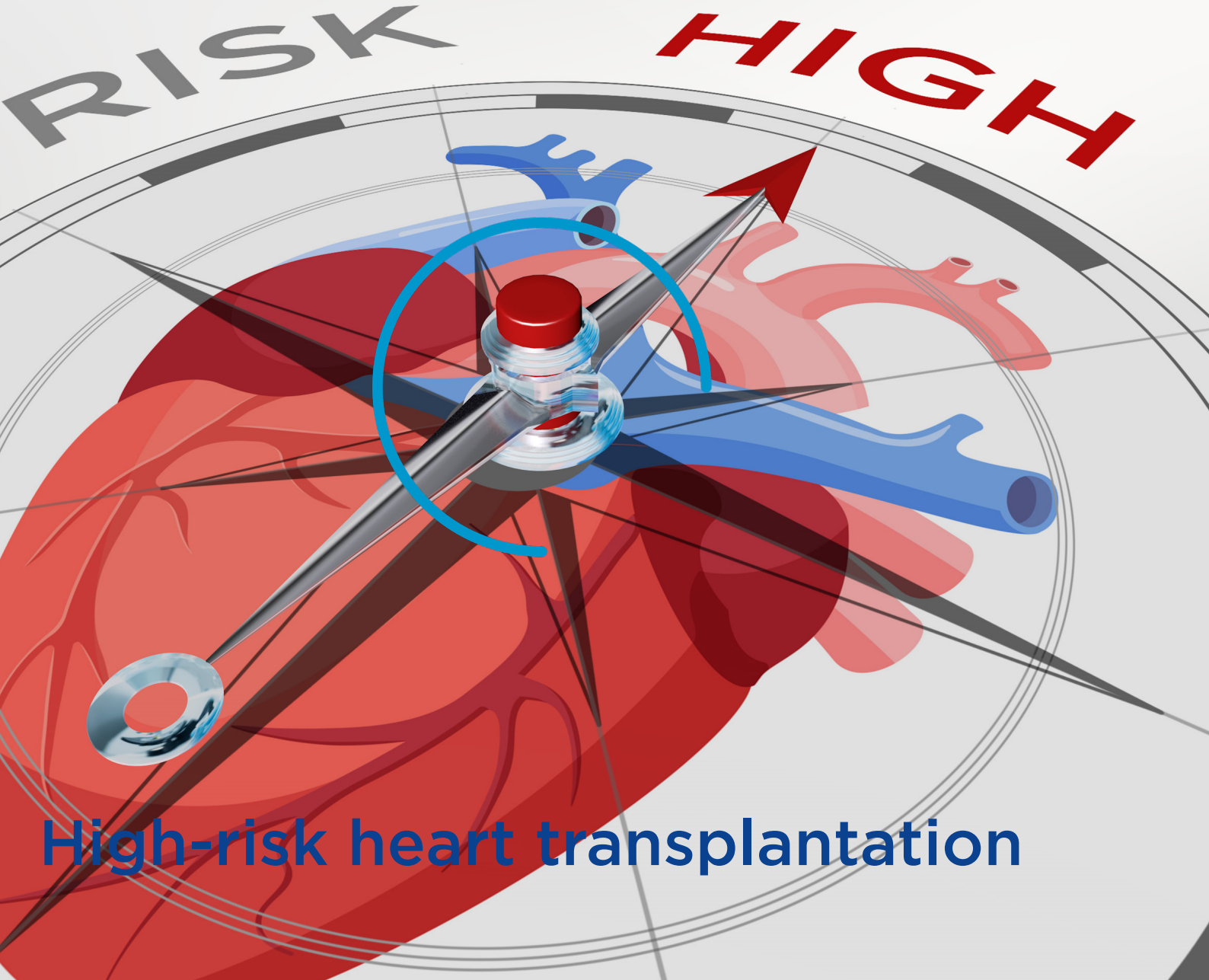
ESOT

Volume 36 | Issue 07
July 2023

Transplant International



High-risk heart transplantation

Transplant International

frontiers | Publishing Partnerships

Transplant International

Editorial Office
Nathan Masters
Sarah Coxon
ti@frontierspartnerships.org

ESOT

Transplant International

Table of contents

Cover Article

Original Research

Brief Research Report

JOIN US!

EDTCO
ESOT

EDTCO ORGAN DONATION CONGRESS 2023

Towards a new era in donor coordination

16 September 2023
Athens, Greece

#ESOT_EDTCO

ESOTCongress
THE INTERNATIONAL TRANSPLANT CONGRESS

16 September

Science Day

Sharing visions, connecting science

ESOTCongress
THE INTERNATIONAL TRANSPLANT CONGRESS

16 September

Education course

Immunosuppression: A critical step in the transplantation journey

#ESOTcongress

ESOT Congress

THE INTERNATIONAL TRANSPLANT CONGRESS

ATHENS | 17-20 SEPTEMBER 2023

Disruptive Innovation, Trusted Care

#ESOTcongress

ESOT | Transplant International | THE PETER MORRIS CENTRE FOR EVIDENCE IN TRANSPLANTATION

TRANSPLANT TRIAL WATCH
published: 27 July 2023
doi: 10.3389/ti.2023.11742

Transplant Trial Watch

John Matthew O'Callaghan [1,2]*

[1]University Hospitals Coventry and Warwickshire, Coventry, United Kingdom, [2]Centre for Evidence in Transplantation, University of Oxford, Oxford, United Kingdom

Keywords: kidney transplant, randomised controlled trial, mTOR inhibitor, cardiovascular outcomes, CMV infection

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

Cardiovascular Outcomes in *De Novo* Kidney Transplant Recipients Receiving Everolimus and Reduced Calcineurin Inhibitor or Standard Triple Therapy: 24-Month Post Hoc Analysis From TRANSFORM Study.

by Sommerer, C., et al. Transplantation 2023 [record in progress].

Aims

This *post hoc* analysis of the TRANSFORM study aimed to compare the effect of everolimus (EVR) and reduced calcineurin inhibitor (rCNI) versus standard triple therapy on cardiovascular disease (CVD) outcomes in *de novo* kidney transplant patients.

Interventions

Participants in the TRANSFORM trial were randomised to either the EVR + rCNI group or the mycophenolic acid (MPA) + standard-exposure CNI (sCNI) group.

Participants

2026 *de novo* kidney transplant recipients.

Outcomes

The main outcomes of interest were the incidence of major adverse cardiac event (MACE), cardiac deaths, time-to-event analysis of MACE, CVD risk factors and levels of metabolic parameters.

Follow-Up

24 months.

CET Conclusion

Everolimus is known to worsen post-transplant dyslipidaemia, but it is not clear that this results in poorer cardiovascular outcomes. This *post hoc* analysis of the TRANSFORM study, which compared

Transplant Trial Watch

OPEN ACCESS

Correspondence:
John Matthew O'Callaghan
ocallaghan.john@gmail.com

Received: *26 June 2023*
Accepted: *17 July 2023*
Published: *27 July 2023*

Citation:
O'Callaghan JM (2023) Transplant Trial Watch. Transpl Int 36:11742. doi: 10.3389/ti.2023.11742

outcomes in kidney transplant patients on standard immunosuppressive therapy vs. a everolimus/reduced tacrolimus regimen, compared the rate of major adverse cardiac events (MACE) between the two groups over a 2 year follow up period. Over 2,000 patients were included in the analysis. Although lipid levels were increased in the everolimus group as expected, the rate of MACEs were not significantly different between the two groups. The authors speculated whether this could be due to cardio-protective effects due to everolimus, which had previously been demonstrated in preclinical studies, and which offset the lipid dysregulation effects. With the potential to reduce posttransplant viral infections, these findings provide further evidence to support everolimus based regimens as a viable alternative to current immunotherapy standard of care.

Trial Registration
Clinicaltrials.gov—NCT01950819.

Funding Source
Industry funded.

RANDOMISED CONTROLLED TRIAL 2

Conversion to mTOR Inhibitor to Reduce the Incidence of Cytomegalovirus Recurrence in Kidney Transplant Recipients Receiving Preemptive Treatment: A Prospective, Randomized Trial.

by Viana, L. A., et al. Transplantation 2023 [record in progress].

Aims
The investigators aim to assess if switching from mycophenolate or azathioprine to sirolimus following first cytomegalovirus (CMV) infection post-transplant reduces recurrence rate of CMV infections.

Interventions
Once randomised the intervention group were abruptly converted from antimetabolite (mycophenolate sodium 720 mg twice daily or azathioprine 2 mg/kg once daily) to sirolimus (5–8 ng/mL), tacrolimus was continued, but the maintained concentrations were lowered to 3–5 ng/mL. The control group continued tacrolimus (5–10 ng/mL) and their antiproliferative agent.

Participants
72 adult kidney transplant recipients who had a treated CMV infection within the first 6 months after transplant.

Outcomes
The primary endpoint was the incidence of recurrent CMV infection within 12 months following randomisation. The secondary endpoints were: Incidence of *de novo* DSA, kidney function, proteinuria, acute rejection, graft loss and death.

Follow-Up
Participants were followed-up for 12 months.

CET Conclusion
This small prospective unblinded randomised control trial demonstrates that conversion to sirolimus form antimetabolite following initial CMV infection has a significant effect to reduce recurrent infection. Within the sirolimus cohort no episodes of CMV infection or disease occurred within the study period. Whereas the control group had a recurrence rate of 43%. All the patients in this study are high risk for CMV with all recipients being CMV positive pre-operative, but received no pre-emptive pharmacological treatment, simply weekly blood monitoring. Upon CMV infection/disease intravenous ganciclovir was commenced and once treated the antimetabolite switch to sirolimus and the study period commenced. Within the study group they saw no significant difference in biopsy confirmed acute rejection, *de novo* DSA, proteinuria, graft survival or death. The generalisability of these findings is limited due to the strict inclusion criteria, within their study period they randomised 72 patients out of a total of 1,309 with a first-treated CMV infection/disease, all of whom were low-immunological risk. The patient cohort and small sample size limits safety and efficacy conclusion as well as conclusion in broader recipient populations, such as D+/R−. While there is insufficient evidence to change current practice it sits along side other trials, some of which multi-centre and larger supporting mTORi in improving CMV related outcomes, although often with higher discontinuation rates.

Jadad Score
3.

Data Analysis
Strict intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
ClinicalTrials.gov—NCT02671318.

Funding Source
Industry funded.

CLINICAL IMPACT SUMMARY

Pre-emptive treatment of CMV after renal transplantation is associated with a considerable risk of later CMV infection recurrence, and further changes to immune suppression and

acute rejection. Sirolimus and other MTORi are associated with lower risk of CMV infection.

The study is a small but interesting one and of good quality. Patients could be included after the first episode of CMV infection or disease, and were then randomised to convert to sirolimus or stay on mycophenolate/azathioprine. Tacrolimus was continued in both groups.

There was no recurrence of CMV in the group randomised to sirolimus, which is in stark contrast to the 43% recurrence in the control arm. Importantly there was no significant difference in the incidence of treated biopsy proven acute rejection within 12 months after randomisation.

This study provides convincing evidence for a potential method to tailoring immune suppression and reduce the risk of further complications for CMV. However, the population was highly selected to be at low risk of rejection, and that needs to be kept in mind.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

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

ESOT | Transplant International

NEWS AND VIEWS
published: 19 July 2023
doi: 10.3389/ti.2023.11670

Understanding the Immunology of Normothermic Machine Perfusion

Menna Ruth Clatworthy[1] *and Christopher John Edward Watson*[1]*

[1]*Department of Medicine, University of Cambridge, Cambridge, United Kingdom,* [2]*Department of Surgery, University of Cambridge, Cambridge, United Kingdom*

Keywords: liver transplantation, normothermic machine perfusion, single cell RNA sequencing, immunology, liver preservation

Our early experience with normothermic liver perfusion (NMP) led us to identify four compartments that might influence the viability of livers [1]. While we and others have tried to characterize the hepatocyte and cholangiocyte compartments [2–5], and have provided some recent insights into adverse factors in the vascular compartment [6, 7], the immune compartment has remained relatively unexplored, until now. The recent paper by Hautz et al. from Schneeberger's group in Innsbruck has thrown important light on immune cell changes during the course of perfusion [8]. This work is significant, as a better understanding of the immunological changes occurring during NMP will inform therapeutic interventions to counter reperfusion injury and improve organ quality. Thus, this paper is an important and welcome landmark dataset.

The paper is divided into two parts, analysis of liver biopsies and analysis of cellular components and cytokines in serial perfusate samples. Single cell RNA seq (scRNAseq) was undertaken on eight livers, generating data on all cellular components of liver biopsies, including immune cells within the vasculature and tissue, comparing liver cellular transcriptomes before and at the end of NMP. They showed that nearly half the immune cells in the liver biopsies were neutrophils (defined by *FCGR3B*-expression), a novel observation, likely related to differences in the techniques used to profile cells. The next most frequent immune cell types were monocyte/macrophages (CD68, 8%). They went on to show that the neutrophils emigrate from the liver during NMP, and many appear to be lost from the circuit and do not recirculate to the liver, although they remain the predominant cell type. This is in contrast to macrophages ($CD68^+$) and T (CD3 and CD4) and B cells (CD79A) whose proportions did not change significantly. They next considered the transcriptional changes occurring in neutrophils and monocyte/macrophages subclusters during NMP; Neutrophil chemokine receptor expression changed such that cells downregulated *CXCR1/2* and upregulated *CXCR4*, the receptor mediating the return of aged neutrophils to the bone marrow for clearance. Their chemokine profile also changed, with marked expression of *CXCL8*/IL8—the major neutrophil recruiting chemokine—during perfusion, suggesting a capacity for autocrine signalling. The authors propose that overall, the transcriptional changes observed in neutrophils during NMP are consistent with a progression to an aged, chronically activated/exhausted neutrophil phenotype. This suggests that the inflammatory response to reperfusion may be exhausted with time on NMP, an important observation. Neutrophil recruiting chemokines (*CXCL2*, *CXCL8*) were among the most upregulated transcripts in kidneys undergoing NMP [9], suggesting similar processes may also be at play in kidneys.

Four clusters of monocyte/macrophages were identified. The M0 cluster that dominated pre-NMP samples expressed high levels of inflammatory markers such as *S100A8/9*, whilst the M3 cluster enriched in post-NMP samples had a more mixed transcriptome, expressing both pro-inflammatory molecules such as *CTSL* (encoding Cathepsin-L, a protease capable of tissue damage) and anti-inflammatory and tolerogenic molecules (for example, *HMOX1*, encoding haemoxygenase-1, which

News & Views

OPEN ACCESS

Correspondence:
Christopher John Edward Watson
cjew2@cam.ac.uk

Received: *10 June 2023*
Accepted: *07 July 2023*
Published: *19 July 2023*

Citation:
Clatworthy MR and Watson CJE (2023) Understanding the Immunology of Normothermic Machine Perfusion. Transpl Int 36:11670. doi: 10.3389/ti.2023.11670

degrades haem and reinforces an M2 macrophage phenotype), with the potential to counter reperfusion injury.

When Hautz et al. looked at the cellular composition of perfusate in 26 livers, they saw a rapid increase in leucocyte numbers after the start of NMP, predominantly neutrophils, NK cells, B cells and monocyte/macrophages [8]. Numbers peaked at 6 h and thereafter declined. They proceeded to immunophenotype the cells and showed that amongst T cells, the initial predominance of CD4 (48%) and CD8 (44%) at 1 h changed over time, becoming predominantly CD4 cells, with more demonstrating a $CD3^+CD4^+FoxP3^+$ Treg phenotype.

Finally, they examined cytokine protein levels in the perfusate. As demonstrated by others, many cytokines are secreted into the perfusate over the duration of perfusion. Among cytokines, they identified IL6 as one of interest, as it was increased in DCD grafts compared with DBD grafts, and was higher in discarded livers relative to those transplanted, with macrophages implicated as the major source in the single cell transcriptomic data. We have independently observed similar associations of perfusate IL6 with adverse clinical outcomes, with the highest levels found in a liver suffering primary non function (Watson, unpublished). These data raise the potential of perfusate IL6 as a viability biomarker, but require larger datasets to confirm. The authors also found TNF to be significantly raised in the perfusate of discarded livers compared to transplanted livers, together suggesting that a heightened innate inflammatory response is associated with worse outcomes, and mirroring bulk transcriptomic data in kidneys undergoing NMP [9].

What can we take away from the paper for clinical use? First, potential targets for intervention have been identified. Immune cells are activated at the start of perfusion, cytokines released, and neutrophils, the most dominant cells, exhausted over time. Targeting DAMPs (damage-associated molecular patterns) or cytokines such as IL1β and IL18 capable of stimulating neutrophil activation may mitigate the initial immune response. Elevated IL6 and TNF were associated with worse outcomes, but these associations remain correlative rather than definitively causative. The authors recommend selective neutralization of cytokines, rather than global cytokine removal, which we have shown to reduce the transcriptional changes associated with delayed graft function in kidneys [9].

Even without removal or neutralization of specific cytokines, the data from Hautz et al. suggest that prolonging perfusion over 6 h may in itself result in an environment more conducive to liver recovery, and therefore decisions regarding viability should be delayed, as suggested by other work from the Innsbruck group which showed that lactate values at 6 h are the most predictive value (paper in preparation). In addition, the emigration of neutrophils during NMP, and their loss on the circuit, may result in a less immunogenic graft.

The literature on cardiopulmonary bypass (CPB) reports a systemic inflammatory response syndrome which is associated with high levels of IL8 [10, 11], and is thought to be related to contact activation of neutrophils exposed to the tubing and oxygenator surfaces of the CPB circuit and the sheer stresses involved [12, 13]. These surfaces may account for the sequestering of neutrophils in NMP. Hence it is possible that it is the mechanical circuit, rather than the liver, that is responsible for the transcriptional changes observed in neutrophils in this study. Therefore another therapeutic avenue to explore would be interventions to the circuit, such as "pacifying" the circuit with high density lipoprotein or albumin before perfusion begins to reduce neutrophil adhesion, or using human albumin in place of Gelofusine [14]; alternatively consideration may be given to altering the oxygenator design [15], to assess whether any of these interventions results in less neutrophil activation and sequestration, and cytokine production.

Overall, this study by Schneeberger's group provides a granular view of cell-specific transcriptional changes occurring in immune populations during liver perfusion, coupled with an assessment of the perfusate, delivering a useful and timely resource for the community and highlights potential therapeutic avenues.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

Author CW has received honoraria from OrganOx Ltd for speaking commitments.

The remaining 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.

REFERENCES

1. Watson CJE, Jochmans I. From "Gut Feeling" to Objectivity: Machine Preservation of the Liver as a Tool to Assess Organ Viability. *Curr Transpl Rep* (2018) 5:72–81. doi:10.1007/s40472-018-0178-9
2. Watson CJE, Kosmoliaptsis V, Pley C, Randle L, Fear C, Crick K, et al. Observations on the *Ex Situ* Perfusion of Livers for Transplantation. *Am J Transpl* (2018) 18:2005–20. doi:10.1111/ajt.14687
3. Watson CJE, Gaurav R, Fear C, Swift L, Selves L, Ceresa CDL, et al. Predicting Early Allograft Function After Normothermic Machine Perfusion. *Transplantation* (2022) 106:2391–8. doi:10.1097/TP.0000000000004263
4. Mergental H, Laing RW, Kirkham AJ, Perera MTPR, Boteon YL, Attard J, et al. Transplantation of Discarded Livers Following Viability Testing with Normothermic Machine Perfusion. *Nat Commun* (2020) 11:2939. doi:10.1038/s41467-020-16251-3
5. van Leeuwen OB, de Meijer VE, Porte RJ. Viability Criteria for Functional Assessment of Donor Livers During Normothermic Machine Perfusion. *Liver Transpl* (2018) 24:1333–5. doi:10.1002/lt.25330
6. Watson CJE, MacDonald S, Bridgeman C, Brais R, Upponi SS, Foukaneli T, et al. D-Dimer Release From Livers During *Ex Situ* Normothermic Perfusion and After *In Situ* Normothermic Regional Perfusion: Evidence for Occult Fibrin Burden Associated with Adverse Transplant Outcomes and Cholangiopathy. *Transplantation* (2023) 107:1311–21. doi:10.1097/TP.0000000000004475

7. Haque O, Raigani S, Rosales I, Carroll C, Coe TM, Baptista S, et al. Thrombolytic Therapy During Ex-Vivo Normothermic Machine Perfusion of Human Livers Reduces Peribiliary Vascular Plexus Injury. *Front Surg* (2021) 8:644859. doi:10.3389/fsurg.2021.644859
8. Hautz T, Salcher S, Fodor M, Sturm G, Ebner S, Mair A, et al. Immune Cell Dynamics Deconvoluted by Single-Cell RNA Sequencing in Normothermic Machine Perfusion of the Liver. *Nat Commun* (2023) 14:2285. doi:10.1038/s41467-023-37674-8
9. Ferdinand JR, Hosgood SA, Moore T, Ferro A, Ward CJ, Castro-Dopico T, et al. Cytokine Absorption During Human Kidney Perfusion Reduces Delayed Graft Function-Associated Inflammatory Gene Signature. *Am J Transpl* (2021) 21:2188–99. doi:10.1111/ajt.16371
10. Gessler P, Pfenninger J, Pfammatter JP, Carrel T, Baenziger O, Dahinden C. Plasma Levels of Interleukin-8 and Expression of Interleukin-8 Receptors on Circulating Neutrophils and Monocytes After Cardiopulmonary Bypass in Children. *J Thorac Cardiovasc Surg* (2003) 126:718–25. doi:10.1016/s0022-5223(03)00685-8
11. Brix-Christensen V, Petersen TK, Ravn HB, Hjortdal VE, Andersen NT, Tonnesen E. Cardiopulmonary Bypass Elicits a Pro- and Anti-Inflammatory Cytokine Response and Impaired Neutrophil Chemotaxis in Neonatal Pigs. *Acta Anaesthesiol Scand* (2001) 45:407–13. doi:10.1034/j.1399-6576.2001.045004407.x
12. Miller BE, Levy JH. The Inflammatory Response to Cardiopulmonary Bypass. *J Cardiothorac Vasc Anesth* (1997) 11:355–66. doi:10.1016/s1053-0770(97)90106-3
13. Radley G, Ali S, Pieper IL, Thornton CA. Mechanical Shear Stress and Leukocyte Phenotype and Function: Implications for Ventricular Assist Device Development and Use. *Int J Artif Organs* (2019) 42:133–42. doi:10.1177/0391398818817326
14. Fontaine E, Warwick R, Sastry P, Poullis M. Effect of Foreign Surface Pacification with Albumin, Aprotinin, Propofol, and High-Density Lipoprotein. *J Extra Corporeal Technol* (2009) 41:3–9.
15. Yildirim F, Amanvermez Senarslan D, Yersel S, Bayram B, Taneli F, Tetik O. Systemic Inflammatory Response During Cardiopulmonary Bypass: Axial Flow Versus Radial Flow Oxygenators. *Int J Artif Organs* (2022) 45:278–83. doi:10.1177/03913988221075043

ESOT | Transplant International

FORUM
published: 24 July 2023
doi: 10.3389/ti.2023.11752

Back to the Future With Co-Stimulation Blockade

Stuart M. Flechner[1*†] *and Klemens Budde*[2†]

[1]*Cleveland Clinic Lerner College of Medicine, Cleveland, OH, United States,* [2]*Department of Nephrology, Charité Universitätsmedizin Berlin, Berlin, Germany*

Keywords: immunosuppression, kidney transplant, belatacept, abatacept, co-stimulation blockade

A Forum discussing:

Conversion From Intravenous In-Hospital Belatacept Injection to Subcutaneous Abatacept Injection in Kidney Transplant Recipients During the First COVID-19 Stay-at-Home Order in France

by Bertrand D, Brunel M, Lebourg L, Scemla A, Lemoine M, Amrouche L, Laurent C, Legendre C, Guerrot D, Anglicheau D and Sberro-Soussan R (2023). Transpl Int 36:11328. doi: 10.3389/ti.2023.11328

The COVID pandemic that first gripped the world in 2020 caused social and economic dislocations; including healthcare in general, and clinical transplantation in particular [1]. Many transplant centers tried to reduce hospital admissions and direct contact between patients and providers, and there was widespread expansion of telehealth and remote medical services. During these times kidney transplant recipients receiving monthly intravenous injections of the co-stimulation blocker Belatacept were identified as high risk for hospital-acquired COVID infection and as needing an alternative to hospital-based i.v. drug injections. While some centers converted patients back to oral immunosuppressive protocols [2] others initiated a specific infection control protocol [3]. In the current issue of Transplantation International two French transplant centers [4] describe an alternative approach and converted 176 patients from maintenance 5 mg i.v. monthly in-center Belatacept to weekly 125 mg subcutaneous Abatacept injections at home. The reason for the drug switch was to reduce the need for patients to travel to the transplant center during the early chaotic period of the COVID pandemic. It is important to note that patients were previously converted to Belatacept because of calcineurin inhibitor (CNI) toxicity (mean eGFR 38 mL/min), and were also given mycophenolate mofetil and steroids. The authors postulated that the alternative CD80/86 co-stimulation blocker Abatacept, administered subcutaneously, could substitute for i.v. Belatacept and provide equivalent immunosuppression using the approved dose for rheumatoid arthritis [5]. After 3 months patients were reconverted to Belatacept, when in-home infusions of Belatacept were again authorized in France. During this short 3-month observation period a low frequency (1%–2%) of changes in graft function, rejection, viral infections, and adverse events were recorded. Injection site reactions were uncommon and not severe. Seven patients (4%) experienced COVID-19 while treated with Abatacept, two developed severe symptoms but all recovered. Importantly, the patients were well informed and felt safe after conversion to Abatacept. In 61% of patients home care nurses did the injections, and approximately half of the patients found Abatacept injections less restrictive because of independence and no hospital attendance. Interestingly, 49% of patients would continue with Belatacept if they had the choice, compared to only 38% with Abatacept. Patients who preferred Belatacept reported that they liked hospital-based reassurance of their status, and disliked weekly injections, nurse dependency, and the risk of forgetting. In summary, this large

Forum

OPEN ACCESS

Correspondence:
Stuart M. Flechner
flechns@ccf.org

†ORCID:
Stuart M. Flechner
orcid.org/0000-0002-7929-5942
Klemens Budde
orcid.org/0000-0002-8172-3918

Received: *28 June 2023*
Accepted: *05 July 2023*
Published: *24 July 2023*

Citation:
Flechner SM and Budde K (2023) Back to the Future With Co-Stimulation Blockade. Transpl Int 36:11752. doi: 10.3389/ti.2023.11752

well-described cohort demonstrates the feasibility, safety, and efficacy of once-weekly subcutaneous injection of Abatacept in kidney transplant recipients previously treated with Belatacept. Although some patients still favored the less frequent but more intrusive hospital-based i.v. method of drug delivery.

Belatacept, a fusion protein of the Fc fragment of a human IgG1 linked to the extracellular domain of CTLA-4 [6] was engineered to overcome slightly weaker binding avidity to CD80/86 of the progenitor molecule Abatacept, which differs just by 2 amino acids. These changes increased *in vitro* T-cell inhibitory activity and demonstrated superior protection from allograft rejection in pre-clinical models but were limited to the i.v. formulation of Belatacept. Extensive clinical trials and follow-ups have confirmed the efficacy of Belatacept for kidney transplantation [6–8], and the drug is often employed as a substitute or conversion agent to overcome intolerance to CNIs. Common reasons for switching include nephrotoxicity, thrombotic microangiopathy, posterior reversible encephalopathy syndrome, reducing cardiovascular risk factors, etc. However, widespread use has been limited by the need for monthly i.v. infusions, production shortages, and concerns of more acute rejection in higher immunological risk patients [9]. Subcutaneous Abatacept fell off the transplant radar but did find a home to treat autoimmune disorders such as rheumatoid arthritis, psoriasis, autoimmune cytopenia, and others [5, 10]. Over the last decade, several small case series and case reports described the use of Abatacept in kidney transplantation for narrow indications such as recurrent focal segmental glomerulosclerosis (FSGS) [10–13], due to unavailability of Belatacept [14] or the lack of venous access [15]. While results on the treatment of recurrent FSGS with Abatacept are conflicting, all the reports confirm overall safety and effective prevention of rejection with Abatacept, comparable to Belatacept. The largest series until now included 9 rescue kidney recipients switched from a CNI to Abatacept when Belatacept was unavailable. This resulted in stable graft function for a median of 82 months (14). Most (8/9) patients were given i.v. Abatacept 10 mg/kg instead of i.v. Belatacept 5 mg/kg, and 1/9 developed a Banff 1A acute rejection. The one patient given subcutaneous Abatacept did well. In a second series, 5 kidney recipients with histologically confirmed CNI nephrotoxicity were switched to subcutaneous Abatacept 125 mg weekly (15). No rejection episodes or DSA appearance were observed after a median of 9 (5–17) months. Two patients did experience reactivation of Cytomegalovirus, which is also seen in Belatacept-treated patients.

Despite all the problems and the enormous burden on the global health system, which persists to this day, the pandemic also brought forward some innovative ideas. Similar to the fruitful lessons learned from remote patient monitoring and telemedicine during the COVID pandemic, the unique experience of conversion to Abatacept during the early COVID outbreak (4) may have rekindled the interest in this agent for kidney transplantation. While the encouraging observations reported herein from a relatively large number of kidney recipients have piqued the interest for further study, the relatively short treatment interval of 3 months and lack of controls necessitates caution and the need for more evidence of safety and efficacy.

With this in mind, one center has embarked on a randomized controlled phase 2b conversion trial from Belatacept to Abatacept, entering kidney transplant recipients stable for at least 2 years on Belatacept and off all CNI drugs for at least 6 months (ClinicalTrials.gov NCT04955366). Such trials should be encouraged and perhaps multi-center collaboration can be envisioned in the near future to seek a proper role for Abatacept in the transplant immunosuppressive drug armamentarium. At a minimum, the availability of Abatacept as a backup would also be a welcomed addition during periods of Belatacept production shortages.

AUTHOR CONTRIBUTIONS

SF and KB participated in the concepts, data collection, writing, and editing of this manuscript. Both agree with the findings and conclusions that were presented.

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.

REFERENCES

1. Papalois V, Kotton CN, Budde K, Torre-Cisneros J, Croce D, Halleck F, et al. Impact of COVID-19 on Global Kidney Transplantation Service Delivery: Interim Report. *Transpl Int* (2022) 35:10302. doi:10.3389/ti.2022.10302
2. Kamar N, Esposito L, Hebral AL, Guitard J, Del Bello A. Specific Organization for In-Hospital Belatacept Infusion to Avoid Nosocomial Transmission During the SARS-CoV-2 Pandemic. *Am J Transpl* (2020) 20(10):2962–3. doi:10.1111/ajt.16074
3. Gouin A, Sberro-Soussan R, Courivaud C, Bertrand D, Del Bello A, Darres A, et al. Conversion From Belatacept to Another Immunosuppressive Regimen in Maintenance Kidney-Transplantation Patients. *Kidney Int Rep* (2020) 5(12):2195–201. doi:10.1016/j.ekir.2020.09.036
4. Bertrand D, Brunel M, Lebourg L, Scemla A, Lemoine M, Amrouche L, et al. Conversion From Intravenous In-Hospital Belatacept Injection to Subcutaneous Abatacept Injection in Kidney Transplant Recipients During the First COVID-19 Stay-at-Home Order in France. *Transpl Int* (2023) 36:11328. doi:10.3389/ti.2023.11328
5. Mohamed Ahamada M, Wu X. Analysis of Efficacy and Safety of Abatacept for Rheumatoid Arthritis: Systematic Review and Meta-Analysis. *Clin Exp Rheumatol* (2023) [Epub ahead of print]. doi:10.55563/clinexprheumatol/2xjg0d
6. Yakubu I, Moinuddin I, Gupta G. Use of Belatacept in Kidney Transplantation: What's New? *Curr Opin Organ Transpl* (2023) 28(1):36–45. doi:10.1097/MOT.0000000000001033
7. Lombardi Y, François H. Belatacept in Kidney Transplantation: What Are the True Benefits? A Systematic Review. *Front Med (Lausanne)* (2022) 9:942665. doi:10.3389/fmed.2022.942665

8. Budde K, Prashar R, Haller H, Rial MC, Kamar N, Agarwal A, et al. Conversion From Calcineurin Inhibitor-to Belatacept-Based Maintenance Immunosuppression in Renal Transplant Recipients: A Randomized Phase 3b Trial. *J Am Soc Nephrol* (2021) 32(12):3252–64. doi:10.1681/ASN.2021050628
9. Vincenti F, Budde K, Grinyo J, Rostaing L, Kirk AD, Larsen CP. Open Letter to Bristol Myers Squibb: Belatacept; We Aren't Done Yet. *Am J Transpl* (2023) [Epub ahead of print]. doi:10.1016/j.ajt.2023.05.033
10. Yu CC, Fornoni A, Weins A, Hakroush S, Maiguel D, Sageshima J, et al. Abatacept in B7-1-Positive Proteinuric Kidney Disease. *N Engl J Med* (2013) 369(25):2416–23. doi:10.1056/NEJMoa1304572
11. Delville M, Baye E, Durrbach A, Audard V, Kofman T, Braun L, et al. B7-1 Blockade Does Not Improve Post-Transplant Nephrotic Syndrome Caused by Recurrent FSGS. *J Am Soc Nephrol* (2016) 27(8):2520–7. doi:10.1681/ASN.2015091002
12. Burke GW, 3rd, Chandar J, Sageshima J, Ortigosa-Goggins M, Amarapurkar P, Mitrofanova A, et al. Benefit of B7-1 Staining and Abatacept for Treatment-Resistant Post-Transplant Focal Segmental Glomerulosclerosis in a Predominantly Pediatric Cohort: Time for a Reappraisal. *Pediatr Nephrol* (2023) 38(1):145–59. doi:10.1007/s00467-022-05549-7
13. Shah Y, Almeshari K, Aleid H, Broering D, Alahmadi I, Ali T. Successful Treatment with Abatacept in Recurrent Focal Segmental Glomerulosclerosis After Kidney Transplant. *Exp Clin Transpl* (2019) 17(1):178–80. doi:10.6002/ect.MESOT2018.P53
14. Badell IR, Karadkhele GM, Vasanth P, Farris AB, 3rd, Robertson JM, Larsen CP. Abatacept as Rescue Immunosuppression After Calcineurin Inhibitor Treatment Failure in Renal Transplantation. *Am J Transpl* (2019) 19(8): 2342–9. doi:10.1111/ajt.15319
15. Uro-Coste C, Atenza A, Heng AE, Rouzaire PO, Garrouste C. Abatacept Rescue Therapy in Kidney Transplant Recipients: A Case Series of Five Patients. *Transpl Int* (2022) 35:10681. doi:10.3389/ti.2022.10681

ESOT | Transplant International

POINT OF VIEW
published: 05 July 2023
doi: 10.3389/ti.2023.11296

Old Age and Frailty in Deceased Organ Transplantation and Allocation–A Plea for Geriatric Assessment and Prehabilitation

*Arved Weimann[1], Marlies Ahlert[2], Daniel Seehofer[3], Tania Zieschang[4] and Mark Schweda[5]**

[1]Department of General, Visceral and Oncological Surgery, St. George Hospital, Leipzig, Germany, [2]Department of Economics, Martin-Luther-University of Halle, Halle, Germany, [3]Department of Visceral, Transplantation, Thoracic and Vascular Surgery, University Hospital Leipzig, Leipzig, Germany, [4]Division of Geriatric Medicine, Department of Health Services Research, School of Medicine and Health Sciences, University of Oldenburg, Oldenburg, Germany, [5]Division of Medical Ethics, Department of Health Services Research, School of Medicine and Health Sciences, University of Oldenburg, Oldenburg, Germany

Due to demographic ageing and medical progress, the number and proportion of older organ donors and recipients is increasing. At the same time, the medical and ethical significance of ageing and old age for organ transplantation needs clarification. Advanced age is associated with the frailty syndrome that has a negative impact on the success of organ transplantation. However, there is emerging evidence that frailty can be modified by suitable prehabilitation measures. Against this backdrop, we argue that decision making about access to the transplant waiting list and the allocation of donor organs should integrate geriatric expertise in order to assess and manage frailty and impairments in functional capacity. Prehabilitation should be implemented as a new strategy for pre-operative conditioning of older risk patients' functional capacity. From an ethical point of view, advanced chronological age *per se* should not preclude the indication for organ transplantation and the allocation of donor organs.

Keywords: old age, frailty, organ allocation, organ transplantation, prehabilitation

Point of View

OPEN ACCESS

***Correspondence:**
Mark Schweda
mark.schweda@uni-oldenburg.de

Received: *14 April 2023*
Accepted: *21 June 2023*
Published: *05 July 2023*

Citation:
Weimann A, Ahlert M, Seehofer D, Zieschang T and Schweda M (2023) Old Age and Frailty in Deceased Organ Transplantation and Allocation–A Plea for Geriatric Assessment and Prehabilitation. Transpl Int 36:11296. doi: 10.3389/ti.2023.11296

INTRODUCTION

In the Eurotransplant region, the trend of increasing age of both donors and recipients of deceased donor organs is evident. Between 2012 and 2021, the share of deceased donors older than 65 years rose from 23.3% to 27.8%. A similar tendency can be shown for recipients. In 2012, 3.88% of the recipients of lungs were older than 65. In 2021, this share amounted to 12.64%. In the same time interval, there was an increase of the respective shares of older recipients of livers from 13.45% to 19.39%, of hearts from 6.68% to 9.29%, and of kidneys from 27.4% to 28.98% [1].

These increases in donor and recipient age raise new questions in public and policy debates on organ donation in the context of old age [2, 3]. Thus, the growing number of older potential organ recipients intensifies concerns about "organ scarcity" and fuels controversies about the efficient use and just distribution of available donor organs between age groups [4, 5]. At the same time, however, older people are discovered as a largely untapped source of donor organs and play an important role in new strategies for a more efficient and fair utilization of available organs. For example, they are targeted as a separate subgroup of kidney donors and recipients in "old for old" programs like the Eurotransplant Senior Program (ESP) [2].

In these and similar debates, assumptions about the medical chances and risks of organ transplantation at advanced age play a crucial role. The prospective medical success of the procedure is a criterion for its medical indication and ethical beneficence at an advanced age. It also factors into the discussion and regulation of the appropriate allocation of donor organs. In this context, advanced chronological age is frequently discussed as a distribution criterion, fostering controversial proposals for age-based rationing of medical resources for the sake of younger age groups and sparking concerns about age discrimination [6, 7].

A central aspect is the functional capacity of older recipients in the context of frailty. The frailty concept describes a syndrome which is associated with ageing and means impairment of functional capacity, physiological reserve, and body resilience. In older people undergoing major surgery, these changes may bear a considerable risk for the development of postoperative complications and prolonged recovery, including limited graft function in the case of organ transplantation. Functional status declines on the waiting list for kidney transplantation and has been shown to be associated with greater mortality and all-cause graft loss [8]. Similar findings have also been reported for liver transplantation [9]. However, recent studies indicate that this risk may be modifiable through adequate preventative measures. Therefore, it may be medically unwarranted and ethically problematic to exclude patients based on chronological age.

Against this backdrop, the contribution discusses the medical assessment and ethical evaluation of the success of organ transplantation in old age. In doing so, we particularly focus on the relevance of frailty for transplant success. We first provide a brief overview of the increasing relevance of old age in organ donation and transplantation, also considering the role of chronological age and frailty in allocation algorithms like the LAS score for lung transplantation. We then highlight the state of research regarding the impact of frailty on transplantation outcomes and review existing evidence that frailty constitutes a modifiable risk factor which can be mitigated by preventative measures. On this basis, we draw conclusions for an adequate treatment of older patients in organ transplantation. These include appropriate score-based risk stratification to achieve transparency for decision making and allocation algorithms of transplant candidates. Before excluding a candidate from transplantation due to age-related functional impairment and frailty, all potential measures of conditioning the patient should be taken into consideration.

OLD AGE AND FRAILTY IN ORGAN TRANSPLANTATION

There is ample evidence that older patients can benefit from organ transplantation. In the US and Europe, a survival advantage for older people (>60 years) vis-a-vis patients on the waiting list who remain on dialysis could be observed [10]. Compared to dialysis, organ transplantation doubles the life expectancy of older people [11]. Survival improves after the first year in patients between 60 and 74 years with a predicted increased life expectancy of 5 years and a 61% reduction in long-term mortality risk [12, 13]. Even in ESP kidney transplantation, the quality of life and the survival rate are significantly better than in patients of the same age who are dialyzed [14].

Nevertheless, older patients pose certain challenges to transplantation medicine. This is due to functional impairment and considerable comorbidity often related to the underlying organ dysfunction. In recent years, frailty has come into consideration as an identifiable preoperative risk factor for the postoperative outcome of organ transplantation [15–20]. The concept describes a syndrome which is associated with ageing and means impairment of functional capacity, physiological reserve, and body resilience. Frailty symptoms are unintended weight loss, exhaustion, weakness, slow gait speed, and low physical activity. They can be summarized in the Fried Frailty Index or other indices that also consider cognitive functioning [21, 22]. While age is the only conventional factor associated with frailty in kidney transplant patients, activities of daily living (ADL), depression scale, education, and health-related quality of life (HRQOL) are independently associated. Poor grip strength, exhaustion, and slowed walking speed are predictors for mortality risk [23]. Moreover, preoperative cognitive function in older people has turned out to be associated with postoperative complication rate and length of hospital stay after major surgery [24].

Frailty is also frequently associated with sarcopenia [25]. The International Working Group on Sarcopenia (IWGS) defines sarcopenia as "age-associated loss of skeletal muscle mass and function." The primary parameter is reduced muscle strength which leads to impaired physical resilience due to reduced muscle quantity or quality [26, 27]. Primary sarcopenia is age-associated, whereas secondary sarcopenia has other causes, e.g., a systemic disease, increased inflammation, decreased physical activity, and inadequate energy and protein intake [27]. Chronic organ failure as the indication for organ transplantation is frequently associated with sarcopenia and frailty [28, 29]. Sarcopenia has been shown to be an independent predictive factor of postoperative complications after liver transplantation for primary liver tumors [30], as well as for major morbidity and mortality after lung and heart transplantation [25, 31]. There is controversial data regarding the correlation of sarcopenia and long-term survival after liver transplantation [30, 32].

A high number of hospital admissions has been observed for kidney transplant candidates during the first year on the waiting list, which is a risk factor for waiting list mortality and lower graft and recipient survival [33]. Most of the symptoms are common across different types of organ failure. A systematic review of frailty in lung transplantation showed a prevalence of frailty of 0%–58% [34]. In kidney transplant recipients, prevalence of frailty is about 11% and has been shown to be associated with dialysis duration [35]. Frailty is a predictor of surgical complications after kidney transplantation [20, 36]. In patients undergoing lung transplantation, frailty was associated with decreased survival and an increased risk of early mortality in a systematic review [34]. The syndrome may be also associated with postoperative delirium and medium-term cognitive decline after transplantation [8, 37]. Furthermore, discharge frailty is also associated with a risk for unplanned rehospitalization [38].

Importantly, a prospective study in kidney transplant recipients showed that pretransplant frailty may improve after an initial decline within 3 months after surgery [39].

Frailty thus constitutes a highly relevant aspect in the consideration of organ transplantation in older adults. Geriatric medicine has developed authoritative expertise and instruments to detect and assess frailty. Comprehensive geriatric assessment (CGA) is a multidimensional, multidisciplinary process which identifies medical, social, and functional needs, and the development of an integrated/coordinated care plan to meet those needs [40]. The instruments used in CGA allow for the identification but also quantification of risk factors, functional capacities and impairments, as well as needs and strengths/resilience of an individual person in his or her environmental setting and goes beyond the determination of frailty status. Importantly, with the help of CGA, modifiable risk factors can be identified and consecutively targeted by interventions such as exercise, nutrition, adaption of medication, or prehabilitation. Components of CGA include assessments regarding medical/physical, psychological/psychiatric (cognition, emotion), functionality, mobility and falls, nutrition, socio-economic aspects through which goal setting, care planning, treatment/rehabilitation as well as discharge planning are tailored for the individual patient [40].

In the context of organ transplantation, a study on incorporating geriatrics and geriatric assessments into kidney transplant evaluation showed that this was feasible and that components of the geriatric assessment, specifically walking speed, falls, dependencies in the Instrumental Activities of Daily Living (IADL) and Activities of Daily Living (ADL), were significantly associated with patients' transplant rate, waiting list placement or removal, and mortality [41]. Another study on using CGA for decision making concerning kidney transplant revealed that geriatricians' recommendations for kidney transplant was influenced by impairments in IADL, physical function, and frailty [42].

OLD AGE AND FRAILTY IN ORGAN ALLOCATION

Old age and frailty also play an important role in the allocation of donor organs. Special transplantation programs for older people have been existing for over 20 years. In the United States, the allocation of kidneys divides donors into standard kidney donor profile index patients (KDPI) and high kidney donor profile index patients (high KDPI). High KDPI kidneys derive from donors older than 60 years and donors 50–59 years with co-morbidities. Participation in this allocation scheme is voluntary and one can choose to be listed for the KDPI kidneys (opt in). The vast majority of patients on the KDPI waiting list are older candidates. For older people, an advantage of this system is that it uses an age-matching formula whereby recipients are entitled to kidneys from donors who are no more than 15 years younger or older [43].

In the Eurotransplant region, organ-specific allocation rules differ with respect to the incorporation of age or functionality-related variables. Age is an explicit criterion in the allocation of kidneys and lungs, whereas variables measuring functionality are only explicitly relevant in lung allocation. The Eurotransplant Senior Program (ESP) was established in 1999 as a special program for kidney transplantation from older donors to older recipients [44]. The program allocates organs between donors and recipients who are 65 years and older [11, 45]. Since 2001, the ESP has become part of the Eurotransplant Kidney Allocation System (ETKAS) [46]. Germany, the Netherlands, and Belgium are the most important contributors [47]. Using regional allocation based on waiting time and blood group only, regardless of HLA match, a short cold ischemic time (CIT) and thus a good primary organ function can be achieved [48]. ESP leads to significantly reduced waiting times and enhances the chance for older patients to receive a renal graft [11].

An example for the role of age and its relation to functionality in organ allocation is the lung allocation score (LAS). The LAS has constituted the basis for the priority rule of lung allocation in Germany since May 2005. The higher a patient's LAS, the higher his or her priority to receive a donor lung. The score is constructed based on empirical data from the United States. It consists of estimates for urgency and expected survival after transplantation at the time an organ is offered. For each patient on the lung waiting list, specific data characterizing the patient and their health status are needed.

The first element of a patient's LAS is an estimate of urgency based on the estimated probabilities to survive from day to day without a transplant during the next year. The second element consists of estimates for day-to-day survival within the year after transplantation. For these estimates, several diagnostic data are used, among them the variables age at the time of offer (depending on the type of diagnosis), functional status (distinguishing between no assistance, some assistance and total assistance) and 6-min walking distance (more than 150 feet or not). Ceteris paribus, the older the patient, the lower their functional status or walking distance, the shorter the expected survival without a transplant, i.e., the higher the estimated urgency. Ceteris paribus, the higher the age at transplantation, the lower the expected survival time after transplantation.

The estimate for transplantation success in the LAS is the difference between the expected survival time with transplant and without transplant. Since both are influenced negatively by age, it is not possible to make a general statement on the dependency of the success measure on age. The LAS itself is constructed as a difference of the measure for success and the expected survival time without a transplant. The higher the urgency, the higher the LAS, the higher the estimated success the higher the LAS. Although a general statement on the ceteris paribus dependency of the LAS on age is not possible, comparing fictional examples shows, e.g., that patients of higher age can achieve a relatively high LAS in case they need no assistance or some assistance. Comparable approaches integrating functional capacity measures are missing for other types of organ transplantation.

From a geriatric point of view, chronological age *per se* should not have too much impact on the allocation score. Instead,

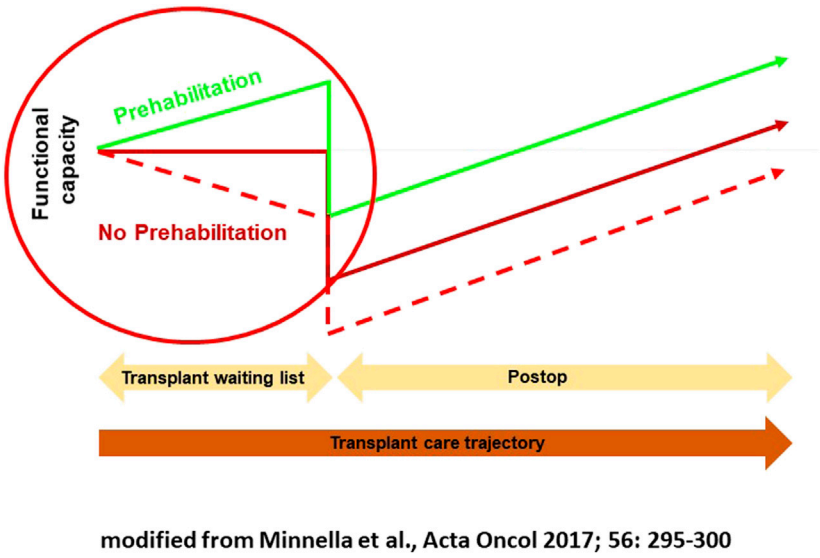


FIGURE 1 | Multimodal prehabilitation.

functional capacity should be given greater weight. Although walking speed is a major predictor of functional decline and mortality in older people [49], the internationally widely used Short Physical Performance Battery (SPPB) [50] additionally measures balance control and lower limb strength (five chair rise). The SPPB has been used to assess physical function in a study on pre-transplant physical function and outcomes after kidney transplant [51]. It was shown to be independently associated with length of hospital stay regardless of age [52, 53] and is also a common measure used in lung transplantation [54]. Generally, in geriatrics, assessment of cognition is important and cognitive impairment is also considered part of a frailty phenotype. However, in a study on frailty measures in patients listed for lung transplantation, cognitive function and depression variables did not strengthen the association with lung transplant waiting list mortality compared with the physical frailty measure [55]. Further research is needed to assess the influence of cognitive impairment on transplantation outcomes.

PREHABILITATION AND TRANSPLANT SUCCESS

Age and functionality represent important factors in the assessment of transplant success. Therefore, they also play a crucial role regarding access to and allocation of donor organs. Thus, it has been shown in a prospective multicenter study that frailty is associated with a lower chance to be listed for kidney transplantation [15]. However, there is increasing evidence that impaired functionality due to frailty may be a modifiable risk factor in older patients. For example, heart-failure associated frailty may be reversible [56]. In a study on lung transplant patients, pre-transplant SPPB increased following pre-habilitation [57].

Preoperative conditioning can improve physical function and nutritional status in high-risk patients before major abdominal surgery and may reduce the rate of complications [58, 59]. So-called trimodal prehabilitation consists mainly of physiotherapy and nutrition therapy as well as psychological intervention. New data suggest that long-term preoperative conditioning performed in appropriate risk patients not only improves physical functions and nutritional status *per se* but can also have positive effects on the postoperative course [60]. The concept developed by a Canadian group with anesthetist Franco Carli is to improve the functional status before the operation in order to attenuate the postsurgical decline, to diminish the risk for a complicated course, and to treat the patient according to an enhanced recovery after surgery (ERAS) protocol (**Figure 1**) [58, 59].

The exact effects of prehabilitation on the postoperative systemic inflammatory response have not been elucidated, yet. When comparing prehabilitation in major surgery with conventional rehabilitation alone, the additional prehabilitation appears to be more effective. A period of four to 6 weeks has been proven efficient in patients undergoing major surgery for cancer. A recent meta-analysis of 22 randomized studies carried out between 1991 and August 2020 showed a significant improvement in functional capacity for patients undergoing major cancer surgery, measured in 6-min walking distance, as well as a significantly shorter hospital stay [60]. In other meta-analyses, a decrease of complication rate with special regard to pulmonary morbidity has been observed [61, 62]. In a recent multicentric randomized clinical study in patients undergoing surgery for colorectal cancer, a 4-week in-hospital supervised multimodal prehabilitation was investigated. 251 patients were analyzed regarding intention-to-treat. The number of severe complications was significantly lower in the treatment group compared to standard care, and prehabilitation patients had significantly fewer medical complications [63].

Similar experience is currently beginning to emerge for the field of organ transplantation. Thus, prehabilitation has been shown feasible prior to kidney transplantation in a Johns Hopkins pilot study of 24 patients [64]. Remote coaching of home exercise has also been proven to be feasible and effective in patients on the waiting list for kidney transplantation [65]. Furthermore, the feasibility of a 12-week home-based prehabilitation was demonstrated in 18 candidates for liver transplantation with an improvement of aerobic and functional capacity, as well as parameters of quality of life. The program included average daily step targets and twice-weekly resistance exercise [66]. Eventually, prehabilitation has also been shown to be effective for improving quality of life and mood status, and reducing dyspnea in patients waiting for lung transplant [67]. Nevertheless, evidence is still limited, especially with regard to duration, modalities and intensity of the program. More systematic research with well-powered randomized trials is needed. Recently, a protocol for a comparative study of frailty in patients on the kidney transplant list regarding the composite of time to death or permanent waiting list withdrawal was published in Canada [68]. Secondary outcomes will include number of hospitalizations and length of stay, and in a subset, changes in frailty severity over time, changes in quality of life, and the probability of being accepted to the waiting list.

CONCLUSION AND OUTLOOK

Old age and frailty play a crucial yet complex role in organ transplantation and allocation. In light of geriatric research, a general equation of advanced chronological age and frailty appears unacceptable. Moreover, there is increasing evidence that frailty constitutes a modifiable risk factor that can be mitigated by suitable prehabilitative measures.

This has important implications for transplantation medicine. First, general chronological age limits for organ transplantation and allocation appear problematic. The functional status and thus the chances and risks of organ transplantation for older patients need to be assessed on an individual basis. When it comes to organ allocation, complex, multifactorial score systems incorporating geriatric scores provide a more accurate, differentiated, and transparent account than general age limits. In both contexts, geriatric medicine can offer suitable professional expertise and validated tools, such as the widely used SPPB that has already been applied in several studies on patients waiting for lung or kidney transplant.

At the same time, the potentials of prehabilitation to mitigate the risks and increase the success rates of organ transplantation for older recipients need further scientific examination and evidence-based practical guidelines. To this end, more systematic data collection and large-scale clinical studies are needed to investigate the effects of prehabilitation and evaluate and compare the outcomes of different prehabilitation measures, especially in the context of organ transplantation for older people, with a focus on health-related quality of life [69]. On this basis, specific guidelines for clinical practice could be formulated.

There are currently no clear recommendations for the organization and implementation of prehabilitation programs. Programs vary widely in terms of duration, content, and frequency of individual measures. For transplant candidates, home- or community-based programs will be most favorable. The special challenge in transplantation patients is the unpredictable time of surgery, and the motivation of the patient for self-managing responsibility. At the same time, motivation and cooperation of the patient in prehabilitation may be considered a predictor of long-term adherence as a basic requirement for transplant success. There are first approaches to prepare and support older transplant recipients for self-management before transplantation, to clarify expectations regarding posttransplant outcome, and to provide support in case of prolonged recovery [70].

Overall, the realization of these recommendations requires a systematic inclusion of geriatric expertise in the relevant studies, organizations, and clinical procedures in the field of transplantation medicine. Geriatric professionals and assessment instruments for frailty like the SPPB should be included on a regular basis in the evaluation of older potential transplant recipients. More research and practical experience is needed regarding the successful involvement of geriatricians in the process of waiting list placements. In addition, state of the art geriatric research should inform the formulation of adequate allocation scores and algorithms for older patients, as well as the development and implementation of suitable prehabilitation programs. This can help to support a more effective utilization of donor organs and prevent ageist stereotypes as well as fears of discrimination of older people in the context of organ transplantation.

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

AW, MA, and MS contributed to conception of the manuscript and wrote the first draft. DS and TZ wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

AW received a research grant for a project on prehabilitation from B. Braun on an institutional account.

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.

