The new kidney allocation policy implemented in March 2021 has replaced the traditional donation service areas (DSAs) boundaries with a single 250-nautical mile circle centered around the donor hospital to decrease geographic disparities in waiting time for deceased donor kidney transplantation (DDKT) (1). Despite the extensive discussion about the policy development and simulation models for potential consequences (2–4), few studies have quantitatively investigated the practical impacts of this redistricting change on transplant center-level and organ procurement organization (OPO)-level practices. An early evaluation of a large rural transplantation program in the East Coast found that the new kidney allocation policy has led to an increase in Kidney Donor Profile Index (KDPI) of donors with longer cold ischemia time (CIT), leading to higher delayed graft function (DGF) rates (5). As a large transplant center located in the Midwestern United States, in this study, we evaluate the impacts of the new allocation policy on our transplant center and its OPO, Mid-American Transplant.

This is a retrospective, cross-sectional analysis of organ offers, allograft outcomes, and attributed costs before and after the change of allocation system. The data from our single transplant center and its OPO between 15 March 2019 and 14 March 2022 was analyzed for three time periods, i.e., pre-allocation era without pandemic (15 March 2019 to 14 March 2020), pre-allocation era with pandemic (15 March 2020 to 14 March 2021), and post-allocation era with pandemic (15 March 2021 to 14 March 2022). For all pre- and post-allocation comparations, data of pre-allocation era with pandemic was used to adjust for the potential impacts of the pandemic.

There were 254, 234, and 224 DDKT performed in our transplant center during three time periods, respectively. No statistically significant difference was found regarding the percentage of imported kidneys, DGF, CIT, and KDPI due to the pandemic (**Figure 1**). Compared to the pre-allocation era with pandemic, the percentage of imported kidneys has increased from 14% to 60% ($p < 0.001$) in the post-allocation era; the percentage of DGF has increased from 21% to 30% ($p < 0.05$). The CIT has increased from an average of 15 h to 20 h ($p < 0.001$). The KDPI has increased from an average of 40% to 50%, with the percentage of KDPI ≥85% increased from 6% to 12% ($p < 0.001$). While the number of transplants performed did not increase, the number of organ offers became extremely voluminous and heavily impacted our ability to perform surgeries the next day

Abbreviations: DSAs, donation service areas; OPO, organ procurement organization; DGF, delayed graft function; CIT, cold ischemia time; KDPI, kidney donor profile index.

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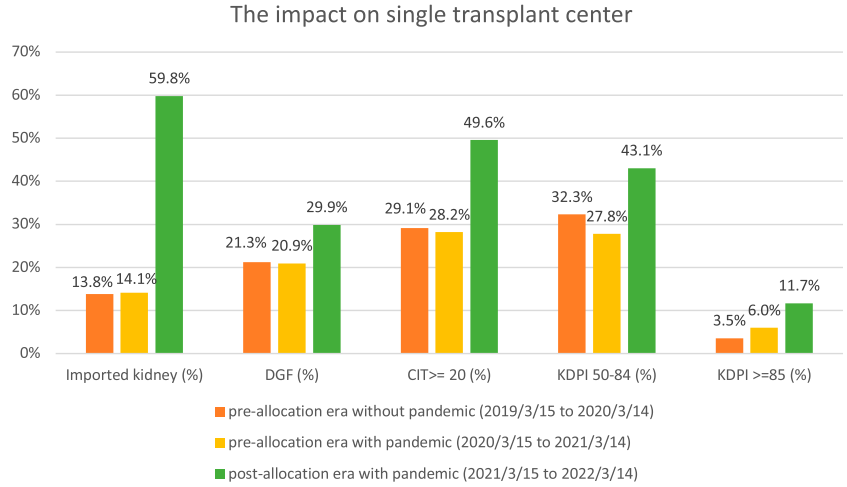

FIGURE 1 | Comparison of the pre- and post-new kidney allocation policy in the kidney transplant center.

after being awake all night reviewing those organ offers. As a result of increased workload and dramatic increase in donors offered in the night during the post-allocation era, our transplant center added 4 new Full-time Equivalent (FTE) positions, with 1 FTE on thoracic offers and 1 FTE on abdominal offers for 24 h periods and having 24 h off.

