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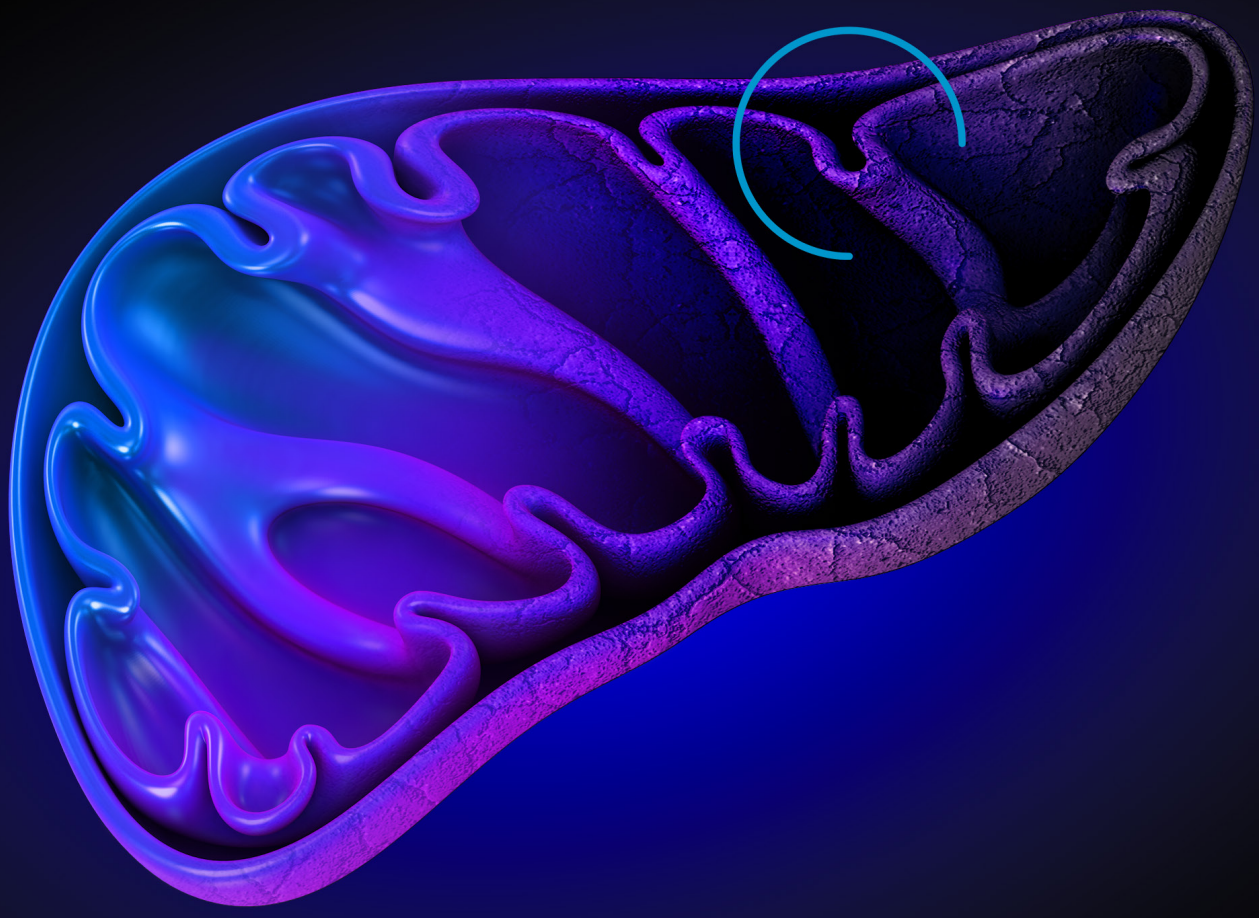

Mitochondria and Graft Viability in Liver Preservation

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Mitochondria and Graft Viability in Liver Preservation

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TRANSPLANT TRIAL WATCH
published: 08 February 2024
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Transplant Trial Watch

Simon R. Knight[1,2]* *and John M. O'Callaghan*[2,3]*

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Keywords: kidney transplantation, hypothermic machine perfusion, randomised controlled trial, lung transplantation, belatacept

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

Perfusate Proteomes Provide Biological Insight Into Oxygenated Versus Standard Hypothermic Machine Perfusion in Kidney Transplantation.

by Mulvey, J. F., et al. Annals of Surgery 2023; 278(5): 676–682.

Aims

The aim of this study was to provide mechanistic insight into biological alterations that occur in deceased donor kidneys during standard non-oxygenated versus oxygenated hypothermic machine perfusion (HMP), using perfusate samples collected in the COMPARE study.

Interventions

In the COMPARE trial, pairs of kidneys donated following circulatory death were randomly assigned to receive either oxygenated HMP or non-oxygenated HMP.

Participants

210 perfusate samples.

Outcomes

The main outcome of this paper was to identify protein changes across durations of perfusion and in relation to 12-month estimated glomerular filtration rate (eGFR).

Follow-Up

12 months.

Transplant Trial Watch

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CET Conclusion

by John O'Callaghan

This well-written report details an analysis of perfusate samples collected during the COMPARE study, an RCT comparing oxygenated with non-oxygenated machine perfusion. Mass spectrometry was used to analyse the proteomic make up of the perfusate fluid. During hypothermic machine perfusion, proteins enter the perfusate system, increasing over time. The authors explored the relation between perfusate proteins and clinical outcomes, with some indication that outcomes such as acute rejection and kidney function at 12 months.

Trial Registration
ISRCTN32967929.

Funding Source
Non-industry funded.

RANDOMISED CONTROLLED TRIAL 2

A Pilot Randomized Controlled Trial of *De Novo* Belatacept-Based Immunosuppression After Lung Transplantation.

by Huang, H. J., et al. Transplantation 2023 [record in progress].

Aims
This study aimed to evaluate the feasibility and inform the design of an RCT investigating the efficacy and safety of belatacept following lung transplantation.

Interventions
Participants were randomly assigned to either continue standard-of-care immunosuppression or switch to belatacept.

Participants
27 lung transplant recipients.

Outcomes
The primary outcome was to assess the feasibility of randomising 80% of eligible patients within 4 h posttransplantation. The primary outcome was later changed to survival following the cessation of treatment with belatacept.

Follow-Up
1 year posttransplantation.

CET Conclusion

by Simon Knight

This pilot study recruited lung transplant recipients at 2 sites, and randomised them to standard immunosuppression (Tac, MMF, Pred) or a belatacept-based regimen (Tac, Belatacept and pred). The hypothesis was that belatacept-based immunosuppression might reduce the incidence of donor-specific antibodies (DSA), leading to a reduction in the risk of chronic lung allograft dysfunction (CLAD). The study was stopped after recruitment of 27 patients due to 3 deaths in the belatacept arm. Causes of death varied—2 patients died from COVID-19 infection, one from CLAD related to infection, one from PTLD, one from pulmonary embolus and one from haemothorax. The authors ascribe 4 of these deaths to viral infections. No differences were seen in incidence of CLAD or development of DSA. It is very difficult to interpret these results given the small numbers, but clearly the authors were correct in stopping the study and switching patients to standard immunosuppression. The relationship of four of the deaths to viral infection would suggest that the immunosuppressive regimen may have contributed, and in the absence of any detectable clinical benefit, the conclusion that this regimen is unsafe in lung transplant recipients seem justified.

Jadad Score
2.

Data Analysis
Strict intention-to-treat analysis.

Allocation Concealment
No.

Trial Registration
ClinicalTrials.gov—NCT03388008.

Funding Source
Non-industry funded.

CLINICAL IMPACT SUMMARY

by Simon Knight

Chronic Lung Allograft Dysfunction (CLAD) is an important long-to medium-term cause of morbidity and mortality following lung transplantation [1]. It results predominantly from chronic immune damage, and is associated with the formation of donor-

specific antibodies (DSA) [2]. Management of CLAD is challenging once established, so most focus is on adequate immunosuppression and prevention of infection to reduce the risk of occurrence [1].

Early studies of belatacept, a T-cell co-stimulation blocker, demonstrated a significantly lower incidence of DSA-formation over 7-year post-transplant compared to a calcineurin-inhibitor-based regimen in kidney transplant recipients [3]. This led the teams in Houston and St. Louis to design a phase 2 pilot study to investigate the impact of belatacept-based immunosuppression on risk of DSA formation and CLAD in lung transplant recipients, reported in Transplantation recently [4].

The study recruited *de novo* lung transplant recipients, and randomised them to standard immunosuppression (ATG, tacrolimus, mycophenolate and prednisone) or to belatacept-based immunosuppression (tacrolimus, belatacept and prednisone). The study was stopped after recruitment of 27 of patients due to excess mortality in the belatacept arm. Overall, five of 13 patients receiving belatacept died, with one additional death after the end of follow-up. At first glance, causes of death appear varied, with two patients dying of COVID-19, one with CLAD, one post-transplant lymphoproliferative disorder (PTLD), one haemothorax and one pulmonary embolus. However, the authors note that four of six deaths had a viral association (viral CLAD, PTLD and COVID-19), with the suggestion that belatacept in this patient population may be associated with increased susceptibility to viral infection and infective complications.

It is hard to draw firm conclusions from a small number of patients, but in the absence of any noticeable difference in DSA formation or development of CLAD, this sobering experience would seem to suggest that the risk of *de novo* belatacept in lung transplant recipients far outweighs any potential theoretical benefit. Other studies have suggested that conversion to belatacept post-transplant might be feasible, but potentially with a higher risk of rejection [5, 6]. Numbers are small and more evidence is needed before belatacept-based strategies for lung recipients can be recommended.

Clinical Impact

4/5.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

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

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IN MEMORIAM
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In Memoriam Sir Roy Yorke Calne December 30th, 1930 to January 6th, 2024

Neville Jamieson *

Retired Transplant Surgeon, Cambridge, United Kingdom

Keywords: in memoriam Roy Yorke Calne, liver transplant, immunosuppression, art, tolerance

See also:

Sir Roy Calne, The Founding President of ESOT
by Oniscu GC (2024). Transpl Int. 37:12790. doi: 10.3389/ti.2024.12790

Sir Roy Calne (1930–2024): Tribute to Founding Father of ELITA, Honouring a Pioneer in European Liver and Intestinal Transplantation
by Hartog H, Germani G, Adam R, and the European Liver and Intestinal Transplant Association (ELITA), a section of ESOT (2024). Transpl Int. 37:12811. doi: 10.3389/ti.2024.12811

In the 1950s and early 1960s the hope of saving lives by carrying out life-saving organ transplants remained an impossible dream outside the special case of kidney transplants between identical twins. This had demonstrated that the technical surgical challenge could be overcome using techniques based on the work of Alexis Carrel at the beginning of the century but the challenges faced by the immune response had defied attempts to overcome them using radiation and the existing pharmacological options.

The challenge of developing solid organ transplantation from a dream to a reality fell to a number of surgeon scientists around the world who went on to be the founders and creators of our specialty. Of these pioneering figures, Roy Calne was a leading star throughout his life. After early unsuccessful experiments using irradiation in kidney transplants carried out at the Royal College of Surgeons Buxton Browne farm in the UK, he pursued an interest in novel chemical immunosuppressive agents triggered by the availability of 6 mercaptopurine and subsequently its oral analogue azathioprine in the laboratory of Joe Murray in Boston. This work was to be the foundation of the development of clinical transplantation into a life-saving reality based on chemical immunosuppression and marked the beginning of an era of clinical organ transplantation.

At this point in his career he was still a trainee but returned to the UK to become a consultant surgeon at the Westminster hospital with Professor Harold Ellis and subsequently moved to become Professor of Surgery at Cambridge University in 1965 at the age of 34—a testimony to the recognition of his early achievements. With his nephrology colleague Dr. David Evans he developed a haemodialysis programme in a dialysis unit close to his home where he also had an office and experimental laboratory, and established a viable clinical renal transplant programme at Addenbrookes Hospital. He followed the work of key transplant researchers around the world and developed a friendly rivalry with Tom Starzl in the United States, following Tom's work with liver transplantation with keen interest and carrying out experimental liver transplants in the laboratory. Early observations of the apparent longer survival of porcine liver transplant recipients were to encourage his lifelong interest in the potential of inducing post transplant tolerance.

He was to go on to carry out the first orthotopic liver transplant in Europe in 1968. In the days before the acceptance of brain stem death testing this was from a DCD donor and because of a significant size

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disparity between donor and recipient also required the use of a novel surgical technique in the form of a "piggy back" caval reconstruction. Surgical innovation was to be a key component of his career and this was an early sign of his ability to adapt to complex surgical circumstances. He established a link with Roger Williams at Kings College Hospital in London which led to the establishment of the Kings/Cambridge joint liver transplant programme which was to continue for many years.

The outcomes of transplantation still offered room for improvement and he continued a keen interest in novel immunosuppressive agents alongside a career long interest in the possibility of achieving the holy grail of tolerance. He attracted scientists to work in his experimental laboratory and together with Dr. David White came across the novel agent Cyclosporin A (later Cyclosporine) which had been developed by Sandoz in Switzerland but was not felt to have any future practical use. They managed to persuade Sandoz this might not be the case and after discovering that the very insoluble agent could be dissolved in olive oil (the suggestion of a Greek visitor working in the laboratory at the time) went on to demonstrate its remarkable immunosuppressive properties allowing its first use in human transplantation in Cambridge in 1978. This led to a step change in kidney transplant outcomes and a renewed interest in transplantation of other organs.

Drug toxicity remained a challenge and novel agents and approaches continued as a major interest with key involvement in the development of Tacrolimus and Sirolimus and with the tempting hints of long term drug free tolerance suggested by experimental observations in murine liver graft recipients acting as a spur.

He maintained a lifelong practice in general surgery and later in his career was part of the first UK combined heart liver and heart lung liver transplant, the development of paediatric liver transplantation and intestinal transplantation. He came to wider public notice outside the transplant community with the transplant of an infant named Ben Hardwick who featured on "That's Life" a popular UK TV programme hosted by Esther Rantzen—Ben was one of the early paediatric transplants in Cambridge in 1984.

Roy Calne was central in all of these events, becoming a leading figure in the newly forming national and international transplantation organisations and publishing more than a thousand papers in a wide range of medical journals together with numerous books on transplantation and general surgery. He was respected and admired by colleagues, locally, nationally and internationally. Over the years surgeons and scientists from around the world came to Cambridge to learn about all aspects of transplantation and work both clinically and in the laboratory before returning home to apply the skills they had learned in Cambridge. They were welcomed and made to feel at home and feel part of the extended family of the Cambridge Transplant Unit attending ward rounds, helping with transplants and attending social events at the Calne family home although these were not infrequently interrupted by the departure of teams (including the host and guests) to carry out transplants at the hospital.

Alongside his career in medicine he had many outside interests. He was a fellow of Trinity Hall in Cambridge and was an active and enthusiastic member of the College fellowship. Sports were important with squash and skiing in the winter and tennis in summer. There was an annual skiing trip jointly with Peter Morris and the Oxford transplant group which featured a Cambridge Oxford ski race featuring both clinical teams and family. A major feature of his life was painting in a variety of styles and themes and his work has featured in many exhibitions and many examples of his work can be found hanging in the corridors of Addenbrookes hospital where he spent his career in addition to featuring in national and international exhibitions. In later life he developed an interest in sculpture and produced a number of impressive bronze figures.

I worked with Roy for much of my career joining the transplant programme for the first time in 1979, an exciting time as this was the year after the first clinical use of Cyclosporine. I had the pleasure of working with him as a trainee and then as a colleague as transplantation developed with the Cambridge unit at its centre. Alongside our UK trainees I met a generation of young transplant clinicians and scientists attracted to Cambridge by Roy Calne's reputation and drive who remain friends and who will remember the charismatic figure of Roy Calne with affection and admiration for his achievements.

His contributions were recognised nationally by the award of a knighthood in 1986, a Fellowship of the Royal Society in 1974, the "Pride of Britain" award in 2014 and internationally by multiple prizes and awards. He received the Lasker DeBakey prize jointly with Tom Starzl in 2012 in recognition of their joint contributions to the field of liver transplantation.

He is survived by his wife Patsy and six children and will remain in the memory of a generation of transplantation surgeons who had the pleasure of meeting him and working alongside him.

Sir Roy Calne, the artist with one of his bronze figures and one of his paintings.

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

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.

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IN MEMORIAM
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Sir Roy Calne, the Founding President of ESOT

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Keywords: in memoriam, in memoriam Roy Yorke Calne, ESOT, organ transplantation, Roy Yorke Calne

See also:

In Memoriam Sir Roy Yorke Calne December 30th, 1930 to January 6th, 2024
by Jamieson N (2024). Transpl Int. 37:12692. doi: 10.3389/ti.2024.12692

Sir Roy Calne (1930–2024): Tribute to Founding Father of ELITA, Honouring a Pioneer in European Liver and Intestinal Transplantation
by Hartog H, Germani G, Adam R and The European Liver and Intestinal Transplant Association (ELITA), a section of ESOT (2024). Transpl Int. 37:12811. doi: 10.3389/ti.2024.12811

On 6th of January, the world of transplantation lost one of its last original pioneers. Sir Roy Calne was a giant of transplantation and his achievements are so numerous and so far reaching that no eulogy could capture his titanic contribution to the field.

The hallmark of Roy Calne's career was innovation. He was a true clinician-scientist, who has shaped many aspects of the emerging field of transplantation, pushed the boundaries with novel surgical techniques, innovative types of transplants but also through cutting edge science. His particular interest in improving the outcome of transplantation and perseverance in pursuing better immunosuppressive therapies became the cornerstone of a new era in transplantation. As a result, transplantation emerged from the experimental stages into main stream clinical care and the field changed forever.

A true visionary, he recognised that making transplantation successful was not just about surgical advances but required collaborations across many disciplines and education of future generations of healthcare practitioners. He fostered this successfully in Cambridge, encouraged countless visitors to adopt this concept and shared his vision nationally and internationally.

It is this foresight and perseverance that in 1982 brought together all European healthcare practitioners under one umbrella that was to become the European Society for Organ Transplantation. It is worth quoting from the archives of ESOT as recorded by Dr Uhlschmid from Zurich: "Following the enthusiastic response from those participating in the Gelin-Memorial Symposium in Gothenburg in November 1981, it was felt that there was a need for a new society to be formed which would represent more accurately the aims and needs of transplantation surgery and surgeons in Europe. A number of European transplant surgeons met and formed a steering committee for the foundation of a society, the proposed name of which would be The European Society of Transplant Surgeons." At the founding assembly which took place on 28th of April 1982, the identity of the new society was discussed, and it was Roy Calne who argued that this should not be yet another surgical society but should involve "all persons actively involved in organ transplantation." The assembly approved the motion and ESTS became ESOT with Roy Calne elected as the first President of the new society.

As a Society we have come a long way since that inaugural assembly, but Roy Calne's philosophy of multi-disciplinarity has remained the key pilar of ESOT to this day. Although he retired from clinical practice many years ago, he never stayed away from the society he helped to shape and was an active contributor to many Congresses, always happy to speak to new generations of transplanters and share his experience. In 2005, ESOT decided to award Honorary Memberships in recognition to contributions

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to the field and there was no more appropriate recipient for this accolade other than Roy Calne together with his friends and fellow pioneers Tom Starzl and Rene Küss.

Over the last 40 years, each ESOT President has had the challenging but rewarding task of building on the legacy of our forebearers and keeping our Society at the forefront of innovation in science and clinical care. This is not an easy mission considering the tall order set by Roy Calne, the Founding President of ESOT!

Sir Roy's passing marks the end of an era but his legacy lives on through countless generations whom he inspired and a robust and visionary Society that he has nurtured.

DATA AVAILABILITY STATEMENT

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

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

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IN MEMORIAM
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Sir Roy Calne (1930–2024): Tribute to a Founding Father of ELITA, Honouring a Pioneer in Liver and Intestinal Transplantation

Hermien Hartog[1], Giacomo Germani[2], Renè Adam[3] and The European Liver and Intestinal Transplant Association (ELITA), a section of ESOT*

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Keywords: liver transplantation, intestinal transplantation, organ transplantation, history, obituary

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Sir Roy Calne, The Founding President of ESOT
by Oniscu GC (2024). Transpl Int 37:12790. doi: 10.3389/ti.2024.12790

It is with great sadness and sympathy that we pay tribute to one of the founding fathers of liver and intestinal transplantation in Europe. As the passing of Sir Roy Calne, may he rest in peace, was announced, an era came to an end of great innovations and the introduction of organ transplantation. We stand on the shoulders of giants, of those who pioneered immunosuppression fearlessly, performed courageous surgery and had the grit and foresight to establish formidable international networks to progress the field of transplantation.

One of the decisive moments for Sir Roy Calne was when, as a young professional, he saw a patient of his own age with end-stage organ failure. He pursued the possibility of organ transplantation and was appointed Professor of Surgery in Cambridge at age 35, recognizing the immense potential of the new immunological discoveries in the early 60 s. His pioneering work on Cyclosporin, Campath and kidney transplantation is history.

Short in stature and with an amiable expression, Sir Roy Calne described himself as a "somewhat rebellious" character [1]. He stood the tide by performing many "firsts"; firsts that were previously dismissed as impossible. He was the first in Europe to prove that patients could survive following a liver transplant. He pioneered intestinal transplantation in the United Kingdom. And took part in the first teams to perform clusters of transplants of heart, liver and lung, and multivisceral plus kidney. He unlocked and believed in the enormous potential that organ transplantation could provide for patients who would otherwise die from organ failure.

Sir Roy Calne also acknowledged the limitations of his profession and more universally, of the human race. In interviews, Sir Roy Calne comes across as someone profoundly touched by the paradoxes of life. While the gift of transplantation gave many individual

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people suffering from lethal disease and their families a new leash of life, he did not leave unmentioned ethical problems in living or deceased organ donation that can occur when power is abused. Being described as a wonderful father to his six children, he examined the problem of world population density in his book "Too many people" [2, 3]. As a surgeon and artist with paintings exhibited in the Science Museum in London, he displayed an outstanding ability to combine candour, humanity, art, and critical thinking [4].

On October 25, 1993, the founding meeting for the European Liver Transplant Association (later ELITA) took place in Rhodes. This meeting was set up by the then Chairs of the ESOT Steering Committee, Professor Jean Bernard Otte and Professor Sir Roy Calne. Eight years prior to this milestone, in 1985, Sir Roy Calne, alongside Professors Henri Bismuth and Rudolph Pichlmayr, initiated the European Liver Transplant Registry (ELTR) in Munich [5]. This registry was established to document all liver transplant procedures across Europe and fostered a collaborative scientific community among European liver transplant centers. We are deeply grateful to Sir Roy Calne for this legacy to establish a successful association of professionals in Europe progressing the potential of liver and intestinal transplantation, which continues until today. We honour his tremendous contributions to science, surgery and medicine in organ transplantation.

Our thoughts are with his wife and children, family and friends.

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

HH drafted the manuscript. RA, TA, LB, DB, EB, MCC, CF, GG, P-DL, SDM, and SN critically revised the manuscript. All authors contributed to the article and approved the submitted version. All authors conceived the manuscript.

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CONFLICT OF INTEREST

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

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NEWS AND VIEWS
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Clinical Pig Heart Xenotransplantation—Where Do We Go From Here?

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Keywords: xenotransplantation, heart, initial clinical studies, outcomes, next steps

The recent sad death of Mr. Lawrence Faucette, the second patient to undergo pig heart transplantation at the University of Maryland at Baltimore (UMB), is a significant setback to the UMB program and, indeed, to all clinical attempts at organ xenotransplantation. However, such disappointments are to be anticipated when pioneering a completely new form of therapy.

The first patient to receive a human heart allotransplant, an operation carried out by Christiaan Barnard in Cape Town in 1967, sadly survived for only 18 days [1], far shorter than the 2-month' survival of Mr. David Bennett, Sr, the first patient to receive a pig heart transplant at UMB [2]. However, Barnard's second patient lived for a remarkable 19 months.

When new surgical treatments are introduced, e.g., open heart surgery, organ transplantation, most of the initial patients offered this novel high-risk treatment are desperately sick with no alternative therapy available to them. If they have a strong desire to live and sufficient courage, they are likely to accept any possible opportunity for prolongation of life, no matter how limited the chances of long-term survival.

This was certainly the situation in which Mr. Bennett and Mr. Faucette found themselves. Both had extremely poor myocardial function with left ventricular ejection fractions of 11%–12% (whereas the normal in a healthy adult should be >50%). For number of reasons, neither was deemed suitable for allotransplantation.

Mr. Bennett had been supported by extracorporeal membrane oxygenation (ECMO) for 6 weeks before undergoing heart transplantation and, as a result of being largely immobilized during this period and previously, was in an advanced state of debility that limited his recovery. Despite intensive physical therapy and good pig heart function for approximately 45 days, he was strong enough to get out of bed on only a single occasion during the 2 months that he survived.

His recovery was not helped by the fact that a dissection of his aorta at the site of the aortic cross-clamp at the time of the heart transplant, almost certainly associated with the fragility of his blood vessel walls because of his debility, required repair. To the surgical team's credit, this was achieved successfully, but the complication resulted in renal failure, for which he required regular dialysis for the remainder of his life. The development of features suggestive of an abdominal infection or other intra-abdominal complication necessitated two laparotomies, undoubtedly contributing to his weakened state.

The very low levels of the immunoglobulins in his blood, again reflecting his prolonged debility, stimulated his medical advisors to administer intravenous immunoglobulin G (IVIg), which very likely contained anti-pig antibodies [3, 4] and may have been a factor in the development of the antibody-mediated rejection from which Mr. Bennett did not recover. In addition, the pig heart was found to harbor latent porcine cytomegalovirus (porcine roseolovirus, pCMV/pRV) whose reactivation and replication may have contributed to inflammation in the organ and to the patient's demise [4–6].

Several aspects of Mr. Bennett's care therefore needed careful reflection and some improvement to prevent complications in future patients. These included 1) removal of

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anti-pig antibodies from IVIg before its administration, and 2) a more sensitive test to determine whether the pig carried pCMV/pRV, but perhaps the most important lesson related to selection of the patient. If a patient is so debilitated that he or she is unlikely to recover full health, then possibly they should not be offered this form of therapy. When one considers the very checkered post-transplant course of Mr. Bennett, it is difficult to conclude that he benefitted in any way from the transplant though his family appreciated the extra time they could spend with him [7].

Because details of Mr. Faucette's post-transplant clinical course have not yet been reported in the literature, we know much less about the factors that might have contributed to his demise, though dialysis was once again required for renal failure and rejection has been mentioned as the cause of death (after 42 days). This is particularly concerning as Mr. Faucette received an anti-CD154mAb-based immunosuppressive regimen (which is known to be more effective than an anti-CD40mAb-based regimen, which was the therapy that Mr. Bennett received), as well as increased complement-inhibitory drugs (a C1-esterase inhibitor followed by a C5 inhibitor, eculizumab). Unless there was a change in the medication schedule that has not been reported yet, if rejection was indeed the cause of graft failure, then there is cause for concern.

Numerous studies in gene-edited pig-to-nonhuman primate (NHP) models, including those at UMB, have provided encouraging data on pig heart [8–12] or kidney [13–15] survival when either an anti-CD40mAb or an anti-CD154mAb has formed the basis of the immunosuppressive regimen. In addition, there is now considerable *in vitro* evidence that strongly suggests that the immune hurdle will be significantly weaker when triple gene-knockout (TKO) pig organs (i.e., organs from pigs in which expression of all three known pig glycan xenoantigens has been deleted) are transplanted into humans than into NHPs [16, 17]. It is therefore disappointing and of concern that both patients might have lost their grafts from rejection.

In both patients, we presume that the presence of preformed anti-pig antibodies was low or had been excluded by pre-transplant testing, and so antibody-mediated rejection should only have occurred following the development of *de novo* anti-pig antibodies, suggesting inadequate immunosuppressive therapy.

In Mr. Bennett's case, the factors that might have resulted in graft failure from rejection are more obvious than in the case of Mr. Faucette. In particular, it has been reported that anti-pig antibody concentrations remained low until postoperative day 47 when, following the administration of IVIg, a sharp increase of anti-pig IgG and, to a lesser extent, IgM was observed, possibly triggering a rejection response. Furthermore, mycophenolate mofetil therapy was discontinued due to pancytopenia from postoperative days 20–50 and instead the patient received tacrolimus from days 20 to 54. Indeed, his severely debilitated state may possibly have influenced the surgical team to reduce the intensity of immunosuppressive therapy to an inadequate level. In addition, the response to the presence of pCMV/pRV in the graft may have had a more detrimental effect on graft function than anticipated. (It has been well-documented that grafts from CMV-positive pigs fail earlier than those from pCMV/pRV -negative pigs [18, 19]).

However, all pioneering efforts are associated with errors and omissions, and it is easy to raise questions in hindsight. Without an initial effort, even if that effort is imperfect, no progress will be made. Hopefully, the causes of graft failure may become more clarified when data on Mr. Faucette's post-transplant course are published.

But what can be done now by the UMB team and by others considering clinical gene-edited pig organ transplantation?

We suggest that the first consideration might be in determining whether pig kidney transplantation should be preferred over pig heart transplantation if only because, if the kidney fails or there are other complications, e.g., life-threatening infection, the pig kidney can be excised, all immunosuppressive therapy can be discontinued, and the patient returned to support by chronic dialysis [20]. At the present time, if pig heart xenotransplantation is justified (because neither allotransplantation nor mechanical support has been deemed possible), there can be no "Plan B"—if the heart fails, the patient will die.

Furthermore, in the experimental laboratory, numerous NHPs have been supported in a healthy condition by pig kidneys for more than a year, and for a maximum of almost 4 years in one case (Adams A, personal communication). In contrast, to our knowledge no NHP has survived while supported by an orthotopically-placed pig heart for >9 months, and failure has uniformly been from antibody-mediated rejection. The expectation that a gene-edited pig heart will support a patient for a prolonged period of time (in excess of a year) is therefore not currently supported by experimental data and may be overly optimistic at the present time. With the current moderately good results of mechanical device support in adults, it is difficult to justify bridging of an adult with a pig heart.

Instead, it has been proposed that xenotransplantation should first be employed as a method of bridging infants with complex life-threatening congenital heart disease, e.g., single ventricle physiology, until a suitable cardiac allograft becomes available [10, 21]. This approach has been suggested because 1) mechanical support devices are relatively rarely successful in infants and neonates, 2) the results of cardiac allotransplantation are better in this age group than of any other organ transplants in any other age group (in part because of their immature immune system and in part because partial or total thymectomy is commonly carried out to gain access to perform the operation), 3) the results of palliative surgery are mixed at best and considered unsatisfactory in many cases, and 4) bridging does not commit the recipient to a life-long dependency on a pig heart with all the "unknowns" with which this is currently associated.

If infants could receive a gene-edited pig heart transplant soon after the birth, this may well maintain life until a cardiac allograft becomes available, which in the United States is an average of approximately 4 months (with a waitlist mortality of 34%) [22, 23]. Even if the recipient becomes sensitized to pig

antigens and produces anti-pig antibodies, the current (limited) evidence is that this would not be detrimental to the outcome of a subsequent cardiac allograft [24]. Furthermore, a successful xenograft would enable the baby to be taken home by the parents, whereas those supported by a mechanical device must remain in an intensive care unit for several months until an allograft becomes available.

As an increasing number of NHPs have been supported by pig hearts for 6 months or longer, this approach seems feasible and may be preferred to destination therapy in adult humans. The experience gained from bridging in infants could enable improvements in management to be made that lead eventually to successful destination therapy.

In summary, perhaps clinical pig xenotransplantation should at present best be directed towards kidney transplantation. Alternatively, bridging infants to cardiac allotransplantation represents an option that needs to be explored further.