REFERENCES

1. Eurotransplant, Statistics Report Library. Quick Start (2023). Available from: https://statistics.eurotransplant.org/(Accessed February 1, 2023).
2. Schweda M, Wöhlke S. Selecting Donors and Recipients. In: Hansen SL, Schicktanz S, editors. *Ethical Challenges of Organ Transplantation: Current Debates and International Perspectives*. Bielefeld: transcript Verlag (2021). p. 227–243.
3. Fazal MS, Gordon EJ, Humbyrd CJ. Current Bioethical Issues in Geriatric Organ Transplantation. *Curr Transplant Rep* (2022) 9:55–62. doi:10.1007/s40472-022-00364-3
4. Cuende N, Cuende JI, Fajardo J, Huet J, Alonso M. Effect of Population Aging on the International Organ Donation Rates and the Effectiveness of the Donation Process. *Am J Transplant* (2007) 7:1526–1535. doi:10.1111/j.1600-6143.2007.01792.x
5. Goldstein DR. The Graying of Organ Transplantation. *Am J Transplant* (2012) 12:2569–2570. doi:10.1111/j.1600-6143.2012.04246.x
6. Reese PP, Caplan AL, Bloom RD, Abt P, Karlawish JH. How Should We Use Age to Ration Health Care? Lessons from the Case of Kidney Transplantation. *J Am Geriatr Soc* (2010) 58:1980–1986. doi:10.1111/j.1532-5415.2010.03031.x
7. Eidelson B. Kidney Allocation and the Limits of the Age Discrimination Act. *Yale L J* (2013) 122:1635.
8. Chu NM, Gross AL, Shaffer AA, Haugen CE, Norman SP, Xue QL, et al. Frailty and Changes in Cognitive Function after Kidney Transplantation. *J Am Soc Nephrol* (2019) 30:336–345. doi:10.1681/ASN.2018070726
9. Engelmann C, Aehling NF, Schob S, Nonnenmacher I, Handmann L, Macnaughtan J, et al. Body Fat Composition Determines Outcomes before and after Liver Transplantation in Patients with Cirrhosis. *Hepatol Commun* (2022) 6:2198–2209. doi:10.1002/hep4.1946
10. Heldal K, Leivestad T, Hartmann A, Svendsen MV, Lien BH, Midtvedt K. Kidney Transplantation in the Elderly—The Norwegian Experience. *Nephrol Dial Transplant* (2008) 23:1026–1031. doi:10.1093/ndt/gfm719
11. Frei U, Noeldeke J, Machold-Fabrizii V, Arbogast H, Margreiter R, Fricke L, et al. Prospective Age-Matching in Elderly Kidney Transplant Recipients—A 5-Year Analysis of the Eurotransplant Senior Program. *Am J Transplant* (2008) 8:50–57. doi:10.1111/j.1600-6143.2007.02014.x
12. Oniscu GC, Brown H, Forsythe JLR. Impact of Cadaveric Renal Transplantation on Survival in Patients Listed for Transplantation. *J Am Soc Nephrol* (2005) 16:1859–1865. doi:10.1681/ASN.2004121092
13. Rao PS, Merion RM, Ashby VB, Port FK, Wolfe RA, Kayler LK. Renal Transplantation in Elderly Patients Older Than 70 Years of Age: Results from the Scientific Registry of Transplant Recipients. *Transplantation* (2007) 83:1069–1074. doi:10.1097/01.tp.0000259621.56861.31
14. Fritsche L, Hörstrup J, Budde K, Reinke P, Giessing M, Tullius S, et al. Old-for-Old Kidney Allocation Allows Successful Expansion of the Donor and Recipient Pool. *Am J Transplant* (2003) 3:1434–1439. doi:10.1046/j.1600-6135.2003.00251.x
15. Haugen CE, Chu NM, Ying H, Warsame F, Holscher CM, Desai NM, et al. Frailty and Access to Kidney Transplantation. *Clin J Am Soc Nephrol* (2019) 14:576–582. doi:10.2215/CJN.12921118
16. Exterkate L, Slegtenhorst BR, Kelm M, Seyda M, Schuitenmaker JM, Quante M, et al. Frailty and Transplantation. *Transplantation* (2016) 100:727–733. doi:10.1097/TP.0000000000001003
17. Bottiger BA, Nicoara A, Snyder LD, Wischmeyer PE, Schroder JN, Patel CB, et al. Frailty in the End-Stage Lung Disease or Heart Failure Patient: Implications for the Perioperative Transplant Clinician. *J Cardiothorac Vasc Anesth* (2019) 33:1382–92. doi:10.1053/j.jvca.2018.08.002
18. Schaenman J, Goldwater D, Malinis M. An Interdisciplinary Approach to the Older Transplant Patient: Strategies for Improving Clinical Outcomes. *Curr Opin Organ Transplant* (2019) 24:504–510. doi:10.1097/MOT.0000000000000662
19. Kodali L, Turner A. When Are You Too Old to Get a Kidney Transplants? *Curr Opin Nephrol Hypertens* (2019) 28:593–599. doi:10.1097/MNH.0000000000000548
20. Schopmeyer L, el Moumni M, Nieuwenhuijs-Moeke GJ, Berger SP, Bakker SJL, Pol RA. Frailty Has a Significant Influence on Postoperative Complications after Kidney Transplantation – a Prospective Study on Short-Term Outcomes. *Transpl Int* (2019) 32:66–74. doi:10.1111/tri.13330
21. Fried L, Tangen C, Walston J, Newman A, Hirsch C, Gottdiener J, et al. Frailty in Older Adults: Evidence for a Phenotype. *The Journals Gerontol Ser A* (2001) 56:M146–M156. doi:10.1093/gerona/56.3.m146
22. Mende A, Riegel AK, Plümer L, Olotu C, Goetz AE, Kiefmann R. Determinants of Perioperative Outcome in Frail Older Patients. *Deutsches Ärzteblatt Int* (2019) 116:73–82. doi:10.3238/arztebl.2019.0073
23. McAdams-DeMarco MA, Ying H, Olorundare I, King EA, Haugen C, Buta B, et al. Individual Frailty Components and Mortality in Kidney Transplant Recipients. *Transplantation* (2017) 101:2126–2132. doi:10.1097/TP.0000000000001546
24. Wobith M, Acikgöz A, Grosser K, Weimann A. Preoperative Cognitive Function in Very Old Patients: Influence on the Complication Rate and Length of Hospitalization. *Chirurg* (2019) 90:930–5. doi:10.1007/s00104-019-01028-2
25. Hsu J, Krishnan A, Lin CT, Shah PD, Broderick SR, Higgins RSD, et al. Sarcopenia of the Psoas Muscles Is Associated with Poor Outcomes Following Lung Transplantation. *Ann Thorac Surg* (2019) 107:1082–1088. doi:10.1016/j.athoracsur.2018.10.006
26. Cruz-Jentoft AJ, Landi F, Schneider SM, Zúñiga C, Arai H, Boirie Y, et al. Prevalence of and Interventions for Sarcopenia in Ageing Adults: a Systematic Review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS). Age and Ageing (2014) 43:748–759. doi:10.1093/ageing/afu115
27. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyere O, Cederholm T, et al. Sarcopenia: Revised European Consensus on Definition and Diagnosis. *Age and ageing* (2019) 48:16–31. doi:10.1093/ageing/afy169
28. Laube R, Wang H, Park L, Heyman JK, Vidot H, Majumdar A, et al. Frailty in Advanced Liver Disease. *Liver Int* (2018) 38:2117–2128. doi:10.1111/liv.13917
29. Duarte-Rojo A, Ruiz-Margáin A, Montaño-Loza AJ, Macías-Rodríguez RU, Ferrando A, Kim WR. Exercise and Physical Activity for Patients with End-stage Liver Disease: Improving Functional Status and Sarcopenia while on the Transplant Waiting List. *Liver Transplant* (2018) 24:122–139. doi:10.1002/lt.24958
30. Valero V, III, Amini N, Spolverato G, Weiss MJ, Hirose K, Dagher NN, et al. Sarcopenia Adversely Impacts Postoperative Complications Following Resection or Transplantation in Patients with Primary Liver Tumors. *J Gastrointest Surg* (2015) 19:272–81. doi:10.1007/s11605-014-2680-4
31. Bibas L, Saleh E, Al-Kharji S, Chetrit J, Mullie L, Cantarovich M, et al. Muscle Mass and Mortality after Cardiac Transplantation. *Transplantation* (2018) 102:2101–2107. doi:10.1097/TP.0000000000002311
32. Englesbe MJ, Patel SP, He KMS, Lynch RJ, Schaubel DE, Harbaugh CBS, et al. Sarcopenia and Mortality after Liver Transplantation. *J Am Coll Surgeons* (2010) 211:271–278. doi:10.1016/j.jamcollsurg.2010.03.039
33. Lynch RJ, Zhang R, Patzer RE, Larsen CP, Adams AB. First-Year Waitlist Hospitalization and Subsequent Waitlist and Transplant Outcome. *Am J Transplant* (2017) 17:1031–1041. doi:10.1111/ajt.14061
34. Montgomery E, Macdonald PS, Newton PJ, Jha SR, Malouf M. Frailty in Lung Transplantation: a Systematic Review. *Expert Rev Respir Med* (2020) 14:219–27. doi:10.1080/17476348.2020.1702527
35. Kosoku A, Uchida J, Iwai T, Shimada H, Kabei K, Nishide S, et al. Frailty Is Associated with Dialysis Duration before Transplantation in Kidney Transplant Recipients: A Japanese Single-center Cross-sectional Study. *Int J Urol* (2020) 27:408–414. doi:10.1111/iju.14208
36. Dos Santos Mantovani M, Coelho de Carvalho N, Archangelo TE, Modelli de Andrade LG, Pires Ferreira Filho S, de Souza Cavalcante R, et al. Frailty Predicts Surgical Complications after Kidney Transplantation. A Propensity Score Matched Study. *PLoS One* (2020) 15:e0229531. doi:10.1371/journal.pone.0229531
37. Haugen CE, Mountford A, Warsame F, Berkowitz R, Bae S, Thomas AG, et al. Incidence, Risk Factors, and Sequelae of Post-kidney Transplant Delirium. *J Am Soc Nephrol* (2018) 29:1752–1759. doi:10.1681/ASN.2018010064
38. Courtwright AM, Zaleski D, Gardo L, Ahya VN, Christie JD, Crespo M, et al. Causes, Preventability, and Cost of Unplanned Rehospitalizations within 30 Days of Discharge after Lung Transplantation. *Transplantation* (2018) 102:838–844. doi:10.1097/TP.0000000000002101

39. McAdams-DeMarco MA, Isaacs K, Darko L, Salter ML, Gupta N, King EA, et al. Changes in Frailty after Kidney Transplantation. *J Am Geriatr Soc* (2015) 63:2152–2157. doi:10.1111/jgs.13657
40. Parker SG, McCue P, Phelps K, McCleod A, Arora S, Nockels K, et al. What Is Comprehensive Geriatric Assessment (CGA)? an Umbrella Review. *Age and ageing* (2018) 47:149–155. doi:10.1093/ageing/afx166
41. Campbell KH, Ahn DJ, Enger F, Zasadzinski L, Tanumihardjo J, Becker Y, et al. Utility of Geriatric Assessments in Evaluation of Older Adults for Kidney Transplantation. *Clin Transplant* (2022) 36:e14813. doi:10.1111/ctr.14813
42. Novais T, Pongan E, Gervais F, Coste MH, Morelon E, Krolak-Salmon P, et al. Pretransplant Comprehensive Geriatric Assessment in Older Patients with Advanced Chronic Kidney Disease. *Nephron* (2021) 145:692–701. doi:10.1159/000517342
43. Veatch RM, Ross LF. *Transplantation Ethics*. Washington: Georgetown University Press (2015). p. 451.
44. Smits JMA, van Houwelingen HC, De Meester J, Persijn GG, Claas FHJ. Analysis of the Renal Transplant Waiting List - Application of a Parametric Competing Risk Method. *Transplantation* (1998) 66:1146–1153. doi:10.1097/00007890-199811150-00006
45. Boesmueller C, Biebl M, Scheidl S, Oellinger R, Margreiter C, Pratschke J, et al. Long-Term Outcome in Kidney Transplant Recipients over 70 Years in the Eurotransplant Senior Kidney Transplant Program: A Single Center Experience. *Transplantation* (2011) 92:210–216. doi:10.1097/TP.0b013e318222ca2f
46. Smits JMA, Persijn GG, Van Houwelingen HC, Claas FHJ, Frei U. Evaluation of the Eurotransplant Senior Program. The Results of the First Year. *Am J Transplant* (2002) 2:664–670. doi:10.1034/j.1600-6143.2002.20713.x
47. Doxiadis ILN, Smits JMA, Persijn GG, Frei U, Claas FHJ. It Takes Six to Boogie: Allocating Cadaver Kidneys in Eurotransplant. *Transplantation* (2004) 77:615–617. doi:10.1097/01.tp.0000103725.72023.d7
48. Bentas W, Jones J, Karaoguz A, Tilp U, Probst M, Scheuermann E, et al. Renal Transplantation in the Elderly: Surgical Complications and Outcome with Special Emphasis on the Eurotransplant Senior Programme. *Nephrol Dial Transplant* (2008) 23:2043–2051. doi:10.1093/ndt/gfm912
49. Studenski S, Perera S, Patel K, Rosano C, Faulkner K, Inzitari M, et al. Gait Speed and Survival in Older Adults. *JAMA: J Am Med Assoc* (2011) 305:50–58. doi:10.1001/jama.2010.1923
50. Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, et al. A Short Physical Performance Battery Assessing Lower Extremity Function: Association with Self-Reported Disability and Prediction of Mortality and Nursing home Admission. *J Gerontol* (1994) 49:M85–M94. doi:10.1093/geronj/49.2.m85
51. Lorenz EC, Cheville AL, Amer H, Kotajarvi BR, Stegall MD, Petterson TM, et al. Relationship between Pre-transplant Physical Function and Outcomes after Kidney Transplant. *Clin Transplant* (2017) 31:e12952. doi:10.1111/ctr.12952
52. Nastasi AJ, Bryant TS, Le JT, Schrack J, Ying H, Haugen CE, et al. Pre-kidney Transplant Lower Extremity Impairment and Transplant Length of Stay: a Time-To-Discharge Analysis of a Prospective Cohort Study. *BMC Geriatr* (2018) 18:246. doi:10.1186/s12877-018-0940-y
53. Courtwright AM, Zaleski D, Tevald M, Adler J, Singer JP, Cantu EE, et al. Discharge Frailty Following Lung Transplantation. *Clin Transplant* (2019) 33:e13694. doi:10.1111/ctr.13694
54. Varughese R, Rozenberg D, Singer LG. An Update on Frailty in Lung Transplantation. *Curr Opin Organ Transplant* (2020) 25:274–279. doi:10.1097/MOT.0000000000000762
55. Montgomery E, Newton PJ, Chang S, Peng W, Jha SR, Wilhelm K, et al. Frailty Measures in Patients Listed for Lung Transplantation. *Transplantation* (2022) 106:1084–1092. doi:10.1097/TP.0000000000003823
56. Macdonald P. Frailty of the Heart Recipient. *Transplantation* (2021) 105:2352–2361. doi:10.1097/TP.0000000000003692
57. Wickerson L, Rozenberg D, Gottesman C, Helm D, Mathur S, Singer LG. Pre-transplant Short Physical Performance Battery: Response to Pre-habilitation and Relationship to Pre- and Early post–lung-transplant Outcomes. *Clin Transplant* (2020) 34:e14095. doi:10.1111/ctr.14095
58. Carli F, Baldini G From Preoperative Assessment to Preoperative Optimization of Frail Older Patients. *Eur J Surg Oncol* (2021) 47:519–523. doi:10.1016/j.ejso.2020.06.011
59. Minnella EM, Bousquet-Dion G, Awasthi R, Scheede-Bergdahl C, Carli F. Multimodal Prehabilitation Improves Functional Capacity before and after Colorectal Surgery for Cancer: a Five-Year Research Experience. *Acta oncologica* (2017) 56:295–300. doi:10.1080/0284186X.2016.1268268
60. Waterland JL, McCourt O, Edbrooke L, Granger CL, Ismail H, Riedel B, et al. Efficacy of Prehabilitation Including Exercise on Postoperative Outcomes Following Abdominal Cancer Surgery: A Systematic Review and Meta-Analysis. *Front Surg* (2021) 8:628848. doi:10.3389/fsurg.2021.628848
61. Hughes MJ, Hackney RJ, Lamb PJ, Wigmore SJ, Christopher Deans DA, Skipworth RJE. Prehabilitation before Major Abdominal Surgery: A Systematic Review and Meta-Analysis. *World J Surg* (2019) 43:1661–1668. doi:10.1007/s00268-019-04950-y
62. Assouline B, Cools E, Schorer R, Kayser B, Elia N, Licker M. Preoperative Exercise Training to Prevent Postoperative Pulmonary Complications in Adults Undergoing Major Surgery. A Systematic Review and Meta-Analysis with Trial Sequential Analysis. *Ann Am Thorac Soc* (2021) 18:678–688. doi:10.1513/AnnalsATS.202002-183OC
63. Molenaar CJL, Minnella EM, Coca-Martinez M, ten Cate DWG, Regis M, Awasthi R, et al. Effect of Multimodal Prehabilitation on Reducing Postoperative Complications and Enhancing Functional Capacity Following Colorectal Cancer Surgery: The PREHAB Randomized Clinical Trial. *JAMA Surg* (2023) 158:572–581. doi:10.1001/jamasurg.2023.0198
64. McAdams-DeMarco MA, Ying H, Van Pilsum Rasmussen S, Schrack J, Haugen CE, Chu NM, et al. Prehabilitation Prior to Kidney Transplantation: Results from a Pilot Study. *Clin Transplant* (2019) 33:e13450. doi:10.1111/ctr.13450
65. Ma X, Zhang Z, Peng M, Yao B, Jiang H, Ji X, et al. Face-to-Face Mentoring, Remotely Supervised Home Exercise Prehabilitation to Improve Physical Function in Patients Awaiting Kidney Transplantation: A Randomized Clinical Trial. *Front Psychol* (2022) 13:831445. doi:10.3389/fpsyg.2022.831445
66. Williams FR, Vallance A, Faulkner T, Towey J, Durman S, Kyte D, et al. Home-Based Exercise in Patients Awaiting Liver Transplantation: A Feasibility Study. *Liver Transplant* (2019) 25:995–1006. doi:10.1002/lt.25442
67. Polastri M, Dell'Amore A, Eden A, Pehlivan E. Does Preoperative Rehabilitation Influence the Quality of Life in Patients Who Are Candidates for Lung Transplant? *Exp Clin Transplant* (2022) 20:543–548. doi:10.6002/ect.2022.0039
68. Tennankore KK, Gunaratnam L, Suri RS, Yohanna S, Walsh M, Tangri N, et al. Frailty and the Kidney Transplant Wait List: Protocol for a Multicenter Prospective Study. *Can J kidney Health Dis* (2020) 7:2054358120957430. doi:10.1177/2054358120957430
69. Kniepeiss D, Wagner D, Pienaar S, Thaler HW, Porubsky C, Tscheliessnigg KH, et al. Solid Organ Transplantation: Technical Progress Meets Human Dignity: a Review of the Literature Considering Elderly Patients' Health Related Quality of Life Following Transplantation. *Ageing Res Rev* (2012) 11:181–187. doi:10.1016/j.arr.2011.06.003
70. Pinter J, Hanson CS, Chapman JR, Wong G, Craig JC, Schell JO, et al. Perspectives of Older Kidney Transplant Recipients on Kidney Transplantation. *Clin J Am Soc Nephrol* (2017) 12:443–453. doi:10.2215/CJN.05890616

ESOT | Transplant International

ORIGINAL RESEARCH
published: 21 July 2023
doi: 10.3389/ti.2023.11089

Heart Transplantation in High-Risk Recipients Employing Donor Marginal Grafts Preserved With *Ex-Vivo* Perfusion

Sandro Sponga[1,2], Igor Vendramin[2], Jawad Salman[3], Veronica Ferrara[1], Nunzio Davide De Manna[2], Andrea Lechiancole[2], Gregor Warnecke[4], Andriy Dralov[2], Axel Haverich[3], Fabio Ius[3], Uberto Bortolotti[2], Ugolino Livi[1,2] and Murat Avsar[3]*

[1]Department of Medicine, University of Udine, Udine, Italy, [2]Cardiothoracic Department, University Hospital of Udine, Udine, Italy, [3]Department of Cardiothoracic, Transplant and Vascular Surgery, Hannover Medical School, Hannover, Germany, [4]Department of Cardiac Surgery, Heidelberg Medical School, Heidelberg, Germany

Extending selection criteria to face donor organ shortage in heart transplantation (HTx) may increase the risk of mortality. *Ex-vivo* normothermic perfusion (EVP) limits ischemic time allowing assessment of graft function. We investigated the outcome of HTx in 80 high-risk recipients transplanted with marginal donor and EVP-preserved grafts, from 2016 to 2021. The recipients median age was 57 years (range, 13–75), with chronic renal failure in 61%, impaired liver function in 11% and previous cardiac surgery in 90%; 80% were mechanically supported. Median RADIAL score was 3. Mean graft ischemic time was 118 ± 25 min, "out-of-body" time 420 ± 66 min and median cardiopulmonary bypass (CPB) time 228 min (126–416). In-hospital mortality was 11% and ≥moderate primary graft dysfunction 16%. At univariable analysis, CPB time and high central venous pressure were risk factors for mortality. Actuarial survival at 1 and 3 years was 83% ± 4%, and 72% ± 7%, with a median follow-up of 16 months (range 2–43). Recipient and donor ages, pre-HTx extracorporeal life support and intra-aortic balloon pump were risk factors for late mortality. In conclusion, the use of EVP allows extension of the graft pool by recruitment of marginal donors to successfully perform HTx even in high-risk recipients.

Keywords: heart transplantation, normothermic machine perfusion, high-risk recipients, donor marginal grafts, *ex-vivo* heart preservation

OPEN ACCESS

Correspondence:
Sandro Sponga
sandro.sponga@asufc.sanita.fvg.it

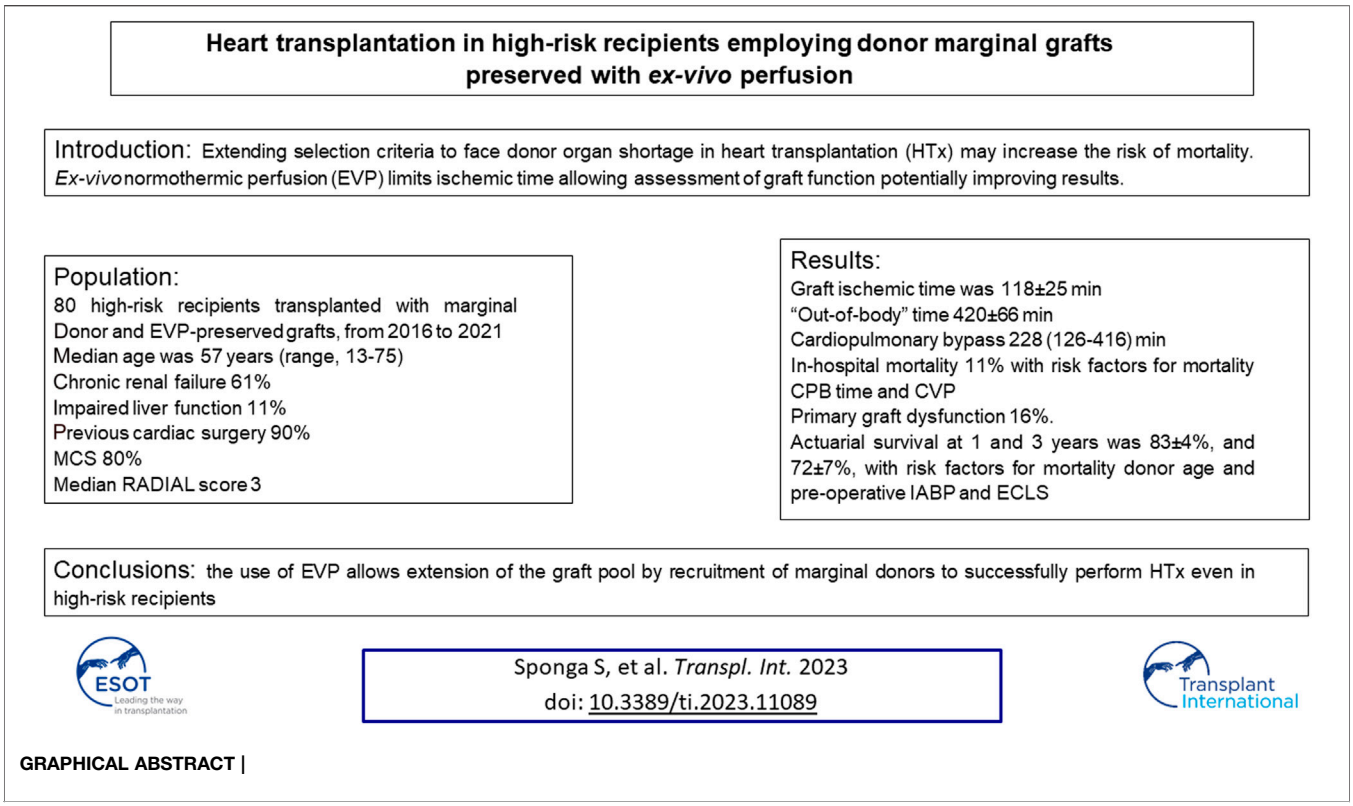
Received: *29 November 2022*
Accepted: *10 July 2023*
Published: *21 July 2023*

Citation:
Sponga S, Vendramin I, Salman J, Ferrara V, De Manna ND, Lechiancole A, Warnecke G, Dralov A, Haverich A, Ius F, Bortolotti U, Livi U and Avsar M (2023) Heart Transplantation in High-Risk Recipients Employing Donor Marginal Grafts Preserved With Ex-Vivo Perfusion. Transpl Int 36:11089. doi: 10.3389/ti.2023.11089

INTRODUCTION

Orthotopic heart transplantation (HTx) is considered the gold standard treatment for patients with advanced refractory heart failure; however, while the demand is growing, the possibility to perform HTx is still limited by the chronic donor shortage [1]. Although expanding donor selection criteria could allow employment of a larger number of organs, it entails an increased risk of early and late mortality [1, 2]. This is particularly relevant in case of high-risk recipients, as those with multiple co-

Abbreviations: CPB, cardio-pulmonary bypass; ECLS, extra-corporeal life support; EVP, *ex-vivo* perfusion; HTx, heart transplantation; MCS, mechanical circulatory support; OCS, Organ Care System™; PGD, primary graft dysfunction; VAD, ventricular assist device; ISHLT, International Society for Heart amd Lung Transplantation.



GRAPHICAL ABSTRACT |

morbidities, a compromised clinical status or requiring pre-HTx mechanical circulatory support (MCS), in whom a more challenging surgical procedure may result in prolonged cold ischemic and bypass times, possibly jeopardizing donor heart viability [3, 4]. Based on these considerations, the use of suboptimal grafts might be a potential solution to increase the donor pool; however, whether high-risk recipients should be transplanted with marginal or regular donors is still a debated issue, which raises ethical problems requiring in some instances both physician and recipient agreement [5]. Marginal donor grafts were used in the past with traditional preservation techniques but since the results have been suboptimal especially in high risk recipients, such grafts are currently not considered for regular patients [6].

Cold static storage is the standard preservation technique of the donor graft, but it may not avoid ischemic and reperfusion injuries when preservation time exceeds 4 h [4], mainly in case of HTx with marginal grafts. In fact, it is well known how a prolonged ischemic time impacts significantly the outcome when combined with other donor and recipient variables [4].

Ex-vivo normothermic perfusion (EVP) is a novel procedure that, maintaining donor grafts in a beating, normothermic condition, limits ischemia-reperfusion injuries, allowing potential recovery of suboptimal organs, also favoring recruitment of longer-distance donors. Moreover, during graft transportation, EVP allows real-time monitoring of graft hemodynamic and metabolic parameters and timely identification of potentially unsuitable hearts [7].

The aim of the present study was to investigate the clinical outcomes of HTx in high-risk recipients who were transplanted only with grafts, selected according to extended donor criteria and preserved with EVP.

PATIENTS AND METHODS

Study Population

All consecutive high-risk patients who underwent HTx with grafts from marginal donors and preserved with EVP at the University Hospital of Udine and Hannover Medical School, from 2016 to 2021, were retrospectively analyzed. Indication for EVP was the same in the two centers and involved in all cases employment of a marginal graft for a high-risk recipient, as subsequently defined. The study was approved by the Institutional Review Board (Clinical Registration Number: 18386, 1 August 2016) and informed consent was waived due to its retrospective nature. The major end-point of the study was the assessment of early and mid-term clinical outcome in terms of survival and major complications after HTx.

Donor Heart Preservation

In the present series EVP was achieved in both participating centers employing the Organ Care System (OCS) (Transmedics Inc., Boston, MA, United States). The OCS perfusion technique has been described in detail previously [8]; briefly, it is instituted by cannulating the aorta and pulmonary artery of the graft and

connecting them to a perfusion circuit with an oxygenator and a pulsatile pump. The beating heart is perfused with warm, oxygenated, nutrient-enriched donor blood mixed with priming solution. Graft function is assessed by continuous monitoring of aortic pressure, coronary flow and lactate trend. Final arterial lactate value (<5 mmol/L), lactate trend, difference between arterial and venous lactate concentration, visual contractility and stability of the perfusion data on OCS are all considered for HTx acceptance of donor grafts. The OCS device was transported either by car, plane or helicopter based on the distance of the donor hospital.

HTx Procedure

Criteria for recipient selection and donor-recipient matching were based on standard guidelines [9–12]. HTx was performed using the bicaval anastomosis technique and immunosuppression therapy was standardized with steroids, calcineurin inhibitors and mycophenolate mofetil. Postoperative and long-term follow-up protocols at the University Hospital of Udine have been previously published and have remained unchanged during the study period [13–17]. In Hannover, all HTx recipients underwent triple immunosuppressive therapy with tacrolimus, prednisolone and mycophenolate mofetil. All patients underwent induction with anti-thymocite globulin on day 3, 4 and 5 after HTx. Endomyocardial biopsies were performed every 2 weeks during the first 3 months of follow-up. Seven patients who showed pre-transplant allosensitization and a positive virtual crossmatch were treated with a perioperative combination of therapeutic plasmapheresis, followed by infusions of Tocilizumab before allograft reperfusion, a single infusion of human immunoglobulins with or without a single infusion of Rituximab.

Definition of Terms

High-risk recipients were defined as those on pre-HTx dependence from inotropic support, pre-HTx implantation of an intra-aortic balloon pump, those bridged to HTx with extracorporeal life support systems (ECLS) or a ventricular assist device (VAD). For ECLS support a Quadrox-i oxygenator and a Cardiohelp centrifugal pump (Getinge, Göteborg, Sweden), were employed. ECLS was used in 26% of patients in one center and in 5% in the other depending on different treatment policies of moderate primary graft dysfunction (PGD). Donors selected according to extended criteria, defined as "marginal," were considered those ≥55 years of age, with a history of drug abuse, cardiac resuscitation or severe prolonged hypotension (>20 min), coronary artery disease, with at least a >30% stenosis of a major coronary branch, expected graft ischemia time ≥4 h, left ventricular ejection fraction <50%, interventricular septum thickness >14 mm, or body surface area difference between donor and recipient >20%. Hemodynamic support of the donor grafts was generally obtained with infusion of ≤0.1 y/kg/min of norepinephrine.

Chronic renal failure was defined by a glomerular filtration rate <50 mL/min/m^2 or a persistent 50% increase of serum creatinine level. Impaired liver function was considered as at least a twofold increase of bilirubin and/or liver enzymes.

PGD was defined according to the International Society for Heart and Lung Transplantation (ISHLT) consensus statement, considering it relevant when grade ≥ moderate [18, 19].

Acute renal failure was defined following the Risk of renal dysfunction, Injury to the kidney, Failure of kidney function, Loss of kidney function, End-stage kidney disease (RIFLE) criteria [20]. The indication for continuous renal replacement therapy was based on persistent oliguria (<0.5 mL/Kg/h) for at least 6 h, a double increase of serum creatinine levels or at least 50% reduction of the glomerular filtration rate within 24 h.

Infections were defined as any episode requiring antibiotic, antiviral or antifungal treatment. Acute rejection was diagnosed, scored and treated following the ISHLT guidelines [21]. Grade ≥ 2 acute rejection or any type of rejection, cellular and/or antibody mediated with hemodynamic impairment was considered as a post-HTx complication and treated accordingly. Coronary allograft vasculopathy was diagnosed by yearly angiographies and defined according to ISHLT classification [22, 23].

For prediction of PGD, the RADIAL score has been used which is calculated on six factors with similar influence, four of which are related to the recipient: Right atrial pressure ≥10 mmHg, Age ≥60 years, Diabetes and Inotropic support dependence while two are related to the donor: Age ≥30 years and Length of total graft ischemic time ≥240 min. The presence of each of these factors in an individual patient adds 1 point to the final PGD predictive score [6].

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation or median and range (min-max) according to the data distribution, after performing the Kolmogorov-Smirnov test for normality. Categorical variables were presented as absolute numbers and percentages. Cumulative overall survival was defined as freedom form all-cause mortality and was determined by the Kaplan-Meier method. Binary logistic regression was used to assess factors for PGD ≥ moderate and in-hospital mortality, while Cox-regression model was used for long-term mortality after HTx. De Long's nonparametric receiver operating characteristic analysis of the area under the curve (AUC) was performed to estimate the accuracy of risk factors that were identified at the univariate analysis and to determine a cut-off value.

Analyses were performed with IBM SPSS Statistics 22 for Microsoft Windows (IBM Corp., Armonk, NY).

RESULTS

Baseline Characteristics

During the study period, out of 88 marginal grafts 80 were employed for HTx in high-risk recipients; 37% were transplanted at the University Hospital of Udine and 71% at the Hannover medical School; 8 (10%) were discarded after being considered unsuitable for HTx through OCS graft assessment because of pathological increase of lactates despite adequate coronary perfusion in most cases; in particular, right ventricle

dysfunction, severe aortic valve regurgitation, coronary anomaly and coronary dissection were detected in four grafts.

Patients Data

Recipient data are shown in **Table 1**. Median age was 57 years (range, 13–75); 80% were males, with a mean body mass index of 26 ± 4 and 12 patients had a weight ≥100 kg and a body mass index ≥30 with a significant size mismatch occurring in 30% of them. Chronic renal failure was present in 61% patients, three of whom where in pre-HTx dialysis and underwent combined heart-kidney transplantation; 11% had impaired liver function and 90% had a previous cardiac operation; 66% were bridged to HTx on long-term VAD with a median support time of 22 months (range, 1–133). In 81% urgent HTx was performed: of these 14% were on short-term ECLS (mean support time of 12 ± 12 days), 12% were dependent from inotropic support and 55% were patients with VAD-related complications. Median RADIAL score was 3 (range, 0–6); a score ≥4 was present in 35% of recipients.

TABLE 1 | Patients data.

	***N* = 80**
Median age, years (range)	57 (13–75)
Male sex, n (%)	64 (80)
Mean BMI	26 ± 4
Diabetes, n (%)	7 (9)
Mean serum creatinine (mg/dL)[a]	1.68 ± 0.58
Median GFR, mL/min/m^2, (range)[a]	38 (16–82)
Median SGOT, UI/L (range)	35 (11–110)
Mean SGPT, UI/L	37 ± 16
Median bilirubin, mg/dL (range)	1.22 (0.29–6.47)
Mean PAP, mmHg	38 ± 13
Median CVP, mmHg (range)	12 (2–28)
Previous cardiac surgery, n (%)	72 (90)
Long-term VAD, n (%)	53 (66)
ECLS, n (%)	11 (14)
Inotropic dependence, n (%)	9 (12)
Urgent HTx, n (%)	65 (81)
Median RADIAL score (range)	3 (0–6)

BMI, body mass index; GFR, Glomerular filtration rate; SGOT, Serum glutamic-oxaloacetic transaminase; SGPT, serum glutamate pyruvate transaminase; INR, international normalized ratio; PAP, systolic pulmonary artery pressure; CVP, central venous pressure; VAD, ventricular assist device; HTx, Heart transplantation; ECLS, Extra-corporeal life support.

[a]*Values reported exclude patients having combined heart and kidney transplantation.*

Donor Data

Donor data are summarized in **Table 2**. Median donor age was 48 years (range, 17–69); 28% were ≥55 years of age, 65% were males, 46% smokers, 29% had a history of alcohol abuse and 39% suffered a previous episode of cardiac arrest. On transthoracic 2D- echo, 6% of grafts had a left ventricular ejection fraction ≤50%, while 21% showed left ventricular hypertrophy; in 4% coronary artery disease was disclosed at angiography revealing 40% stenosis of the left anterior descending in two cases and stenoses of 45% and 50% of left anterior descending and right coronary artery in another, respectively.

Early Outcomes

Mean graft ischemic time was 118 ± 25 min, mean EVP time 289 ± 62 min, mean total "out-of-body" time 420 ± 66 min and median cardiopulmonary bypass (CPB) time 228 min (range 126–416 min). Three patients underwent combined heart-kidney transplantation (**Table 3**).

Post-operative data are shown in **Table 4**. In-hospital mortality was 11%. Causes of early deaths were septic (n = 2) or haemorrhagic shock (n = 2), pancreatitis (n = 2), multi-organ failure (n = 2), and ECLS-related complications (n = 1). Postoperatively, 15 patients (19%) needed *de novo* ECLS support, because of ≥ moderate PGD in 13 patients (16%), vasoplegia syndrome in 1 and respiratory insufficiency in 1. Moreover, two patients needed veno-venous ECLS for pulmonary complications while an intra-aortic balloon was implanted in three patients. Two patients were assisted with ECLS for <24 h and 8 for <72 h.

Median intensive care unit stay was 6 days (range, 1–123); median mechanical assisted ventilation time was 29 h (range, 3 h to 110 days) with 26% of patients requiring >72 h of ventilation and 18% a tracheostomy. Median hospital stay was 36 days (range, 3–236), during which 64% of patients needed dialysis for acute renal failure, 24% had new onset of atrial fibrillation and 11% acute rejection grade ≥2 (combined with antibody-mediated rejection 2 in two cases); 19% needed sternal re-entry for bleeding and 1% had a stroke.

At univariable analysis, CPB time resulted a risk factor for both ≥moderate PGD (p = 0.001) and in-hospital mortality (p = 0.031).

In addition, high pre-HTx central venous pressure (CVP) was also a risk factor for hospital mortality (p = 0.050); PGD ≥ moderate and in-hospital mortality predictions of the CPB time showed AUC of 0.82 and 0.73 with cutoff values of 246 and 272 min, respectively. The predictive role of CVP regarding in-hospital mortality showed an AUC of 0.69, with a cutoff value of 15 mmHg (**Table 5**).

Follow-Up Data

During a median follow-up of 16 months (range, 2–43 months), eight patients died. The causes of late mortality are shown in **Table 6**. Actuarial survival at 1 and 3 years HTx was 83% ± 4% and 72% ± 7% (**Figure 1**). The rate of grade ≥2R acute rejection episodes and coronary allograft vasculopathy during follow-up was 6% and 10%, respectively.

At univariable analysis, recipient age (p = 0.021), pre-HTx ECLS (p = 0.010), donor age (p = 0.031) were reported to be risk-factors for late mortality (**Table 5**).

DISCUSSION

HTx represents the gold standard surgical treatment of end-stage heart failure with improved early and late post-HTx outcomes; however, the rate of PGD continues to be relatively high [8]. The interaction of donor, recipient and procedural factors may predispose to this life-threatening complication which represents the leading cause of 30-day mortality post-HTx [9].

TABLE 2 | Donor data.

	***N* = 80**
Median age, years (range)	48 (17–69)
Age ≥55 years, n (%)	22 (28)
Male sex, n (%)	52 (65)
LVEF ≤50%, n (%)	5 (6)
Mean LVEF, %	56 ± 10
Mean LV diastolic diameter, cm	4.6 ± 0.5
Mean LV systolic diameter, cm	3.4 ± 1.3
Median IVS, mm (range)	12 (7–16)
LV hypertrophy, n (%)	17 (21)
Cardiac arrest/prolonged severe hypotension, n (%)	31 (39)
Mean CPR time, min	28 ± 15
CAD, n (%)	3 (4)
Diabetes, n (%)	1 (1)
Smoking habit, n (%)	37 (46)
Alcohol abuse, n (%)	23 (29)

LVEF, left ventricular ejection fraction; LV, left ventricle; IVS, interventricular septum; CPR, Cardio-pulmonary resuscitation; CAD, coronary artery disease.

TABLE 3 | Intra-operative data.

	***N* = 80**
Mean EVP time, min	289 ± 62
Mean ischemic time, min	118 ± 25
Mean out of body time, min	420 ± 66
Median CPB time, min (range)	228 (126–416)
Combined heart-kidney transplantation	3

EVP, Ex-vivo *perfusion; CPB, Cardio-pulmonary by-pass.*

TABLE 4 | Post-operative data.

	***N* = 80**
Overall complications, n (%)	69 (86)
Moderate/severe PGD, n (%)	13 (16)
ECLS, n (%)	17 (21)
Pre-Htx ECLS	2
IABP, n (%)	3 (4)
Median MAV time, hours (range)	29 (3–2,649)
MAV >72 h, n (%)	21 (26)
Tracheostomy, n (%)	14 (18)
Revision for bleeding, n (%)	15 (19)
Need for CRRT, n (%)	51 (64)
Stroke, n (%)	1 (1)
Atrial fibrillation, n (%)	19 (24)
Median ICU stay (days, range)	6 (1–123)
Median hospital stay (days, range)	36 (3–236)
In-hospital mortality, n (%)	9 (11)

PGD, primary graft dysfunction; ECLS, extra corporeal life support; IABP, Intra-aortic balloon pump; MAV, mechanical assisted ventilation; CRRT, continuous renal replacement therapy; ICU, intensive care unit.

TABLE 5 | Results of univariable analysis.

	Odds ratio	**95% CIs**	***p*-value**
Risk factors for ≥moderate PGD			
CPB time	1.019	1.007–1.032	0.001
Risk factors for in-hospital mortality			
CPB time	1.001	1.001–1.023	0.031
Pre-HTx CVP	1.155	1.000–1.333	0.050
Risk factors for late mortality	Hazard ratio	95% CIs	*p*-value
Recipient age	6.619	1.331–32.904	0.021
Pre-HTx ECLS	6.183	1.542–24.798	0.010
Donor age	1.089	1.088–1.177	0.031

PGD, primary graft dysfunction; CPB, cardiopulmonary bypass; HTx, Heart transplantation; CVP, central venous pressure; ECLS, extracorporeal life support; IABP, Intra-aortic balloon pump.

The improvement of medical and interventional therapies, as well as the widespread use of MCS systems, have led to consider as potential candidates for HTx a subset of patients with end-stage heart failure with increasing age and multiple comorbidities. Based on the data reported by the ISHLT registry in 2019, in the last decade, 50% of HTx recipients have had a prior cardiac surgery, 39% were dependent on inotropic support and 5% had history of dialysis before HTx [10]. In the present series, 90% of recipients had a previous cardiac surgery, 61% chronic renal failure and 12% were on inotropic support, while three patients in dialysis pre-HTx underwent a combined heart and kidney transplantation. Therefore, these recipients were considered at high risk of developing PGD, 32% of them having a RADIAL score ≥ 4. In this study we have used the RADIAL score to predict post-HTx PGD since we believe it a simple, still reliable and easy-to-use tool particularly when data collection involves International centers; other scores, reported to have a more predictive accuracy, either consider a large variety of factors, including many intraoperative data, which were not available for our analysis, or analyzes only the recipient-related risk factors [20, 21].

Among high-risk patients a specific group that could particularly benefit of EVP are those requiring MCS as a bridge to HTx [14]. In this series the rate of patients on MCS was much higher than that reported in the ISHLT registry, 66% being on VAD and 14% on ECLS. Although currently long-term VAD recipients are not uniformly considered as high-risk patients, data from the European Registry of Mechanical Circulatory Support (EUROMACS) recognize VAD as a risk factor for post-HTx mortality as also confirmed by some reports from the United States [24, 25]. Particularly, among our patients many required urgent HTx because of life threatening complications related to the long-term VAD implantation. HTx in patients after long-term assistance with VAD is more complex and technically demanding, due to the presence of coarse adhesions and bleeding due to anticoagulation which often require prolonged CPB times. In the present study a longer CPB time, generally required for more complex redo procedures such as those in patients with VAD, has been found to be a risk factor at univariable analysis for both hospital mortality and PGD as also confirmed by others [19]. Similar problems have been encountered also in ECLS bridged patients, due to their generally more critical hemodynamic conditions and frequent multiorgan impairment. Furthermore, also a high CVP has been found to be a risk factor for early death as also recognized in the RADIAL score system; indeed, the effects of a high CVP, mainly on the splancnic district, are well

TABLE 6 | Long-term complications.

	Survivors *n* = 71
Median follow-up, months (range)	16 (2–43)
Rejection grade ≥2R, n (%)	5 (6)
CAV, n (%)	7 (10)
Late mortality, n (%)	8 (10)
Cardiac	2
Infection	3
Stroke	1
Cerebral hemorrhage	1
Neoplasia	1

CAV, coronary allograft vasculopathy.

known, conditioning the patient status pre-HTx and thus the outcome [8].

Considering the chronic donor shortage, patients at higher risk and with multiple comorbidities generally have a reduced probability to be considered as suitable HTx candidates. Similarly, also obese patients, who are more likely to have a significant donor/recipient size mismatch, may have less chances to be transplanted; moreover, size mismatch becomes a significant risk factor particularly when associated to other high-risk characteristics of both recipients or donors [12, 26]. In our study 30% of patients had a significant donor/recipient size mismatch; however, this data has not been analyzed separately because of the small number of cases generally associated with other important risk factors.

To more effectively address the issue of organ shortage, donor criteria have been extended, enlarging the available donor pool, but potentially also increasing the risk of adversely affecting the outcomes after HTx by employing suboptimal grafts. In performing a donor risk analysis, the use of the Eurotransplant donor heart risk score has been suggested to facilitate donor risk assessment allowing for more appropriate matching of extended criteria donor hearts [27]. However, it does not include among the donor factors considered the ischemia time, which is one of the most important variables, the one we actually tried to restrain with the use of OCS. Furthermore, the variables included in our analysis are those commonly used in other similar studies including the more recent Expand trial [28].

In the effort to increase the donor pool by using also marginal donors, alternative techniques of graft protection, such as the OCS, have been suggested yielding gratifying results [13]. It has been demonstrated that the time-dependent negative impact of ischemia on graft function depends on the donor age since prolonged ischemia is poorly tolerated by grafts from older donors [29]. Indeed, EVP provides a better myocardial protection, not only by allowing to limit the graft ischemic time, but also to assess and potentially recondition the donor heart [13–17]. Analysis of histological biopsy samples confirms that cardiomyocyte damage was either stable or even improved after reperfusion following HTx in EVP supported hearts, while after cold storage preservation donor grafts showed at histology worsening of myocardial damage after reperfusion [12]. Cardiomyocyte degeneration and edema increased after 6 h of support and, therefore, OCS perfusion longer than 8 h should be avoided [17]. This is supported by others who demonstrated that the length of time on OCS was a strong predictor of PGD [19]. Thus, employment of the OCS, the only device currently available for normothermic *ex-vivo* heart perfusion, could have an important role in graft protection limiting the rate of PGD and PGD-related mortality, especially when critical recipients receive hearts from high-risk donors [13, 30–34].

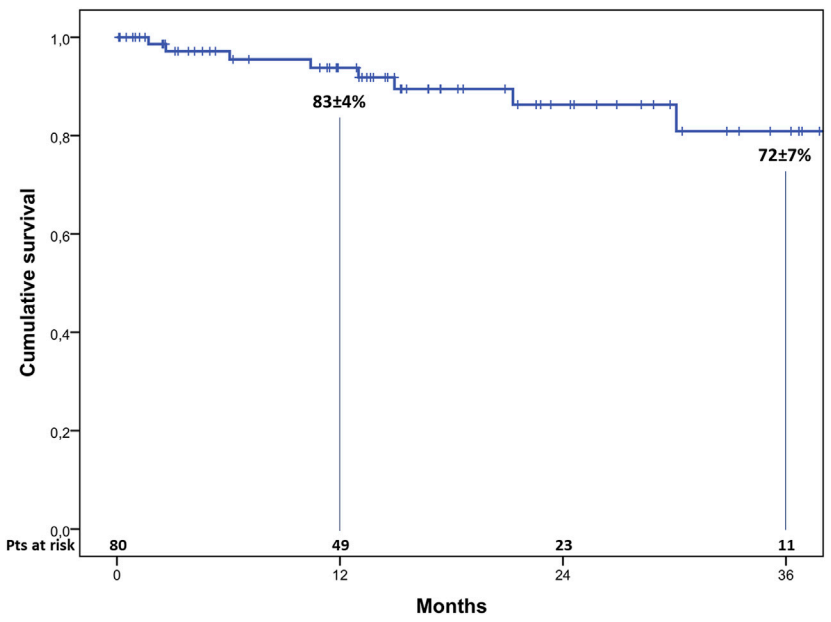


FIGURE 1 | Actuarial 3-year survival after heart transplantation.

Morbidity and mortality of the present experience are worse than those previously observed in both Institutions after HTx using standard donors in low risk recipients [35, 36]; however, they are quite comparable to those previously reported by García Sáez et al. who observed excellent short-term outcomes with the OCS in high-risk patient transplanted with marginal donors, despite the higher age of donors in our series [30, 35, 36]. Our results are also similar to those reported by the ISHLT registry [12] and may be explained not only by reduction of the ischemic times and better graft preservation, but also by the possibility of identifying, during EVP, unsuitable marginal grafts [7, 15]. Indeed, in the present experience 10% of donor grafts were discarded because timely detection of cardiac pathologies diagnosed owing to the OCS system.

Despite good early survival many patients experienced postoperative complications with a consequently extended intensive care unit stay. An increased need for chest re-entry for postoperative bleeding was observed mainly in patients undergoing HTx on VAD or reoperations, frequently associated to coagulation disorders. Also a high rate of renal failure and respiratory insufficiency requiring dialysis or prolonged mechanical ventilation was observed. It must be underlined that the high incidence of dialysis reflects the policy of one of the two centers, which in case of postoperative worsening of kidney function preferred to sustain it with early replacement therapy. Furthermore, the high number of patients requiring post-HTx ECLS implantation (21%) is related to a more liberal use of ECLS owing to the greater safety of such systems in the current era. This reflects differences in the centers policy, since one Institution preferred to employ early ECLS for a limited time, generally from <24 to 72 h, in patients with signs predicting the possible onset of even moderate PGD. The rate of PGD in our study was quite acceptable, being 16%, not much higher than what reported in the Expand trial [28] and by García Sáez et al [30], despite the different profiles of patients analyzed in such studies, regular recipients in the first and younger donor age in the second.

Study Limitations

This study has certainly some limitations mostly represented by its retrospective nature and those pertaining to any multicenter collaboration. In this specific study only two centers were involved minimizing potential biases in patient selection and treatment; however, some differences in each center policy, especially in postoperative immunosuppressive treatment and follow-up protocols, have emerged, which might have had an impact on patient outcomes but with negligible influence on PGD and perioperative complications. Moreover, the number of patients enrolled in this study was limited due to the specific patient characteristics, both of donor and recipients, thus precluding to select a control group for comparison.

Conclusion

Our study, dealing with a very complex setting represented by marginal donors, very high-risk recipients and expected long ischemic time, indicates that EVP appears to be an effective tool in reducing overall donor graft ischemic time and allowing continuous evaluation of graft function and viability during transportation. This should provide adequate grafts for high-risk recipients who would be otherwise excluded from the possibility of a HTx. Nevertheless, based on the gratifying results observed in the present study, we advocate the employment of EVP using OCS technology as a promising and valid tool to further extend the donor pool, to successfully perform HTx even in high-risk settings.

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 Comitato Etico Unico Regionale—CEUR FVG. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

SS, JS, GW, AH, UL, and MA contributed to conception and design of the study. JS, VF, ND, AL, AD, and FI contributed to data curation. SS, JS, VF, GW, and IV performed the statistical analysis. SS, VF, ND, and UB wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

REFERENCES

1. Khush KK. Donor Selection in the Modern Era. *Ann Cardiothorac Surg* (2018) 7:126–34. doi:10.21037/acs.2017.09.09
2. Chan JL, Kobashigawa JA, Reich HJ, Ramzy D, Thottam MM, Yu Z, et al. Intermediate Outcomes with *Ex-Vivo* Allograft Perfusion for Heart Transplantation. *J Heart Lung Transpl* (2017) 36:258–63. doi:10.1016/j.healun.2016.08.015
3. Fukushima N. Current Status and Future Aspects of *Ex Vivo* Allograft Perfusion for Heart Transplantation. *J Heart Lung Transpl* (2017) 36:247–9. doi:10.1016/j.healun.2017.01.002
4. Ardehali A, Esmailian F, Deng M, Soltesz E, Hsich E, Naka Y, et al. *Ex-vivo* Perfusion of Donor Hearts for Human Heart Transplantation (PROCEED II):

A Prospective, Open-Label, Multicentre, Randomised Non-Inferiority Trial. *Lancet* (2015) 385:2577–84. doi:10.1016/S0140-6736(15)60261-6
5. Petrini C. Organ Transplantation From Marginal and Non-Standard Risk Donors: Ethical Requisites for Consent From Recipients. *Ann Ist Super Sanità* (2017) 53:350–3. doi:10.4415/ANN_17_04_13
6. Hess NR, Ziegler LA, Zaczorowski DJ. Heart Donation and Preservation: Historical Perspectives, Current Technologies, and Future Directions. *J Clin Med* (2022) 11:5762. doi:10.3390/jcm11195762
7. Sponga S, Napgal D, Beltrami AP, Ferrara V, Nalon S, Finato N, et al. Coronary Dissection Discovered During *Ex-Vivo* Organ Preservation: Avoiding a Fatal Complication. *Ann Thorac Surg* (2017) 104:e383–4. doi:10.1016/j.athoracsur.2017.05.087
8. Segovia J, Cosío MDG, Barceló JM, Bueno MG, Pavía PG, Burgos R, et al. RADIAL: A Novel Primary Graft Failure Risk Score in Heart Transplantation. *J Heart Lung Transpl* (2011) 30:644–51. doi:10.1016/j.healun.2011.01.721
9. Mehra MR, Canter CE, Hannan ME, Semigran MJ, Uber PA, Baran DA, et al. The 2016 International Society for Heart Lung Transplantation Listing Criteria for Heart Transplantation: A 10-year Update. *J Heart Lung Transpl* (2016) 35:1–23. doi:10.1016/j.healun.2015.10.023
10. Copeland H, Knezevic I, Baran DA, Rao V, Pham M, Gustafsson F, et al. Donor Heart Selection: Evidence-Based Guidelines for Providers. *J Heart Lung Transpl* (2023) 42:7–29. doi:10.1016/j.healun.2022.08.030
11. Trivedi JR, Cheng A, Ising M, Lenneman A, Birks E, Slaughter MS. Heart Transplant Survival Based on Recipient and Donor Risk Scoring: A UNOS Database Analysis. *ASAIO J* (2016) 62:297–301. doi:10.1097/MAT.0000000000000337
12. Krush KK, Cherich WS, Chambers DC, Harhay MO, Hayes D, Jr, Hsich E, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation; Thirty-Sixth Adult Heart Transplantation Report – 2019: Focus Theme: Donor and Recipient Size Matching. *J Heart Lung Transpl* (2019) 38:1056–66. doi:10.1016/j.healun.2019.08.004
13. Sponga S, Bonetti A, Ferrara V, Beltrami AP, Isola M, Vendramin I, et al. Preservation by Cold Storage vs *Ex Vivo* Normothermic Perfusion of Marginal Donor Hearts: Clinical, Histopathological and Ultrastructural Features. *J Heart Lung Tranplant* (2020) 39:1048–16. doi:10.1016/j.healun.2020.08.021
14. Sponga S, Benedetti G, De Manna ND, Ferrara V, Vendramin I, Lechiancole A, et al. Heart Transplant Outcomes in Patients with Mechanical Circulatory Support: Cold Storage Versus Normothermic Perfusion Organ Preservation. *Interact Cardiovasc Thorac Surg* (2021) 32:476–82. doi:10.1093/icvts/ivaa280
15. Benedetti G, Sponga S, Vendramin I, Nalli C, Lechiancole A, Bortolotti U, et al. *Ex Vivo* Normothermic Perfusion: A New Preservation Strategy for a Donor Heart with a Myocardial Bridge? *Transpl Int* (2020) 33:1555–6. doi:10.1111/tri.13707
16. Bonetti A, Sponga S, Livi U, Ortolani F. A Case of Dramatic Sarcomere Disarray in a Marginal Donor Heart Explanted Soon After Cardiac Arrest: Possible Rearrangement After *Ex Vivo* Perfusion. *Transplantation* (2021) 105:e111–2. doi:10.1097/TP.0000000000003795
17. Sponga S, Vendramin I, Bortolotti U, Livi U. *Ex Vivo* Donor Heart Preservation in Heart Transplantation. *J Card Surg* (2021) 36:4836. doi:10.1111/jocs.15978
18. Kobashigawa J, Zuckermann A, Macdonald P, Leprince P, Esmailian F, Luu M, et al. Report From a Consensus Conference on Primary Graft Dysfunction After Cardiac Transplantation. *J Heart Lung Transpl* (2014) 33:327–40. doi:10.1016/j.healun.2014.02.027
19. Sing A, Sing S, Banner RN, Rushton S, Simon AR, Berry C, et al. ISHLT Primary Graft Dysfunction Incidence, Risk Factors, and Outcome: A UK National Study. *Transplantation* (2019) 103:336–43. doi:10.1097/TP.0000000000002220
20. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P, Acute Dialysis Quality Initiative workgroup. Acute Renal Failure - Definition, Outcome Measures, Animal Models, Fluid Therapy and Information Technology Needs: The Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* (2004) 8:204–12. doi:10.1186/cc2872
21. Stewart S, Winters GL, Fishbein MC, Tazelaar HD, Kobashigawa J, Abrams J, et al. Revision of the 1990 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Heart Rejection. *J Heart Lung Transpl* (2005) 24:1710–20. doi:10.1016/j.healun.2005.03.019
22. Mehra MR, Crespo-Leiro MG, Dipchand A, Ensminger SM, Hiemann NE, Kobashigawa JA, et al. International Society for Heart and Lung Transplantation Working Formulation of a Standardized Nomenclature for Cardiac Allograft Vasculopathy-2010. *J Heart Lung Transpl* (2010) 29:717–27. doi:10.1016/j.healun.2010.05.017
23. Weiss ES, Allen JG, Arnaoutakis GJ, George TJ, Russel SD, Shah AS, et al. Creation of a Quantitative Recipient Risk Index for Mortality Prediction After Cardiac Transplantation (IMPACT). *Ann Thorac Surg* (2011) 92:914–21. doi:10.1016/j.athoracsur.2011.04.030
24. Akin S, Soliman O, de By TMMH, Muslem R, Tijissen JGP, Schoenrath AS, et al. Causes and Predictors of Early Mortality in Patients Treated with Left Ventricular Assist Device Implantation in the European Registry of Mechanical Circulatory Support (EUROMACS). *Intens Care Med* (2020) 46:1349–60. doi:10.1007/s00134-020-05939-1
25. Shekar K, Gregory SD, Fraser JF. Mechanical Circulatory Support in the New Era: An Overview. *Crit Care* (2016) 16:66. doi:10.1186/s13054-016-1235-3
26. Weiss ES, Allen JC, Russell SD, Shah AS, Conte JV. Impact of Recipient Body Mass Index on Organ Allocation and Mortality in Orthotopic Heart Transplantation. *J Heart Lung Transpl* (2009) 28:1150–7. doi:10.1016/j.healun.2009.06.009
27. Smits JM, De Pauw M, de Vries E, Rachmel A, Meiser B, Laufer G, et al. Donor Scoring System for Heart Transplantation and the Impact on Patient Survival. *J Heart Heart Lung Transpl* (2012) 31:387–97. doi:10.1016/j.healun.2011.11.005
28. Schroder JN, Shah A, Anyanwu A, D'Alessandro D, Streuber M, Mudy K, et al. Increasing Utilization of Extended Criteria Donor After Brain Death (DBD) Hearts Seldomly Used for Transplantation in the U.S. Due to Limitation of Ischemic Cold Storage - 2-Year Results of the OCS Heart EXPAND Prospective Multi-Center Trial (OCS Heart EXPAND). *J Heart Lung Transpl* (2022) 41:S73. doi:10.1016/j.healun.2022.01.167
29. Russo MJ, Chen JM, Sorabella RA, Martens TP, Garrido M, Davies RR, et al. The Effect of Ischemic Time on Survival After Heart Transplantation Varies by Donor Age: An Analysis of the United Network for Organ Sharing Data Base. *J Thorac Cardiovasc Surg* (2007) 133:554–9. doi:10.1016/j.jtcvs.2006.09.019
30. García Sáez D, Zych B, Sabashnikov A, Bowles CT, De Robertis F, Mohite PN, et al. Evaluation of the Organ Care System in Heart Transplantation with an Adverse Donor/Recipient Profile. *Ann Thorac Surg* (2014) 98:2099–105. discussion 2105-6. doi:10.1016/j.athoracsur.2014.06.098
31. Chen Q, Singer-Englar T, Kobashigawa JA, Roach A, Emerson D, Megna D, et al. Long-term Outcomes After Heart Transplantation Using *Ex Vivo* Allograft Perfusion in Standard Risk Donors: A Single-center Experience. *Clin Transpl* (2022) 14:e14591. doi:10.1111/ctr.14591
32. Dang Van S, Gaillard M, Laverdure F, Thes J, Venhard JC, Fradi M, et al. *Ex Vivo* Perfusion of the Donor Heart: Preliminary Experience in High-Risk Transplantations. *Arch Cardiovasc Dis* (2021) 114:715–26. doi:10.1016/j.acvd.2021.07.003
33. Pinnelas R, Kobashigawa JA. *Ex Vivo* Normothermic Perfusion in Heart Transplantation: A Review of the TransMedics Organ Care System. *Future Cardiol* (2022) 18:5–15. doi:10.2217/fca-2021-0030
34. Fleck TPK, Ayala R, Kroll J, Siepe M, Schibilsky D, Benk C, et al. *Ex Vivo* Allograft Perfusion for Complex Pediatric Heart Transplant Recipients. *Ann Thorac Surg* (2021) 112:1275–80. doi:10.1016/j.athoracsur.2020.12.025
35. Sponga S, Deroma L, Sappa R, Piani D, Lechiancole A, Spagna E, et al. Recipient Age Impact on Outcome After Cardiac Transplantation: Should it Still Be Considered in Organ Allocation? *Interact Cardiovasc Thorac Surg* (2016) 23:573–9. doi:10.1093/icvts/ivw184
36. Fischer S, Strueber M, Haverich A. Clinical Cardiac and Pulmonary Transplantation: The Hannover Experience. *Clin Transpl* (2000) 2000:311–6.