For the OPO, the average sequence number for all kidneys accepted for three time periods was 759, 534, and 1,491, respectively. This dramatic increase was driven by expedited kidney allocation, which was a response to the significant decline in kidney utilization and increased discards experienced in the post-allocation period. The number of kidneys exported also increased from 134 in pre-allocation era to 261 in the post-allocation era. In anticipation of increased offers and increased import organs, the OPO hired one additional Organ Import Coordinator (OIC) and one additional Organ Recovery Coordinator (ORC). The OIC handles the incoming organ offers for the transplant centers and assists with planning and logistics. The ORC is the preservationist, who also cannulates and pumps imported kidneys and monitors them for a while before sending them to the transplant center. Additionally, compared to the pre-allocation era with pandemic, the percentage of imported kidneys increased from 10% to 32% in the post-allocation era ($p < 0.001$). As the percentage of imported kidney increases, the cost of kidneys increases accordingly. The cost of transportation of a local donor to a local transplant center was $60 or less, whereas it takes between $600 and $1500, on average, when shipping a kidney across the country. For local kidneys, CIT increased from an average of 16 h to 19 h ($p < 0.001$); the percentage of pumped kidneys decreased from 60% to 52% ($p < 0.05$).

Our analyses show that the implementation of new kidney allocation policy has posed an additional operational and financial burden to our transplant center and its local OPO. Our results were consistent with the findings of Rohan et al. (5) and the anticipations about the complexity and unintended detrimental consequences of the new kidney allocation (6, 7). While this single transplant center analysis needs to be interpreted carefully, it remains unknown if these changes would continue to be the new norm or would regress after reaching a new equilibrium. Continuous monitoring the efficiency and evaluating the impacts of the new allocation policy in different regions in the United States are warranted.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

TA and GM designed the study, acquired data, interpreted data, and revised the paper critically. MJ analyzed and interpreted data, drafted the paper, and revised it critically. RR, S-HC, and JW revised the paper critically.

CONFLICT OF INTEREST

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

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LETTER TO THE EDITOR
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SARS-CoV-2 Omicron BA.1/ BA.2 Neutralization up to 8 Weeks After PrEP With Sotrovimab or Cilgavimab/Tixagevimab

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Keywords: COVID-19, kidney transplantation, antiviral, prophylaxis, SARS-CoV2

Dear Editors,

Pre-exposition prophylaxis (PrEP) with monoclonal antibodies (mab) against severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) is used to prevent coronavirus disease 2019 (COVID-19) in high-risk individuals with insufficient response to vaccination (1, 2). Currently, cilgavimab/tixagevimab (Evusheld, AstraZeneca) remains the only mab combination approved for PrEP. A significant reduction of *in vitro* neutralization capacity against the Omicron BA.1 variant (B1.1.529) was observed for most mabs including cilgavimab/tixagevimab. A high rate of breakthrough infections including severe disease following cilgavimab/tixagevimab was observed for BA.1 (3). Sotrovimab (Xevudy, VIR Biotechnology GlaxoSmithKline) retained substantial *in vitro* neutralization capacity against BA.1, and a half-life of 48.8 days made it a candidate for an off-label use as PrEP in high-risk individuals. However, sotrovimab has shown a significantly reduced *in vitro* neutralization capacity against the Omicron BA.2 sub-lineage while cilgavimab/tixagevimab retained strong activity (4).

We used sotrovimab in an off-label indication as PrEP in kidney transplant recipients (KTR) without neutralizing antibodies after at least three COVID-19 vaccine doses at our institution (Medical University of Vienna, Austria) beginning in January 2022 (following the emergence of BA.1), and PrEP was changed to cilgavimab/tixagevimab in March 2022 (following the emergence of BA.2). PrEP was provided to KTR with antibody levels <264 BAU/mL following three vaccinations and no previous history of COVID-19.

In the present analysis, we longitudinally assessed the *in vivo* neutralization capacity of sotrovimab (n = 20) against BA.1 as well as BA.2 (proof of principle for sotrovimab as PrEP), and cilgavimab/tixagevimab (n = 30) against BA.2 (following the emergence of BA.2) for up to 8 weeks after PrEP (baseline characteristics are provided in **Supplementary Table S1**). Patients either received 1) 500 mg of sotrovimab intravenously (between January 12 and January 19, 2022) or 2) 300 mg of cilgavimab/tixagevimab by intramuscular injection (between March 4 and March 9, 2022). All patients were followed at the outpatient department of the Division of Nephrology and Dialysis at the Medical University in Vienna (IRB# 1362/2020; 1612/2021). Serum samples were collected at 4 and 8 weeks after antibody administration in all patients as well as 1 hour (only sotrovimab) and 2 weeks (both sotrovimab and cilgavimab/tixagevimab) in a subgroup of patients. Variant-specific live virus neutralization tests (NT) were performed with BA.1 and BA.2 variants. Detailed methods are provided in the supplementary material (5, 6). NT titers of serum samples ≥10 were considered positive. Neutralization titers are reported as median and Q1 and Q3.