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

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

CONFLICT OF INTEREST

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

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The Predictive Value of Graft Viability and Bioenergetics Testing Towards the Outcome in Liver Transplantation

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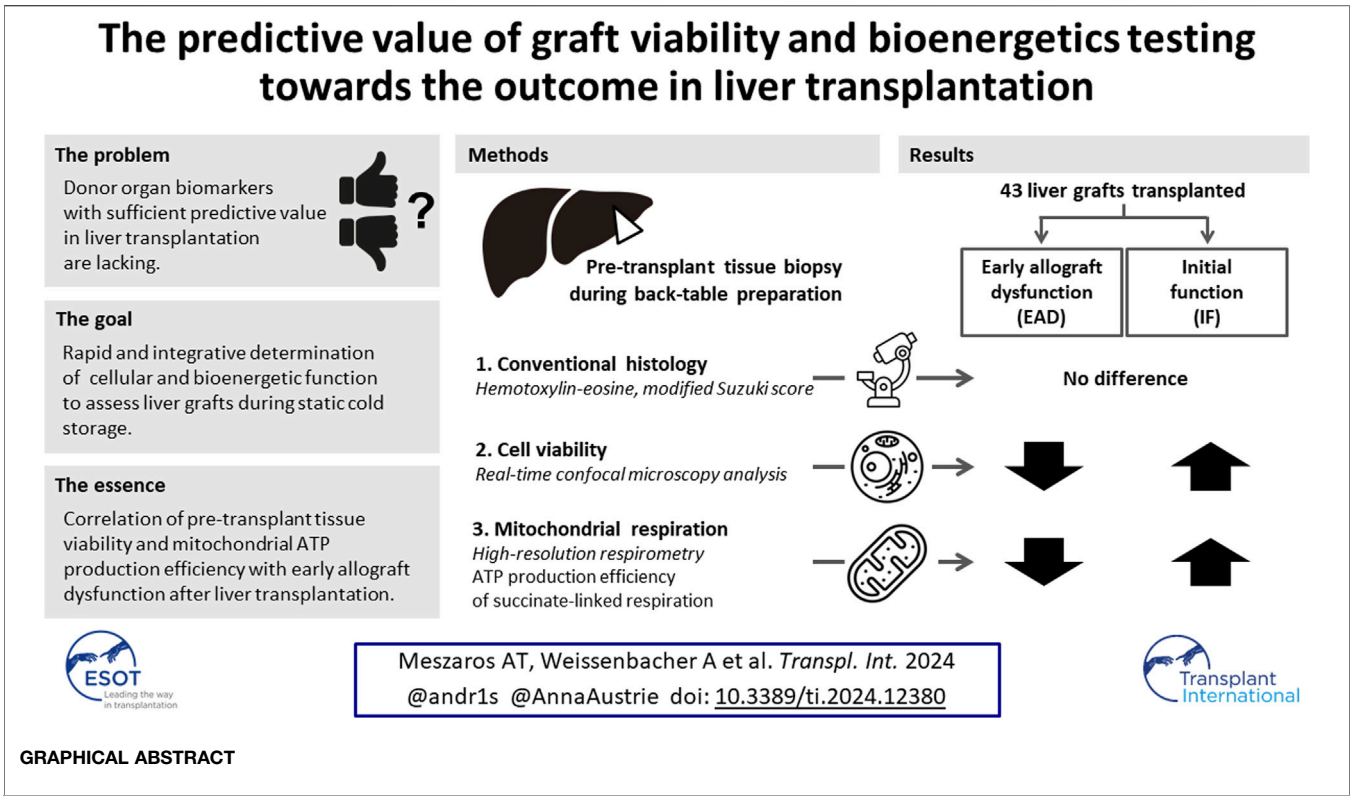
Donor organ biomarkers with sufficient predictive value in liver transplantation (LT) are lacking. We herein evaluate liver viability and mitochondrial bioenergetics for their predictive capacity towards the outcome in LT. We enrolled 43 consecutive patients undergoing LT. Liver biopsy samples taken upon arrival after static cold storage were assessed by histology, real-time confocal imaging analysis (RTCA), and high-resolution respirometry (HRR) for mitochondrial respiration of tissue homogenates. Early allograft dysfunction (EAD) served as primary endpoint. HRR data were analysed with a focus on the efficacy of ATP production or *P-L* control efficiency, calculated as 1-*L*/*P* from the capacity of oxidative phosphorylation *P* and non-phosphorylating respiration *L*. Twenty-two recipients experienced EAD. Pre-transplant histology was not predictive of EAD. The mean RTCA score was significantly lower in the EAD cohort (–0.75 ± 2.27) compared to the IF cohort (0.70 ± 2.08; $p = 0.01$), indicating decreased cell viability. *P-L* control efficiency was predictive of EAD (0.76 ± 0.06 in IF vs. 0.70 ± 0.08 in EAD-livers; $p = 0.02$) and correlated with the RTCA score. Both RTCA and *P-L* control efficiency in biopsy samples taken during cold storage have predictive capacity towards the outcome in LT. Therefore, RTCA and HRR should be considered for risk stratification, viability assessment, and bioenergetic testing in liver transplantation.

Keywords: liver, transplantation, static cold storage, mitochondria, high-resolution respirometry, real-time confocal imaging

INTRODUCTION

The limited number of organ donors and the low number of livers of deceased donors with optimal organ quality are key restricting factors in liver transplantation (LT). While the indications for LT are increasing, many technical aspects and tools for the assessment of graft quality have not changed [1, 2]. The outcomes have been improving steadily with LT survival rates reaching 90% after the first year [3], but up to 20% of patients are dying while waiting or being removed from the liver transplant waiting list due to the scarcity of available organs [2, 4–6]. Current efforts to enlarge the donor pool



GRAPHICAL ABSTRACT

and to increase organ utilization is the inclusion of livers from extended criteria donors (ECD), from donors after circulatory death (DCD), steatotic organs, and livers with longer cold and warm ischemia times [1, 7, 8]. Accepting such pre-injured organs for LT is afflicted with an increased risk of morbidity and mortality [9–12]. The above grafts are more susceptible to temperature fluctuations, ischemia and re-oxygenation (ischemia-reperfusion injury, IRI). Furthermore, the activation of damage associated molecular pattern proteins (DAMPs) in the donor (as a response of brain death) during reperfusion results in secretion of proinflammatory cytokines triggering inflammation and consecutive damage of the liver [9, 13].

In addition to the inflammatory response, the impairment of mitochondrial function during IRI is considerable. Oxygen deprivation, ATP depletion, and the enhanced generation of reactive oxygen species (ROS) during reperfusion can alter the bioenergetic status and mitochondrial integrity [9, 13–15]. While many mechanisms contributing to IRI and the subsequent organ dysfunction are known, further details and their immediate clinical implications remain to be elucidated. Both the assessment of cell viability and bioenergetic function have merit in the search for biomarkers with such predictive value.

Previously, Martins *et al.* described a clear relationship between IRI and impaired mitochondrial respiration in liver transplantation. In a murine model, mild hypothermia was protective against loss of mitochondrial membrane potential [14]. A correlation was demonstrated in a clinical trial between LT and mitochondrial function, aminotransferase peaks, and arterial lactate levels [15]. More recently, a correlation between mitochondrial injury and the outcome in LT has been suggested. Hypothermic oxygenated machine perfusion (HOPE) may improve cellular bioenergetics and flavin mononucleotide (FMN) was proposed as a biomarker, a shedding product of the mitochondrial Complex I monitored in the perfusate during machine perfusion [16, 17]. While the analysis of metabolic products such as FMN may indicate mitochondrial damage, it does not allow for evaluation of the actual bioenergetic capacity.

In addition to the bioenergetic function, the assessment of cell viability and damage during and after LT may help to predict the fate of an organ. Real-time confocal analysis (RTCA) of tissue samples was found to have predictive value toward the occurrence of delayed graft function in kidney biopsies [18]. This method was validated in a murine liver warm ischemia model [19] and its applicability for characterization of cell viability during clinical liver NMP was recently demonstrated [20].

We have previously assessed mitochondrial respiration during normothermic machine perfusion (NMP) of the liver and found a predictive capacity towards the outcome after LT [20]. The aim of the present study was to evaluate the relevance and capacity of RTCA and HRR in clinical LT after static cold storage (SCS). We hypothesized that both cell viability assessment and evaluation of mitochondrial respiratory function provide integrative assessments of subcellular and cellular function and damage to liver grafts. Our goal was to employ methods for rapid assessment without the need for isolation of mitochondria or tissue fixation. Our results confirm a correlation between RTCA and mitochondrial function and the outcome after LT.

MATERIALS AND METHODS

Clinical Trial Design

Based on a previously established technology with RTCA [19, 21, 22], a prospective, single arm, observational clinical trial was conducted at the Medical University of Innsbruck between October 2017 and October 2019. The study was approved by the institutional review board of the Medical University of Innsbruck (vote number 1025/2017). All patients participating in the trial signed the respective informed consent form.

All liver grafts stemmed from donors after brain death and none of the livers underwent machine perfusion.

Forty-three consecutive patients were included in this trial. Recipient, donor, and transplant characteristics were collected and collated. Early allograft dysfunction (EAD) served as primary endpoint, Model for Early Allograft Function (MEAF [23]) Liver Graft Assessment Following Transplantation (L-GrAFT [24, 25]), graft and patient survival, length of stay and biliary complications served as secondary endpoints. EAD was defined as the presence of one or more of i) bilirubin $\geq$10 mg·dL^{-1} on day seven after transplantation, ii) international normalized ratio (INR) $\geq$ 1.6 on day seven, and iii) alanine (ALT) or aspartate aminotransferases (AST) > 2000 IU·L^{-1} within the first 7 days after liver transplantation [26].

Sampling and Preparing Liver Biopsies for Real-Time Live Confocal Imaging

Liver wedge biopsies were taken during the back-table preparation. All biopsy samples were placed in HTK solution (Custodiol®, Dr. Franz Köhler Chemie GmbH, Bensheim, Germany) on ice for transportation prior to analysis.

Real-time live confocal microscopy assessment was performed using the following live stains: Wheat germ agglutinin conjugate (WGA; Molecular Probes, Eugene, OR, United States; 10 µg·mL^{-1} final concentration) visualizes the tissue morphology, SYTO®16 (Molecular Probes; final concentration 5 µM) visualizes all nuclei and propidium iodide (PI) (Molecular Probes; final concentration 500 nM) the nuclei of dead cells [20]. Incubation time was 15 min at 37°C. Real-time live confocal imaging was performed in eight-well chambered cover glasses (Nalge Nunc International). Images were acquired with a spinning disk confocal system (UltraVIEW VoX; Perkin Elmer, Waltham, MA) connected to a Zeiss Axio Observer Z1 microscope (Zeiss, Oberkochen, Germany) and visualized employing the Volocity software (Perkin Elmer) using a ×10 objective. Time for readout was approximately 5 min per sample.

High-Resolution Respirometry

High-resolution respirometry (HRR, O2k, Oroboros Instruments, Innsbruck, Austria) was applied to assess mitochondrial respiration. All measurements were carried out in O2k-chambers of 2 mL at 37°C under constant stirring at 750 rpm [27]. Data were acquired at intervals of 2 s and analysed with the DatLab software (Datlab 7.4, Oroboros Instruments, Innsbruck, Austria). Besides monthly instrumental background calibrations, before each experiment, air-calibration was performed with MiR05 mitochondrial respiration medium (MiR05-Kit, Oroboros Instruments, Innsbruck, Austria). The finally prepared medium consists of 0.5 mM EGTA, 3 mM $MgCl_2 \bullet 6\ H_2O$, 60 mM lactobionic acid, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM D-sucrose, 1 g·L^{-1} essentially fatty acid free bovine serum albumin. Twenty mg of liver tissue was dissected on a cooled plate at 4°C, weighted, and subsequently homogenized in 4°C MiR05 using a PBI-Shredder O2k-Set (Oroboros Instruments, Innsbruck, Austria) according to the manufacturer's instructions. 2-mL tissue homogenate with a final concentration of 1 mg wet mass·mL^{-1} was immediately added into each of the O2k-chambers. Chemicals for the pre-defined substrate-uncoupler-inhibitor titration (SUIT) protocols were titrated using glass microsyringes (Oroboros Instruments, Innsbruck, Austria). The SUIT protocols (i; corresponding SUIT-025[1]) and (ii; corresponding SUIT-006 O2 mt D047[2]) are defined in the **Supplementary Tables S1, S2**. Each titration step was carried out after respiration reached a steady state. Measurements were performed in technical duplicates.

Respiration rates were expressed as O_2 flux per wet mass tissue [pmol O_2·s^{-1}·mg^{-1}].

Three substrate pathways delivering convergent electron flow to the electron transport system were investigated. The fatty acid oxidation (FAO)-pathway F was determined in the presence of octanoylcarnitine and a low concentration of malate, the NADH-pathway N with the substrates pyruvate, glutamate, and malate. The succinate-linked pathway S was assessed after inhibiting the mitochondrial Complex I with rotenone and adding succinate. In addition to studying these pathways separately, the combined pathways FNS feeding electrons into the coenzyme Q-junction were investigated to reconstitute the tricarboxylic acid cycle function of the living cell and determine possible additive effects [28]. For further details, see **Supplementary Tables S1, S2**.

Respiratory capacities were normalized to an internal reference rate for each measurement to determine flux control ratios (*FCR*) for evaluation of SUIT protocol (i). For SUIT protocol (ii) the coupling states LEAK (*L*), OXPHOS (*P*), and OXPHOS(c) (*Pc*), were evaluated [28]. LEAK, a dissipative component of respiration, was measured in the presence of the mitochondrial Complex I inhibitor rotenone and reducing substrate succinate without ADP (rate S_L). The respiratory capacity of oxidative phosphorylation (OXPHOS) was assessed in the presence of succinate, 5 mM ADP, and 10 mM inorganic phosphate in MiR05 (S_P). Finally, cytochrome *c* was added to test the integrity of the mitochondrial outer membrane, obtaining the rate S_{Pc}. Based on the above, the following control efficiencies were calculated for the succinate pathway: *P-L* control efficiency (1-*L*/*P*), to evaluate the efficiency of ATP production in the succinate pathway, and cytochrome *c* control efficiency (j_c = 1-*P*/*Pc*) to evaluate the damage to the mitochondrial outer membrane [28].

[1]https://wiki.oroboros.at/index.php/SUIT-025
[2]https://wiki.oroboros.at/index.php/SUIT-006

Real-Time Confocal Analysis

In each liver biopsy, 10 optical sections of 1 µm were analysed. Cell viability and matrix architecture of the liver were quantified by counting events (one event is either a viable or a non-viable cell) and groups which comprise i) total count of cells, irrespective of the localization; ii) cells from the central vein area; iii) cells from the portal triad area.

For each group, the number (total count) of viable cells was divided by the number of non-viable cells (total count) with following possible results: (+1) for highly viable biopsies/areas with more viable than non-viable cells; (0) for biopsies/areas in which the number of viable cells equals the one of non-viable cells; (−1) for those in which the number of non-viable cells outnumbers the one of viable cells. For each biopsy, a score was calculated consisting of two central vein areas and one portal triad area resulting in a maximum of +3 points in the best or −3 in the worst-case scenario.

Histopathological Assessment

After completion of live confocal imaging, the liver biopsy was placed and fixed in Millonig's solution and processed for paraffin embedding. Four µm thick sections were stained by haematoxylin and eosin as per standard protocols. Light microscopy observations were carried out on a Nikon Eclipse 50i microscope (Nikon Corporation, Japan). Histological assessment was performed according to a modified Suzuki score [29] based on necrosis, steatosis, inflammation, fibrosis and vascular changes (**Supplementary Table S3**). An overall histopathologic score indicated i) normal liver tissue or only mild histopathologic alterations—score 3 (subscores 0 or 1), ii) moderate histopathologic alterations—score 2 (at least one subscore 2), or iii) severe histopathologic alterations—score 1 (at least one subscore 3). Slide scanning was performed on an Olympus VS120 microscope and evaluated using Olympus OlyVIA software.

Statistical Analysis

The statistical testing was done with Graph Pad Prism 9 and IBM® SPSS® Statistics Version 25. A p-value of <0.05 was considered as statistically significant. Biopsy results (RTCA, histology scores and HRR), recipient, donor and transplant factors were analysed using parametric and non-parametric tests (including Spearman rank correlation). The RTCA score and *P-L* control efficiency were adjusted for clinically relevant parameters and evaluated in uni- and multivariate logistic regression analyses.

RESULTS

Patient Demographics and Early Allograft Dysfunction

Table 2 depicts the demographics and the transplant data of 43 liver transplants stratified for EAD (N = 22, 51.2%) and initial function (IF, N = 21) following liver transplantation. The proportion of ECD was numerically, but not statistically higher in the cohort developing EAD (18/22, 81.1%) compared to patients with IF (13/21, 61.9%), p = 0.27. Recipients with EAD received livers from donors with a significantly higher BMI (28.05 ± 6.29 $kg{\cdot}m^{-2}$ in EAD vs. 24.6 ± 4.16 $kg{\cdot}m^{-2}$ in IF, mean ± SD; p = 0.031). The Liver and the Eurotransplant donor risk indices (DRI) were comparable between the groups. The anastomosis time was significantly longer in EAD-patients compared to patients with IF livers (47.64 ± 9.86 min in EAD vs. 40.57 ± 5.92 min in IF-patients, p = 0.02). Patients developing EAD had significantly higher mean MEAF-scores (6.68 ± 1.3, compared to liver recipients with IF, 4.77 ± 1.41, p < 0.0001). The mean L-GrAFT score was −0.63 ± 1.13 and corresponded with EAD (−0.26 ± 1.21 in EAD vs. −1.11 ± 0.82 in IF, p = 0.015).

Technical Feasibility

RTCA and scoring in fresh liver wedge biopsy samples collected from donor livers after static cold storage was completed in approximately 30 min. HRR took 90 min including sample preparation. Hence, the two methods which were carried out simultaneously proved to be feasible for immediate assessment albeit requiring availability of staff and respective expertise at the point in time. Both assessments required <40 mg tissue sample (wet mass), and, if the technology is available, have a cost per analysed sample of ca. 200 EUR.

Characterization of Mitochondrial Function in Human Liver Samples

In a first step, we analysed the capacity of mitochondrial oxidative phosphorylation (OXPHOS) in liver crude homogenates for the NADH-linked, fatty acid oxidation (FAO), and succinate pathways (**Figure 1A**; **Table 1**). In the biopsies after SCS in the whole cohort (N = 43), respiration was highest for succinate-linked OXPHOS (40.4 ± 12.6 $pmol{\cdot}s^{-1}{\cdot}mg\ wet\ mass^{-1}$), with a considerable variation between grafts. In contrast, respiration was markedly lower for the FAO and NADH pathways (10.5 ± 4.3 and 4.3 ± 3.0 $pmol{\cdot}s^{-1}{\cdot}mg\ wet\ mass^{-1}$, respectively). No difference was found between the EAD and IF groups.

Next, we calculated the flux control ratios (*FCR*) as the single pathway capacities relative to the maximum OXPHOS respiration reached with the combination of all substrates. As shown in **Figure 1B**; **Table 1**, succinate-linked respiration alone was sufficient to saturate OXPHOS capacity. This pattern of pathway control reflects an incomplete additivity [28]. Thus, our detailed analysis of mitochondrial function and calculation of the coupling control efficiencies focused on the S pathway.

Early Allograft Dysfunction, RTCA, HRR, and Histology

The assessment of RTCA and *P-L* coupling control efficiency revealed significant differences between EAD and IF livers: The

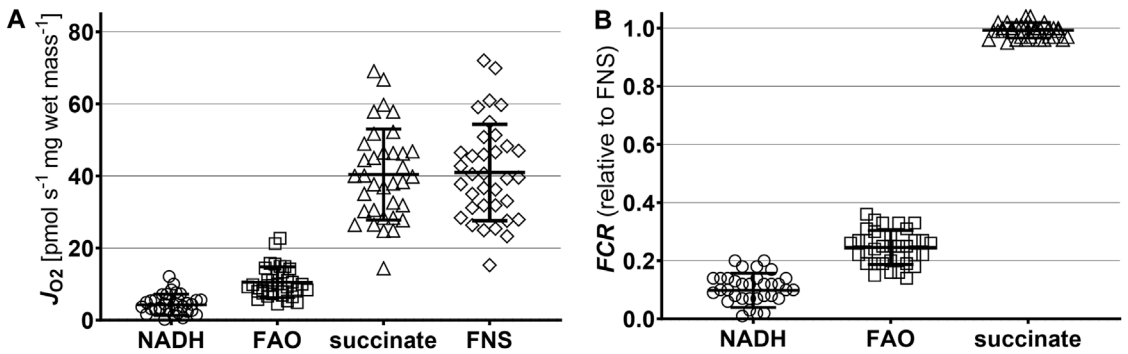


FIGURE 1 | Pathway control analysis of mitochondrial respiration after static cold storage. **(A)** Respiration in the OXPHOS state with saturating ADP concentration (5 mM). Pyruvate (5 mM), malate (2 mM), and glutamate (10 mM) were the substrates for the N-linked pathway (NADH). Octanoylcarnitine (0.5 mM) and malate (0.1 mM) supported fatty acid oxidation (FAO). Rotenone (0.5 μM) and succinate (10 mM) were added to assess the S-linked pathway (succinate). FNS represents the OXPHOS capacity with all pathways converging at the Q-junction [28]. **(B)** Relative contributions of the three mitochondrial pathways, expressed as flux control ratios (*FCR*). *FCR* of the F-, N-, and S-linked pathways were calculated relative to the maximum OXPHOS capacity reached after addition of substrates of all three pathways. Results are shown as individual values, mean ± SD.

TABLE 1 | High-resolution respirometry.

	EAD (*N* = 22)	IF (*N* = 21)	*p*-value
Characteristics (mean ± SD)			
Pathway control: OXPHOS capacity per mg wet mass			
FAO pathway F_P [pmol·s^{-1}·mg^{-1}]	10.75 ± 4.37	10.26 ± 4.23	ns
NADH pathway N_P [pmol·s^{-1}·mg^{-1}]	4.12 ± 2.88	4.43 ± 3.09	ns
Succinate pathway S_P [pmol·s^{-1}·mg^{-1}]	42.94 ± 12.62	40.04 ± 13.19	ns
Convergent pathway FNS_P [pmol·s^{-1}·mg^{-1}]	43.50 ± 13.25	40.61 ± 14.06	ns
FAO pathway *FCR* (relative to FNS)	0.25 ± 0.06	0.24 ± 0.06	ns
NADH pathway *FCR* (relative to FNS)	0.09 ± 0.06	0.11 ± 0.06	ns
Succinate pathway *FCR* (relative to FNS)	0.99 ± 0.03	0.99 ± 0.03	ns
Coupling control: Succinate pathway characteristics			
LEAK respiration S_L [pmol·s^{-1}·mg^{-1}]	12.07 ± 5.64	9.67 ± 3.67	ns
OXPHOS capacity S_P [pmol·s^{-1}·mg^{-1}]	40.76 ± 16.26	38.83 ± 11.19	0.94
Cytochrome *c* control efficiency, 1-S_P/S_{Pc}	0.17 ± 0.12	0.11 ± 0.09	0.12
***P-L* control efficiency, 1-S_L/S_P**	**0.70 ± 0.08**	**0.76 ± 0.06**	**0.02**

Statistically significant differences are bold.

mean RTCA score was significantly lower in the EAD cohort (0.75 ± 2.27 compared to 0.70 ± 2.08 in the IF cohort; $p = 0.01$), indicating a decreased cell viability. In agreement with the RTCA results, the *P-L* control efficiency was significantly better and predictive of IF (mean *P-L* control efficiency of 0.76 ± 0.06 in IF-livers vs. 0.70 ± 0.08 in EAD-livers; $p = 0.02$; **Table 2**; **Figures 2**, **3**). The MEAF score correlated negatively with the RTCA score; $p = 0.01$, Spearman's rho correlation coefficient −0.407; a lower viability correlated with a higher risk of liver dysfunction. Nonparametric correlation analysis showed that RTCA and *P-L* control efficiency are closely linked: $p = 0.005$, Spearman's rho correlation coefficient was 0.493. When RTCA score and *P-L* control efficiency were adjusted for recipient and donor age as strongest confounders, the significance of both RTCA and OXPHOS coupling was confirmed (**Table 3**).

In contrast, histology did not differ between EAD and IF, although there was a trend towards better overall scores in the IF group (**Table 2**; **Figure 4**). Accordingly, none of the individual histopathological features such as necrosis, steatosis, inflammation, and vasculitis correlated with the outcome.

Graft and Patient Survival

The 90-day mortality was 7.0% (3/43; 2 after EAD); 90-day graft loss was 4.7% (2/43; 1 after EAD). Eight patients (8/43, 18.6%, 6 after EAD) died during the follow up; three patients (3/43, 7.0%, 2 after EAD) had to undergo a re-transplant within the first year after transplant. The succinate-linked OXPHOS capacity was predictive for patient survival in the univariate Cox regression analysis.

Overall graft loss and patient death were numerically higher in the EAD group, but not significantly different in comparison to IF livers; graft loss after EAD 4/22 (18.2%) vs. IF 1/21 (4.8%), $p = 0.18$; death after EAD 6/22 (27.3%) vs. 3/21 (14.3%), $p = 0.31$. Re-transplantation was the only risk factor independently predictive

TABLE 2 | Demographics and transplant factors of liver transplant recipients with analyzed biopsies (RTCA, HRR, histology).

	EAD (*N* = 22)	IF (*N* = 21)	*p*-value
Characteristics			
Donor age, [y] (mean ± SD)	52.45 ± 15.46	48.33 ± 15.65	0.40
Donor BMI [kg·m^{-2}] (mean ± SD)	**28.05 ± 6.29**	**24.60 ± 4.16**	**0.03**
Extended criteria donor (ECD)—(*N*, %)	18 (81.8%)	13 (61.9%)	0.27
Age >65 years	5 (27.8%)	2 (15.4%)	
BMI >30 kg·m^{-2}	4 (22.2%)	2 (15.4%)	
Macrovesicular steatosis >30%	5 (27.8%)	1 (7.7%)	
ICU-stay >7 days	2 (11.1%)	1 (7.7%)	
Infection serology	2 (11.1%)	1 (7.7%)	
Hypernatremia (Na-peak >165 mEq·L^{-1})	1 (5.6%)	1 (7.7%)	
Aspartate aminotransferase >90 U·L^{-1}	5 (27.8%)	5 (38.5%)	
Alanine aminotransferase >105 U·L^{-1}	3 (16.7%)	4 (30.8%)	
Total bilirubin >3 mg·dL^{-1}	3 (16.7%)	4 (30.8%)	
LDRI (mean ± SD)	1.60 ± 0.34	1.51 ± 0.28	0.33
ET-DRI (mean ± SD)	1.75 ± 0.42	1.60 ± 0.28	0.19
Recipient age [y] (median, min-max)	60.23 ± 10.39	59.24 ± 9.29	0.48
Recipient BMI [kg·m^{-2}] (mean, SD)	26.61 ± 5.10	25.72 ± 5.26	0.59
Prior transplantation (*N*, %)	1 (4.6%)	2 (9.5%)	
MELD score (mean ± SD)	16.41 ± 7.41	18.80 ± 8.00	0.38
Cold ischemia time [h] (mean ± SD)	8.41 ± 1.99	7.82 ± 2.26	0.5
Anhepatic time [min] (mean ± SD)	61.05 ± 19.54	52.29 ± 16.03	0.05
Anastomosis time [min] (mean ± SD)	**47.64 ± 9.86**	**40.57 ± 5.92**	**0.02**
Length of hospital stay [days] (median, IQR)	18.5 (15, 23.5)	19 (14.5, 28.5)	0.61
ICU stay after LT [days] (median, IQR)	4.5 (3, 7)	4 (2.5, 9.5)	0.81
RTCA score	**–0.75 ± 2.27**	**0.70 ± 2.08**	**0.01**
High-resolution respirometry			
OXPHOS capacity S_P [pmol·s^{-1}·mg wet mass^{-1}]	40.76 ± 16.26	38.83 ± 11.19	0.94
Cytochrome *c* control efficiency, 1-S_P/S_{Pc}	0.17 ± 0.12	0.11 ± 0.09	0.12
***P-L* control efficiency, 1-S_L/S_P**	**0.70 ± 0.08**	**0.76 ± 0.06**	**0.02**
Histology	2.52 ± 0.68	2.85 ± 0.49	0.06
Necrosis	0.41 ± 0.96	0.33 ± 0.73	0.87
Steatosis	0.64 ± 0.85	0.43 ± 0.60	0.61
Inflammation	0.65 ± 0.59	0.30 ± 0.47	0.08
Fibrosis	none	none	n.a
Vasculitis	0.20 ± 0.41	0.20 ± 0.41	1.000

Statistically significant differences are bold.

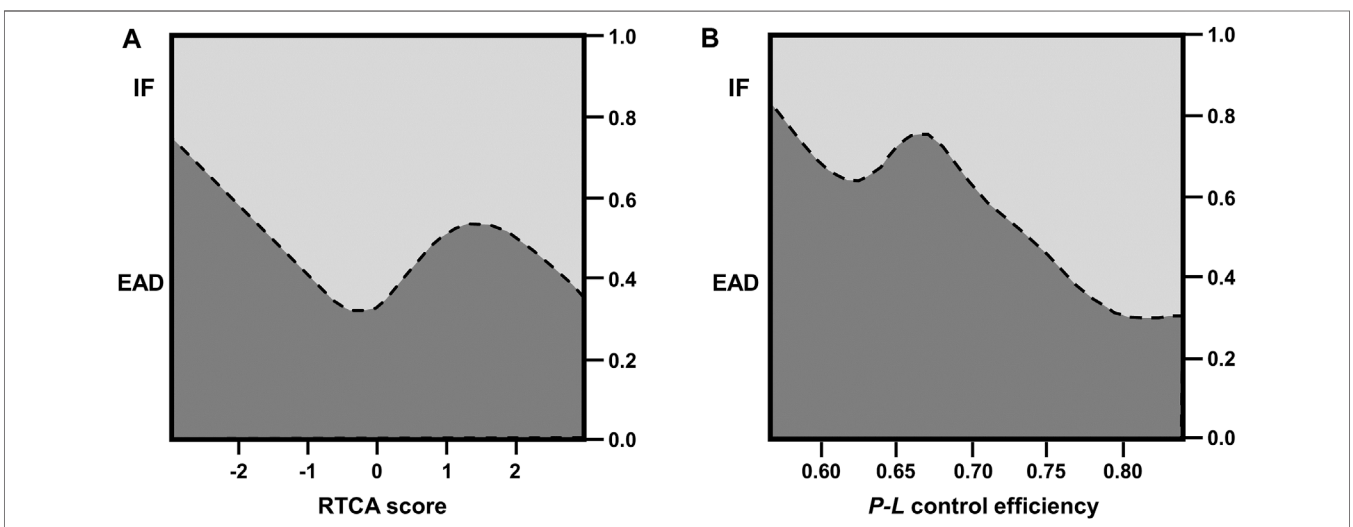


FIGURE 2 | Conditional density plots of **(A)** RTCA score and **(B)** *P-L* coupling control efficiency of succinate pathway as factors impacting EAD.

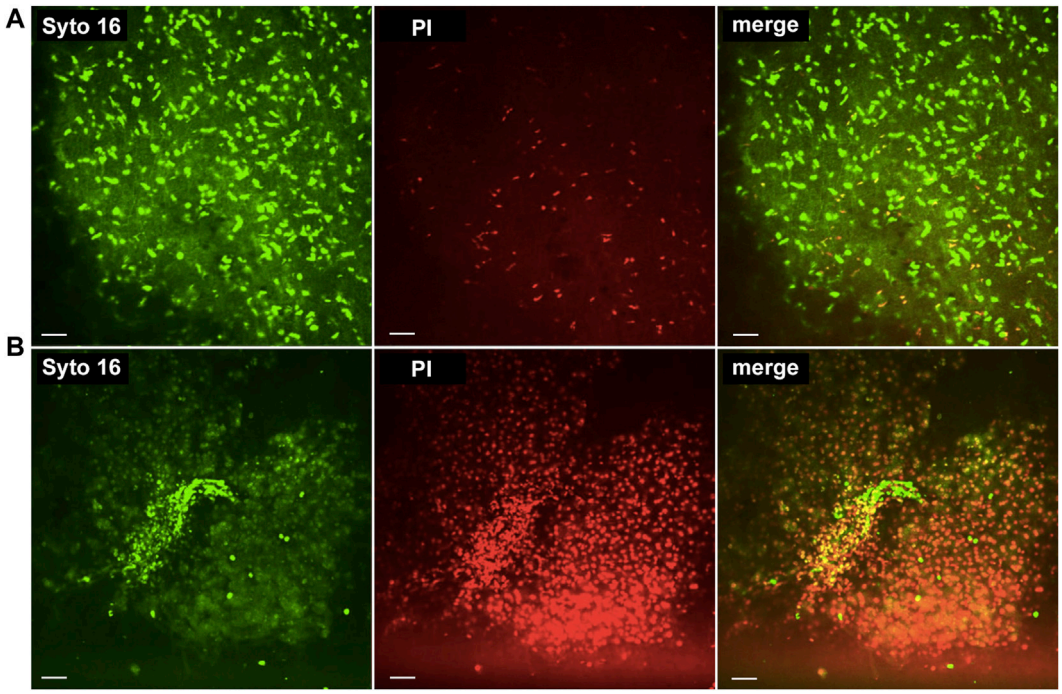


FIGURE 3 | Real-time confocal microscopy. **(A)** RTCA score +3: 54-year-old female, non-steatotic liver; cause of death was traumatic head injury; cold ischemia time 8 h 12 min. Liver recipient did not experience early allograft dysfunction. **(B)** RTCA score −3: 53-year-old, female, non-steatotic liver; cause of death was intracerebral bleeding; cold ischemia time 9 h 32 min. Liver recipient suffered early allograft dysfunction. We used the following stains: i) Syto16®, which stains nuclei in dead and living cells, ii) propidium iodide (PI), which is only taken up by nuclei of dead cells; the merged image demonstrates the relation of stained cells within the tissue.