ESOT | Transplant International

ORIGINAL RESEARCH
published: 24 July 2023
doi: 10.3389/ti.2023.11328

Conversion From Intravenous In-Hospital Belatacept Injection to Subcutaneous Abatacept Injection in Kidney Transplant Recipients During the First COVID-19 Stay-at-Home Order in France

Dominique Bertrand[1]†, Mélanie Brunel[2]†, Ludivine Lebourg[1], Anne Scemla[2], Mathilde Lemoine[1], Lucile Amrouche[2], Charlotte Laurent[1], Christophe Legendre[2], Dominique Guerrot[1,3], Dany Anglicheau[2] and Rebecca Sberro-Soussan[2]*

[1]Department of Nephrology, Kidney Transplantation and Hemodialysis, Rouen University Hospital, Rouen, France, [2]Department of Kidney Transplantation, Hôpital Necker-Enfants Malades, Assistance Publique-Hôpitaux de Paris, Université Paris Cité, Paris, France, [3]INSERM U1096, University of Rouen Normandy, Rouen, France

The first COVID-19 stay-at-home order came into effect in France on 17 March 2020. Immunocompromised patients were asked to isolate themselves, and outpatient clinic visits were dramatically reduced. In order to avoid visits to the hospital by belatacept-treated kidney transplant recipients (KTRs) during the initial period of the pandemic, we promptly converted 176 KTRs at two French transplant centers from once-monthly 5 mg/kg in-hospital belatacept infusion to once-weekly 125 mg subcutaneous abatacept injection. At the end of follow-up (3 months), 171 (97.16%) KTRs survived with a functioning graft, 2 (1.14%) had died, and 3 (1.70%) had experienced graft loss. Two patients (1.1%) experienced acute T cell–mediated rejection. Nineteen patients (10.80%) discontinued abatacept; 47% of the KTRs found the use of abatacept less restrictive than belatacept, and 38% would have preferred to continue abatacept. Mean eGFR remained stable compared to baseline. Seven patients (3.9%) had COVID-19; among these, two developed severe symptoms but survived. Only one patient had a *de novo* DSA. Side effects of abatacept injection were uncommon and non-severe. Our study reports for the first time in a large cohort that once-weekly injection of abatacept appears to be feasible and safe in KTRs previously treated with belatacept.

Keywords: COVID-19, kidney transplantation, belatacept, abatacept, CNI toxicity

OPEN ACCESS

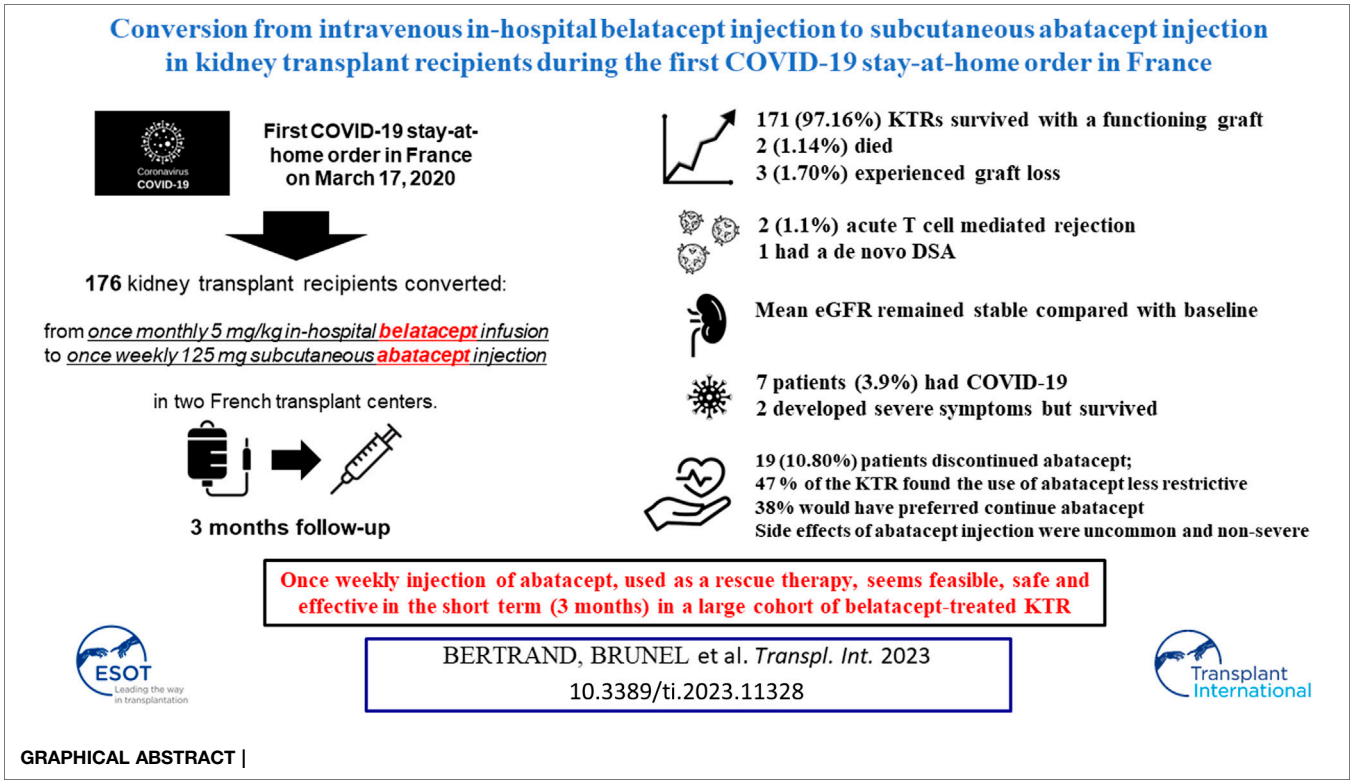
Correspondence:
Dominique Bertrand
dominique.bertrand@chu-rouen.fr

†These authors have contributed equally to this work and share first authorship

Received: *02 March 2023*
Accepted: *05 July 2023*
Published: *24 July 2023*

Citation:
Bertrand D, Brunel M, Lebourg L, Scemla A, Lemoine M, Amrouche L, Laurent C, Legendre C, Guerrot D, Anglicheau D and Sberro-Soussan R (2023) Conversion From Intravenous In-Hospital Belatacept Injection to Subcutaneous Abatacept Injection in Kidney Transplant Recipients During the First COVID-19 Stay-at-Home Order in France. Transpl Int 36:11328. doi: 10.3389/ti.2023.11328

Abbreviations: CNI, calcineurin inhibitor; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; KTR, kidney transplant recipient; COVID-19, coronavirus disease 2019; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; CMV, cytomegalovirus; TCMR, T cell–mediated rejection.



GRAPHICAL ABSTRACT |

INTRODUCTION

Belatacept is a fusion protein composed of the heavy chain constant region of human IgG1 linked to the extracellular domain of human cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) that selectively inhibits T-cell activation through costimulation blockade. Since its approval by the U.S. Food and Drug Administration and the European Medicines Agency in 2011, belatacept has become widely used in kidney transplantation as an alternative to calcineurin inhibitors (CNIs) for maintenance immunosuppression [1, 2]. Belatacept is used as a *de novo* immunosuppressive therapy after kidney transplantation, but also as a conversion from calcineurin inhibitors [1, 2] in cases of CNI toxicity and/or side effects [3]. Belatacept should be administered intravenously every month under the supervision of a healthcare provider.

Coronavirus disease 2019 (COVID-19) has been particularly deleterious in kidney transplant recipients (KTRs), with a very high risk of severe disease associated with a high mortality rate [4, 5]. During the first wave of the pandemic, a lockdown order came into effect in France on 17 March 2020. Immunocompromised patients were asked to isolate themselves and outpatient clinic visits were dramatically reduced. Patients who have converted to belatacept for CNI toxicity are suspected to be at high risk of opportunistic infections [6, 7]. In order to avoid frequent clinic visits by belatacept-treated KTRs and prevent SARS-CoV-2 contamination during the initial pandemic period, and also to release some institutional resources to care for COVID-19-infected KTRs, we searched for a temporary alternative solution to monthly administration of belatacept. CNI conversion appeared to be a safer option because i) patients could take their treatment orally at home; ii) most of them had previously received tacrolimus or cyclosporine before belatacept conversion; and iii) CNIs significantly reduce acute rejection rates. However, this solution was not generalizable to all of our patients, due to some having a low estimated glomerular filtration rate (eGFR) or history of CNI toxicity and/or intolerance [8].

Abatacept is also genetically constructed by fusion of the external domain of human CTLA-4 to the heavy chain constant region of human IgG1. This drug was the predecessor of its higher-affinity evolution, belatacept, which was engineered to contain two amino acid substitutions to bind its ligands CD80 and CD86 with greater potency for use in kidney transplantation. Abatacept was approved by the Food and Drug Administration for use in adults with rheumatoid arthritis in 2005 [9] and in children with juvenile idiopathic arthritis in 2008 [10], and it can be used intravenously or subcutaneously [11]. Data on the use of abatacept after kidney transplantation are very scarce, but the results of a preclinical study using a primate kidney transplant model [12] and of a small report on nine patients [13] seemed reassuring.

Considering these results, and replicating the protocol used for rheumatoid arthritis [11], we believed that once-weekly subcutaneous injection of abatacept could be a safe, effective, and logistically feasible alternative to belatacept during the COVID-19 pandemic. Here, we report on a cohort of patients

from two transplant centers who received abatacept during the initial stay-at-home order in France. Our aim was to assess short-term graft and patient outcomes, kidney allograft function, immunological features, and tolerance and safety of abatacept maintenance to provide a rationale for belatacept avoidance in the event of a prolonged crisis, and as an alternative in KTRs with problematic vascular access; these findings have even greater relevance in the current period of belatacept shortage.

MATERIAL AND METHODS

Patients

A total of 176 KTRs receiving belatacept as a conversion protocol at two French transplant centers (Necker University Hospital and Rouen University Hospital) were converted to abatacept during the COVID-19 pandemic (**Figure 1**). All patients were 18 or older, had received either a living or a deceased donor kidney transplant, and had received no prior or concurrent non-renal solid organ transplant. Patient characteristics and biological data were collected from electronic medical records. According to French law (loi Jardé), because this was an anonymous retrospective study, institutional review board approval was not required.

Immunosuppression

Patients had been initially converted from CNI to belatacept according to the protocol published in phase II and III conversion studies. Belatacept was then maintained at 5 mg/kg every 4 weeks in all KTRs. For abatacept conversion, patients received subcutaneous injection of 125 mg abatacept once weekly at home, initiated 1 month after the last belatacept infusion. The remaining components of maintenance immunosuppression were not modified while the patients were on abatacept.

Follow-Up

Patients were followed up 3 months after the first injection while on abatacept therapy. After 3 months, patients were switched back to belatacept because the French administration authorized the in-home infusion of belatacept in the context of the COVID-19 pandemic. Kidney allograft function was assessed on day 0 and at 3 months, using plasma creatinine and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [14] for eGFR in KTRs with a functioning graft. BK virus and cytomegalovirus (CMV) viral loads were measured. The tolerance and safety of abatacept maintenance were evaluated using a specific questionnaire.

Anti-HLA Antibody Testing

The presence of anti–HLA-A, -B, -Cw, -DR, -DQ, and -DP donor-specific antibodies (DSAs) was analyzed using single-antigen flow bead assays (One Lambda, Inc., Canoga Park, CA) performed using a Luminex platform on serum samples at time of transplantation, every year or at the time of any biopsy, and 3 months after abatacept conversion. The presence of DSAs was defined by a median fluorescence intensity (MFI) ≥500. The number, class, specificities, and MFI of each anti-HLA DSA were recorded.

Histologic Phenotyping of Kidney Allograft Biopsies

During the 3 months under abatacept, graft biopsies were performed only for cause. Biopsies were graded using the 2017 Banff classification [15]: C4d staining was performed by immunohistochemistry on paraffin sections or immunofluorescence on frozen sections and graded from 0 to 3 by the percentage of peritubular capillaries with linear staining.

Statistical Analysis

Continuous variables were summarized in the form of means with SDs or medians with IQRs, and they were compared using Mann-Whitney or t-tests, as appropriate. Categorical variables were summarized in the form of numbers with proportions, and they were compared using Fisher's exact test. We used STATA (version 14, Data Analysis and Statistical Software) and R (version 3.2.1; R Foundation for Statistical Computing) to carry out descriptive analyses. A p-value <0.05 was considered significant.

RESULTS

Baseline Characteristics of Converted KTRs

A total of 176 patients from two transplant centers were converted to abatacept during the early stages of the COVID-19 pandemic in France (March 2020). Of these, 19 patients (10.80%) discontinued abatacept: 12/116 patients (10.34%) at Necker Hospital and 7/60 (11.67%) at Rouen Hospital ($p = 0.8$). Detailed reasons for abatacept discontinuation are presented in **Figure 1**. The remaining 157 patients (89.20%) were reassessed 3 months after conversion. KTR characteristics are listed in **Table 1**.

Conversion data are outlined in **Table 2**. Belatacept indications were similar between centers, but belatacept conversion occurred later in Rouen. All except two patients (2.5 and 4 months) were converted to abatacept beyond the first 6 months after transplant.

Patient and Graft Outcomes After Conversion

At the end of follow-up, 171 patients (97.16%) survived with a functioning graft, 2 (1.14%) died, and 3 (1.70%) experienced graft loss. Causes of death were vascular (stroke) and infectious (invasive aspergillosis with CMV disease). Graft loss only occurred in patients with chronic allograft dysfunction and severe renal impairment at baseline (eGFR <20 mL/min/1.73 m^2). These patients returned to hemodialysis and underwent premature discontinuation of abatacept. No biopsy was performed.

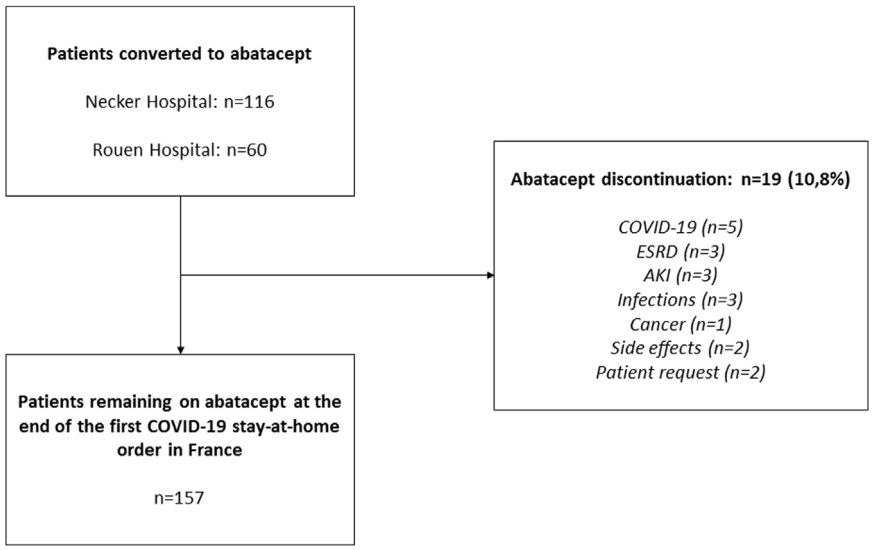


FIGURE 1 | Flow Chart. AKI, acute kidney injury; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; SLKT, simultaneous liver–kidney transplant.

TABLE 1 | Transplant recipients' demographic and baseline characteristics.

	All patients *n* = 176	Necker *n* = 116	Rouen *n* = 60	*p*-value
Recipient				
Age, yr, median (IQR)	57 (44–66)	54.5 (43–65)	59.5 (47–68)	0.096
Men, *n* (%)	111 (63.07)	70 (60.34)	41 (68.33)	0.326
ESKD causes, *n* (%)				
Diabetes/hypertension	38 (21.59)	18 (15.52)	20 (33.33)	0.092
Glomerulonephritis	37 (21.02)	22 (18.97)	15 (25.00)	
Interstitial nephritis	19 (10.80)	15 (12.93)	4 (6.67)	
Polycystic kidney disease	27 (15.34)	19 (16.38)	8 (13.33)	
Uropathy	13 (7.39)	11 (9.48)	2 (3.33)	
Other	8 (4.55)	6 (5.17)	2 (3.33)	
Unknown	34 (19.32)	25 (21.55)	9 (15.00)	
Previous kidney transplant, *n* (%)	20 (11.36)	16 (13.79)	4 (6.67)	0.212
Donor				
Age, yr, median (IQR)	62 (51–71)	61.5 (50–71.5)	62 (52–70.5)	0.815
Men, *n* (%)	86 (48.86)	59 (50.86)	27 (45.00)	0.526
Deceased donor, *n* (%)	153 (86.93)	95 (81.90)	58 (96.67)	*0.005*
Preformed anti-HLA DSAs[a], *n* (%)				
Class I		6 (5.26)		
Class II		13 (11.40)		
Class I/II		2 (1.75)		
Induction treatment[a], *n* (%)				
Thymoglobulin®	62 (35.43)	43 (37.39)	19 (31.67)	0.285
Basiliximab	109 (62.29)	68 (59.13)	41 (68.33)	
None	4 (2.29)	4 (3.48)	0 (0.00)	

DSA, donor-specific antibody; ESKD, end-stage kidney disease; HLA, human leukocyte antigen.
[a]*Missing data: preformed DSAs, two patients; induction treatment, one patient.*
Italic values indicate statistically significant between the two groups.

Eight patients (4.55%) developed an acute kidney injury requiring a graft biopsy under abatacept. Detailed histologic findings are presented in **Table 3**. Two patients experienced acute T cell–mediated rejection (TCMR). The first of these patients experienced a grade Ib TCMR (Biopsy#4) 2 months after abatacept conversion and was successfully treated with a high dose of intravenous steroids. Abatacept was stopped and belatacept was resumed. The second experienced a grade IIb TCMR (Biopsy#7) 1.5 months after abatacept initiation; this was successfully treated with steroids. Belatacept was also resumed,

TABLE 2 | Conversion data.

	All patients *n* = 176	Necker *n* = 116	Rouen *n* = 60	*p*-value
Time of belatacept conversion post-Tx, mo, median (IQR)	17 (5–57)	13.1 (3–44)	30 (9–104)	*0.001*
Belatacept indication, *n* (%)				
CAD – IFTA	139 (78.98)	86 (74.14)	53 (88.33)	0.133
CNI toxicity	24 (13.64)	18 (15.52)	6 (10.00)	
TMA	10 (5.68)	9 (7.76)	1 (1.67)	
De novo	3 (1.70)	3 (2.59)	0 (0.00)	
Time of abatacept conversion post-Tx, mo, median (IQR)	60 (32–95)	55 (32–85)	66 (32–123)	0.184
Time of abatacept conversion post-belatacept, mo, median (IQR)	25 (11–48)	30 (15–51)	19 (6–42)	*0.008*
Immunosuppression regimen, *n* (%)				
Abatacept/Mycophenolic acid/Prednisone	108 (61.36)	88 (75.86)	20 (33.33)	*< 0.001*
Abatacept/Azathioprine/Prednisone	14 (7.95)	13 (11.21)	1 (1.67)	
Abatacept/Everolimus/Prednisone	4 (2.27)	3 (2.59)	1 (1.67)	
Abatacept/Mycophenolic acid	26 (14.77)	1 (0.86)	25 (41.67)	
Abatacept/Azathioprine	2 (1.14)	0 (0.00)	2 (3.33)	
Abatacept/Everolimus	1 (0.57)	0 (0.00)	1 (1.67)	
Abatacept/Prednisone	20 (11.36)	11 (9.48)	9 (15.00)	
Abatacept	1 (0.57)	0 (0.00)	1 (1.67)	
Maintenance drug doses, median (IQR)				
Mycophenolic acid, mg/d	720 (450–720)	720 (540–1,080)	720 (360–720)	0.077
Azathioprine, mg/d	100 (75–125)	100 (75–150)	100 (50–100)	0.576
Everolimus trough level, ng/mL	5.1 (5–7.1)	5 (3–7.1)	6.25 (5.1–7.4)	0.248
Prednisone (mg/d)	7.5 (5–10)	10 (5–10)	7.5 (5–10)	*0.021*
Time on abatacept, mo, median (IQR)		2.9 (2.8–3.7)		
Number of abatacept infusions, median (IQR)		12 (11–16)		

CAD, chronic allograft dysfunction; CNI, calcineurin inhibitor; IFTA, interstitial fibrosis and tubular atrophy; TMA, thrombotic microangiopathy; Tx, transplant.
Italic values indicate statistically significant between the two groups.

but the patient developed severe invasive aspergillosis with CMV disease and died. A third patient was diagnosed with chronic antibody-mediated rejection (Biopsy#4). Abatacept was pursued and eGFR remained stable.

Among the 157 patients receiving abatacept at the end of follow-up, mean eGFR remained stable compared with baseline (38.0 ± 18.9 mL/min/1.73 m^2 versus 38.1 ± 19.4 mL/min/1.73 m^2, $p = 0.8$) (**Figure 2**), as did proteinuria/creatininuria ratio (0.56 ± 0.65 g/g versus 0.58 ± 0.85 g/g, $p = 0.6$). Only one patient had a *de novo* DSA, without a history of antibody-mediated rejection.

Tolerance and Safety of Abatacept

One patient (0.57%) experienced CMV disease of the gastrointestinal tract under abatacept, which resolved with a single course of ganciclovir therapy for 3 weeks and MPA discontinuation; additionally, three patients (1.71%) contracted an asymptomatic CMV viremia (>3 log copies/mL), of whom two had already had CMV viremia under belatacept. One patient had a low-level BK viremia without nephritis. Seven patients (3.98%) experienced COVID-19 under abatacept; among these, two developed severe and critical symptoms but survived. Five KTRs developed other non-severe viral infections: one simplex herpes virus and one zoster herpes virus infection; one adenovirus cystitis; one norovirus colitis; and one gastroenteritis. Bacterial infections occurred in 14 KTRs: 10 non-severe urinary tract infections, one bacteremia, one pneumonia, one *clostridium difficile* colitis, and one *campylobacter* colitis. Finally, fungal infections occurred in two patients: one case of invasive aspergillosis and one extensive dermatophytosis.

Side effects of abatacept injection were uncommon and non-severe. These are reported in **Table 4**. The results of the quality of life survey are depicted in **Figure 3**.

DISCUSSION

To the best of our knowledge, we report here for the first time in a large cohort of KTRs the feasibility, safety, and efficacy of conversion from once-monthly intravenous infusion of belatacept to once-weekly subcutaneous injection of abatacept as a maintenance immunosuppression regimen. Tolerance was excellent and side effects were very uncommon in this fragile population. eGFR remained stable during the follow-up period, and cases of biopsy-proven TCMR after abatacept conversion were rare (1.1%). In comparison, conversion from belatacept to another immunosuppressive treatment in cases of CNI toxicity or intolerance is associated with a decrease in eGFR, as recently reported by [8]. In this cohort of 44 KTRs from five French transplantation centers, who were converted from maintenance belatacept to another regimen because of a complication (n = 28), by patient request, or due to belatacept shortage (n = 13), mean eGFR decreased from 44.2 ± 16 mL/min per 1.73 m^2 at conversion from belatacept to 35.7 ± 18.4 mL/min per 1.73 m^2 at last follow-up ($p = 0.0002$). Of note, eGFR decreased more severely in patients who were converted to CNIs.

As an alternative approach, we could have increased the spacing of the belatacept injections from 4 to 8 weeks, as reported by [16]; however, although the result was not statistically significant, rates of BPAR were twofold higher in

TABLE 3 | Banff classification of kidney graft biopsies performed under abatacept treatment.

Biopsy#	t	i	g	ah	v	ti	iIFTA	cg	ci	ct	cv	mm	cpt	C4d
1	0	0	0	2	0	0	0	0	0	0	2	0	0	0
2	0	0	1	2	0	1	1	0	2	2	1	1	0	0
3	0	0	1	3	0	0	1	3	2	2	3	1	2	0
4	3	2	0	2	0	2	3	0	1	1	1	0	1	0
5	1	0	0	2	0	0	1	0	2	2	1	0	0	0
6	0	0	0	2	0	0	2	0	1	1	2	0	0	0
7	3	1	0	1	2	2	3	0	2	2	2	1	0	0
8	0	0	0	3	0	1	2	0	3	3	2	0	0	0

ah, arteriolar hyaline thickening; cg, transplant glomerulopathy; ci, interstitial fibrosis; cpt, peritubular capillary inflammation; ct, tubular atrophy; cv, arterial fibrous intimal thickening; g, glomerulitis; i, interstitial inflammation; t, tubulitis; v, endarteritis.

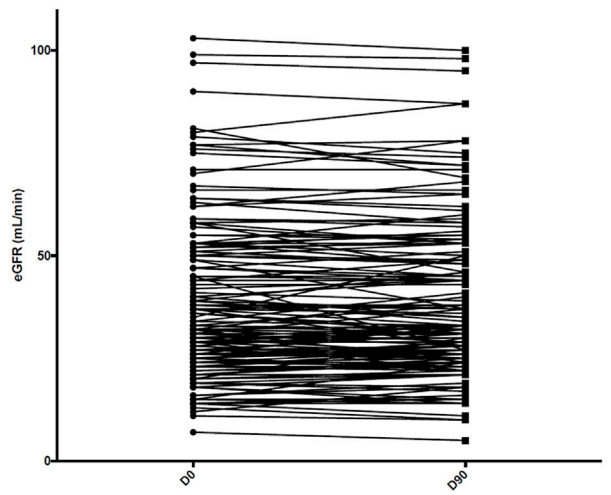


FIGURE 2 | Kidney allograft function assessed at day 0 (D0) and at 3 months (D90) among the 157 patients receiving abatacept at the end of follow-up. eGFR: estimated glomerular filtration rate.

TABLE 4 | Side effects under abatacept treatment.

	Yes	No	Missing
Infusion site reaction, *n* (%)	7 (3.98)	150 (85.23)	19 (10.80)
Arthralgia, *n* (%)	25 (14.20)	131 (74.43)	20 (11.36)
Erythema, *n* (%)	7 (3.98)	151 (85.80)	18 (10.23)
Abdominal pain, *n* (%)	16 (9.09)	144 (81.82)	16 (9.09)
Diarrhea, *n* (%)	20 (11.36)	140 (79.55)	16 (9.09)
Nausea/vomiting, *n* (%)	10 (5.68)	149 (84.66)	17 (9.66)
Stomatitis, *n* (%)	6 (3.41)	152 (86.36)	18 (10.23)
Cough, *n* (%)	11 (6.25)	148 (84.09)	17 (9.66)
Headache, *n* (%)	19 (10.80)	139 (78.98)	18 (10.23)

patients administered belatacept every 8 weeks vs. every 4 weeks. Another alternative would have been to pursue in-hospital belatacept infusions with a specific infection control protocol, as reported by Kamar et al. [17]. Nevertheless, these measures were very restrictive and time-consuming, and they did not fully rule out the risk of nosocomial transmission of SARS-CoV-2. Our in-home attitude is also retrospectively supported by the low humoral and cellular immunogenicity induced by SARS-CoV-2 vaccination in belatacept-treated KTRs [18, 19], related to their profoundly immunocompromised condition and to their high risk of opportunistic infection [6, 7] and severe COVID-19.

Data on the use of abatacept after kidney transplantation are very scarce. Abatacept is a genetically constructed by fusion of the external domain of human CTLA-4 to the heavy chain constant region of human IgG1. This drug was the predecessor of its higher-affinity variant belatacept, which was engineered to contain two amino acid substitutions to bind its ligands CD80 and CD86 with greater potency for use in kidney transplantation. Apprehension toward its use after kidney transplantation is therefore related to its supposedly insufficient immunosuppressive capacity. The data reported in the present study are quite reassuring, with a low risk of rejection when abatacept is used in a conversion protocol beyond the first 6 months after kidney transplant. Although preclinical non-human primate (NHP) studies have shown superior results with belatacept in a kidney transplant model [20], abatacept has also exhibited efficacy in a kidney transplant model [12], as well as in an NHP allogeneic islet transplant model, as a *de novo* monotherapy or in combination with CD154- specific blockade [21]. It has also since been effectively used in the clinical setting to treat rheumatoid arthritis and, more recently, other autoimmune disorders [9]. While belatacept may be indeed more potent and the preclinical data on abatacept warrant caution regarding its immunosuppressive strength for the purpose of inhibiting alloreactivity, preclinical data from murine and primate models alike have proven not to be entirely predictive of clinical outcomes or directly translatable to humans [22]. Abatacept has been used after kidney transplantation for recurrence of focal and segmental glomerulosclerosis [23], but most publications are case reports [24, 25] and its effectiveness is widely debated [26, 27]. Recently; [13], have reported on a series of 9 CNI-intolerant transplant recipients who were converted to abatacept early after transplant as a form of rescue immunosuppression during periods of belatacept shortage. A retrospective review revealed successful allograft salvage and 100% patient and graft survival (median 115 months) after conversion to abatacept. Patients received intravenous abatacept for a median duration of 82 months with stable, long-term renal allograft function, a single cellular rejection episode, and no clinically apparent protective immunity concerns. Furthermore, CD86 receptor saturation levels (a

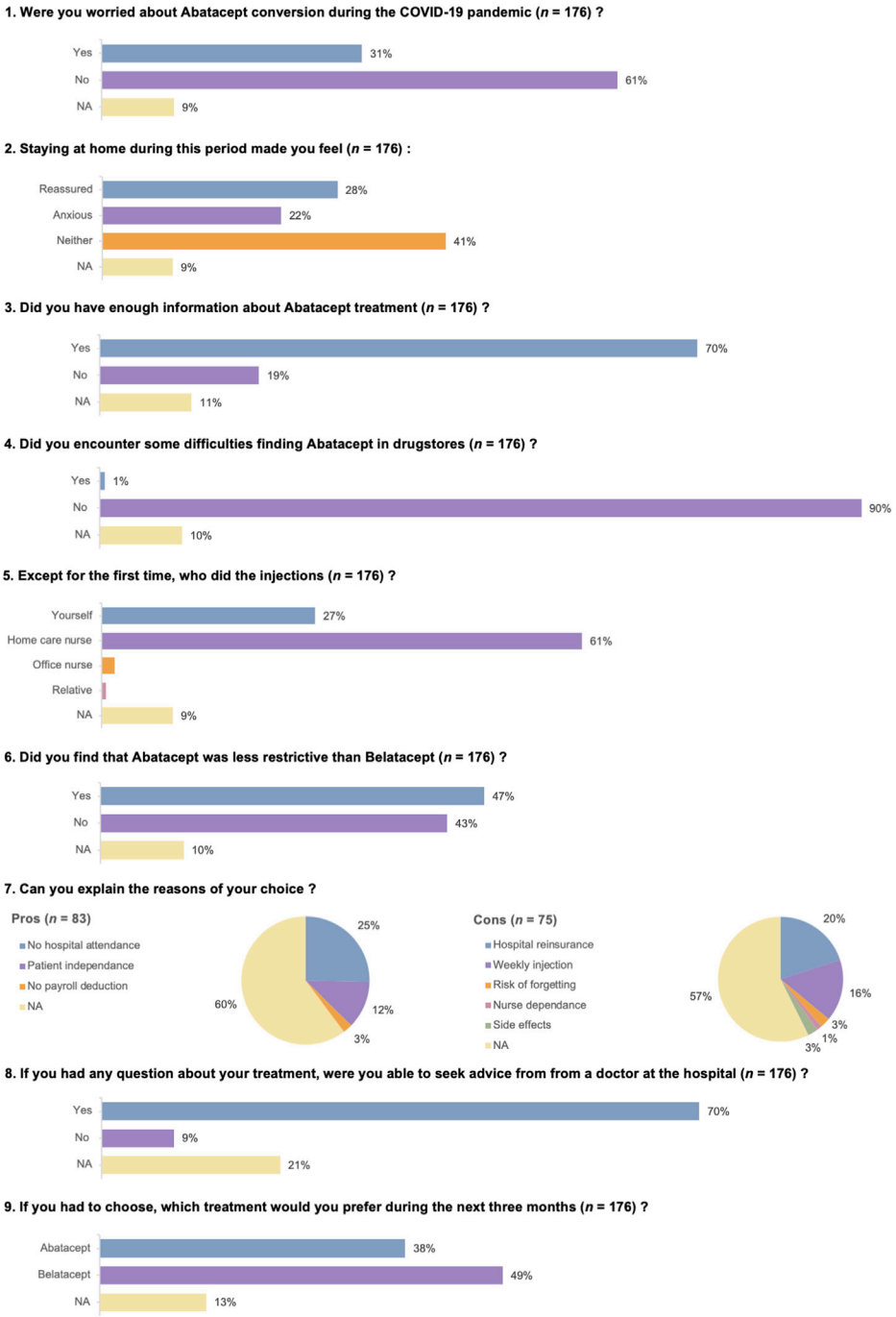


FIGURE 3 | Quality of life survey.

pharmacodynamic measure of costimulation blockade proposed to correlate with inhibition of alloresponses [28]) did not differ between belatacept- and abatacept-treated patients tested after infusion. Although abatacept was originally formulated as an infusion, it is now available in a subcutaneous formulation, which has equal safety and efficiency in rheumatoid arthritis patients [11]. Nevertheless, only one patient was treated with once-weekly subcutaneous injections of abatacept, although this patient was treated under this regimen for 16 months without complications. Very recently, Uro-Coste et al. reported their experience with abatacept injection in 5 KTRs, suggesting that weekly subcutaneous administration of 125 mg abatacept may be an effective alternative to belatacept [29]. The data presented here on the use of subcutaneous injection of abatacept in a large cohort could be very useful for patients with CNI toxicity or intolerance and in need of conversion to belatacept but with poor vascular

access, like many end-stage renal disease patients. This approach could also represent a solution in cases of belatacept shortage. Costimulation blockade with abatacept could potentially have a logistical advantage over belatacept in kidney transplant recipients. Nevertheless, our niche experience over 3 months does not allow us to make definitive assertions as to the potential benefits mentioned above.

Our work has several limitations. The short duration of our follow-up period (3 months) prevents us from drawing a firm conclusion on the risks of rejection and infection in patients treated with abatacept as a maintenance therapy. Nevertheless, the median half-life of belatacept is reported to be 8 days (range: 3.1–11.9) [30], and the very low incidence of TCMR during the weeks following abatacept initiation can be taken into account and is quite reassuring. Because of the ethical issues related to data scarcity on abatacept safety, and the possibility of in-home belatacept infusion, we could not accept the risk of continuing to pursue abatacept therapy once the first wave of SARS-CoV-2 had ended. The absence of a control group receiving ongoing belatacept injection is also problematic. However, our main goal was to avoid frequent hospital visits by immunocompromised KTRs during the initial period of the SARS-CoV-2 pandemic. Under these conditions, we chose to treat as many patients as possible with abatacept. A clinical trial NCT04955366 (https://clinicaltrials.gov/) was developed to answer the question of whether patients can be safely converted from monthly belatacept IV infusions to subcutaneous abatacept injections without a decrease in kidney function. The results of this study will be available in late 2024 or in 2025. In the meantime, the message of our work is not to treat all belatacept-converted patients with subcutaneous abatacept, as reflected by the questionnaire completed by the patients: 49% preferred in-hospital belatacept, and 43% did not find that abatacept was less restrictive than belatacept. Nevertheless, we would like to point out that before the COVID-19 pandemic, all patients on belatacept were receiving infusions in hospital. As a result of these treatment sessions, they were closely monitored on a monthly basis. The pandemic has taken patients away from the hospital, making them very anxious at times and most probably explaining the rather low acceptance rates and the rather high discontinuation rate over this short period. In this context of in-home subcutaneous abatacept injection, close monitoring with, for example, regular blood draws or regular teleconsultation could be reassuring for patients and represent an additional safety measure.

In conclusion, our study demonstrates for the first time, in a large cohort of belatacept-treated KTRs, that once-weekly injection of abatacept, used as a rescue therapy, appears to be feasible, safe, and effective in the short term (3 months). The current context of belatacept shortage makes this report even more important.

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. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MB, RS-S, DA, and DB designed the study; MB, RS-S, and DB collected data; MB and DB analyzed the data; MB and DB wrote the paper; and all authors provided feedback and critical review.

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.

REFERENCES

1. Vincenti F, Rostaing L, Grinyo J, Rice K, Steinberg S, Gaite L, et al. Belatacept and Long-Term Outcomes in Kidney Transplantation. *N Engl J Med* (2016) 374(4):333–43. doi:10.1056/NEJMoa1506027
2. Durrbach A, Pestana JM, Florman S, Del Carmen Rial M, Rostaing L, Kuypers D, et al. Long-Term Outcomes in Belatacept- Versus Cyclosporine-Treated Recipients of Extended Criteria Donor Kidneys: Final Results From BENEFIT-EXT, a Phase III Randomized Study. *Am J Transpl* (2016) 16(11):3192–201. doi:10.1111/ajt.13830
3. Budde K, Prashar R, Haller H, Rial MC, Kamar N, Agarwal A, et al. Conversion From Calcineurin Inhibitor to Belatacept-Based Maintenance Immunosuppression in Renal Transplant Recipients: A Randomized Phase 3b Trial. *J Am Soc Nephrol* (2021) 32:3252–64. doi:10.1681/ASN.2021050628
4. Caillard S, Anglicheau D, Matignon M, Durrbach A, Greze C, Frimat L, et al. An Initial Report From the French SOT COVID Registry Suggests High Mortality Due to COVID-19 in Recipients of Kidney Transplants. *Kidney Int* (2020) 98(6):1549–58. doi:10.1016/j.kint.2020.08.005
5. Caillard S, Chavarot N, Francois H, Matignon M, Greze C, Kamar N, et al. Is COVID-19 Infection More Severe in Kidney Transplant Recipients? *Am J Transpl Off J Am Soc Transpl* (2021) 21(3):1295–303. doi:10.1111/ajt.16424
6. Bertrand D, Chavarot N, Gatault P, Garrouste C, Bouvier N, Grall-Jezequel A, et al. Opportunistic Infections After Conversion to Belatacept in Kidney Transplantation. *Nephrol Dial Transpl* (2020) 35(2):336–45. doi:10.1093/ndt/gfz255
7. Chavarot N, Divard G, Scemla A, Amrouche L, Aubert O, Leruez-Ville M, et al. Increased Incidence and Unusual Presentations of CMV Disease in Kidney Transplant Recipients After Conversion to Belatacept. *Am J Transpl* (2021) 21:2448–58. doi:10.1111/ajt.16430
8. Gouin A, Sberro-Soussan R, Courivaud C, Bertrand D, Del Bello A, Darres A, et al. Conversion From Belatacept to Another Immunosuppressive Regimen in Maintenance Kidney-Transplantation Patients. *Kidney Int Rep* (2020) 5(12):2195–201. doi:10.1016/j.ekir.2020.09.036

9. Kremer JM, Westhovens R, Leon M, Di Giorgio E, Alten R, Steinfeld S, et al. Treatment of Rheumatoid Arthritis by Selective Inhibition of T-Cell Activation With Fusion Protein CTLA4Ig. *N Engl J Med* (2003) 349(20): 1907–15. doi:10.1056/NEJMoa035075
10. Ruperto N, Lovell DJ, Quartier P, Paz E, Rubio-Pérez N, Silva CA, et al. Abatacept in Children With Juvenile Idiopathic Arthritis: A Randomised, Double-Blind, Placebo-Controlled Withdrawal Trial. *Lancet Lond Engl* (2008) 372(9636):383–91. doi:10.1016/S0140-6736(08)60998-8
11. Genovese MC, Covarrubias A, Leon G, Mysler E, Keiserman M, Valente R, et al. Subcutaneous Abatacept Versus Intravenous Abatacept: A Phase IIIb Noninferiority Study in Patients With an Inadequate Response to Methotrexate. *Arthritis Rheum* (2011) 63(10):2854–64. doi:10.1002/art.30463
12. Kirk AD, Harlan DM, Armstrong NN, Davis TA, Dong Y, Gray GS, et al. CTLA4-Ig and Anti-CD40 Ligand Prevent Renal Allograft Rejection in Primates. *Proc Natl Acad Sci U S A* (1997) 94(16):8789–94. doi:10.1073/pnas.94.16.8789
13. Badell IR, Karadkhele GM, Vasanth P, Farris AB, Robertson JM, Larsen CP. Abatacept as Rescue Immunosuppression After Calcineurin Inhibitor Treatment Failure in Renal Transplantation. *Am J Transpl* (2019) 19(8): 2342–9. doi:10.1111/ajt.15319
14. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A New Equation to Estimate Glomerular Filtration Rate. *Ann Intern Med* (2009) 150(9):604–12. doi:10.7326/0003-4819-150-9-200905050-00006
15. Haas M, Loupy A, Lefaucheur C, Roufosse C, Glotz D, Seron D, et al. The Banff 2017 Kidney Meeting Report: Revised Diagnostic Criteria for Chronic Active T Cell-Mediated Rejection, Antibody-Mediated Rejection, and Prospects for Integrative Endpoints for Next-Generation Clinical Trials. *Am J Transpl* (2018) 18(2):293–307. doi:10.1111/ajt.14625
16. Vincenti F, Blancho G, Durrbach A, Grannas G, Grinyó J, Meier-Kriesche HU, et al. Ten-year Outcomes in a Randomized Phase II Study of Kidney Transplant Recipients Administered Belatacept 4-weekly or 8-weekly. *Am J Transpl* (2017) 17(12):3219–27. doi:10.1111/ajt.14452
17. Kamar N, Esposito L, Hebral AL, Guitard J, Del Bello A. Specific Organization for In-Hospital Belatacept Infusion to Avoid Nosocomial Transmission During the SARS-CoV-2 Pandemic. *Am J Transpl* (2020) 20(10):2962–3. doi:10.1111/ajt.16074
18. Bertrand D, Hamzaoui M, Lemée V, Lamulle J, Hanoy M, Laurent C, et al. Antibody and T Cell Response to SARS-CoV-2 Messenger RNA BNT162b2 Vaccine in Kidney Transplant Recipients and Hemodialysis Patients. *J Am Soc Nephrol* (2021) 32:2147–52. doi:10.1681/ASN.2021040480
19. Chavarot N, Ouedrani A, Marion O, Leruez-Ville M, Vilain E, Baaziz M, et al. Poor Anti-SARS-CoV-2 Humoral and T-Cell Responses After 2 Injections of mRNA Vaccine in Kidney Transplant Recipients Treated With Belatacept. *Transplantation* (2021) 105:e94–e95. doi:10.1097/TP.0000000000003784
20. Larsen CP, Pearson TC, Adams AB, Tso P, Shirasugi N, Strobert E, et al. Rational Development of LEA29Y (Belatacept), a High-Affinity Variant of CTLA4-Ig With Potent Immunosuppressive Properties. *Am J Transpl* (2005) 5(3):443–53. doi:10.1111/j.1600-6143.2005.00749.x
21. Levisetti MG, Padrid PA, Szot GL, Mittal N, Meehan SM, Wardrip CL, et al. Immunosuppressive Effects of Human CTLA4Ig in a Non-Human Primate Model of Allogeneic Pancreatic Islet Transplantation. *J Immunol* (1997) 159(11):5187–91. doi:10.4049/jimmunol.159.11.5187
22. Kirk AD. Crossing the Bridge: Large Animal Models in Translational Transplantation Research. *Immunol Rev* (2003) 196:176–96. doi:10.1046/j.1600-065x.2003.00081.x
23. Yu CC, Fornoni A, Weins A, Hakroush S, Maiguel D, Sageshima J, et al. Abatacept in B7-1-Positive Proteinuric Kidney Disease. *N Engl J Med* (2013) 369(25):2416–23. doi:10.1056/NEJMoa1304572
24. Mühlbacher T, Amann K, Mahling M, Nadalin S, Heyne N, Guthoff M. Successful Long-Term Management of Recurrent Focal Segmental Glomerulosclerosis After Kidney Transplantation With Costimulation Blockade. *Clin Kidney J* (2021) 14(6):1691–3. doi:10.1093/ckj/sfaa267
25. Burke GW, Chandar J, Sageshima J, Ortigosa-Goggins M, Amarapurkar P, Mitrofanova A, et al. Benefit of B7-1 Staining and Abatacept for Treatment-Resistant Post-Transplant Focal Segmental Glomerulosclerosis in a Predominantly Pediatric Cohort: Time for a Reappraisal. *Pediatr Nephrol Berl Ger* (2023) 38(1):145–59. doi:10.1007/s00467-022-05549-7
26. Kristensen T, Ivarsen P, Povlsen JV. Unsuccessful Treatment With Abatacept in Recurrent Focal Segmental Glomerulosclerosis After Kidney Transplantation. *Case Rep Nephrol Dial* (2017) 7(1):1–5. doi:10.1159/000454947
27. Delville M, Baye E, Durrbach A, Audard V, Kofman T, Braun L, et al. B7-1 Blockade Does Not Improve Post-Transplant Nephrotic Syndrome Caused by Recurrent FSGS. *J Am Soc Nephrol* (2016) 27(8):2520–7. doi:10.1681/ASN.2015091002
28. Latek R, Fleener C, Lamian V, Kulbokas E, 3rd, Davis PM, Suchard SJ, et al. Assessment of Belatacept-Mediated Costimulation Blockade Through Evaluation of CD80/86-Receptor Saturation. *Transplantation* (2009) 87(6): 926–33. doi:10.1097/TP.0b013e31819b5a58
29. Uro-Coste C, Atenza A, Heng AE, Rouzaire PO, Garrouste C. Abatacept Rescue Therapy in Kidney Transplant Recipients: A Case Series of Five Patients. *Transpl Int* (2022) 35:10681. doi:10.3389/ti.2022.10681
30. Shen J, Townsend R, You X, Shen Y, Zhan P, Zhou Z, et al. Pharmacokinetics, Pharmacodynamics, and Immunogenicity of Belatacept in Adult Kidney Transplant Recipients. *Clin Drug Investig* (2014) 34(2):117–26. doi:10.1007/s40261-013-0153-2

ESOT | Transplant International

ORIGINAL RESEARCH
published: 17 July 2023
doi: 10.3389/ti.2023.11381

Post-Transplant Surveillance and Management of Chronic Active Antibody-Mediated Rejection in Renal Transplant Patients in Europe

Lionel P. E. Rostaing[1], Georg A. Böhmig[2], Ben Gibbons[3] and Muhammed Mahdi Taqi[3]**

[1]Service de Néphrologie, Hémodialyse, Aphérèses et Transplantation Rénale, CHU Grenoble-Alpes, Grenoble, France, [2]Clinical Department of Nephrology and Dialysis, University Clinic for Internal Medicine III, Medical University of Vienna, Vienna, Austria, [3]Bryter Inc., New York, NY, United States

Antibody mediated rejection (ABMR) is the leading cause of immune-related allograft failure following kidney transplantation. Chronic active ABMR (CABMR) typically occurs after one-year post-transplant and is the most common cause of late allograft failure. This study was designed to assess common practices in Europe for post-transplant surveillance 1 year after kidney transplant, as well as the diagnosis and management of CABMR. A 15-minute online survey with 58 multiple choice or open-ended questions was completed by EU transplant nephrologists, transplant surgeons and nephrologists. Survey topics included patient caseloads, post-transplant routine screening and treatment of CABMR. The results indicated that observing clinical measures of graft function form the cornerstone of post-transplant surveillance. This may be suboptimal, leading to late diagnoses and untreatable disease. Indeed, less than half of patients who develop CABMR receive treatment beyond optimization of immune suppression. This is attributable to not only late diagnoses, but also a lack of proven efficacious therapies. Intravenous Immunoglobulin (IVIG), steroid pulse and apheresis are prescribed by the majority to treat CABMR. While biologics can feature as part of treatment, there is no single agent that is being used by more than half of physicians.

Keywords: kidney transplant, antibody-mediated rejection, chronic active, post-transplant surveillance, CABMR

Correspondence:
Lionel P. E. Rostaing
lrostaing@chu-grenoble.fr
Muhammed Mahdi Taqi
mahdi.taqi@bryter-us.com

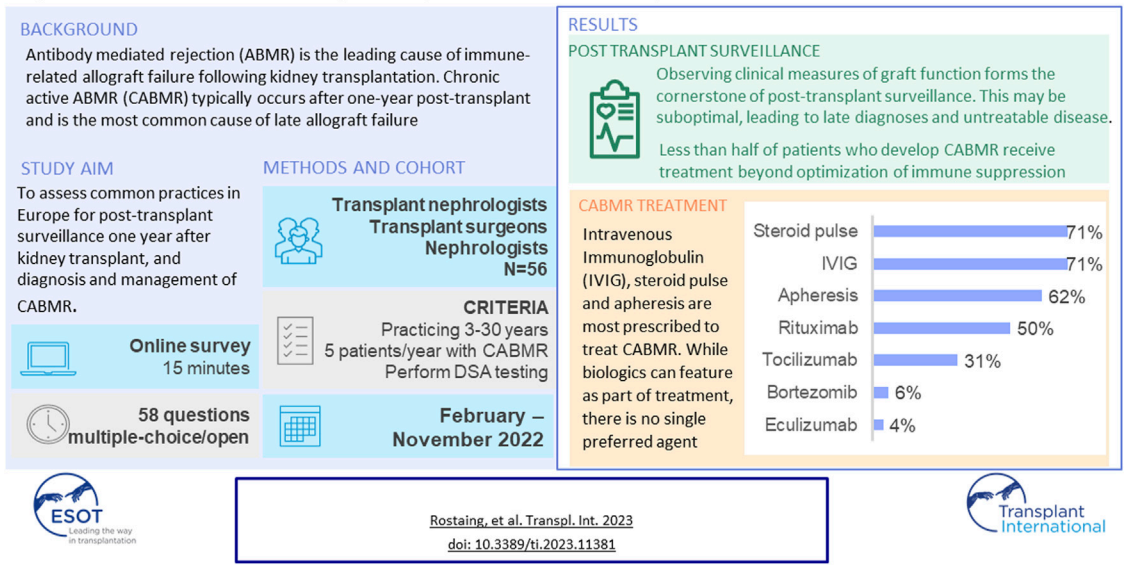
Received: *21 March 2023*
Accepted: *27 June 2023*
Published: *17 July 2023*

Citation:
Rostaing LPE, Böhmig GA, Gibbons B and Taqi MM (2023) Post-Transplant Surveillance and Management of Chronic Active Antibody-Mediated Rejection in Renal Transplant Patients in Europe. Transpl Int 36:11381. doi: 10.3389/ti.2023.11381

INTRODUCTION

Antibody mediated rejection (ABMR) is the leading cause of immune-related allograft failure following kidney transplantation [1, 2]. Although the pathophysiological pathways that give rise to ABMR are yet to be fully elucidated, it is understood that B cell and plasma cell activation leads to generation of donor-specific antibodies (DSAs), which bind to human leukocyte antigen (HLA) or non-HLA molecules expressed on endothelial cells within the kidney allograft [1, 3, 4]. Chronic active antibody mediated rejection (CABMR) typically occurs after one-year post-transplant and manifests as a slower decline in graft function than acute ABMR. Risk for negative outcomes is higher for those who develop CABMR, including graft loss or death [4].

CABMR is characterized by evidence of both chronic disease (interstitial fibrosis, tubular atrophy, transplant glomerulopathy) and active disease components (glomerulitis, peritubular capillaritis) [5]. It is the leading cause of late allograft failure; within 2 years of diagnosis, over 75% of those with CABMR lose their graft [6].



GRAPHICAL ABSTRACT |

Maintenance immunosuppression starting prior to or immediately post-transplant is required in order to prevent immune-related graft injury (including CABMR). Despite maintenance immunosuppression, CABMR continues to be a challenge. The reasons why current immunosuppression protocols fail to prevent the development of CABMR are not yet fully understood, however contributing factors have been identified: patient non-adherence with immunosuppression [2, 7], and insufficient immunosuppression [2].

Post-surveillance monitoring is crucial to ensure optimized maintenance immunosuppression and to detect signs of graft dysfunction. While consensus guidelines on the management of patients post-transplant exist [8], these have not been updated to discuss recent advancements in testing (DSA testing and cell-free DNA testing) and the case for protocol biopsies. Clinical measures of graft function (eGFR, serum creatinine, proteinuria), monitoring DSAs, and biopsies are typically used for surveillance. In recent studies, donor-derived cell-free DNA in peripheral blood has gained interest as a potential non-invasive biomarker following demonstration of ABMR association with serum concentrations of donor-derived cell-free DNA [7].

Treatment options for CABMR are limited beyond optimization of immune suppression. A relative lack of evidence for specific treatments means that there is no current consensus on CABMR management in Europe. IVIG, apheresis and corticosteroids are widely used treatment options. There is also evidence to support the use of biologics. These include B-cell targeting agents (e.g., rituximab [9, 10]), complement targeting agents (e.g., eculizumab), and more recently, agents targeting IL-6 pathways, (e.g., tocilizumab [11]).

This research was designed to assess common practices in Europe for post-transplant monitoring of patients receiving a renal allograft 1 year after transplant. Focus was placed on monitoring for CABMR, and how patients with CABMR are typically managed once diagnosed.

MATERIALS AND METHODS

52 transplant nephrologists, nephrologists and transplant surgeons were recruited by email invitation through targeted lists provided by ESOT, then screened and profiled to ensure a good representation of the European market was achieved. Physicians must have been in practice for 3–30 years, see a minimum of 5 patients/year with CABMR and perform DSA testing post-transplant to qualify. In addition to study-specific screening criteria, respondents were screened to ensure that they are not affiliated with any industry partners. A full breakdown of sample demographics is shown in **Table 1**. Physicians completed a 15-minute online survey with 58 multiple choice or open-ended questions grouped into sections: patient caseloads, post-transplant routine screening, treatment of CABMR and demographic questions.

Data was aggregated and described using the mean and range. In order to determine whether findings were statistically significant, we used *t*-test for parametric data and chi-squared for non-parametric data. A p-value ≤ 0.05 was considered statistically significant.

TABLE 1 | Table showing respondent profile breakdown (N = 52).

		Total (*n* =)
All respondents		52
Specialty	Transplant nephrologist	41
	Nephrologist	9
	Transplant surgeon	2
Gender	Male	29
	Female	20
	Prefer not to say	2
Length of time in practice	Less than 3 years	1
	3–10 years	13
	11–20 years	17
	21+	21
Hospital type	Teaching/university hospital	46
	General hospital	4
	Private hospital	1
Number of renal transplant patients followed up with per year	5–50 patients	6
	51–100 patients	23
	101+ patients	23
Country of practice	Italy	10
	France	9
	Spain	5
	United Kingdom	4
	Netherlands	4
	Germany	3
	Greece	3
	Belgium	2
	Croatia	2
	Poland	2
	Portugal	2
	Austria	1
	Czech Republic	1
	Sweden	1
	Switzerland	1
	Bosnia and Herzegovina	1
	Montenegro	1

RESULTS

Post-Transplant Monitoring in the 1st Year Post-Transplant

Proteinuria and serum creatinine are tested frequently in the first-year post-transplant: 88% assess creatinine and 79% assess proteinuria every 1–3 months, rising to 100% assessing creatinine and proteinuria every 1–6 months.