All individuals receiving sotrovimab retained neutralization capacity against the BA.1 variant for 4 weeks follow up (FU), and all but one individual still exhibited neutralization capacity against

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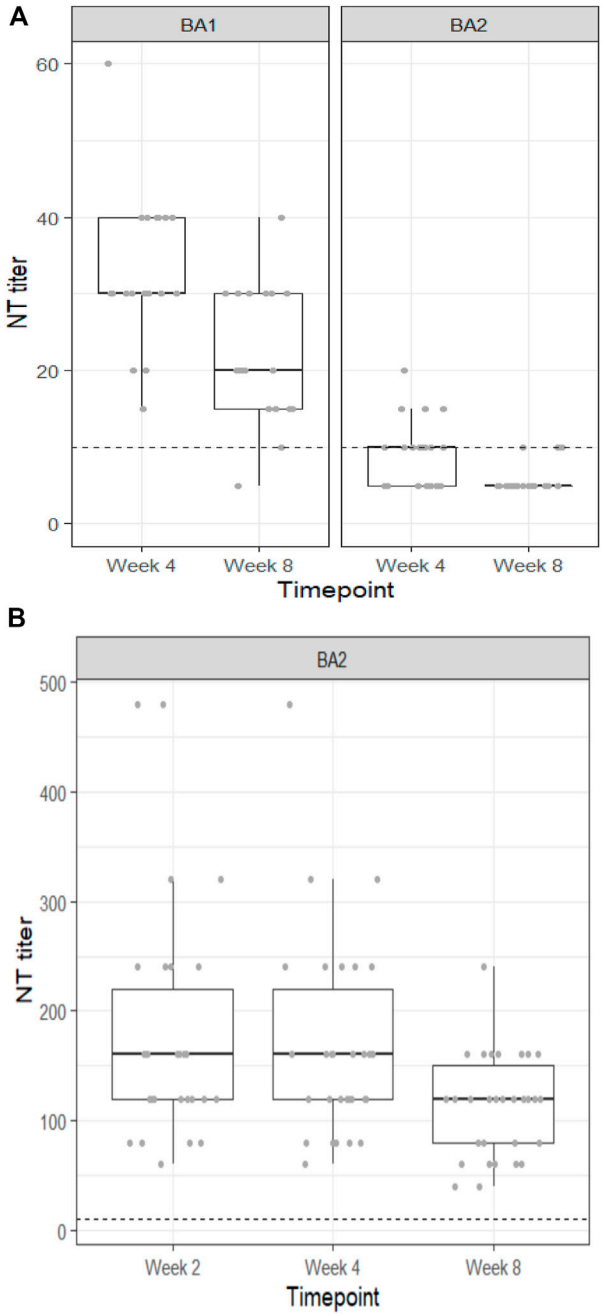

FIGURE 1 | (A) Neutralization titers of patient sera against the SARS-CoV-2 Omicron BA.1 and BA.2 variants at four and 8 weeks after prophylactic infusion of sotrovimab. **(B)** Neutralization titers of patient sera against the SARS-CoV-2 Omicron BA.2 variant at 2, 4, and 8 weeks after prophylactic infusion of cilgavimab/tixagevimab.

BA.1 at 8 weeks FU. Median NT titers decreased from 30 (Q1, Q3: 30, 40) at 4 weeks to 20 (Q1, Q3: 15, 30) at 8 weeks FU (**Figure 1A**). In contrast, neutralizing capacity against the BA.2 variant in serum was only present in 60% at 4 weeks and further decreased to 15% at 8 weeks FU. In line, median NT titers against the BA.2 variant were also significantly lower (10 [Q1, Q3: <10, 10] and <10 [Q1, Q3: <10, <10] at 4 weeks and 8 weeks of FU, respectively; **Figure 1A**). However, analysis in the subgroup with measurements at 1 h and 2 weeks after sotrovimab infusion showed that all individuals had initially achieved neutralization capacity against BA.2 (40 [Q1, Q3: 30, 45] and 20 [Q1, Q3: 19, 30] at 1 h and 2 weeks, respectively).