TABLE 3 | RTCA score and *P-L* control efficiency as factors impacting EAD—adjusted for age.

Model A	**Wald**	**Odds ratio**	**95% CI**	***p-value***
Recipient age	0.126	0.987	0.921–1.059	*0.723*
Donor age	0.846	1.024	0.974–1.076	*0.358*
RTCA score	**3.886**	**0.736**	**0.542–0.998**	***0.049***
Model B	**Wald**	**Odds Ratio**	**95% CI**	***p-value***
Recipient age	0.268	0.979	0.905–1.060	*0.610*
Donor age	0.070	0.994	0.948–1.041	*0.791*
***P-L* control efficiency 1-S_L/S_P**	**3.918**	**0.000**	**0.000–0.893**	***0.048***

RTCA, real-time confocal analysis.
EAD, early allograft dysfunction.
Statistically significant differences are bold.

for graft survival in the univariate Cox regression analysis; p = 0.04, HR 19.3, Wald 4.4. Univariate and multivariate analyses for patient survival are displayed in **Tables 4**, **5**. The most important and independent factor for patient survival was also re-transplantation; p = 0.001, HR 105.2, Wald 10.279.

DISCUSSION

In this prospective clinical pilot trial, we assessed liver biopsies using HRR and RTCA during SCS. We found both methods applicable, clinically feasible and more meaningful for the short-term outcome after LT when compared to standard haematoxylin and eosin histology of pre-implantation biopsies. Our approach with analysis during SCS was designed to mimic a pre-transplant decision-making process, similar as aided by routine frozen section histology.

Martins and co-workers previously evaluated mitochondrial function as a possible tool to determine graft quality before LT [15]. Their comprehensive study design included measurement of mitochondrial respiration, mitochondrial membrane potential, and intracellular ATP content. In the present clinical trial, we

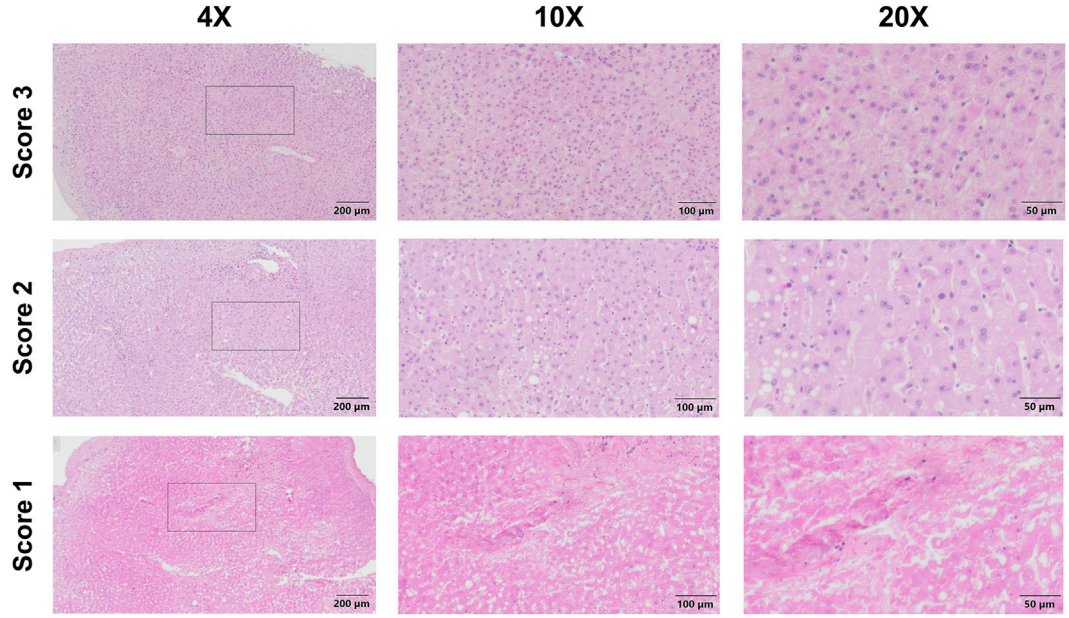


FIGURE 4 | Histopathology of liver biopsy samples prior to liver transplantation. Representative images for overall histopathology score 3 (normal liver tissue, first row), score 2 (moderate changes including steatosis and mild periportal and parenchymal inflammation, second row), and score 1 (severe changes including extensive necrosis, third row) are shown.

TABLE 4 | Univariate Cox regression analysis—patient survival.

Characteristic	Wald	HR	95% CI	*p-value*
Recipient age [y]	**4.43**	**0.943**	**0.892–0.996**	**0.035**
Recipient BMI [kg·m^{-2}]	0.139	1.026	0.896–1.176	0.71
Recipient sex	0.547	0.553	0.115–2.662	0.46
BAR score	0.086	1.018	0.905–1.144	0.8
Prior Tx	**12.776**	**66.01**	**6.635–656.759**	**< 0.001**
Donor age in [y]	0.005	1.001	0.960–1.044	0.945
Donor BMI [kg·m^{-2}]	0.02	0.991	0.882–1.115	0.887
Donor sex	0.114	0.797	0.214–2.973	0.736
Extended criteria donor (ECD)	1.282	3.326	0.415–26.637	0.258
ET-DRI	3.269	3.56	0.899–14.100	0.071
LDRI	2.347	5.469	0.622–48.090	0.126
Cold ischemia time [h]	1.734	1.004	0.998–1.009	0.19
Anastomosis time [min]	0.451	1.023	0.958–1.093	0.5
RTCA score	1.236	1.196	0.872–1.641	0.266
Succinate-linked OXPHOS capacity S_P [pmol·s^{-1}·mg wet mass^{-1}]	**4.955**	**1.054**	**1.006–1.104**	**0.03**
P-L control efficiency 1-S_L/S_P	1.344	0.003	0.000–56.131	0.25
Steatosis in zero biopsy	0.268	1.237	0.554–2.764	0.6
Necrosis in zero biopsy	0.332	0.742	0.267–2.054	0.6
EAD	0.768	1.859	0.464–7.440	0.381
Graft loss	**8.995**	**7.657**	**2.025–28.958**	**0.003**

HR, hazard ratio; CI, confidence interval; BAR, balance of risk; ET DRI, eurotransplant donor risk index; LDRI, liver donor risk index; RTCA, real-time confocal analysis. Statistically significant differences are bold.

aimed at validating HRR as a rapid assay requiring only a small tissue sample mass. We found that *P-L* control efficiency of the succinate pathway measured before transplantation correlates with EAD. The results of our study are based on a limited number of liver transplants which calls for caution in the interpretation of statistical tests. However, mitochondrial function was aligned with the RTCA score and correlated with the clinical endpoints, indicating that we are measuring a true and relevant phenomenon.

Recently, Weissenbacher *et al.* [18] published the predictive value of the RTCA score for delayed graft function (DGF) in kidney pre-implantation biopsies. The added value and information of the RTCA in this study is the quantification of the acute and ischemia-related cellular damage in addition to pre-

TABLE 5 | Multivariate Cox regression analysis—patient survival.

Characteristic	Wald	HR	95% CI	*p-value*
Recipient age [y]	0.624	0.965	0.884–1.054	0.429
Prior transplantation	**10.279**	**105.188**	**6.108–1811.585**	**0.001**
Succinate–linked OXPHOS capacity S_P [pmol·s^{-1}·mg wet mass^{-1}]	0.209	1.018	0.944–1.097	0.65
Graft loss	2.724	6.494	0.704–59.878	0.099

HR, hazard ratio; CI, confidence interval.
Statistically significant differences are bold.

existing injury as characterized by histology. Similar to kidney transplantation, standard histology failed to predict the initial function in LT. The degree of steatosis and necrosis in the pre-implantation biopsy did not correlate with early allograft function. While this might be attributable to the limited sample size, it also relates to the fact that microscopic structural damage is a parameter with limited value towards the decision to transplant or discard an individual organ. The added value of RTCA together with mitochondrial assessment is due to the fact, that these techniques measure, display and quantify the additional acute injury at cellular and subcellular levels.

For HRR, only 2 mg of sample (wet mass) per measurement were required. This is an order of magnitude less when compared to a recent study employing other methods for functional assessment of mitochondrial respiration [15] and speaks towards the feasibility of this approach in clinical settings. Instead of time-consuming isolation of mitochondria for HRR, a liver homogenate was prepared using a tissue shredder. This preparation method takes less than 5 min, requires a small sample size, and contains all mitochondrial subpopulations [30]. Mitochondrial respiration was assessed at 37°C, ruling out temperature-dependent deviations for the extent and mechanism of mitochondrial coupling.

The *P-L* control efficiency (ratio of net to total OXPHOS capacity) for the succinate pathway was calculated and used as a statistically more robust parameter compared to the classical respiratory acceptor control ratio (RCR), the State 3/State 4 flux ratio [28]. Importantly, the OXPHOS state is defined by saturating ADP and inorganic phosphate concentrations while State 3 only indicates high ADP and inorganic phosphate concentrations, which are not necessarily saturating.

While RTCA and mitochondrial function were predictive of postoperative organ function, they were not predictive for graft or patient survival. In contrast, the OXPHOS capacity of the mitochondrial succinate pathway was found predictive of patient survival in the univariate analysis. This parameter is closely related to the tissue viability and mitochondrial mass concentration. Recently, our group demonstrated the predictive value of mitochondrial respiratory capacity in a setting of clinical NMP and LT [20]. In agreement with the published trial, we herein establish the feasibility and value of a functional mitochondrial measurement in standard cold storage and LT.

Whereas the assessment requires fresh tissue, the considerable advantage of HRR and RTCA is the rapid process. For HRR, the tiny tissue sample is rewarmed to 37°C and resupplied with oxygen, mimicking physiological temperatures, thus enabling mitochondrial performance testing despite sampling during SCS.

The high percentage of EAD in the cohort is primarily not a result of a bias due to the relatively low number of transplanted livers. As demonstrated by Fodor *et al.* [31], the rate of EAD in our center raised over the last years, mainly because of increasing acceptance of ECD grafts. Indeed, in the present study cohort, 72% of the liver grafts stemmed from ECD donors. Recent developments with pre-transplant machine perfusion are promising ways to reduce EAD.

In summary, tissue analysis by RTCA and HRR shed light into viability and bioenergetic performance of SCS liver allografts and can be applied to anticipate EAD. Our results further enhance the understanding and relevance of bioenergetic function in liver ischemia and transplantation and provide the basis for further consideration of these parameters as biomarkers in LT. These observations confirm previous studies and serve to underline the feasibility of RTCA and mitochondrial functional tests as tools for liver quality assessment prior to transplantation [15].

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

The studies involving humans were approved by the Institutional Review Board of the Medical University of Innsbruck. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RO, AW, SS, and EG established the LBC study setup and design and coordinated the team. MS coordinated the HRR measurements. AM, AW, and MS analysed the data and wrote the manuscript together with SS. RO, AM, MS, TE-B, MH, JU, CM, BR, JH, BZ, TH, TR, CM, MM, TK, BC, and EG participated in data collection, data analysis and critical revision of the

manuscript. TK and EG contributed reagents, analytic tools and participated in data analysis. HU participated in data analysis and statistical support. DÖ, JT, and SS supported the study, contributed research advice, and revised the manuscript critically. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

EG is founder and CEO of Oroboros Instruments.

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.12380/full#supplementary-material

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GLOSSARY

ALT alanine aminotransferase

AST aspartate aminotransferase

ATP adenosine triphosphate

BAR balance of risk

BMI body mass index

DAMP damage associated molecular pattern

DCD donation after circulatory death

EAD early allograft dysfunction

ECD extended criteria donors

ET-DRI Eurotransplant donor risk index

F FAO pathway

FAO fatty acid oxidation

FCR flux control ratio

FMN flavin mononucleotide

HOPE hypothermic oxygenated machine perfusion

HR hazard ratio

HRR high-resolution respirometry

IF initial function

IRI ischemia-reperfusion injury

L LEAK respiration

LDRI Liver donor risk index

LEAK the state of non-phosphorylating resting respiration in the absence of ADP

L-GrAFT Liver Graft Assessment Following Transplantation

LT liver transplantation

MEAF Model for Early Allograft Function

N NADH-linked pathway

OXPHOS oxidative phosphorylation

P respiratory OXPHOS rate in the presence of kinetically saturating ADP

PI propidium iodide

ROS reactive oxygen species

RTCA real-time confocal imaging analysis

S succinate-linked pathway

SCS static cold storage

SUIT Substrate-Uncoupler-Inhibitor Titration

WGA wheat germ agglutinin

ESOT | Transplant International

ORIGINAL RESEARCH
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Clinical Impact and Risk Factors of Seizure After Liver Transplantation: A Nested Case-Control Study

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Seizures are a frequent neurological consequence following liver transplantation (LT), however, research on their clinical impact and risk factors is lacking. Using a nested case-control design, patients diagnosed with seizures (seizure group) within 1-year post-transplantation were matched to controls who had not experienced seizures until the corresponding time points at a 1:5 ratio to perform survival and risk factor analyses. Seizures developed in 61 of 1,243 patients (4.9%) at median of 11 days after LT. Five-year graft survival was significantly lower in the seizure group than in the controls (50.6% vs. 78.2%, respectively, $p < 0.001$) and seizure was a significant risk factor for graft loss after adjusting for variables (HR 2.04, 95% CI 1.24–3.33). In multivariable logistic regression, body mass index <23 kg/m^2, donor age ≥45 years, intraoperative continuous renal replacement therapy and delta sodium level ≥4 mmol/L emerged as independent risk factors for post-LT seizure. Delta sodium level ≥4 mmol/L was associated with seizures, regardless of the severity of preoperative hyponatremia. Identifying and controlling those risk factors are required to prevent post-LT seizures which could result in worse graft outcome.

Keywords: seizure, liver transplantation, hyponatremia, sodium, neurologic complication

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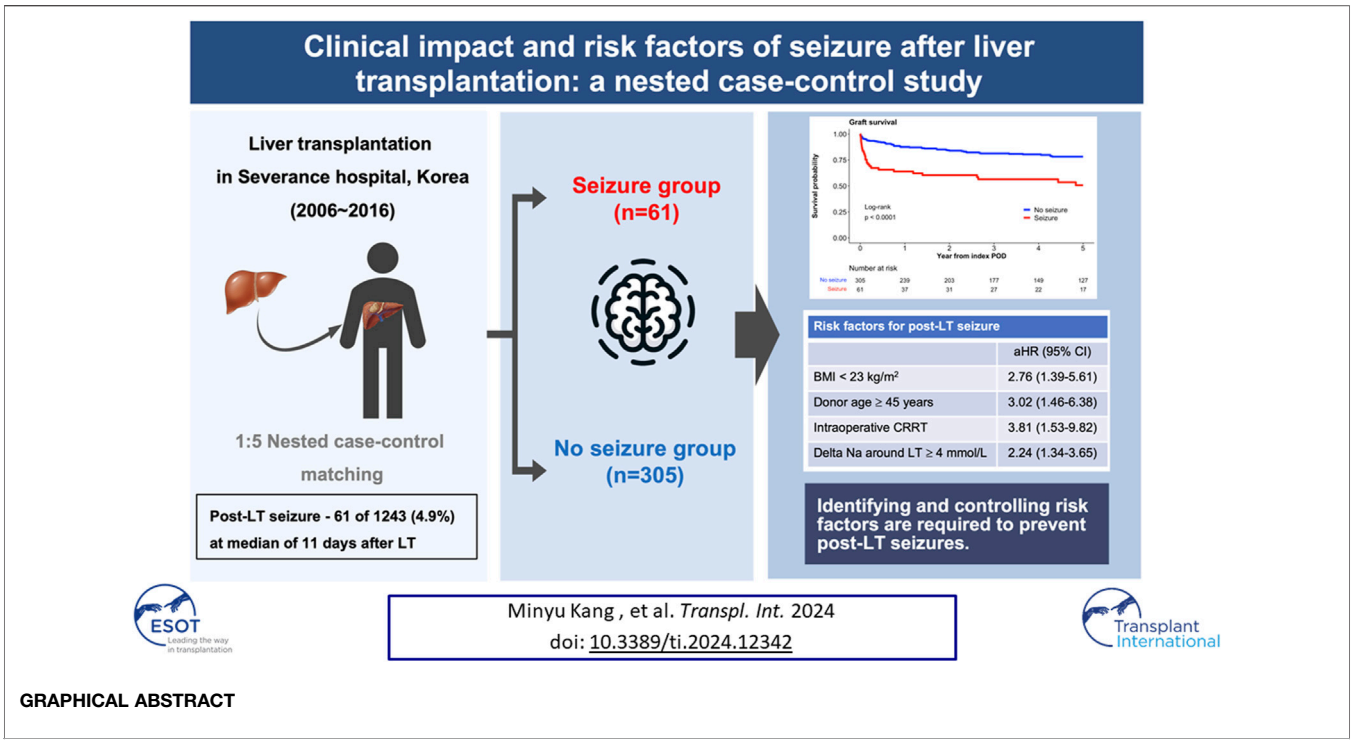
INTRODUCTION

Liver transplantation (LT) is a life-saving procedure for various end-stage liver diseases and hepatocellular carcinoma. Despite significant advancements in surgical techniques and postoperative care, LT patients are susceptible to a range of complications, with neurological events being particularly concerning [1, 2]. Among these, seizures stand out both for their frequency and their impact on patient outcomes [3, 4].

Seizures occur in approximately 10% of LT recipients, a rate notably higher than in other postoperative scenarios [5]. The etiology of these seizures is multifactorial, often involving systemic infections and rapid shifts in electrolyte and osmotic balances. In some instances, seizures are secondary to other neurological events like ischemic strokes or brain hemorrhages [5]. Notably, while

Abbreviations: BMI, body mass index; CI, confidence interval; DOS, demyelinating osmotic syndrome; CRRT, continuous renal replacement therapy; CVA, cerebrovascular accident; HR, hazard ratio; IQR, interquartile range; LT, liver transplantation; MELD, model for end-stage liver disease; NA, sodium; OR, odds ratio; POD, postoperative day; PS, propensity score.



GRAPHICAL ABSTRACT

demyelinating osmotic syndrome (DOS) and other brain imaging abnormalities can accompany these seizures, they can also occur with no apparent imaging anomalies.

This higher incidence of seizures in LT patients can be attributed to various factors inherent to the transplantation process. Pre-existing conditions like hepatic encephalopathy and hyponatremia in LT candidates have been recognized as contributing factors [6, 7]. Post-transplant, the complex interplay of immunosuppressive therapy, particularly with the widespread use of tacrolimus, infection, and metabolic disturbances, creates a conducive environment for neurological complications [7].

The impact of seizures on LT outcomes has not been extensively studied, especially in the context of long-term survival. Existing studies, albeit limited in volume, indicate a significant association between post-transplant seizures and early mortality [8–10]. However, the long-term implications of these seizures and their specific risk factors remain inadequately explored. This retrospective study aimed to determine the clinical impact and risk factors of seizures after LT.

MATERIALS AND METHODS

Study Population

Among 1,385 LTs performed between July 2005 and December 2021 at Severance Hospital, Korea, where living donor LT is predominant [11]. Patients aged <18 years ($n = 120$), those with a history of seizures before LT ($n = 15$), and those with missing data ($n = 7$) were excluded from the study. From the 1,243 eligible patients who underwent LT, those diagnosed with seizures at 1-year post-transplantation upon consultation with a neurologist were recruited. The causes of seizures were identified using blood tests and brain imaging studies and categorized according to the presence and type of abnormalities in imaging studies.

Nested Case-Control Design

Patients diagnosed with seizures (seizure group) were matched to controls (no-seizure group) at a 1:5 ratio using a nested case-control design (**Figure 1**). Possible control individuals who had not experienced seizures (regardless of the possibility of seizures in the future) were randomly sampled at the corresponding time point (index postoperative day [POD]) when seizures developed in the seizure group. The year of LT was matched during the sampling process to ensure a comparable follow-up duration. Patients selected for the no-seizure group at certain time points were reused as potential controls at the subsequent sampling time for the seizure group unless seizures had not occurred before then. These procedures were conducted with *ccwc* function of *Epi* package (version 2.47.1) in R. The resulting left-truncated data were followed from the index POD until death, retransplantation, 5 years after sampling, or June 2022, whichever came first. If the sampled controls experienced seizures thereafter, they were censored during seizure development for the survival analyses.

Data Collection

Baseline characteristics of the recipient and donor and transplant factors were retrieved from the institutional LT database. In addition, data on pretransplant cerebrovascular accident (CVA), pretransplant dialysis, and intraoperative continuous renal replacement therapy (CRRT) among the sampled cohort

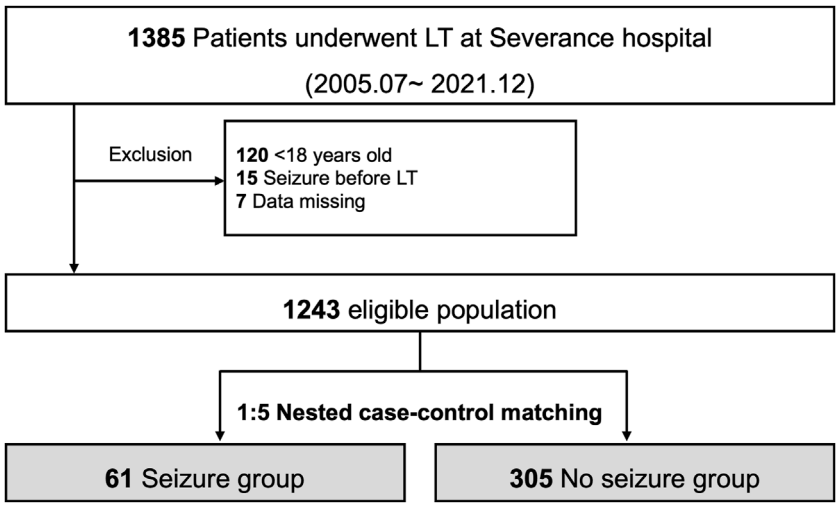


FIGURE 1 | Study flow for nested case-control study.

were collected from electronic medical records. Pretransplant sodium (Na) level was categorized according to severity, and delta Na level was calculated as the difference between Na levels measured closest to the time of surgery among those recorded before and after the LT.

After applying a nested case-control design, various laboratory results at the index POD of each patient were merged with those of a matched cohort. In addition, the use of each immunosuppressant was defined as prescriptions over 50% of post-transplant days before the index POD. To analyze the association between tacrolimus exposure and seizures, tacrolimus trough levels before the index POD were estimated. Data regarding surgical complications, rejection, and sepsis before the index POD were also collected. Graft loss was defined as patient death or retransplantation.

Statistical Analysis

According to their normality, data were presented as a number (percentage) for categorical variables and as a median (interquartile range [IQR]) for continuous variables. The chi-square test or Wilcoxon rank-sum test was used, as necessary, to compare the seizure group with the control group. Graft survival following the index POD was compared between the two groups using the Kaplan–Meier curve and log-rank test to examine the clinical impact of seizures. The relationship between seizures and graft survival was assessed using univariate and multivariate Cox regression analyses. For more robustness, graft survival was compared between matched population upon deciles of propensity score (PS) which was calculated using all baseline variables [12]. The matching was considered adequately balanced when the standardized mean differences between the groups were below 0.1 [13].

Risk factors for posttransplant seizures were examined using logistic regression analysis. Significant continuous variables were entered into the model after categorization with cutoff values determined by the Yuden Index, which were analyzed using the pROC package of R software [14, 15]. Considering the relatively small number of events compared to the number of variables we intended to evaluate, multivariable logistic and Cox regression models were created using the backward stepwise method. All analyses were performed using the R statistical package, version 4.2.0 for macOS,[1] with the threshold for significance set at $p < 0.05$.

Ethic Approval

This study was performed in accordance with the Declaration of Helsinki and Declaration of Istanbul and was approved by the Institutional Review Board at Severance Hospital, Yonsei University Health System (IRB No. 4-2023-1567). Informed consent was not required because of the study's retrospective design.

RESULTS

Of the 1,243 eligible patients, 61 (4.9%) experienced seizures within 1 year after LT (**Figure 1**). The median time from LT to seizure was 11 (IQR: 6–26) days, and 47 of the 61 (77.0%) patients developed seizures within 30 days after LT (**Supplementary Figure S1**). Among the 61 patients with seizures, 14 (22.9%) showed structural abnormalities on brain imaging. The brain structural abnormalities identified included cerebral hemorrhage (n = 3), cerebral infarction (n = 5), posterior reversible encephalopathy syndrome (n = 3), DOS (n = 2), and hypoxic brain damage (n = 1). In the nested case-control design, 305 control patients were matched with the seizure group patients.

[1]http://cran.r-project.org

TABLE 1 | Baseline characteristics.

Variables	Seizure (*n* = 61)	No seizure (*n* = 305)	*P*
Age	53 (45–63)	54 (47–59)	0.941
Sex, female	13 (21.3)	94 (30.8)	0.181
BMI, kg/m^2	22.6 (20.7–24.5)	23.9 (22.0–26.3)	0.007
Year of LT			1.000
2012–2015	27 (44.3)	135 (44.3)	
2016–2018	21 (34.4)	105 (34.4)	
2019–2021	13 (21.3)	65 (21.3)	
Hypertension	9 (14.8)	75 (24.6)	0.133
Diabetes mellitus	17 (27.9)	88 (28.9)	1.000
Cardiovascular disease	8 (13.1)	25 (8.2)	0.327
Underlying liver disease			0.084
Viral	32 (52.5)	172 (56.4)	
Alcoholic	24 (39.3)	75 (24.6)	
Others	5 (8.2)	58 (19.0)	
HCC	25 (41.0)	149 (48.9)	0.326
Pretransplant MELD	23 (15–32)	15 (10–24)	<0.001
Pretransplant stay			<0.001
Out-patient day	24 (39.3)	161 (52.8)	
Ward	26 (42.6)	130 (42.6)	
Intensive care unit	11 (18.0)	14 (4.6)	
Refractory ascites	20 (32.8)	54 (17.7)	0.012
Encephalopathy			0.001
No	32 (52.5)	231 (75.7)	
Mild	20 (32.8)	49 (16.1)	
Moderate to severe	9 (14.8)	25 (8.2)	
Re-transplantation	1 (1.6)	6 (2.0)	1.000
ABO incompatibility	5 (8.2)	51 (16.7)	0.135
Donor type			0.012
Living	31 (50.8)	209 (68.5)	
Deceased	30 (49.2)	96 (31.5)	
Donor age	47 (32–54)	37 (27–47)	0.005
Donor sex, female	14 (23.0)	104 (34.1)	0.121
Donor BMI	22.3 (20.5–24.7)	22.9 (21.1–24.7)	0.277
Operation time, min	594 (472–660)	592 (504–699)	0.284
RBC transfusion, L	2.7 (1.2–4.5)	1.2 (0.6–2.4)	<0.001
Pretransplant CVA	4 (6.6)	3 (1.0)	0.017
Pretransplant dialysis	14 (23.0)	23 (7.5)	0.001
Intraoperative CRRT	22 (36.1)	23 (7.5)	<0.001
Pretransplant hyponatremia			0.007
Normal (≥135 mmol/L)	39 (63.9)	242 (79.3)	
Mild (130–134 mmol/L)	13 (21.3)	36 (11.8)	
Moderate (126–129 mmol/L)	3 (4.9)	19 (6.2)	
Severe (<126 mmol/L)	6 (9.8)	8 (2.6)	
Delta Na around LT[a], mmol/L	5 (4–7)	3 (2–6)	<0.001

BMI, body mass index; CRRT, continuous renal replacement therapy; CVA, cerebrovascular accident; HCC, hepatocellular carcinoma; LT, liver transplantation; MELD, model for end-stage liver disease; RBC, red blood cell.

[a]*Difference of Na between before and after LT, within 24 h.*

Baseline Characteristics

As shown in **Table 1**, the age and sex were similar in the seizure and non-seizure groups. The seizure group showed lower BMI than did the control group (22.6 [IQR: 20.7–24.5] kg/m^2 vs. 23.9 [IQR: 22.0–26.3] kg/m^2, p = 0.007). There were no differences in the incidence of hypertension, diabetes mellitus, or cardiovascular disease between the groups. The frequency of the underlying liver diseases was not statistically different between the groups; however, the seizure group had a higher proportion of patients with alcoholic liver disease than did the control group (39.3% vs. 24.6%). Pretransplant model for end-stage liver disease (MELD) score (23 [IQR: 15–32] vs. 15 [IQR: 10–24], p < 0.001) was higher, and pretransplant intensive care unit (ICU) stay (18.0% vs. 4.6%, p < 0.001) was more frequent in the seizure group than in the control group. Encephalopathy before LT was more severe in the seizure group than in the control group (p = 0.001). The seizure group showed a higher proportion of deceased donor LT (49.2% vs. 31.5%, p = 0.005) and an advanced donor age (47 [IQR: 32–54] years vs. 37 [IQR: 27–47] years, p = 0.005) than did the control group. The seizure group received more red blood cell transfusion (2.7 [IQR: 1.2–4.5] vs. 1.2 [IQR: 0.6–2.4] L, p < 0.001), had more pretransplant CVAs (6.6% vs. 1.0%, p = 0.017), and received more pretransplant dialysis (23.0% vs. 7.5%,

TABLE 2 | Information at index POD.

Variables[a]	Seizure (*n* = 61)	No seizure (*n* = 305)	P
Total bilirubin, mg/dL	3.1 (1.1–8.3)	1.3 (0.8–2.6)	<0.001
AST, IU/L	43 (27–79)	33 (21–74)	0.110
ALT, IU/L	54 (21–79)	57 (20–133)	0.274
Creatinine, mg/dL	0.9 (0.6–1.4)	0.8 (0.6–1.2)	0.269
BUN, mg/dL	27.8 (18.6–47.3)	20.3 (14.5–31.7)	<0.001
Albumin, mg/dL	3.1 (2.9–3.4)	3.4 (3.1–3.7)	<0.001
Glucose, mg/dL	156 (134–184)	138 (113–181)	0.014
Na, mmol/L	138 (135–142)	138 (136–140)	0.955
White blood cell, 10^3/μL	6.9 (4.5–12.1)	6.2 (4.6–9.0)	0.171
Hemoglobin, g/dL	9.5 (8.3–10.5)	10.1 (8.9–11.3)	0.004
Platelet, 10^3/μL	63 (45–126)	110 (67–165)	<0.001
Use of immunosuppressants[b]			
Tacrolimus	60 (98.4)	298 (97.7)	1.000
Mycophenolate mofetil	23 (37.7)	150 (49.2)	0.134
mTOR inhibitor	4 (6.6)	17 (5.6)	1.000
Steroid	53 (86.9)	269 (88.2)	0.943
Tacrolimus trough level, ng/dL[b]			
Mean	6.6 (4.5–8.7)	7.3 (5.4–10.3)	0.100
Standard deviation	2.9 (2.1–4.3)	2.5 (1.5–3.9)	0.287
Maximum	11.1 (7.7–18.0)	11.3 (7.8–17.8)	0.826
Variance	7.7 (5.5–11.0)	8.0 (5.5–12.7)	0.214
Coefficient of variance	8.5 (4.3–18.9)	6.4 (2.2–15.4)	0.287
Prior rejection	3 (4.9)	34 (11.1)	0.215
Prior bile duct complication	4 (6.6)	22 (7.2)	1.000
Prior vascular complication	4 (6.6)	5 (1.6)	0.070
Reoperation	14 (23.0)	59 (19.3)	0.640
Sepsis	10 (16.4)	21 (6.9)	0.029

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; mTOR, mammalian target of rapamycin; POD, post-operative day.
[a]*Values were acquired from LT to index POD in each patients.*
[b]*Use of each immunsuppressants was defined as prescription at over 50% of post-transplant days before index POD.*

$p = 0.001$) and intraoperative CRRT (36.1% vs. 7.5%, $p < 0.001$) than did the control group. Severe pretransplant hyponatremia was observed in the seizure group (9.8% vs. 2.6%, $p = 0.007$), and delta Na (5 [IQR: 4–7] vs. 3 [IQR: 2–6], $p < 0.001$) was also higher in the seizure group than in the control group.