Frequency of DSA testing was found to vary by patient type. Physicians are more likely to routinely assess pre-sensitized patients for *de novo* DSA (81% of physicians report doing so at least once a year) than patients who are not pre-sensitized (67% assess these patients at least once a year) ($t(55) = -2.00$, $p = 0.03$). 27% and 19% indicate that they do not routinely test for *de novo* DSA in non pre-sensitized and pre-sensitized patients (respectively) after the 1st year post-transplant. The One Lambda Luminex® platform assay is the most used DSA testing method—utilized by 69% of physicians—followed by the Immucor Luminex® platform assay (29%).

Surveillance (protocol) biopsies are not routinely performed by physicians. Only 27% perform them routinely at 6–12 months post-transplant and significantly less perform them routinely after 1 year (15%, $t(51) = -2.58$, $p = 0.05$).

Use of cell free DNA testing is not widespread with only 13% of physicians using the test for a small portion of their patients.

See **Figure 1** for a summary of post-surveillance tests performed in the 1st year post-transplant, and their frequency.

Prevention and Treatment of CABMR

Mycophenolate mofetil (MMF) tacrolimus and glucocorticoids are the maintenance immunosuppressive treatments primarily used to prevent immune-mediated rejection. MMF and tacrolimus are used by the entire sample (100%), and 94% use glucocorticoids (see **Figure 2** for other maintenance treatments used).

Beyond optimization of immune suppression, additional treatment is not received by around half (52%) of all CABMR patients; the reasons why are listed in **Figure 3**. The advanced severity of disease is the primary reason for this (67%). Other factors include: a lack of proven/efficacious therapies (61%), the belief that disease can be controlled with immunosuppression optimization alone (33%), patient refusal of treatment (16%) and disease severity not warranting further treatment (12%). Of the patients who do not receive treatment beyond optimization of immunosuppression, on average 57% achieve adequate disease control.

The current therapies used for the treatment of CABMR are illustrated in **Figure 4**. IVIG, steroid pulse and apheresis are most commonly used with 71%, 71% and 62% respectively using these therapies. Of those prescribing steroid pulse treatment, 92% prescribe between 5 mg/kg and 10 mg/kg. An average of 3 doses (SD = 1.63) are prescribed. Of those prescribing IVIg, 74% of physicians prescribe a dosage of 1 mg/kg or less. An average of 4 doses (SD = 4.13) are prescribed.

Biologics are not used as frequently; rituximab is the most widely used (50% prescribe this treatment), followed by tocilizumab (31%), bortezomib (6%) and eculizumab (4%).

DISCUSSION

Post-Transplant Surveillance

Previously, poorly HLA-matched transplants and transplantation of poorer quality organs [12] were too high risk for transplantation. Recent advancements in transplant science, including preservation and tolerance techniques, are allowing for transplantation of these suboptimal organs. These types of transplants are at greater risk of post-transplant complications [12]; therefore, the need for effective surveillance post-transplant has increased.

Our findings indicate that post-transplant surveillance in Europe centers on clinical measures of graft function (proteinuria and creatinine levels) and, in most cases, testing for *de novo* DSA (dnDSA). Risk factors for graft failure were identified as proteinuria and increased creatinine [13, 14]. Detection of dnDSA is considered both a marker and a contributor of ongoing alloimmunity; this is evidenced by an increased rate of estimated glomerular filtration rate (eGFR)

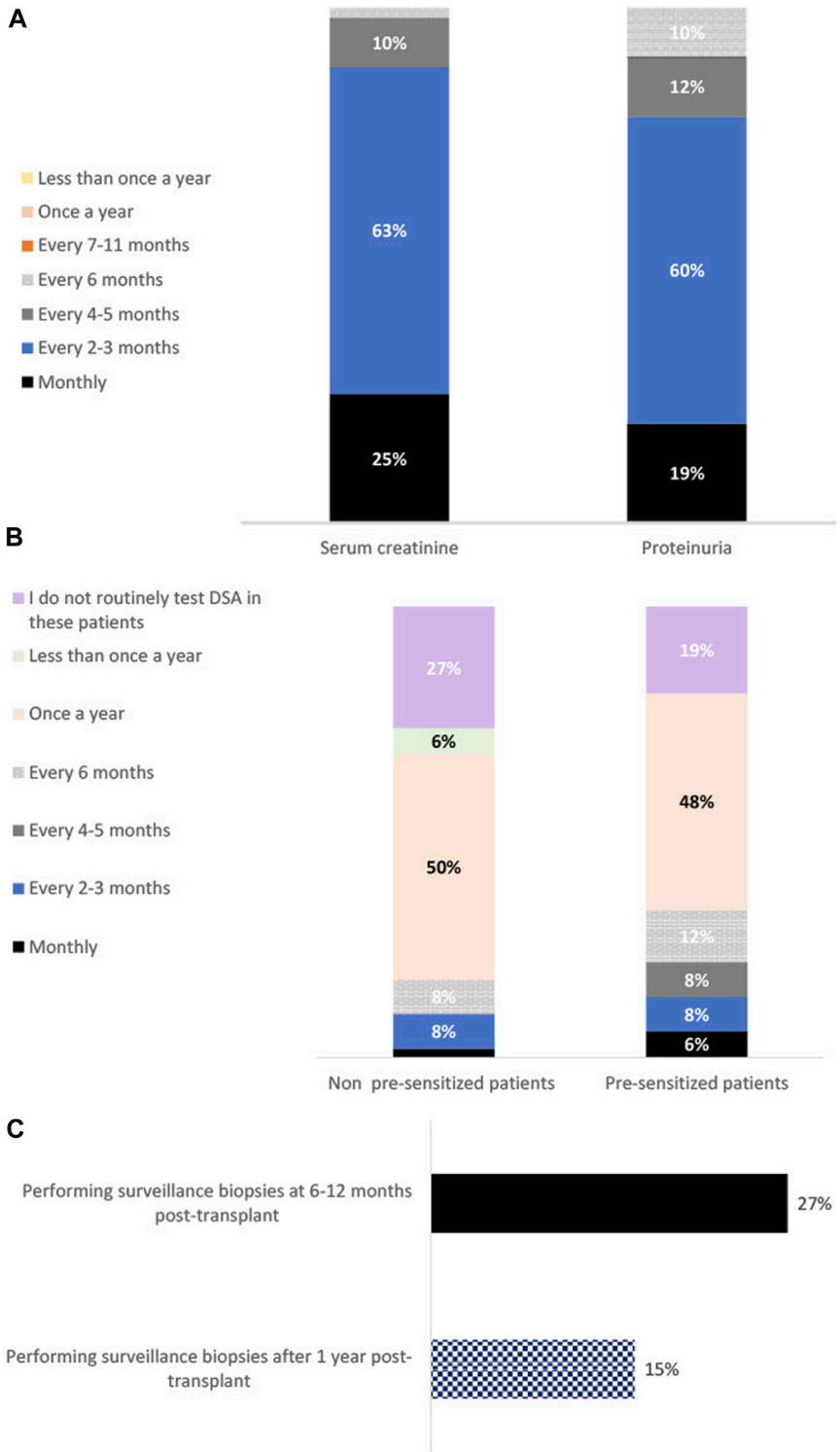


FIGURE 1 | Bar charts showing percentage using each surveillance method 1 year post-transplant, and their frequency of usage. Panel **(A)** shows the percentage of physicians testing for serum creatinine and proteinuria at each time interval (n = 52). Panel **(B)** shows the percentage of physicians carrying out DSA testing at each time interval in patients that are not sensitized (determined by lack of detectable DSA) at transplantation and those that are pre-sensitized at transplantation (n = 52). Panel **(C)** shows the percentage of physicians using surveillance kidney allograft biopsies at 6–12 months post-transplant vs. 1 year post transplant (n = 52).

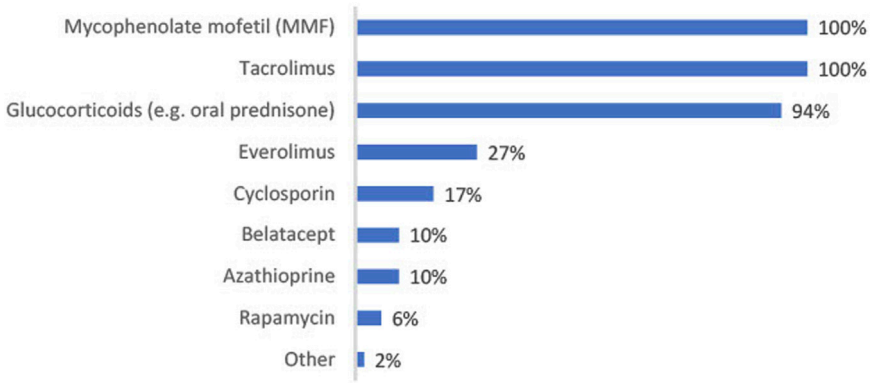


FIGURE 2 | Bar chart showing percentage of physicians using each maintenance immunosuppressive treatment post-transplant (*n* = 52).

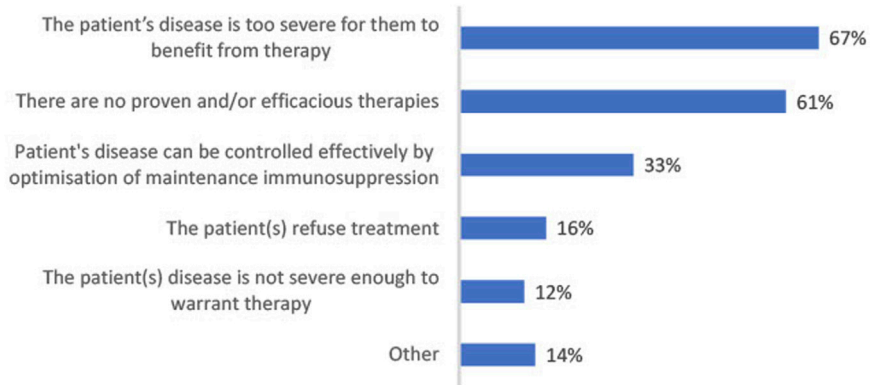


FIGURE 3 | Bar chart showing most frequent reasons for not prescribing further treatment for CABMR beyond optimization of immune suppression given by those who said at least some of their patients receive no treatment except for maintenance immunosuppression (*n* = 49).

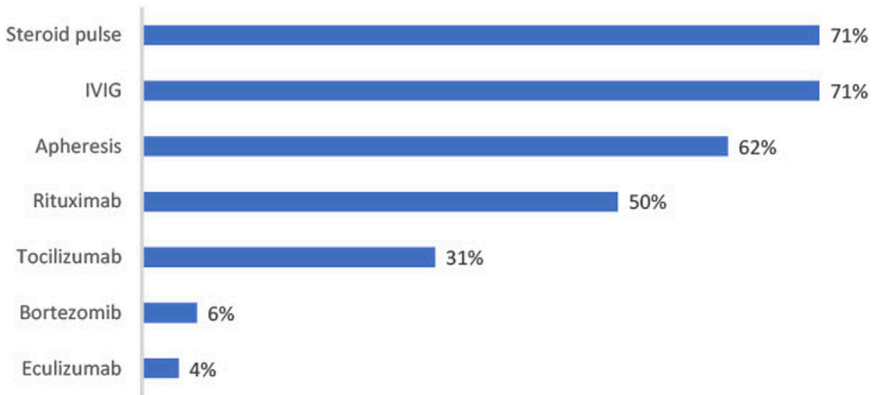


FIGURE 4 | Figure shows percentage of physicians prescribing each treatment for CABMR (*n* = 52).

decline even before the detection of dnDSA, followed by an accelerated decline after detection of dnDSA [15].

In our introduction we alluded to a lack of guidance regarding surveillance assessment of DSA levels; this is reflected in how testing is implemented in clinical practice. Likelihood of testing and frequency of testing is variable after the first-year post-transplant and is influenced by patient type. More physicians are testing sensitized patients than non-sensitized patients. Indeed, those with pre-existing DSAs are at greater risk for CABMR than patients without DSAs at transplantation [4, 16].

Proteinuria, creatinine and DSA testing appear to be the primary methods for detecting CABMR, although this may be suboptimal. Over half of patients do not receive treatment beyond optimized maintenance immunosuppression; in most cases, this is a result of their disease being too severe to benefit from treatment. Proteinuria and creatinine testing only indicate broad graft function and are not sufficient markers to diagnose subclinical antibody mediated rejection or CABMR alone. eGFR, serum creatinine, or proteinuria levels are not noticeably impacted by subclinical ABMR until extensive morphological damage has occurred [17]. An irreversible loss of function may be experienced by patients before they can be treated for any immune-related graft issues [18]. Additionally, the importance of DSA testing is still a topic of debate, as histologic changes consistent with AMR can still be observed in those with no detectable DSA [15, 19].

Currently, biopsies are the most accurate way to evaluate graft health by identifying two main types of lesions: lesions related to tissue damage and function and lesions related to immune suppression. For CABMR, biopsies are the only way to obtain a definitive diagnosis. Additionally, biopsies are the only accurate method of detecting subclinical rejection, which left unaddressed can lead to loss of graft function and/or total graft loss [15, 17]. Protocol biopsies at 3 months post-transplant can improve 5-year transplant survival rates according to recent findings [18].

Despite the unmatched diagnostic value that biopsies provide, physicians may be reluctant to perform them without specific cause. In the present study, surveillance biopsies at 6–12 months post-transplant are routinely performed by only 27% of physicians, decreasing to 15% performing them one-year post-transplant. This reluctance to perform protocol biopsies may be because their risk-benefit is still unclear [18, 20, 21]. A lack of proven treatments in the category may be leading physicians to feel there is no merit in conducting routine invasive procedures.

Donor-derived cell free DNA testing is of increasing interest as a potential biomarker for CABMR [7, 22] due to its non-invasive nature and the potential to facilitate earlier treatment by detecting subclinical allograft injury. Currently, cell free DNA testing is used by only 13% of physicians surveyed. While the efficacy of cell free DNA testing as a biomarker for ABMR continues to be shown by growing evidence [22, 23], it remains to be seen what role it will play in the future of post-transplant surveillance.

Treating CABMR

Plasma exchange, IVIG, and steroids for treatment, with the possible addition of rituximab in the setting of dnDSA, were all recommended in recent consensus guidelines for the treatment of CABMR [24]. Our findings are consistent with these guidelines. IVIG, steroids pulses and apheresis are being prescribed by the majority of physicians. Rituximab is being prescribed by half of physicians, and this might be due to a lack of evidence of efficacy and an increase in risk of pneumonia associated with its usage (when combined with IVIg and steroids vs. IVIg and steroids alone) [25].

Despite some evidence for complement targeting treatments for CABMR, optimal regimens have not yet been identified. The level of improvement seen when using these additional agents has not been clinically significant enough to substantially change the treatment paradigm. Further research is needed to determine whether the benefit seen is patient subtype specific, and to facilitate personalization of treatment protocols.

IL-6 targeting strategies for the treatment of CABMR [11] are receiving growing interest due to the role of IL-6 in inflammatory processes and the maturation and activation of T cells, B cells and plasma cells [11]. There is preliminary evidence to support the use of the IL-6R targeting agent tocilizumab in the desensitization of patients with pre-existing DSA prior to transplant and the treatment of CABMR [26], however further randomized controlled trials are required to support these findings. Our study found that 31% of surveyed physicians are prescribing it in the CABMR setting despite the lack of evidence from randomized and controlled trials.

Ultimately, while physicians appear to be aligned on the usage of steroids, apheresis and IVIg for CABMR, further investigation is required for consensus on biologics. IL-6 targeting agents have potential; clazakizumab, an anti-IL6 targeting agent, was assessed in a recent phase 2 study, and evidence was found for modulation of DSA, stabilization of glomerular filtration rate (GFR), and a manageable safety profile [27]. The phase 3 IMAGINE trial assessing the efficacy and safety of clazakizumab is currently ongoing. The unmet need for proven efficacious therapies could be addressed by positive outcomes.

Study Limitations

A limitation of the present study is that a low proportion of responding physicians are based in high volume centers. Fewer CABMR patients are treated at low volume centers, and physicians employed there have less experience in treating this relatively rare patient type. Subsequently, findings may not be generalizable to how the majority of CABMR patients are treated.

The results may not accurately represent current practices within countries due to the small number of physicians that responded from each country; however, the inclusion of respondents from a range of over 15 European countries provides an understanding of attitudes towards post-transplant surveillance and CABMR treatment across the continent.

CONCLUSION

Clinical measures (proteinuria and creatinine levels) of graft dysfunction and DSA testing are currently the cornerstone of post-transplant surveillance. While there is evidence to support their usage, late diagnoses and consequently poor treatment outcomes may be caused by their inability to detect subclinical signs of rejection. Earlier detection of CABMR could occur through surveillance biopsies, but they are not widely used. Cell-free DNA testing is also still in its infancy. When CABMR develops, around half of patients receive treatment beyond maintenance immunosuppression. This is attributable to late-stage diagnoses but also a lack of effective therapies. IVIG, apheresis and steroids are the main treatments prescribed by physicians to treat CABMR,

with half prescribing rituximab. Other biologics may be prescribed, but a lack of sufficient evidence is likely limiting their usage.

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 authors.

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 participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LR and GB were involved in survey instrument design and finalizing the manuscript. BG and MT were involved in fielding the research and preparing the abstract.

FUNDING

An unrestricted grant from CSL Behring funded this research.

CONFLICT OF INTEREST

MT and BG are full-time employees of Bryter Inc. BG holds shares in Bryter Limited, its parent company.

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.

ACKNOWLEDGMENTS

The authors gratefully acknowledge CSL Behring for providing an unrestricted grant for funding this research. The authors would also like to thank Devi May, Chief Executive Officer of the European Society of Organ Transplantation (ESOT) for her help in facilitating the distribution of the online questionnaire. Operational support in programing and hosting the online questionnaire and processing the survey data was provided by Bryter Limited.

REFERENCES

1. El-Zoghby ZM, Stegall MD, Lager DJ, Kremers WK, Amer H, Gloor JM, et al. Identifying Specific Causes of Kidney Allograft Loss. *Am J Transpl* (2009) 9(3): 527–35. doi:10.1111/j.1600-6143.2008.02519.x
2. Sellarés J, de Freitas DG, Mengel M, Reeve J, Einecke G, Sis B, et al. Understanding the Causes of Kidney Transplant Failure: the Dominant Role of Antibody-Mediated Rejection and Nonadherence. *Am J Transpl* (2012) 12(2):388–99. doi:10.1111/j.1600-6143.2011.03840.x
3. Gaston RS, Cecka JM, Kasiske BL, Fieberg AM, Leduc R, Cosio FC, et al. Evidence for Antibody-Mediated Injury as a Major Determinant of Late Kidney Allograft Failure. *Transplantation* (2010) 90(1):68–74. doi:10.1097/TP.0b013e3181e065de
4. Wiebe C, Gibson IW, Blydt-Hansen TD, Karpinski M, Ho J, Storsley LJ, et al. Evolution and Clinical Pathologic Correlations of De Novo Donor-specific HLA Antibody post Kidney Transplant. *Am J Transpl* (2012) 12(5):1157–67. doi:10.1111/j.1600-6143.2012.04013.x
5. Loupy A, Haas M, Roufosse C, Naesens M, Adam B, Afrouzian M, et al. The Banff 2019 Kidney Meeting Report (I): Updates on and Clarification of Criteria for T Cell- and Antibody-Mediated Rejection. *Am J Transpl* (2020) 20(9): 2318–31. doi:10.1111/ajt.15898
6. Redfield RR, Ellis TM, Zhong W, Scalea JR, Zens TJ, Mandelbrot D, et al. Current Outcomes of Chronic Active Antibody Mediated Rejection - A Large Single center Retrospective Review Using the Updated BANFF 2013 Criteria. *Hum Immunol* (2016) 77(4):346–52. doi:10.1016/j.humimm.2016.01.018
7. Mayer KA, Doberer K, Tillgren A, Viard T, Haindl S, Krivanec S, et al. Diagnostic Value of Donor-Derived Cell-free DNA to Predict Antibody-Mediated Rejection in Donor-specific Antibody-Positive Renal Allograft Recipients. *Transpl Int* (2021) 34:1689–702. doi:10.1111/tri.13970
8. Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO Clinical Practice Guideline for the Care of Kidney Transplant Recipients. *Am J Transpl* (2009) 9(3):S1–155. doi:10.1111/j.1600-6143.2009.02834.x
9. Smith RN, Malik F, Goes N, Farris AB, Zorn E, Saidman S, et al. Partial Therapeutic Response to Rituximab for the Treatment of Chronic Alloantibody Mediated Rejection of Kidney Allografts. *Transpl Immunol* (2012) 27(2-3):107–13. doi:10.1016/j.trim.2012.08.005
10. Kulkarni S, Kirkiles-Smith NC, Deng YH, Formica RN, Moeckel G, Broecker V, et al. Eculizumab Therapy for Chronic Antibody-Mediated Injury in Kidney Transplant Recipients: A Pilot Randomized Controlled Trial. *Am J Transpl* (2017) 17(3):682–91. doi:10.1111/ajt.14001
11. Jordan SC, Ammerman N, Choi J, Kumar S, Huang E, Toyoda M, et al. Interleukin-6: An Important Mediator of Allograft Injury. *Transplantation* (2020) 104(12):2497–506. doi:10.1097/TP.0000000000003249
12. Kato K, Takeuchi A, Akashi K, Eto M. Cyclophosphamide-Induced Tolerance in Allogeneic Transplantation: From Basic Studies to Clinical Application. *Front Immunol* (2020) 10:3138. doi:10.3389/fimmu.2019.03138
13. Schinstock CA, Stegall M, Cosio F. New Insights Regarding Chronic Antibody-Mediated Rejection and its Progression to Transplant Glomerulopathy. *Curr Opin Nephrol Hypertens* (2014) 23(6):611–8. doi:10.1097/MNH.0000000000000070
14. Maraghi E, Rahimi Foroushani A, Younespour S, Rostami Z, Einollahi B, Eshraghian MR, et al. Longitudinal Assessment of Serum Creatinine Levels on Graft Survival after Renal Transplantation: Joint Modeling Approach. *Nephro-urology monthly* (2016) 8(4):e37666. doi:10.5812/numonthly.37666
15. Parajuli S, Joachim E, Alagaramoorthy S, Blazel J, Aziz F, Garg N, et al. Subclinical Antibody-Mediated Rejection after Kidney Transplantation: Treatment Outcomes. *Transplantation* (2019) 103(8):1722–9. doi:10.1097/TP.0000000000002566
16. Aubert O, Loupy A, Hidalgo L, Duong van Huyen JP, Higgins S, Viglietti D, et al. Antibody-Mediated Rejection Due to Preexisting versus De Novo Donor-specific Antibodies in Kidney Allograft Recipients. *J Am Soc Nephrol* (2017)) 28(6):1912–23. doi:10.1681/ASN.2016070797
17. Filippone EJ, Farber JL. The Problem of Subclinical Antibody-Mediated Rejection in Kidney Transplantation. *Transplantation* (2021) 105(6): 1176–87. doi:10.1097/TP.0000000000003543
18. Terrec F, Noble J, Naciri-Bennani H, Malvezzi P, Janbon B, Emprou C, et al. Protocol Biopsies on De Novo Renal-Transplants at 3 Months after Surgery: Impact on 5-Year Transplant Survival. *J Clin Med* (2021) 10(16):3635. doi:10.3390/jcm10163635
19. Sablik KA, Clahsen-van Groningen MC, Looman CWN, Damman J, Roelen DL, van Agteren M, et al. Chronic-active Antibody-Mediated Rejection with or without Donor-specific Antibodies Has Similar Histomorphology and Clinical

Outcome - a Retrospective Study. *Transpl Int* (2018) 31(8):900–8. doi:10.1111/tri.13154
20. Huang Y, Farkash E. Protocol Biopsies: Utility and Limitations. *Adv Chronic Kidney Dis* (2016) 23(5):326–31. doi:10.1053/j.ackd.2016.09.002
21. Tanabe T. The Value of Long-Term Protocol Biopsies after Kidney Transplantation. *Nephrology* (2014) 19:2–5. doi:10.1111/nep.12253
22. Huang E, Sethi S, Peng A, Najjar R, Mirocha J, Haas M, et al. Early Clinical Experience Using Donor-Derived Cell-free DNA to Detect Rejection in Kidney Transplant Recipients. *Am J Transpl* (2019) 19(6):1663–70. doi:10.1111/ajt.15289
23. Thongprayoon C, Vaitla P, Craici IM, Leeaphorn N, Hansrivijit P, Salim SA, et al. The Use of Donor-Derived Cell-free DNA for Assessment of Allograft Rejection and Injury Status. *J Clin Med* (2020) 9(5):1480. doi:10.3390/jcm9051480
24. Schinstock CA, Mannon RB, Budde K, Chong AS, Haas M, Knechtle S, et al. Recommended Treatment for Antibody-Mediated Rejection after Kidney Transplantation: The 2019 Expert Consensus from the Transplantion Society Working Group. *Transplantation* (2020) 104(5):911–22. doi:10.1097/TP.0000000000003095
25. Joachim E, Parajuli S, Swanson KJ, Aziz F, Garg N, Mohamed M, et al. Treatment of Chronic Active Antibody-Mediated Rejection with Pulse Steroids, IVIG, with or without Rituximab Is Associated with Increased Risk of Pneumonia. *Transplant Direct* (2021) 7(1):e644. doi:10.1097/TXD.0000000000001080
26. Cabezas L, Jouve T, Malvezzi P, Janbon B, Giovannini D, Rostaing L, et al. Tocilizumab and Active Antibody-Mediated Rejection in Kidney Transplantation: A Literature Review. *Front Immunol* (2022) 13:839380. doi:10.3389/fimmu.2022.839380
27. Doberer K, Duerr M, Halloran PF, Eskandary F, Budde K, Regele H, et al. A Randomized Clinical Trial of Anti-IL-6 Antibody Clazakizumab in Late Antibody-Mediated Kidney Transplant Rejection. *J Am Soc Nephrol* (2021) 32(3):708–22. doi:10.1681/ASN.2020071106

ESOT | Transplant International

ORIGINAL RESEARCH
published: 14 July 2023
doi: 10.3389/ti.2023.11373

The Independent Effects of Kidney Length and Vascular Plaque on Ten-Year Outcomes of Extended Criteria Donor Kidney Transplants

Bekir Tanriover[1], Darren Stewart[2], Layla Kamal[3], Muhammad Saeed[3], Matthew Cooper[4], Julia Foutz[5], Harrison McGehee[5] and Gaurav Gupta[3]*

[1]Division of Nephrology, University of Arizona, Tucson, AZ, United States, [2]Department of Surgery, New York University Langone Health, New York City, NY, United States, [3]Division of Nephrology, Virginia Commonwealth University, Richmond, VA, United States, [4]Medical College of Wisconsin, Milwaukee, WI, United States, [5]United Network for Organ Sharing, Richmond, VA, United States

The independent effects of deceased donor kidney length and vascular plaque on long-term graft survival are not established. Utilizing DonorNet attachments from 4,480 expanded criteria donors (ECD) recovered between 2008 and 2012 in the United States with at least one kidney biopsied and transplanted, we analyzed the relationship between kidney length and vascular plaques and 10-year hazard of all-cause graft failure (ACGF) using causal inference methods in a Cox regression framework. The composite plaque score (range 0–4) and the presence of any plaque (yes, no) was also analyzed. Kidney length was modeled both categorically (<10, 10–12, >12 cm) as well as numerically, using a restricted cubic spline to capture nonlinearity. Effects of a novel composite plaque score 4 vs. 0 (HR 1.08; 95% CI: 0.96, 1.23) and the presence of any vascular plaque (HR 1.08; 95% CI: 0.98, 1.20) were attenuated after adjustment. Likewise, we identified a potential nonlinear relationship between kidney length and the 10-year hazard of ACGF, however the strength of the relationship was attenuated after adjusting for other donor factors. The independent effects of vascular plaque and kidney length on long-term ECD graft survival were found to be minimal and should not play a significant role in utilization.

Keywords: kidney anatomy, length, vascular plaque, expanded criteria donor, deceased donor

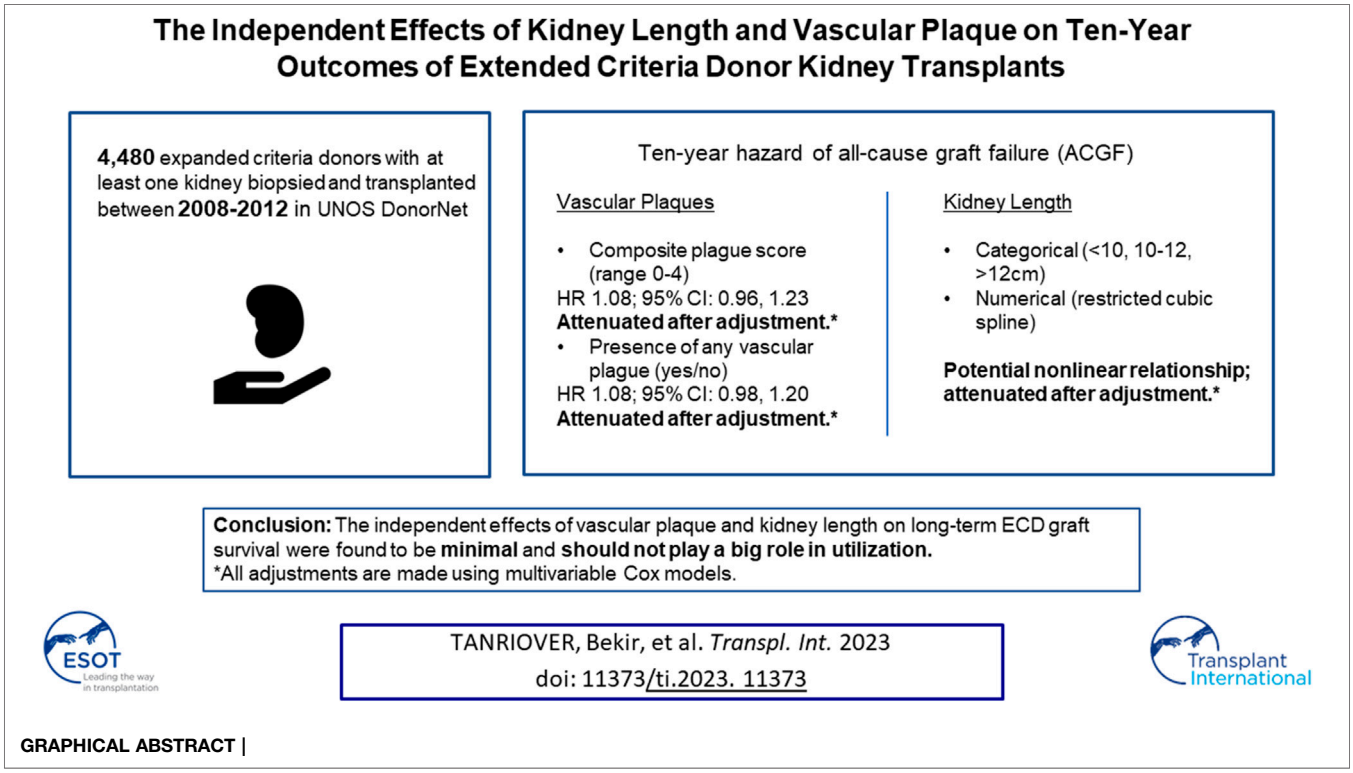
OPEN ACCESS

Correspondence:
Bekir Tanriover
btanriover@arizona.edu

Received: *19 March 2023*
Accepted: *05 July 2023*
Published: *14 July 2023*

Citation:
Tanriover B, Stewart D, Kamal L, Saeed M, Cooper M, Foutz J, McGehee H and Gupta G (2023) The Independent Effects of Kidney Length and Vascular Plaque on Ten-Year Outcomes of Extended Criteria Donor Kidney Transplants. Transpl Int 36:11373. doi: 10.3389/ti.2023.11373

Abbreviations: ACGF, all-cause graft failure; AS, arteriosclerosis; BARETO, Biopsy Anatomy & Resistance Effects on Transplant Outcomes; DGF, delayed graft function; DRR, doubly robust regression; ECD, expanded criteria donors; GS, glomerulosclerosis; IF, interstitial fibrosis; IPW, inverse probability weighting; KDPI, Kidney Donor Profile Index; OPTN, Organ Procurement and Transplantation Network; PNF, primary non-function; Redcap, Research Electronic Data Capture; Rho, Spearman rank correlation coefficient; UNOS, United Network for Organ Sharing.



GRAPHICAL ABSTRACT |

INTRODUCTION

The assessment of deceased donor kidney anatomy (specifically regarding kidney length and vascular plaque) can influence whether kidneys are transplanted or ultimately discarded [1, 2]. Surgical evaluation can provide valuable information regarding kidney size (length and weight) [3, 4], atherosclerosis (vascular plaques affecting aorta and renal artery) [2, 5], anatomical variations (number of donor renal arteries and ureters) [6], injuries, renal tumors, infarcts, thrombosis, scarring, and *ex-vivo* organ perfusion. The surgical appraisal is particularly critical for extended criteria donors (ECD), which have comprised 20.4% of deceased donor pool with an average kidney donor profile index (KDPI) of 85% and a disproportionately high discard rate (exceeding 50%) during the past decade in the United States (U.S.) [7–10].

In initial results from our BARETO (Biopsy, Anatomy & Resistance Effects on Transplant Outcomes) study [11], we reported the independent effects of procurement biopsy findings on long-term renal graft survival in ECD transplants. In the study cohort, across four GS categories (0%–5%, 6%–10%, 11%–15%,16%–20%, 21+%), donor characteristics, as expected, included following noteworthy comorbidities: older age (the mean age range from 59.4 ± 5.8 to 60.2 ± 6.1 years), a relatively high prevalence of hypertension (from 74.7% to 84.9%) and diabetes (15.2%–27.5%), and vascular atherosclerotic plaques (arterial and aortic soft/hard plaques from 52.9% to 62.2% and 87.6%–89.3%, respectively). In another recent study, among 11,795 KDPI>85% kidneys recovered for transplant in the U.S., 56.4% of kidneys (n = 6,214) were discarded, with biopsy findings (mainly glomerulosclerosis [GS], interstitial fibrosis [IF], arteriosclerosis [AS]) (n = 2,747, 44.2%) and unspecified anatomical abnormalities (n = 342, 5.5%) reported as reasons for discard [9, 12]. It is expected that transplant decision-makers regularly face assessments of macroscopic (atherosclerotic soft and hard plaques) and microscopic vasculopathy (AS, GS, IF) in older donors with multiple comorbidities. Aortic and renal arterial plaques may make arterial anastomosis challenging and increase the risk for vascular complications (bleeding, thrombosis, and dissection), limit blood flow by causing luminal stenosis, and can adversely affect long-term renal outcomes [5, 13]. In addition, extrinsic atherosclerosis (as manifested by aortic and/or renal artery plaque formation) can result in progression of chronic kidney disease, and could also represent involvement of renal microvasculature [14].

Aging kidneys, typified in ECDs, undergo anatomical and physiological changes as a part of true renal physiological senescence and common diseases (hypertension, diabetes, obesity, atherosclerosis). These changes increase progressively with age and include nephrosclerosis (comprising AS, GS, IF, tubular atrophy and arterial hyalinosis), decline in number of functional glomeruli and glomerular filtration rate (GFR) [15, 16]. Kidney length and total volume remain stable until very old age (>70 years-old), but renal cortical parenchymal volume could be predisposed to decrease with aging [17], hypertension and atherosclerosis [18], and have important implications for inferior renal transplant outcomes, especially, in the setting of small donor kidney length and volume compared to recipient size [4, 19, 20]. Smaller kidney size (length< 10 cm) is associated with older age (decreased nephron mass due nephrosclerosis),

shorter height, lower BMI, hypertension and atherosclerosis, while larger kidney size (>12 cm) is generally observed in younger donor age, taller height, higher body mass index (BMI> 30 kg/m^2), diabetes (hyperfiltration), and congested kidneys (resulting from tissue injury/edema and poor perfusion during deceased donor recovery) [2].

A recent analysis (an abstract presented at the American Transplant Congress in 2022) studied the relationship between kidney anatomy findings (length, severe arterial plaque, hard plaque, cyst/discoloration, infarcted areas, fat cleaned, and subcapsular hematoma) and kidney utilization in a cohort of adult deceased kidney donors with at least one kidney recovered and relevant DonorNet attachments identified using the Organ Procurement and Transplantation Network (OPTN) database in 2019 (N = 9,433) [21]. In a multivariable logistic regression adjusted for KDPI and biopsy status, they reported an increased odds of discard with presence of severe arterial plaque (odds ratio [OR] 1.63; 95% confidence interval [CI]: 1.03, 2.59) and hard arterial plaque (OR 2.03; 95% CI: 1.48, 2.80). The authors also showed a U-shaped relationship with kidney length and discard (kidney length [OR 0.36; CI: 0.23, 0.56] and kidney length squared [OR 1.04; CI: 1.02, 1.06]).

In this study, we analyzed the relationship between kidney length and vascular plaque (aortic plaque and arterial plaque) reported in attachments uploaded to DonorNet and 10-year hazard of all-cause graft failure. We hypothesized a nonlinear relationship between kidney length and graft failure risk. We also surmised that the presence and type (hard, soft; aortic, arterial) of vascular plaque would be associated with higher graft failure risk.

MATERIALS AND METHODS

We primarily utilized the same study cohort from our previous BARETO study and the materials and method section mirrored those described in the publication [11]. This study used data from the Organ Procurement and Transplantation Network. The OPTN data system includes data on all donors, wait-listed candidates, and transplant recipients in the US, submitted by members of the OPTN. The Health Resources and Services Administration (HRSA), U.S. Department of Health and Human Services, provides oversight to the activities of the OPTN contractor. Data, including DonorNet® attachments, were released to the United Network for Organ Sharing (UNOS) by the OPTN after Institutional Review Board (IRB) approval from Virginia Commonwealth University Ethics Board. The study was therefore been performed in accordance with the ethical standards laid down in an appropriate version of the 2000 Declaration of Helsinki[1] as well as the Declaration of Istanbul 2008[2]. The IRB granted a waiver of consent due to retrospective observational nature of the analysis.

In the United States, when a patient is diagnosed with brain death in a hospital, donor hospitals collaborate with Organ Procurement Organizations (OPOs) in their respective donor service areas (DSAs). There are over 1,000 donor hospitals and 57 OPOs regulated by the Centers for Medicare and Medicaid Services (CMS). OPOs are responsible for tasks such as obtaining consent, transmitting donor data to the United Network for Organ Sharing (UNOS) through a web portal called UNet, procuring organs, and delivering them to transplant centers. Evaluation of deceased donor kidneys is conducted by surgical recovery teams consisting of transplant surgeons and OPO donation coordinators. OPOs use a platform called DonorNet to upload and modify deceased donor information, including anatomy and biopsy data saved as PDFs. However, there are over 25 different forms used by OPOs for kidney anatomy and pathology reporting, leading to potential variability and subjectivity in the assessment process due to different protocols, expertise levels, and available resources among OPOs. Efforts to standardize the process continue.

DonorNet PDF attachments were manually reviewed and biopsy and anatomy findings entered into the Research Electronic Data Capture (REDCap) [22] database according to a protocol (**Supplementary Figure S1**) aligned with the Banff Histopathological Consensus criteria [23] for 4,480 extended criteria donors (ECD) recovered from 2008–2012 with at least one kidney reported as having been biopsied and transplanted. Of these, 3,957 (88.3%) had at least one kidney transplanted, and an anatomy attachment found. Among these transplanted donors, 3,006 (76.0%) had both kidneys transplanted, while 951 (24.0%) had just one kidney transplanted. Since the exposure variables in the broader BARETO study include not only anatomy but also biopsy findings, ECD donors, which we found to be almost universally biopsied (93.2%), were chosen to avoid confounding by indication [24] resulting from for cause biopsies [23].

The three anatomy dimensions reported with high frequency on attachments were aortic plaque (99.7% reported), arterial plaque (99.3% reported), and kidney length (99.5% reported). For kidneys with multiple anatomy attachments (1.0% reported), we chose for analysis the attachment with the fewest missing or unknown data elements among these three variables (aortic plaque, arterial plaque, and kidney length). Due to the high correlation between aortic and arterial plaque (**Supplementary Tables S1, S2**), it was judged infeasible to reliably estimate the independent effect of each type of plaque adjusting for the other. Instead, we created a new exposure variable—the composite plaque score (range 0–4)—by adding the degree of aortic (hard = 2, soft = 1, none = 0) and arterial (hard = 2, soft = 1, none = 0) plaques. The presence of any plaque (yes, no) was also analyzed.

The primary study outcome was all-cause graft failure up to 10 years post-transplant, which was analyzed by the Kaplan-Meier method for aortic plaque, arterial plaque, plaque score, plaque presence, and kidney length. Plaque score, plaque presence, and kidney length were further analyzed using Cox regression and causal inference methods to serve the study's central aim of characterizing the independent associations between these three exposure variables and long-term graft

[1]https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/

[2]http://multivu.prnewswire.com/mnr/transplantationsociety/33914/docs/33914-Declaration_of_Istanbul-Lancet.pdf

survival. Our primary findings were derived using doubly robust regression (DRR) [25], which combines the strengths of propensity-score based inverse probability weighting (IPW) [26] and multiple regression to adjust for potential confounding. DRR weights were based on covariate balancing propensity scores (CBPS) [27]. Unadjusted results, as well as results based on IPW alone and multiple regression alone, are provided for comparison. Following Stensrud and Hernan [28], we interpret the hazard ratio estimates from this study as reflecting the weighted average of the true hazard ratios during the 10 years after transplant.

Statistical inference was derived by bootstrapping the entire DRR process, including single-imputation of missing data using the MICE algorithm [29, 30] (**Supplementary Figure S2**), with 1,000 bootstrap iterations and percentile-based 95% confidence intervals. **Supplementary Tables S1–S4** show the degree of missingness for each covariate. Kidney length was modeled both categorically as well as numerically, using a restricted cubic spline to capture nonlinearity. Pointwise (kidney length of 9 cm, 9.5 cm, 10 cm, . . ., 14 cm) confidence intervals were generated using the bootstrap process.

Potentially confounding covariates were chosen for inclusion by relying on previous literature; subject matter expertise; clinical hypothesis generation; exposure variable vs. covariate correlation analysis; and a philosophy of erring on the side of inclusion while leveraging opportunities for parsimony. A total of seventeen covariates were ultimately included in each set of models: ten donor characteristics; two recipient factors; donor/recipient sex matching; cold ischemic time; pumped (yes/no); kidney length and percent glomerulosclerosis for plaque score and plaque presence; and kidney-specific aortic and arterial plaque for kidney length (**Supplementary Tables S1–S4**).

Numerical and graphical correlation analysis was used to assess the relationship between kidney length and donor height, weight, BMI, and KDPI. We used R Software Version 4.1.0, including WeightIt, cobalt, CBPS, mice, survival, rms, and lme4 packages.

RESULTS

Effect of Aortic Plaque on Outcomes

Unadjusted graft survival based on the Kaplan-Meier method revealed a statistically significant relationship between the degree of aortic plaque (p = 0.002, **Figure 1A**), but not arterial plaque (p = 0.26, **Figure 1B**), and 10-year graft survival. Unadjusted graft survival was significantly lower (p = 0.03) for higher plaque score values in an apparent, albeit weak, dose-response relationship (**Figure 1C**). Similarly, graft survival was significantly lower (p = 0.003) for any plaque compared to no plaque (**Figure 1D**).

Several notable associations were found between plaque score and potentially confounding factors—donor age (p < 0.001), KDPI (p < 0.001), donor BMI (p = 0.03), donor height (p < 0.001), donor gender (p < 0.001), donor race/ethnicity (p < 0.001),

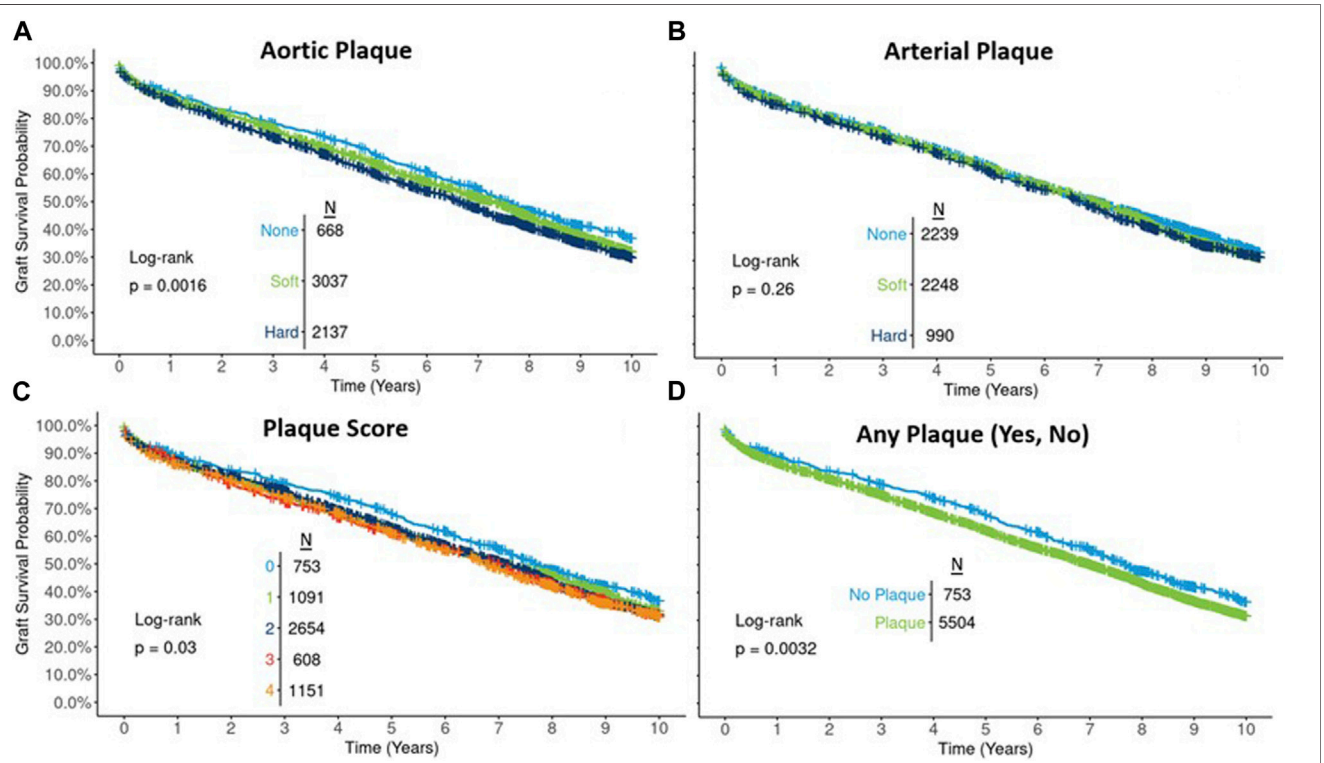


FIGURE 1 | Ten-year Kaplan-Meier graft survival by type of aortic plaque **(A)**, arterial plaque **(B)**, plaque score **(C)**, and presence of any plaque **(D)**.

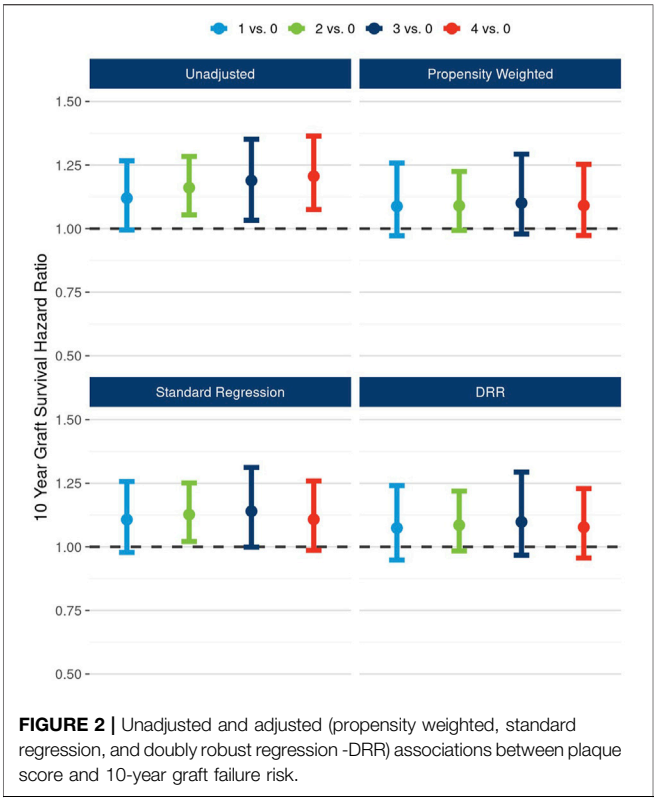


FIGURE 2 | Unadjusted and adjusted (propensity weighted, standard regression, and doubly robust regression -DRR) associations between plaque score and 10-year graft failure risk.

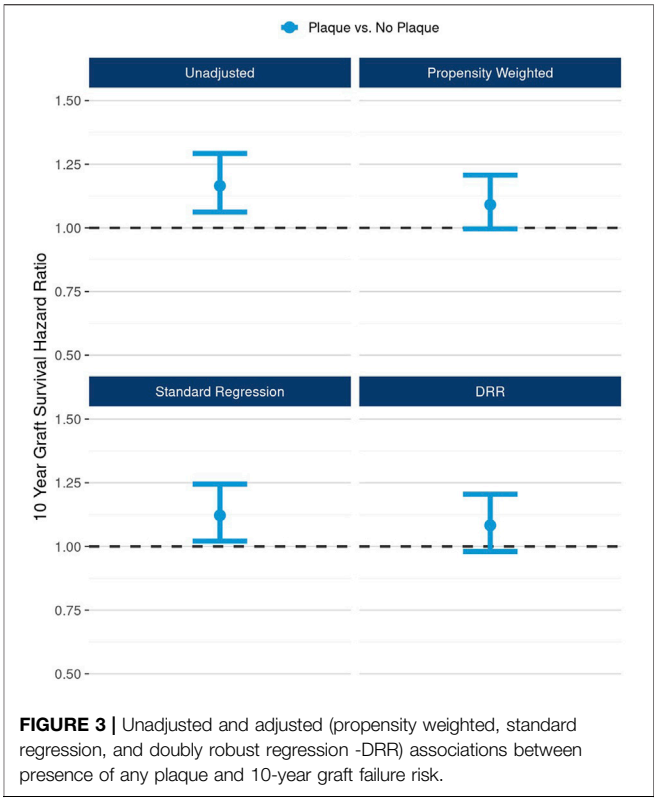


FIGURE 3 | Unadjusted and adjusted (propensity weighted, standard regression, and doubly robust regression -DRR) associations between presence of any plaque and 10-year graft failure risk.

donor hypertension ($p = 0.02$), donor diabetes ($p < 0.001$), kidney length ($p < 0.001$), recipient estimated post-transplant survival (EPTS) ($p < 0.001$), cold ischemic time ($p = 0.001$), pumped ($p < 0.001$), donor-recipient mismatch ($p < 0.001$), and percent glomerulosclerosis ($p < 0.001$) (**Supplementary Table S3**).

In Cox proportional hazards modeling, after accounting for the associations between plaque score and potential confounders, the 10-year hazard of graft failure for plaque scores 4 vs. 0 attenuated and was no longer significant: unadjusted HR 1.21 (95% CI: 1.08, 1.36), DRR-adjusted HR 1.08 (95% CI: 0.96, 1.23). The mild dose-response relationship evident in the unadjusted results was also attenuated in adjusted analyses (**Figure 2**).

Likewise, after accounting for the associations between the presence of any plaque and potential cofounders, the 10-year hazard of graft failure approached but did not reach statistical significance: unadjusted HR 1.17 (95% CI: 1.06, 1.29), DRR-adjusted HR 1.08 (95% CI: 0.98, 1.20) (**Figure 3**).

Effect of Kidney Length on Outcomes

Unadjusted survival curves suggest a possible nonlinear relationship between kidney length and long-term graft survival, as the best outcomes were observed for mid-range (10–12 cm) kidneys. However, this relationship did not reach statistical significance ($p = 0.09$, **Figure 4**). A correlation analysis revealed a moderate to strong positive relationship between kidney length and donor height (Spearman rank correlation coefficient (rho) = 0.34, **Figure 5A**) and donor weight (rho = 0.41, **Figure 5B**) and statistically significant but weak correlations between kidney length and donor BMI (rho = 0.27, **Figure 5C**) and KDPI (rho = −0.14, **Figure 5D**).

In DRR analysis, the hypothesized nonlinear relationship between length and graft survival was still evident, however effects were not statistically significant: ≤10 cm vs.10–12 cm (HR 1.06; 95% CI: 0.98, 1.16), >12 cm vs. 10–12 cm (HR 1.07; 95% CI: 0.97, 1.18) (**Figure 6**). Unadjusted analysis of continuous kidney length modeled nonlinearly revealed a statistically significant increasing hazard as kidney length rose from about 12 cm to 14 cm; however, this pattern was no longer apparent in fully adjusted DRR analysis, suggesting the nonlinear relationship is, if not entirely, explained by correlations with other donor characteristics (**Figure 7**).

Love plots [31] (**Supplementary Figures S3–S5**) indicated highly successful covariate balancing among exposure groups after weighting, with all standardized differences falling near or below 0.1 [32].

DISCUSSION

After rigorous statistical adjustment for confounding, this study has revealed that the associations between 10-year hazard of graft failure and vascular plaque (analyzed through both a composite plaque score and simply presence vs. absence) approached, but did not reach, statistical significance. The residual effect of plaque presence was significant, suggesting that there may very well be a real, albeit modest, effect that our sample sizes just were not large

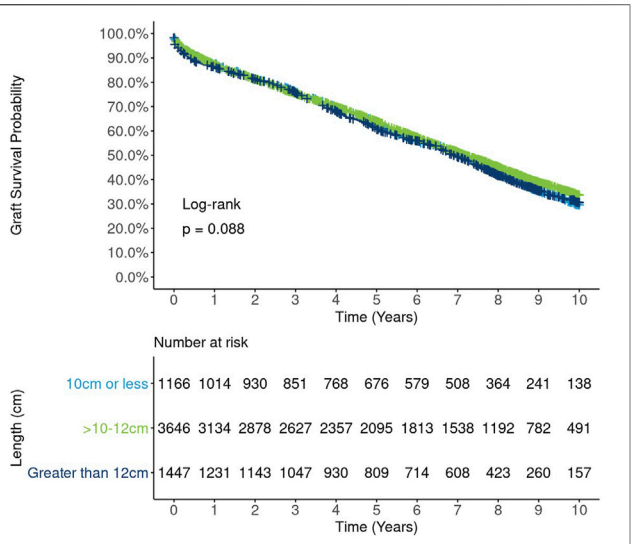


FIGURE 4 | Ten-year Kaplan-Meier graft survival by kidney length.

enough to detect. Still, the key message from this study is that any effect of plaque is small, and thus this parameter should not play a key role in organ utilization decisions. Likewise, the nonlinear relationship that was hypothesized for kidney length and the 10-year hazard of all-cause graft failure indeed manifested, however it is largely or entirely explained by other donor factors, and the residual effects are modest in size and did not reach statistical significance.

The specificity in reporting vascular plaques on the UNOS DonorNet is inadequate. While the descriptions of these plaques are offered in two distinct locations - the aorta and the arteries - and in two types - soft and hard, there is an absence of information regarding their size and extent. This omission hinders differentiation of vascular plaques found in the aortic patch from those located in the renal artery orifice/lumen. In addition, there are currently no objective measurements or standardized scoring system regarding assessment of vascular plaques nor a body of evidence concerning their effects on deceased donor kidney quality and transplant outcomes. Naturally, it is expected that presence of vascular plaque may lead to difficult anastomoses resulting in intra/post-operative complications, such as bleeding and thrombosis, and these plaques can also be interpreted as proxies for intra-renal histological arteriosclerosis.

In one of the few studies published on the topic, Keijback et al. analyzed the data of the kidneys (donor age >50 years old) recovered for transplant (N = 2,610; 2,239 transplanted [85.8%] and 371 discarded [14.2%]) between 2000 and 2015 in the Netherlands as a part of the Eurotransplant system, where renal artery macroscopic arteriosclerosis data were available [5]. Their study revealed that the macroscopic arteriosclerosis commonly occurred, 68% in the transplanted kidneys (none 31%, mild 9%, moderate 46%, massive 13%) and 79% in the

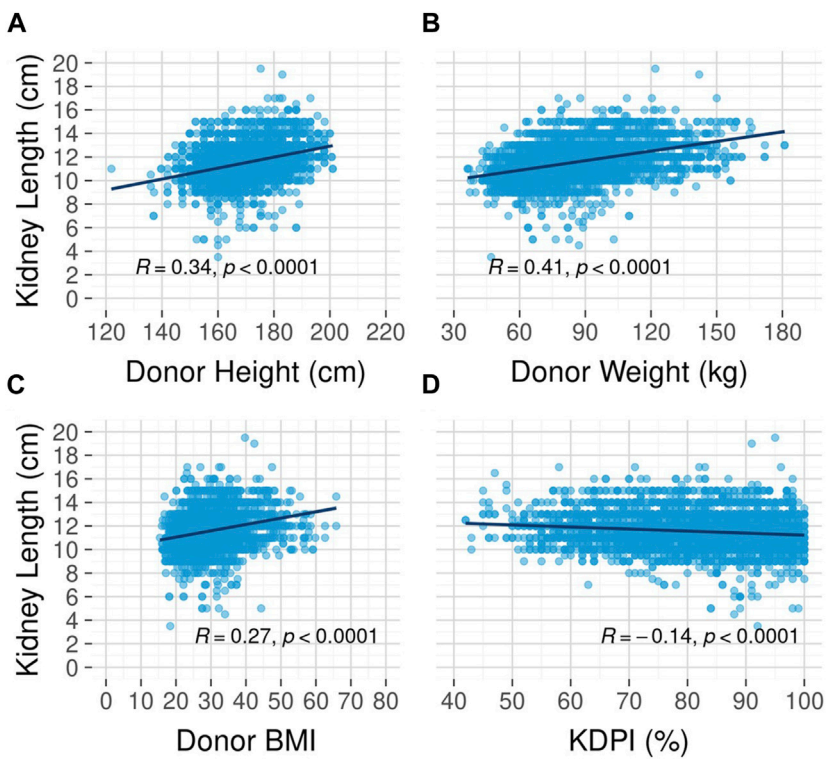


FIGURE 5 | Correlation between kidney length and donor height **(A)**, weight **(B)**, body mass index **(C)**, and kidney donor profile index **(D)**.