Patients receiving cilgavimab/tixagevimab had significantly higher neutralization titers against BA.2 than those receiving sotrovimab at all time points: 160 [Q1, Q3: 120, 220], 160 [Q1, Q3: 120, 220] and 120 [Q1, Q3: 80, 150] at 2, 4, and 8 weeks, respectively (**Figure 1B**).

We could show that sotrovimab retains neutralization capacity against BA.1 for at least 8 weeks while only having a limited neutralization activity against the BA.2 variant. Cilgavimab/tixagevimab on the contrary shows strong *in vivo* neutralization of BA.2 for at least 8 weeks. Our data support that PrEP has to be adapted based on immune-evasion characteristics of pre-dominant SARS-CoV-2 variants.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics committee of the Medical University of Vienna. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KS, LW, AH, RO, and RR-S conceptualized the study. KS, AH, and JC performed the analyses. AH and RR-S wrote the draft of the manuscript. All authors approved the mansucript.

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.2022.10906/full#supplementary-material

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TRANSPLANT QUIZ
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A Kidney Transplant Recipient on Prophylactic Eculizumab Presenting With Myalgia

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Keywords: Eculizumab, atypical hemolytic uremic syndrome, acute kidney injury, renal allograft, myalgia

CASE REPORT

A 50-year-old renal transplant recipient presented with 1 week history of myalgia and fever. His past medical history was remarkable for a deceased donor kidney transplant 16 months prior to presentation. The cause of renal disease leading to end stage kidney disease (ESKD) prior to his transplant was atypical haemolytic uraemic syndrome (aHUS) with thrombotic microangiopathy (TMA) on the native kidney biopsy (also had features of advanced interstitial fibrosis and tubular atrophy). Subsequent investigations confirmed heterozygous complement factor I mutation. His treatment included single-agent immunosuppression with tacrolimus and fortnightly prophylactic Eculizumab, since his transplant.

On admission, he was normotensive and oligo-anuric with dark urine. A COVID-19 rapid test was positive and was confirmed with nasopharyngeal RT-PCR (no previous vaccination). He had missed one dose of Eculizumab. Laboratory tests revealed significant AKI with a creatinine of 1450 umol/l (baseline 180 umol/l), urea 41.1 mmol/l, LDH 12300U/L, CRP 264 mg/l (**Figure 1**). His haemoglobin, platelet count and haptoglobin levels were normal without fragments on a blood film and complement levels were normal. He was treated with intravenous fluids, broad spectrum antibiotics and dexamethasone according to the local protocol at the time. He did not respond to volume expansion and required intermittent haemodialysis. In view of the on-going need for dialysis more than 2 weeks following his admission a kidney graft biopsy was performed (**Figures 2A,B**, **3**).

TEST QUESTIONS

(1) Which patients are recommended to have prophylactic Eculizumab prior to kidney transplant?
   (a) Isolated membrane co-factor (MCP) protein mutations
   (b) Persistently negative Factor H autoantibodies
   (c) Pathogenic variant in *CFH* or *CFI*
   (d) Pathogenic variant in a non-complement pathway gene, eg., DGKε
   (e) Previous graft loss due to allograft rejection

(2) Which maintenance immunosuppression agent should be avoided in patients at risk of recurrent aHUS?
   (a) Ciclosporin
   (b) Tacrolimus
   (c) Sirolimus
   (d) MMF
   (e) Azathioprine

Transplant Quiz

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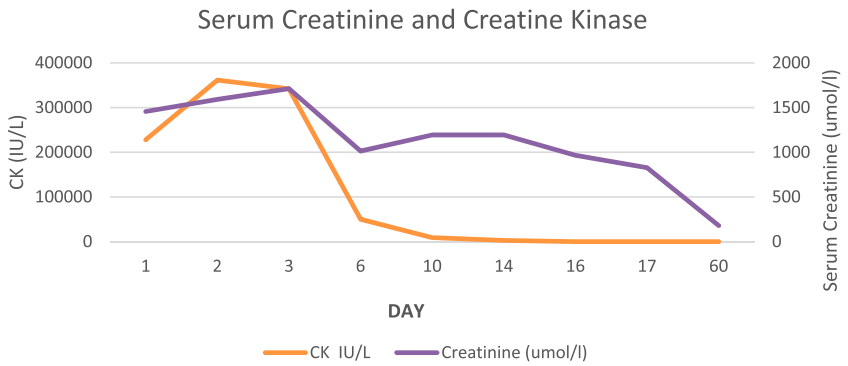

FIGURE 1 | Serum creatinine (umol/l) and creatine kinase (IU/L).