Information at Index POD

At matched index POD, the seizure group showed higher total bilirubin (3.1 [IQR: 1.1–8.3] mg/dL vs. 1.3 [IQR: 0.8–2.6] mg/dL, $p < 0.001$), blood urea nitrogen (27.8 [IQR: 18.6–47.3] mg/dL vs. 20.3 [IQR: 14.5–31.7] mg/dL, $p < 0.001$), and glucose (156 [IQR: 134–184] mg/dL vs. 138 [IQR: 113–181] mg/dL, $p = 0.014$, **Table 2**) levels than did the control group. Albumin level was lower in the seizure group than in the control group (3.1 [IQR: 2.9–34] mg/dL vs. 3.4 [IQR: 3.1–3.7] mg/dL, $p < 0.001$). Hemoglobin level (9.5 [IQR 8.3–10.5] g/dL vs. 10.1 [IQR 8.9–11.3] g/dL, $p = 0.004$) and platelet count (63 [IQR 45–126] × 10^3/μL vs. 110 [67–165] × 10^3/μL, $p < 0.001$) were also lower in the seizure group than in the control group. The use of each immunosuppressant and tacrolimus trough level were similar between the groups in terms of the mean, standard deviation, maximum variance, and coefficient of variance. The rates of post-LT complications, such as rejection, bile duct complications, vascular complications, and reoperation, prior to the index POD were also similar between the groups, except for sepsis, which was higher in the seizure group than in the control group (16.4% vs. 6.9%, $p = 0.029$).

Seizure and Graft Survival

Among 366 matched population, 86 patients (23.5%) experienced graft loss (85 death and 1 retransplantation) within 5 years after index POD. In the Kaplan-Meier analysis, graft survival rate after the index POD was significantly lower in the seizure group than in the control group (63.9%, 56.4%, and 50.6% at 1, 3, and 5 years, respectively, in the seizure group vs. 87.5%, 81.4%, and 78.2% at 1, 3, and 5 years, respectively, in the no-seizure group, $p < 0.001$, **Figure 2**). In uni- and multivariable Cox regression models, post-LT seizure was an independent risk factor for graft loss in the matched cohort (hazard ratio [HR]: 2.04, 95% CI: 1.24–3.33, **Supplementary Table S1**). When the seizure group was matched with those who did not experience seizure on PS, hazardous effect of seizure on the graft survival was also observed (**Supplementary Figure S2**), although all variables were balanced between two groups (**Supplementary Table S2**).

Infection, graft failure, and HCC recurrence were three most common causes of death in both groups (**Supplementary Figure S3**). Among them, infectious death was significantly higher in the seizure group than the control group (18.0% vs. 5.9%, $p = 0.003$, **Supplementary Table S3**).

Risk Factors for Seizure After LT

In uni- and multivariable logistic regressions (**Table 3**), the independent risk factors for post-LT seizures were BMI <23 kg/m^2 (odds ratio [OR]: 2.76, 95% CI: 1.39–5.61), donor age ≥45 years (OR: 3.02, 95% CI: 1.46–6.38),

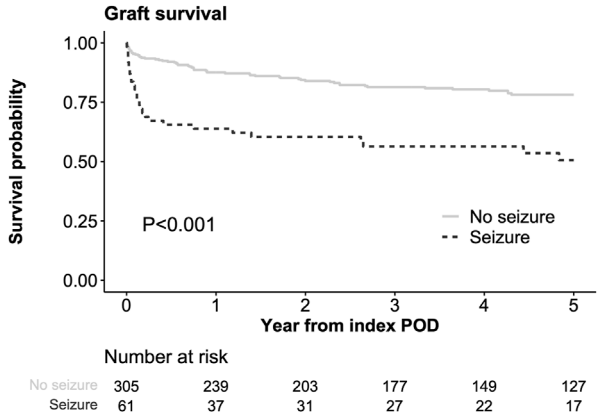


FIGURE 2 | Comparison of graft survival from index POD Index POD was the time of seizure occurrence in the seizure group and the corresponding POD in the matched control groups.

intraoperative CRRT (OR: 3.81, 95% CI: 1.53–9.82), and delta Na ≥4 mmol/L (OR: 5.38, 95% CI: 2.55–12.3). Among laboratory values at index POD, total bilirubin level ≥2.5 mg/dL (OR: 2.50, 95% CI: 1.21–5.24) and albumin level <3.5 mg/dL (OR: 6.75, 95% CI: 2.13–28.4) emerged as independent risk factors for post-LT seizures. When we performed sensitivity analysis only including seizures without structural abnormality, same risk factors were observed (**Supplementary Table S4**).

Delta Na and Seizure Risk in Pre-LT Hyponatremia Subgroups

In the subgroup without pre-LT hyponatremia (≥135 mmol/L), the incidence of seizures was significantly higher when delta Na was ≥4 mmol/L than when it was <4 mmol/L (21.2% vs. 7.4%, $p = 0.002$; **Table 4**). In the subgroup with pre-LT hyponatremia (<135 mmol/L), post-transplant seizures occurred more frequently when the delta Na was ≥4 mmol/L than when it was <4 mmol/L, although this was not significant owing to the small effect size (30.2% vs. 13.6%, $p = 0.215$). Delta Na ≥4 mmol/L was significantly associated with post-transplant seizure after adjustment of other risk factors in both subgroups with (OR: 5.16, 95% CI: 2.12–14.1) or without pre-LT hyponatremia (OR: 11.2, 95% CI: 1.79–120, **Supplementary Table S5**).

DISCUSSION

This study demonstrated the clinical impact of post-LT seizures and their associated risk factors using a retrospective nested case-control design. Among the matched population, the incidence of seizure was significantly associated with a low graft survival rate, even after adjusting for baseline covariates and various information at matched time points after LT. Furthermore, we demonstrated various risks factors for seizure including change of Na, which contains clinical implication for the prevention of seizure after LT.

LT candidates often have complications such as hepatic encephalopathy and hyponatremia, and patients with high MELD scores and acute/acute-on-chronic liver failure are often ICU stay- or ventilator-dependent [6, 16–18]. In addition to conditions before LT, various post-LT factors, such as electrolyte alteration, infection, and medications, including CNI, could cause a higher incidence of seizures [5]. Reports of seizures after other non-brain surgeries are fewer than those on the incidence of seizures after cardiac surgery (2.7%); the incidence of seizures in patients who underwent LT is higher than that in patients who underwent other surgeries [19]. Post-LT seizures can cause prolonged ICU stay and ventilator use and can be a critical cause of worsening LT outcomes, regardless of the

TABLE 3 | Risk factor analyses for seizure after LT.

Variables	Univariable		Multivariable[a]	
	OR (95% CI)	*P*	OR (95% CI)	*P*
BMI < 23 kg/m^2	2.13 (1.23–3.75)	0.008	2.76 (1.39–5.61)	0.004
Alcoholic	1.99 (1.11–3.52)	0.019	1.77 (0.85–3.66)	0.123
Pretransplant MELD ≥18	2.90 (1.64–5.23)	<0.001	0.72 (0.30–1.70)	0.466
Encephalopathy				
No	—		—	
Mild	2.95 (1.54–5.55)	<0.001	1.48 (0.61–3.46)	0.375
Moderate to severe	2.60 (1.07–5.91)	0.027	1.99 (0.64–6.08)	0.227
Donor age ≥ 45 years	2.91 (1.66–5.12)	<0.001	3.02 (1.46–6.38)	0.003
Pretransplant CVA	7.06 (1.52–36.6)	<0.001	3.33 (0.63–19.3)	0.155
RBC transfusion ≥ 2L	3.63 (2.06–6.54)	<0.001	1.24 (0.59–2.57)	0.570
Intraoperative CRRT	6.92 (3.52–13.6)	<0.001	3.81 (1.53–9.82)	0.005
Delta Na around LT ≥ 4 mmol/L	3.56 (1.93–6.96)	<0.001	5.38 (2.55–12.3)	<0.001
Laboratory results at index POD				
Total bilirubin ≥ 2.5 mg/dL	4.57 (2.59–8.23)	<0.001	2.50 (1.21–5.24)	0.014
Albumin < 3.5 mg/dL	6.06 (2.40–20.4)	<0.001	6.75 (2.13–28.4)	0.003

BMI, body mass index; CRRT, continuous renal replacement therapy; CVA, cerebrovascular accident; HCC, hepatocellular carcinoma; LT, liver transplantation; MELD, model for end-stage liver disease; RBC, red blood cell.

[a]*Model was established by backward stepwise method and only variables included in the model were presented.*

TABLE 4 | Incidence of seizure by pretransplant hyponatremia and delta Na around LT.

	PreLT Na ≥ 135 (*n* = 281)			PreLT Na < 135 (*n* = 85)		
	Delta Na ≥ 4 (*n* = 132)	Delta Na < 4 (*n* = 149)	P	Delta Na ≥ 4 (*n* = 63)	Delta Na < 4 (*n* = 22)	P
Group			0.002			0.215
No seizure	104 (78.8%)	138 (92.6%)		44 (69.8%)	19 (86.4%)	
Seizure	28 (21.2%)	11 (7.4%)		19 (30.2%)	3 (13.6%)	

liver graft function. This could be supported by higher infectious death in the seizure group (18.0%) than in the controls (5.9%) among our study population. Therefore, identifying the risk factors for seizures after LT and managing modifiable factors can improve LT outcomes. This study suggests that management strategies, including limiting excessive Na replacement before and after LT, can prevent LT mortality due to seizures.

Approximately 23% of adult patients who experience their first epileptic seizure reportedly show abnormalities on brain imaging [20]. The prognosis after seizures worsens when structural problems are present [21]. Seizures in patients who underwent LT have also been reported to be accompanied by structural abnormalities such as stroke, DOS, and PRES, but few studies have focused on the seizure itself or examined the proportion of structural abnormalities [2, 5, 22, 23]. In the LT population in this study, seizures occurred without imaging abnormalities in 83.6% of patients with seizures. The overall incidence rate was 4.9%, and most cases (77.0%) occurred within 30 days of surgery. This is thought to be because patients who underwent LT have different risks and mechanisms of seizure occurrence compared with those of the general population. Therefore, it is important to identify LT-specific seizure risk factors and the correct modifiable factors.

The grade of encephalopathy before LT has been reported as an important risk factor for neurological complications in previous studies [6, 24, 25]. Patients with alcoholic liver cirrhosis are prone to Wernicke's encephalopathy with associated seizures [26, 27]. However, in this study, an increase in Na concentration before and after LT was an important risk factor, regardless of these factors. The occurrence of DOS due to rapid Na correction to 10–12 mmol/L within 24 h in transplant patients with hyponatremia is a well-known complication [22]. However, this study showed that even a mild increase in sodium (≥4 mmol/L) increases the risk of seizures regardless of the severity of pretransplant hyponatremia. More research is required to determine whether focused management of perioperative Na alterations can successfully prevent post-transplant seizures.

A low BMI was an independent risk factor for seizures after LT. Low BMI was considered an indicator of sarcopenia in our LT population, which has been shown to be related to neurological disorders such as dementia, ischemic stroke, depression, and cognitive impairment in recent studies [28]. Dopaminergic dysfunction, neuronal hypoexcitability, brain atrophy, and neuromuscular junction dysfunction are the regulatory processes associated with the pathophysiology of sarcopenia. Although evidence is insufficient, various hormonal and electrophysiological changes in patients with sarcopenia can lead to a high incidence of post-LT seizures [29]. Furthermore, low BMI represents malnutrition which may enhance the tacrolimus induced neurotoxicity [30]. Although sarcopenia and seizure risk have not yet been directly studied, and sarcopenia was not directly measured in this study, its association with neuropsychiatric complications is clear; therefore, it is thought that the control and treatment of sarcopenia to prevent seizures are also important.

Intraoperative CRRT is a strong risk factor for seizures after LT. In contrast to the typical seizure prevalence of 1%, patients with renal failure reported a lifetime seizure prevalence of 9% due to uremia and electrolyte disturbances [31]. Patients receiving hemodialysis are more likely to experience seizures not only because of a higher chance of hypotension and electrolyte imbalance but also because electrolytes are cleared more quickly from their blood than from their cerebral spinal fluid [32]. Because most patients undergoing CRRT have acute or acute-on-chronic liver failure, the underlying cirrhosis itself may be severe, and the seizure risk may be increased. Current hypoalbuminemia was also a significant risk factor for seizures. Hypoalbuminemia also reflects the severity of the liver failure, which is indicated by an MELD score of 3.0 [33]. Nevertheless, the association between albumin level and seizure risk should be evaluated in future studies.

Retrospective features could have resulted in a selection bias between patients who experienced seizures and controls in this study. In addition, the actual type of seizure was not identified, and the difference between seizures with or without imaging abnormalities was not validated owing to the relatively small number of patients in the seizure group. Finally, our results should be interpreted with caution because the living donor LT-dominant population usually undergoes surgery in an elective setting.

Despite these limitations, this nested case-control study demonstrated that seizure occurrence could be related with low graft survival rate. In addition to low BMI, advanced donor age, intraoperative CRRT, total bilirubin and albumin levels, and perioperative Na change ≥4 mmol/L significantly increased post-LT seizures. Identifying and controlling those risk factors may aid in the prevention of post-LT seizures.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by the Institutional Review Board at Severance Hospital, Yonsei University Health System. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because of the study's retrospective design.

AUTHOR CONTRIBUTIONS

D-GK has full access to all aspects of the study and takes responsibility for the integrity of the data and accuracy of the data analysis; MK, H-HK, and D-GK participated in the research design; SY, MC, E-KM, JL, DJ, and MSK participated in performing the research; MK, H-HK, MC, E-KM, and SY participated in the data acquisition; MK, H-HK, and D-GK participated in the statistical analysis; MK and H-HK participated in the writing of the paper; and D-GK supervised the study. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.12342/full#supplementary-material

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ORIGINAL RESEARCH
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Virtual Physical Prehabilitation in Lung Transplant Candidates: A Proof-of-Concept Study

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This study aimed to preliminary test the effectiveness of 12-week virtual physical prehabilitation program followed by a maintenance phase. The main objective was to estimate the extent to which it affects exercise capacity, frailty, lower limb strength and health-related quality of life (HRQOL) in lung transplant candidates. The program offered supervised strengthening exercises, independent aerobic exercises and weekly phone calls (maintenance phase). Primary outcome was the six-minute walk distance (6MWD). Secondary outcomes: the Short Physical Performance Battery (SPPB), five-times sit-to-stand test (5STS), the St George's Respiratory Questionnaire (SGRQ) for HRQOL. Twenty patients were included (mean age 57.9; 6 women/14 men); fourteen completed the prehabilitation program and 5 completed the maintenance phase. There was no statistically significant improvement in 6MWD, SPPB or SGRQ after the 12-week program. Most patients either maintained or improved the 6MWT and SPPB scores. There was a significant improvement in the 5STS. After the maintenance phase, most patients either improved or maintained their scores in all outcomes except for the sub-score of symptoms in the SGRQ. A 12-week virtual physical prehabilitation program with a 12-week maintenance phase can help lung transplant candidates improve or maintain their physical function while waiting for transplantation.

Keywords: lung transplantation, prehabilitation, telerehabilitation, exercise, rehabilitation

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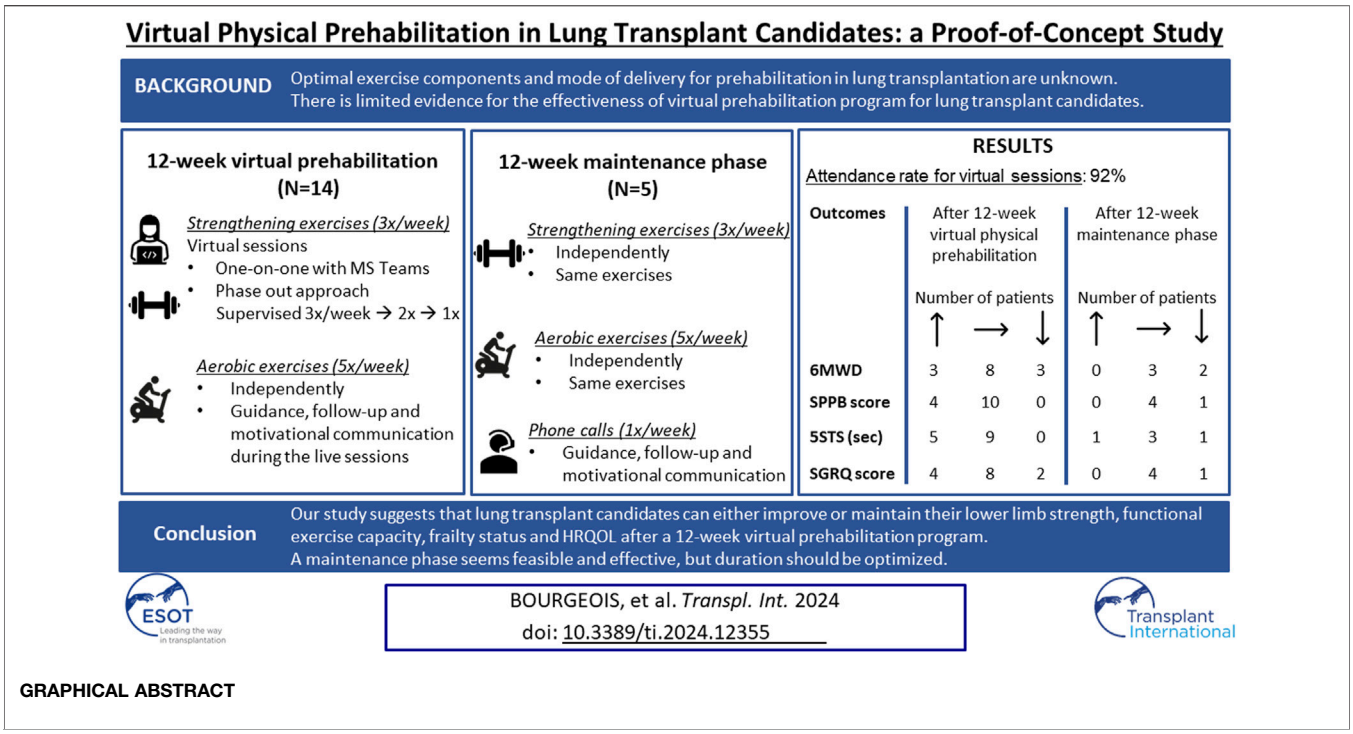
INTRODUCTION

Individuals with advanced lung disease, including lung transplant candidates, present symptoms of dyspnea, decreased exercise capacity and muscle strength and are commonly frail; all of which impact their daily activities and societal roles [1, 2]. Limitations in exercise capacity in these individuals can negatively impact their clinical outcomes prior to and after lung transplantation [3]. For example, functional exercise capacity [assessed using the 6-min walk test (6MWT)] has been associated with mortality in patients awaiting lung transplantation [3] and following lung transplantation [3]. Frailty is also an important clinical factor as it has been shown to be associated with greater disability and delisting pre-lung transplant [4].

Although prehabilitation is recommended for lung transplant candidates to improve their physical and psychological health prior to the surgery and to obtain a faster recovery post-



GRAPHICAL ABSTRACT

transplant [5], there is a very limited number of randomized controlled trials of exercise interventions in lung transplant candidates [6–8] and thus, the evidence is still scarce [5, 9]. A recent consensus statement on prehabilitation for solid organ transplantation candidates [9] stated that the optimal exercise components and mode of delivery for prehabilitation in lung transplantation are unknown.

Prehabilitation interventions included in the published literature are center-based [6, 10–13] or a mix of center-based with home-based [14–17]. Center-based programs may not be the optimal mode of delivery as transplant candidates may be waiting for their transplant in a distant location from the transplant centres. Unsupervised home-based exercises are feasible but may affect patients' engagement and adherence [18, 19]. Technology tools incorporated into home-based delivery models have the potential to enhance uptake, adherence and communication between patients and providers as well as improve the efficiency for patient monitoring for safety and effectiveness [20]. During COVID-19 pandemic, many programs switched from center-based to virtual rehabilitation and continue to use this mode of delivery [21]. However, there is limited evidence for the effectiveness of virtual prehabilitation program for lung transplant candidates. Layton et al. [22] performed a 12-week home-based rehabilitation via an app in lung transplant candidates, but only patients with cystic fibrosis were included. Singer et al. [19] performed an 8-week home-based intervention through a mobile health application targeting frail lung transplant candidates, however, the intervention was mostly unsupervised, and the authors excluded patients with pulmonary hypertension.

In 2020, our team completed a retrospective study which examined the changes in functional exercise capacity in lung transplant candidates who had received counselling to perform exercises at home with no supervision [18]. This study demonstrated that the majority of the lung transplant candidates who performed the exercises at home were able to either increase or maintain their 6MWD during the waiting list period [18]. Due to its retrospective nature and the limited outcome measures included, a formal prospective evaluation of such program is required. Following the ORBIT model for Developing Behavioral Treatments for Chronic Diseases [23], our first prospective evaluation will be a proof-of-concept study which will determine if our improved intervention deserves more rigorous and costly testing using a randomized controlled trial.

The aim of this study is to preliminary test the effectiveness of an improved home-based exercise program with supervision and use of technology. The specific objectives are 1) to estimate the extent to which a 12-week virtual physical prehabilitation program affect exercise capacity, frailty, functional leg strength and health-related quality of life (HRQOL) in lung transplant candidates; 2) to estimate the extent to which any improvement in outcomes is maintained after a 12-week maintenance phase; 3) to assess the safety and acceptability of the improved intervention.

PATIENTS AND METHODS

This was a prospective longitudinal sequential study with three times points. The reporting of the findings is based on the CONSORT checklist extension for feasibility trials [24] and the Consensus on Exercise Reporting Template (CERT) [25].

The study was conducted at the Centre Hospitalier de l'Université de Montréal (CHUM) (Montreal, Quebec, Canada) between November 2021 and February 2023. The study was approved by the University of Montreal Health Centre Research Ethics Board.

Participants

We recruited consecutive men or women (aged ≥18 years) who were being assessed to be listed for lung transplantation at the CHUM. Participants had to speak English or French and technologically capable of connecting (either independently or through household members) with an online videoconferencing platform. A tablet was lent to participants who did not have one. We excluded patients who were: 1) planning to be listed on the emergency waiting list as they would very likely not complete our intervention, 2) participating in a structured exercise program (hospital-based or home-based) and 3) hospitalized for any reason during the assessment for eligibility or waiting for the lung transplant. We also excluded patients who had pre-existing or newly identified cardiac, musculoskeletal, or neurological condition that could affect their exercise performance or otherwise render prehabilitation participation unsafe and patients who had pre-existing or newly identified significant cognitive impairment. The recruitment was made by the physiotherapist from the Lung Transplant Program. Medical clearance was given by a respirologist. Participants did not receive remuneration for this study other than being allowed to keep the fitness tracker used for the study.

Intervention

The intervention was delivered by Willkin, an incorporated company that offers specialized kinesiologist services. The intervention consisted of a 12-week virtual physical prehabilitation program (induction phase) and a 12-week maintenance phase with independent home exercises.

The exercise program during the induction phase included lower and upper body strengthening (3 times/week) as well as independent aerobic exercises (5 times/week). The strengthening exercises consisted of functional exercises for lower extremities and weight exercises for upper extremities with existing home equipment (e.g., dumbbells, elastics or bottles/cans). No exercise equipment was given to patients. The strengthening exercises were administered through a screen interface over the Microsoft Teams video conferencing platform and lasted around 30 min. All live video sessions were performed in a one-on-one manner and sessions were not recorded.

The supervised sessions followed a phase-out approach to encourage progressive autonomy and long-term adherence to prescribed exercises. There were three supervised live video sessions/week during weeks 1–4; two supervised live video sessions/week during weeks 5–8 (and one independent session/week) and one supervised live video session/week during weeks 9–12 (and two independent sessions/week). The target intensity for strengthening exercises was a moderate intensity (rating of 3-4) on the Borg 0–10 scale [26] for dyspnea though initial intensities varied by patient. Training progression was tailored to each patient and were accomplished by a combination of repetition and/or set increases and by prescribing increasingly difficult exercises. The modifications were guided by participant feedback with the Borg scale improvements at the beginning and end of each session. Supplemental oxygen was titrated based on the initial 6MWT and patients were using the prescribed oxygen when doing the exercises. The exercise session was stopped if saturation dropped below 85%. Participants received a pulse oximeter if they did not have one.

Guidance and motivational communication were offered during the live sessions to encourage participants to perform the independent aerobic exercises. Recommendations were for at least 30 min of exercise 5 times per week, which could be done with a treadmill or stationary bike if available at home or walking in a mall or outdoors. A moderate-intense level with maintaining 3-4 in the Borg 0–10 scale [26] was recommended. As a safety measure, each participant wore a pulse oximeter for point-of-care heart rate and oxygen saturation information at each supervised or independent session.

After the 12-week virtual physical prehabilitation phase, patients were encouraged to maintain their exercise program that were prescribed previously (aerobic and strengthening) independently for 12 weeks. During this maintenance phase, they received weekly phone calls from the kinesiologist with motivational messages to keep them engaged. If patients were not transplanted within the 24-week period of the intervention, the physiotherapist of the Lung Transplant Program continued the follow-up according to the current standard of practice.

Outcome Measures

Participants were assessed by the physiotherapist of the Lung Transplant Program. The outcomes were collected before (T0) and after the 12-week virtual physical prehabilitation phase (T1) and at the end of the maintenance phase (T2) (except for the acceptability outcome which was assessed at T2 only).

Descriptive Measures

We collected age, sex, body mass index, primary pulmonary diagnosis, oxygen requirements, comorbidities, lung and cardiac function.

Primary Outcome Measure

The primary outcome was functional exercise capacity (distance in meters) measured using the 6MWT according to the American Thoracic Society guidelines [27] for directives and encouragement. The predicted value of the 6MWT was calculated using the formula from normative data of healthy Canadians aged 45–85 years: 6MWD = 970.7 + (−5.5 × age) + (56.3 × gender), where females = 0, males = 1 [28].

Oxygen saturation and dyspnea [measured using the BORG scale 0–10 (26)] was assessed before, during and immediately after the 6MWT. The number of rests during the test was recorded. Oxygen requirement during the test was recorded as flow rate and delivery system and then converted to the estimated fraction of inspired oxygen (FiO_2) using a suggested conversion table [29].

Secondary Outcome Measures

Physical frailty was measured using the Short Physical Performance Battery (SPPB) [30]. The SPPB measures lower extremity function and is considered as a surrogate measure of physical frailty in adult lung transplant candidates [4, 31]. It has been found to have similar construct validity to the Fried Frailty Phenotype Index [31]. In lung transplant candidates, the SPPB has been categorized as frail (≤7/12), pre-frail [8, 9], and non-frail ≥10 [4]. The SPPB consists of three sub-tests scored from 0–4: standing balance, 4-m gait speed test and 5-repetition sit-to-stand (5STS) [32]. A score of 4 indicates the highest level of performance and 0 indicates inability to complete the task [32]. The results of the five-times sit-to-stand (5STS) component were also presented separately as a measure of functional lower limb strength [33].

Health-related quality of life (HRQOL) was assessed using the St George's Respiratory Questionnaire (SGRQ). The SGRQ measures disease impact on overall health, daily life, and perceived wellbeing in individuals with chronic lung diseases including lung transplant candidates [34]. Adherence was used using a multi-modal strategy as suggested by the World Health Organization [35]. Adherence to the exercise program was monitored using two diaries and a fitness tracker (AK1980, China) which was used as a pedometer to record steps. To reach the objectives of 30 min of exercise per day, we used a proposed calculation of 3000 steps for 30 min of walking [36] as a decision for adherence. Participants recorded their number of steps daily in a document and recorded their unsupervised exercise sessions in another document including the duration of aerobic training, type of strengthening exercises including number of sets and repetitions and series. Both documents were in paper format and were retrieved at the end of the study, The acceptability of the intervention was assessed using a semantic differential scale that consisted of 16 questions graded on a 7-point Likert scale with a total possible score of 48. For analysis, answers with grades 1 to 3 were classified as being in agreement with the statement, 0 classified as neutral and −1 to −3 as being in disagreement. Adverse outcome, costs in Canadian dollars of therapist hours and equipment were documented.

Analysis

Based on the data from our retrospective study [18], we required a sample size of 5 to achieve a power of 80% and a level of significance of 5% (two sided) for detecting a mean difference of 85.8 m in the 6MWT between pre- and post-intervention, assuming that the SD of the difference is 42.8 m (the minimal clinically important difference (MCID) in this population is 30 m [37]). However, to be powered for our secondary outcome (frailty measured by the SPPB), 11 patients were required (based on data from Wickerson et al. [4]; mean difference of 1 point and standard deviation of 1). To account for a 15% refusal rate [38] and loss to follow-up (patients who would eventually be transplanted before the end of the intervention), we planned to include 20 patients. Normality of the data was tested with the Shapiro-Wilk Test. Paired t-tests were performed to examine the changes in the outcomes pre (T0) vs post induction phase (T1) in normally distributed data. Wilcoxon rank test was used if the normality of the data distribution was not obtained. Due to the small number of participants completing the maintenance phase, the data on the difference between the end of the induction phase (T1) and the end of maintenance phase (T2) were reported descriptively. All p-values are two-tailed, and values < 0.05 were considered statistically significant. We calculated the effect size (ES) of each outcome using the Cohen's d calculation and degree of ES [39]. We analyzed the changes in each outcome related to the MCID of each of them. We used the following MCID: 30m for the 6MWD (37), 1.7 s for the 5STS [40], 8 for the SGRQ [41, 42] and 1 for the SPPB [43]. Statistical analysis was performed using SPSS software (SPSS, version 26.0; IBM).

RESULTS

Participant Characteristics

Fifty-three patients referred for lung transplantation between November 2021 and August 2022 were assessed for eligibility. After applying the inclusion and exclusion criteria, twenty-four patients (45%) were offered to enter the study and 20 accepted to participate. Fourteen patients completed the induction phase (70% and five completed the maintenance phase (25%) (**Figure 1**). During the virtual prehabilitation phase, three patients were transplanted before the reassessment and three patients were excluded for medical reasons (**Figure 1**). Of the 14 participants who started the maintenance phase, 8 were transplanted before the final assessment and one was excluded as he was no longer a candidate for transplant. Baseline characteristics of the 20 patients are presented in the **Table 1**. When comparing the 14 patients who

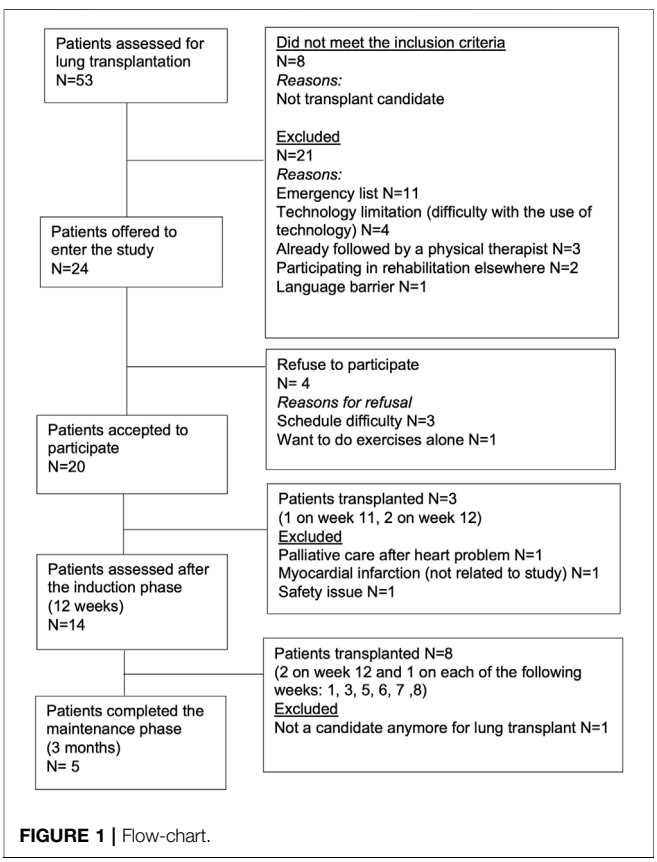


FIGURE 1 | Flow-chart.

TABLE 1 | Participant characteristics at baseline.