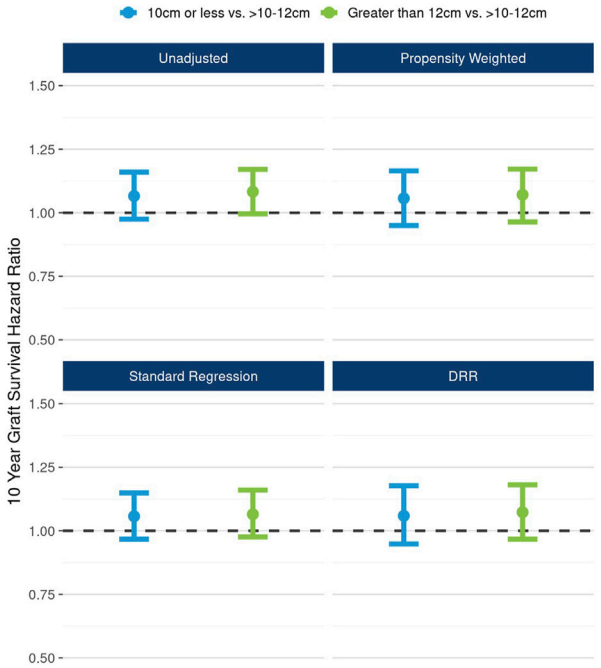


FIGURE 6 | The unadjusted and adjusted (propensity weighted, standard regression, and doubly robust regression -DRR) associations between kidney length and 10-year graft failure risk.

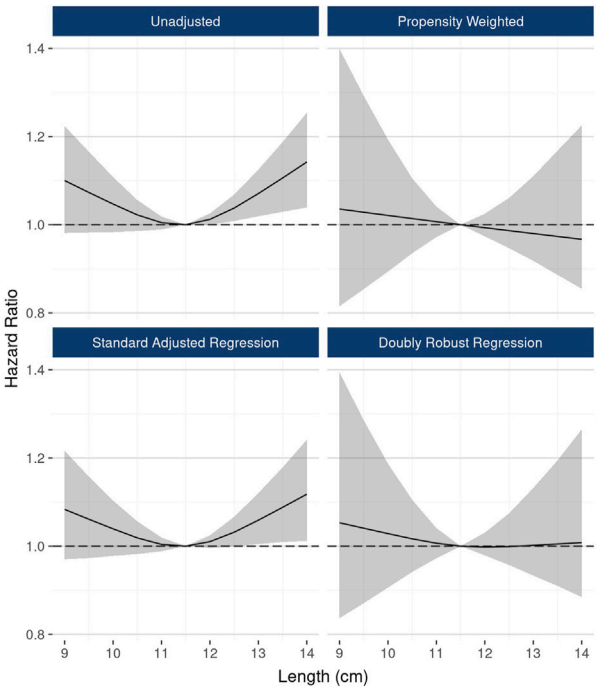


FIGURE 7 | The associations between continuous kidney length and 10-year graft failure risk, modeled as a restricted cubic spline.

discarded kidneys (none 22%, mild 13%, moderate 31%, massive 35%), and increased the risk of discard by 36% (odds ratio [OR], 1.36; 95% confidence interval [CI] 1.02–1.80, *p*-value = 0.03). However, compared to the no vascular lesion category, the macroscopic arteriosclerosis (any degrees) was not associated with delayed graft function (DGF), estimated glomerular filtration rate at 1-year post-transplant, and death censored graft failure during the study period. Early vascular complications leading to graft failure (primary non-function-PNF) among the kidneys with moderate to massive arteriosclerosis were rare and did not differ compared to the kidneys without vascular lesions. These insignificant findings on the outcomes could at least partly be due to small sample size, i.e., insufficient power to detect subtle differences. Among the subgroup of kidney transplant recipients who had a pre-implantation allograft biopsy (n = 109), Keijback et al. showed that there was no correlation between macroscopic renal arteriosclerosis and histological arteriosclerosis (specifically, vascular fibrous intimal thickening and arteriolar hyalinosis). Still, a bias regarding the effect of arteriosclerosis could be introduced in their conclusions because they did not analyze the relationship between macroscopic and microscopic arteriosclerosis correlation among discarded kidneys. In our study, we also observed that the procurement biopsy findings (interstitial fibrosis, vascular changes, and to some extent glomerulosclerosis) did not deteriorate with the presence of higher degree of aortic and arterial plaques (**Supplementary Table S1**). In turn, we cautiously suggest that vascular plaques should not be viewed as surrogate for intra-renal chronic vascular changes and related histological findings.

The presence of vascular hard stenotic plaques may imply a contraindication to deceased donor transplantation. Depending on the availability of the Carrel aortic patch, the location of hard stenotic plaque (involving ostium and extending into renal artery), length of plaque free renal artery (safe anastomosis generally requiring main renal artery 1.5 cm or longer), either resection of a segment of artery/Carrel patch containing plaque (permitting end to side anastomosis in a similar fashion performed in living donor kidney transplantation) or eversion endarterectomy can be successfully performed as a rescue procedure but requires increased technical expertise [33].

Studies analyzing the relationship between kidney length and short/long-term graft outcomes are limited. Tierie et al. conducted a prospective pilot study (N = 166) to predict the effect of systematic procurement surgical assessment (16 donor variables related to kidney temperature, anatomy [length and width], atherosclerosis, perfusion, and overall quality) on short term graft outcomes (DGF or PNF vs. immediate function, 1-year graft failure or eGFR< 50 mL/min/1.73 m^2 vs. functioning graft or eGFR>50 mL/min/1.73 m^2) [2]. In multivariable logistic regression analysis, a larger kidney width (>6 cm) and the poor quality of perfusion (suggestive of congested and edematous kidneys) were associated with DGF/PNF. A larger kidney length (>12 cm), lower first donor creatinine and KDPI predicted a functioning

graft or eGFR>50 mL/min/1.73 m^2 at 1-year. In contrast, our analysis revealed that the kidney length>12 cm was not associated with better long-term graft survival.

Some may have legitimate concern that these donor anatomy parameters are subject to significant measurement errors (kidney length measurement with perinephric fat and lack of kidney volume assessment accounting for the three-dimensional nature of the kidney) and can have subjectivity (lack of length, location, and extension of stenotic hard vascular plaque). However, despite their imperfections, analyzing these parameters' associations with outcomes is meaningful and relevant since they are used in clinical practice to influence kidney utilization decision making. Moreover, OPTN policy requires transplant centers to update their "Kidney Minimum Acceptance" criteria annually in which the kidney anatomy section includes questions regarding both vascular plaque (considering a kidney from a donor with soft or hard plaque in the renal artery described as mild, moderate, severe) and kidney length (considering a donor kidney that is 2 or more centimeters smaller that the kidney on the opposite site) [34]. Despite known limitations, semi-quantitative assessment is a commonly applied, accepted and undisputable part of the kidney allocation system, as encountered with procurement kidney biopsy reporting, the Banff Histopathological Consensus Criteria for preimplantation kidney biopsies similarly classify IF, tubular atrophy, AS, arteriolar hyalinosis, acute tubular necrosis findings in four categories (none, mild, moderate, and severe).

Evaluating kidney size is a multifaceted process, and it would be erroneous to base it entirely on the measurement of bipolar length for an accurate estimation of kidney volume. One must bear in mind that a kidney of lesser length might compensate with a greater width, hence maintaining a similar overall volume and nearly equivalent split function. Therefore, when sizing a kidney, it's essential to take into account the substantial disparity in sizes between the two kidneys from the same donor, rather than concentrating exclusively on their total length. This consideration could potentially clarify the observed absence of correlation between length and our outcome of interest.

Ensuring compatibility between the donor kidney size and the metabolic demand of the recipient is vital in kidney transplantation. While there are general guidelines in place, individual factors also play a significant role. Elements like body size, age, and overall health status ought to be considered during the evaluation of kidney size. For instance, a small kidney may not suffice for a large, young recipient due to inadequate kidney function. Conversely, the same small kidney might be appropriate for an older recipient with a reduced body size and metabolic requirement. By taking into account these factors, the transplanted kidney's capacity to meet the recipient's needs can be maximized, thereby enhancing the likelihood of a successful transplant outcome.

Our study has strengths and limitations. Using national registry data provided large sample sizes for increased statistical power. We also applied rigorous causal inference methods adjusting for numerous potential confounders. Utilizing DonorNet attachments reflects the real-world framework. Our use of a 2008–2012 cohort allowed us to analyze the effects of kidney length and macroscopic/microscopic vascular disease on 10-year hazard of all-cause graft failure, a meaningful outcome to patients. Even so, it is plausible that unmeasured variables and selection bias related to kidney utilization (transplant vs. discard) may affect the results. Smaller sample sizes for the most extreme values of the three renal anatomy dimensions could have decreased statistical power. The reported data on luminal narrowing in renal artery was not specific (arterial plaque <50%, >50% or circumferential not quantified). Aortic plaque usually involves the distal aorta but sometimes can involve aorta at origin of renal arteries. Presence of aortic plaque at renal artery orifice and its' extension into renal hilum are also not available. Plaque assessment is a subjective and can vary between surgeons based on experience which may introduce a selection bias. Lastly, we analyzed the effect of individual kidney length measurement on the outcome but not the effect of significant length asymmetry between two mate kidneys.

Despite these limitations, our data suggest that any effect of vascular plaques on the 10-year hazard of all-cause graft failure is small, which should justify a diminished influence on decision-making regarding organ utilization. Secondly, vascular plaques should not be viewed as surrogate for intra-renal chronic vascular histological findings. Finally, though a nonlinear relationship between kidney length and long-term outcomes is evident, it is explained by other pre-measured and reported donor factors and thus should not be 'double-counted' when weighing factors in organ acceptance decisions.

Carefully quantifying the independent effects of prognostic parameters on outcomes meaningful to patients and their providers has the potential to improve transplant decision-making and organ utilization. Standardized OPTN data collection on renal anatomy data may improve decision-making and allow for more robust future analyses, like what the OPTN has in the works for biopsy findings like standardized forms and electronic data capture.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The data are not publicly available due to privacy or ethical restrictions. Requests to access these datasets should be directed to GG, gaurav.gupta@vcuhealth.org.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Virginia Commonwealth University. The IRB granted a waiver of consent due to the retrospective observational nature of the analysis.

AUTHOR CONTRIBUTIONS

BT, MS, MC, and LK participated in the writing of manuscript. GG participated in research design and the writing of the manuscript. DS, JF, and HM participated in research design, the performance of the research, data analysis and in the writing of manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by a grant from the Mendez National institute of Transplantation Foundation.

CONFLICT OF INTEREST

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

ACKNOWLEDGMENTS

The data reported here have been supplied by UNOS as the contractor for the Organ Procurement and Transplantation Network (OPTN). The interpretation and reporting of these 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 U.S. Government. We are also grateful for these contributions: Virginia Commonwealth University students Perray Saravanene, Rym Yusfi, Shirley Yu, Charmy Patel, Ohm Tripathi, and Farhan Rasheed entered biopsy and anatomy data into REDCap; Duke University Sociology Professor Steve Vaisey provided valuable guidance along the way on doubly robust regression; Noah Greifer of Johns Hopkins provided troubleshooting assistance with the R. WeightIt package.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11373/full#supplementary-material

REFERENCES

1. Dare AJ, Pettigrew GJ, Saeb-Parsy K. Preoperative Assessment of the Deceased-Donor Kidney: from Macroscopic Appearance to Molecular Biomarkers. *Transplantation* (2014) 97(8):797–807. doi:10.1097/01.TP.0000441361.34103.53
2. Tierie EL, Roodnat JI, Dor F. Systematic Surgical Assessment of Deceased-Donor Kidneys as a Predictor of Short-Term Transplant Outcomes. *Eur Surg Res* (2019) 60(3-4):97–105. doi:10.1159/000501602
3. Tan JC, Paik J, Chertow GM, Grumet FC, Busque S, Lapasia J, et al. Validity of Surrogate Measures for Functional Nephron Mass. *Transplantation* (2011) 92(12):1335–41. doi:10.1097/TP.0b013e31823705ef
4. Tanriover B, Fernandez S, Campenot ES, Newhouse JH, Oyfe I, Mohan P, et al. Live Donor Renal Anatomic Asymmetry and Posttransplant Renal Function. *Transplantation* (2015) 99(8):e66–74. doi:10.1097/TP.0000000000000599
5. Keijbeck A, Veenstra R, Pol RA, Konijn C, Jansen N, van Goor H, et al. The Association between Macroscopic Arteriosclerosis of the Renal Artery, Microscopic Arteriosclerosis, Organ Discard, and Kidney Transplant Outcome. *Transplantation* (2020) 104(12):2567–74. doi:10.1097/TP.0000000000003189
6. Husain SA, King KL, Robbins-Juarez S, Adler JT, McCune KR, Mohan S. Number of Donor Renal Arteries and Early Outcomes after Deceased Donor Kidney Transplantation. *Kidney360* (2021) 2(11):1819–26. doi:10.34067/KID.0005152021
7. Tanriover B, Mohan S, Cohen DJ, Radhakrishnan J, Nickolas TL, Stone PW, et al. Kidneys at Higher Risk of Discard: Expanding the Role of Dual Kidney Transplantation. *Am J Transpl* (2014) 14(2):404–15. doi:10.1111/ajt.12553
8. Stewart DE, Garcia VC, Rosendale JD, Klassen DK, Carrico BJ. Diagnosing the Decades-Long Rise in the Deceased Donor Kidney Discard Rate in the United States. *Transplantation* (2017) 101(3):575–87. doi:10.1097/TP.0000000000001539
9. Ozer Y, Kaplan S, Sandikci B, Gupta G, Tanriover B. Increased Rates of Kidney Discard in the Era of COVID-19 and Recent KAS Policy Implementation. *Transplantation* (2022) 106:e503–e506. doi:10.1097/TP.0000000000004321
10. Cecka JM, Gritsch HA. Why Are Nearly Half of Expanded Criteria Donor (ECD) Kidneys Not Transplanted? *Am J Transpl* (2008) 8(4):735–6. doi:10.1111/j.1600-6143.2007.02071.x
11. Stewart DEFJ, Kamal L, Weiss S, McGehee HS, Cooper M, Gupta G The Independent Effects of Procurement Biopsy Findings on 10-year Outcomes of Extended Criteria Donor Kidney Transplants. *KI Rep* (2022) 7(8):1850–65. doi:10.1016/j.ekir.2022.05.027
12. Reese PP, Harhay MN, Abt PL, Levine MH, Halpern SD. New Solutions to Reduce Discard of Kidneys Donated for Transplantation. *J Am Soc Nephrol* (2016) 27(4):973–80. doi:10.1681/ASN.2015010023
13. Woestenburg A, Sennesael J, Bosmans JL, Verbeelen D. Vasculopathy in the Kidney Allograft at Time of Transplantation: Impact on Later Function of the Graft. *Transplantation* (2008) 85(7):S10–8. doi:10.1097/TP.0b013e318169c311
14. Kon V, Linton MF, Fazio S. Atherosclerosis in Chronic Kidney Disease: the Role of Macrophages. *Nat Rev Nephrol* (2011) 7(1):45–54. doi:10.1038/nrneph.2010.157
15. Glassock RJ, Rule AD. The Implications of Anatomical and Functional Changes of the Aging Kidney: with an Emphasis on the Glomeruli. *Kidney Int* (2012) 82(3):270–7. doi:10.1038/ki.2012.65
16. Denic A, Mathew J, Lerman LO, Lieske JC, Larson JJ, Alexander MP, et al. Single-Nephron Glomerular Filtration Rate in Healthy Adults. *N Engl J Med* (2017) 376(24):2349–57. doi:10.1056/NEJMoa1614329
17. Piras D, Masala M, Delitala A, Urru SAM, Curreli N, Balaci L, et al. Kidney Size in Relation to Ageing, Gender, Renal Function, Birthweight and Chronic Kidney Disease Risk Factors in a General Population. *Nephrol Dial Transpl* (2020) 35(4):640–7. doi:10.1093/ndt/gfy270
18. Paivansalo MJ, Merikanto J, Savolainen MJ, Lilja M, Rantala AO, Kauma H, et al. Effect of Hypertension, Diabetes and Other Cardiovascular Risk Factors on Kidney Size in Middle-Aged Adults. *Clin Nephrol* (1998) 50(3):161–8.
19. Molnar MZ, Streja E, Kovesdy CP, Shah A, Huang E, Bunnapradist S, et al. Age and the Associations of Living Donor and Expanded Criteria Donor Kidneys with Kidney Transplant Outcomes. *Am J Kidney Dis* (2012) 59(6):841–8. doi:10.1053/j.ajkd.2011.12.014
20. Lepeytre F, Delmas-Frenette C, Zhang X, Lariviere-Beaudoin S, Sapir-Pichhadze R, Foster BJ, et al. Donor Age, Donor-Recipient Size Mismatch, and Kidney Graft Survival. *Clin J Am Soc Nephrol* (2020) 15(10):1455–63. doi:10.2215/CJN.02310220
21. Placona AMMC, McCharen K, Shean B, Stuart M. The Association between Renal Anatomy Data and Kidney Utilization [abstract]. *Am J Transpl* (2022) 22(3). Available at: https://atcmeetingabstracts.com/abstract/the-association-between-renal-anatomy-data-and-kidney-utilization/ (Accessed October 7, 2022).
22. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research Electronic Data Capture (REDCap)-Aa Metadata-Driven Methodology

and Workflow Process for Providing Translational Research Informatics Support. *J Biomed Inform* (2009) 42(2):377–81. doi:10.1016/j.jbi.2008.08.010

23. Liapis H, Gaut JP, Klein C, Bagnasco S, Kraus E, Farris AB, 3rd, et al. Banff Histopathological Consensus Criteria for Preimplantation Kidney Biopsies. *Am J Transpl* (2017) 17(1):140–50. doi:10.1111/ajt.13929
24. Kyriacou DN, Lewis RJ. Confounding by Indication in Clinical Research. *JAMA* (2016) 316(17):1818–9. doi:10.1001/jama.2016.16435
25. Funk MJ, Westreich D, Wiesen C, Sturmer T, Brookhart MA, Davidian M. Doubly Robust Estimation of Causal Effects. *Am J Epidemiol* (2011) 173(7):761–7. doi:10.1093/aje/kwq439
26. Austin PC, Stuart EA. Moving towards Best Practice when Using Inverse Probability of Treatment Weighting (IPTW) Using the Propensity Score to Estimate Causal Treatment Effects in Observational Studies. *Stat Med* (2015) 34(28):3661–79. doi:10.1002/sim.6607
27. Imai K, Ratkovic M. Covariate Balancing Propensity Score. *J R Stat Soc Ser B (Statistical Methodology)* (2014) 76(1):243–63. doi:10.1111/rssb.12027
28. Stensrud MJ, Hernan MA. Why Test for Proportional Hazards? *JAMA* (2020) 323(14):1401–2. doi:10.1001/jama.2020.1267
29. Shao J, Sitter RR. Bootstrap for Imputed Survey Data. *J Am Stat Assoc* (1996) 91(435):1278–88. doi:10.1080/01621459.1996.10476997
30. White IR, Royston P, Wood AM. Multiple Imputation Using Chained Equations: Issues and Guidance for Practice. *Stat Med* (2011) 30(4):377–99. doi:10.1002/sim.4067
31. Greifer N *Covariate Balance Tables and Plots: a Guide to the Cobalt Package* (2020). Available at: https://cran.r-project.org/web/packages/cobalt/vignettes/cobalt.html (Accessed October 10, 2020).
32. Austin PC. Balance Diagnostics for Comparing the Distribution of Baseline Covariates between Treatment Groups in Propensity-score Matched Samples. *Stat Med* (2009) 28(25):3083–107. doi:10.1002/sim.3697
33. Khan MA, El-Hennawy H, Jones KC, Harriman D, Farney AC, Rogers J, et al. Eversion Endarterectomy of the Deceased Donor Renal Artery to Prevent Kidney Discard. *Clin Transpl* (2018) 32(6):e13275. doi:10.1111/ctr.13275
34. Narvaez JRF, Nie J, Noyes K, Leeman M, Kayler LK. Hard-to-place Kidney Offers: Donor- and System-Level Predictors of Discard. *Am J Transpl* (2018) 18(11):2708–18. doi:10.1111/ajt.14712

ESOT | Transplant International

ORIGINAL RESEARCH
published: 26 July 2023
doi: 10.3389/ti.2023.11306

Evidence for Alloimmune Sinusoidal Injury in *De Novo* Nodular Regenerative Hyperplasia After Liver Transplantation

Mylène Sebagh[1,2,3]*, *Funda Yilmaz*[4], *Ilias Kounis*[2,3,5], *Faouzi Saliba*[2,3,5], *Cyrille Feray*[2,3,5], *Jean-Luc Taupin*[6], *Daniel Cherqui*[2,3,5], *Daniel Azoulay*[2,3,5], *Didier Samuel*[2,3,5], *Audrey Coilly*[2,3,5], *Antony-Jake Demetris*[7] *and Desley Neil*[8]

[1]*Laboratoire d'Anatomopathologie, AP-HP Hôpital Paul-Brousse, Villejuif, France,* [2]*Inserm, Unité 1193, Université Paris-Saclay, Villejuif, France,* [3]*Université Paris-Saclay, Villejuif, France,* [4]*Ege University Organ Transplantation Center, Department of Pathology, School of Medicine, Ege University, Bornova, Izmir, Türkiye,* [5]*Centre Hépato-Biliaire, AP-HP Hôpital Paul-Brousse, Villejuif, France,* [6]*Département d'Immunologie and d'Histocompatibilité, AP-HP Hôpital Saint-Louis, Paris, France,* [7]*Division of Transplantation, Medical Center, University of Pittsburgh, Pittsburgh, PA, United States,* [8]*Cellular Pathology, Queen Elizabeth Hospital, Birmingham, United Kingdom*

Posttransplant nodular regenerative hyperplasia (NRH) mostly remains unexplained. Microvascular injury due to antibody-mediated rejection (AMR) is suspected, but lack of donor specific antibody (DSA) testing makes it difficult to prove. Centered around a 1-year period of routine DSA testing, concomitant protocol, and indicated posttransplant liver biopsies (LB), recipients with NRH (n = 18) were compared with a matched control group (n = 36). All index, previous, and subsequent LB were reviewed. Both groups were similar in terms of demographics, timing of index LB, and DSA. In the index LB, the NRH group had higher sinusoidal C4d positivity (p = 0.029) and perisinusoidal fibrosis (p = 0.034), both independently associated with NRH (p = 0.038 and 0.050, respectively). Features of "possible" chronic AMR were detected in 28.5% of the NRH group without a known cause and 0% of the control group (p = 0.009). The NRH group had more preceding indicated LB with increased incidence of rejection and biliary obstruction pattern. In the follow-up histology, overall, sinusoidal and portal C4d positivity, sinusoidal microvasculitis, and perisinusoidal fibrosis were also higher (all $p < 0.050$). In conclusion, we provide evidence towards the hypothesis that some cases of posttransplant NRH are related to preceding active and persistent AMR. Large multicenter studies with protocol DSA testing are required to confirm.

Keywords: liver transplantation, nodular regenerative hyperplasia, Banff criteria, chronic antibody-mediated rejection, pathology, C4d

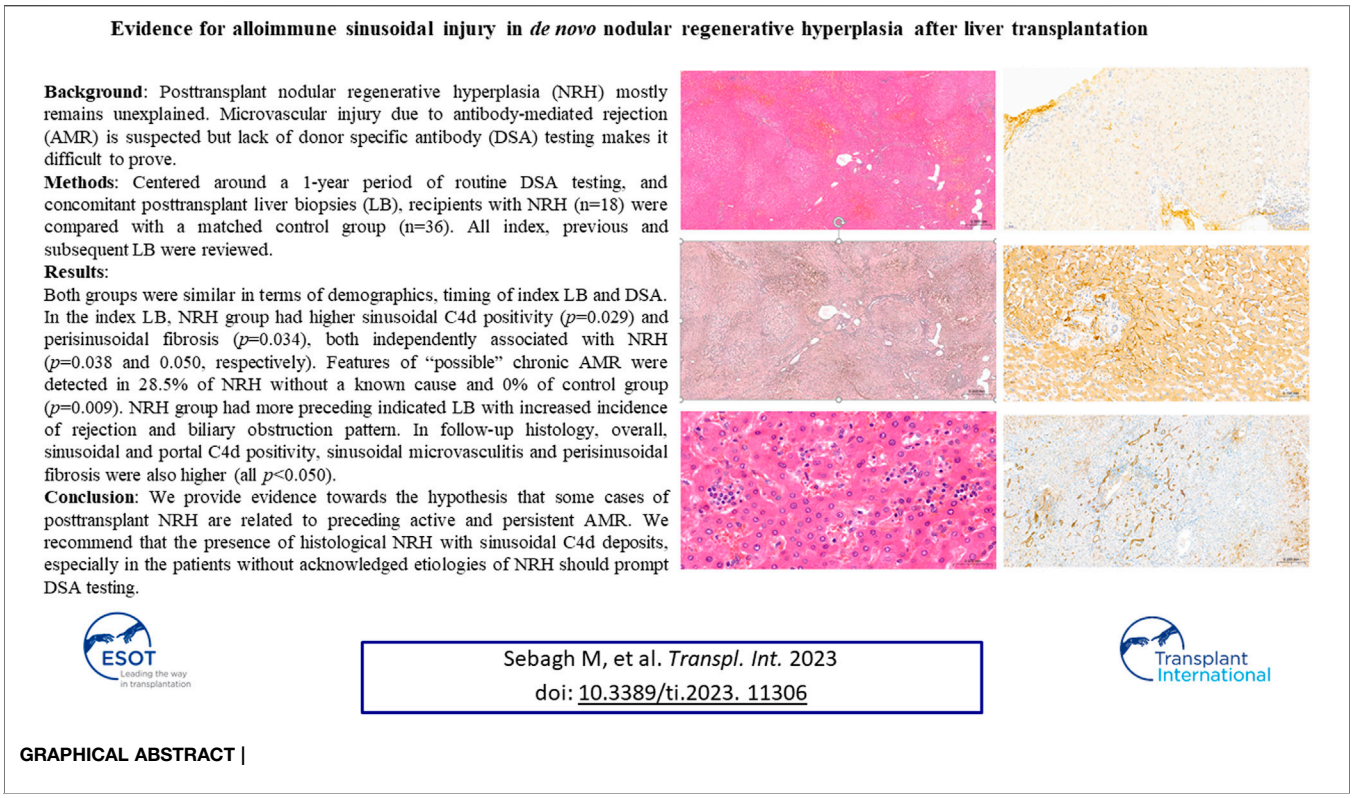
OPEN ACCESS

Correspondence:
Mylène Sebagh
mylene.sebagh@aphp.fr

Received: *25 February 2023*
Accepted: *29 June 2023*
Published: *26 July 2023*

Citation:
Sebagh M, Yilmaz F, Kounis I, Saliba F, Feray C, Taupin J-L, Cherqui D, Azoulay D, Samuel D, Coilly A, Demetris A-J and Neil D (2023) Evidence for Alloimmune Sinusoidal Injury in De Novo Nodular Regenerative Hyperplasia After Liver Transplantation. Transpl Int 36:11306. doi: 10.3389/ti.2023.11306

Abbreviations: aAMR, acute antibody-mediated rejection; cAMR, chronic antibody-mediated rejection; DSA, donor specific antibodies; HSC, hepatic stellate cells; LB, liver biopsies; LT, liver transplantation; LFT, liver function tests; LSEC, liver sinusoidal endothelial cells; MFI, mean fluorescence intensity; MHC, major histocompatibility complex; NRH, nodular regenerative hyperplasia; PSVD, porto-sinusoidal vascular disease; TCMR, T cell-mediated rejection; α-SMA, α-smooth muscle actin.



GRAPHICAL ABSTRACT |

INTRODUCTION

Nodular regenerative hyperplasia (NRH) is characterized by the diffuse transformation of the liver parenchyma into regenerative nodules with little-to-no perisinusoidal fibrosis [1]. In native livers, NRH is generally attributable to abnormalities in intrahepatic blood flow in small portal vein branches or hepatic venous drainage, and during the early stages of biliary tract disorders before more blatant cholangiopathic changes become obvious. NRH is associated with an ever-growing group of extrahepatic diseases and therapeutic agents including various immunological disorders, hematopoietic diseases, solid organ and bone marrow transplantation, and treatment with immunosuppressive or chemotherapeutic agents [2–4]. NRH can be asymptomatic and severe cases can show evidence of portal hypertension. Serum alkaline phosphatase and gamma glutamyl transpeptidase levels can be mildly elevated, but serum transaminase levels are usually normal [3].

The development of NRH following liver transplantation (LT) is not well documented, with only a few case reports [5–8] and three retrospective series in adult recipients [9–11]. NRH is seen with increasing frequency and with increased graft longevity [12, 13]. Most cases are asymptomatic, diagnosed on protocol liver biopsies (LB); however, some cases require retransplantation due to portal hypertension. Of the etiologies for NRH in native liver, azathioprine and vascular issues are higher up on the list of differential causes, however the number of unexplained cases remains high [11]. Porto-sinusoidal vascular disease (PSVD), which produces NRH is reported as an uncommon cause of recurrent disease after LT [14]. Chronic antibody-mediated rejection (cAMR), less well defined than acute antibody-mediated rejection (aAMR), is suspected by the Banff group to be one of the likely causes of NRH [15, 16]. Based on limited prior studies published before the era of donor specific antibodies (DSA), it is difficult to document this relationship and establish criteria for AMR-related NRH. Few centers do protocol LB and DSA testing. HLA DSA testing is haphazard, hepatologist-dependent, and non-HLA DSA is even less frequently tested. In the most recent series [11], based on a chart review without looking for histological clues to the potential etiology, no unifying risk factors were found, but the data pointed towards an immune-mediated process in the development of NRH.

The aim of this study was to get more concrete evidence for the relationship between AMR and the development of NRH, based on a detailed histological assessment for features of AMR from protocol and indicated LB taken during a 1-year window of protocol HLA DSA testing. The biopsies preceding and subsequent to the index LB were examined to look at sequential changes.

PATIENTS AND METHODS

Study Population

In our center, DSA testing is not routinely conducted, except for patients on the waiting list for retransplantation or with

unexplained graft dysfunction. For the purpose of the current study, case identification was restricted to 2014 as this was the sole period where DSA testing was performed systematically as part of a parallel study to know the incidence of DSA in the LT population. Patients were initially selected from the Pathology Department database using search criteria prospectively coded for a histological diagnosis of NRH made in 2014. Biopsy-proven NRH patients from this period, with synchronous DSA testing, were included (**Supplementary Figure S1**). The control group was formed by matching each NRH patient with two non-NRH patients who were biopsied in the nearest timeframe (just before and just after a given NRH patient). The study was conducted in accordance with the Declaration of Helsinki and French law for medical research. Free and informed consent was obtained for all the patients included.

Regular assessment includes a clinical, biochemical, and serological screening and calcineurin inhibitors doses at least every 6 months. LB were either "indicated" due to clinical and/or biochemical reasons or part of the systematic posttransplantation "protocol" at 1, 2, 5, 10, 15, and 20 years, independently of the donor and recipient HLA typing. Regarding immunosuppression, induction therapy such as IL-2 receptor antibodies (basiliximab) and anti-thymocyte globulin was used in patients with kidney dysfunction and those at higher immunological risk (retransplantation, immune-mediated liver disease, multiorgan recipient, highly sensitized) compared with essentially all other recipients who are considered lower immunological risk. A maintenance immunosuppression regimen is usually based on steroids (tapered and stopped between 3 and 6 months after LT), calcineurin inhibitors (tacrolimus or cyclosporine, mainly in HCV positive patients) and mycophenolate mofetil.

Pathology Studies

The specimens were routinely paraffin-embedded and stained with hematoxylin-eosin-safran and Picrosirius. Index, previous, and subsequent LB and/or explants were reviewed by two experienced liver transplant pathologists (FY and MS) blinded to the clinical status. Disagreements between the two readers were minor and resolved by consensus meeting. The diagnosis of NRH was based on diffuse transformation of the liver parenchyma (confirmed by Gordon Sweet's silver staining for reticulin, **Figure 1**) into regenerative nodules. Particular attention has been paid to histological features of AMR, as described elsewhere [15]. In short, histopathological pattern of injury consistent with aAMR mainly includes portal changes (i.e., microvascular endothelial cell hypertrophy, capillary and inlet venule dilatation, microvasculitis, edema, and ductular reaction). Among them, microvasculitis is the histopathological "signature" of aAMR. It can also affect the sinusoids (Table 4 of the Banff document [15]). Histopathological pattern of injury consistent with cAMR includes both unexplained and mononuclear portal and/or perivenular inflammation with interface and/or perivenular necro-inflammatory activity, and portal/periportal, sinusoidal and/or perivenular fibrosis. Portal microvasculitis is potentially observed in cAMR.

Here, the presence of monocyte/macrophage clusters of more than 5 cells within dilated sinusoids in most inflamed areas randomly in the lobules was named as "sinusoidal microvasculitis."

Immunostaining

Immunostaining for C4d (rabbit monoclonal A24-T Biotech, Kosice, Slovakia) was evaluated in the compartments defined by the Banff group in portal veins and portal capillaries [15], but also in the centrilobular veins and sinusoidal endothelial cells. C4d immunostaining was scored as negative (score 0), minimal (<10%, score 1), focal (10%–50%, score 2) and diffuse (>50%, score 3) of structures in the index LB and last follow up histology in both groups (**Figure 2**).

In the NRH group, a panel (CD3, CD20, CD4, CD8, and CD68) was performed for immunotyping the sinusoidal infiltrate in the cases with sinusoidal microvasculitis. Changes in liver sinusoidal endothelial cell (LSEC) and hepatic stellate cell (HSC) phenotype were studied by comparing similarly-sized portal tracts, central veins, and sinusoids in the index LB versus last follow up histology stained for CD34 (mouse monoclonal, QBE-10, DAKO) and α-smooth muscle actin (α-SMA, mouse monoclonal, 1A4; DAKO). The expression of major histocompatibility complex (MHC) class II antigen (mouse monoclonal CR3/43; DAKO (MO775, Carpinteria, CA), the putative target of Class II DSA, was assessed by compartment.

Assays for Anti-HLA Antibodies

Donors were typed for the HLA system using commercially available serological methods (One Lambda, Inc., Canoga Park, CA). Loci A, B, DP, DQ, and DR were typed. Blood samples were harvested from the recipients at the time and after the index LB. Recipient anti-HLA antibodies were retrospectively analyzed by Luminex with the LABScreen single antigen class I and single antigen class II beads (LS1A04 and LS2A01, respectively; One Lambda, Inc.), after neutralization of the complement interference phenomenon using ethylene diamine tetraacetic acid before treatment of the serum for all the samples that were found positive using the screening assay (LSM12; One Lambda, Inc.). Normalized mean fluorescence intensity (MFI) values of DSA were reported, using the baseline formula from the Fusion® software. The specificities for both class I and II HLA antibodies were considered significant for MFI >1,000 in accordance with the cutoff values used in LT [17].

Statistical Analysis

NRH and control patients were compared in terms of demographic data, histopathological features, immunostaining for C4d, and DSA. Student's t-test was used for continuous variables, whereas the chi-squared test or Fisher exact test (for small numbers) was applied to analyze categorical variables. The variables were first considered under univariate analysis. Those with $p < 0.15$ (because of the small sample size) were then tested by logistic regression analysis. A p-value of <0.05 was considered to be significant.

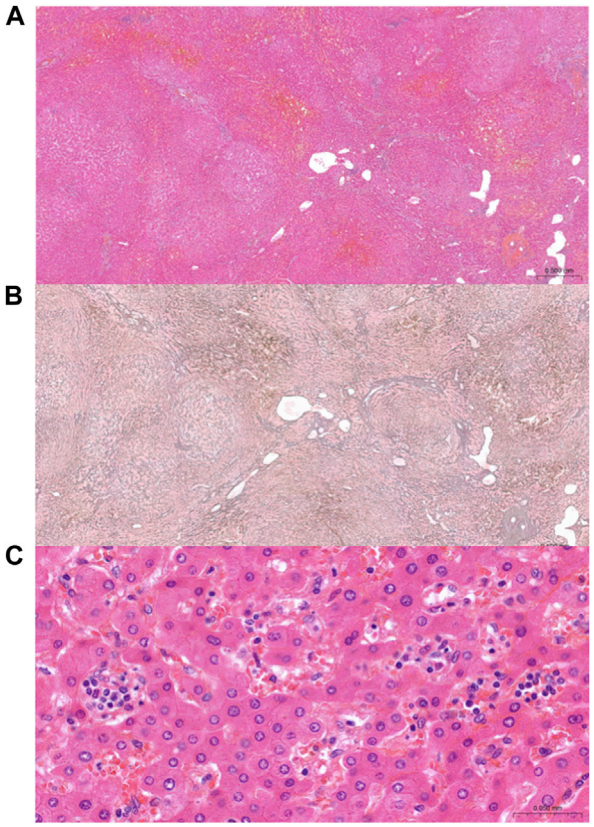


FIGURE 1 | Representative images of NRH and "sinusoidal microvasculitis." Alternating widened and atrophic hepatic plates in a nodular architecture, consistent with NRH on Hematoxylin eosin staining **(A)** and Gordon Sweet's silver staining **(B)**. At high magnification, so-called "sinusoidal microvasculitis" **(C)** by analogy with portal capillaritis, defined by the presence of marginated monocytes/macrophages within dilated sinusoids.

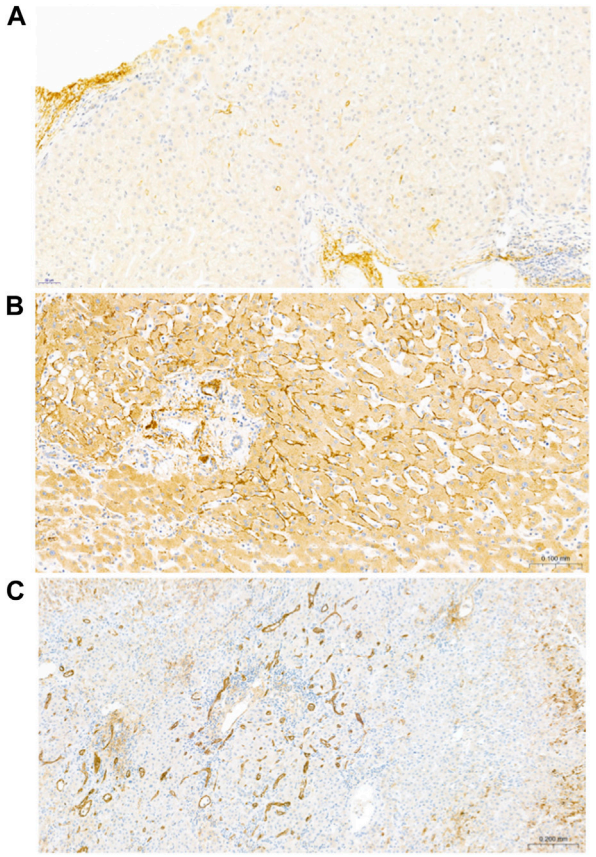


FIGURE 2 | Representative images of C4d in patients with NRH. **(A)** An example of minimal (<10% staining) C4d positivity in an index LB with NRH. C4d positivity is observed within the sinusoidal microvasculature. **(B)** Focal (10%–50% staining) C4d positivity within the sinusoidal microvasculature in an explant with NRH. C4d deposition on the elastic fibers of arterioles was regarded as a positive internal control. **(C)** Diffuse (>50% staining) C4d positivity in an explant with NRH. C4d positivity is observed within the portal venules, capillaries and inlet venules, and sinusoids.

RESULTS

Patients

During the study period, a total of 356 LB were performed in 329 liver transplant patients (**Supplementary Figure S1**). Twenty-three (6.4%) patients were diagnosed with NRH in 23 (6.9%) LB. Among them, five patients were excluded due to lack of synchronous DSA testing. The study included 18 NRH patients and 36 matched controls. **Table 1** gives patients' characteristics. There were 14 males and 4 females, with a mean age of 50.7 + 11.6 years at the time of the initial LT. None of the patients were infected by HIV. The majority of patients were transplanted for cirrhosis. None were transplanted for NRH. None of the patients had systemic diseases, prothrombotic status, or hematological disorders. One patient concomitantly underwent kidney transplantation for chronic interstitial nephropathy and one underwent heart transplantation 4 years after LT for amyloidosis-related cardiac insufficiency.

Regarding immunosuppression, induction therapy was used in eight patients. Fourteen (77%) patients were on maintenance immunosuppression with tacrolimus, three (16%) with cyclosporine and one (5%) with everolimus. Sixteen (89%) received mycophenolate mofetil (MMF) and one (n°14) received azathioprine since LT (10 years ago). There was no change in immunosuppression following the diagnosis of NRH.

Both groups were similar in terms of demographics such as sex, mean age at the time of LT, native disease, induction therapy (8/36 versus 3/18) and maintenance immunosuppression, which was mainly based on tacrolimus (24/36 versus 14/18) and MMF (24/36 versus 16/18). The two groups did not differ in terms of mean posttransplant timing of index LB (6.4 ± 6.0 versus 6.2 ± 6.3 years), and indication for index LB (clinically indicated in 3/36 versus 4/18, or protocol LB in 33/36 versus 14/18). Regardless of the indication for index LB, abnormal LFT were significantly less frequent in the control group (p = 0.036).

Graft loss was higher in the NRH group (5/18 versus 0/36, p = 0.003). Patients were retransplanted at a mean time of 8.1 ± 5.9 years after LT and at a mean interval of 2.1 ± 0.9 years after the diagnosis of NRH. The reason for retransplantation was related to NRH complicated by portal hypertension (refractory ascites in 3,

TABLE 1 | Characteristics of the liver transplant patients from NRH and control groups.

	NRH group (*n* = 18)	Control group (*n* = 36)	*P*[#]
Gender (M/F)	14/4	20/16	0.142
Mean age at initial transplantation	50.7 ± 11.6	51.4 ± 11.5	0.690
Native disease			
Alcoholic cirrhosis	2 (11.1)	8 (22.2)	0.466
HCV-cirrhosis	4 (22.2)	12 (33.3)	0.532
HBV-cirrhosis	3 (16.7)	8 (22.2)	0.733
NASH-cirrhosis	1 (5.6)	0 (0.0)	
Fulminant hepatitis	1 (5.6)	3 (8.3)	
Amyloidotic neuropathy	3 (16.7)	1 (2.8)	
Primary biliary cholangitis	0	3	
Primary sclerosing cholangitis	1	1	
Biliary atresia	2	0	
Tyrosinemia	1	0	
Concomitant HCC	5 (27.8)	7 (19.4)	0.506
Chemotherapy for prevention of HCC recurrence	2/5	0/7	
Immunosuppression			
Induction therapy	3 (16.7)	8 (22.2)	0.733
Maintenance regimen			
Tacrolimus	14 (77.8)	24 (66.7)	0.532
Cyclosporine	3 (16.7)	10 (27.8)	0.506
Everolimus	1 (5.6)	2 (5.6)	
Mycofenolate mofetil	16 (88.9)	24 (66.7)	0.105
Azathioprine	1 (5.6)	0 (0.0)	
Index LB			
Mean post-transplant time (years)	6.2 ± 6.3	6.4 ± 6.0	0.640
Nature of the index LB			
Indicated LB	4 (22.2)	3 (8.3)	0.182
Routine LB	14 (77.8)	33 (91.7)	0.204
Abnormal LFTs at the time of the index LB regardless of its nature	11 (61.1)	10 (27.8)	**0.036**
Cholestasis	7	4	
AST and/or ALT elevation	0	2	
Both	4	4	
Surgical complications	3	5	1.000
Biliary stenosis	1 (5.6)	2 (5.6)	
Portal vein thrombosis	2 (11.1)	0 (0.0)	
Arterial stenosis	0 (0.0)	2 (5.6)	
Arteriolo-venous fistula	0 (0.0)	1 (2.8)	
Follow up			
Available histological follow-up	14 (77.8)	25 (69.4)	0.748
Follow-up course			
Death	1 (5.6)[a]	1 (2.8)[b]	1.000
Retransplantation	5 (27.8)	0 (0.0)	**0.003**

Values presented as n (%), Liver biopsies (LB), HCC, hepatocellular carcinoma; LB, liver biopsy; LFTs, Liver Function Tests.

[#]*p ≤ 0.05 was considered statistically significant (in bold).*

[a]*Death due to sepsis.*

[b]*Death due to colon carcinoma.*

variceal bleeding in 2). Patient survival was similar in both groups.

Pathological Results

Previous LB in Both Groups

Biopsies prior to the index LB were performed in 15 NRH patients and 26 control patients who underwent 47 and 76 LB, respectively. The mean and median number of previous LB were 2.3 ± 2 and 2 (range: 0–8) in the NRH group, and 2 ± 2 and 2 (range: 0–6) in the control group. The number of indicated LB was significantly higher in the NRH group (32/47 versus 27/76, $p = 0.001$) (**Table 2**).

Among overall previous LB to index ones, features consistent with aAMR were present in one patient of the NRH group and in none of the control group. T cell-mediated rejection (TCMR) was more common in the NRH group (22% versus 8%) without reaching significance. Ductopenic rejection was observed in one NRH patient and one control patient. The pattern of biliary obstruction was significantly more common in the NRH group ($p = 0.033$). This was observed only in the NRH group and in the absence of abnormalities of the biliary imaging. The presence of sinusoidal microvasculitis was similar between both groups.

TABLE 2 | Comparison of main histopathological features, C4d immunostaining, and DSA between NRH and control groups.

	n (%)		
	NRH *n* = 18	**Controls *n* = 36**	**P[#]**
Previous liver biopsies (LB)	47	76	
Median (range)/Mean number of LB	2 (0–8)/2.3 + 2	2 (0–6)/2 ± 2	1.000
Number of patients with at least one previous LB	15 (83.3)	26 (72.2)	0.506
Number of protocol/indicated LB	15/32	49/27	**0.001**
Rejection	7	4	**0.029**
TCMR	4	3	
Chronic ductopenic rejection	1	1	
Plasma cell rich rejection	1	0	
aAMR	1	0	
Biliary obstruction pattern	3	0	**0.033**
Sinusoidal microvasculitis	4	5	0.461
Index liver biopsies			
TCMR	0 (0.0)	2 (5.6)	0.547
Chronic ductopenic rejection	2 (11.1)	4 (11.1)	1.000
C4d positivity	8 (44.4)	8 (22.2)	0.119
Portal compartment	3 (16.7)	5 (13.8)	1.000
Sinusoidal compartment	7 (38.9)	4 (11.1)	**0.029**
Centrilobular vein	0 (0.0)	2 (5.6)	1.000
Sinusoidal microvasculitis	6 (33.3)	5 (13.9)	0.150
Perisinusoidal fibrosis	6 (33.3)	5 (13.9)	0.150
Perisinusoidal fibrosis unrelated to NASH	5 (27.8)	2 (5.6)	**0.034**
Last histological follow up	14 (77.8)	25 (69.4)	0.748
TCMR	1 (7.1)	0 (0.0)	0.358
Chronic ductopenic rejection	2 (14.2)	2 (8.0)	0.61
C4d positivity	10 (71.4)	1 (4.0)	<**0.001**
Portal compartment	5 (35.7)	1 (4.0)	**0.021**
Sinusoidal compartment	10 (71.4)	0 (0.0)	<**0.001**
Centrilobular vein	0 (0.0)	0 (0.0)	1.000
Sinusoidal microvasculitis	7 (50.0)	3 (12.0)	**0.019**
Perisinusoidal fibrosis	5 (35.7)	4 (16%)	0.238
Perisinusoidal fibrosis unrelated to NASH	3 (21.4)	0 (0.0)	**0.039**
DSA at the time of index liver biopsies			
Positive DSA	6 (33.3)	12 (33.3)	1.000
More than one DSA	4 (22.2)	2 (5.6)	0.087
Class II DSA	5 (27.8)	11 (30.6)	1.000
High MFI (>1,000) class II DSA	5 (27.8)	7 (19.4)	0.506
Class I DSA	3 (16.7)	1 (2.8)	0.102
High MFI (>1,000) class I DSA	1 (5.6)	1 (2.8)	1.000
Chronic AMR	4 (22.2)	0	**0.009**

TCMR, T cell-mediated rejection; AMR, antibody-mediated rejection; DSA, donor specific antibodies; MFI, mean fluorescence intensity; NRH, nodular regenerative hyperplasia.
[#]*p ≤ 0.05 was considered statistically significant (in bold).*

NRH Group

On the index LB, NRH was confirmed in all of them. Ductopenic rejection was concomitant to NRH in two and TCMR in none. No portal capillaritis was observed. Sinusoidal microvasculitis was observed in six (33%) LB. Perisinusoidal fibrosis was observed in six (33%) LB, of whom five were unrelated to NASH. No microthrombotic changes were observed in sinusoids or portal venules. Immunostaining for C4d was positive in eight (44.4%) LB. Positivity was observed only in portal venules in one (minimal), only in sinusoids alone in five (minimal in four and focal in one, respectively) and in both in two LB (focal in one and diffuse in one, respectively).

On last follow up histology available in 14 cases including 9 LB and 5 explants, we observed persistence of NRH. No microthrombotic changes were observed in sinusoids. Two explants showed portal venopathy. The global incidence of C4d deposits and sinusoidal microvasculitis (71.4% and 50%, respectively) increased as compared with the index LB (44% and 33%, respectively) without reaching significance. For a given patient, C4d deposits, either in portal tracts or sinusoidal, increased in 50% of patients in the follow-up LB after the index LB. The sinusoidal infiltrate contained abundant lymphocytes of predominantly CD3/CD8 phenotype in all cases with sinusoidal microvasculitis. We detected a shift toward a pathogenic phenotype in HSC and LSEC (**Figure 3**): Over time, there was an increase in CD34 expression in peri-portal, sinusoidal, or peri-venular regions, and in α-SMA expression diffusely. MHC Class II staining increased dramatically, predominantly in the sinusoidal compartment (**Figure 4**).

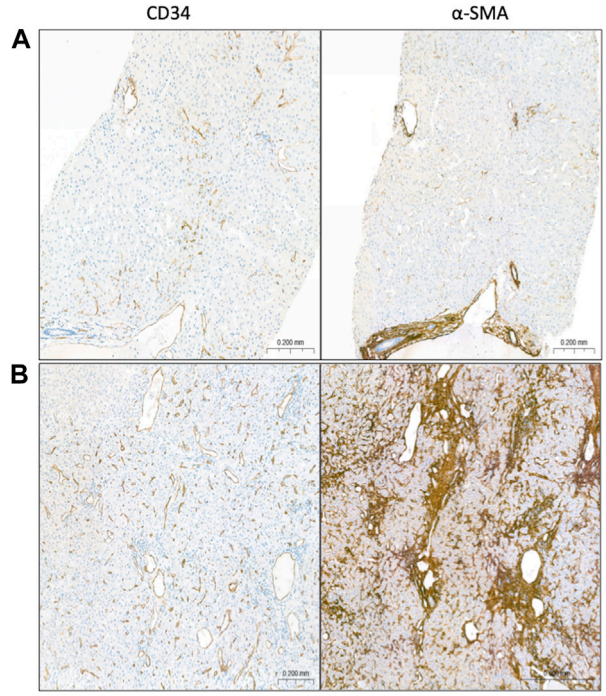


FIGURE 3 | Shift toward a pathogenic phenotype in HSC and LSEC over time. By comparing similarly-sized portal tracts, central veins, and sinusoids in the index LB **(A)** versus last follow up histology **(B)**, there was an increase in CD34 expression in peri-portal, sinusoidal, or peri-venular regions, and in α-SMA expression diffusely.

Control Group

On the index LB, the main pathological diagnoses were as follows: normal in six, steatosis/non-alcoholic steatohepatitis in nine, ductopenic rejection in four, TCMR in three, sinusoidal congestion in four, chronic hepatitis in nine, and biliary obstruction in one. No NRH changes were observed. No portal capillaritis was observed. Sinusoidal microvasculitis was observed in five (14%) LB. Perisinusoidal fibrosis was observed in five (14%) LB, of whom two were unrelated to NASH. Immunostaining for C4d was positive in eight (22%) LB. Positivity was observed only in sinusoids in 3 (minimal in 1 and focal in 2, respectively), only in portal venules in 2 (minimal), in both portal venules and sinusoids in 1 (minimal), and in both portal and centrilobular venules in 2 LB (minimal).

Last histology showed no NRH changes and no significant fibrosis, and only one (1/25) had minimal portal C4d deposit.

Comparison of Main Histological and Immunohistochemical C4d Features of Index LB and Subsequent Histology Between Groups

There is a trend towards increased sinusoidal microvasculitis in the NRH group in the index LB (33% versus 14%) and previous LB (27% versus 19%), however this does not reach statistical significance (using univariate analysis). Sinusoidal C4d positivity and perisinusoidal fibrosis in index LB were higher in NRH group (p = 0.029 and 0.034, respectively). Under multivariate analysis, sinusoidal C4d deposits and perisinusoidal fibrosis were independently associated with NRH (p = 0.038 and 0.050, respectively).

In follow-up histology, higher sinusoidal microvasculitis, perisinusoidal fibrosis, and overall, sinusoidal and portal C4d positivity (all p < 0.050) were observed in the NRH group. Multivariate analysis was not possible because the number of patients with follow up histology was not sufficient.

DSA at the Time of the Index LB

DSA were present in six (33%) patients of the NRH group in whom four had more than one DSA type (**Table 2**). Five patients exhibited class II DSA in significant MFI in all of them. Three patients had class I DSA in significant MFI in one and approaching significance in two of them. Two patients exhibited both class I and II DSA. Twelve (33%) patients of the control group had positive DSA in whom two had more than one DSA type. One patient had significant class I DSA. Eleven patients had class II DSA, in significant MFI in seven of them. There was no difference between the groups in terms of DSA (presence, number, class, and level of MFI).

Diagnosis of cAMR

No patient from the control group could be classified as cAMR as they did not meet the histopathological features at index and follow-up.

In the NRH group, four patients (**Supplementary Table S1**, n°1, 3, 7, 11) with mainly sinusoidal and/or portal vascular C4d positivity, portal and/or perisinusoidal fibrosis in index LB and DSA positivity were classified as cAMR. Three of them were retransplanted. Explant livers showed progression of the histopathological features detected in the index LB, especially perisinusoidal fibrosis and sinusoidal microvasculitis. DSA in terms of type and level of MFI were comparable to those present at the time of the index LB. The fourth patient developed ascites and had no follow-up biopsy and no DSA testing. In these patients, no other causes to explain clinical and morphological findings and acknowledged etiologies of NRH were found. These four patients represented 22.2% (4/18) of all the NRH patients and 28.5% (4/14) of the NRH patients without acknowledged etiologies of NRH.

Three additional patients with NRH (n°2, 4, 15) had the histopathological criteria and C4d immunostaining consistent with cAMR in follow-up, but DSA were not tested in one patient (n°15) and remained negative in the two retransplanted patients (n°2, 4). In these two latter patients, portal vein thrombosis has been discovered after the diagnosis of NRH on the index LB in one (pt n°2), and before the index LB indicated for ascites in the other. Both explants found portal vein thrombosis and portal venopathy.