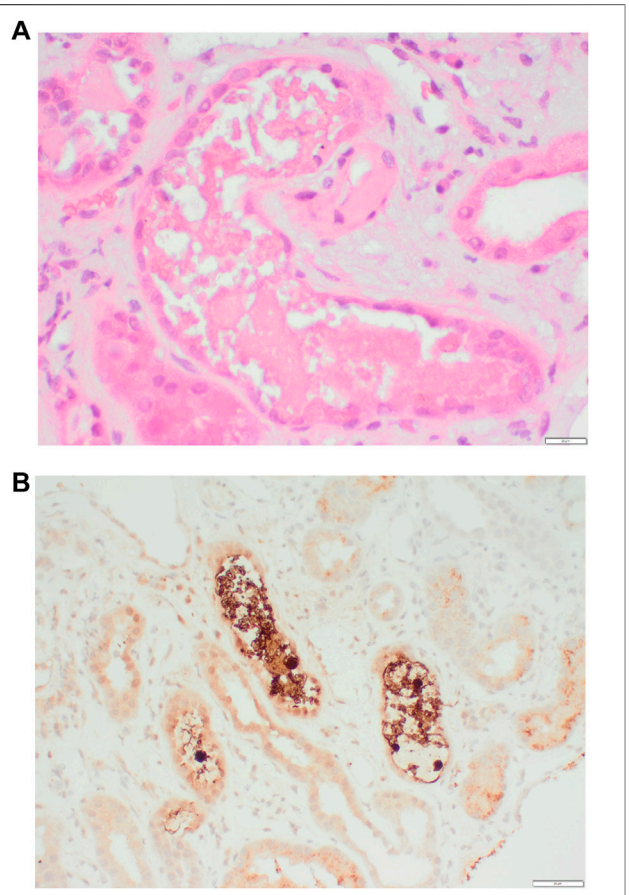

FIGURE 2 | (A) Granular "stringy" and "beaded" cast suspicious for myoglobulin and **(B)** Positive immunohistochemistry (brown) in myoglobin casts.

(3) What is the histological finding from renal biopsy in **Figure 2A,B**?
   (a) Oxalate crystals
   (b) Myoglobin casts (rhabdomyolysis)
   (c) Cholesterol emboli
   (d) Acute Thrombotic microangiopathy
   (e) Rejection

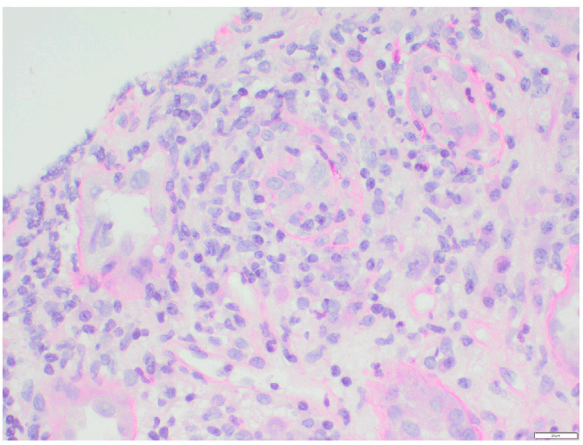

FIGURE 3 | Tubulitis with between 5 and 10 intraepithelial lymphocytes per tubular cross section and discontinuity of the tubular basement membrane (t3).

(4) What is the most significant histological finding from renal biopsy in **Figure 3**?
   (a) Glomerulitis
   (b) Tubulitis
   (c) Peritubular capillaritis
   (d) Acute thrombotic microangiopathy
   (e) Intimal arteritis

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional

requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

OS and RJ wrote the first draft. RJ and AK performed the literature search. AS provided the histopathology images and reviewed the manuscript. AK reviewed, revised and wrote the final manuscript.

CONFLICT OF INTEREST

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

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APPENDIX

Answers and Discussion

Question 1

The correct answer is c.