	Patients included (N = 20)
Age (years)	57.9 ± 11.0
Sex: Female/Male [n (%)]	6 (30)/14 (70)
Primary diagnosis [n (%)]	
ILD	9 (45)
COPD	6 (30)
CF	2 (10)
Retransplant	1 (5)
PAH	1 (5)
Sclerodermia	1 (5)
Comorbidities (>1 patient) [n (%)]	
Gastroesophageal reflux disease	6 (30)
Hypertension	4 (20)
Osteoporosis	4 (20)
Dyslipidemia	4 (20)
Anxiety	4 (20)
Coronary heart disease	2 (10)
Diabetes	2 (10)
Anemia	2 (10)
Clinical characteristics	
BMI (kg/m^2)	23.2 ± 4.4
Home oxygen at rest (% FiO_2)	23.7 ± 4.4
Home oxygen at exercise (% FiO_2)	31.1 ± 10.3
FEV1 (% pred)	43.0 ± 22.0
FVC (% pred)	52.6 ± 12.9
DLCO (% pred)	65.5 ± 27.0
LVEF (%)	54.5 ± 10.7
PAP (mmHg)	54.4 ± 30.3
Outcome measures at baseline	
6MWT	
6MWD (m)	342.7 ± 70.0
Percentage predicted 6MWD (%)	50.2 ± 12.3
Borg max (/10)	5.8 ± 1.4
HR max (bpm)	112.8 ± 15.8
FiO_2 during test (%)	33.5 ± 9.9
SPPB	
Total score (/12)	11.4 ± 0.9
Balance score (/4)	4.0 ± 0.2
4MGS score (/4)	3.9 ± 0.4
5STS score (/4)	3.6 ± 0.7
5STS (sec)	10.3 ± 2.3
SGRQ	
Symptoms score (/100)	61.3 ± 19.8
Activities score (/100)	82.1 ± 13.0
Impacts score (/100)	56.3 ± 21.6
Total score (/100)	65.1 ± 17.2

Values are [mean ± SD] if not mentioned otherwise.
Abbreviations: ILD, interstitial lung diseases; COPD, chronic obstructive lung disease; CF, cystic fibrosis; PAH, pulmonary arterial hypertension; BMI, Body-Mass Index; FiO2, fraction inspired oxygen; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; DLCO, diffusing capacity of the lungs for carbon monoxide; LVEF, left ventricular ejection fraction; PAP, pulmonary arterial pressure; 6MWT, six-minute walk test; 6MWD, six-minute walk distance; HR, heart rate; SPPB, short physical performance battery; 5STS, five time sit-to-stand; 4MGS, 4-m gait speed; SGRQ, St George's Respiratory Questionnaire.

completed the virtual prehabilitation phase with the 6 patients who did not complete it, no statistically significant differences in their baseline characteristics were found.

Changes After the 12-week Virtual Physical Prehabilitation

Changes in outcomes after the 12-week virtual physical prehabilitation phase are presented in **Table 2**. No statistically significant difference was noted in the 6MWT, in distance in meters or % of predicted distance. There was no statistically significant difference in the Borg scale level at the end of the walking test but there was statistically significant increase in the FiO_2 used during the 6MWT (mean change of 7.1%, $p = 0.012$, ES = 0.53) (The decision to increase the FiO_2 during the 6MWT was made by the clinical team). We found a statistically significant decrease in the 5STS test (mean change of 1.4 s, $p = 0.009$, ES = 0.61). There was no statistically significant difference in the SPPB score ($p = 0.059$) or in the SGRQ total score and the 3 sub-scores. When we examine the effect size, we can see a trend in improvement in the SPPB score, decrease in the Borg scale during the 6MWT as well as improvement in the impact sub-score of the SGRQ. When the changes in the 6MWT was compared to its MCID, we noted that 11 patients either improved or maintained their 6MWT scores. As for the other outcomes, the majority of the patients improved or maintained their scores in all the outcomes (**Figure 2**).

Changes After the Maintenance Phase

Five patients completed the maintenance phase. Reasons for non-completion are shown in **Figure 1**. Changes in outcomes (compared to the 12-week prehabilitation follow-up) for each patient individually after the maintenance phase are presented in **Table 3**. We observed a mean decrease of 32.4 m in the 6MWD with an increase in the Borg scale and an increase in the FiO_2. Compared with the MCID for each outcome, the majority of the patients improved or maintained their scores in all the outcomes except for the sub-score of symptoms in the SGRQ where 3 patients declined their score (**Figure 3**).

Adherence

The average attendance rate of the virtual sessions for the 14 participants who completed the 12-week virtual physical prehabilitation phase was 91.9% (range 75%–100%). Four patients completed the diary for the independent exercise sessions, three patients partially and 7 patients did not complete the diary. The main reason for not completing the diary was that they forgot to record their sessions or lost the diary. As for the daily steps diary, six patients completed it correctly, one patient partially and 7 patients did not complete it. Patients provided the same reasons for not completing the daily steps diary. The average steps per day ranged from 1,296 to 5,901 in the 8 patients that filled out the daily steps diary. Using the proposed cut-off of 3,000 steps, only 4 patients had adequate adherence to the aerobic exercises.

Adverse Events

No adverse events were reported during the live training sessions, independent sessions at home or walking.

Cost

In our study, two kinesiologists spent approximately 480 h to deliver the exercise program to the participants. At $40 Canadian dollars/

TABLE 2 | Changes in outcomes after the 12-week virtual physical prehabilitation.

	Baseline (T0) (N = 14)	*Post 12 weeks (T1) (N = 14)*	*Mean change*	*p-value*	*Effect size*
6MWT					
6MWD (m)	357.4 [315.6–399.1] *356 (254–464)*	359.6 [305.7–413.5] *359 (192–528)*	2.21 [−25.7–21.2]	.842	0.03
% predicted 6MWD	53.0 [45.9–60.1] *52.5 (36.0–75.0)*	53.5 [44.6–62.4] *49.0 (27.0–78.0)*	0.5 [−4.1–3.1]	.770	0.04
Borg max (/10)	5.6 [4.7–6.4] *6 (3–8)*	5.0 [3.9–6.1] *4 (2–9)*	-0.6 [−1.9–0.7]	.358	0.36
HR max (bpm)	111.5 [102.7–120.3] *114 (82–129)*	110.5 [103.0–118.0] *110 (81–128)*	−1.0 [−4.5–2.5]	.549	0.07
% FiO_2 during test[#]	33.6 [27.9–39.4] *30 (21–55)*	40.8 [31.2–50.4] *36 (21–75)*	7.1 [1.9–12.4]	.012*	0.53
SPPB score					
Total score[#]	11.4 [10.8–12.0] *12 (9–12)*	11.8 [11.5–12.0] *12 (11–12)*	0.4 [−0.1–0.9]	.059	0.56
Balance score[#]	3.9 [3.8–4.0] *4 (3-4)*	4.0 [4.0–4.0] *4 (4-4)*	0.1 [−0.1–0.2]	.320	0.52
4MGS score[#]	3.9 [3.6–4.0] *4 (3-4)*	3.9 [3.8–4.0] *4 (3-4)*	0.1 [−0.1–0.2]	.317	0.22
5STS score[#]	3.6 [3.1–4.0] *4 (2–4)*	3.9 [3.6–4.0] *4 (3-4)*	0.3 [−0.1–0.6]	.102	0.52
5STS (sec) [#]	10.0 [8.5–11.5] *9.3 (7.2–12.1)*	8.6 [7.5–9.8] *8.1 (6.2–13.2)*	−1.4 [−2.3–−0.5]	.009*	0.61
SGRQ					
Symptoms Score	56.4 [45.3–67.4] *57.6 (26.2–83.8)*	53.5 [40.1–66.9] *53.4 (0–92.8)*	−2.8 [−11.0–5.3]	.465	0.13
Activities score[#]	81.4 [74.2–88.5] *79.5 (53.4–100)*	83.9 [75.4–92.3] *89.2 (41.6–100)*	2.5 [−6.6–11.6]	.314	0.19
Impacts score	52.3 [39.6–65.1] *55.9 (11.7–80.5)*	46.3 [36.2–56.4] *53.9 (16.1–70.2)*	−6.0 [−14.4–2.3]	.144	0.30
Total score	62.0 [52.1–71.9] *65 (30.7–84.5)*	59.1 [50.2–67.9] *65 (26.2–76.1)*	−2.9 [−10.2–4.4]	.401	0.18

Paired samples t*-test were used for normally distributed data.*
Wilcoxon rank test was used for non-normally distributed data (see # above).
Mean [95% CI].
Median (Min-Max).
$p < 0.05$.
Effect size was calculated with Cohen d.
Abbreviations: 6MWT, six-minute walk test; 6MWD, six-minute walk distance; HR, heart rate; FiO2, fraction inspired oxygen; SPPB, short physical performance battery; 5STS, five time sit-to-stand; 4MGS, 4-m gait speed; SGRQ, St George's Respiratory Questionnaire.

hour, this represents a total budget of $19,200 Canadian dollars. As for the evaluation session by the physiotherapist, we calculated a total of 1.5 h per participant, for a total of 30 h. At a salary of $50 Canadian dollars/hour, this represents a total of $1,500 Canadian dollars. We bought for $3,500 worth of equipment: pulse oximeters, tablets, fitness trackers. We did not need to lend any tablets and only loaned just one oximeter. The overall cost of our intervention in 20 individuals was $24,200 Canadian dollars. This led to a cost per patient of $1,210 Canadian dollars.

Acceptability

Seventeen participants completed the acceptability questionnaire. The average score of the acceptability questionnaire was high at 45.5 (range 33–48, SD 3.8; maximal score is 48). See **Table 4** for more details on the questions and answers of the acceptability questionnaire.

DISCUSSION

This prospective longitudinal study demonstrated that a 12-week virtual prehabilitation program can improve lower limb strength as measured by the 5STS and maintain exercise capacity, frailty status and HRQOL in lung transplant candidates. A 12-week maintenance phase can either improve or maintain these outcomes. There was a high drop-out rate in the maintenance phase due mainly to intercurrent transplantation. The prehabilitation program was well accepted by patients and had a high attendance rate and no adverse events.

Although most of the participants were able to improve or maintain their 6MWD after the 12-week virtual physical prehabilitation phase, there was no statistically significant improvement in this outcome. Similarly, Singer et al. [19] did

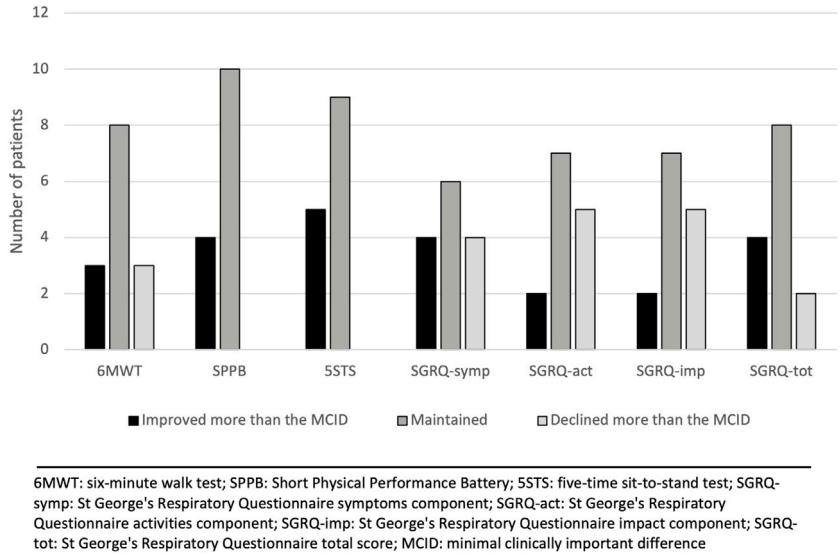


FIGURE 2 | Changes in outcomes after the 12-week virtual physical prehabilitation phase relative to the minimal clinically important difference (MCID) of each outcome.

not demonstrate improvement in 6MWD after an unsupervised home-based exercise training delivered via a mobile device to frail lung transplant candidates. In addition, Layton et al. [22] did not find improvements in 6MWD after a telerehabilitation offered to lung transplant candidates with cystic fibrosis. In contrast, prospective studies that offered hospital-based prehabilitation programs to lung transplant candidates have shown significant improvements in the 6MWD after the period of prehabilitation [6, 11, 44, 45]. This discrepancy could be because the hospital-based programs were able to offer a more intense aerobic exercise with supervision. In our study, the aerobic component of the program was not supervised. Most of the patients did not complete the diary to record the number of aerobic exercise sessions, therefore, we are unable to determine whether patients adhered to this part of the program. We also observed that the amount of oxygen that participants required to perform the 6MWT after the intervention was higher than what it was required before intervention which reflects a higher hypoxemia and may represent a progression of the underlying disease [46, 47]. Considering the progressive nature of the end-stage lung disease, maintaining the functional walking capacity of transplant candidates during the waiting time is a good outcome.

There was no statistically significant change in the SPPB after the prehabilitation, however, the mean change was close to reach statistical significance ($p = 0.059$). This is in line with the findings by Singer et al. [19] which found no statistically significant difference in the SPPB after the prehabilitation program even though their patients had lower SPPB scores at baseline than our patients (mean of 9.7 vs. 11.4) [19]. Byrd et al. [45] showed a statistically significant improvement in the SPPB after a 1 month outpatient rehabilitation in lung transplant candidates and the effect size of their cohort was similar to our study (0.54 vs. 0.56). The high SPPB scores at baseline in our study is explained by the fact that patients with limited functional status and who are frail are normally not listed for transplantation in our centre. This aligns with the recent consensus document for the selection of lung transplant candidates where frailty is considered a risk factor and limited functional status as an absolute contraindication if there is no potential for rehabilitation [48]. However, perhaps the frail patients could be the ones to target with prehabilitation as they are the ones that would benefit the most so that they can be considered for transplantation.

We found statistically significant improvement in one component of the SPPB, the 5STS. As the virtually one-on-one sessions focused on strengthening exercises and attendance to these sessions was high, this result was expected. Wickerson et al. [4] showed in an hospital-based outpatient program an improvement in the 5STS component of the SPPB after 6 weeks of prehabilitation. Byrd et al. [45] also found an improvement in 5STS after 4 weeks of inpatient prehabilitation. As quadriceps strength has been associated with intensive care length of stay and exercise capacity [49], increasing lower limb strength may positively impact post-transplant outcomes.

There was no significant improvement in HRQOL in our study. The symptom and impact components of the SGRQ as well as the total score improved, but not enough to reach statistical significance. In lung transplant candidates, all domains of quality of life are affected to some degree but physical functioning appears to be more affected than mental health [50]. As the goal of exercise training is to improved physical function, which in fact we observed in our study, one would expect that the HRQOL in our participants would improve. However, a decline in HRQOL in transplant candidates can occur due to fatigue, loss of self-esteem, anxiety and depression related to the prognosis of end-stage disease which might outweigh the effect of exercise [51]. Some studies of exercise interventions in lung transplant

TABLE 3 | Changes in outcomes in each patient after the 12-week maintenance phase.

	6MWT					SPPB score	5STS (sec)	SGRQ			
	6MWD (m)	% pred 6MWD	Borg max (/10)	HR max (bpm)	% FiO_2 during test			Symptoms Score	Activities score	Impacts score	Total score
Patient 1											
T1	316	49	6	102	36	12	7.9	63.9	80.5	70.2	72.5
T2	306	48	7	93	51	12	6.6	45.4	73	66.7	65.4
Change	*–10*	*–1*	*1*	*–9*	*15*	*0*	*–1.34*	*–18.5*	*–7.5*	*–3.5*	*–7.1*
Patient 2											
T1	356	49	6	114	21	11	13.2	92.8	92.5	61.5	76.1
T2	351	48	5	106	28	10	16.5	86.4	92.5	64.4	76.6
Change	*–5*	*–1*	*–1*	*–8*	*7*	*–1*	*3.35*	*–6.4*	*0*	*2.9*	*0.5*
Patient 3											
T1	437	67	3	112	50	12	7.6	36.7	72.2	39.2	49.1
T2	372	57	6	106	75	12	5.9	56.3	92.5	46.6	62.1
Change	*–65*	*–10*	*3*	*–6*	*25*	*0*	*–1.7*	*19.6*	*20.3*	*7.4*	*13*
Patient 4											
T1	490	76	3	108	75	12	9.9	0	41.6	25.7	26.2
T2	462	71	3	113	75	12	9.3	11.1	17.3	24.1	20
Change	*–28*	*–5*	*0*	*5*	*0*	*0*	*–0.6*	*11.1*	*–24.3*	*–1.6*	*–6.2*
Patient 5											
T1	362	58	5	125	44	12	7.6	53.4	92.5	55.1	66.5
T2	308	49	7	127	44	12	8.8	68.4	92.5	49.6	65.7
Change	*–54*	*–9*	*2*	*2*	*0*	*0*	*1.2*	*15*	*0*	*–5.5*	*–0.8*
All											
T1 mean	392.2	59.8	4.6	112.2	45.2	11.8	9.2	49.4	75.9	50.3	58.1
T1 SD	70.0	11.7	1.5	8.5	19.9	0.4	2.4	34.3	21.0	17.8	20.6
T2 mean	359.8	54.6	5.6	109.0	54.6	11.6	9.4	53.5	73.6	50.2	58.0
T2 SD	63.7	9.9	1.7	12.4	20.4	0.9	4.2	28.1	32.6	17	21.9
Mean Change	–32.4	–5.2	1	–3.2	9.4	–0.2	0.2	4.2	–2.3	–0.1	–0.1

T1: value after 12-week virtual physical prehabilitation phase.

T2: value after maintenance phase.

Abbreviations: 6MWT, six-minute walk test; 6MWD, six-minute walk distance; HR, heart rate; FiO2, fraction inspired oxygen; SPPB, short physical performance battery; 5STS, five time sit-to-stand; SGRQ, St George's Respiratory Questionnaire; SD, standard deviation.

candidates have shown improvements in HRQOL [11, 13, 15], others in some components only [12, 14, 16]. In contrast, Li et al. [10] have noted a decline in all component of the SGRQ. In addition, a systematic review of exercise interventions in solid organ transplantation has not shown improvement in HRQOL when comparing intervention with control groups [52]. Finally, the non-difference in the SGRQ might be because we were not powered to see a difference in this outcome.

There was a high number of patients who underwent transplant during our study and therefore did not complete the post-interventions assessments. Three patients were transplanted during the 12-week virtual physical prehabilitation phase and eight during the 12-week maintenance phase. The duration of the induction phase was informed by the findings of a systematic review on exercise in solid organ transplant candidates [52]. Studies that reported improvements in exercise capacity had an exercise program duration longer than 10 weeks [52]. Additionally, many studies in lung transplant prehabilitation have used a 12-week program [9, 52]. The loss of patients during the induction phase might have prevented us from seeing differences in some outcomes. Also, we were not able to see any difference and perform complete analysis of the maintenance phase as 8 of the remaining 14 patients after the induction phase were transplanted before the end of this phase. The intervention duration was designed based on experience without knowing the recent change in the waiting time. Indeed, the increasing number of lung transplants in our centre in the last few years significantly decreased the waiting time for transplant (from 12 months to 4 months in average).

In this study, we calculated that cost of our intervention per patient was $1,210 Canadian dollars for the healthcare system. As our study was not aimed to perform cost analysis, some expenses were not recorded thoroughly and a comparison between our

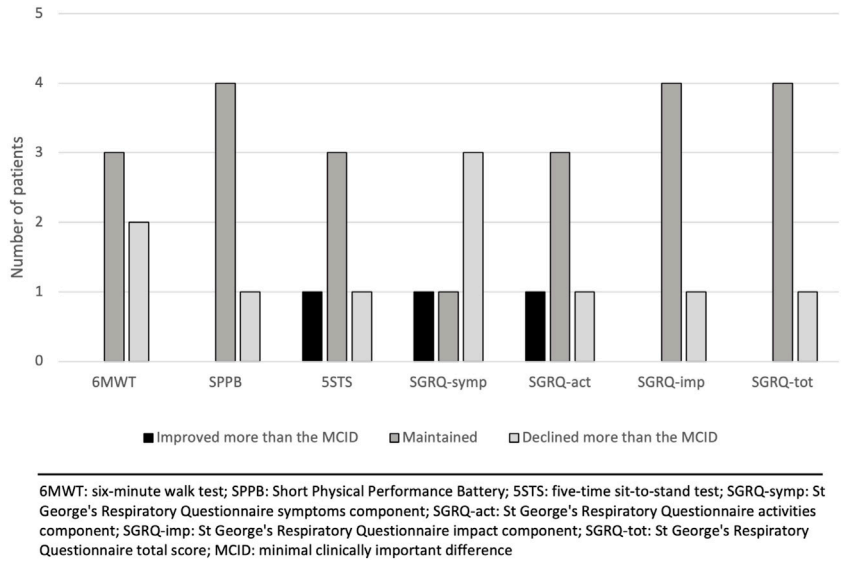


FIGURE 3 | Changes in outcomes after the maintenance phase relative to the minimal clinically important difference (MCID) of each outcome.

TABLE 4 | Responses of the acceptability questionnaire.

Questions from questionnaire	Agree with this sentence (% of participants)
To offer this exercise program for people awaiting lung transplantation is	
• Good idea	17 (100%)
• Pleasing	17 (100%)
• Easy[a]	16 (94%)
• Helpful	17 (100%)
• Simple[b]	16 (94%)
My family and/or friends liked that I participated in the exercise program[a]	16 (94%)
The exercises provided in the program were relevant to me	17 (100%)
I see the need for this virtual home-based exercise program in my life	17 (100%)
I think I benefited from this exercise program	17 (100%)
I felt confident to perform all exercises without assistance	17 (100%)
It was easy to learn how to perform the exercises	17 (100%)
It was easy to connect with the physiotherapist via Teams[b]	16 (94%)
I would recommend this virtual home-based exercise program to others	17 (100%)
The length of the program was good	17 (100%)
The number of exercises was good	17 (100%)
I intend to continue to do the exercises even after the program has finished	17 (100%)

N = 17.
[a]*1 participant was neutral for this item.*
[b]*1 participant disagree with this item.*

standard care could not been done. However, a systematic review by Grigorovich et al. [53] on economic analysis of home-based telerehabilitation found that telerehabilitation may result in similar or lower costs as in-person rehabilitation. The social impact and expenses for patients should also be considered. During our intervention, patients could stay at home until the date of transplant as opposed to travelling or moving closer to a transplant or rehabilitation centre to perform the prehabilitation program.

One of the strengths of this study includes the one-on-one virtual strengthening sessions with the presence of a kinesiologist while other studies [19, 22] included applications with videos of exercises. The one-on-one sessions allowed direct supervision of the participants to adjust exercises and monitor vitals. As non-adherence to home-based rehabilitation can reach 50%, strategies to provide direct feedback, monitor symptoms and performance of exercises can improve self-efficacy and increase adherence to exercises as patient feel better supported [54]. Another strength is the inclusion of a maintenance phase after the induction period. As transplant date is not known, limiting an intervention to a certain amount of time could mean that the patients would have to maintain exercises for a longer period than 12 weeks.

Interestingly, our recruitment rate was higher (83%) than those in Layton et al. (72%) and Singer et al. (65%). A possible explanation could be that our recruitment was made by the physiotherapist of the Lung Transplant Program. Since our study was conducted after the start of the COVID-19 pandemic, it may also be that patients and their families were more familiar with teleconferencing platforms at that time. Finally, we included participants with a wide range of diagnoses (ILD, CF, COPD, PAH, sclerodermia, and one patient for retransplantion).

There are several limitations to this study. First, our final sample size may have limited us from reaching statistically significance in our outcomes. The absence of a control group limited us from drawing a definitive conclusion on the effectiveness of the intervention. However, as the main goal of this study was to preliminary test our prehabilitation program following the ORBIT model, adding a control group in this phase was not recommended [23]. Another limitation was the uncertain adherence to the program. There was a larger number of diary non-completion for independent sessions which makes it difficult to know the exact frequency of the walking program, especially during the maintenance phase. Also, although we provided an exercise booklet and directives to the participants about the strengthening exercises, the number of sets and repetitions were not recorded. In future trials, the therapist should ask participants every week what they did for their independent sessions and record it with more details. During the maintenance phase, the same information can be asked during the weekly phone calls. Although the inclusion of a maintenance phase after the induction period was a strength of our study, 64% of the patients dropped out during this period, mainly because of intercurrent transplantation. The optimal duration for the maintenance phase should be determined in future trials. Finally, this study participants are not reflective of the entire lung transplant candidate's population. We excluded patients that were on the emergency list for transplantation since they were more likely to be transplanted before the end of the induction phase or because a large proportion of them were hospitalized before the transplant.

CONCLUSION

Our study suggests that lung transplant candidates can either improve or maintain their lower limb strength, functional exercise capacity, frailty status and HRQOL after a 12-week virtual prehabilitation program that is safe and acceptable to patients. Offering a maintenance phase seems feasible and effective, but optimal duration of this phase should match the transplant wait times of each center. Whether this prehabilitation program can impact post-transplant outcomes still needs further study.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by the University of Montreal Health Centre Research Ethics Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TJ-F, NB, KP, LL, and CP: designed the study; NB: analyzed data; NB and TJ-F: wrote the paper. KP (patient partner), LL, and CP: critically revised the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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ORIGINAL RESEARCH
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Multi-Centre UK Analysis of Simultaneous Pancreas and Kidney (SPK) Transplant in Recipients With Type 2 Diabetes Mellitus

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90% of the UK diabetic population are classified as T2DM. This study aims to compare outcomes after SPK transplant between recipients with T1DM or T2DM. Data on all UK SPK transplants from 2003–2019 were obtained from the NHSBT Registry (n = 2,236). Current SPK transplant selection criteria for T2DM requires insulin treatment and recipient BMI < 30 kg/m^2. After exclusions (re-transplants/ambiguous type of diabetes) we had a cohort of n = 2,154. Graft (GS) and patient (PS) survival analyses were conducted using Kaplan-Meier plots and Cox-regression models. Complications were compared using chi-squared analyses. 95.6% of SPK transplants were performed in recipients with T1DM (n = 2,060). Univariate analysis showed comparable outcomes for pancreas GS at 1 year (p = 0.120), 3 years (p = 0.237), and 10 years (p = 0.196) and kidney GS at 1 year (p = 0.438), 3 years (p = 0.548), and 10 years (p = 0.947). PS was comparable at 1 year (p = 0.886) and 3 years (p = 0.237) and at 10 years (p = 0.161). Multi-variate analysis showed comparable outcomes in pancreas GS (p = 0.564, HR 1.221, 95% CI 0.619, 2.406) and PS(p = 0.556, HR 1.280, 95% CI 0.563, 2.911). Comparable rates of common complications were demonstrated. This is the largest series outside of the US evaluating outcomes after SPK transplants and shows similar outcomes between T1DM and T2DM recipients. It is hoped dissemination of this data will lead to increased referral rates and assessment of T2DM patients who could benefit from SPK transplantation.

Keywords: equitable access, diabetes mellitus type 2, simultaneous kidney pancreas transplantation, United Kingdom, outcomes

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†RO is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis

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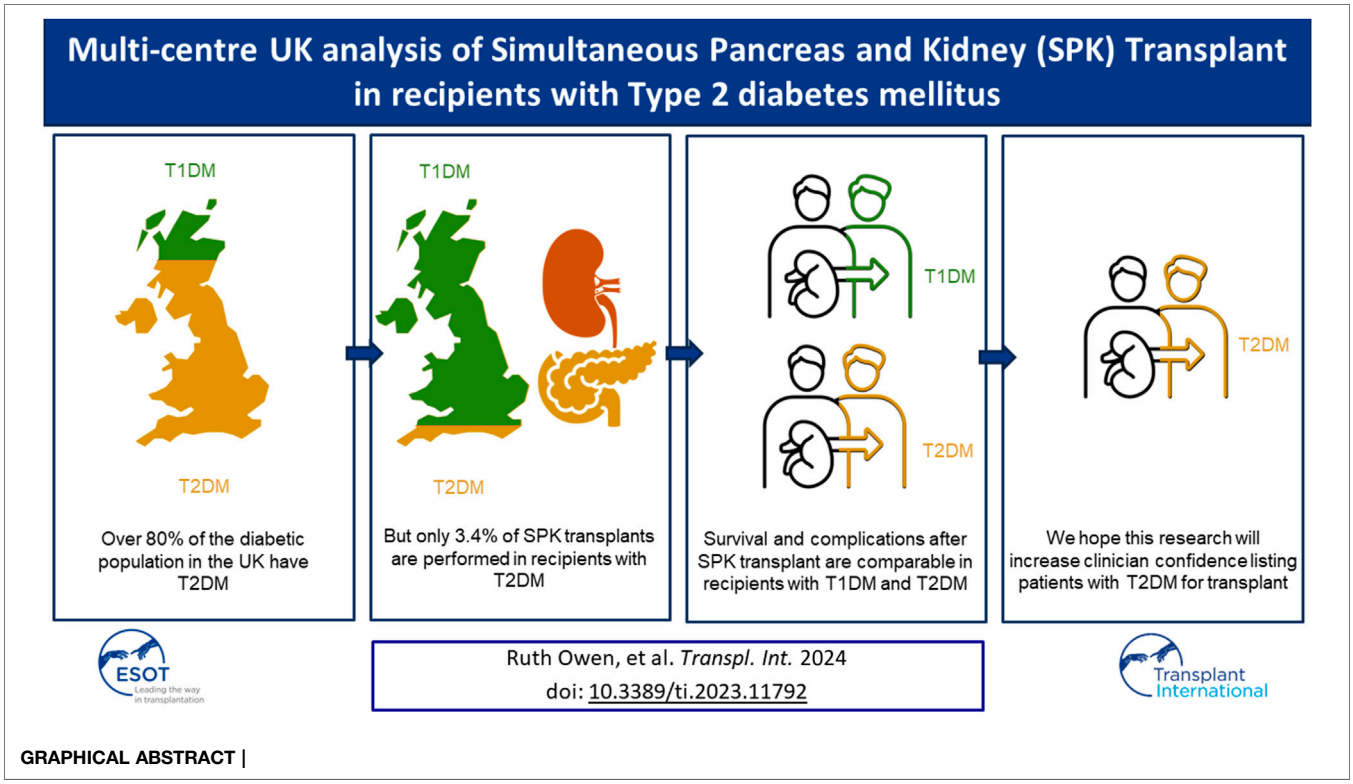
Abbreviations: BMI, Body Mass Index; BAME, Black, Asian and Minority Ethnic; CIT, Cold Ischaemia Time; CVA, Cerebrovascular Accident; DBD, Donor Brain Death; DCD, Donor Circulatory Death; IPTR, International Pancreas Transplant Registry; ITA, Islet Transplant Alone; KTA, Kidney Transplant Alone; MI, Myocardial infarction; NHSBT, National Health Service Blood and Transplant; NICE, National Institute of Health and Care Excellence; PAK, Pancreas after Kidney; PTA, Pancreas Transplant Alone; PTDM, Post Transplantation Diabetes Mellitus; SIK, Simultaneous Islet and Kidney Transplant; SPK, Simultaneous Pancreas and Kidney; T1DM, Type one diabetes mellitus; T2DM, Type two diabetes mellitus; UTI, Urinary tract infection; UK, United Kingdom; UNOS, United Network for Organ Sharing; WIT, Warm Ischaemia Time.



GRAPHICAL ABSTRACT |

INTRODUCTION

4.9 million people in the United Kingdom (UK) have diabetes characterised by progressive loss of beta-cell mass and/or function. There are broadly two main classifications of diabetes mellitus; Type 1 (T1DM) and Type 2 (T2DM) but sometimes it is difficult to precisely distinguish between the two. The first simultaneous pancreas and kidney transplant was performed in 1966 and initially reserved for patients with T1DM [1]. As the techniques and indications have evolved it was soon realised that some patients with T2DM would also benefit [2–5].

Approximately 90% of the diabetic population have been classified as T2DM compared to only 8% with T1DM [6]. Previously it was thought that T1DM was a disease with onset always in the young, whereas T2DM affected only older adults who were overweight. However, with increasing understanding about diabetes, binary classification of T1DM and T2DM has become increasingly difficult [7]. Studies using historic data comparing various cohorts of diabetic patients is therefore subject to different interpretations when considering the complexities of categorisation. The complex aetiology also makes planning the best management of these patients challenging when they are referred for beta-cell replacement therapy. T2DM is an extremely heterogenous disease. For example, life threatening severe hypoglycaemic unawareness is rare in T2DM but more common in patients with T1DM. Consequently, both Pancreas transplant alone (PTA) and Islet transplant alone (ITA), indicated in the UK solely for recurrent life-threatening hypoglycaemia has never been undertaken for T2DM patients. In the current study outcomes after SPK, as opposed to solitary pancreas transplantation, were investigated in patients with T2DM.

The current UK listing criteria for SPK in a potential T2DM recipient includes; 1) the need for insulin treatment and dependence 2) a BMI of ≤30 kg/m^2 and 3) patients must be receiving dialysis or have a GFR ≤20 mLs/min [8]. The presence of C-peptide is not an absolute contraindication because of inaccuracies in evaluation in patients with renal failure [9]. In essence potential T2DM recipients need to be fit for surgery, not overtly obese, on insulin treatment with end stage renal disease. Numerous previous studies have shown that patient survival after SPK transplant is superior to those patients on dialysis or those having deceased donor kidney transplant alone (KTA) [10–13].

The aim of this study was to compare outcomes in the NHSBT database between patients with either T2DM or T1DM after SPK transplantation.

PATIENTS AND METHODS

NHS Blood and Transplant UK registry data was obtained for all simultaneous pancreas and kidney (SPK) transplants that took place between 2003–2019, n = 2,236. Cases where the aetiology of diabetes was missing or had been classified as "other" rather than specifically Type 1 or Type 2 diabetes were excluded, as were

recipients who had received a re-transplant, resulting in a final cohort of n = 2,154. The type of diabetes was predefined by the centre listing the patient for transplant.