DISCUSSION

Our study is the first that attempts to investigate the relationship between posttransplant NRH and AMR. Despite inconsistent routine DSA testing overall, thanks to a 1-year period of routine DSA testing together with a long-standing system of protocol LB, we were able to look at a subset of our liver transplant patients with NRH. In comparing our cohort of

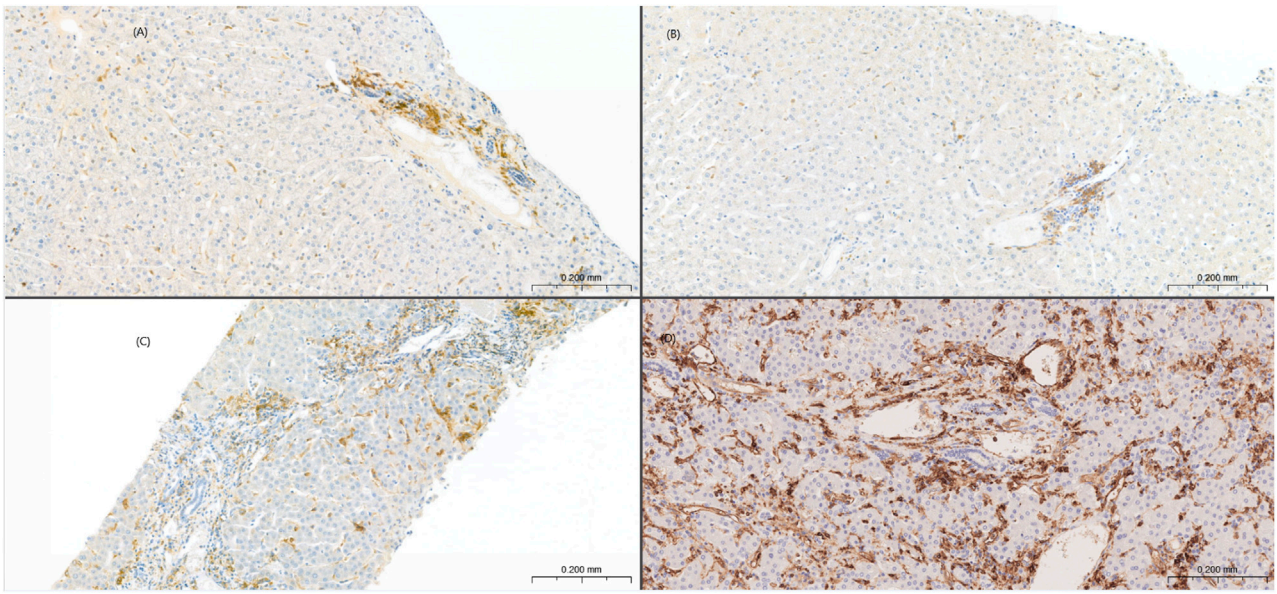


FIGURE 4 | Sinusoidal MHC class II overexpression in NRH. Low MHC class II expression limited focally on sinusoidal endothelium in the index **(A)** and last follow up LB **(B)** from a control patient. Sinusoidal MHC class II overexpression in the index **(C)** and last follow up LB **(D)** from a NRH patient. Portal-based dendritic cells served as internal positive controls.

posttransplant NRH to the control group, we found that the patients who develop NRH had more preceding indicated LB with an increased incidence of rejection diagnosis, including TCMR and features suspicious for AMR, or biliary features which are also a recognized feature of AMR, after exclusion of biliary obstruction. At the time of the index LB, the diagnosis of cAMR could be made in a subset of our NRH patients according to the current 2016 Banff criteria [15]. In addition to these, currently classifiable as cAMR, we identified an association with a form of progressive antibody-mediated sinusoidal injury consisting of persistent sinusoidal microvasculitis and sinusoidal C4d staining, evolving from the previous LB to the index LB and follow-up histology. These findings argue for the addition of NRH to the features of cAMR and should prompt DSA testing, especially in the absence of acknowledged etiologies of NRH.

Due to the short time period of routine DSA testing, the present study has several limitations, including its retrospective design, small sample size, and the inability to test some antibodies such as anti-endothelial antibodies at that time. The strengths of our study include a single-center experience, uniform diagnostic methods, and attentive post-LT care including performance of protocol LB.

As expected, the majority of NRH cases had no known associated risk factor for the development of NRH (14/18), with some requiring retransplantation for the consequences of non-cirrhotic portal hypertension/PSVD. Graft loss/retransplantation was higher (28%) in the NRH group than in the control group (0%) in whom no patient developed PSVD or cirrhosis. A significantly higher sinusoidal microvasculitis in follow up histology, and sinusoidal C4d accumulation and perisinusoidal fibrosis in the index LB and follow-up histology, were observed in the NRH group. First, a sinusoidal lymphocyte infiltrate has not been reported in NRH after LT but the case reports and the series did not provide sufficient histological data to be certain that this is the case. A sinusoidal microvasculitis has rarely been described in native livers with NRH either in the non-transplant setting or after transplantation of organs other than the liver. Ziol et al [18] described intrasinusoidal infiltrate composed of cytotoxic CD8+T-lymphocytes in 32% of patients with NRH. The T-cells were located near atrophic liver cell plates and adjacent to endothelial cells exhibiting evidence of apoptosis. The authors suggested the contribution of T-lymphocyte cytotoxicity against endothelial cells as a pathophysiologic mechanism in NRH with intrasinusoidal infiltrate as well as in NRH without intrasinusoidal infiltrates since the previous repeated LB demonstrated lymphocyte infiltration that decreased up to its complete disappearance. In contrast, in our study, higher sinusoidal microvasculitis was observed in the NRH group but only on the last follow up histology, and not prior to the development of the NRH. The late lymphocyte recruitment argues that it did not cause NRH. We ruled out the potential confounding factors such as recurrent disease, adverse drug reactions, and severe TCMR [19, 20]. We did not identify sinusoidal microthrombi in any case of NRH with or without infiltrate, although we would postulate that these have occurred previously at a time when a biopsy was not taken or that they were so subtle that they were not detected by standard staining [21]. Microthrombi have been reported as a result of endothelial injury/activation related to aAMR in liver graft [22, 23], analogous to a thrombotic microangiopathy seen as part of AMR in renal biopsies [24]. In native livers with PSVD, portal venopathy (identified in 2 of our NRH patients) is thought to result from previous microthrombotic events [25], which are attributed to recurrence in patients transplanted with common variable immunodeficiency for this indication [14].

Second, higher sinusoidal C4d deposits on index and last histology were observed in the NRH group. There is no data of C4d accumulation in native livers with NRH, as C4d immunostaining is not performed in these cases. It can be argued that sinusoidal C4d deposits can be "nonspecific" due to C4d binding to collagen around diseased sinusoids because of increased perisinusoidal fibrosis. We ruled out this hypothesis: Sinusoidal C4d deposits and perisinusoidal fibrosis were independently associated with NRH under multivariate analysis. In addition, we demonstrated MHC class II overexpression in sinusoids while native and graft liver display low MHC class II expression, limited predominantly to occasional portal capillaries and focally on sinusoidal endothelium as previously reported [26–29]. It is of note that, in the eight NRH patients and eight controls with C4d positivity, DSA was negative in four and in two, respectively. The principal targets of the humoral immune response are the highly polymorphic HLA antigens, but studies have also implicated antibodies directed against non-HLA autoantigens such as angiotensin type 1 receptor, perlecan, and collagen in the process of AMR [30, 31]. Unfortunately, none of our patients had available data regarding non-HLA antibodies to address this question.

Third, perisinusoidal fibrosis is not specific to NRH after LT, which can occur in the late stages of NRH in native livers, irrespective of the cause. The abnormal CD34 expression of LSEC reflects capillarization, lack of fenestration, and formation of an organized basement membrane, which are permissive for HSC activation, related-α-SMA positivity, and fibrosis [27, 28]. Irrespective of the etiologies, initial endothelial injury promotes the phenotypic changes in LSEC and HSC. The MHC class II overexpression could reflect an injury of immune-mediated nature.

The features of cAMR are described as low-grade chronic inflammation, progressive fibrosis, and microvascular C4d deposition in patients with (near) normal LFT and DSA positivity [15, 16, 32]. The denomination into «probable» and «possible» cAMR depends on the C4d score. From these actual 2016 Banff criteria, it is of note that: 1) the possible category: "DSA not available, equivocal, or negative," present for the classification of aAMR is not defined for cAMR. It is the reason for which our NRH patients with other criteria for cAMR in follow-up but negative DSA in two and non tested-DSA in one were finally not classified as cAMR. In the two retransplanted patients, explants additionally showed portal venopathy, this feature being a part of AMR but also being due to the portal vein thrombosis; 2) C4d deposits are located into portal tracts. In a multicenter study [33], sinusoidal C4d deposits were rare and difficult to identify. One team twice reported sinusoidal C4d deposits as an indication of antibody-mediated response in liver allografts [34, 35]; 3) regarding progressive fibrosis, atypical fibrosis patterns have emerged including perisinusoidal and perivenular fibrosis [15, 36]; 4) low-grade chronic inflammation affects portal tracts and/or perivenular areas and "portal capillaritis" is potentially observed. Sinusoidal microvasculitis may be the morphological equivalent of the portal capillaritis; 5) the Banff group admitted that cAMR suffers from a lack of specific/typical features, and additionally suspected a spectrum of liver allograft injuries including non-inflammatory fibrosis, low-grade inflammation, biliary strictures, v-lesion, and NRH as histopathological features of cAMR [15, 29, 36, 37]. It is not clear if these injuries should be associated with all the established Banff criteria or whether their presence alone is sufficient for a diagnosis of AMR. Irrespective of the above caveats, by strictly applying the actual 2016 Banff criteria, four NRH patients (22.2% of all NRH patients and 28.5% of those where other likely causes of NRH were excluded) could be classified as "possible" cAMR, the most striking histological features were within the sinusoids: sinusoidal microvasculitis, sinusoidal C4d deposits, and perisinusoidal fibrosis.

The following question is raised: "is this just a co-incidence or is there a direct and causal relationship between AMR and NRH?" The comparison between both groups showed at least a significant association between both conditions. We believe this is related to AMR/DSA and not a non-specific response to circulating HLA antibodies, as there is no evidence that circulating HLA antibodies—when not donor specific (e.g., transplant of a different organ who develop antibody or sensitization following transfusion)—are linked to the development of NRH. For the development of AMR, the "second-hit" hypothesis has been proposed, summarized in Figure 1 from the review by Kim et al. [32] as follows: Injury in the liver allograft upregulates class II expression that facilitates class II DSA binding. Complement fixing antibodies may activate complement. Antibodies with Fc binding receptors may facilitate antibody dependent cellular cytotoxicity explaining the presence of sinusoidal lymphocytes. This demonstrates a sinusoidal localization of each step, supporting the sinusoidal and architectural changes, just as we observed (i.e., C4d deposits, MHC class II overexpression and microvasculitis). This also highlights a dynamic phenomenon: Here, follow up histology versus index LB showed increased sinusoidal and portal C4d deposits, and late onset of sinusoidal microvasculitis. Previous LB to index ones more often displayed the pattern of biliary obstruction in NRH patients without imaging abnormalities in the biliary tree. Biliary features are suspected to be a part of AMR, possibly due to the involvement of peribiliary plexus [15, 29, 36, 37]. Such cases can be speculated as presenting indirect evidence of previous AMR. Previous LB also displayed more rejection. However, features consistent with aAMR were observed in only one NRH patient. Since not all of our patients were biopsied before the index LB, there is the possibility that subclinical "indolent" AMR may have been underdiagnosed during the process. Taken together, we believe that a subset of posttransplant NRH is the result of a form of cAMR with prominent sinusoidal features. This is consistent with the known association of immunological/inflammatory causes of PSVD in native livers and the NRH development [4, 25]. The current Banff criteria for the diagnosis of cAMR in allograft livers require an active/acute component to be present, this definition will miss the cases that have architectural changes and scarring related to previous acute and acute on chronic components, but at the time of biopsy, in particular for protocol biopsies, have no active component. There may be a need to revise the classification to three groups, as has been done in renal transplantation [24], changing the current cAMR to chronic active AMR and then adding a cAMR category where there is no active component, but with documented evidence of previous acute or chronic active AMR.

In conclusion, we reported a subgroup of posttransplant NRH cases (28.5% of the NRH group without a known cause of NRH) with concomitant features consistent with "possible" cAMR

according to the current 2016 Banff criteria. The presence of prominent sinusoidal findings led us to suspect the contribution of antibody-mediated sinusoidal injury in the NRH development. Further multicenter studies, with more complete DSA testing, are needed to confirm these findings. To limit costs and potentially pick up AMR at a treatable time point, we recommend that the presence of histological NRH with sinusoidal C4d deposits, especially in the patients without acknowledged etiologies of NRH should prompt DSA testing. The difficulties we have had with classifying cases and the prominent sinusoidal changes warrant a review of the Banff AMR criteria.

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 study was conducted in accordance with the Declaration of Helsinki and French law for medical research. Free and informed consent was obtained for all the patients included.

AUTHOR CONTRIBUTIONS

MS: conceptualization, methodology, formal analysis, investigation, data curation writing, original draft, and writing; FY: conceptualization, methodology, formal analysis, investigation, original draft, and writing; IK: investigation; FS: review and editing visualization; CF: statistic, J-LT: investigation; DC: review and editing visualization; DA: review and editing visualization; DS: review and editing visualization; AC: review and editing visualization, supervision; A-JD: supervision; DN: supervision, review, and editing. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11306/full#supplementary-material

Supplementary Figure S1 | Flowchart of the included patients. Patients were initially selected from the Pathology Department database using search criteria prospectively coded for a histological diagnosis of NRH made in 2014. Biopsy-proven NRH patients from this period, with synchronous DSA testing were included. The control group was formed by matching each NRH patient with two non-NRH patients who were biopsied in the nearest timeframe (just before and just after a given NRH patient).

Supplementary Table S1 | Histopathological features, immunostaining for C4d and DSA in the NRH group.

REFERENCES

1. Steiner PE. Nodular Regenerative Hyperplasia of the Liver. *Am J Pathol* (1959) 35:943–53. doi:10.3748/wjg.v17.i11.1400
2. Wanless IR. Micronodular Transformation (Nodular Regenerative Hyperplasia) of the Liver: a Report of 64 Cases Among 2,500 Autopsies and a New Classification of Benign Hepatocellular Nodules. *Hepatology* (1990) 11(5):787–97. doi:10.1002/hep.1840110512
3. Naber AH, Van Haelst U, Yap SH. Nodular Regenerative Hyperplasia of the Liver: an Important Cause of portal Hypertension in Non-cirrhotic Patients. *J Hepatol* (1991) 12(1):94–9. doi:10.1016/0168-8278(91)90916-y
4. Northup PG, Garcia-Pagan JC, Garcia-Tsao G, Intagliata NM, Superina RA, Roberts LN, et al. Vascular Liver Disorders, Portal Vein Thrombosis, and Procedural Bleeding in Patients with Liver Disease: 2020 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* (2021) 73(1):366–413. doi:10.1002/hep.31646
5. Sebagh M, Farges O, Samuel D, Bismuth H, Reynès M. Nodular Regenerative Hyperplasia of the Liver Following Orthotopic Liver Transplantation. *Transpl Proc* (1995) 27(4):2510–1. doi:10.1002/hep.1840200114
6. Duvoux C, Kracht M, Lang P, Vernant JP, Zafrani ES, Dhumeaux D. Nodular Regenerative Hyperplasia of the Liver Associated with Azathioprine Therapy. *Gastroenterol Clin Biol* (1991) 15(12):968–73. doi:10.1186/s12969-022-00690-x
7. Coelho R, Rodriguez S, Rodrigues-Pinto E, Silva R, Lopes J, Macedo G. Nodular Regenerative Hyperplasia after Liver Transplantation Complicated with Inferior Vena Cava Stenosis: a Clue for Etiopathogenesis? *J Gastrointestin Liver Dis* (2015) 24(3):383–5. doi:10.15403/jgld.2014.1121.243.cho
8. Alhosh R, Genyk Y, Alexopoulos S, Thomas D, Zhou S, Yanni G, et al. Hepatopulmonary Syndrome Associated with Nodular Regenerative Hyperplasia after Liver Transplantation in a Child. *Pediatr Transpl* (2014) 18(5):E157–160. doi:10.1111/petr.12281
9. Gane E, Portmann B, Saxena R, Wong P, Ramage J, Williams R. Nodular Regenerative Hyperplasia of the Liver Graft after Liver Transplantation. *Hepatology* (1994) 20:88–94. 1 Pt 1. doi:10.1016/0270-9139(94)90138-4
10. Devarbhavi H, Abraham S, Kamath PS. Significance of Nodular Regenerative Hyperplasia Occurring De Novo Following Liver Transplantation. *Liver Transpl* (2007) 13(11):1552–6. doi:10.1002/lt.21142
11. Chen AK, Lunow-Luke T, Yamaguchi S, Praglin C, Agudelo E, Mehta N, et al. Nodular Regenerative Hyperplasia after Liver Transplant; It's All in the Presentation. *Front Surg* (2022) 9:876818. doi:10.3389/fsurg.2022.876818
12. Hübscher SG. What Is the Long-Term Outcome of the Liver Allograft? *J Hepatol* (2011) 55(3):702–17. doi:10.1016/j.jhep.2011.03.005
13. Sebagh M, Samuel D, Antonini TM, Coilly A, Degli Esposti D, Roche B, et al. Twenty-year Protocol Liver Biopsies: Invasive but Useful for the Management of Liver Recipients. *J Hepatol* (2012) 56(4):840–7. doi:10.1016/j.jhep.2011.11.016
14. Magaz M, Giudicelli-Lett H, Nicoară-Farcău O, Rajoriya N, Goel A, Raymenants K, et al. Liver Transplantation for Porto-Sinusoidal Vascular Liver Disorder: Long-Term Outcome. *Transplantation* (2022) 107:1330–40. doi:10.1097/tp.0000000000004444
15. Demetris AJ, Bellamy C, Hübscher SG, O'Leary J, Randhawa PS, Feng S, et al. 2016 Comprehensive Update of the Banff Working Group on Liver Allograft Pathology: Introduction of Antibody-Mediated Rejection. *Am J Transpl* (2016) 16(10):2816–35. doi:10.1111/ajt.13909

16. Demetris AJ, Zeevi A, O'Leary JG. ABO-Compatible Liver Allograft Antibody-Mediated Rejection: an Update. *Curr Opin Organ Transpl* (2015) 20(3): 314–24. doi:10.1097/MOT.0000000000000194
17. Jucaud V, Shaked A, DesMarais M, Sayre P, Feng S, Levitsky J, et al. Prevalence and Impact of De Novo Donor-specific Antibodies during a Multicenter Immunosuppression Withdrawal Trial in Adult Liver Transplant Recipients. *Hepatology* (2019) 69(3):1273–86. doi:10.1002/hep.30281
18. Ziol M, Poirel H, Kountchou GN, Boyer O, Mohand D, Mouthon L, et al. Intrasinusoidal Cytotoxic CD8+ T Cells in Nodular Regenerative Hyperplasia of the Liver. *Hum Pathol* (2004) 35(10):1241–51. doi:10.1016/j.humpath.2004.06.016
19. Siddiqui I, Selzner N, Hafezi-Bakhtiari S, Marquez MA, Adeyi OA. Infiltrative (Sinusoidal) and Hepatitic Patterns of Injury in Acute Cellular Rejection in Liver Allograft with Clinical Implications. *Mod Pathol* (2015) 28(9):1275–81. doi:10.1038/modpathol.2015.84
20. Sawada T, Shimizu A, Kubota K, Fuchinoue S, Teraoka S. Lobular Damage Caused by Cellular and Humoral Immunity in Liver Allograft Rejection. *Clin Transpl* (2005) 19(1):110–4. doi:10.1111/j.1399-0012.2004.00310.x
21. Neil DAH. CD31 Highlights Platelet-Rich Microthrombi. *Histopathology* (2009) 54(3):387–8. doi:10.1111/j.1365-2559.2008.03215.x
22. Hübscher SG, Adams DH, Buckels JA, McMaster P, Neuberger J, Elias E. Massive Haemorrhagic Necrosis of the Liver after Liver Transplantation. *J Clin Pathol* (1989) 42(4):360–70. doi:10.1136/jcp.42.4.360
23. Halle-Smith JM, Hall LA, Hann A, Hartog H, Perera MTPR, Neil DAH. Seventh Day Syndrome Revisited: Early Recognition of the Clinical Syndrome and an Evolving Understanding of its Etiology. *Front Transpl* (2022) 1:913584. doi:10.3389/frtra.2022.913584
24. Roufosse C, Simmonds N, Clahsen-van Groningen M, Haas M, Henriksen KJ, Horsfield C, et al. A 2018 Reference Guide to the Banff Classification of Renal Allograft Pathology. *Transplantation* (2018) 102(11):1795–814. doi:10.1097/TP.0000000000002366
25. De Gottardi A, Rautou PE, Schouten J, Rubbia-Brandt L, Leebeek F, Trebicka J, et al. Porto-sinusoidal Vascular Disease: Proposal and Description of a Novel Entity. *Lancet Gastroenterol Hepatol* (2019) 4(5):399–411. doi:10.1016/S2468-1253(19)30047-0
26. Terada T, Nakanuma Y, Hoso M, Obata H. Expression of HLA-DR Antigen on Hepatic Vascular Endothelial Cells in Idiopathic portal Hypertension. *Clin Exp Immunol* (1991) 84(2):303–7. doi:10.1111/j.1365-2249.1991.tb08165.x
27. Demetris AJ, Bellamy COC, Gandhi CR, Prost S, Nakanuma Y, Stolz DB. Functional Immune Anatomy of the Liver-As an Allograft. *Am J Transpl* (2016) 16(6):1653–80. doi:10.1111/ajt.13749
28. Feng S, Demetris AJ, Spain KM, Kanaparthi S, Burrell BE, Ekong UD, et al. Five-year Histological and Serological Follow-Up of Operationally Tolerant Pediatric Liver Transplant Recipients Enrolled in WISP-R. *Hepatology* (2017) 65(2):647–60. doi:10.1002/hep.28681
29. O'Leary JG, Cai J, Freeman R, Banuelos N, Hart B, Johnson M, et al. Proposed Diagnostic Criteria for Chronic Antibody-Mediated Rejection in Liver Allografts. *Am J Transpl* (2016) 16(2):603–14. doi:10.1111/ajt.13476
30. Zhang Q, Reed EF. The Importance of Non-HLA Antibodies in Transplantation. *Nat Rev Nephrol* (2016) 12(8):484–95. doi:10.1038/nrneph.2016.88
31. O'Leary JG, Demetris AJ, Philippe A, Freeman R, Cai J, Heidecke H, et al. Non-HLA Antibodies Impact on C4d Staining, Stellate Cell Activation and Fibrosis in Liver Allografts. *Transplantation* (2017) 101(10):2399–409. doi:10.1097/TP.0000000000001853
32. Kim PTW, Demetris AJ, O'Leary JG. Prevention and Treatment of Liver Allograft Antibody-Mediated Rejection and the Role of the "Two-hit Hypothesis". *Curr Opin Organ Transpl* (2016) 21(2):209–18. doi:10.1097/MOT.0000000000000275
33. Neil DAH, Bellamy CO, Smith M, Haga H, Zen Y, Sebagh M, et al. Global Quality Assessment of Liver Allograft C4d Staining during Acute Antibody-Mediated Rejection in Formalin-Fixed, Paraffin-Embedded Tissue. *Hum Pathol* (2018) 73:144–55. doi:10.1016/j.humpath.2017.12.007
34. Kozlowski T, Andreoni K, Schmitz J, Hayashi PH, Nickeleit V. Sinusoidal C4d Deposits in Liver Allografts Indicate an Antibody-Mediated Response: Diagnostic Considerations in the Evaluation of Liver Allografts. *Liver Transpl* (2012) 18(6):641–58. doi:10.1002/lt.23403
35. Kozlowski T, Rubinas T, Nickeleit V, Woosley J, Schmitz J, Collins D, et al. Liver Allograft Antibody-Mediated Rejection with Demonstration of Sinusoidal C4d Staining and Circulating Donor-specific Antibodies. *Liver Transpl* (2011) 17(4):357–68. doi:10.1002/lt.22233
36. O'Leary JG, Demetris AJ, Friedman LS, Gebel HM, Halloran PF, Kirk AD, et al. The Role of Donor-specific HLA Alloantibodies in Liver Transplantation. *Am J Transpl* (2014) 14(4):779–87. doi:10.1111/ajt.12667
37. Stevenson HL, Prats MM, Isse K, Zeevi A, Avitzur Y, Ng VL, et al. Isolated Vascular "V" Lesions in Liver Allografts: How to Approach This Unusual Finding. *Am J Transpl* (2018) 18(6):1534–43. doi:10.1111/ajt.14708

ESOT | Transplant International

ORIGINAL RESEARCH
published: 29 June 2023
doi: 10.3389/ti.2023.11332

Association of Procurement Time With Pancreas Transplant Outcomes in Brain-Dead Donors

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

Department of Transplantation and Liver Surgery, Helsinki University Hospital and the University of Helsinki, Helsinki, Finland

A brain-death-induced cytokine storm damages organs in an organ donor. However, a longer time period between declaration of brain death and organ procurement (procurement interval) is associated with improved outcomes in kidney, liver, heart, and lung transplantation. The aim of this study was to find the optimal procurement interval for pancreas transplantation. Association of procurement interval with pancreas graft outcomes was analyzed using multivariable models adjusted for variables possibly affecting procurement interval and outcomes. Altogether 10,119 pancreas transplantations were included from the Scientific Registry of Transplant Recipients. The median follow-up was 3.2 (IQR 1.01–6.50) years. During the first year, 832 (9.0%) grafts were lost, including 555 (6.0%) within the first 30 days. Longer procurement interval was associated with increased death-censored graft survival in a multivariable model (HR 0.944 95% CI 0.917–0.972, per 10-h increase, $p < 0.001$). A decreasing hazard of graft loss was observed also with 1-year, but not with 30-day graft survival. During 1-year follow-up, 953 (12.1%) patients had an acute rejection, and longer procurement interval was also associated with less acute rejections (OR 0.937 95% CI 0.900–0.976, per 10-h increase, $p = 0.002$) in the multivariable model. In conclusion, longer procurement interval is associated with improved long-term outcomes in pancreas transplantation.

Keywords: graft survival, organ procurement, brain death, pancreas allograft function, early graft loss

OPEN ACCESS

Correspondence:
Ilkka Helanterä
ilkka.helantera@helsinki.fi

Received: *03 March 2023*
Accepted: *12 June 2023*
Published: *29 June 2023*

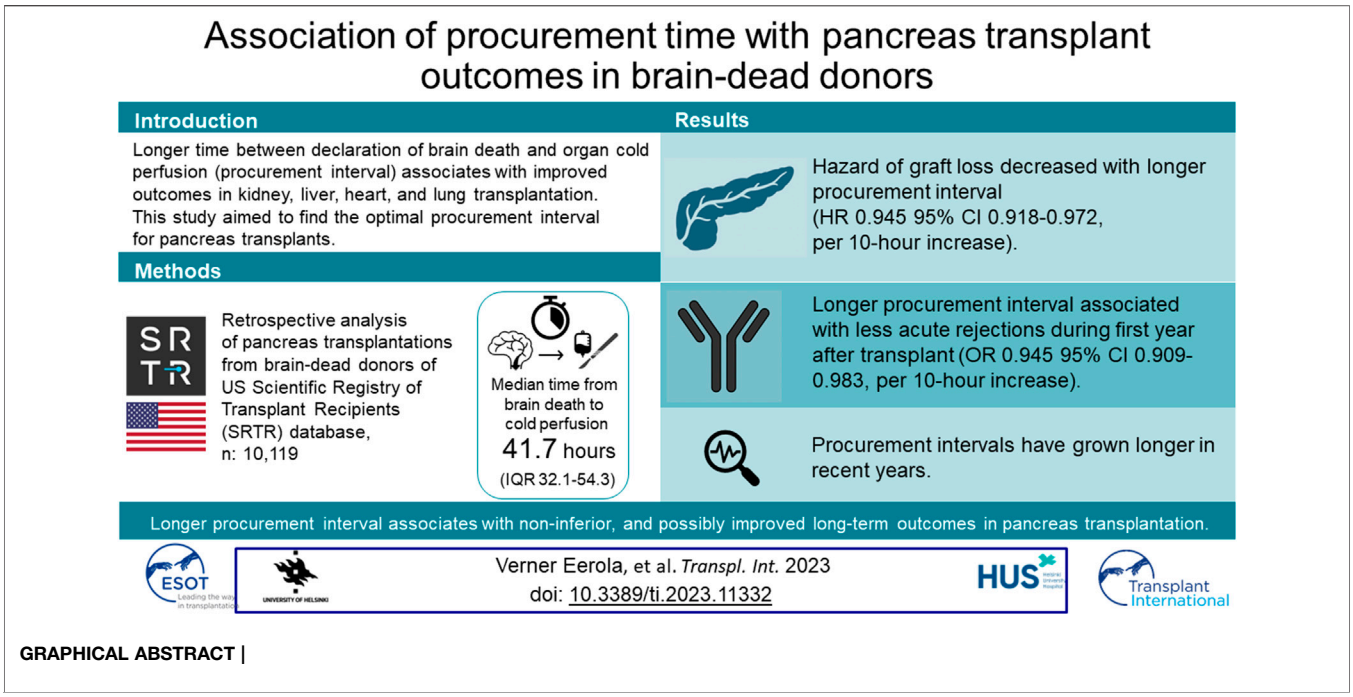
Citation:
Eerola V, Sallinen V, Lempinen M and Helanterä I (2023) Association of Procurement Time With Pancreas Transplant Outcomes in Brain-Dead Donors. Transpl Int 36:11332. doi: 10.3389/ti.2023.11332

INTRODUCTION

As practically all pancreas allografts are obtained from deceased donors, and as there continues to be hesitation regarding using pancreata from donors after circulatory death, roughly 97% of transplanted pancreata are affected by brain death and the resulting "cytokine storm" [1, 2]. The following hemodynamic instability, possible organ sensitization, and blood coagulation disorders have led to cell damage and ischaemia in various organ systems in animal and human studies [3–6].

To minimize this possible damage, some transplant centers have aimed to minimize time to procurement; although, in recent decades, evidence to support continuous organ injury is sparse, and donor losses from hemodynamic instability are rare [7–9]. Interestingly, a publication in

Abbreviations: CPRA, calculated panel-reactive antibodies; DAG, directed acyclic graph; HR, hazard ratio; IQR, interquartile range; OPTN, organ procurement and transplantation network; OR, odds ratio; PAK, pancreas after kidney transplantation; PTA, pancreas transplant alone; SPK, simultaneous pancreas and kidney transplantation; SRTR, scientific registry of transplant recipients.



GRAPHICAL ABSTRACT |

1994 identified that prolonging procurement interval after donor brain death was associated with increased risk of pancreas graft thrombosis and graft loss [10].

In other retrospective studies of kidney, lung, liver, and heart transplantation, waiting before procurement seems beneficial, at least up to 50 h after brain death [11–18]. Organ reactions to brain death can differ and finding the optimal time for procurement of pancreas grafts could improve transplantation logistics and outcomes as pancreas transplants suffer from the highest incidence of non-immunologic complications of all solid organ transplants—often leading to graft loss [1, 19].

This study aimed to analyze the association of procurement interval (time from declaration of brain death to organ cold perfusion) with pancreas allograft survival and acute rejections in a retrospective cohort from the United States.

MATERIALS AND METHODS

This study used data from the Scientific Registry of Transplant Recipients (SRTR). The SRTR data system includes data on all donors, wait-listed candidates, and transplant recipients in the US, submitted by the members of the Organ Procurement and Transplantation Network (OPTN). The Health Resources and Services Administration (HRSA), U.S. Department of Health and Human Services provides oversight to the activities of the OPTN and SRTR contractors.

Pancreas transplantations from brain-dead donors recorded in the SRTR database in the US between January 2010 and September 2021 were included. Follow-up was recovered for all patients from SRTR Standard Analysis Files. Living and donation after circulatory death (DCD) donors were excluded.

This study was approved by the Institutional Review Board of Helsinki University Hospital (HUS/459/2018) and SRTR. The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism" and the Declaration of Helsinki.

Variables

Procurement interval was defined as the time between the declaration of brain death and the start of *in situ* cold perfusion. Brain death is generally diagnosed according to strict criteria, which include having a cause of death, testing for the absence of brainstem-, and pain reflexes, and apnea. Additional testing proceeds from uncertainty of any of the above [20].

The following donor data were gathered: the time of declaration of brain death, start time of cold perfusion in organ procurement surgery, location, donor age, gender, cause of death, body mass index and history of resuscitation, inotrope use, hypertension, and diabetes. The obtained recipient and transplantation data included recipient age, gender, body mass index, history of hypertension, human leukocyte antigen mismatches, calculated panel-reactive antibodies (CPRA), time in dialysis, previous transplants, graft cold ischemia time, organ location, acute rejection episodes before discharge from hospital and before follow-ups, and graft survival during the follow-up.

Endpoints

Death-censored graft survival (measured as centers' reporting to follow-up forms) was chosen as the primary dependent outcome measure. Secondary endpoints were graft survival at 30 days after the operation, graft survival 1 year after the operation and acute rejections during the first year after transplantation. The

definition of pancreas graft failure has evolved from center-specific definitions of either a degree of insulin-independence or C-peptide production, to (from 2018 onwards) a uniform measurement of either: 1. Removal of graft, 2. Patient waitlisted for retransplantation or islet transplantation, 3. Patient death, or 4. Recipients total insulin need is ≥0.5 units/kg/day for 90 consecutive days [1]. This definition has been criticized of having a large cut-off of insulin dosage [21]. In this study a center-specific report of pancreas graft "loss" is accepted as a meaningful endpoint as the cohort is large.

Statistical Analysis

Characteristics of data in the tables are reported with median and interquartile range (IQR) for continuous data and frequencies with percentages for categorical data. Number of patients with missing values are stated in **Table 1**. Tertiles of procurement interval were used to divide the data into groups in **Table 1** for assessment of uneven distribution of variables and allograft quality.

Confounder analysis for the multivariable model was constructed as a directed acyclic graph (DAG) [22]. The DAG (**Figure 1**) presents our team's theory of factors possibly affecting procurement intervals and confounding the associations. Cox proportional hazards models and logistic regression were used for analysis of the association of procurement interval with endpoints and for covariate adjustment. Donor age, donor BMI, donor location as local or shared, stroke as cause of donor death, and recipient's HLA mismatches, retransplantation, and CPRA were identified as confounders. The very few missing variables were estimated to be randomly distributed allowing complete-case analyses.

The associations were modelled as both linear and non-linear. Potentially non-linear associations were checked for by using restricted cubic spline functions between procurement interval and endpoints, as logistic and Cox regression models require the assumption of linearity for continuous data. The associations are modelled in the figures as non-linear for realization of confidence intervals and data visualization. Linear associations were reported using hazard ratio (HR) and odds ratio (OR) with a 95% confidence interval (CI), and significance of non-linearity was reported with *p*-values. All associations were linear in the final results.

Bias was addressed by the DAG, including all transplantations, testing endpoints for non-linearity, confounder adjustment, and sensitivity analyses for including only SPK recipients and for comparing different eras.

The significance level was set at 5% and analyses were carried out as two-tailed. Analyses were performed using R software, utilizing survival and rms packages (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patients

From 2010 to September 2021, 11,919 pancreas transplantations were performed and recorded to the SRTR database in the United States. The final cohort of complete cases included 8,046 simultaneous pancreas and kidney (SPK) transplantations and 2,073 pancreas transplant alone (PTA) or pancreas after kidney (PAK) transplantations, as 311 DCD, 57 transplantations with unreliable procurement interval (>120 h), 449 pediatric recipient transplantations and 983 transplantations with missing brain death time, survival status or follow-up time were excluded.

Median procurement interval was 41.7 h (IQR 32.1–54.3). The distribution is provided in **Figure 2**. **Table 1** summarizes the characteristics of donors, transplantations, patients, endpoints, and missing values. For description of data, the characteristics are divided by procurement interval tertiles into **Table 1** and outcomes into **Table 2**.

Graft Survival

During the median follow-up period of 3.2 years, 1,764 (17.4%) grafts were lost and 986 (9.7%) patients died. Altogether 593 (5.9%) were lost during the first 30 days and 832 (9.0%) during the first year. In a univariable Cox regression model the association of procurement interval with death-censored graft survival was linear (non-linearity $p = 0.88$, **Figure 3**) and significant (HR 0.944 95% CI 0.917–0.972 per 10-h increase, $p < 0.001$). The association remained independent in the adjusted model (HR 0.944 95% CI 0.917–0.972 per 10-h increase, $p < 0.001$). For graphical purposes in the Kaplan-Meier curve (**Figure 4**) the cohort was divided into tertiles for unadjusted interpretation of survival.

In the adjusted model, longer procurement interval was associated with better 1-year graft survival (HR 0.923 95% CI 0.885–0.962 per 10-h increase, $p < 0.001$), but 30-day graft survival was not associated with procurement interval (HR 0.964 95% CI 0.920–1.009 per 10-h increase, $p = 0.118$). As procurement intervals grew longer with time in the cohort, transplant year was added to the 1-year adjusted model as a confounder. In this model, transplant year and the interaction between procurement interval and transplant year were significant, and a longer procurement interval remained significantly associated with improved 1-year graft survival (HR 0.952 95%CI 0.911–0.995 per 10-h increase, $p = 0.030$).

A composite endpoint of graft and patient survival was associated with procurement interval, similarly to death-censored graft survival (**Supplementary Tables S2, S3**).

Acute Rejections

During the study period, 953 (12.1%) patients had an acute rejection episode before 1-year of follow-up. In a univariable logistic regression model the association of procurement interval with acute rejection within 1 year was significant (OR 0.938 95% CI 0.901–0.977 per 10-h increase, $p = 0.002$) and linear (non-linearity $p = 0.96$, **Figure 5**). When adjusted, longer procurement interval was associated with less acute rejections within 1 year (OR 0.937 95% CI 0.900–0.976 per 10-h increase, $p = 0.002$, **Supplementary Figure S1**).

Sensitivity Analyses

Simultaneous Pancreas and Kidney (SPK) Transplantations

When only SPK transplantations were included, the analyzed associations remained equally significant as in the full cohort during different follow-up periods for graft survival and acute

TABLE 1 | Characteristics of donors and recipients of pancreas transplants performed between January 2010 and September 2021 in the US and recorded to the SRTR database and divided to tertiles by procurement interval.

Variable	Median and interquartile range or n (valid %). N: 10,119	Missing (%)	1st n: 3,365 (33.33%) (0–35.25 h)	2nd n: 3,387 (33.35%) (35.25–49.37 h)	3rd n: 3,367 (33.33%) (49.37 h->)
			Median (IQR) or n (%)	Median (IQR) or n (%)	Median (IQR) or n (%)
Donor Age, years	23 (18–29)	0 (0%)	23 (18–30)	23 (18–30)	23 (19–29)
Donor BMI, kg/m^2	23.6 (21.2–26.2)	0 (0%)	23.7 (21.3–26.3)	23.5 (21.1–26.0)	23.7 (21.0–26.3)
Donor, male	7,006 (69.2%)	0 (0%)	2,300 (68.4%)	2,369 (69.9%)	2,337 (69.4%)
Donor Hypertension	448 (4.4%)	43 (0.4%)	156 (4.6%)	160 (4.7%)	132 (3.9%)
Donor Cause of Death, stroke	1,215 (12.0%)	0 (0%)	443 (13.2%)	388 (11.5%)	384 (11.4%)
Donor organ yield[a]	5 (5–6)	0 (0%)	5 (5–6)	5 (5–6)	5 (5–6)
Local donor	7,157 (70.7%)	0 (0%)	2,450 (72.8%)	2,377 (70.2%)	2,330 (69.2%)
Machine perfusion used for SPK kidneys	1,235 (15.4%)	3 (0.0%)	451 (17.3%)	381 (14.1%)	403 (14.7%)
Inotrope use for donor	4,687 (46.3%)	15(0.1%)	1,835 (54.5%)	1,556 (45.9%)	1,296 (38.5%)
≥3 inotropes during procurement	65 (0.6%)	5,665 (56.0%)	40 (1.7%)	19 (1.3%)	6 (0.8%)
Recipient Age, years	42 (35–49)	0 (0%)	42 (35–49)	42 (35–49)	42 (35–49)
Recipient BMI, kg/m^2	25.3 (22.7–28.2)	82 (0.8%)	25.3 (22.6–28.2)	25.2 (22.7–28.0)	25.4 (22.7–28.3)
Recipient Gender, male	5,964 (58.9%)	0 (0%)	1,984 (59.0%)	2,006 (59.2%)	1,974 (58.6%)
Recipient Hypertension	3,554 (81.6%)	5,762 (56.9%)	1,654 (80.6%)	1,153 (82.2%)	747 (82.7%)
Previous Transplants	1,316 (13.0%)	0 (0.0%)	528 (15.7%)	429 (12.7%)	359 (10.7%)
HLA[b] mismatches	5 (4–5)	5 (0%)	5 (4–5)	5 (4–5)	5 (4–5)
Recipient CPRA[c] >20%	1,911 (18.9%)	5 (0%)	599 (17.8%)	642 (19.0%)	670 (19.9%)
Transplant type[d],					
SPK	8,046 (79.5%)	0 (0%)	2,600 (77.3%)	2,700 (79.7%)	2,746 (81.6%)
PAK	970 (9.6%)	0 (0%)	407 (12.1%)	300 (8.9%)	263 (7.8%)
PTA	1,103 (10.9%)	0 (0%)	358 (10.6%)	387 (11.4%)	358 (10.6%)
Follow-up Time, years	3.2 (1.0–6.5)	0 (0%)	5.1 (1.9–8.0)	3.4 (1.1–6.0)	2.0 (0.8–4.3)
Transplant year	2015 (2012–2018)	0 (0%)	2013 (2011–2016)	2016 (2013–2018)	2018 (2015–2020)

[a]*Number of organs donated (kidney, kidney, liver, pancreas, intestine, lungs, heart).*
[b]*Human leukocyte antigen.*
[c]*Calculated panel-reactive antibodies.*
[d]*SPK, simultaneous pancreas and kidney transplantation; PAK, pancreas after kidney; PTA, pancreas transplant alone.*

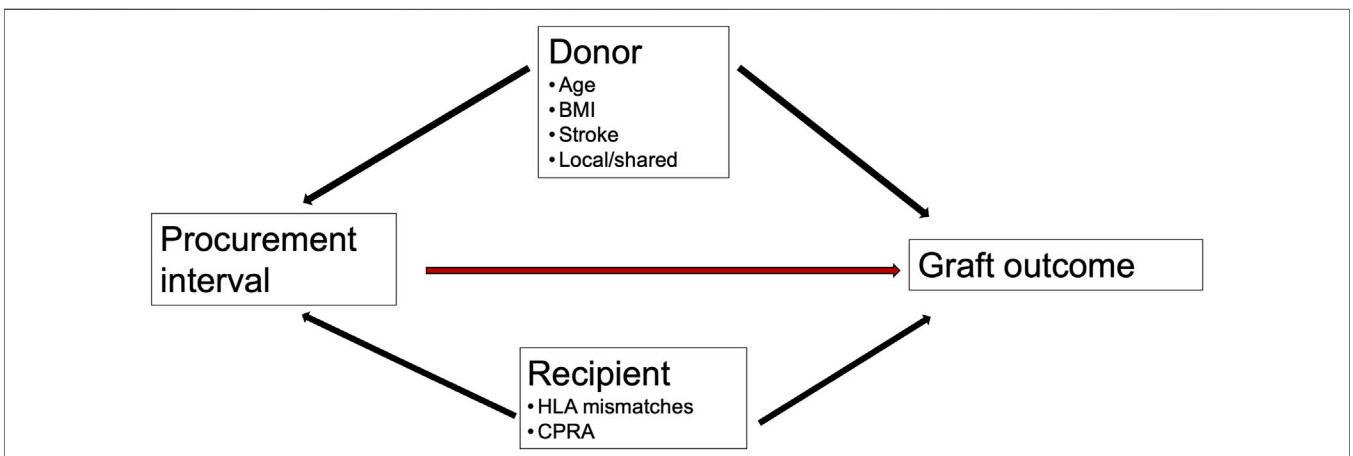


FIGURE 1 | Directed acyclic graph. Confounders of association of procurement interval (time from brain death to organ cold perfusion) with outcomes.

rejections (**Figure 6**). Procurement interval was beneficially associated with 1-year graft survival of SPK-kidneys in the adjusted analyses (HR 0.897 95% CI 0.814–0.989 per 10-h increase, p = 0.029), but not significantly associated with delayed graft function (HR 1.019 95% CI 0.970–1.069 per 10-h increase, p = 0.460, **Supplementary Tables S2, S3**).

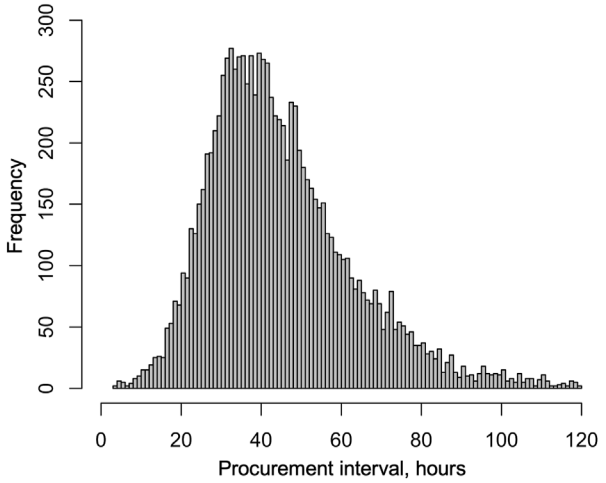


FIGURE 2 | Distribution of procurement intervals in brain-dead donors in the US from January 2010 to September 2021.

Transplant Year

Procurement intervals grew longer during the cohort period (**Table 1**) and thus the cohort was divided in two for an additional sensitivity analysis. Pre-2016 group had 5,115 patients and the post-2016 group included 5,004 patients. The associations of procurement interval with pancreas graft survival in these sub-groups are summarised in **Figure 6**. The association of procurement interval with acute rejections within 1 year was nonsignificant for both time periods when the cohort was divided by 2016 (**Figure 6**).

Inotropes

Donor inotrope use at the start of the procurement operation decreased with longer procurement interval (**Table 1**). Inotrope use was available for 99.8% of the cohort, and was considered on its own (as a surrogate for donor instability) and with procurement interval. In Cox regression univariable analyses, inotrope use was not significantly associated with graft survival (HR 1.070 95% CI 0.972–1.175 per 10-h increase, p = 0.168) or 1-year graft survival (HR 1.109 95% CI 0.968–1.271 per 10-h increase, p = 0.137). For 30-day graft survival the association was slightly significant (HR 1.268 95% CI 1.073–1.499 per 10-h increase, p = 0.005). When the association of procurement interval with outcomes was adjusted with inotrope use the association of procurement interval remained significant (i.e., for 1-year graft survival HR 0.925 95% CI 0.887–0.964 per 10-h increase, $p < 0.001$). The association also remained significant when the interaction between procurement interval and inotrope use was considered (for 1-year graft survival HR 0.873 95% CI 0.822–0.927 per 10-h increase, $p < 0.001$). The association of procurement interval with 30-day graft survival became significant when inotrope use and the interaction with procurement interval were analyzed (adjusted model HR 0.922 95% CI 0.857–0.991 per 10-h increase, p = 0.028).

DISCUSSION

In this study, longer procurement interval was associated with improved long-term pancreas graft survival and fewer rejections within 1 year. Most importantly, a longer procurement interval posed no additional risk.

Potential donors are seldom lost to cardiovascular collapse, possibly due to improved donor management protocols [7–9]. Earlier dogma of fast procurement might have been due to "unstable" donors with undoubtedly worse outcomes when organ perfusion has been compromised. In earlier settings, longer time after brain death may indeed have posed a risk for transplant. However, it has been proposed that if organ perfusion is kept stable, the organs can recover from the first hit of brain death, and are better prepared for cold ischemia (i.e., the two-hit theory first suggested by Kunzendorf et al [13]) as the autonomous and cytokine storm seems to "cool down" in the hours following brain death [23–25].

This is outlined in recent retrospective studies which point to benefit in outcomes from longer procurement intervals in kidneys, livers, hearts, or lungs [11–18]. This study is in concordance with these studies. The slightly improved graft survival associated with longer procurement intervals could reflect the two-hit theory. Other factors possibly outlining this

TABLE 2 | Outcomes of pancreas transplants performed between January 2010 and September 2021 in the US and recorded to the SRTR database, divided to tertiles by procurement interval.

Outcome	Median and interquartile range or n (valid %). *N*: 10,119	Missing (%)	1st *n*: 3,365 (33.33%) (0–35.25 h)	2nd *n*: 3,387 (33.35%) (35.25–49.37 h)	3rd *n*: 3,367 (33.33%) (49.37 h->)
			Median (IQR) or n (%)	Median (IQR) or n (%)	Median (IQR) or n (%)
Graft Loss[a] <30 days[b]	5.9%	0%	6.4%	5.8%	5.4%
Graft Loss[a] <1 year[b]	9.1%	0%	10.2%	9.0%	8.0%
Acute Rejection, Before Discharge	164 (1.6%)	14 (0.1%)	63 (1.9%)	53 (1.6%)	48 (1.4%)
Acute Rejection, First Year[c]	953 (12.1%)	1,388 (15.0%)	368 (13.3%)	329 (12.2%)	256 (10.5%)

[a]*Death-censored graft survival defined as center reporting to follow-up form.*
[b]*Kaplan-Meier estimated survival percentages with standard errors of 0.004 (30-day)-0.005 (1-year).*
[c]*Of 9,280 cases with at least 1 year of follow-up.*

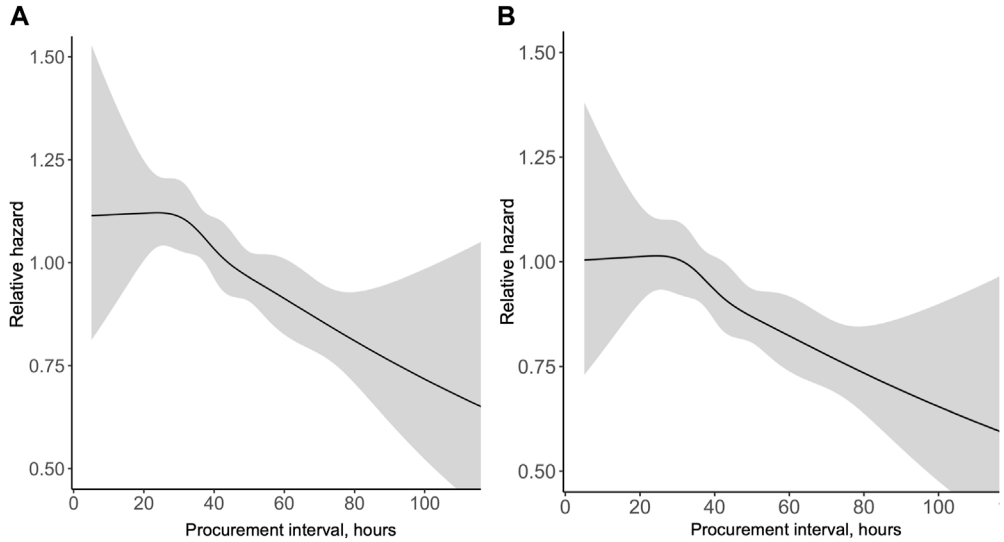


FIGURE 3 | Univariable **(A)** and multivariable **(B)** association of procurement interval with relative hazard of pancreas graft loss [**(A)** Relative to median, **(B)** Relative to minimum interval].

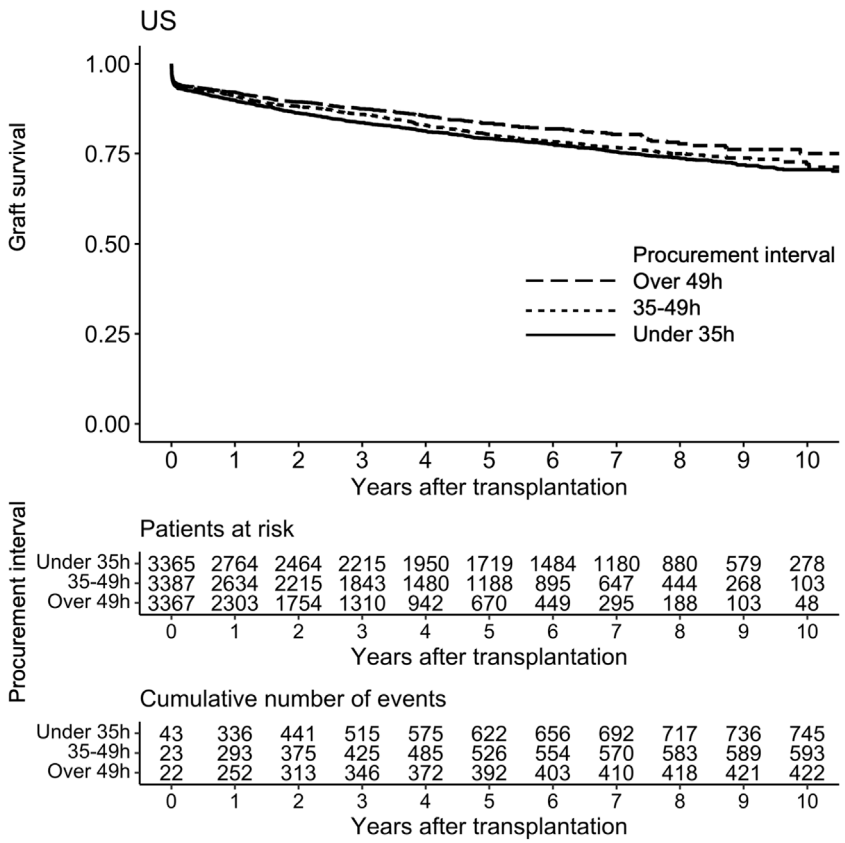


FIGURE 4 | Kaplan-Meier curve of graft survival of pancreas transplant tertiles of procurement interval (time between brain death and cold perfusion).

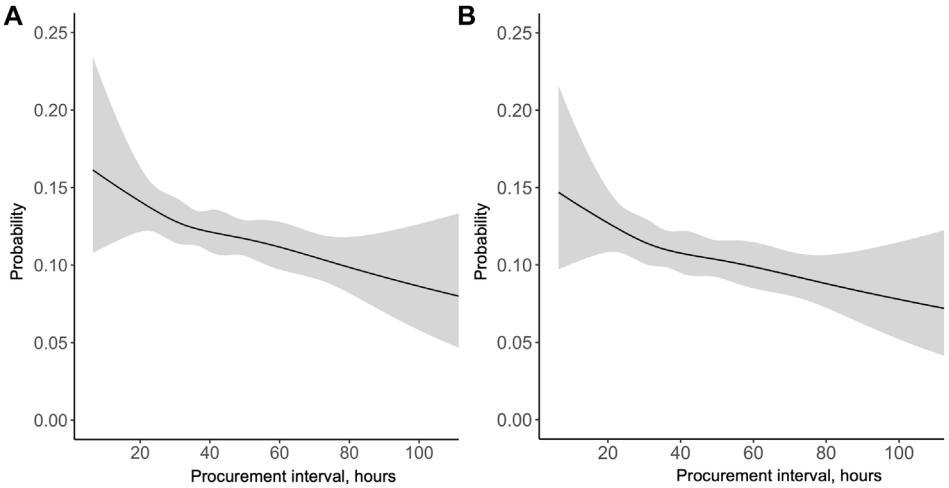


FIGURE 5 | Probability of acute rejection in the first year after pancreas transplantation by procurement interval in the **(A)** univariable and **(B)** multivariable model.

suggested improvement in graft survival could be related to preconditioning initiated by cytokines that activate expression of protective genes upon brain death [26–30]. Earlier studies may have been confounded by fewer multiorgan-donors pooling into shorter brain death times as time-consuming donor testing non-randomly distributes healthier donors (with better outcomes) into longer intervals. This study shows that the phenomenon seems to exist in the healthiest organ donors—typically multiorgan pancreas donors—as well.

Donor inotrope use decreased with procurement interval, which could be expected with cytokine storm cooling and stabilization of the donor. It could also serve as a surrogate marker for instability. Interestingly, in sensitivity analyses the use of inotropes in the management of the organ donor was not significantly associated with pancreas graft outcomes. However, dichotomous inotrope use before procurement operation is probably insufficient as a marker for donor instability. Unfortunately, the use of three or more inotropes was only reported for a few patients.

Acute rejections have not been associated with procurement times in other organs in earlier studies. The reason for this discrepancy remains unclear, but may on one hand be related

Subgroup	Nonlinearity p	Univariable HR	Multivariable HR (95%CI)
Graft survival, SPK only	0.884	0.937	0.939 (0.906-0.972)
1-year graft survival, SPK only	0.701	0.921	0.924 (0.880-0.971)
30-day graft survival, SPK only	0.915	0.952	0.958 (0.908-1.011)
1-year acute rejection, SPK only	0.771	0.930	0.930 (0.886-0.976)
Graft survival <2016	0.375	0.973	0.975 (0.938-1.013)
Graft survival >=2016	0.197	0.933	0.934 (0.888-0.981)
1-year graft survival <2016	0.417	0.978	0.980 (0.925-1.039)
1-year graft survival >=2016	0.058	0.895	0.896 (0.839-0.957)
30-day graft survival <2016	0.336	1.028	1.030 (0.961-1.104)
30-day graft survival >=2016	0.086	0.935	0.936 (0.873-1.003)
1-year acute rejection <2016	0.548	0.981	0.983 (0.928-1.042)
1-year acute rejection >=2016	0.660	0.956	0.957 (0.898-1.020)

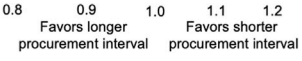


FIGURE 6 | Sensitivity analyses of procurement interval's association per 10-h increase with endpoints in subgroups. Univariable confidence intervals provided in **Supplementary Table S1**.

to different granularity of reporting acute rejections to large registries, and on the other hand relate to variable sensitization of different organs during the process of brain death [31].

Procurement intervals grew longer during the period of the study cohort, and possibly better donor treatment practices during the later years are associated with the better outcomes of longer procurement intervals. Therefore, sensitivity analyses by transplantation year were conducted, which showed the association of a longer procurement interval with improved graft survival to be significant only in recent years, and the association of less acute rejections to dissipate.

The difference in procurement intervals between Europe and the US is notable, which may arise from scheduling procurement during office hours and more time consuming consent obtainment in the United States (Nijboer). Obviously much less concern about longer procurement intervals exists in the United States.

This study is an observational registry analysis and therefore cannot prove causality. Retrospective studies can be susceptible to non-random allocation and confounding, and residual bias, which cannot be completely overcome by multivariable adjustment or sensitivity analyses. However, the use of the multivariable model and non-linear associations, together with the sensitivity analyses, provide greater confidence in the conclusion. The start of the procurement interval was defined by the declaration of brain death, although the exact time or progression of the fatal brain insult cannot be known. Similarly, the urgency of obtaining the diagnosis of brain death and declaration time and thus the start of the interval can vary between different centers and practises. However, this variance should be balanced in a large cohort. Graft failure for pancreas allografts has been uniformly defined in the US only from 2020 onwards, which can also have an influence on our findings. A lack of definition for pancreas graft function could have resulted in variation in reporting complications. Still, a large cohort can alleviate many of these concerns.