A typical HUS accounts for 10% presentations of HUS, with 70% of the patients have either a genetic or acquired defect in the regulation of the alternative pathway of complement activation. Around 50% of these patients either die or develop ESKD within 1 year of diagnosis. The risk of recurrence post transplantation is high (up to 60%) with poor graft outcomes. Eculizumab, a humanised Anti-C5 monoclonal antibody, has been shown to be effective for treatment of aHUS, in both native and kidney transplant patients. Current recommendations include the use of prophylactic Eculizumab in those individuals deemed medium or high risk of recurrence. These include patients with previous early aHUS recurrence, pathogenic variants in *CFH* or gene re-arrangements involving *CFH* and Factor H-related proteins, gain of function pathogenic variants in *CFB* or C3, pathogenic variant in *CFI* and those without identified mutation or with persistent low-titre anti-CFH antibody. Patients with the pathogenic variants of Membrane Cofactor Protein (MCP) have the lowest recurrence risk and no recurrence is seen in those with absent DGKε function (1).

Question 2

The correct answer is c.

Early use of mTOR inhibitors is an independent risk factor for the development of TMA (2). With regards to other immunosuppressive agents, there is conflicting evidence of their association with aHUS recurrence. Although, calcineurin inhibitors (CNIs), have been associated with *de novo* TMA, avoiding CNIs did not reduce the risk of recurrence of aHUS. Nonetheless, their use has the established advantage in reducing allograft rejection. Tacrolimus has a lower rate of post-transplant TMA incidence, hence is recommended as per transplant protocol published in 2021 by the National Renal Complement Therapeutics Centre (NRCTC) UK (3).

Question 3

The correct answer is b.

The figure demonstrates myglobin casts secondary to COVID-19 associated rhabdomyolysis. The patients' CK on presentation was 350000 IU/l with anuric AKI and uraemia needing dialysis. Rhabdomyolysis refers to the lysis of striated muscle with glomerular filtration of myoglobin, which binds Tamm-Horsfall proteins leading to cast formation and distal tubular obstruction. The morphology of the myoglobin casts ranged from slightly brown granular casts by hematoxylin and eosin, to beaded globular casts that stained brightly fuchsinophilic with Masson trichrome and partially argyrophilic with silver methenamine. Calcium oxalate and cholesterol crystals dissolve during routine histological preparation. Calcium oxalate is seen as intratubular crystals of multicoloured birefringence under polarised light, with classic fan-shaped morphology. Cholesterol crystal emboli are usually defined by empty, biconvex and needle-shaped clefts, whereas in frozen sections, the crystals are birefringent under polarised light. Diagnostic features of TMA include fibrin thrombi within glomerular capillary loops in the acute stage and double contouring of glomerular basement membrane and intimal proliferation of arterioles in the chronic stage.

We have identified 38 case reports and series of COVID-19 associated rhabdomyolysis with detailed clinical data, including 54 individual cases, on MEDLINE. 80% (n = 43) were male, 18% (n = 10) female, and 2% (n = 1) unspecified. Median age was 54 (19–88) years. There was high burden of comorbidities, including Hypertension (41%, n = 22), pre-existing CKD (11%, n = 6), and Diabetes (19%, n = 10). 11% (n = 6) were taking statin. CK was elevated at presentation in 50% (n = 27), with a mean of 70,049 IU/L. CK peaked at presentation in 33% (n = 18) and peaked after presentation in 65% (n = 35). 54% (n = 29) of patients had AKI, with 69% (20/29) evident at presentation. 33% (n = 18) required haemofiltration and 3.7% (n = 2) remained dialysis-dependent after discharge. A large retrospective study of 1014 hospitalised patients with COVID-19 showed a 2.2% incidence of rhabdomyolysis. Peak CK levels >1000IU/L were an independent risk factor for in-hospital death (HR = 6.46, 95%CI:3.02–13.86) (4).

Rhabdomyolysis should be considered in all patients with COVID-19, presenting with AKI. A biopsy could be pursued if alternative diagnoses are considered or renal function is not improving. Whether rhabdomyolysis is a marker of severe disease; existing as part of a multi-system inflammatory disorder or a driver of mortality in itself, remains unclear.

Question 4

The correct answer is b.

Figure 3 shows lymphocytic infiltration of the tubules (tubulitis). Our patient was diagnosed with COVID-19 associated rhabdomyolysis and concomitant T cellmediated rejection (Type 1B). Without a biopsy the diagnosis of TCMR would have been missed. Early treatment with steroids and subsequent addition of MMF (4 weeks post COVID-19), improved renal function back to his previous baseline (**Figure 1**) and the patient is dialysis independent.

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