Recipient characteristics; age, sex, body mass index (BMI—categorised by the WHO classification) [14], ethnic group (categorised as white or BAME—black, Asian and minority ethnic), waiting time for transplant, pre-transplant insulin requirements and dialysis status were analysed for variations between our two cohorts. Donor characteristics; age, sex, ethnic group, donor type (DBD/DCD), warm ischaemic time (WIT) and cold ischaemic time (CIT) were also analysed for variation.

Recipient survival and death-censored pancreas and kidney graft survival were analysed at 1, 3, 5 and 10 years. Pancreas graft failure was defined by the recipient follow-up centre based on the resumption of insulin treatment. Kidney graft survival is defined as resumption of dialysis.

We further delineated our groups by BMI into; T1DM < 30 kg/m^2, T1DM > 30 kg/m^2, T2DM < 30 kg/m^2 and T2DM > 30 kg/m^2 and performed recipient survival and death-censored pancreas and kidney graft and patient survival at 10 years. We also further delineated our groups by ethnic group into; T1DM-White, T1DM-BAME, T2DM-White, T2DM-BAME.

Any patient outside standard listing criteria is discussed through an exemptions panel. Expert opinion within this group guided potential listing.

Common complications after pancreas transplant were analysed between our two cohorts including; incidence of post-operative myocardial infarction (MI), cerebrovascular accident (CVA), anastomotic leak, urinary tract infection (UTI), systemic infection (further delineated into bacterial, viral or fungal), pancreatitis, rejection at 3 months and resumed insulin use at 1 year.

This study aims and methodology were submitted to the NHS Blood and Transplant Research Advisory Group (RAG) and approved prior to gaining access to the registry data.

Statistical Analysis

Recipient characteristics were delineated by aetiology of diabetes and stratified by age, sex, body mass index (BMI), ethnic group, waiting time on the transplantation list, pre-transplantation insulin requirement, and dialysis status. These are all reported as percentages or means ± standard deviation. Donor characteristics were also delineated by aetiology of diabetes and stratified by age, sex, ethnic group, donor type (DBD/DCD), WIT and CIT and were reported as percentages or means ± standard deviation.

Univariate analysis of pancreas graft, kidney graft and patient survival were performed using Kaplan Meier survival plots and p-values derived from the log-rank test. A cox regression model was used for multivariable survival analysis. Our multivariable model was built using variables that had previously been reported to have a detrimental impact on graft or patient survival (cold ischaemic time, dialysis status). The incidence of common post-operative complications underwent chi-squared analysis. All analyses were performed using GraphPad Prism 9.0 and IBM SPSS statistics version 28. All tests were two-sided and p values <0.05 were considered significant.

TABLE 1 | Number of SPK transplants performed per year.

Year	T1DM	T2DM
	n = 2,060	n = 94
2004	62 (98.41%)	1 (1.59%)
2005	86 (97.73%)	2 (2.27%)
2006	114 (94.21%)	7 (5.79%)
2007	174 (97.21%)	5 (2.79%)
2008	136 (96.45%)	5 (3.55%)
2009	132 (95.65%)	6 (4.35%)
2010	133 (99.25%)	1 (0.75%)
2011	140 (95.89%)	6 (4.11%)
2012	150 (95.54%)	7 (4.46%)
2013	158 (93.49%)	11 (6.51%)
2014	147 (96.08%)	6 (3.92%)
2015	138 (94.52%)	8 (5.48%)
2016	126 (91.97%)	11 (8.03%)
2017	132 (95.65%)	6 (4.35%)
2018	131 (94.24%)	8 (5.76%)
2019	101 (96.19%)	4 (3.81%)

T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

RESULTS

The majority, (95.6%) of simultaneous pancreas and kidney (SPK) transplants were performed in recipients with Type 1 diabetes mellitus (T1DM) (n = 2,060). Only 3.4% (n = 94) of SPK transplants have been performed between 2003 and 2019 in recipients with type 2 diabetes mellitus (T2DM). Over the past 15 years we have seen an increasing trend in the percentage of SPK transplants being performed in T2DM recipients (1.6% in 2004 to 5.8% in 2018), **Table 1**. However, numbers remain comparatively small when compared to T1DM recipients. The median follow-up of all patients in this study was 1900 days, which was until death in 193 patients (8.96%).

Clinical Characteristics of Recipients

Recipients with T1DM and T2DM were comparable in terms of time on the waiting list and pre-transplant insulin requirements. Recipients with T2DM were more likely to be older (p < 0.0001***), male (p < 0.0001***), have a higher BMI (p = 0.0223*), be from BAME communities (p < 0.0001***), **Table 2**. Our dataset contained 176 recipients with a BMI >30 kg/m^2. 168 (95.1%) had T1DM and 8 (4.9%) had T2DM. Those patients outside standard criteria are evaluated within an exemptions committee.

Clinical Characteristics of Donors

Donors were comparable with no statistically significant parameters found between our two cohorts when analysing for donor sex, age, ethnic group and donor type (DBD/DCD). Warm ischaemic time (WIT) and cold ischaemic time (CIT) were also similar between the two groups, **Table 3**.

TABLE 2 | Recipient characteristics.

Recipient characteristic	T1DM	T2DM	*p*-value
Age (years)	41.88 ± 8.33	47.46 ± 787	<0.0001****
Sex (%)			<0.0001****
Male	1,189 (57.7%)	75 (79.8%)	
Female	871 (42.3%)	19 (20.2%)	
BMI (Range)	24.77 ± 3.85 (10–36.9)	25.84 ± 3.76 (19.7–34.4)	0.0223*
Ethnic Group (%)			<0.0001****
White	1,863 (91.1%)	41 (43.1%)	
BAME	182 (8.9%)	54 (56.8%)	
Waiting time for transplant (days)	424.8 ± 369.2	372.9 ± 332.6	0.144
Pre-transplantation Insulin Requirement (units)	44.84 ± 19.39	44.76 ± 21.33	0.974
Dialysis status (%)			0.052
Haemodialysis	693 (33.9%)	40 (42.5%)	
Peritoneal	515 (25.1%)	15 (15.9%)	
Not on dialysis	838 (41.0%)	39 (41.4%)	

Data shown as number or mean ± SD or percentage. BAME, Black, Asian and minority ethnic.
** p ≤ 0.05, **** p ≤ 0.0001.*

TABLE 3 | Donor characteristics.

Donor characteristic	T1DM	T2DM	*p*-value
Age (years)	34.88 ± 13.44	36.91 ± 13.86	0.166
Sex (%)			0.198
Male	1,038 (50.4%)	41 (43.6%)	
Female	1,021 (49.6%)	53 (56.4%)	
Ethnic Group (%)			0.842
White	1,892 (93.9%)	84 (93.3%)	
BAME	124 (6.1%)	6 (6.7%)	
Donor Type (%)			0.562
DBD	1,692 (82.1%)	75 (79.8%)	
DCD	368 (17.9%)	19 (20.2%)	
Warm Ischaemic Time (mins)	45.39 ± 71.61	53.15 ± 83.81	0.420
Cold Ischaemic Time (mins)	709.3 ± 252.6	756.3 ± 224.6	0.059

Data shown as number + percentage. BAME, Black, Asian and minority ethnic; DBD, donation after brainstem death; DCD, donation after circulatory death.

Univariate Analysis of the Impact of Diabetes Aetiology on Graft and Patient Survival

Death-censored survival analyses were performed using Kaplan Meier plots and revealed no statistically significant difference in pancreas graft survival at 1 year (p = 0.120), 3 years (p = 0.316), 5 years (p = 0.451), or 10 years (p = 0.196), **Figure 1**. There were also comparable rates of kidney graft survival at 1 year (p = 0.438), 3 years (p = 0.548), 5 years (p = 0.920), and 10 years (p = 0.947), **Figure 1**. When analysing patient survival, again no statistically significant difference was seen at 1 year (p = 0.886), or 3 years (p = 0.237), **Figure 1**. There was a statistically significant difference at 5 years (p = 0.028*), which showed poorer survival in those with T2DM. This trend was not borne out long-term as survival rates were comparable at 10 years (p = 0.161). p values and percentage survival were amalgamated into **Table 4**.

A further analysis was performed further stratifying the two diabetes groups into those with a BMI ≤30 kg/m^2, and those with a BMI >30 kg/m^2. A complete case analysis was used and cases without information pertaining to BMI were excluded. In total 176 (8.2%) recipients had a BMI >30 kg/m^2. Of the 176, 168 (95.5%) had T1DM and 8 (4.5%) had T2DM, **Table 5**. Although numbers are small in T2DM patients there was no statistically significant difference in pancreas graft (p = 0.200) or kidney graft (p = 0.684) survival was found between these groups. However, a statistically significant decrease in patient survival was seen in our recipients with T2DM and a BMI >30 compared with the other categories. (p = 0.002**), **Figure 2**.

We also delineated our diabetes groups by ethnicity and found comparable outcomes for pancreas graft survival (p = 0.224), kidney graft survival (p = 0.873) and patient (p = 0.866) survival, **Figure 3**.

Multivariate Analysis of the Impact of Diabetes Aetiology on Graft and Patient Survival

It is important to understand the impact of the type of diabetes within the context of multiple donor and recipient factors. As such, a multivariate analysis was built, including parameters known to influence recipient outcomes. Diabetes type in this multivariate analysis showed no statistically significant impact on pancreas graft survival (HR 1.221, 95% CI 0.619–2.406, p = 0.564) **Table 6**, kidney graft survival (HR 0.953, 95% CI 0.372–2.439, p = 0.920), **Table 7**, or patient survival (HR 1.280, 95% CI 0.565–2.911, p = 0.556) **Table 8**. The multivariate did show that recipient age (HR 0.965, 95% CI 0.951–0.980, p < 0.001), recipient BMI (HR 1.049, 95% CI 1.016–1.082, p = 0.004) and donor age (HR 1.008, 95% CI 1.008–1.029, p < 0.001) were statistically significant variables that affected pancreas graft survival. Recipient age also statistically significantly affected kidney graft survival (HR 0.973, 95% CI 0.955–0.991, p = 0.003) and patient survival (HR 1.042, 95% CI 1.024–1.061, p < 0.001).

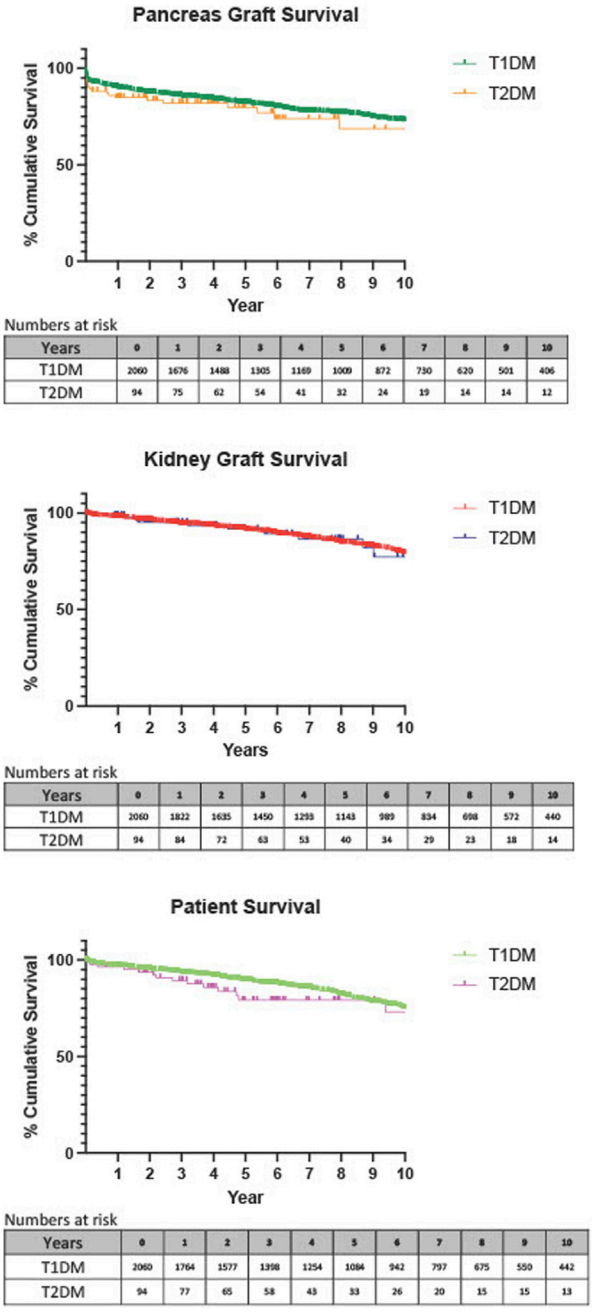


FIGURE 1 | Death-censored Kaplan-Meier analysis of 1 year pancreas graft, kidney graft and patient survival, T1DM recipients compared with T2DM recipients.

Incidence of Complications Stratified by Diabetes Aetiology

Complications after transplantation can pose a significant burden on the recipient as well as affect survival outcomes. Common complications after pancreas transplant, including incidence of post-operative myocardial infarction (MI), cerebrovascular accident (CVA), anastomotic leak, urinary tract infection (UTI), systemic infection (further delineated into bacterial, viral or fungal), pancreatitis, rejection at 3 months and resumed insulin use at 1 and 5 years. There was no statistically significant difference between recipients with T1DM or T2DM between any of the above parameters, **Table 9**. Incidence of graft failure caused by vascular thrombosis was analysed. 184 grafts failed in the T1DM group within 120 days of transplant. Of these 61 (33%) were due to vascular thrombosis. In the T2DM group 11 grafts failed and 2 (18%) were due to vascular thrombosis.

DISCUSSION

In the UK categorisation of diabetes is primarily a clinical diagnosis. For a diagnosis of T1DM, the current criteria includes; hyperglycaemia (random plasma glucose >11 mmol) with one or more of the following features; ketosis, rapid weight loss, age <50, BMI < 25 kg/m^2 and/or a personal/family history of autoimmune disease [15]. For a diagnosis of T2DM, the patient should have persistent hyperglycaemia (inferred using a HbA1c > 48 mmol/mol as a surrogate marker) [16], symptoms of; polyuria, polydipsia, unexplained weight loss, recurrent infections or tiredness in the context of known risk factors (i.e., obesity, family history, ethnicity, metabolic syndrome) and the absence of T1DM features (i.e., rapid onset, young age, insulin dependence, ketoacidosis). Unlike other countries, biomarkers such as auto-antibodies or c-peptide are not routinely used for diagnosis or classification in the United Kingdom. The National Institute of Health and Care Excellence (NICE) guidance on diagnosis of diabetes (updated in 2022) recommends using clinical features to make the diagnosis of diabetes, to not routinely use C-peptide, and if using diabetes-specific autoantibodies to take into account the false negative rate of this test [15].

Irrespective of how a patient's diabetes is classified the unifying result is hyperglycaemia which leads to down-stream micro and macrovascular complications. Early detection and changing medical management of diabetes mellitus undoubtedly helps delay the onset of complications associated with hyperglycaemia however, retinopathy, vasculopathy and nephropathy still remain serious and common afflictions in these patients [6]. Pancreas transplant remains the only realistic, long-term insulin-independent treatment for diabetes [10]. During 2020 there were 198 patients on the UK SPK transplant waiting list compared to 16 who need simultaneous islet and kidney (SIK) transplant [17]. Although the indications are the same the patient groups are likely to be different in terms of associated co-morbidities. SPK transplant is the favoured treatment in those that are fit when considering long-term insulin independence, this also applies to those patients with T2DM.

This study has shown comparable death censored pancreas graft survival and kidney graft survival after simultaneous pancreas and kidney transplants regardless of type of diabetes with the caveat that the diagnosis and type of diabetes was pre-defined by the listing centre using the UK criteria highlighted above. This study has also shown comparable patient survival at 1 and 3 years regardless of diabetes type. Interestingly at 5 years we see a statistically significant

TABLE 4 | Percentage graft and patient survival at 1, 3, 5, and 10 years.

		T1DM	T2DM	*p*-Value
Pancreas Graft Loss	1 year	89.9% (95% CI 91.1–88.5) *n* = 1,676	84.9% (95% CI 90.7–75.8) *n* = 75	0.120
	3 years	85.4% (95% CI 86.9–83.7) *n* = 1,305	82.0% (95% CI 88.6–72.2) *n* = 54	0.316
n = number at risk	5 years	81.8% (95% CI 83.5–79.9) *n* = 1,009	79.8% (95% CI 87.2–69.0) *n* = 32	0.451
	10 years	72.7% (95% CI 75.1–70.1) *n* = 406	68.7% (95% CI 80.8–51.8) *n* = 12	0.196
Kidney Graft Survival	1 year	97.6% (95% CI 98.2–96.8) *n* = 1822	98.9% (95% CI 99.8–92.3) *n* = 84	0.438
	3 years	92.2% (95% CI 93.2–91.0) *n* = 1,450	95.2% (95% CI 98.2–87.7) *n* = 63	0.548
n = number at risk	5 years	91.3% (95% CI 92.6–89.8) *n* = 1,143	91.7% (95% CI 96.3–82.1) *n* = 40	0.920
	10 years	78.9% (95% CI 81.3–76.2) *n* = 440	77.2% (95% CI 88.3–58.3) *n* = 14	0.947
Patient Survival	1 year	96.8% (95% CI 97.5–95.5) *n* = 1764	96.5 (95% CI 98.8–89.6) *n* = 77	0.886
	3 years	93.2% (95% CI 94.3–91.9) *n* = 1,398	89.3% (95% CI 94.5–79.6) *n* = 58	0.237
n = number at risk	5 years	89.4% (95% CI 90.8–87.8) *n* = 1,084	79.2% (95% CI 87.6–66.2) *n* = 33	0.028*
	10 years	74.8% (95% CI 77.4–71.9) *n* = 442	73.1% (95% CI 85.0–54.7) *n* = 13	0.161

Data shown as percentage with 95% confidence intervals. Number at risk depicts how many recipients with follow up at that time period.
** p ≤ 0.05.*

TABLE 5 | WHO classification of recipient BMI.

Category	BMI (kg/m^2)	T1DM	T2DM
		***n* = 1,614**	***n* = 72**
Underweight	16–18.5	39 (2.42%)	1 (1.39%)
Normal	18.5–25	853 (52.85%)	29 (40.28%)
Overweight	25–30	554 (34.32%)	34 (47.22%)
Obese	>30	168 (10.41%)	8 (11.11%)

Data shown as mean ± SD or percentage.

decrease in T2DM patient survival when compared to their T1DM counterparts. This trend is not borne out at 10 years which again shows no statistically significant difference in patient survival. We believe this may be partly explained by the older, heavier T2DM having a poorer initial patient survival and the younger, lighter T2DM recipients surviving out to 10 years.

In 2020, an American single-centre study (*n* = 323) demonstrated comparable outcomes in terms of pancreas graft survival (death censored) and incidence of post transplantation diabetes mellitus (PTDM) between recipients with T2DM (*n* = 39) compared with T1DM (*n* = 284). Patients in this study were defined as T1DM and T2DM using clinical assessment at the time of initial evaluation as well as utilising a novel scoring system assessing; pre-transplant insulin requirement, pre-transplant fasting c-peptide levels (assigning a score of +2 if C-peptide <0.5 ng/L, −1 if 0.5–2 ng/L and −2 if >2 ng/L), family history and the presence of diabetes-associated antibodies. A score from −9 to +9 was created, and a negative score correlated with T2DM and a positive score with T1DM [18]. This scoring system was not used in our study.

The largest reported study to date (*n* = 6,756), utilised the United Network for Organ Sharing (UNOS) database. Again, the majority of patients (90.8%, *n* = 6,141) had T1DM and only 8.2% of SPK transplant's were performed in T2DM (*n* = 582). This study also showed no statistically significant difference in graft and patient survival in patients with T2DM [19]. Type of diabetes was predefined by the listing centre, and no further information

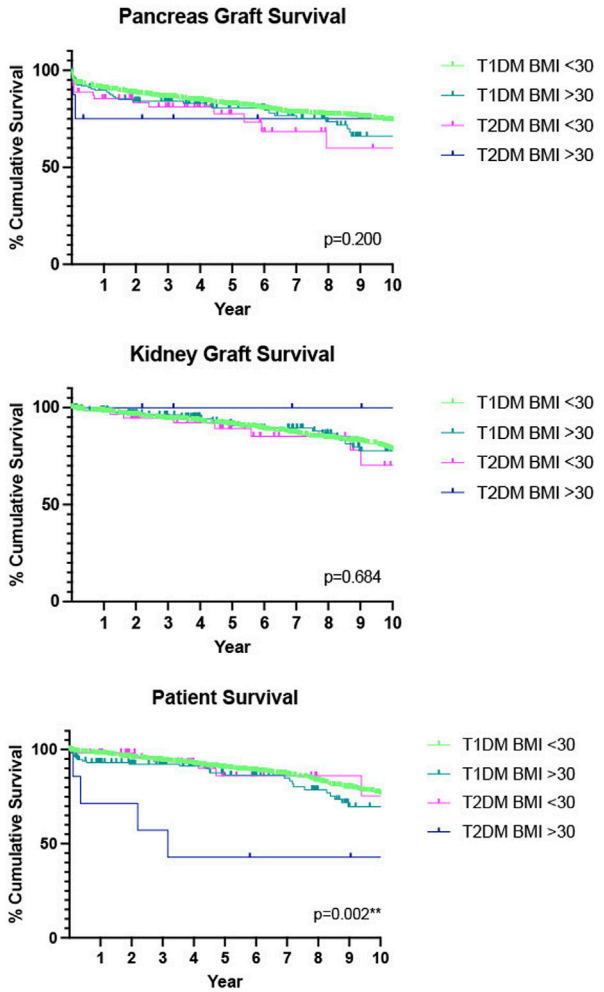


FIGURE 2 | Death-censored Kaplan-Meier analysis of 10 years pancreas graft, kidney graft and patient survival, T1DM recipients compared with T2DM recipients, further stratified by BMI. *n* = 528 had missing data for BMI so were not included in this analysis.

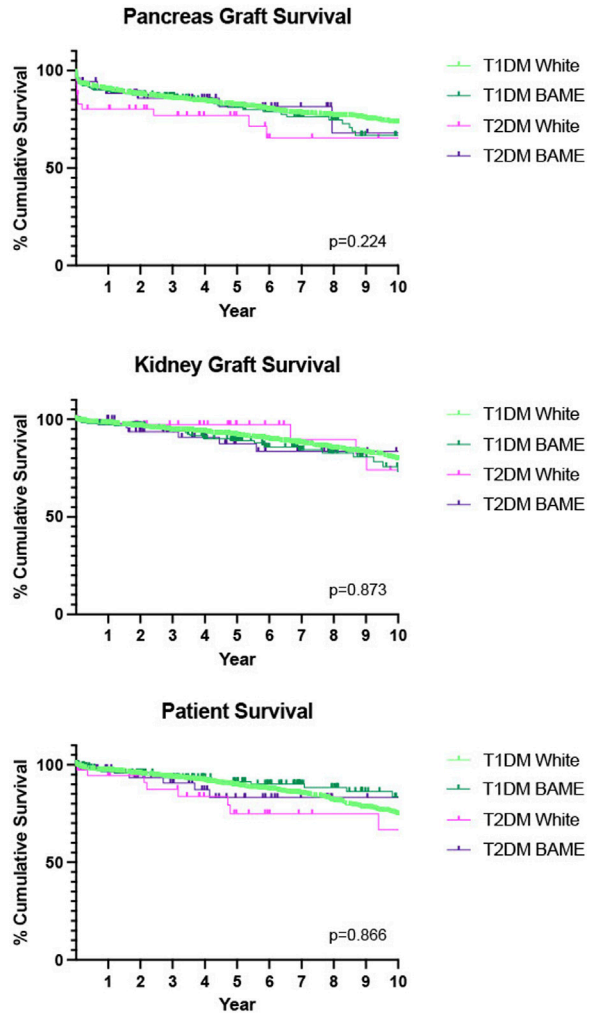


FIGURE 3 | Death-censored Kaplan-Meier analysis of 10 years pancreas graft, kidney graft and patient survival, T1DM recipients compared with T2DM recipients, further stratified by ethnicity. W-White, BAME–Black, Asian and minority ethnic.

regarding this was offered in this publication which makes it harder to draw any more useful detail between the two cohorts.

A smaller single centre study was reported in 2013 by an Austrian group (n = 248) comparing T1DM undergoing SPK transplant (n = 195) with T2DM SPK transplant (n = 21) and T2DM kidney transplant alone (KTA) (n = 32) [20]. They defined T2DM using detectable C-peptide levels. This study demonstrated comparable rates of graft survival between T1DM and T2DM recipients undergoing SPK. A statistically significant difference in patient survival was seen when comparing T2DM recipients (both SPK and KTA) with T1DM who underwent SPK ($p < 0.001$). This finding contrasts with the other literature discussed. It is also important to note this paper does not differentiate KTA by donor brain death (DBD), donor circulatory death (DCD) or living related donor (LRD) making it difficult to interpret. However, there is a large American study utilising a National Registry for T2DM patients (n = 37,117) where T2DM recipients were shown to have better statistically

TABLE 6 | Multivariable analysis of pancreas graft survival.

Variable	Hazards ratio	95.0% CI	*p*-value
Recipient Age	0.965	(0.951–0.980)	<0.001***
Recipient BMI	1.049	(1.016–1.082)	0.004**
Recipient Sex	0.850	(0.665–1.087)	0.195
Recipient Ethnicity	0.808	(0.533–1.226)	0.316
Dialysis Status	1.162	(0.812–1.073)	0.284
Type of Diabetes	1.221	(0.619–2.406)	0.564
Donor Age	1.019	(1.008–1.029)	<0.001***
Donor BMI	0.995	(0.960–1.032)	0.787
Donor Sex	0.935	(0.729–1.200)	0.598
Donor Ethnicity	0.928	(0.580–1.485)	0.757
Donor Type (DCD Vs. DBD)	0.917	(0.660–1.274)	0.605
Warm Ischaemic Time (mins)	1.000	(0.999–1.001)	0.844
Cold Ischaemic Time (mins)	1.001	(1.000–1.002)	0.010

BMI, body mass index; DBD, donation after brainstem death; DCD, donation after circulatory death.

** p ≤ *0.01*, *** p ≤ *0.001*.

TABLE 7 | Multivariable analysis of kidney graft survival.

Variable	Hazards ratio	95.0% CI	*p*-value
Recipient Age	0.973	(0.955–0.991)	0.003
Recipient BMI	1.015	(0.974–1.058)	0.472
Recipient Sex	0.729	(0.545–0.975)	0.033
Recipient Ethnicity	0.939	(0.568–1.552)	0.806
Dialysis Status	1.129	(0.840–1.517)	0.420
Type of Diabetes	0.953	(0.372–2.439)	0.920
Donor Age	1.013	(1.000–1.025)	0.045
Donor BMI	1.000	(0.958–1.044)	0.991
Donor Sex	1.057	(0.780–1.432)	0.722
Donor Ethnicity	0.953	(0.486–1.867)	0.887
Donor Type (DCD Vs. DBD)	1.183	(0.801–1.748)	0.398
Warm Ischaemic Time (mins)	1.001	(1.000–1.002)	0.122
Cold Ischaemic Time (mins)	1.024	(0.976–1.073)	0.336

BMI, body mass index; DBD, donation after brainstem death; DCD, donation after circulatory death.

TABLE 8 | Multivariable analysis of patient survival.

Variable	Hazards ratio	95.0% CI	*p*-value
Recipient Age	1.042	(1.024–1.061)	<0.001**
Recipient BMI	1.017	(0.981–1.055)	0.349
Recipient Sex	1.229	(0.920–1.641)	0.163
Recipient Ethnicity	0.488	(0.246–0.965)	0.039
Dialysis Status	1.840	(0.692–0.967)	0.788
Type of Diabetes	1.280	(0.563–2.911)	0.556
Donor Age	1.009	(0.997–1.021)	0.164
Donor BMI	0.988	(0.947–1.030)	0.558
Donor Sex	0.900	(0.667–1.213)	0.487
Donor Ethnicity	0.728	(0.372–1.428)	0.356
Donor Type (DCD Vs. DBD)	0.818	(0.523–1.279)	0.379
Warm Ischaemic Time (mins)	1.000	(0.998–1.001)	0.839
Cold Ischaemic Time (mins)	1.001	(1.000–1.001)	0.117

BMI, body mass index; DBD, donation after brainstem death; DCD, donation after circulatory death.
*** p ≤ 0.01.*

TABLE 9 | Analysis of common complications after SPK transplants.

Complications three month follow up	T1DM	T2DM	*p*-value
	***n* = 2,060**	***n* = 94**	
Myocardial Infarction	15	1	0.715
Cerebrovascular Accident	9	0	0.520
Anastomotic Leak	64	2	0.574
UTI	97	9	0.804
Systemic Infection			
- Viral	9	1	0.371
- Bacterial	66	5	0.176
- Fungal	12	0	0.453
Pancreatitis	49	2	0.895
Rejection at 3 months	133	5	0.556
One year follow up	*n* = 1,084	*n* = 33	
Resumed insulin use at 1 year	113	3	0.401
Five year follow up	*n* = 442	*n* = 13	
Resumed insulin use at 5 years	104	1	0.163

UTI, urinary tract infection.

significant patient survival and kidney allograft survival after SPK when compared to those receiving a KTA alone, irrespective of whether the kidney was from a deceased donor or living donor [21].

A further single centre study in Argentina ($n = 45$), showed no statistically significant difference in patient survival when comparing T1DM ($n = 35$) to T2DM ($n = 11$) after SPK. They classified patients type of diabetes clinically; those who were diagnosed in childhood, with a lower BMI and requiring immediate insulin treatment were classified as T1DM whereas patients who were diagnosed with diabetes aged >30 years/old and with metabolic features were classified as T2DM.

A final study from Washington classifying diabetes by C-peptide >/<0.8 ng/mL ($n = 136$) showed comparable outcomes between their Type and Type 2 recipients. They state that C-peptide status does not influence outcomes after SPK transplant and this treatment option should be offered regardless of their C-peptide level [22].

Whilst the majority (90%) of the UK diabetic population have T2DM, only 3.4% of this population had an SPK. Other countries have comparable proportions of T2DM; in the US 91% of the diabetic population have T2DM [23], in Germany 90%–95% [24] and 90% in the Netherlands [25]. In 2010 the International Pancreas Transplant Registry, IPTR (which receives data from both UNOS and Eurotransplant) showed 8% of SPK's were performed in patients presumed to have T2DM [26, 27]. Despite comparable proportions of T2DM within these national populations one can extrapolate that the percentage of SPKs performed in recipients with T2DM in the UK is well below that of our American and other European counterparts [3, 28]. However we accept there is no uniform consensus on the criteria used for a diagnosis of T2DM which could explain this observation.

From 2019–2021 a consensus group was formed to deliberate on current pancreas transplant outcomes in an effort to provide evidence to support current practice (28), After removal of duplicate papers and by applying exclusion criteria, 31 studies regarding SPK in T2DM patients were reviewed. The consensus concluded that SPK transplant improved both quality of life and long-term survival in suitable T1DM and T2DM recipients. For T2DM, the authors state that evidence is insufficient to suggest SPK transplant provides greater survival when compared with living donor kidney transplant alone. We did not analyse solitary kidney transplants in this study and to our knowledge this analysis has never been done. It would be interesting to see if SPK transplant is better than PAK transplant with a living donor kidney for patients with T2DM. Numbers in our national dataset would be too small for a useful comparison.

From our cohort we can see that those patients who received a pancreas transplant with T2DM were more likely to be older, male and with a higher BMI. Factors associated with the development of T2DM are obesity and smoking [29–31] which have been typically associated with a male cohort [32, 33]. Currently the UK SPK transplant patient selection policy contains a selection criteria of a BMI <30 kg/m^2 for T2DM recipients and does not define a BMI restriction for those with T1DM [8]. Although there is some selectively amongst UK

centres where >30 kg/m^2 may be considered as a relative contra-indication by some.

In total 8.2% (n = 176) of our cohort had a BMI > 30 kg/m^2. A previous study from our group showed that whilst BMI does affect outcomes, those who received a pancreas transplant (SPK, PTA and PAK) with a BMI > 30 kg/m^2 had comparable outcomes with recipients with a BMI < 30 kg/m^2 and concluded that assigning a cut off of <30 kg/m^2 as a gatekeeper to pancreas transplantation had the potential to prevent good candidates accessing this treatment option [18,34]. For our study we delineated BMI by type of diabetes to better analyse the data in the context of this selection policy. 8.5% (n = 8) of the SPK transplants performed in T2DM had a BMI > 30 kg/m^2. As this goes against the standard selection policy their case went to an exemptions panel prior to being placed on the waiting list and so were excellent candidates in terms of other parameters. We saw no statistically significant difference in overall graft survival, however recipients with T2DM and a BMI > 30 kg/m^2 had poorer patient survival than the other categories. Whilst numbers are small in our T2DM > 30 kg/m^2 group, this would suggest that the combination of T2DM and obesity is of concern. Many T2DM patients are not obese and, in this study, have been shown to have comparable outcomes. Those with T1DM showed comparable graft and patient survival outcomes independent of BMI.