A possible selection bias follows from longer procurement times distributing to more recent years. We sought to limit this with sensitivity analyses, which do not point to the effect resulting from better care in the later years, but did show significantly better results with a longer procurement interval only for later years in terms of graft survival, and no significance for acute rejections. Also, other short-term complications, such as graft thrombosis and leaks would have been of interest in this study, but are unfortunately outside the scope of the used registry data.

A concern in optimizing other organs by lengthening the procurement interval is that more pancreas grafts would become edematous or firm and lead to more discard of the pancreas grafts. Before suggesting delaying procurement, information on discard rates with a longer procurement interval would be insightful.

Future studies with randomization by procurement interval would be welcomed but could prove ethically challenging and unwarranted since so many other factors weigh first in organ procurement. Also, studies on how many donors are lost to cardiovascular collapse during the current era of donor management protocols would be of interest. These studies would have clinical implications for transplantation logistics and patient outcomes.

In conclusion, based on this study, pancreas procurement from a brain-dead donor can be postponed if needed, and a longer procurement interval may lead to better outcomes.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because Restrictions apply to the availability of the data based on the current data use agreements with SRTR. Other data are available from the corresponding author upon reasonable request. Requests to access the datasets should be directed to SRTR, srtr@srtr.org.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of Helsinki University Hospital (HUS/459/2018). 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

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

FUNDING

This study was supported by grants from Finnish Kidney Foundation (to VE) and Finska Läkaresällskapet and Helsinki University Research Grants (to VS).

CONFLICT OF INTEREST

IH reports receiving consulting fees from Novartis, Hansa Biopharma, and Takeda, and research funding from MSD and Hansa Biopharma outside the submitted work.

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.

ACKNOWLEDGMENTS

The data reported here have been supplied by the Hennepin Healthcare Research Institute (HHRI) as the contractor for the

Scientific Registry of Transplant Recipients (SRTR). The interpretation and reporting of these data are the responsibility of the author(s) and in no way should be seen as an official policy of or interpretation by the SRTR or the U.S. Government.

REFERENCES

1. Kandaswamy R., Stock P. G., Miller J., Skeans M. A., White J., Wainright J., et al. OPTN/SRTR 2020 Annual Data Report: Pancreas. *Am J Transpl* (2022) 22:137–203. doi:10.1111/AJT.16979
2. Shahrestani S., Webster A. C., Lam V. W. T., Yuen L., Ryan B., Pleass H. C. C., et al. Outcomes from Pancreatic Transplantation in Donation after Cardiac Death: A Systematic Review and Meta-Analysis. *Transplantation* (2017) 101(1):122–30. doi:10.1097/TP.0000000000001084
3. Bos E. M., Leuvenink H. G. D., van Goor H., Ploeg R. J. Kidney Grafts from Brain Dead Donors: Inferior Quality or Opportunity for Improvement? *Kidney Int* (2007) 72(7):797–805. doi:10.1038/SJ.KI.5002400
4. Pratschke J., Weiss S., Neuhaus P., Pascher A. Review of Nonimmunological Causes for Deteriorated Graft Function and Graft Loss after Transplantation. *Transpl Int* (2008) 21:512–22. doi:10.1111/j.1432-2277.2008.00643.x
5. Obermaier R., Dobschuetz E., Keck T., Hopp H. H., Drognitz O., Schareck W., et al. Brain Death Impairs Pancreatic Microcirculation. *Am J Transplant* (2004) 4(2):210–5. doi:10.1046/j.1600-6143.2003.00317.x
6. Dziodzio T., Biebl M., Pratschke J. Impact of Brain Death on Ischemia/reperfusion Injury in Liver Transplantation. *Curr Opin Organ Transplant* (2014) 19:108–14. doi:10.1097/MOT.0000000000000061
7. Salim A., Martin M., Brown C., Belzberg H., Rhee P., Demetriades D. Complications of Brain Death: Frequency and Impact on Organ Retrieval. *Am Surg* (2006) 72(5):377–81. doi:10.1177/000313480607200502
8. Inaba K., Branco B. C., Lam L., Salim A., Talving P., Plurad D., et al. Organ Donation and Time to Procurement: Late Is Not Too Late. *J Trauma* (2010) 68(6):1362–6. doi:10.1097/TA.0b013e3181db30d3
9. Mckeown D. W., Bonser R. S., Kellum J. A. Management of the Heartbeating Brain-Dead Organ Donor. *Br J Anaesth* (2012) 108:96–107. S1. doi:10.1093/bja/aer351
10. Douzdjian V., Gugliuzza K. G., Fish J. C. Multivariate Analysis of Donor Risk Factors for Pancreas Allograft Failure after Simultaneous Pancreas-Kidney Transplantation. *Surgery* (1995) 118(1):73–81. doi:10.1016/S0039-6060(05)80012-1
11. Eerola V., Helanterä I., But A., Lempinen M., Mäkisalo H., Nordin A., et al. The Association of Time to Organ Procurement on Short-And Long-Term Outcomes in Kidney Transplantation. *Clin J Am Soc Nephrol* (2021) 16(3):427–36. doi:10.2215/CJN.11420720
12. Nijboer W. N., Moers C., Leuvenink H. G. D., Ploeg R. J. How Important Is the Duration of the Brain Death Period for the Outcome in Kidney Transplantation? *Transpl Int* (2010) 24(1):14–20. doi:10.1111/j.1432-2277.2010.01150.x
13. Kunzendorf U., Hohenstein B., Oberbarnscheid M., Muller E., Renders L., Schott G. E., et al. Duration of Donor Brain Death and its Influence on Kidney Graft Function. *Am J Transplant* (2002) 2(3):292–4. doi:10.1034/j.1600-6143.2002.20316.x
14. Ergün M., Özdemir-van Brunschot D. M. D., Donders R., Hilbrands L. B., Hoitsma A. J., Warlé M. C. Prolonged Duration of Brain Death Was Associated with Better Kidney Allograft Function and Survival: A Prospective Cohort Analysis. *Ann Transpl* (2019) 24:147–54. doi:10.12659/AOT.913869
15. Guner M., Pirat A., Zeyneloglu P., Karaaslan P., Sevmis S., Colak T., et al. Effect of the Interval between Organ Donor Brain Death and Organ Harvesting on Kidney Graft Function after Transplantation. *Transpl Proc* (2007) 39(4):837–41. doi:10.1016/J.TRANSPROCEED.2007.02.021
16. Jawitz O. K., Raman V., Barac Y., Mulvihill M. S., Moore C., Choi A. Y., et al. Impact of Donor Brain Death Duration on Outcomes after Lung Transplantation. *Ann Thorac Surg* (2019) 108:1519–26. doi:10.1016/j.athoracsur.2019.05.026
17. Jawitz O. K., Raman V., Barac Y. D., Anand J., Patel C. B., Mentz R. J., et al. Influence of Donor Brain Death Duration on Outcomes Following Heart Transplantation: A United Network for Organ Sharing Registry Analysis. *J Thorac Cardiovasc Surg* (2019) 4:1345–53.e2. doi:10.1016/j.jtcvs.2019.04.060
18. Eerola V., Helanterä I., Åberg F., Lempinen M., Mäkisalo H., Nordin A., et al. Timing of Organ Procurement from Brain-Dead Donors Associates with Short- and Long-Term Outcomes after Liver Transplantation. *Transpl Int* (2022) 0:10364. doi:10.3389/TI.2022.10364
19. Harbell J. W., Morgan T., Feldstein V. A., Roll G. R., Posselt A., Kang S. M., et al. Splenic Vein Thrombosis Following Pancreas Transplantation: Identification of Factors that Support Conservative Management. *Am J Transplant* (2017) 17(11):2955–62. doi:10.1111/AJT.14428
20. Greer D. M. Determination of Brain Death. *New Engl J Med* (2021) 385:2554–61. doi:10.1056/NEJMcp2025326
21. Stratta R. J., Farney A. C., Fridell J. A. Analyzing Outcomes Following Pancreas Transplantation: Definition of a Failure or Failure of a Definition. *Am J Transpl* (2022) 22(6):1523–6. doi:10.1111/AJT.17003
22. Suttorp M. M., Siegerink B., Jager K. J., Zoccali C., Dekker F. W. Graphical Presentation of Confoundingin Directed Acyclic Graphs. *Nephrol Dial Transplant* (2015) 30:1418–23. doi:10.1093/ndt/gfu325
23. Danobeitia J. S., Sperger J. M., Hanson M. S., Park E. E., Chlebeck P. J., Roenneburg D. A., et al. Early Activation of the Inflammatory Response in the Liver of Brain-Dead Non-human Primates. *J Surg Res* (2012) 176(2):639–48. doi:10.1016/J.JSS.2011.10.042
24. Contreras J. L., Eckstein C., Smyth C. A., Sellers M. T., Vilatoba M., Bilbao G., et al. Brain Death Significantly Reduces Isolated Pancreatic Islet Yields and Functionality *In Vitro* and *In Vivo* after Transplantation in Rats. *Diabetes* (2003) 52(12):2935–42. doi:10.2337/DIABETES.52.12.2935
25. Pérez López S., Otero Hernández J., Vázquez Moreno N., Escudero Augusto D., Álvarez Menéndez F., Astudillo González A. Brain Death Effects on Catecholamine Levels and Subsequent Cardiac Damage Assessed in Organ Donors. *J Heart Lung Transpl* (2009) 28(8):815–20. doi:10.1016/J.HEALUN.2009.04.021
26. Damman J., Bloks V. W., Daha M. R., Van Der Most J. P., Sanjabi B., van der Vlies P., et al. Hypoxia and Complement-And-Coagulation Pathways in the Deceased Organ Donor as the Major Target for Intervention to Improve Renal Allograft Outcome. *Transplantation* (2015) 99(6):1293–300. doi:10.1097/TP.0000000000000500
27. van der Hoeven J. A. B., Moshage H., Schuurs T., Nijboer M., Van Schilfgaarde R., Ploeg R. J. Brain Death Induces Apoptosis in Donor Liver of the Rat. *Transplantation* (2003) 76(8):1150–4. doi:10.1097/01.TP.0000080983.14161.95
28. Schuurs T. A., Morariu A. M., Ottens P. J., T Hart N. A., Popma S. H., Leuvenink H. G. D., et al. Time-dependent Changes in Donor Brain Death Related Processes. *Am J Transplant* (2006) 6(12):2903–11. doi:10.1111/j.1600-6143.2006.01547.x
29. Weiss S., Kotsch K., Francuski M., Reutzel-Selke A., Mantouvalou L., Klemz R., et al. Brain Death Activates Donor Organs and Is Associated with a Worse I/R Injury after Liver Transplantation. *Am J Transplant* (2007) 7(6):1584–93. doi:10.1111/j.1600-6143.2007.01799.x
30. Michel S. G., Madariaga M. L. L., LaMuraglia G. M., Villani V., Sekijima M., Farkash E. A., et al. The Effects of Brain Death and Ischemia on Tolerance Induction Are Organ-specific. *Am J Transplant* (2018) 18(5):1262–9. doi:10.1111/ajt.14674
31. Ritschl P. V., Ashraf M. I., Oberhuber R., Mellitzer V., Fabritius C., Resch T., et al. Donor Brain Death Leads to Differential Immune Activation in Solid Organs but Does Not Accelerate Ischaemia-Reperfusion Injury. *J Pathol* (2016) 239(1):84–96. doi:10.1002/path.4704

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11332/full#supplementary-material

ESOT | Transplant International

ORIGINAL RESEARCH
published: 21 July 2023
doi: 10.3389/ti.2023.11394

Physical Examination of Potential Deceased Organ and Tissue Donors: An Overview of the European Landscape

Akila Chandrasekar[1], Richard Lomas[1], Jacinto Sánchez-Ibáñez[2], Mar Lomero[3], Arlinke Bokhorst[4], Margarida Ivo Da Silva[5], Esteve Trias[6,7], Alicia Pérez Blanco[8], Beatriz Domínguez-Gil[8] and Marta López-Fraga[3]*

[1]Tissue and Eye Services, NHS Blood and Transplant, Liverpool, United Kingdom, [2]Tissue Establishment and Cryobiology unit, A Coruña University Hospital, Coruña, Spain, [3]European Directorate for the Quality of Medicines and Healthcare (EDQM), Strasbourg, France, [4]National Office for Hemovigilance and Biovigilance (TRIP), Leiden, Netherlands, [5]National Coordination for Transplantation, Instituto Portugues do Sangue e da Transplantacao, Lisbon, Portugal, [6]Hospital Clinic of Barcelona, Barcelona, Spain, [7]Leitat Technological Center, Barcelona, Spain, [8]Organización Nacional de Trasplantes, Ministerio de Sanidad, Madrid, Spain

Physical examination (PE) of donors is essential to identify potential risks to the safety and efficacy of donated organs and tissues and is mandatory in the EU. However, no detailed guidance is available as to how PE should be performed. Health authorities (HA) and health professionals (HP) in member states of the European Committee on Organ Transplantation of the Council of Europe (CD-P-TO) and observer countries completed surveys relating to the regulatory requirements for PE and the professional practice of PE in their countries for organ and tissue donors. The HA survey addressed regulatory aspects, and the HP survey addressed professional practices, training, and respondents' opinions on the value of PE. These surveys revealed significant inter-country variation in the regulatory approach to PE and the performance of PE by professionals. Most respondents opined that PE was important and yielded valuable information in identifying contraindications to donation. There is no consensus at a regulatory or professional level as to how PE should be performed on organ and tissue donors. There is a requirement for agreed best practice guidelines in this area.

Keywords: organ donation, donor assessment, donor screening, physical examination, tissue and cornea donation

OPEN ACCESS

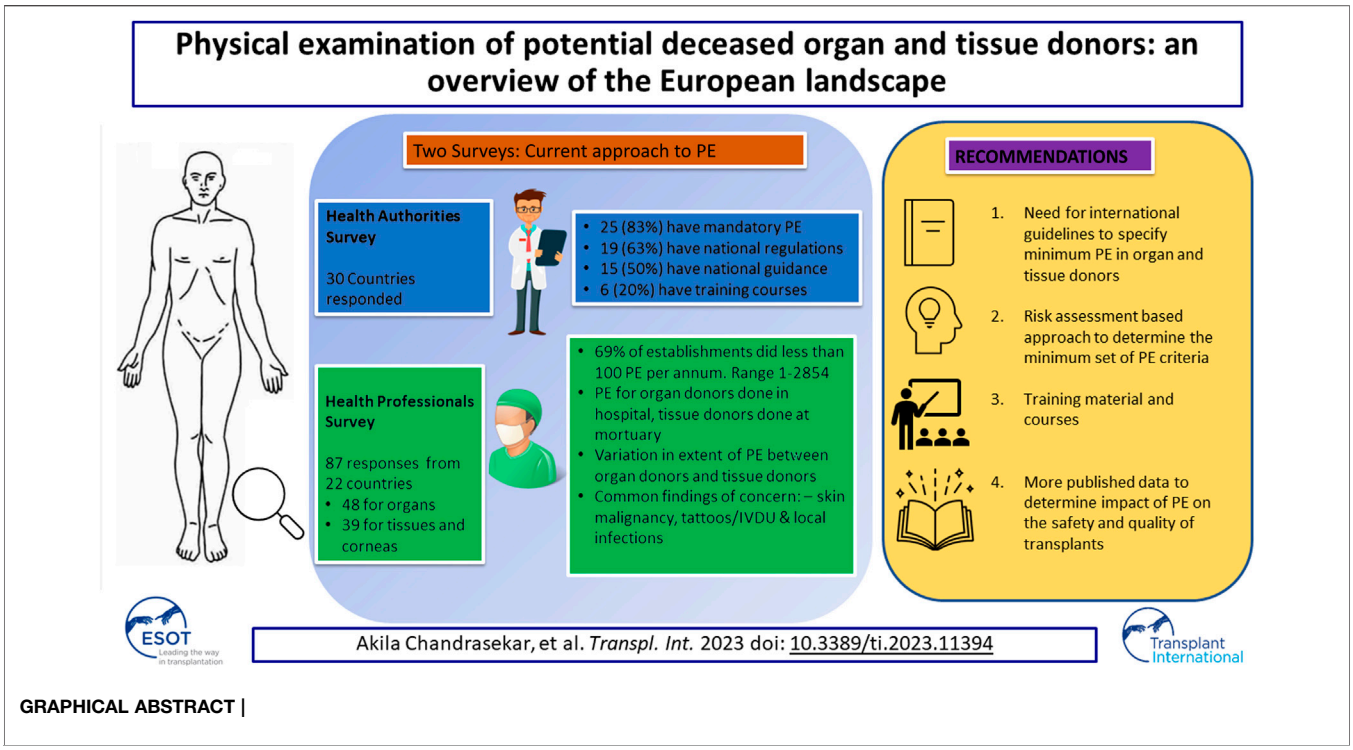
Correspondence:
Akila Chandrasekar
akila.chandrasekar@nhsbt.nhs.uk

Received: *24 March 2023*
Accepted: *30 June 2023*
Published: *21 July 2023*

Citation:
Chandrasekar A, Lomas R, Sánchez-Ibáñez J, Lomero M, Bokhorst A, Ivo Da Silva M, Trias E, Pérez Blanco A, Domínguez-Gil B and López-Fraga M (2023) Physical Examination of Potential Deceased Organ and Tissue Donors: An Overview of the European Landscape. Transpl Int 36:11394. doi: 10.3389/ti.2023.11394

INTRODUCTION

Organizations/establishments that are active in the field of tissue and organ procurement from deceased donors perform a comprehensive donor assessment to mitigate the risk of transmission of infection and disease from donors to recipients and to optimize the quality and safety of donated material in order to maximize the probability of good clinical outcomes. Physical examination (PE) is used in conjunction with review of medical records, medical history obtained from referring professionals, interviews with donor families, information from general practitioners, autopsy reports (if applicable), and screening tests, as an essential part of this comprehensive donor evaluation. It should be noted that PE performed to evaluate the suitability of an individual to donate organs and tissues differs significantly from PE performed on a living individual during a standard medical examination. When considering donation, PE focusses on indications that relate specifically to the safety and quality of donated material rather than indicators of a patient's health.



GRAPHICAL ABSTRACT |

Directive 2006/17/EC (technical requirements for the donation, procurement and testing of human tissues and cells) [1] states in Annex I "Selection criteria for donors are based on an analysis of the risks related to the application of the specific cells/tissues. Indicators of these risks must be identified by physical examination . . . ," and Annex IV states "...in the case of a deceased donor [...] a physical examination of the body must be performed to detect any signs that may be sufficient in themselves to exclude the donor, or which must be assessed in the light of the donor's medical and personal history." and that these findings must be recorded. Similarly, Directive 2010/45/EU (standards of quality and safety of human organs intended for transplantation) [2] states "Information from a potential donor's medical history, physical examination and complementary tests should be collected for the adequate characterization of the organ and the donor."

Thus, at EU level all donor coordinators, organ procurement organizations and tissue establishments (TEs) adhering to these requirements are obliged to perform a documented PE prior to procurement. However, neither directive specifies the content of the PE, who should perform the PE, or how it should be performed and documented. The focus of the PE of a potential tissue or organ donor differs from the medical examination performed on the same individual during admission to hospital as a patient. PE should therefore be performed in all cases of donation of tissues or organs in order to systematically identify evidence of infections or diseases that could be transmitted through organs, tissues and corneas and pose a risk to the transplant recipient, as well as to better assess the quality of the donated substance [3]. The findings of PE complement the comprehensive clinical data collected on each potential donor [4].

The EDQM "Guide to the quality and safety of organs for transplantation" [5] and "Guide to the quality and safety of tissues and cells for human application" [6] provide basic guidance on what to look for in the PE of deceased organ and tissue donors. In general, the objective of PE is to identify physical manifestations of disorders that could be an indication for a condition listed in the exclusion criteria for donation. There are, however, only a very limited number of studies [7] that have evaluated what PE should consist of, how it should be performed, and the added value of physical findings noted in relation to the final donor evaluation.

For this reason, the European Committee on Organ Transplantation of the Council of Europe (CD-P-TO)[1]

[1]The European Committee on Organ Transplantation (CD-P-TO) is the steering committee in charge of organ, tissue and cell donation and transplantation activities at the European Directorate for the Quality of Medicines and Healthcare (EDQM) of the Council of Europe. As of March 2023, the CD-P-TO is composed of 37 members (Albania, Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Republic of Moldova, Montenegro, Netherlands, North Macedonia, Norway, Poland, Portugal, Romania, Serbia, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, Turkiye, Ukraine, and the United Kingdom) and 20 observers [Armenia, Canada, Georgia, Israel, United States, Council of Europe Committee on Bioethics, DTI Foundation, European Association of Tissue and Cell Banks, European Commission, European Eye Bank Association, European Society for Blood and Marrow Transplantation, European Society for Organ Transplantation, European Society of Human Reproduction and Embryology, Eurotransplant, Scandiatransplant, South-Europe Alliance for Transplants, The Transplantation Society, United Network for Organ Sharing, World Health Organization (WHO), and the World Marrow Donors Association].

conducted a survey to determine the current practices for performing PE and the regulatory approach in Council of Europe (CoE) member states, with a view to developing guidance on best practice.

METHODS

Two different English language survey questionnaires were prepared: one to investigate the regulatory framework (legally binding and non-legally binding documents) governing the PE of organ and tissue donors to be completed by health authorities (HAs) and the second to capture the actual practices of health professionals (HPs) performing PE of organ and tissue donors. A CD-P-TO working group was set up to develop and validate the questions for the surveys. Both final survey questionnaires were piloted in a limited number of member states, using CD-P-TO representatives as contact points, to evaluate their content and the use of English language terminology prior to wider circulation. The final surveys were circulated to member states via their CD-P-TO representatives, who disseminated them nationally. Responses were gathered electronically using an online survey tool (Surveymonkey.com). Prior to analysis, all responses were reviewed to remove any invalid responses—for example, instances where respondents had submitted a partial response prior to providing a full response at a later date. In total, five incomplete responses from the HP survey were removed.

The HA survey consisted of 10 questions (Q), Q1–Q5 to gather country-specific general/demographic information and Q6–Q10 to collect information on regulations in place related to the practice of PE (**Supplementary Datasheet S1**). The HP survey contained 35 questions divided into five sections. The first 6 (Q1–Q6) concerned respondents' profiles and the next 10 (Q7–Q16) were related to their organization, followed by questions relating to their practices for performing PE (Q17–Q25), training (Q26–Q31) and a final section on their personal views about the value of PE (Q32–35). A distinction was made between responses from those who perform PE on organ donors, multi-tissue donors and cornea donors because of the differences in donor selection criteria. The same HP questionnaire was used in all cases, but a separate response was requested for each type of donor (**Supplementary Datasheet S2**). Some organizations that responded to the HP survey were responsible for PE for different types of donors. In these cases, the organization was asked to submit a separate response for each type of donor.

RESULTS

The surveys were distributed among representatives in CD-P-TO member [37] and observer [5] countries. Seven member countries (Albania, Latvia, Malta, North Macedonia, Norway, Turkiye and Ukraine) and one observer country (United States) did not respond to either the HA or the HP survey, and one observer country (Armenia) was excluded from the analysis after responding that they did not currently have a deceased donor program. In total, 33 of 42 countries (79%) responded to one or both of the surveys as shown in **Table 1**.

Multiple responses to the HP survey were received from some countries, as discussed below.

HA Survey

Thirty responses (70% response rate) were received for the HA survey (**Table 1**); 83% of respondents, representing 25 countries, declared that PE is mandatory in their countries for either organs or tissues, or for both. However, only 63% (19 countries) have national regulations related to PE. Fifteen of the 30 respondents (50%) have national guidance documents related to PE; however, 55% (16 countries) reported that they do not have a uniform template/model (form) in their country to record the findings of the PE. The majority of respondents (21 countries, 70%) noted that they had no specific training course covering aspects related to the PE of tissue donors.

HP Survey

There were 87 responses from 22 countries for the HP survey, 48 related to organ donors, 16 to tissue donors, and 23 to cornea donors, as shown in **Table 1**. More than half of respondents (46, 54%) identified themselves as donor/transplant coordinators, 19 (22%) as medical directors/assistant directors/Responsible Persons, 11 (13%) as transplant surgeons, 9 (11%) had other job titles, such as retrieval team leader or member, and 2 did not provide their job title. They had various roles in the organ or tissue donation and transplantation pathway as shown in **Figure 1** (some had multiple roles).

The survey included responses from organizations/establishments involved in one or more activities. Some organizations (29, 33%) had responsibilities for both organ/tissue procurement and for processing and banking as a TE. In total, 119 responses were received from 87 individual respondents, with 71 (82%) from organ/tissue procurement organizations and hospitals responsible for donor consent, medical history, and procurement, 42 (48%) from TEs responsible for procurement, processing, storage, and distribution and 6 (7%) from TEs who have agreements with external organizations to perform procurement. These organizations/establishments facilitated between 1 and 2,854 donations in one calendar year. These responses were categorized into establishments performing <100 PEs, 101–500 PEs and >501 PEs (**Table 2**). The majority (69%) of responding establishments performed <100 PEs per year. PE of organ donors was always (100%) done in a hospital setting, whereas PE of tissue donors was mainly (74%) done in a mortuary setting. All of the PEs of organ donors and most of those of tissue and cornea donors are done by those with medical or nursing qualifications. A small proportion (14%) of PE of tissue and cornea donors are done by individuals with non-medical/nursing qualifications (**Table 2**).

In 2019, 60 (71%) HPs who responded had performed one or more PEs and 25 (29%) had not performed any; 2 respondents did not answer this question (**Table 2**). Four respondents (8%) reported that total body CT scan is routinely performed as part of the PE of organ donors and 21 (44%) that is done in selected

TABLE 1 | CD-P-TO PE survey responses.

Country	HA	Organ	Tissue	Cornea
Austria	1	2	2	2
Belgium	1	1		
Bulgaria	1	1	1	1
Croatia	1	2		
Cyprus	1			
Czech Republic		3		
Denmark	1			
Estonia	1	2		
Finland		2		
France	1	1		1
Germany	1	0	1	2
Greece		1		
Hungary	1	2		1
Ireland	1			
Italy	1	5	3	4
Lithuania	1	2		
Luxembourg	1			1
Moldova	1	1	2	
Montenegro	1			
Netherlands	1			
Poland	1	1		
Portugal	1	7	1	4
Romania	1			
Serbia	1	1		
Slovak Republic	1	3	1	
Slovenia	1			
Spain	1	4	2	4
Sweden	1	1	1	1
Switzerland	1	3		1
United Kingdom	1	3	2	1
Canada	1			
Georgia	1			
Israel	1			
Total	30	48	16	23

cases, for example, donors aged over 50 and donors with suspected malignancy. Routine use of CT scan was not reported in any tissue or cornea donor responses, with only 11% reporting use in selected cases (**Table 2**).

Carrying Out PE

The most common response for time taken to complete the PE was 5–15 min, reported in 42% of responses for organ donors and 53% of responses for tissue and cornea donors (**Table 2**). There were differences between organ, tissue, and cornea donors in terms of the number of persons present (**Table 3**), techniques used (**Table 4**) and the PE process (**Table 5**). The responses in the HA survey were compared with those of the HP survey to determine whether there is a variation in PE practice existing between countries with and without national guidelines. Using lymph node palpation in organ donors as a comparator, in countries with guidelines, 63% of responses reported that they always palpated lymph nodes and 33% reported that they sometimes did this, compared to 42% and 33% in countries without national guidelines.

For cornea donors, in 58% of responses PE was done by a single person, in comparison to 43% for tissue donors and 23% for organ donors. For tissue and cornea donors, visual inspection was always done as part of the donor PE. Auscultation and percussion are not applicable because they are not possible in deceased tissue and cornea donors unless done during organ donation assessment prior to death. In total, 58% (25/43) of respondents for organs and 79% (29/36) of tissue and cornea respondents reported identifying anomalies during PE that prevented donation from proceeding at that point, or that had resulted in subsequent rejection of the procured organs/tissues. Evidence of suspected malignancy—the three most common being melanoma, abnormal lymph nodes, and breast lesion—was the main reason that prevented organ donation, skin lesion/tattoo/evidence of IV drug use were the main reasons that prevented tissue donation and corneal infection/scar/ulcer were the main reasons that prevented cornea donation. Options available for escalation should an abnormal finding be detected are shown in **Table 6**. Practical issues that form barriers to performing a detailed PE of deceased tissue and cornea donors are shown in **Figure 2**, the most commonly reported being rigor mortis.

Training

Almost all (82 of 87) respondents answered the question relating to training; 52 (63%) reported that they had received some kind of specific training in how to perform a donor PE. In this group, the training was primarily "on the job" practical training delivered by colleagues. Other respondents reported that training was provided by external bodies from outside of their organization. Training was delivered mainly by practical simulation, reading the SOP and/or visual presentations. Most respondents considered their training as either "very" or "extremely" valuable (**Table 7**).

General Opinion

The views of the respondents on the value of and reasons for PE are shown in **Tables 8–10**. Opinions varied depending on the type of donor that was evaluated: 16% of HPs performing PEs of tissue or cornea donors considered that PE was not valuable or slightly valuable, compared to only 2% of HPs performing PEs of organ donors.

DISCUSSION

There are very few published articles relating to PE of deceased donors [7], and this is the first multi-national survey to date that has explored current practices for performing PE of potential organ, tissue, and cornea donors, soliciting feedback from both HAs and HPs. Responses to the HA survey showed that, while PE is mandatory in the majority (83%) of countries, many respondents reported that there were no nationally mandated standards or protocols for performing PE. This indicates that the performance of PE could vary between establishments and that the outcome of the donor selection process may differ within the same country. It was also evident from responses to the HP survey that there was intra-country variation in the practices for performing PE on organ and tissue donors, however, there are

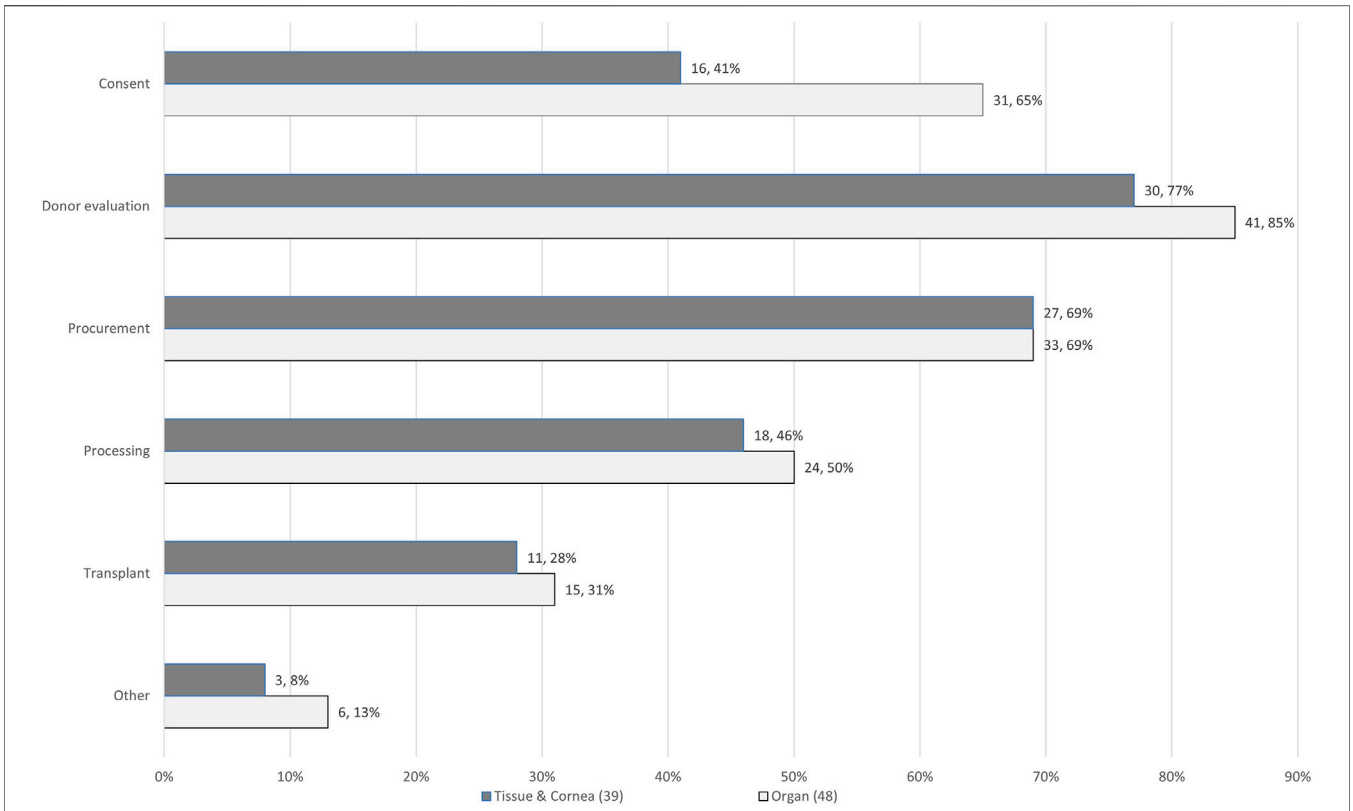


FIGURE 1 | Roles of HPs (87 respondents) completing the survey.

insufficient individual responses from different countries to draw firm conclusions in this area. Without established protocols or guidelines it is also difficult for HAs to assess whether organs or tissues meet quality standards. In order to safeguard donor selection outcomes, it is therefore necessary to establish uniform protocols for PE for different types of donors. An international body, such as the EDQM in collaboration with HAs and HPs, could play a key role in developing standardized protocols for PE based on the analysis of available national standards, relevant literature and data, relevant risk factors that indicate rejection criteria and limitations that are present for deceased donors.

Responses to the HP survey came from a wide range of organizations of different types and sizes, which was reflected in the number of PEs performed by each organization as a whole, and by individuals completing the survey. This broad spread of responses gives a valuable insight into real-life practices. For analysis, responses relating to organ donors were compared with responses relating to tissue and cornea donors, although any clear differences between responses for tissue and cornea donors are also highlighted and discussed. A previous national survey carried out in Australia [8] was targeted at organ and tissue coordinator nurses, while our survey was open to anyone performing PEs. For both organs and tissues/corneas, more than 2/3 of responses were from individuals with a medical background.

When reviewing the responses relating to PE techniques, taking into account all responses, the results of this survey are broadly comparable to the aforementioned Australian survey. However, if the separate responses relating to organ and tissue/cornea donors are considered, there are clear differences: while observation is performed consistently in almost all donors, palpation is done in only 58% of tissue donors compared to 88% of organ donors, and auscultation and percussion are rarely performed for tissue/cornea donors because it does not give relevant information post-mortem. This would explain the observed tendency for PE of tissue/cornea donors to take a shorter amount of time than PE of organ donors (**Table 2**). There are differences between organ and tissue/cornea donors in the frequency with which different types of examination methods are used (**Table 5**). This is not surprising, and probably reflects the different circumstances under which PE is performed for these types of donors, such as whether the PE is done pre- or post-mortem, and the number of individuals present to perform PE. For example, where a PE is being performed by a single person, as is common with corneal donors, it is not practical to turn a donor and examine the dorsal surface. Similarly, if PE is performed post-mortem after rigor mortis has set in, as is often the case with tissue and cornea donors, techniques such as opening and examining the oral cavity or palpating the lymph nodes may be impractical. However, even for organ donors, there was no consistent approach regarding the examination methods used. The extent to which these differences lead to differences in the quality and safety of the final organ or tissue remains to be investigated. It

TABLE 2 | Details of activity in the organizations/establishments.

		Organ (O) (48)	Tissue/Cornea (TC) (39)
When is PE routinely performed in the organization/establishment?	During/after donor medical assessment	46 (96%)	12 (31%)
	Prior to procurement (after refrigeration)	0	25 (64%)
	Not performed in our establishment	2 (4%)	2 (5%)
Number of deceased donor PEs performed in 2019 in the organization/ establishment (Range: 1–2,854) No response (6): O:3 + TC:3	Low (1–100)	37 (82%)	19 (53%)
	Medium (101–500)	4 (9%)	12 (33%)
	High (501 and above)	4 (9%)	5 (14%)
Number of HPs who performed PEs in the organization/establishment in 2019 (Range: 1–320) No answer (12): O:6 + TC:6	Low (1–10)	22 (52%)	22 (67%)
	Medium (11–100)	15 (36%)	10 (30%)
	High (101 and above)	5 (12%)	1 (3%)
Number of PEs performed in 2019 by HPs completing the survey questionnaire (Range: 0–630) No answer (2) O:1 + TC:1	Not performed	9 (19%)	16 (42%)
	1–100	36 (77%)	18 (47%)
	Above 101	2 (4%)	4 (11%)
Describe the setting where the donor PE is performed No answer (1) TC:1	Hospital setting (ICU/operating theatre)	48 (100%)	19 (50%)
	Hospital mortuary	11 (23%)	28 (74%)
	Forensic department	1 (2%)	9 (24%)
	Other	1 (2%)	4 (10%)
Who performs the donor PE in your establishment?	HP in charge of the donor (GP, hospital physician, nurse, etc.)	32 (67%)	12 (32%)
	Organ or tissue coordinator	34 (71%)	18 (47%)
	Professional from the procurement team of the TE	12 (25%)	24 (63%)
	Pathologist/forensic examiner	6 (13%)	8 (21%)
	Other	0	1 (3%)
Basic qualifications of the HP performing the donor PE No answer (1) TC:1	Medical	32 (67%)	26 (68%)
	Nursing	16 (33%)	7 (18%)
	Graduate (e.g., science degree) or similar professional qualifications	0	3 (8%)
	Other (please specify)	0	2 (6%)
Does your establishment/organization use total body CT scan as a routine examination for tissue/organ donors? No answer (1) TC:1	Yes, always	4 (8%)	0
	Yes, in selected cases	21 (44%)	4 (11%)
	No, not used	23 (48%)	34 (89%)
Time to complete PE No answer (10) O:5, TC:5	Less than 5 min	2 (5%)	7 (20%)
	5–15 min	18 (42%)	18 (53%)
	16–30 min	14 (32%)	5 (15%)
	31–60 min	6 (14%)	2 (6%)
	More than 60 min	3 (7%)	2 (6%)

TABLE 3 | No of people present to perform PE on an individual donor.

	1	2	3	>3	Total responses	No response
Organ	10 (23%)	22 (51%)	5 (12%)	6 (14%)	43	5
Tissue	6 (43%)	7 (50%)	0 (0%)	1 (7%)	14	2
Cornea	11 (58%)	7 (37%)	0 (0%)	1 (5%)	19	4
Total	27 (36%)	36 (48%)	5 (7%)	7 (9%)	76	11

TABLE 4 | Techniques used in performing PE.

	Organ donors (44)		Tissue/cornea donors (36)	
	Yes	No (or NA)	Yes	No (or NA)
Observation	43 (98%)	1 (2%)	36 (100%)	0
Auscultation	27 (64%)	15 (36%)	2 (6%)	31 (94%)
Palpation	37 (88%)	5 (12%)	19 (58%)	14 (42%)
Percussion	17 (41%)	24 (59%)	3 (10%)	30 (90%)

should be noted that non-invasive internal scanning performed in organ donors can add value to the PE, but it is not a substitute for visual inspection to note external findings.

Where an abnormal finding was identified during PE, respondents performing PE of organ donors much more frequently reported that they had options for further investigation, such as biopsy for histopathological investigation, other non-invasive examinations such as CT, MRI, or X-ray, or obtaining a second opinion from a colleague (**Table 6**). The most likely reasons for this difference are the clinical setting in which the PE is performed, and the risk-benefit profile of organ and tissue/cornea transplantation. It was notable that taking of biopsies was less common with cornea donors than with tissue donors. This perhaps reflects the fact that most malignancies are not a contraindication for cornea

TABLE 5 | PE Process: When donor PE is performed, do you?

	Organ (46)				Tissue/Cornea (35)			
	Always	Sometimes	Never	Total	Always	Sometimes	Never	Total
Open and examine the oral cavity	21	15	6	42	7	12	13	32
	50%	36%	14%		22%	38%	40%	
Inspect/examine the genital area	31	9	1	41	21	5	7	33
	76%	22%	2%		64%	15%	21%	
Turn the donor to examine the back	28	12	1	41	16	8	10	34
	68%	29%	3%		47%	24%	29%	
Palpate the lymph nodes	23	13	5	41	8	6	18	32
	56%	32%	12%		25%	19%	56%	
Palpate the breast tissue	23	11	6	40	7	6	19	32
	58%	27%	15%		22%	19%	59%	
Palpate the abdomen	28	6	8	42	7	8	17	32
	67%	14%	19%		22%	25%	53%	
Check for evidence of intravenous drug use	40	1	0	41	33	1	1	35
	98%	2%			94%	3%	3%	

TABLE 6 | What options are available to you in your practice if you identify an abnormal finding?

	Organ (43)		Tissue (14)		Cornea (21)		Tissue and cornea (35)	
	No.	%	No.	%	No.	%	No.	%
Document the findings and proceed/stop	33	77	14	100	19	90	33	94
Ask a colleague to examine the donor for a second opinion	33	77	8	57	7	29	15	43
Phone a senior colleague from your team and describe your findings to obtain advice	26	60	9	64	11	52	20	57
Take a photograph and send it to an external expert (e.g., skin specialist)	23	53	4	29	7	33	11	31
Take a biopsy for histopathology examination	31	72	8	57	4	19	12	34
Other tests or non-invasive examinations (CT, MRI, Xray)	35	81	1	7	2	10	3	9
Review medical notes and/or contact general practitioner	33	77	10	71	11	52	21	60
Other (please provide details)	5	12	2	14	1	5	3	9

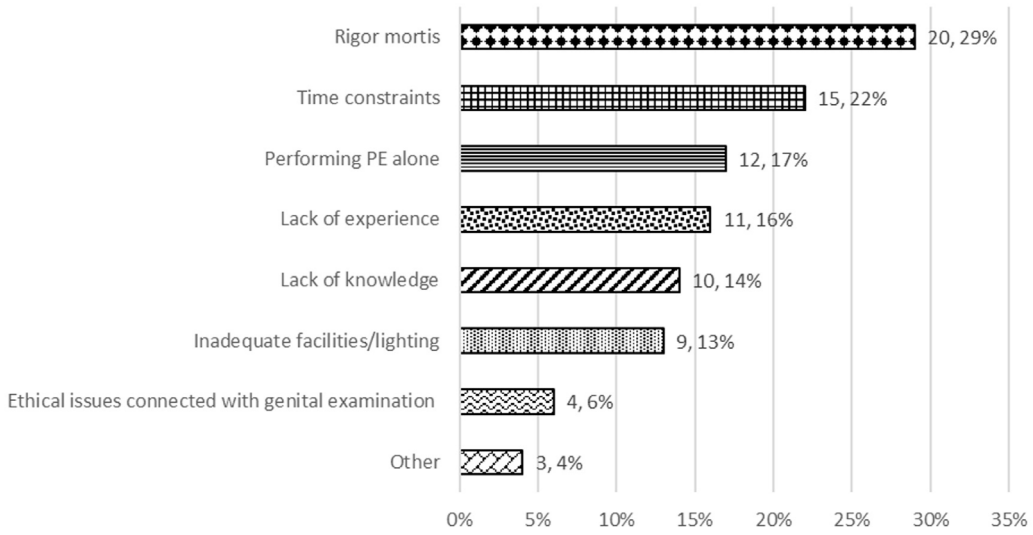


FIGURE 2 | For deceased tissue and cornea donors only: common issues in your practice that are barriers to performing a detailed PE.

donation. One retrospective study on potential tissue donors [9] reported that quickly identifying and taking biopsies of suspicious lesions without needing to interpret the findings to determine donor eligibility at the time of procurement could be beneficial for the time management of procurement teams. Of 561 biopsies taken from abnormal findings identified in the PE during the

TABLE 7 | Training.

		Organ (O)	Tissue/ Cornea (TC)
Have you received any specific training in how to perform a donor PE for organ/tissue/cornea donors?	Yes	26 (58%)	26 (70%)
No response (5): O:3 + TC:2	No	19 (42%)	11 (30%)
If you have received training, when did this take place	During my degree studies	9 (30%)	5 (18%)
No response (29): O:18 + TC:11	Provided by external bodies outside the organization	11 (37%)	10 (36%)
	Before starting to work in my establishment (during induction, including theory)	6 (20%)	9 (32%)
	Case-by-case training by another colleague during my working practice	20 (67%)	16 (57%)
	Other	4 (13%)	3 (11%)
How was the training delivered?	Reading the SOP	14 (47%)	16 (59%)
No answer (30): O:18 + TC:12	PowerPoint presentation	12 (40%)	15 (56%)
	eLearning course	6 (20%)	7 (26%)
	Practical simulation	24 (80%)	21 (78%)
	Other	4 (13%)	4 (15%)
Did the training include how to document PE findings?	Yes	17 (57%)	22 (81%)
No answer (30): O:18 + TC:12	No	13 (43%)	5 (19%)
Describe the value of the training for your daily work?	Extremely valuable	14 (47%)	16 (62%)
No answer (31): O:18 + TC:13	Very valuable	9 (30%)	7 (27%)
	Moderately valuable	4 (13%)	3 (11%)
	Slightly valuable	1 (3%)	
	Not valuable at all	2 (7%)	
Competency	Training updates (How often? Please provide details)	11 (67%)	19 (73%)
No answer (31): O:18 + TC:13	Audit	9 (30%)	7 (27%)
	Peer-review practice	13 (43%)	11 (42%)
	Task-based training using SOPs	10 (33%)	10 (38%)
	Other	5 (17%)	

TABLE 8 | Opinion of respondents on the value of the PE in the evaluation of deceased donors.

	Organ (43)		Tissue (15)		Cornea (22)		Tissue and cornea (37)	
	Number	%	Number	%	Number	%	Number	%
Extremely valuable	17	40%	10	67%	6	27%	16	43
Very valuable	17	40%	3	20%	7	32%	10	27
Moderately valuable	8	18%	0		5	23%	5	14
Slightly valuable	1	2%	1	7%	3	14%	4	11
Not valuable at all	0		1	7%	1	5%	2	5%

TABLE 9 | Top 3 most important reasons selected for doing a PE prior to organ and tissue/cornea donation.

	Organ (43)		Tissue and cornea (37)	
	No.	%	No.	%
To identify the cause of death	13	30	5	14
To identify potential medical contraindications	39	91	29	78
To exclude high-risk individuals (e.g., social risks)	34	79	31	84
To confirm information available from other sources	24	56	18	49
To comply with regulations and guidelines	12	28	19	51
Transplant centers are interested in the donor PE	9	21	1	3
Not important, as the PE is of limited value for tissue donors, including cornea donors	0		4	11%

study period (January 2005 to March 2010), the results showed that the tissue did not need to be rejected in 552 (98.4%) cases; the procured tissue from only 9 (1.6%) donors was discarded due to the biopsy results (five for malignancy and four for infection).

In general, the most common abnormal findings reported in PE of tissue donors related to superficial skin findings, such as suspicious injection marks or skin lesions, and for cornea donors, corneal lesions. This is consistent with the

TABLE 10 | On a scale of 1–10, with 1 indicating no importance and 10 indicating extreme importance, what is the value of abnormal findings in the donor PE in prevention of donor recipient disease transmission (safety) or graft quality?

	Mean score
Tissue/Cornea donors: Donor-recipient transmission	7.4 (29)
Tissue/Cornea donors: Graft Quality	6.3 (30)
Organ donors: Donor-recipient transmission	9.0 (27)
Organ donors: Graft Quality	6.2 (25)

Figures given as mean score with number of responses in brackets.

PE techniques used for these types of donor, as discussed earlier. It is also consistent with the observations reported in the systematic review [7], where the authors found that almost all articles discussing PE findings that may pose higher risk included findings such as jaundice, tattoos, body piercing, nonmedical injection sites, signs of sexually transmitted infections, scars, oral thrush, and skin lesions, all of which can be identified by visual inspection during a PE.

A significant proportion (37%) of the HP respondents doing PE had not received specific training, higher than was reported in the Australian survey (23%). Of those who received training, 82% felt it was extremely or very valuable. It is important to define the content of training and competency assessment programs taking into consideration the limitations of doing PE after rigor mortis. It is also essential to agree upon the minimum set of physical signs to assess during PE. For example, Van Wijk et al. [10] used a risk assessment-based approach based on the Failure Mode and Effects Analysis model. In their study, 106 signs that could be identified in PE were scored on different criteria, considering available control measures specified in the EU Tissue and Cell Directive [1] or other sources. They proposed risk management procedure to identify minimal necessary content of PE in potential tissue donors and suggested that signs of advanced infection with HIV, hepatitis B/C and syphilis can be omitted, since these contraindications will be detected by the required serological testing. When further defining these issues, the limitations for performing PE should also be taken in consideration, e.g., when only one person is performing the procurement, as is common with cornea donors, and is unable to turn the donor.

Despite the discussed limitations of PE in deceased donors, the majority of respondents (75%) felt that it very or extremely valuable, with the identification of potential medical contraindications to donation and the exclusion of high-risk individuals given as the most important reasons for performing PE. For both organ and tissue/cornea donors, a similar level of importance was accorded to the value of PE for evaluating graft quality, whilst responders for organ donation placed a higher level of importance on the value of PE for preventing donor to recipient disease transmission.

Donor to recipient disease transmission remains a fortunately rare event following organ or tissue transplantation. In EU members states, there is a requirement of establishing a system for the reporting and management of Serious Adverse Reactions and Events imposed by Directive 2010/53/EU, and this is reiterated in the EDQM guides. The Notify Library [11] was established by the World Health Organisation and the Italian National Transplant Centre, with the collaboration of the EU funded project SOHO V&S (Vigilance and Surveillance of Substances of Human Origin and serves to collate reports of adverse events resulting from transplant/transfusion of medical products of human origin. It is imperative that these events are reported and systematically audited.

The limitations of this study must be acknowledged. Firstly, a response to the HA survey was received from only 34 (81%) of the CD-P-TO member and observer countries surveyed. Responses to the HP survey were received from 22 (52%) of the countries surveyed, with some countries submitting multiple responses. The survey was circulated to HPs via CD-P-TO representatives of their countries, therefore responses received may not have included all organisations undertaking PE. The profile of the respondents also varied. The outcomes therefore may not represent a systematic response. It should also be considered that survey was performed to determine the requirement for and practice of PE; it does not attempt to make any determination regarding the best practice of PE.

CONCLUSION

This is the first survey that has analyzed the differences in the PE between deceased organ and tissue/cornea donors. The HP survey highlighted wide variations in practice and the HA survey demonstrated the absence of international standards in this area. It is likely that the variations in practice demonstrated in this survey are due to discrepancies in training and education, and the lack of standardized guidelines. We strongly suggest that international guidelines be developed to specify the minimum requirements for PE in organ and tissue donors, to be accompanied by appropriate training materials.

Given the limited published literature, it is difficult to determine the added value and effectiveness of PE in contributing to the safety and quality of organs and tissue grafts for clinical use. A risk assessment-based approach, similar to that described by van Wijk et al. [10], could be useful for developing a minimum set of physical assessment criteria, and practical guidelines. More published data relating to the impact of PE on donor deferral would certainly be of value. More importantly, the survey has demonstrated the need to differentiate PE of organ donors from PE of tissue and cornea donors, and to apply a risk-based approach when developing guidance. One size does not fit all!

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

AC proposed the concept of the study and designed the study with JS-I and ML. RL designed the online surveys and analyzed the results with AC. The first draft of the manuscript was written by AC and RL with input from JS-I and ML. All authors provided feedback on the study and questionnaire design and reviewed and commented on drafts of the manuscript. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11394/full#supplementary-material

REFERENCES

1. EUR-Lex. *Commission Directive 2006/17/EC of 8 February 2006 Implementing Directive 2004/23/EC of the European Parliament and of the Council as Regards Certain Technical Requirements for the Donation, Procurement and Testing of Human Tissues and Cells* (2006). Available from: http://data.europa.eu/eli/dir/2006/17/oj (Accessed October 28, 2022).
2. EUR-Lex. *Directive 2010/45/EU of the European Parliament and of the Council of 7 July 2010 on Standards of Quality and Safety of Human Organs Intended for Transplantation* (2010). Available from: http://data.europa.eu/eli/dir/2010/53/oj (Accessed October 28, 2022).
3. Beele H, Van Wijk M, Bokhorst A, Van Geyt C. Physical Examination of the Potential Tissue Donor, what Does Literature Tell Us? *Cell Tissue Bank* (2009) 10(3):253–7. doi:10.1007/s10561-008-9116-x
4. Van Geyt C, Van Wijk M, Bokhorst A, Beele H. Physical Examination of the Potential Tissue Donor, what Do European Tissue banks Do? *Clin Transpl* (2010) 24(2):259–64. doi:10.1111/j.1399-0012.2009.01089.x
5. EDQM, Council of Europe. *Guide to the Quality and Safety of Organs for Transplantation*. 8th ed. Strasbourg: EDQM (2022). Available from: https://freepub.edqm.eu/publications/PUBSD-88/detail (Accessed February 20, 2023).
6. EDQM, Council of Europe. *Guide to the Quality and Safety of Tissues and Cells for Human Application*. 5th ed. Strasbourg: EDQM (2022). Available from: https://freepub.edqm.eu/publications/AUTOPUB_17/detail (Accessed February 20, 2023).
7. Holloway JAC, Ranse K, Currie M, Jamieson M, van Haren F. An Integrative Review of the Physical Examination Performed on Deceased Potential Organ and Tissue Donors. *Prog Transpl* (2019) 29(1):84–94. doi:10.1177/1526924818817029
8. Holloway JAC, Ranse K, Bail K, Jamieson M, Van Haren F. Practice and Attitudes of Donor Coordinator Roles Regarding Physical Examination of Potential Organ and Tissue Donors in Australia. *Transpl Direct* (2019) 5(8):e471. doi:10.1097/TXD.0000000000000906
9. Singh S, Blevins MB, Wakeman M, Bergevin M. The Utility of Recovery Biopsies in Determining Donor Suitability. *Cell Tissue Bank* (2012) 13(4):565–7. doi:10.1007/s10561011-9272-2
10. van Wijk MJ, van Geyt C, Laven ABH, Beele H, Bokhorst AG. Physical Examination of Potential Tissue Donors: Results of a Risk Management Procedure to Identify the Critical Elements of the Physical Examination. *Cell Tissue Bank* (2012) 13(4):547–63. doi:10.1007/s10561-011-9270-4
11. NOTIFY Library. *Notify Project* (2023). Available from: https://www.notifylibrary.org (Accessed May 23, 2023).

ESOT | Transplant International

BRIEF RESEARCH REPORT
published: 05 July 2023
doi: 10.3389/ti.2023.11110

Assessing Tissue Transmission of Hepatitis C Virus From Viremic Donor to Seronegative Kidney Transplant Recipients: A Case Series

Antonio Franco[1]*, *Carla Gosalvez*[2,3], *Adelina Gimeno*[2], *Migul Trigueros*[3], *Noelia Balibrea*[1] *and Francisco Javier Perez Contreras*[1]

[1]Department of Nephrology, Hospital General Universitario Dr. Balmis, Alicante, Spain, [2]Department of Microbiology, Hospital General Universitario Dr Balmis, Alicante, Spain, [3]Department of Pathology, Hospital General Universitario Dr Balmis, Alicante, Spain

The transmission of hepatitis C virus from viremic donors to seronegative recipients of kidney transplantation is well documented. Pre-transplant administration of direct-acting antivirals prevents viremia, but the seroconversion rate is high. We studied the transmission of the virus through the transplanted tissue by determining viral RNA in 15 kidneys from 8 deceased viremic donors, 5 males and 3 females aged 52.3 ± 15 years. HIV positive donors and active intravenous drugs abusers were discarded to avoid possible window periods in the virus transmission. Recipients, 9 males and 6 females aged 52.7 ± 18 years, were treated with glecaprevir/pibrentasvir for 8 weeks and received immunosuppression with thymoglobulin, tacrolimus, sirolimus and prednisone. Hepatitis C Virus was detected in 9 of the 15 histological samples analyzed but viremia was detected in no recipient at day 1 and 7 post–transplantation and 12 weeks after the treatment. However, 13 of the 15 recipients had seroconverted within 1 month. In conclusion, Hepatitis C virus was detected in a significant proportion of tissue of kidney grafts from viremic donors, but treatment with direct-acting antivirals avoids the transmission of the virus from donor to recipient. Then Donor pools should be expanded.