Our study also found that those patients with T2DM receiving a transplant were much more likely to be from a BAME (Black, Asian and minority ethnic) community rather than their T1DM counterparts. This is not unsurprising because from epidemiological studies we know that UK BAME communities have a 3–5× higher prevalence of T2DM with an earlier age of onset [28, 35, 36]. We further analysed our dataset to better understand the role ethnicity played in recipient outcomes. We found no statistically significant difference in either graft or patient survival regardless of ethnicity.

An analysis of common complications after pancreas transplantation was performed. This included incidence of myocardial infarction, cerebrovascular accident, anastomotic leaks, urinary tract infections, systemic infections, pancreatitis, graft rejection at 3 months and resumed insulin use (at 1 year and also 5 years). Both T1DM and T2DM have been associated with an increased incidence of infection [37], poorer wound healing [38] and thrombotic events [39, 40]. When compared against each other we found comparable rates of all complications regardless of type of diabetes.

Study Limitations

This study is limited by certain factors. Our T2DM cohort was a small highly selected group (3.4%) relative to the UK's overall population of T2DM patients. However, as we had 96 patients we believe this to be sufficiently high to allow some useful conclusions to be made.

The type of diabetes was predefined by recipient centres using clinical judgement as described above. Unlike other countries objective measurements such as C-peptide levels and presence of antibodies were not routinely utilised. This limits conclusions in terms of the effect of residual C peptide may have on clinical outcomes.

When analysing BMI, there were only 8 recipients with T2DM *and* a BMI > 30kg/m^2, so we advise caution when interpreting these results. It is important to consider that many of the patients in this study are on dialysis and potentially could have large fluctuations in pre-operative weight, although in general we assume a dry weight is documented and recorded. Recipient weight was taken once at the time of listing, rather than serial measurements and so may be influenced by their dialysis schedule.

A final limitation is the missing data present in post-operative complications, a common problem when utilising large databases. A complete-case analysis was chosen as our statistical method to deal with this.

This is one of the largest studies ever performed and the only study from a UK population. It supports the findings of other national, and international studies. Our study is unique as common complications after SPK transplant were also analysed, as well as the impact of BMI and ethnicity delineated by type of diabetes.

In summary, we have found no statistically significant differences in death censored pancreas graft survival, kidney graft survival or patient survival when delineating by diabetes type which is consistent with previous studies research. Despite this evidence it should be noted SPK is rarely performed for T2DM patients, more so in the UK than several other countries. We have shown that fit patients with T2DM who are insulin dependent, not overtly obese (BMI < 30 kgm^2 although this would subject to opinion) and who are uraemic will do well with SPK. We were unable to draw useful conclusion regarding C peptide status in terms of clinical outcome.

We believe there needs to be a clear consensus on listing criteria and the diagnosis of T2DM to ensure that eligible patients are being referred for SPK transplant and are not excluded by questionable listing criteria. We also believe further research is needed within the UK population to better understand the disparity in percentage of T2DM patients receiving a SPK transplant.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Access to the Dataset must be sought from the NHS Blood and Transplant UK registry team. Requests to access these datasets should be directed to statistical.enquiries@nhsbt.nhs.uk.

ETHICS STATEMENT

Study aims and methodology were submitted to the NHS Blood and Transplant Research Advisory Group (RAG) and approved prior to gaining access to the registry data.

AUTHOR CONTRIBUTIONS

RO: participated in research design, participated in the writing of the manuscript, participated in data analysis, reviewed and revised the manuscript. HC: participated in data analysis, reviewed and revised the manuscript. CC: participated in research design, reviewed and revised the manuscript. ST: participated in data analysis, reviewed and revised the manuscript. ET: participated in data analysis, participated in the writing of the manuscript, reviewed and revised the manuscript. DM: reviewed and revised the

manuscript. JS: participated in the writing of the manuscript, reviewed and revised the manuscript. CW: participated in research design, participated in the writing of the manuscript, participated in data analysis, reviewed and revised the manuscript. SW: participated in research design, participated in the writing of the manuscript, participated in data analysis, reviewed and revised the manuscript.

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AUTHOR DISCLAIMER

The views expressed are those of the authors and not necessarily those of the National Health Service, the NIHR, the department of health or NHSBT.

CONFLICT OF INTEREST

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

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Beyond the First Year: Epidemiology and Management of Late-Onset Opportunistic Infections After Kidney Transplantation

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Late opportunistic infections (OI) occurring beyond the first year after kidney transplantation (KT) are poorly described and not targeted by prophylactic strategies. We performed a ten-year retrospective monocentric cohort study describing epidemiology, risk factors and impact of late OI occurring 1 year after KT. We included clinically symptomatic OI requiring treatment besides BK virus nephropathy. Control groups included early OI occurring in the first year after KT, and KT recipients without OI since KT and alive with a functional allograft at 1 year. Among 1066 KT recipients, 185 (19.4%) presented a first episode of OI 21.0 (8.0–45.0) months after KT: 120 late OI (64.9%) and 65 early OI (35.1%). Late OI were mainly viral ($N = 83$, 69.2%), mostly herpes zoster (HZ) ($N = 36$, 43.4%). Pneumocystis represented most late fungal infections ($N = 12$/25, 48%). Compared to early OI, we reported more pneumocystis ($p = 0.002$) and less invasive aspergillosis ($p = 0.01$) among late OI. Patients with late OI were significatively younger at KT (54.0 ± 13.3 vs. 60.2 ± 14.3 years, $p = 0.05$). Patient and allograft survival rates between late OI and control groups were similar. Only age was independently associated with mortality. While late OI were not associated with higher mortality or graft loss, implementing prophylactic strategies might prevent such infections.

Keywords: kidney transplant, herpes zoster, opportunistic infections, transplant infectious disease, pneumocystis

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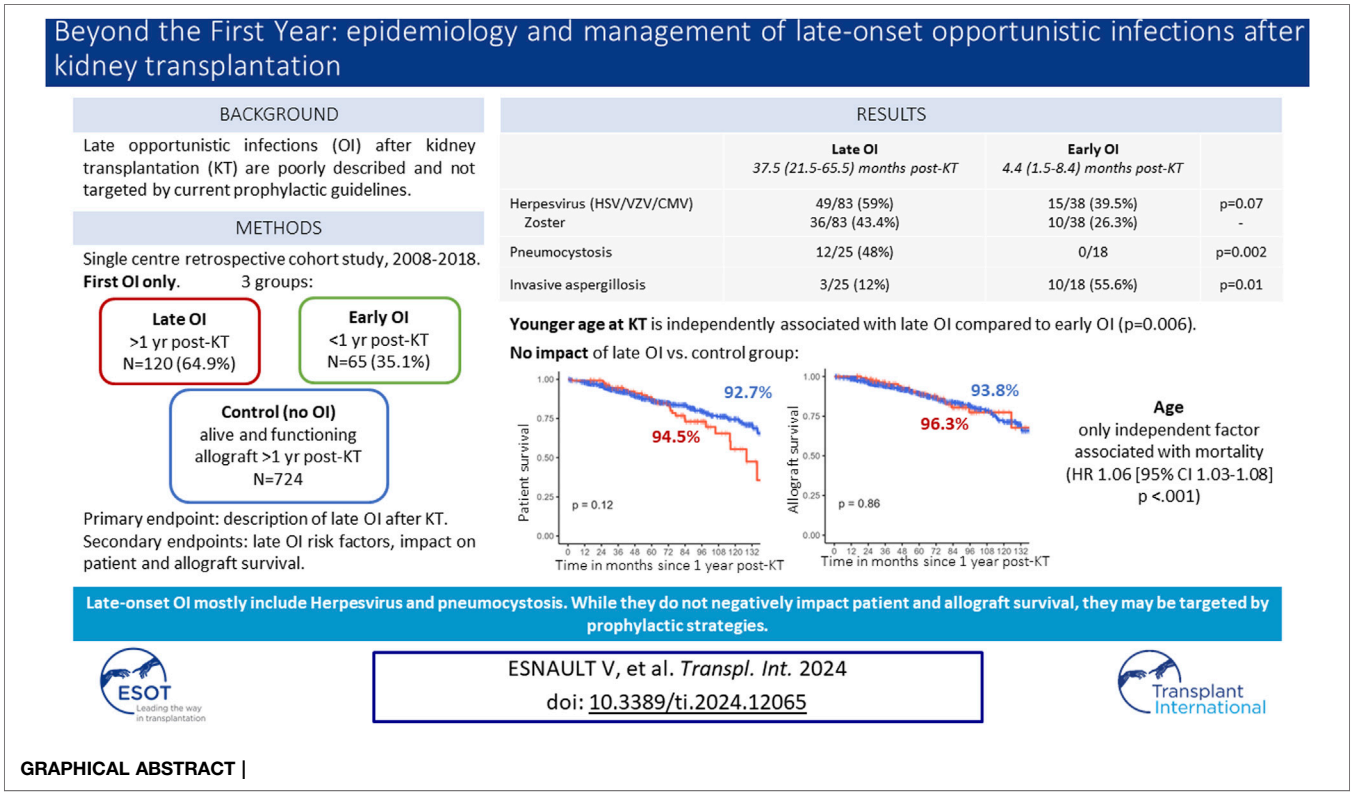
Abbreviations: ALC, absolute lymphocyte count; CMV, cytomegalovirus; ECD, extended criteria donor; eGFR, estimated glomerular filtration rate; EOI, early opportunistic infection; HZ, herpes zoster; KT, kidney transplantation; KTR, kidney transplantation recipient; LOI, late opportunistic infection; PJP, *Pneumocystis jirovecii* pneumonia; VZV, varicella zoster virus.



GRAPHICAL ABSTRACT |

INTRODUCTION

Kidney transplantation (KT) remains the best treatment of end-stage renal disease with better quality-of-life and longer survival than dialysis [1]. While management of kidney transplant recipients (KTR) has improved patient and allograft survival over the last decades, infections remain a major concern and represent the second cause of death within the first year post-KT [2].

Occurrence of opportunistic infections (OI) after transplantation may be considered as an inappropriate net state of immunosuppression for a given patient, resulting from a complex interaction of numerous factors among which the nature of the immunosuppressive therapy is essential [3]. OI affect up to 25% of KTR [2]. Historically, the first 6–12 months after KT represented the period most at-risk for OI, in relation with intensive immunosuppressive regimens including induction [4]. With the implementation of universal antimicrobial prophylaxis within the first year after KT, incidence of *Pneumocystis jirovecii* pneumonia (PJP) and cytomegalovirus (CMV) disease have dropped [5–7]. However, we and others have reported increasing rates of OI beyond the first 12 months after KT [8, 9], possibly related to a rising proportion of older and comorbid KTR, and identification of extended criteria donor as an independent risk factor of OI [9]. New immunosuppressive agents such as belatacept have also been associated with susceptibility to some OI including CMV disease and PJP [10, 11]. While preventive strategies are well defined within the first year after transplantation, the place for antimicrobial prophylaxis beyond 1-year post-transplantation is still lacking in current guidelines [12]. Moreover, no study comparing the epidemiology of late versus early OI is available but is mandatory to determine relevant prophylaxis.

In this context, we conducted a monocentric retrospective cohort study to describe late OI characteristics in KTR and assess the impact of such infections on patient and kidney allograft survivals.

MATERIALS AND METHODS

Study Design and Patients

We conducted a single centre retrospective cohort study. All adult KTR engrafted between January 2008 and December 2018 in Henri Mondor Hospital (Créteil, France) were eligible apart from primary allograft non-function within 30 days after KT and combined transplantation.

All patients received infectious prophylaxis according to international guidelines [13]. CMV prophylaxis consisted in valganciclovir for intermediate (R+ treated with lymphodepleting agents) and high-risk patients (D+/R–) within 3 and 6 months after KT, respectively [7]. PJP prophylaxis consisted in trimethoprim-sulfamethoxazole (TMP-SMX) for 12 months after KT [6].

As occurrence of an OI indicates an inappropriate net state of immunosuppression [3], it may frequently lead to

modification of the immunosuppressive regimen. Consequently, the first OI represents a tipping point in KTR care, and we only included the first OI for each patient. KTR with a first OI occurring beyond the first year post-transplantation were included in the late OI group (LOI). We chose the cut-off of 12 months after KT as TMP-SMX is withdrawn at this time point in our centre. Two control groups were defined: 1) KTR with a first OI occurring within the first year after KT (early OI, EOI); 2) KTR with no history of OI since transplantation and alive with a functioning allograft for at least 1 year after KT (no-OI group).

OI Definition and Collection

In the absence of a standardized list of OI in solid organ transplant recipients, definition of OI was based on the 1993 revised classification system for OI in the setting of human immunodeficiency virus (HIV) infection [14], on international guidelines [6, 7], and the concertation of two senior Infectious Disease and Kidney Transplantation specialists. We analysed symptomatic OI requiring therapy without restriction to hospitalized OI, except for BK virus nephropathy (BKVN) for which no treatment is available. The following pathogens and infections were considered (a complete definition of OI is provided in **Supplementary Data**):

- -Bacteria: *Nocardia* sp., *Mycobacterium tuberculosis* and non-tuberculous mycobacteria, *Listeria monocytogenes*, *Legionella pneumophila*.
- -Virus: severe herpes simplex virus (HSV) infections (encephalitis, pneumonitis or other organ involvement requiring appropriate antiviral treatment); severe varicella-zoster virus (VZV) infections [encephalitis, pneumonitis, herpes zoster (HZ) requiring appropriate antiviral treatment]; hepatitis B (HBV) reactivation, hepatitis E infection (HEV); CMV disease; Human-Herpes virus 8 (HHV8)-associated Kaposi sarcoma; JC virus-associated progressive multifocal leukoencephalopathy (PML); chronic norovirus infection; disseminated and severe localized adenovirus disease; histologically proven BK virus-associated nephropathy (BKVN) (i.e., no presumptive BKVN).
- -Fungi: invasive candidiasis and rare yeast disseminated infections such as *Trichosporon* spp.; *Cryptococcus neoformans*; invasive mold diseases (aspergillosis, mucormycosis, fusariosis); *Pneumocystis jirovecii* pneumonia.
- -Parasites: *Toxoplasma gondii*, *Microsporidium* sp, *Cryptosporidium* sp, *Leishmania* sp.

OI were identified in our local KT database, which prospectively collects patient data from registration on KT waiting list to engraftment, as well as every significant in- and out-patient event occurring afterwards. All events are implemented by a clinical research associate specialized in KT. OI characteristics were retrospectively collected from patients' medical records and independently reviewed and validated by two Infectious Diseases specialists.

Outcomes

The primary endpoint was description of late OI after KT. Secondary endpoints were 1) risk factors of late OI compared to early OI after KT and 2) impact of late OI on overall survival and kidney allograft survival after KT. Allograft loss was considered if dialysis was needed or if the estimated glomerular filtration rate (eGFR) was below 15 mL/min/1.73 m^2.

Covariates

We collected data about KTR characteristics [age, sex, underlying nephropathy, extended criteria donor (ECD), biological data] and immunosuppressive therapy (induction and maintenance regimen, rejection therapy). ECD was defined as a donor older than 60 years or between 50 and 60 years, with two of the three following criteria: 1) hypertension; 2) pre-retrieval serum creatinine >1.50 mg/dL; and 3) cerebrovascular cause of brain death [15]. Delayed graft function was considered in case of haemodialysis within the first 7 days after KT. The eGFR was estimated using CKD-EPI formula [16]. Acute rejection episodes were histologically proven and analysed according to updated Banff classification [17]. Our local immunosuppressive protocol is provided in **Supplementary Methods**. Conversion to belatacept as maintenance regimen was considered for analysis if treatment had been initiated at least 1 month before the first OI episode.

Statistical Analysis

Continuous variables were described by mean (standard deviation, SD) or median (interquartile range, IQR) as appropriate and categorical variables by number and percentage. We used t-test or Wilcoxon test for continuous variables and Chi-2 or Fisher exact tests for categorical variables. Logistic regression models were performed for multivariable analyses, which included all variables with a p-value ≤0.2 in univariable analysis.

In the primary analyses, we studied late OI and early OI groups. Patients were followed from the first OI episode to allograft loss, death from any cause or until 31st December 2020 (end of study period) whichever occurred first. Overall survival, allograft survival and survival without allograft loss were described with Kaplan Meier curves, using the occurrence of the first OI as baseline. Hazard ratios were estimated by Cox model regressions which included variables known to be associated with both patient and allograft survival: sex and age. Proportional-hazards assumption was formally tested by using Schoenfeld residuals. As BKVN is an independent risk factor of graft loss, we then performed a sensitivity analysis excluding patients with BKVN.

We also performed a secondary analysis comparing survival rates between late OI and no-OI groups for which baseline was set at 1-year post-transplantation, i. e., conditional survival analysis beyond 1 year.

A p-value <0.05 was considered significant. Tests were two-tailed. To account for multiple testing, we used Benjamini-Hochberg method as appropriate. Statistical analyses were carried out using R 3.6.2.

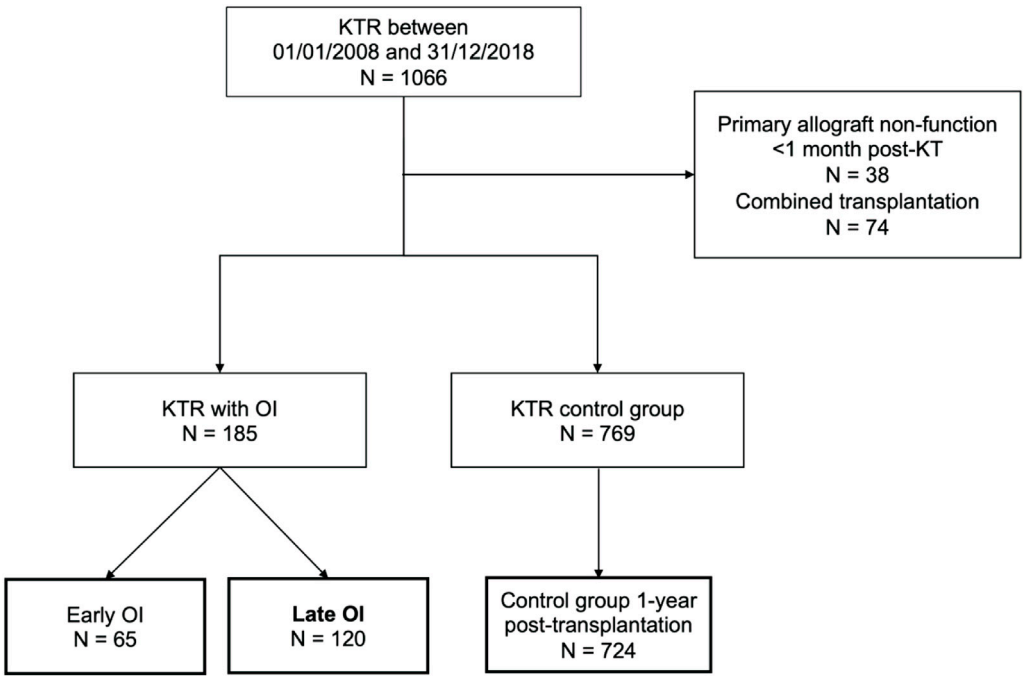


FIGURE 1 | Flowchart of the patients included in the study. Opportunistic infections (OI) were divided into early OI (occurring within the first year after kidney transplantation) and late OI (occurring beyond the first year post-transplantation). The control group included 724 kidney transplant recipients (KTR) without OI who were alive with a functional kidney allograft at least 1 year after transplantation.

RESULTS

Between January 2008 and December 2018, 1066 KT were performed in our centre and 954 KTR were included in the study (**Figure 1**). Among those, 185 (19.4%) presented a first OI in a median time of 21.0 (8.0–45.0) months after KT: 120 late OI (64.9%) and 65 early OI (35.1%). The control group with no history of OI included 724 KTR alive up to 1 year after KT.

Late and early OI occurred 37.5 (21.5–65.5) and 4.4 (1.5–8.4) months after KT, respectively. Most late OI were viral (N = 83, 69.2%) and fungal (N = 25, 20.8%) infections, while bacterial and parasitic OI were scarce (N = 5, 4.2% and N = 7, 5.8%, respectively) (**Table 1**). Among late OI, leading viral infections were HZ (N = 36, 43.4%), BKVN (N = 15, 18.1%) and CMV disease (N = 11, 13.3%). Fungal infections consisted mainly in PJP (N = 12, 48%) and invasive candidiasis (N = 7, 28%). Other invasive fungal infections included invasive aspergillosis (N = 3, 12%), cryptococcosis (N = 2, 8%) and mucormycosis (N = 1, 4%). Compared to early OI, we reported significatively more PJP (N = 12, 48% vs. N = 0, p = 0.002) occurring in a median time of 51.7 (23.8–76.5) months, and significatively less invasive aspergillosis among late OI (N = 3, 12% vs. N = 10, 55.6%, p = 0.01, respectively). Invasive aspergillosis occurred 4.0 (1.3–8.5) months after KT among early OI.

Characteristics of KTR from the late OI and early OI groups are presented in **Table 2**. KTR with a first OI occurring beyond 12 months post-allograft were significatively younger at the time of KT (54.0 ± 13.3 vs. 60.2 ± 14.3 years, p = 0.05). Induction and maintenance immunosuppressive regimens were similar in both groups. Occurrence of acute rejection in the year preceding the OI was significantly less reported among the late OI group (4.2% vs. 18.5%, p = 0.04). There was no difference between the two groups in terms of occurrence of CMV viremia prior to the OI, new-onset diabetes after transplantation or switch to belatacept (**Table 2**). Among PJP episodes, lymphocytopenia (defined by an absolute lymphocyte count [ALC] <1,000/mm^3) in the year preceding the OI was reported in 6/8 (75%) KTR with available data.

In multivariable analysis, younger age at KT (p = 0.006) and less acute rejection within the year before OI (p = 0.003) were significantly associated with late OI compared to early OI (**Table 3**).

Mean follow-up was of 68.7 (±37.1) months. Overall and allograft survivals in late and early OI groups are presented in **Figures 2A, B**. Thirty-six months after OI, overall survival rates were 78.7% in the late OI group and 74.5% in the early OI group (p = 0.6). OI-related mortality occurred in 5/60 (8.33%) patients: 2/27 (7.4%) in the early OI group (one *Aspergillus fumigatus* endocarditis and one Kaposi sarcoma), and 3/33 (9.0%) patients in the late OI group (two PJP and one CMV disease). Thirty-six months after OI, allograft survival rates in the late OI and early OI groups were similar (84.3% and 85.2%, respectively, p = 0.99). As BKVN may influence kidney allograft survival, we performed kidney allograft survival analysis excluding those OI; results were similar (85.1% and 88.2%, respectively, p = 0.70). Compared to early OI, occurrence of late OI was not associated with mortality (adjusted HR 1.22 [95% CI 0.69–2.17], p = 0.49). Age was an

TABLE 1 | Description of late opportunistic infections and comparison to early opportunistic infections.

	Total *N* = 185	Late opportunistic infection *N* = 120	Early opportunistic infection *N* = 65	*p*-value
Type of infection				0.33
Viral, *N* (%)	121 (65.4)	83 (69.2)	38 (58.5)	
Fungal, *N* (%)	43 (23.2)	25 (20.8)	18 (27.7)	
Bacterial, *N* (%)	11 (5.9)	5 (4.2)	6 (9.2)	
Parasitic, *N* (%)	10 (5.4)	7 (5.8)	3 (4.6)	
Viral infections	*N* = 121	*N* = 83	*N* = 38	
Herpesviridae (CMV/HSV/VZV)	64 (52.9)	49 (59.0)	15 (39.5)	0.07
Herpes zoster	46 (38.0)	36 (43.4)	10 (26.3)	—
CMV disease	15 (12.4)	11 (13.3)	4 (10.5)	—
BK virus nephropathy	27 (22.3)	15 (18.1)	12 (31.6)	0.16
Norovirus/adenovirus	12 (9.9)	7 (8.4)	5 (13.2)	0.51
HBV/HEV	7 (5.8)	6 (7.2)	1 (2.6)	0.43
HHV8	11 (9.1)	6 (7.2)	5 (13.2)	0.32
Fungal infections	*N* = 43	*N* = 25	*N* = 18	
Aspergillus spp.	13 (30.2)	3 (12.0)	10 (55.6)	0.01
Pneumocystis jirovecii pneumonia	12 (27.9)	12 (48.0)	0 (0.0)	0.002
Candida spp.	11 (25.6)	7 (28.0)	4 (22.2)	0.74
Cryptococcosis	6 (14.0)	2 (8.0)	4 (22.2)	0.22
Mucormycosis	1 (2.3)	1 (4.0)	0 (0.0)	
Bacterial infections	*N* = 11	*N* = 5	*N* = 6	
Legionella	5 (45.5)	3 (60.0)	2 (33.3)	
Nocardia spp.	2 (18.2)	0 (0.0)	2 (33.3)	
Tuberculosis	3 (27.3)	1 (20.0)	2 (33.3)	
Non-tuberculous mycobacteria	1 (9.1)	1 (20.0)	0 (0.0)	
Parasitic infections	*N* = 10	*N* = 7	*N* = 3	
Microsporidiosis	2 (20.0)	2 (28.6)	0 (0.0)	
Cryptosporidiosis	5 (50.0)	3 (42.8)	2 (66.7)	
Toxoplasmosis	3 (30.0)	2 (28.6)	1 (33.3)	

CMV, cytomegalovirus; HSV, herpes simplex virus; VZV, varicella-zoster virus; HBV, hepatitis B virus; HEV, hepatitis E virus.

independent factor of mortality (aHR 1.06 [95% CI 1.03–1.08], $p < 0.001$). Gender was not associated with mortality (male sex aHR 1.24 [95% CI 0.67–2.30], $p = 0.50$).

To specifically assess the impact of late OI on overall and allograft survivals, we compared late OI to the no-OI control group. Characteristics from both groups were similar (**Supplementary Table S1**). We reported no difference in conditional survival rates at 36 months between the two groups (94.5% vs. 92.7%, respectively, $p = 0.12$) (**Figures 2C, D**). Compared to the control group, late OI were not associated with mortality (aHR 1.16 [95% CI 0.76–1.78], $p = 0.49$) while age was (aHR 1.08 [95% CI 1.07–1.10], $p < 0.001$). Kidney allograft survival was similar in both groups (LOI 96.3% vs. no-OI 93.8%, $p = 0.86$).

Finally, we specifically analysed the 33 (17.8%) KTR with OI converted from calcineurin inhibitors to belatacept. Among those, 21 (63.6%) presented a first episode of OI after initiation of belatacept: 14 (66.6%) late OI and 7 (33.3%) early OI (**Supplementary Table S2**). Time between conversion and occurrence of OI was 10.7 (2.6–22.0) months: 17.1 (11.2–24.8) months for LOI and 2.6 (2.3–2.7) months for EOI. Among converted patients with late OI, viral infections were predominant ($N = 9$, 64.3%), mostly HZ and CMV disease, followed by fungal infections ($N = 4$, 28.6%), all PJP.

DISCUSSION

In this large retrospective monocentric study spanning across 10 years of KTR follow-up, we reported for the first time the description of late OI occurring beyond 1 year after transplantation. PJP and HZ were the most frequently identified late OI. Occurrence of a first OI beyond 12 months post-KT was associated with younger age at transplantation. Conversely, we showed that early OI episodes were more frequent in older KTR and associated with rejection. Late-onset OI had no deleterious impact on either patient or allograft survivals.

We only analysed the first OI for each patient, considering that the first occurrence of an OI represents a tipping point in KTR management that require immunosuppression adaptations because of an inappropriate net state of immunosuppression [3]. Those modifications may lead to *de novo* donor-specific antibodies (DSA) development, antibody-mediated rejection with negative impact on kidney allograft survival [18]. Here, we reported an incidence of OI of 19% consistent with the literature (10%–25%) [8, 19]. We consider that our database captured all OI after KT, including episodes treated outside the hospital system for which the transplantation centre was systematically reached to discuss specific treatment and immunosuppression management. While our results highlight the efficacy of codified infectious prophylaxis in reducing the

TABLE 2 | Patients characteristics at the time of kidney transplantation and during follow-up.

	Total	Late opportunistic infection *N* = 120	Early opportunistic infection *N* = 65	*p*-value
Recipient				
Age, mean ± SD	56.2 ± 14.0	54.0 ± 13.3	60.2 ± 14.3	0.05
Male sex, *N* (%)	124 (67.0)	75 (62.5)	49 (75.4)	0.34
Dialysis before KT, *N* (%)	168 (92.3)	106 (90.6)	62 (95.4)	0.70
Time on dialysis (years), median (IQR)	4.2 (2.2–6.2)	4.3 (2.2–6.6)	4.1 (2.2–6.0)	0.84
History of non-kidney SOT, N (%)	4 (2.2)	0 (0.0)	4 (6.2)	0.07
Diabetes[a], *N* (%)	39 (26.2)	18 (19.6)	21 (36.8)	0.13
Underlying nephropathy				0.44
Glomerulopathy, *N* (%)	27 (14.8)	19 (16.2)	8 (12.3)	
Diabetes, *N* (%)	26 (14.3)	11 (9.4)	15 (23.1)	
Genetic, *N* (%)	18 (9.9)	13 (11.1)	5 (7.7)	
Autoimmune disease, *N* (%)	6 (3.3)	3 (2.6)	3 (4.6)	
Other, *N* (%)	45 (24.7)	31 (26.5)	14 (21.5)	
Unspecified, *N* (%)	60 (33.0)	40 (34.2)	20 (30.8)	
Biological characteristics				
Leukocytes (/mm^3), median (IQR)	6200 (5200–7900)	6200 (5300–7900)	6100 (5200–7800)	0.95
Lymphocytes (/mm^3), median (IQR)	1,300 (1,000–1,700)	1,300 (1,000–1,750)	1,200.0 (825–1,500)	0.41
Lymphocytes <1,000/mm^3, *N* (%)	41 (24.3)	24 (22.4)	17 (27.4)	0.84
VIH, *N* (%)	6 (3.3)	5 (4.3)	1 (1.5)	0.70
VHC, *N* (%)	6 (3.4)	3 (2.6)	3 (4.7)	0.88
Donor				
Age, mean ± SD	60.0 ± 15.1	58.5 ± 15.0	62.8 ± 14.8	0.21
Living donor, *N* (%)	14 (7.7)	12 (10.3)	2 (3.1)	0.41
Extended criteria donor, *N* (%)	105 (57.7)	59 (50.4)	46 (70.8)	0.07
Kidney transplantation				
DSA, *N* (%)	34 (21.9)	23 (23.0)	11 (20.0)	0.95
CMV serostatus (D/R), *N* (%)				1
D−/R−	12 (6.6)	8 (6.8)	4 (6.2)	
D−/R+	62 (34.1)	39 (33.3)	23 (35.4)	
D+/R−	23 (12.6)	16 (13.7)	7 (10.8)	
D+/R+	85 (46.7)	54 (46.2)	31 (47.7)	
Cold ischemia time (hours) median (IQR)	15.9 (12.6–20.1)	15.2 (12.0–20.0)	16.8 (14.0–21.0)	0.07
Induction therapy				
Anti-CD25 mAbs, *N* (%)	92 (50.5)	63 (53.8)	29 (44.6)	0.62
Polyclonal antithymocyte globulin, *N* (%)	87 (47.8)	51 (43.6)	36 (55.4)	0.41
Maintenance immunosuppressive regimen				
Calcineurin inhibitors, *N* (%)				
Ciclosporin	35 (19.2)	25 (21.4)	10 (15.4)	0.70
Tacrolimus	160 (87.9)	105 (89.7)	55 (84.6)	0.70
Mycophenolate mofetil, *N* (%)	156 (85.7)	100 (85.5)	56 (86.2)	1
mTOR inhibitors, *N* (%)	25 (13.7)	15 (12.8)	10 (15.4)	0.95
Corticosteroids, *N* (%)	182 (100)	117 (100)	65 (100)	
During follow-up				
Rejection treated within the year preceding OI, *N* (%)	17 (9.2)	5 (4.2)	12 (18.5)	0.04
CMV viremia, *N* (%)	50 (27.0)	35 (29.2)	15 (23.1)	0.72
New-onset diabetes, *N* (%)	51 (27.6)	34 (28.3)	17 (26.2)	0.99
Switch to belatacept, *N* (%)	21 (11.4)	14 (11.7)	7 (10.8)	1

IQR, interquartile range; KTR, kidney-transplant recipient; CMV, cytomegalovirus; DSA, donor specific antibodies; mAbs, monoclonal antibodies; mTOR, mammalian target of rapamycin; SD, standard deviation; SOT, solid organ transplant.
[a]*Significant missing data (36/185, 19.5%).*

TABLE 3 | Risk factors of late opportunistic infections compared to early opportunistic infections.