Keywords: kidney transplantation, hepatitis C virus, viremic donor, transmission, seronegative recipient

OPEN ACCESS

***Correspondence:**
Antonio Franco
franco_ant@gva.es

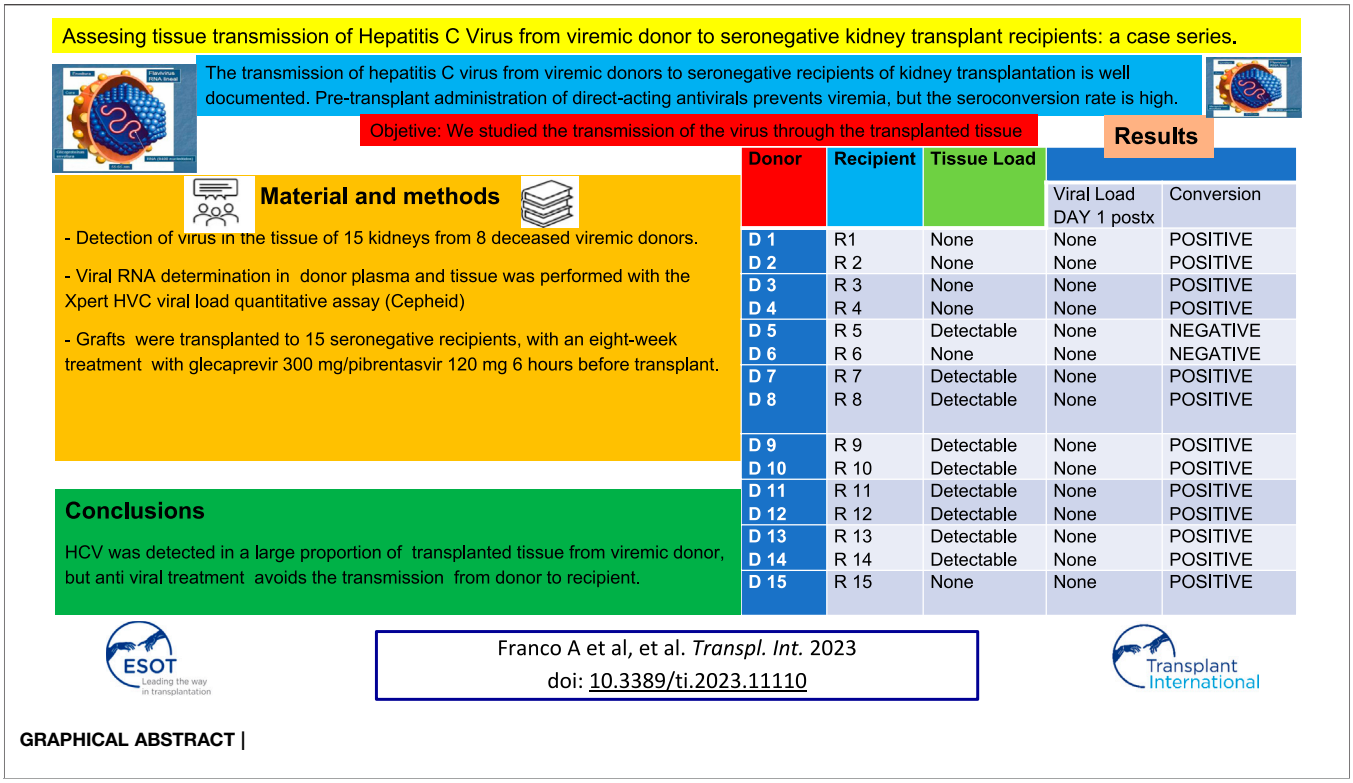
Received: *06 December 2022*
Accepted: *15 June 2023*
Published: *05 July 2023*

Citation:
Franco A, Gosalvez C, Gimeno A, Trigueros M, Balibrea N and Perez Contreras FJ (2023) Assessing Tissue Transmission of Hepatitis C Virus From Viremic Donor to Seronegative Kidney Transplant Recipients: A Case Series. Transpl Int 36:11110. doi: 10.3389/ti.2023.11110

INTRODUCTION

Transmission of hepatitis C virus (HCV) from viremic donor to seronegative recipient via kidney transplantation is well documented [1, 2]. Administration of direct-acting antivirals (DAA) to the recipient pre-transplant prevents post-transplant viremia and possibly transmission of infection [3, 4]; however, starting treatment in the post-transplant period prevents neither viremia nor the consequent transmission [5, 6]. In addition to detecting viral particles in different extrahepatic compartments, including kidney tissue, several studies have also observed viral replication [7–13], which could enable the transmission of the infection in the absence of viremia through the tissue of kidney grafts.

The aim of this study was to assess HCV transmission through the tissue of kidney grafts from viremic donors to seronegative recipients treated with DDA.



GRAPHICAL ABSTRACT |

MATERIALS AND METHODS

Study Design and Population

This study is a case series on adult kidney transplant recipients from HCV viremic donors undergoing transplantation from March 2018 and December 2019 in the Hospital General of Alicante (Spain).

We determined the presence of HCV in the tissue of 15 kidneys from 8 deceased viremic donors (one graft was not included in the study because it was transplanted to a seropositive recipient). HIV positive donors and active intravenous drugs abusers were discarded to avoid possible window periods in the virus transmission.

Procedures

The grafts were transplanted to 15 seronegative recipients, who started an eight-week course of treatment after breakfast with glecaprevir 300 mg/pibrentasvir 120 mg 6 h prior to transplantation.

Plasma viral load was determined in donors within a few hours prior to transplantation, and in recipients at day 1 and 7 post–transplantation and 12 weeks after the treatment. Antibodies against HCV were measured in the serum of transplant patients 1 month after transplant.

Tissue samples were extracted in the operating room for diagnostic purposes using an 18G biopsy needle (Biopince full core biopsy instrument). Tissue cylinders were 18 mm–22 mm long and 1 mm thick. They were paraffinized for pre-transplantation histological study and then deparaffinized for virus detection. The deparaffinized tissue was washed three times with sterile saline and homogenized with 2 mL of sterile distilled water.

Viral RNA determination in donor plasma and tissue was performed with the Xpert HVC viral load quantitative assay (Cepheid), which has a quantification range of 10 IU/mL to 4–6 IU/mL. The Xpert HCV Viral Load Assay is a polymerase chain amplification (PCR)-based assay with no need for batch processing of samples and with a result available in 105 min. This test was used in donor samples because it allows quick results on demand. In biopsy samples it was used due to its higher sensitivity. This method employs a reverse transcription polymerase chain reaction technique (RT-PCR) that uses fluorescence to detect and quantify the RNA of HCV genotypes 1 to 6.

In recipients, HCV viral load was determined using RT-PCR with the HCV COBAS AmpliPrep/COBAS TaqMan quantitative technique, v2.0, whose lower detection limit is 15 IU/mL. This test is designed for batch testing of multiple specimens within a run. Antibodies against HCV were measured in the serum of transplant patients using the Roche Diagnostics Elecsys Anti-HCV II assay.

Ethic Issues

The present study was performed n accordance with the Declaration of Helsinki and was consistent with the Principles of the Declaration of Istambul on Organ Trafficking and Transplant Tourism. This study received approval from the Hospital of Alicante Review Board (record 2021-04, 28 April 2021).

TABLE 1 | Donor and recipient characteristics and outcome at 3 years.

	Donor	Recipient
Age (years)	52.3 ± 15	52.7 ± 18
Male gender	5/8: 62.5%	9/15: 60%
Virus Genotipe 1a	4	na
1b	3	
3	1	
Blood Group A	2	4
B	1	2
AB	0	0
0	5	9
End stage cause	na	
glomerular disease		2
interstitial nephritis		4
Lupus		2
polycystic disease		3
Hypertension		1
diabetes nephropathy		3
Graft. Survival at 3 years	na	93.3%
Patient survival at 3 years	na	93.3%

Na, No applicable.

The strategy of kidney transplant from viremic and non-viremic hepatitis C positive donors into negative recipients was conducted under The Spanish consensus document coordinated by the National Transplant Organization (ONT) [14]. All recipients had signed an informed consent when were enroled in the waiting list.

Statistical Analysis

This is a descriptive analysis. Categorical data are shown as absolute numbers and their frequencies, and metrics used for continuous data with relative dispersión.

RESULTS

Table 1 shows donor and recipient demographics and post-transplant outcomes at 3 years.

All recipients received immunosuppression with thymoglobulin, tacrolimus, sirolimus and prednisone.

Table 2 shows the plasma viral load in the donors pre-transplant and in the recipients on day 1 and 7 post-transplant and 12 weeks after treatment, the serological results against HCV at 1 month, and the detection of HCV in the transplanted tissue. Plasma viral load in all donors was significant, and in recipients it was undetectable on days 1 and 7 post-transplantation and 12 weeks after treatment. However, 13 of the 15 recipients had seroconverted within 1 month. HCV was detected in 9 of the 15 histological samples analyzed. In all cases where viral RNA was detected, the concentration was less than 10 IU per tissue sample.

All recipients completed treatment with DAA without reported adverse events or treatment interruptions. We do not modify DAA posology during treatment. The interactions of DAA with Tacrolimus and sirolimus were managed with the monitorization of their plasmatic levels.

DISCUSSION

In our study, seroconversion occurred in most kidney transplant recipients with viremic donors, despite the absence of viremia in the immediate post-transplant period (**Table 2**). These results are consistent with previous reports [3, 4] and indicate that seronegative recipients had contact with the virus.

The presence of HCV in the extrahepatic tissue of viremic patients has scarcely been studied, but several authors have described it in gastrointestinal mucosa [7], lymph nodes [8, 9], bone marrow [10], the central nervous system [11], the pancreas, heart, and even kidney [12, 13]. In addition, some authors have observed the presence of negative-polarity RNA in extrahepatic tissue [7, 12, 15], which acts as an intermediary in the replication of the virus, confirming the possibility of transmitting the active infection [15].

The HCV detection rate in different extrahepatic tissues is low, and obviously lower than that detected in the liver [15] according to the authors, these findings suggest that levels of HCV infection and replication at the extrahepatic level are low, but still sufficient to transmit infection [15].

In our study, we detected viral RNA in most of the donor kidney tissue samples by means of a quantitative RT-PCR, Xpert HVC Viral Load (Cepheid). We did not look for evidence of viral replication, which has already been demonstrated by other authors [7, 12, 15]. In all cases where viral RNA was detected, the concentration was under 10 IU/mL.

We chose this technique because it is easy to use and very sensitive, with a detection limit below 10 IU/mL. As Wrobleswska et al. [16] have described, the volume of the samples determines the sensitivity of the technique. The number of viral particles in extrahepatic tissues is very low [13, 16], and in our case, the samples were small.

The technique used is commercially available and validated for serum and plasma. Currently, there is no validated technique for tissue [13].

Yan et al. assessed HCV by *postmortem* RT-PCR of extrahepatic tissue in nine patients with severe viral hepatitis, detecting its presence in the kidney, pancreas, heart, and intestine in all cases, and its replication by negative-polarity RNA in some, including the kidney; authors concluded that HCV can infect and replicate in various extrahepatic tissues [12].

Gelpi et al. [17] reported an experience similar to ours, but with different and perhaps complementary results. These authors ruled out the presence of occult HCV infection by kidney graft biopsy in three seronegative recipients who had not taken DAA when receiving the transplant from seropositive, non-viremic donors. This is a different case from our study, in that our recipients did receive treatment because donors were viremic. Taken together, these studies provide a more complete picture on the silent transmission of HCV through kidney tissue, with no transmission occurring with non-viremic donors and transmission in the case of viremic donors [18].

These data are corroborated by Shike et al. [13], who studied HCV in the kidney tissue of 14 seropositive donors rejected for transplantation. In the 11 donors with a positive plasma load, investigators detected viral RNA by RT-PCR in 16 of the 22 kidneys (72.7%), data similar to ours. However, in the

TABLE 2 | Plasma viral load in donors pre-transplant and in recipients on day 1 and 7 post-transplant and 12 weeks after treatment, serological results against virus at 1 month, and detection of virus in the transplanted tissue.

Donor	Recipient	Donor plasma viral Load (UI/mL)	Recipient plasma viral load (UI/mL)			Kidney viral load	Seroconversion
			Day 1	Day 7	12 weeks post transplant		
D 1	R1	4,000	UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	POSITIVE
D 2	R 2		UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	POSITIVE
D 3	R 3	470,000	UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	POSITIVE
D 4	R 4		UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	POSITIVE
D 5	R 5	1,400,000	UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	DETECTABLE	NEGATIVE
D 6	R 6		UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	NEGATIVE
D 7	R 7	1,300,000	UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	DETECTABLE	POSITIVE
D 8	R 8		UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	DETECTABLE	POSITIVE
D 9	R 9	180,000	UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	DETECTABLE	POSITIVE
D 10	R 10		UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	DETECTABLE	POSITIVE
D 11	R 11	1,300,000	UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	DETECTABLE	POSITIVE
D 12	R 12		UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	DETECTABLE	POSITIVE
D 13	R 13	4,500,000	UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	DETECTABLE	POSITIVE
D 14	R 14		UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	DETECTABLE	POSITIVE
D 15	R 15	450,000	UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	UNDETECTABLE	POSITIVE
D16	R 16						

remaining three donors with a negative plasma load, none of the six grafts showed viral RNA, in keeping with Gelpi's results [17]. Compared to our study, Shike et al.'s [13] has the advantage of having a large amount of tissue on which the virus could be detected, since it included kidneys rejected for transplantation instead of on graft cylinders for transplantation.

In our group, viral RNA was detected in D5 kidney tissue, but not in D6, from the same donor (**Table 2**), Shike's experience is similar to ours, as they also detected the virus in the tissue of one but not both kidneys in two of their donors [13]. These authors [13], like us, attribute the cases of non-detection of the virus in tissue to the low viral load present in extrahepatic tissues [16] and to the lower sensitivity of the tissue detection technique [13].

Shike et al. [13] establish a correlation between the amount of viral load in the donor's plasma and the presence of virus in the tissue, concluding that the higher the viral plasma load, the more likely the virus will be detected in tissue and the larger the amount. Data from our study seem to confirm this conclusion: in the cases where no virus was detected in tissue (D1, D2, D3, D4, D15), the viral plasma load remained under 500,000 IU/mL (**Table 2**).

The viral concentration in tissue was below the technique's linear range. However, the detection of viral RNA in most grafts suggests that HCV could be detected in all grafts from viremic donors if the tissue samples were larger, as in the study by Shike [13].

In spite of detecting HVC in plasma and tissue from the donors, the treatment with DAA avoided the transmission of the virus from donor to recipient. As a matter of fact, recipients from viremic donors starting DAA pre-transplant do not present viral replication at any time or only a very low one, but starting DAA after transplant show viral transmission in all the cases. Then, starting DAA pre-transplant should be mandatory [18].

The main strength of our study is the detection of the virus in both, donors through plasma viral load and tissue detection, and in recipients, through plasma viral load and subsequent serology. In contrast, Shike's study was limited to detection in the donor [13].

The study's primary limitation is the lack of a validated technique for tissue. The second limitation is the small amount of tissue available for virus detection.

In conclusion, HCV was detected in a large proportion of transplanted tissue from viremic donors, which could facilitate its transmission to recipients, but treatment with DAA avoids the transmission of the virus from donor to recipient. Then Donor pools should be expanded.

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 Ethics Comitee Hospital Dr. Balmis Alicante, Spain. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

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

REFERENCES

1. Levitsky J, Formica RN, Bloom RD, Charlton M, Curry M, Friedewald J, et al. The American Society of Transplantation Consensus Conference on the Use of Hepatitis C Viremic Donors in Solid Organ Transplantation. *Am J Transpl* (2017) 17(11):2790–802. doi:10.1111/ajt.14381
2. Bowring MG, Shaffer AA, Massie AB, Cameron A, Desai N, Sulkowski M, et al. Center-Level Trends in Utilization of HCV-Exposed Donors for HCV-Uninfected Kidney and Liver Transplant Recipients in the United States. *Am J Transpl* (2019) 19(8):2329–41. doi:10.1111/ajt.15355
3. Durand CM, Bowring MG, Brown DM, Chattergoon MA, Massaccesi G, Bair N, et al. Direct-acting Antiviral Prophylaxis in Kidney Transplantation from Hepatitis C Virus-Infected Donors to Noninfected Recipients an Open-Label Nonrandomized Trial. *Ann Intern Med* (2018) 168:533–40. doi:10.7326/M17-2871
4. Franco A, Moreso F, Sancho A, Esforzado N, Paul J, Llorente S, et al. Protocol for Optimizing the Use of Kidneys from Donors with Seropositivity for Hepatitis C Virus in Seronegative Recipients. *Transpl Proc* (2021) 53:2655–8. doi:10.1016/j.transproceed.2021.09.013
5. Reese PP, Abt PL, Blumberg EA, Van Deerlin VM, Bloom RD, Potluri VS, et al. Twelve-month Outcomes after Transplant of Hepatitis C–Infected Kidneys into Uninfected Recipients a Single-Group Trial. *Ann Intern Med* (2018) 169:273–81. doi:10.7326/M18-0749
6. Molnar MZ, Nair S, Cseprekal O, Yazawa M, Talwar M, Balaraman V, et al. Transplantation of Kidneys from Hepatitis C-Infected Donors to Hepatitis C-Negative Recipients: Single center Experience. *Am J Transpl* (2019) 19:3046–57. doi:10.1111/ajt.15530
7. Russelli G, Pizzillo P, Iannolo G, Barbera F, Tuzzolino F, Liotta R, et al. HCV Replication in Gastrointestinal Mucosa: Potential Extra-hepatic Viral Reservoir and Possible Role in HCV Infection Recurrence after Liver Transplantation. *PLoS One* (2017) 12(7):e0181683. doi:10.1371/journal.pone.0181683
8. Pal S, Sullivan DG, Kim S, Lai KK, Kae J, Cotler SJ, et al. Productive Replication of Hepatitis C Virus in Perihepatic Lymph Nodes *In Vivo*: Implications of HCV Lymphotropism. *Gastroenterology* (2006) 130:1107–16. doi:10.1053/j.gastro.2005.12.039
9. Antonucci F, Cento V, Sorbo MC, Manuelli MC, Lenci I, Sforza D, et al. HCV-RNA Quantification in Liver Bioptic Samples and Extrahepatic Compartments, Using the Abbott RealTime HCV Assay. *J Virol Methods* (2017) 246:1–7. doi:10.1016/j.jviromet.2017.04.001
10. Radkowski M, Kubicka J, Kisiel E, Cianciara J, Nowicki M, Rakela J, et al. Detection of Active Hepatitis C Virus and Hepatitis G Virus/GB Virus C Replication in Bone Marrow in Human Subjects. *Blood* (2000) 95:3986–9. doi:10.1182/blood.v95.12.3986
11. Radkowski M, Wilkinson J, Nowicki M, Adair D, Vargas HE, Ingui C, et al. Search for Hepatitis C Virus Negative-Strand RNA Sequences and Analysis of Viral Sequences in the Central Nervous System: Evidence of Replication. *J.Virol.* (2002) 76:600–8. doi:10.1128/jvi.76.2.600-608.2002
12. Fu-Ming M, An-Shen C, Hao F, Zhao X, Gu C, Zhao L, et al. Hepatitis C Virus May Infect Extrahepatic Tissues in Patients with Hepatitis C. *World J Gastroenterol* (2000) 6:805–11. doi:10.3748/wjg.v6.i6.805
13. Shike H, Kadry Z, Imamura-Kawasawa Y, Greene W, Riley T, Nathan HM, et al. Hepatitis C Virus (HCV) RNA Level in Plasma and Kidney Tissue in HCV Antibody-Positive Donors: Quantitative Comparison. *Clin Transpl* (2018) 32(9):e13358. doi:10.1111/ctr.13358
14. SATOT. *Documento de consenso para la valoracion de donantes con serologia positive para el virus de la hepatitis C* (2022). Available from: http://wwwont.es/infesp/DocumentosDeConsenso/Documento%20Consenso%20Valoraci%C3%B3n%20Donantes%20Virus20C_ABRIL2019.pdf (Accessed August 10, 2022).
15. Yan FM, Hao F, Zhao LB, Gu CH, Chen AS, Zhao XP, et al. Study of Expression of Hepatitis C Virus Antigens and Viral Replication in Extrahepatic Tissues. *Zhonghua Ganzangbing Zazhi* (2000) 8:40–2.
16. Wrobleswska A, Krzysztof PB, Sikorska K. Occult Infection with Hepatitis C Virus: Looking for Clear-Cut Boundaries and Methodological Consensus. *J Clin Med* (2021) 10:5874. doi:10.3390/jcm10245874
17. Gelpi R, Rodríguez-Villar C, Paredes D, Roque R, Ruiz A, Adalia R, et al. Safety of Hepatitis C Virus (HCV)-treated Donors for Kidney Transplantation Excluding Occult HCV Infection through Kidney Biopsies. *Transpl Int* (2018) 31(8):938–9. doi:10.1111/tri.13270
18. Franco A, Moreso F, Sola E, Beneyto I, Esforzado N, Gonzalez Roncero F, et al. Outcome of Kidney Transplants from Viremic and Non-viremic Hepatitis C Virus Positive Donors into Negative Recipients: Results of the Spanish Registry. *J Clin Med* (2023) 12:1773. doi:10.3390/jcm12051773

ESOT | Transplant International

BRIEF RESEARCH REPORT
published: 30 June 2023
doi: 10.3389/ti.2023.11172

The Perspectives of General Nephrologists Toward Transitions of Care and Management of Failing Kidney Transplants

Tarek Alhamad[1], Haris Murad[1], Darshana M. Dadhania[2], Martha Pavlakis[3], Sandesh Parajuli[4], Beatrice P. Concepcion[5], Neeraj Singh[6], Naoka Murakami[7], Michael J. Casey[8], Mengmeng Ji[1], Michelle Lubetzky[9], Ekamol Tantisattamo[10], Omar Alomar[1], Arman Faravardeh[11], Christopher D. Blosser[12], Arpita Basu[13], Gaurav Gupta[14], Joel T. Adler[9], Deborah Adey[15], Kenneth J. Woodside[16], Song C. Ong[17], Ronald F. Parsons[13] and Krista L. Lentine[18]*

[1]John T. Milliken Department of Medicine, Washington University in St. Louis, Saint Louis, MO, United States, [2]Department of Transplantation Medicine, Weill Cornel Medicine - New York Presbyterian Hospital, New York, NY, United States, [3]Department of Medicine, Beth Israel Deaconess Medical Center and Harvard University, Boston, MA, United States, [4]Department of Medicine, University of Wisconsin - Madison, Madison, WI, United States, [5]Department of Medicine, University of Chicago, Chicago, IL, United States, [6]John C. McDonald Regional Transplant Center, Willis Knighton Health System, Shreveport, LA, United States, [7]Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, United States, [8]Department of Medicine, Medical University of South Carolina, Charleston, SC, United States, [9]Division of Abdominal Transplantation, Department of Surgery and Perioperative Care, Dell Medical School, University of Texas at Austin, Austin, TX, United States, [10]Department of Medicine, University of California, Irvine, Orange, CA, United States, [11]SHARP Kidney and Pancreas Transplant Center, San Diego, CA, United States, [12]Department of Medicine, Seattle Children's Hospital, University of Washington, Seattle, WA, United States, [13]Department of Medicine, Emory University, Atlanta, GA, United States, [14]Department of Medicine, Virginia Commonwealth University, Richmond, VA, United States, [15]Department of Medicine, University of California, San Francisco, San Francisco, CA, United States, [16]Department of Surgery, University of Michigan, Ann Arbor, MI, United States, [17]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, United States, [18]Center for Abdominal Transplantation, Saint Louis University, Saint Louis, MO, United States

OPEN ACCESS

Correspondence:
Tarek Alhamad
talhamad@wustl.edu

Received: *05 January 2023*
Accepted: *14 June 2023*
Published: *30 June 2023*

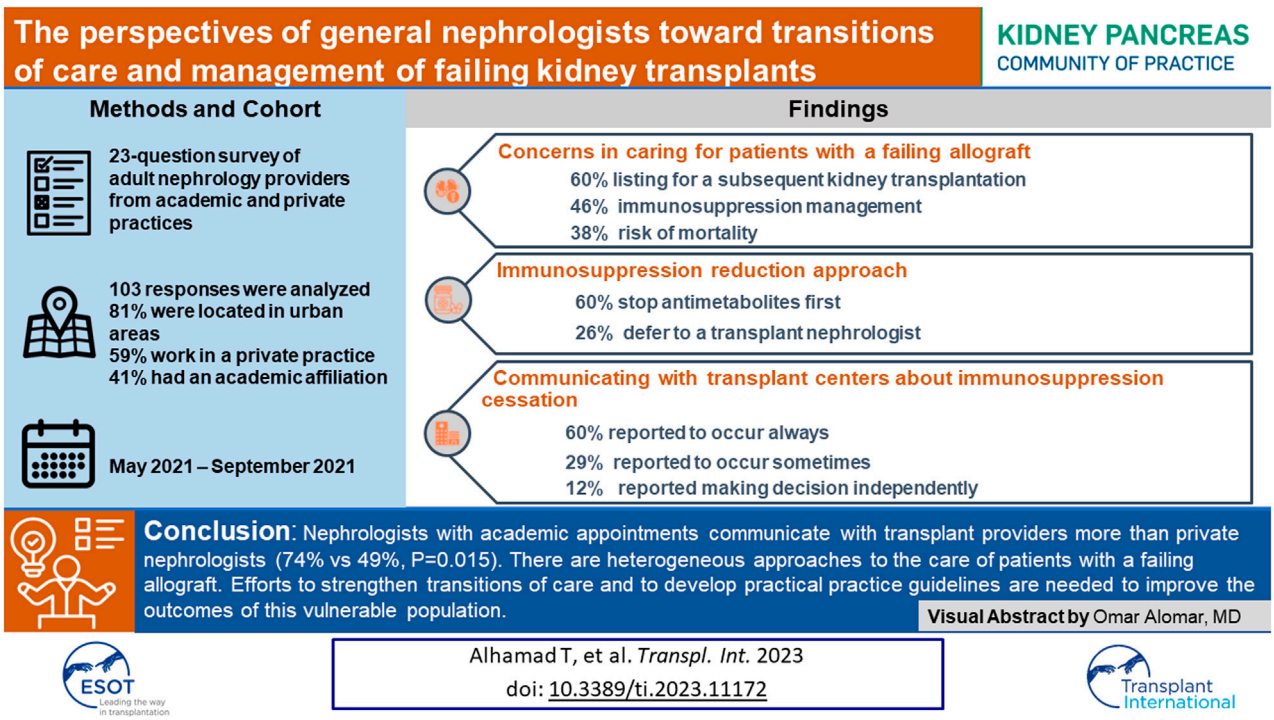
Citation:
Alhamad T, Murad H, Dadhania DM, Pavlakis M, Parajuli S, Concepcion BP, Singh N, Murakami N, Casey MJ, Ji M, Lubetzky M, Tantisattamo E, Alomar O, Faravardeh A, Blosser CD, Basu A, Gupta G, Adler JT, Adey D, Woodside KJ, Ong SC, Parsons RF and Lentine KL (2023) The Perspectives of General Nephrologists Toward Transitions of Care and Management of Failing Kidney Transplants. Transpl Int 36:11172. doi: 10.3389/ti.2023.11172

The management of failing kidney allograft and transition of care to general nephrologists (GN) remain a complex process. The Kidney Pancreas Community of Practice (KPCOP) Failing Allograft Workgroup designed and distributed a survey to GN between May and September 2021. Participants were invited via mail and email invitations. There were 103 respondents with primarily adult nephrology practices, of whom 41% had an academic affiliation. More than 60% reported listing for a second kidney as the most important concern in caring for patients with a failing allograft, followed by immunosuppression management (46%) and risk of mortality (38%), while resistant anemia was considered less of a concern. For the initial approach to immunosuppression reduction, 60% stop antimetabolites first, and 26% defer to the transplant nephrologist. Communicating with transplant centers about immunosuppression cessation was reported to occur always by 60%, and sometimes by 29%, while 12% reported making the decision independently. Nephrologists with

Abbreviations: AST, American Society of Transplantation; KPCOP, Kidney Pancreas Community of Practice; CKD, chronic kidney disease; CNIs, calcineurin inhibitors; KRAFT, Kidney Recipients with Allograft Failure - Transition of Care; MMF, mycophenolate mofetil.

academic appointments communicate with transplant providers more than private nephrologists (74% vs. 49%, p = 0.015). There are heterogeneous approaches to the care of patients with a failing allograft. Efforts to strengthen transitions of care and to develop practical practice guidelines are needed to improve the outcomes of this vulnerable population.

Keywords: re-transplantation, failing kidney allograft, transition of care, immunosuppression management, multidisciplinary team



GRAPHICAL ABSTRACT |

INTRODUCTION

Kidney transplants have a limited life span, with a median half-life ranging from 9 to 12 years [1, 2]. In fact, 11.9% of patients on the kidney transplant waiting list have had a prior failed transplant [3]. These patients are at a higher risk of morbidity and mortality compared to patients who are on dialysis without a previous failed transplant [4–6]. This increased risk is thought to be due to a combination of immunocompromised status, as well as a chronic inflammatory state which leads to increased infectious and cardiovascular complications, amongst others [7–9].

Effective transitions of care between providers are an ongoing challenge in chronic kidney disease (CKD) [10]. Timely referral of patients with failing allografts to general nephrologists is crucial to begin appropriate CKD care. Based on the care model, CKD care may not be the focus of some transplant centers. This includes vascular access planning, anemia management, and a well-timed transition to dialysis. Patients with a failing allograft face many challenges, including high risk of depression and social challenges that would add more difficulties in their care.

The American Society of Transplantation Kidney Pancreas Community of Practice (AST-KPCOP) established a workgroup to study Kidney Recipients with Allograft Failure–Transition of Care (KRAFT) to understand the current data and practice patterns related to the management of recipients with a failing allograft. A recent survey of transplant providers performed by this group reflected the common transition-related practices of transplant providers and highlighted the substantial heterogeneity in several aspects of the care of patients with allograft failure [9]. To date, the practice patterns of general nephrology providers related to this important area of kidney patient care has not been studied. Hence, we conducted a survey to assess practice patterns and priorities of general nephrology providers regarding patients with a failing or failed kidney allograft.

METHODS

Survey Design

This survey was performed by AST-KPCOP's KRAFT workgroup. The survey questions were developed collaboratively and the instrument was piloted with general nephrologists as well as among KPCOP workgroup members. Where needed, the wording of the questions was adjusted for clarity. The final survey comprised 23 questions, including two related to management of failing allografts, six related to the comfort of and approach to tapering immunosuppression, three related to perceived risks and benefits associated with tapering immunosuppression, four related to communications and referrals to transplant centers, three related to the management of rejection in a failed allograft, and five related to program description which included practice type, size, and location. Main concerns in the care of patients with a failing kidney allograft and factors linked to tapering off immunosuppression were graded on a semi-quantitative scale: very important, intermediate importance, and not very important.

Survey Administration and Participants

The survey was approved by the Washington University in St. Louis Institutional Review Board and approved by the Education Committee of AST and KPCOP for distribution. The survey was built into the SurveyMonkey tool and distributed in May 2021 via mail to the members (MD, NP, AP) of the American Society of Nephrology (ASN), electronic links by individual email invitations to general nephrologists in academic and private institutions, and by posting the link in the KPCOP HUB. The survey pool was closed on 1 September 2021. Responses were recorded anonymously. Zip codes were used to examine the distribution of responses in the US.

ArcMap 10.8 was used to geocode the location of respondents using 5-digit zip code locator. The Zip codes of the respondents were then linked to a reference map of 2020 Census Bureau's urban area classification to identify the rural and urban areas. Chi-square test was performed for comparison of responses across practice type and geographical area.

RESULTS

Survey Participants

There were 66 responses who received the invitations from the mailing cards to the ASN members and 56 responses from electronic links from individual email invitations and through the link in the KPCOP HUB. In total, we registered a total of 122 responses. We excluded two responses from providers that were primarily pediatrics and 17 responses from providers whose practice included more than 50%, transplant patients. Amongst the remaining 103 responses, 98 responses were from the US, one from Canada, and four from other countries (2 Pakistan, 1 Belgium, and 1 Singapore).

Of the 103 responses who practiced primarily adult nephrology, 41% had an academic affiliation. 23% practiced in a small private practice with one to four nephrologists, 16% practiced in a medium-size practice with five to ten nephrologists, and 20% practiced in a large private practice with more than 10 nephrologists. Most participating nephrologists were located within urbanized areas.

Patients With a Failing or Failed Kidney Allograft

More than half (n = 57) reported that only 1%–5% of dialysis patients in their practice had failed kidney allografts, one third reported 6%–10%, eight reported 11%–20%, one reported more than 20%, and only three reported that their practice did not include any failed or failing kidney allografts.

Communication and Transition of Care With a Failing Allograft

Please see **Figure 1** for all responses and results. When asked how often patients with a failing allograft get referred back to general nephrologists by their transplant center, 39% reported always (**Figure 1A**). Regarding discussing the transition of care with the transplant team for patients with a failed allograft, 26% of respondents reported always (**Figure 1B**).

In terms of referral for another transplant before return to dialysis, 24% of respondents reported that more than 50% of the patients with a failing allograft were referred (**Figure 1C**). The majority (77%) of respondents were more likely to refer a patient with a failing allograft than a patient with native CKD for another transplant (**Figure 1D**).

Immunosuppression Approach With a Failing Allograft

Respondents were asked if they feel comfortable managing immunosuppression for patients with a failing allograft (not on dialysis). 22% felt very comfortable, 60% felt comfortable unless complications developed, and 18% were uncomfortable and would need guidance.

In terms of the initial approach for reduction of immunosuppression in a patient with a failing allograft, a majority, 60% of respondents would stop antimetabolites first, 26% would defer to transplant nephrologist, 8% would stop calcineurin inhibitors first, and 6% would stop prednisone first.

Reasons and Concerns for Maintaining Immunosuppression

Approximately 30% of respondents believed that continuing immunosuppression in a patient who has started dialysis would increase the risk of having adverse events and/or mortality, 35% did not think continuing immunosuppression increases risks, and the remaining 35% were not sure. None of the respondents would stop all immunosuppression when a patient with a failing allograft starts dialysis, 55% would taper off immunosuppression, while 46% would continue immunosuppression.

Compared to care for those with native kidney CKD stage 4–5, more than 60% of respondents reported that listing for a second

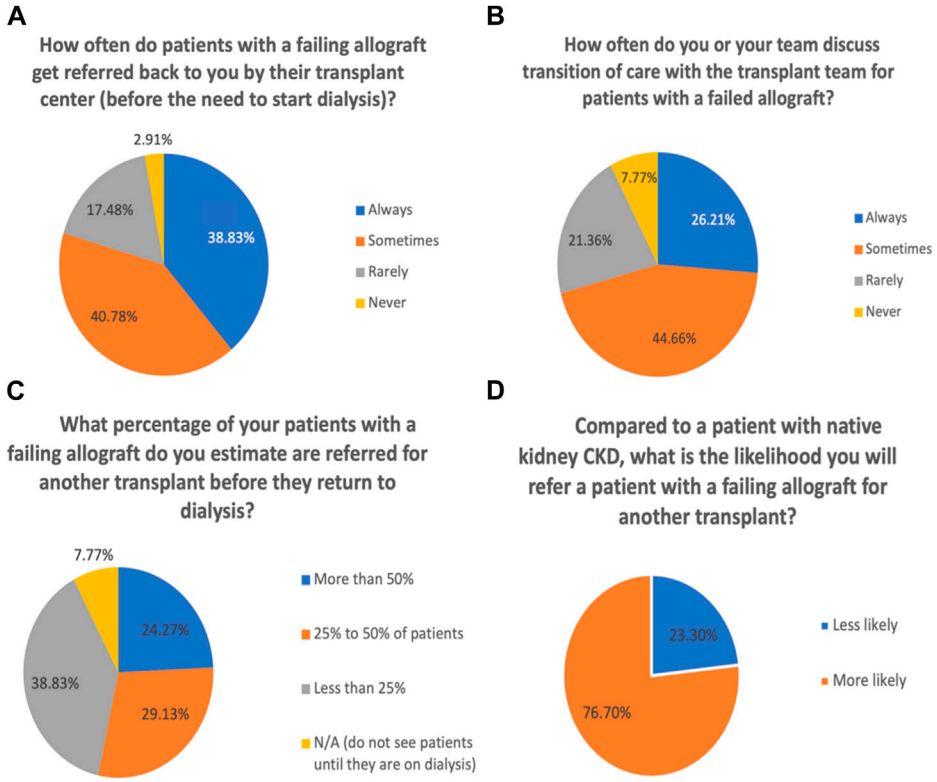


FIGURE 1 | Communications with transplant nephrology and referral patterns for patients with failing allografts. **(A)** Referrals back to general nephrologists before starting dialysis. **(B)** Discussion of transition of care with transplant teams. **(C)** Rate of referrals for another transplant before return to dialysis. **(D)** Referrals of patients with failing allograft to transplant compared to patients with native CKD.

kidney is the most important concern for patients with a failing allograft, followed by immunosuppression management (46%). Other responses could be seen in **Figure 2A**.

The respondents were asked to rate the importance of reasons for maintaining immunosuppression in a failing allograft. About 65% reported that minimizing the risk of allosensitization for a subsequent transplant is the most important factor in their decision to maintain immunosuppression, followed by preventing allograft rejection (52%) (**Figure 2B**).

Immunosuppression Reduction When Starting Dialysis

Respondents were asked if they communicate with transplant centers regarding immunosuppression cessation in patients with a failing allograft. About 60% reported always communicating, 29% reported sometimes, and 12% reported that they made the decision themselves. Among 93 respondents who manage immunosuppression, 73% monitor calcineurin inhibitor levels, and 72% monitor urine output in deciding when to stop immunosuppression.

The respondents were asked if their approach towards immunosuppression in a failed allograft would be different when the patient's waiting time for a kidney is less than 1 year (e.g., availability of a living donor) compared to longer waiting times. 48% of respondents indicated they would keep patients at a more intensive regimen if the patient's waiting time for a kidney is <1 year; 10% said that the presence of a living donor would not alter their immunosuppression plans; 43% said that they would defer to transplant nephrology.

We examined the essential factors that influenced clinicians' decisions for tapering off immunosuppression. More than 75% reported that the likelihood of receiving another transplant is the most important factor in their decision to taper off immunosuppression, followed by side effects of medications (63%). The least important factors were reported to be urine output and the age of the patient (**Figure 2C**).

Referral for Allograft Nephrectomy

Regarding the initial approaches to a patient with signs/symptoms of rejection in a failed allograft (multiple choice question), the majority would increase the steroid dose (56%) and defer to the transplant provider (56%) (**Figure 3A**).

To be more specific, we asked about the timing of referral for an allograft nephrectomy (multiple choice question). 64% referred patients for nephrectomy if there are persistent signs or symptoms of rejection (e.g., allograft tenderness, hematuria) despite medical therapy (**Figure 3B**).

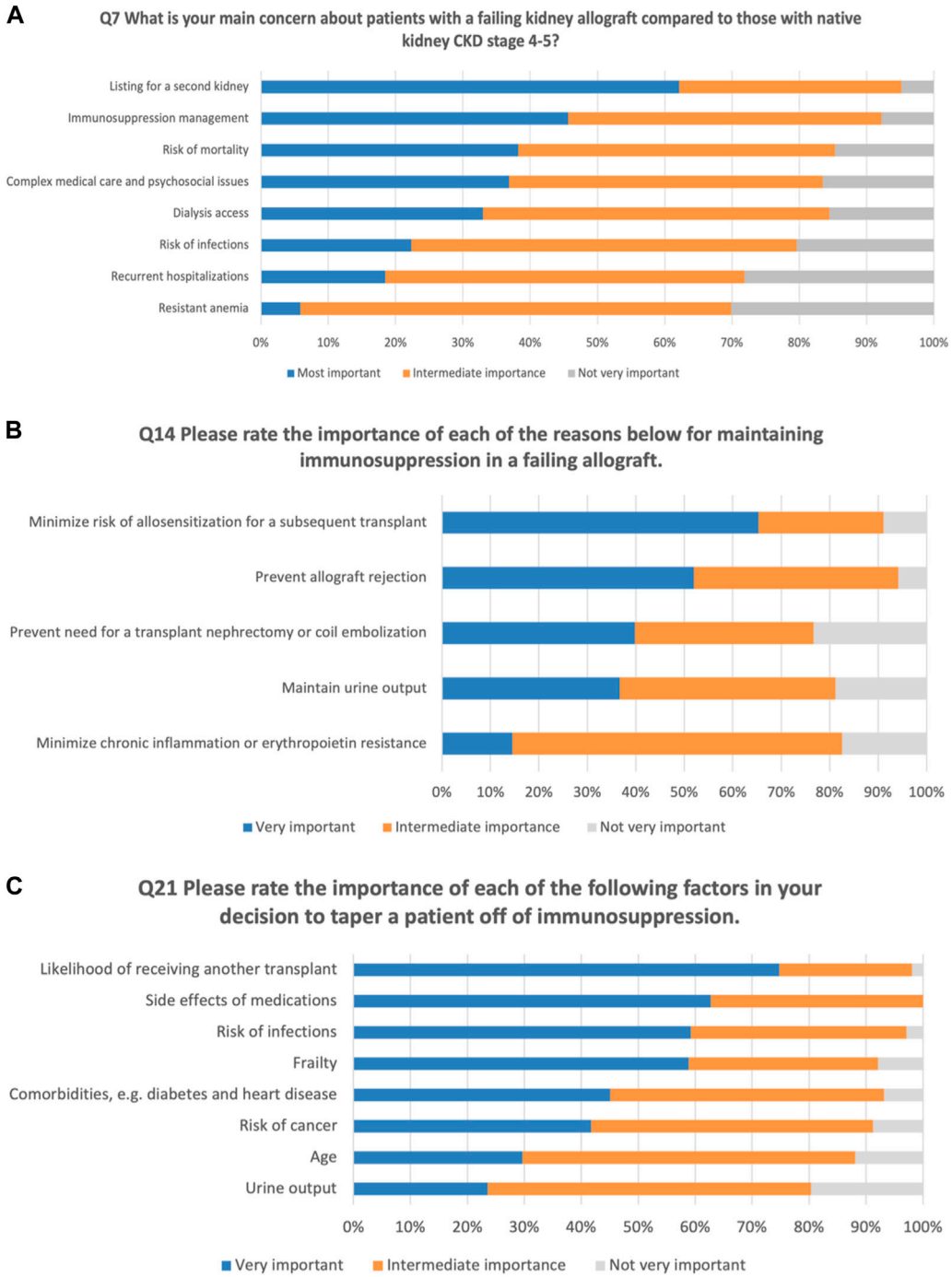


FIGURE 2 | Perception of general nephrologists on the management of patients with failing kidney allograft. **(A)** Concerns about patients with failing allografts compared to native CKD. **(B)** Importance of selected factors in maintaining immunosuppression. **(C)** Importance of selected factors in the decision to taper immunosuppression.

For adjusting immunosuppression when a patient is scheduled for an allograft nephrectomy, 62% defer and discuss with transplant provider, 18% reported a week or two after the surgery, 17% reported immediately after the surgery (**Figure 3C**).

Differences Across Affiliation Type and Geographical Area

Compared to respondents with a private affiliation, respondents who had an academic affiliation reported a

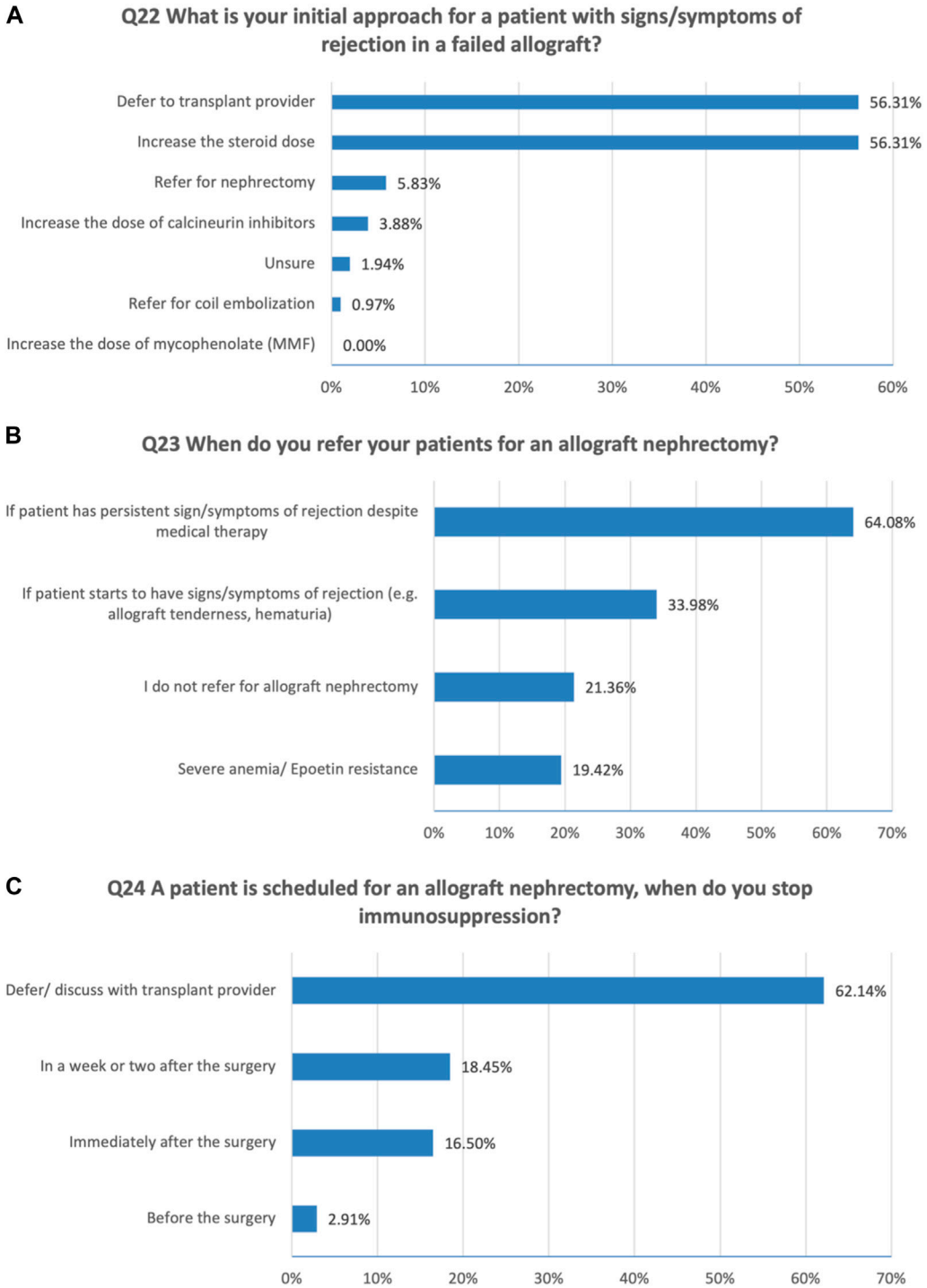


FIGURE 3 | Medical and surgical management of failing kidney allograft. **(A)** Initial approaches to a patient with signs/symptoms of rejection in a failed allograft. **(B)** Timing of referral for an allograft nephrectomy. **(C)** Immunosuppression cessation approaches when a patient is scheduled for an allograft nephrectomy.

higher percentage of dialysis patients with failed kidney allografts in their practice. Respondents with an academic affiliation were more likely to communicate with transplant centers regarding immunosuppression cessation and less likely to monitor urine output (**Table 1**). For the initial approach for a patient with signs/symptoms of rejection in a failed allograft, respondents with an academic affiliation were more likely to defer to the transplant provider.

Compared to respondents living in rural areas, respondents living in urban areas were more likely to communicate with

TABLE 1 | Responses by affiliate and location.

Questions	Responses	Affiliate			Location		
		Private (*n* = 61) (%)	Academic (*n* = 42) (%)		Rural (*n* = 18) (%)	Urban (*n* = 79) (%)	
What percentage of dialysis patients in your practice have failed kidney allografts?	1%–5%	65.6	40.5	$p = 0.016$	72.2	51.9	$p = 0.581$
	11%–20%	1.6	16.7		5.6	7.6	
	6%–10%	29.5	38.1		22.2	35.4	
	>20%	1.6	0.0		0.0	1.3	
	None	1.6	4.8		0.0	3.8	
How often do you or your team discuss transition of care with the transplant team for patients with a failed allograft?	Never	9.8	4.8	$p = 0.794$	5.6	8.9	$p = 0.053$
	Rarely	19.7	23.8		44.4	15.2	
	Sometimes	44.3	45.2		33.3	48.1	
	Always	26.2	26.2		16.7	27.8	
How comfortable do you feel managing immunosuppression with a failing allograft (patient not on dialysis)?	Very comfortable	23.0	21.4	$p = 0.236$	11.1	22.8	$p = 0.226$
	Comfortable unless complications develop	63.9	52.4		77.8	55.7	
	Not comfortable and need guidance	13.1	26.2		11.1	21.5	
What is your initial approach for reduction of immunosuppression in a patient with a failing allograft?	Stop antimetabolites first	67.2	50.0	$p = 0.108$	61.1	58.2	$p = 0.427$
	Stop calcineurin inhibitors first	9.8	4.8		16.7	6.3	
	Stop prednisone first	4.9	7.1		5.6	6.3	
	Defer to transplant nephrologist	18.0	38.1		16.7	29.1	
Do you communicate with transplant centers regarding Immunosuppression cessation?	Yes, always	49.2	73.8	$p = 0.015$	27.8	65.8	$p = 0.004$
	Sometimes	32.8	23.8		61.1	22.8	
	No, I make the decision myself	18.0	2.4		11.1	11.4	
If you manage immunosuppression, do you monitor calcineurin inhibitor levels?	Yes	73.8	54.8	$p = 0.066$	88.9	60.8	$p = 0.063$
	No	21.3	28.6		11.1	26.6	
	Not applicable	4.9	16.7		0.0	12.7	
If you manage immunosuppression, do you monitor urine output?	Yes	70.5	57.1	$p = 0.029$	61.1	64.6	$p = 0.151$
	No	26.2	23.8		38.9	22.8	
	Not applicable	3.3	19.0		0.0	12.7	

transplant centers regarding immunosuppression cessation. No differences were found for responses to other questions across affiliation type and geographical area.

DISCUSSION

This contemporary survey of primarily U.S. adult general nephrologists highlights challenges and practice patterns in the care of patients with failing allografts. Our data reflect the perspectives of general nephrologists who practice in private and academic settings and demonstrate a wide range of practices in the management of a failing allograft. Such heterogeneity speaks to the need for clear and accessible recommendations to guide collaborative co-management of this important and vulnerable patient group.

While listing patients with failing allografts for a subsequent transplant was the primary concern of the majority of nephrologists in this survey, more than a third were cognizant of the increased medical complexity, mortality, and complex psychosocial issues amongst patients with a failing allograft, which are well established in literature [4–6, 11]. It is clear that this patient population needs special attention which may present an additional burden and challenge for busy practices.

Our study highlights that only 39% of respondents noted that patients were always referred back to general nephrologists before being started on dialysis after allograft failure. Establishing care with a general nephrologist before dialysis could improve the patient's experience and transition in a psychologically challenging time. Furthermore, well-planned transitions of care can help in early dialysis access creation, which in turn can reduce morbidity and mortality [12]. This transition should ideally not be an abrupt change in care teams but is perhaps best suited for a period of co-management between the transplant team and general nephrology, with visits alternating between both practices. This transition needs to incorporate a multidisciplinary team approach and includes nurses, dieticians, pharmacists, and social workers. Educational programs of dialysis modalities need to be part of the clinic. Champion access and the involvement of surgeons and radiologists are important to increase the rates of fistulas. A key strategy for this transition is to start early in the post-transplant

course, so general nephrologists maintain their relationships with their patients.

Notably, only 24% of respondents reported that more than half of their patients with a failing allograft were referred for re-transplantation. The majority (77%) of nephrologists felt that patients with a failing allograft were more likely to be referred for transplant as compared to patients with native kidney CKD. Transplant centers have a mutual interest in referring appropriate candidates back for re-transplantation. Based on that, transplant centers do frequently relist patients before going back to dialysis. This is consistent with the finding that there were higher rates of referral for relisting for failed allografts than native CKD patients. It is also possible that patients with failed allografts would try to avoid going back to dialysis and could themselves be more active in trying to get relisted for re-transplantation. Notably, a recent study utilizing the Austrian Dialysis and Transplant Registry showed that the survival benefit of a second kidney transplant is conditional on the wait time since the loss of the first graft, highlighting the necessity of early referral [13].

In terms of communication regarding the transition of care for patients with failed allografts, our data demonstrated that 29% of the general nephrologists rarely or never discussed the care with transplant providers. Urban and academic nephrologists reported significantly better communication with transplant centers regarding immunosuppressant cessation than rural and private nephrologists.

Similar to transplant providers surveyed in an earlier KRAFT survey, general nephrologists felt that minimizing the risk of sensitization for a subsequent transplant is the most important reason to continue immunosuppression medications [9]. The strategy of slowly tapering off immunosuppression has been shown to reduce sensitization following graft failure [14, 15]. Similar to transplant providers, general nephrologists endorsed a strong preference towards first stopping antimetabolites when reducing immunosuppression.

Our study has several limitations. First, our participants represent a subset of the nephrology community and their responses may not be generalizable to the other clinicians. Second, we were not able to calculate the percentage of responses to the survey due to the fact that the survey was posted online through the HUB.

In summary, this survey of general nephrology clinicians highlights a wide range of concerns in the care of patients with failing allografts. Clear areas of opportunity exist for better communication with transplant centers, early referral for a subsequent transplant, and early involvement of general nephrology providers in the care of patients with failing allografts. This study highlights the need to establish and disseminate best practice recommendations, along with structured programs for early involvement of nephrologists in the care of patients with failing allografts and for building a strong network of communication with transplant centers.

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 survey was approved by the Washington University in St. Louis Institutional Review Board and approved by the Education Committee of AST and KPCOP for distribution.

AUTHOR CONTRIBUTIONS

TA designed the study, acquired data, interpreted data, revised the paper critically, gave final approval of the version to be published and agrees to be accountable for all aspects of the work with regard to its accuracy and integrity. MJ analyzed and interpreted data, drafted the paper, revised it critically, gave final approval of the version to be published and agrees to be accountable for all aspects of the work with regard to its accuracy and integrity. All coauthors interpreted data, revised the paper critically, gave final approval of the version to be published and agrees to be accountable for all aspects of the work with regard to its accuracy and integrity. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

ACKNOWLEDGMENTS

The authors would like to acknowledge Daniel Coyne, MD, Tingting Li, MD, and Anitha Vijayan, MD at Washington University in St. Louis for the review of the survey. We also thank the survey respondents for sharing their valuable time and perspectives in completing the survey, and members of the AST Education Committee for their review and feedback.

REFERENCES

1. Lamb KE, Lodhi S, Meier-Kriesche HU. Long-term Renal Allograft Survival in the United States: a Critical Reappraisal. *Am J Transpl* (2011) 11(3):450–62. doi:10.1111/j.1600-6143.2010.03283.x
2. Wekerle T, Segev D, Lechler R, Oberbauer R. Strategies for Long-Term Preservation of Kidney Graft Function. *Lancet* (2017) 389(10084):2152–62. doi:10.1016/S0140-6736(17)31283-7
3. Hart A, Lentine KL, Smith JM, Miller JM, Skeans MA, Prentice M, et al. OPTN/SRTR 2019 Annual Data Report: Kidney. *Am J Transpl* (2021) 21(2): 21–137. doi:10.1111/ajt.16502

4. Fuquay R, Teitelbaum I. Care of the Patient after Renal Allograft Failure: Managing the Present and Planning for the Future. *Am J Nephrol* (2012) 36(4): 348–54. doi:10.1159/000342626
5. Gill JS, Abichandani R, Kausz AT, Pereira BJ. Mortality after Kidney Transplant Failure: the Impact of Non-immunologic Factors. *Kidney Int* (2002) 62(5):1875–83. doi:10.1046/j.1523-1755.2002.00640.x
6. Brar A, Markell M, Stefanov DG, Timpo E, Jindal RM, Nee R, et al. Mortality after Renal Allograft Failure and Return to Dialysis. *Am J Nephrol* (2017) 45(2): 180–6. doi:10.1159/000455015
7. Lopez-Gomez JM, Perez-Flores I, Jofre R, Carretero D, Rodríguez-Benitez P, Villaverde M, et al. Presence of a Failed Kidney Transplant in Patients Who Are on Hemodialysis Is Associated with Chronic Inflammatory State and Erythropoietin Resistance. *J Am Soc Nephrol* (2004) 15(9):2494–501. doi:10.1097/01.ASN.0000137879.97445.6E
8. Ayus JC, Achinger SG. At the Peril of Dialysis Patients: Ignoring the Failed Transplant. *Semin Dial* (2005) 18(3):180–4. doi:10.1111/j.1525-139X.2005.18304.x
9. Alhamad T, Lubetzky M, Lentine KL, Edusei E, Parsons R, Pavlakis M, et al. Kidney Recipients with Allograft Failure, Transition of Kidney Care (KRAFT): A Survey of Contemporary Practices of Transplant Providers. *Am J Transpl* (2021) 21(9):3034–42. doi:10.1111/ajt.16523
10. Kalantar-Zadeh K, Kovesdy CP, Streja E, Rhee CM, Soohoo M, Chen JLT, et al. Transition of Care from Pre-dialysis Prelude to Renal Replacement Therapy: the Blueprints of Emerging Research in Advanced Chronic Kidney Disease. *Nephrol Dial Transplant* (2017) 32(2):ii91–8. doi:10.1093/ndt/gfw357
11. Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY, et al. Comparison of Mortality in All Patients on Dialysis, Patients on Dialysis Awaiting Transplantation, and Recipients of a First Cadaveric Transplant. *N Engl J Med* (1999) 341(23):1725–30. doi:10.1056/NEJM199912023412303
12. Oliver MJ, Rothwell DM, Fung K, Hux JE, Lok CE. Late Creation of Vascular Access for Hemodialysis and Increased Risk of Sepsis. *J Am Soc Nephrol* (2004) 15(7):1936–42. doi:10.1097/01.asn.0000131524.52012.f8
13. Kainz A, Kammer M, Reindl-Schwaighofer R, Strohmaier S, Petr V, Viklicky O, et al. Waiting Time for Second Kidney Transplantation and Mortality. *Clin J Am Soc Nephrol* (2022) 17(1):90–7. doi:10.2215/CJN.07620621
14. Augustine JJ, Woodside KJ, Padiyar A, Sanchez EQ, Hricik DE, Schulak JA. Independent of Nephrectomy, Weaning Immunosuppression Leads to Late Sensitization after Kidney Transplant Failure. *Transplantation* (2012) 94(7): 738–43. doi:10.1097/TP.0b013e3182612921
15. Casey MJ, Wen X, Kayler LK, Aiyer R, Scornik JC, Meier-Kriesche HU. Prolonged Immunosuppression Preserves Nonsensitization Status after Kidney Transplant Failure. *Transplantation* (2014) 98(3):306–11. doi:10.1097/TP.0000000000000057

Transplant International

Official journal of the European Society for Organ Transplantation

Editorial Office

Avenue du Tribunal Fédéral 34
CH – 1005 Lausanne
Switzerland

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

tieditorialoffice@frontierspartnerships.org
frontierspartnerships.org/journals/transplant-international

frontiers | Publishing Partnerships