	Univariable analysis		Multivariable analysis	
	OR [95% CI]	*p*-value	ORa [95% CI]	*p*-value
Age	0.97 [0.94–0.99]	0.005	0.97 [0.94–0.99]	0.006
Male sex	0.54 [0.27–1.05]	0.08	0.64 [0.31–1.29]	0.22
Rejection treated within the year preceding OI, *N* (%)	0.19 [0.06–0.55]	0.003	0.18 [0.05–0.52]	0.003

OI, opportunistic infection; CMV, cytomegalovirus.

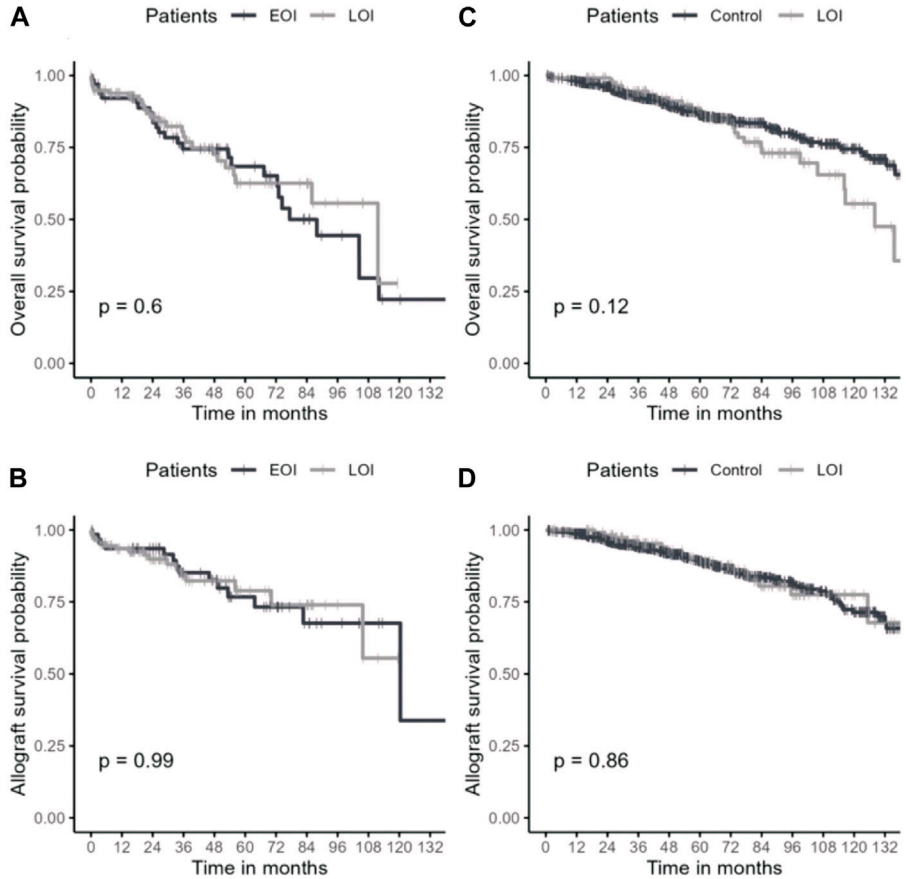


FIGURE 2 | Overall survival and kidney allograft survival in late OI and control groups. Overall survival **(A)** and kidney allograft survival **(B)** after OI episode. Overall survival **(C)** and kidney allograft survival **(D)** after 1 year of transplantation. OI: opportunistic infection; Early/Late OI: Opportunistic infections occurring within the first-year post-transplantation (EOI) and after the first-year post-transplantation (LOI); Control: kidney transplant recipient without opportunistic infection alive with a functional allograft at least 1 year after transplantation.

burden of early OI after transplantation, late-onset OI were actually predominant in our study and are currently not targeted by preventive strategies [12]. We confirmed previous data suggesting two incidence peaks of OI after KT, one within 6 months and one up to 3 years post-allograft [8, 19].

The majority of late OI were viral infections, mainly HZ. We and others had already emphasized HZ predominance after KT, which occurs in up to 10% of KTR, mostly beyond the second year post-transplantation [9, 20]. Age >50 years and steroids have been identified as risk factors, while CMV prophylaxis might be protective [20]. HZ complications are described in almost a quarter of KTR, especially in those >50 years old, and include postherpetic neuralgia, disseminated disease and cranial nerve involvement [20]. In older adults, zoster vaccine markedly reduces HZ incidence and morbidity as well as postherpetic neuralgia, regardless of VZV serology status [21–23]. In France, zoster vaccination using a live attenuated vaccine is currently recommended in all adults older than 65 years [24]. The future availability of the inactivated zoster vaccine in France should enable vaccination after KT as well [25]. Until then, we suggest that live attenuated zoster vaccination should be implemented systematically among patients awaiting KT, regardless of age.

In our study, the second predominant late OI was PJP, which occurred over 4 years after KT. No PJP was described during the first year post-transplantation, as expected with universal TMP-SMX prophylaxis prescribed for 12 months in our centre [6, 25]. Previous studies focusing on late PJP described a high burden of disease between 1 and 2 years post-transplantation occurring after prophylaxis discontinuation, with identification of an ALC <1,000/mm^3 in the year prior to PJP as a risk factor [12, 26, 27]. Consecutively, resuming TMP-SMX prophylaxis in case of ALC <1,000/mm^3 or maintaining life-long TMP-SMX should be discussed and evaluated in further studies [12].

Additionally, we reported a rare but significant predominance of invasive aspergillosis during the first-year post-transplantation. Incidence rate of invasive aspergillosis after KT is around 0.5%–4% and most episodes occur early, with negative impact on patient and allograft survival [28–32]. Whether the infection develops as a consequence of pre-transplantation colonisation or not remains unclear. Risk factors of invasive aspergillosis after KT include

high and prolonged duration of corticosteroids, dialysis requirement after transplantation and duration of pretransplant haemodialysis [30, 32–34]. Currently, no prophylactic strategy is recommended before kidney transplantation or in the following year [35]. While there is no place for antifungal prophylaxis in this population, we suggest that non-invasive preventive strategies, such as systematic pre-transplantation sinus CT-scan to detect pauci- or asymptomatic *Aspergillus* sinusitis, should be evaluated in prospective studies. Indeed, non-invasive fungal sinusitis represents a significant risk factor of invasive fungal infections in immunocompromised individuals [36].

We also found an association between older age at transplantation and early OI. Aged KTR experience increased rates of infection probably due to immune senescence [37]. Several specific mechanisms have been described, such as accelerated aging of the $CD8^+$ T cell after CMV infection and immune senescence of innate T cells [38]. Chronic kidney disease can also accelerate immune aging [39]. Our result may be the consequence of immune system exhaustion combined with the required immunosuppression. History of rejection in the year preceding the OI was also significantly more frequent in the early OI group, reflecting that increasing immunosuppression in the early period is a potent risk factor for OI.

The subgroup analysis of KTR switched from CNI to belatacept was performed *a priori* considering the higher risk of OI recently described in those KTR [10, 11]. As proportion of belatacept-switched KTR was similar in both OI groups, our data suggested that belatacept was not a risk factor of late OI. However, the small sample size prevented any definitive conclusion.

We recognize our study's limits, the first one being its monocentric retrospective design. However, this ensured homogeneous immunosuppressive regimens—even if no CNI level information was available—rejection management and infectious prophylaxis over a ten-year period. Despite the lack of a standardized classification of OI in non-HIV immunocompromised patients, we included OI based on their clinical and therapeutic impact and validated this selection by a consensus of experienced specialists, in the light of current literature. For instance, we only included CMV disease (as defined by the evidence of CMV infection with attributable symptoms [7]), for which treatment is mandatory; we did not consider isolated CMV DNAemia for which neither therapy nor systematic surveillance are recommended after KT [7]. Analysing the first OI only for each KTR prevented us from identifying risk factors of recurrent OI. Finally, future research should focus on all severe community infections, considering that bacterial infections represent the most common cause of infection-related mortality and appropriate prophylactic strategies are still limited.

CONCLUSION

Late-onset OI are currently predominant after kidney transplantation among young KTR and mostly include HZ and PJP. While we did not report a negative impact of late OI on patient and allograft survival, preventive strategies should be discussed and evaluated in prospective cohort studies.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The study involving human participants were reviewed and approved by the local ethics committee (IRB #00003835). Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

GM, VE, and MM conceived the study, collected patient data, performed the interpretation of the data, made critical revisions for intellectual content, and wrote the manuscript. LH and BP performed the statistical analysis and the interpretation of data. VF, SF, CA, CC, SG, PA, and AM contributed to study design, to data collection, analysis, and critical revisions for intellectual content. 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.2024.12065/full#supplementary-material

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Higher Donor Age and Severe Microvascular Inflammation Are Risk Factors for Chronic Rejection After Treatment of Active Antibody-Mediated Rejection

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Recent developments in intensive desensitization protocols have enabled kidney transplantation in human leukocyte antigen (HLA)-sensitized recipients. However, cases of active antibody-mediated rejection (AABMR), when they occur, are difficult to manage, graft failure being the worst-case scenario. We aimed to assess the impact of our desensitization and AABMR treatment regimen and identify risk factors for disease progression. Among 849 patients who underwent living-donor kidney transplantation between 2014 and 2021 at our institution, 59 were diagnosed with AABMR within 1 year after transplantation. All patients received combination therapy consisting of steroid pulse therapy, intravenous immunoglobulin, rituximab, and plasmapheresis. Multivariable analysis revealed unrelated donors and preformed donor-specific antibodies as independent risk factors for AABMR. Five-year death-censored graft survival rate was not significantly different between patients with and without AABMR although 27 of 59 patients with AABMR developed chronic AABMR (CABMR) during the study period. Multivariate Cox proportional hazard regression analysis revealed that a donor age greater than 59 years and microvascular inflammation (MVI) score (g + ptc) ≥4 at AABMR diagnosis were independent risk factors for CABMR. Our combination therapy ameliorated AABMR; however, further treatment options should be considered to prevent CABMR, especially in patients with old donors and severe MVI.

Keywords: antibody-mediated rejection, Banff classification, graft survival, kidney transplantation, treatment outcomes

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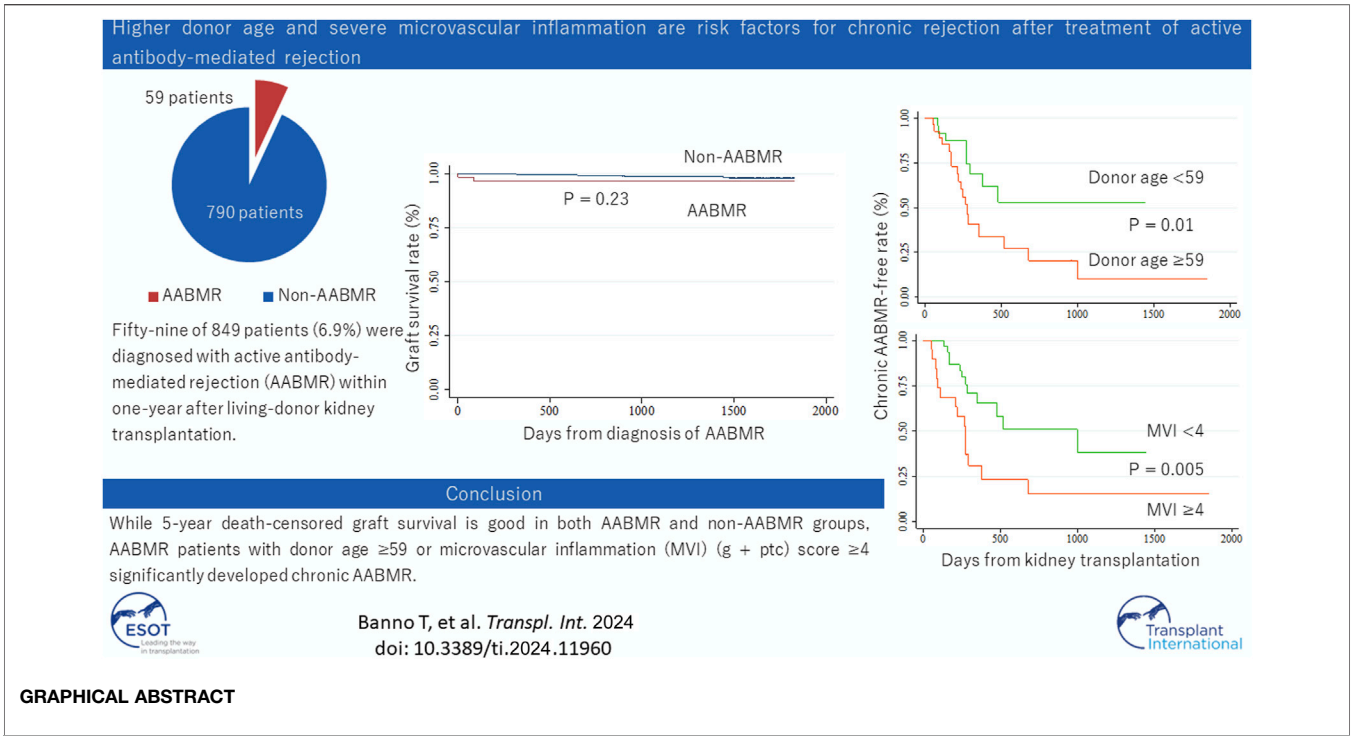
INTRODUCTION

Long-term graft survival has steadily improved over the past decades owing to advances in the care of transplant recipients [1]. Acute allograft rejection rates have also steadily decreased due to the use of immunosuppressive regimens targeting early rejection. In the current era, the incidence of acute rejection has decreased from rates exceeding 50% during the 1970s to between 10% and 20%. However, the situation is entirely different for patients who have sensitization against human leukocyte antigens (HLA).



GRAPHICAL ABSTRACT

Moreover, the number of HLA incompatibility adversely affects graft outcomes although the introduction of modern immunosuppression has lessened the degree of this impact over time [2]. AABMR, which is associated with HLA mismatch, HLA incompatibility, and blood group incompatibility, is an independent risk factor for death-censored graft failure [3]. Moreover, graft survival is significantly worse, especially from chronic allograft nephropathy, in those with AABMR than in those with acute rejection without evidence of AABMR [4]. Despite the development of immunosuppressive therapies over the decades, AABMR remains a cause of declining long-term graft survival.

Although several studies on treatments for AABMR have been reported, including plasmapheresis, IVIG, steroid pulse therapy, and anti-CD20 monoclonal antibody (rituximab) administration [5–12], a consensus on the therapeutic strategy for AABMR remains elusive. Furthermore, chronic AABMR (CABMR), characterized by transplant glomerulopathy (the result of remodeling glomeruli and microvascular injury), is unlikely to be reversed by current therapies [13].

In Japan, intravenous immunoglobulin (IVIG) was approved as a desensitization regimen in 2019 and is now covered by public health insurance. Thereafter, the number of kidney transplantation cases in highly HLA-sensitized recipients increased. Therefore, we are concerned that the number of cases of severe AABMR has increased and resulted in poorer graft outcomes. Although it had not been covered by the insurance, we have used high-dose IVIG for the desensitization and the AABMR treatment since before 2019. We conducted this study to evaluate the treatment outcomes, including impact on the Banff score, and identify risk factors for CABMR development in patients with living-donor kidney transplantation, including HLA-incompatible recipients.

MATERIALS AND METHODS

Ethics Statements

This study was approved by the Health Sciences Institutional Review Board (IRB) of Tokyo Women's Medical University Hospital (approval number: 4460-R), and the procedures followed were in accordance with the ethical standards of the local IRB and with the Helsinki Declaration of 1975, as revised in 2013. Informed consent was waived because patient data were extracted as anonymized data.

Study Design and Participants

This single-center retrospective study included a recent patient cohort including HLA-sensitized recipients. Between 2014 and 2021, 894 kidney transplantations were performed at Tokyo Women's Medical University Hospital, including 849 living-donor and 45 deceased-donor transplantations. Protocol allograft biopsies were routinely performed 3 months and 1 year after kidney transplantation. For-cause allograft biopsies were also performed in patients with delayed graft function, serum creatinine level elevation, increased proteinuria, and *de novo* donor-specific antibody (DSA) detection. Among the 849 patients with living-donor kidney transplantation, 59 were diagnosed with AABMR within 1 year of kidney transplantation.

Follow-up allograft biopsies were conducted approximately 6 months after treatment.

Patient Monitoring

Data were collected from patients' medical records. All patients were examined for HLA compatibility with complement-dependent cytotoxicity (CDC) crossmatch (XM), flow cytometry crossmatch (FCXM), or solid-phase immunoassay (SPI) using a single antigen bead assay (LABScreen™ single antigen beads, One Lambda, Canoga Park, CA). Serum creatinine levels, estimated glomerular filtration rate (eGFR), and the presence of proteinuria 6 months after treatment (after) were compared with those at diagnosis (before). eGFR was calculated using revised equations for eGFR from serum creatinine in Japan as follows: eGFR (mL/min/1.73 m^2) = 194 × serum creatinine (−1.094) × age (−0.287) [×0.739 (if female)] [14]. Anti-HLA antibodies were screened using LABScreen™ single antigen beads 1 year after transplantation or when ABMR was suspected. We defined positive DSA when an anti-HLA antibody to the donor was detected by SPI.

Transplant Biopsy and Pathological Diagnosis

Renal allograft biopsies were performed using an ultrasound-guided percutaneous technique, and two cores were collected per biopsy using a 16-gauge needle. Histomorphology was evaluated in formalin-fixed paraffin-embedded sections using a standard methodology. Pathological diagnosis was retrospectively reviewed and uniformed according to the Banff criteria 2019 as stated below [15].

Active ABMR

1. Histological evidence of acute tissue injury, which may include one or more of the following:
   - Microvascular inflammation (MVI) (g > 0 and/or ptc >0), in the absence of recurrent or *de novo* glomerulonephritis, although in the presence of acute T-cell mediated rejection (TCMR), borderline infiltrate, or infection, ptc ≥1 alone is not sufficient and g must be ≥1
   - Intimal or transmural arteritis.
   - Acute thrombotic microangiopathy, in the absence of any other cause.
   - Acute tubular injury, in the absence of any other apparent cause.
2. Evidence of current/recent antibody interaction with vascular endothelium, including one or more of the following:
   - Linear C4d staining in peritubular capillaries or medullary vasa recta.
   - At least moderate MVI ([g + ptc] ≥ 2) in the absence of recurrent or *de novo* glomerulonephritis, although in the presence of acute TCMR, borderline infiltrate, or infection, ptc ≥2 alone is not sufficient, and g must be ≥1.
   - Increased expression of gene transcripts/classifiers in the biopsy tissue is strongly associated with ABMR if thoroughly validated.
3. Serologic evidence of circulating DSA. C4d staining substitutes for DSA in cases without DSA. Patients negative for both DSA and C4d were classified into suspected AABMR. Non-HLA antibodies were not routinely examined in the current study.

Chronic Active ABMR

1. Morphologic evidence of chronic tissue injury, including one or more of the following:
   - Transplant glomerulopathy (cg > 0) if there is no evidence of chronic thrombotic microangiopathy or chronic recurrent/*de novo* glomerulonephritis, including changes evident by electron microscopy alone.
   - Severe peritubular capillary basement membrane multilayering.
   - Arterial intimal fibrosis of new onset, excluding other causes.
2. Identical to criterion 2 for active ABMR, as stated above.
3. Identical to criterion 3 for active ABMR, as stated above, including a strong recommendation for DSA testing whenever criteria 1 and 2 are met.

Immunosuppressive Regimen and Desensitization

Patients undergoing kidney transplantation at our institution started a triple immunosuppressive regimen including a calcineurin inhibitor (tacrolimus), an anti-proliferative agent (mycophenolate mofetil), and steroid (methylprednisolone) 7 days before transplantation as immunosuppression induction. Furthermore, the non-depleting anti-CD25 monoclonal antibody (basiliximab) was routinely induced twice: on the day of transplantation and postoperative day 4. ABO blood type-incompatible patients received additional desensitization with rituximab (200 mg/body) and plasmapheresis (2–4 times) until the anti-blood type IgG and IgM antibody titers decreased to <1:32, according to our protocol as we have reported before [16, 17]. Regarding HLA-incompatible kidney transplantation, the desensitization in patients with mean fluorescence intensity (MFI) of DSA <3,000 and negative for CDC and FCXM was performed according to ABO blood type-incompatible kidney transplantation. High-dose IVIG (2 g/kg) is added to HLA-incompatible patients with MFI of DSA ≥3,000 or positive for CDC or FCXM, in addition to ABO blood type-incompatible kidney transplantation protocol [18–20]. Maintenance immunosuppression included tacrolimus (trough value of approximately 5 ng/mL), mycophenolate mofetil acid (500–750 mg), and methylprednisolone (2–4 mg).

Treatments for Active Antibody-Mediated Rejection

All the patients with AABMR were treated with methylprednisolone administration at 500 mg for two consecutive days, except patients with subclinical AABMR with diabetes or other complications. Patients diagnosed

TABLE 1 | Patient characteristics with living-donor kidney transplantation.

	Total	AABMR[1]	Non-AABMR	*p*-value
N	849	59	790	
Recipient age at transplantation (years), mean (SD[2])	49.1 (13.3)	55.1 (9.3)	48.6 (13.5)	<0.001
Donor age at transplantation (years), mean (SD)	59.6 (10.1)	57.4 (8.3)	59.8 (10.2)	0.08
Recipient sex				
Male, *n* (%)	558 (65.7)	33 (55.9)	525 (66.5)	0.10
Female, *n* (%)	291 (34.3)	26 (44.1)	265 (33.5)	
Donor sex				
Male, *n* (%)	289 (34.0)	25 (42.4)	264 (33.4)	0.16
Female, *n* (%)	560 (66.0)	28 (53.8)	532 (66.7)	
Relation of donor				
Relative, *n* (%)	450 (53.0)	11 (18.6)	439 (55.6)	<0.001
Unrelated, *n* (%)	399 (47.0)	48 (81.4)	351 (44.4)	
ABO-incompatible transplantation, *n* (%)	250 (29.5)	24 (40.7)	226 (28.6)	0.051
Number of kidney transplantations				
Primary, *n* (%)	770 (90.7)	48 (81.4)	722 (91.4)	0.01
Multiple, *n* (%)	79 (9.3)	11 (18.6)	68 (8.6)	
Complement-dependent cytotoxicity				
T-cell positive, *n* (%)	0 of 845 (0)	0 of 59 (0)	0 of 786 (0)	
B-cell positive, *n* (%)	8 of 845 (0.9)	3 of 59 (5.1)	5 of 786 (0.6)	0.001
Flow cytometry crossmatch				
T-cell positive, *n* (%)	35 of 843 (4.2)	14 of 59 (23.7)	21 of 784 (2.7)	<0.001
B-cell positive, *n* (%)	13 of 843 (1.5)	6 of 59 (10.2)	7 of 784 (0.9)	<0.001
Presence of preformed DSA[3], *n* (%)	131 of 833 (15.7)	35 of 59 (59.3)	96 of 774 (12.4)	<0.001
Allograft weight (grams), mean (SD)	176.9 (44.6)	188.7 (48.2)	176.0 (44.3)	0.04
Warm ischemia time (minutes), mean (SD)	3.6 (1.1)	3.5 (0.9)	3.6 (1.2)	0.37
Total ischemia time (minutes), mean (SD)	72.9 (25.4)	77.6 (24.1)	72.5 (25.5)	0.14
Follow-up period (days), median (IQR[4])	1,544 (903–2,356)	1,365 (714–2,237)	1,549 (917–2,363)	0.15

AABMR[1], active antibody-mediated rejection; SD[2], standard deviation; DSA[3], donor-specific antibody; IQR[4], interquartile range.

with for-cause biopsy (clinical AABMR) or those with protocol biopsy (subclinical AABMR) with eGFR <25 mL/min, MVI (g + ptc) score ≥4, or positive for *de novo* DSA were considered for IVIG administration/plasma pheresis, which has been known to improve graft survival [21–24], when patients agreed after giving informed consent. Rituximab administration was considered when CD19+B cells remained detectable.

Statistical Analysis

Continuous variables are expressed as mean and standard deviation or median and interquartile range (IQR), while categorical variables are expressed as percentages. Independent continuous variables were analyzed using the *t*-test for normally distributed data and the Wilcoxon rank-sum test for non-normally distributed data, and categorical variables were analyzed using the Pearson χ-square test. Paired t-tests and Wilcoxon signed-rank tests were used to analyze dependent continuous variables. McNemer's test was used for dependent categorical variables. Univariable and multivariable Cox proportional hazard regression models were used to assess the hazard risk. Continuous variables were converted into categorical variables in Cox proportional hazard regression analysis. Kaplan-Meier curves and Log-rank tests were generated to compare the time until an event occurs between the different groups. Statistical significance was set at $p < 0.05$. Analyses were performed using Stata, version 15.1 (Stata Corp. LP, College Station, TX, United States).

RESULTS

Patient Background Characteristics

Table 1 presents the patient background characteristics. Fifty-nine of 849 patients with living-donor kidney transplantations (6.9%) developed AABMR or suspected AABMR (AABMR group) within 1 year of kidney transplantation. The recipient age and rate of unrelated donors were significantly higher in the AABMR group than in the non-AABMR group. Patients in the AABMR group showed a higher immunological risk compared to those in the non-AABMR group (higher rate of history of kidney transplantation, positivity for CDC-XM, FCXM, and SPI). The patients with ABO incompatibility showed a trend of higher frequency in the AABMR group though the difference did not reach statistical significance ($p = 0.051$). AABMR was diagnosed 90 days (IQR: 3–105) after kidney transplantation. Twenty-seven patients (45.8%) with AABMR were diagnosed by for-cause biopsy findings and the remaining 32 (54.2%) were diagnosed by protocol biopsy results. Out of 59 AABMR patients, 36 (61.0%) had preformed DSA, with 8 in class 1, 19 in class 2, and 9 in both classes. Seventeen (28.8%) of 59 in the AABMR group had *de novo* DSA, with 2 in class 1 and 15 in class 2. The immunodominant MFI values were 1900 (1,247–10,843) for preformed DSA and 3,181 (1,541–4,713) for *de novo* DSA. Of the 59 patients, 16 (27.1%) did not show either preformed or *de novo* DSAs. However, eight patients were positive for C4d staining, which could substitute for DSA

TABLE 2 | Cox proportional hazard regression analysis of variables associated with active antibody-mediated rejection within 1 year after kidney transplantation.

		Univariate				Multivariate			
		HR[1]	95% CI[2]		*p*-value	HR	95% CI		*p*-value
Recipient age	<50 years	reference				reference			
	≥50 years	2.72	1.51	4.90	0.001	1.04	0.53	2.05	0.90
Donor age	<60 years	reference							
	≥60 years	0.77	0.46	1.30	0.34				
Recipient sex	Female	reference							
	Male	0.66	0.39	1.11	0.12				
Donor sex	Female	reference							
	Male	1.54	0.91	2.59	0.12				
Relation of donor	Relative	reference				reference			
	Unrelated	5.58	2.82	11.0	<0.001	4.48	2.05	9.79	<0.001
ABO compatibility	Compatible	reference							
	Incompatible	1.53	0.90	2.61	0.12				
Number of transplantations	Primary	reference				reference			
	Multiple	2.44	1.26	4.70	0.008	1.99	1.01	3.91	0.05
CDC[3] for B cells	Negative	reference							
	Positive	6.84	2.14	21.9	0.001				
FCXM[4] for T cells	Negative	reference							
	Positive	9.25	5.05	16.9	<0.001				
FCXM for B cells	Negative	reference							
	Positive	8.17	3.26	20.5	<0.001				
Solid–phase immunoassay	Negative	reference				reference			
	Positive	9.72	5.70	16.6	<0.001	7.05	4.16	12.4	<0.001

HR[1], hazard ratio; CI[2], confidence interval; CDC[3], complement-dependent cytotoxity; FCXM[4], flow cytometry crossmatch.

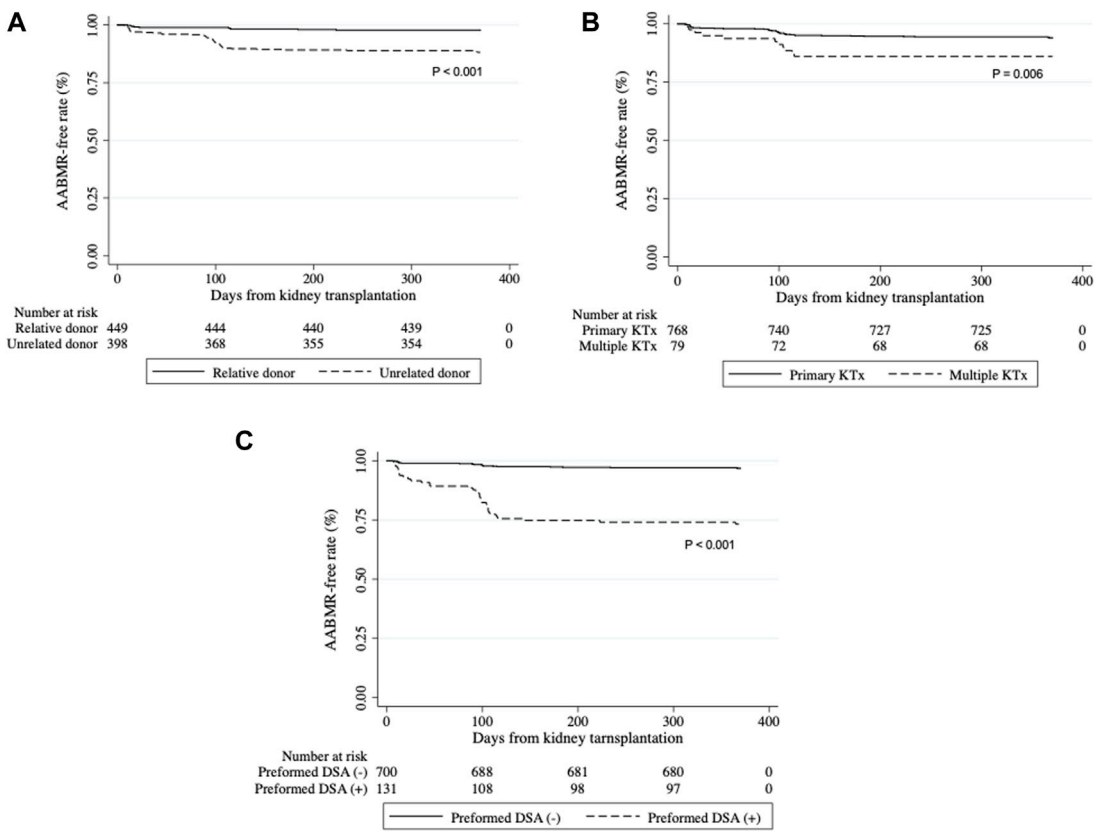


FIGURE 1 | Development of active antibody-mediated rejection (AABMR) within 1 year after kidney transplantation Kaplan-Meier curves for AABMR-free survival between **(A)** relative and unrelated donors, **(B)** primary and multiple kidney transplantation (KTx), and **(C)** positive and negative for preformed donor-specific antibody (DSA). *p*-values calculated by Log-rank tests were shown.

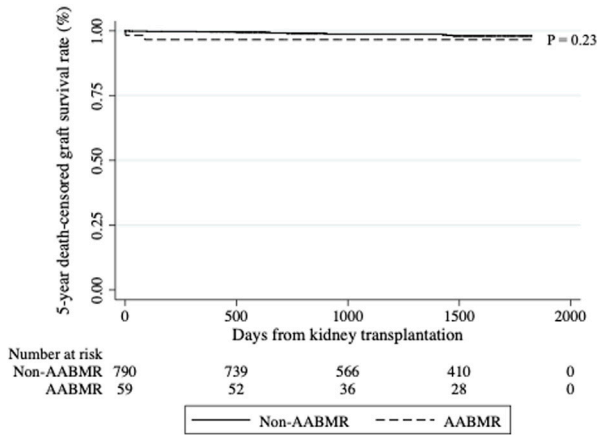


FIGURE 2 | Five-year death-censored graft survival rate between active antibody-mediated rejection (AABMR) and non-AABMR groups. The graft survival rates were compared between patients with AABMR within 1 year of kidney transplantation and those without it. The five-year death-censored graft survival rates did not differ between the groups. Kaplan-Meier curves depicting the five-year death-censored graft survival rate from kidney transplantation were presented, and the *p*-value was calculated using the Log-rank test.

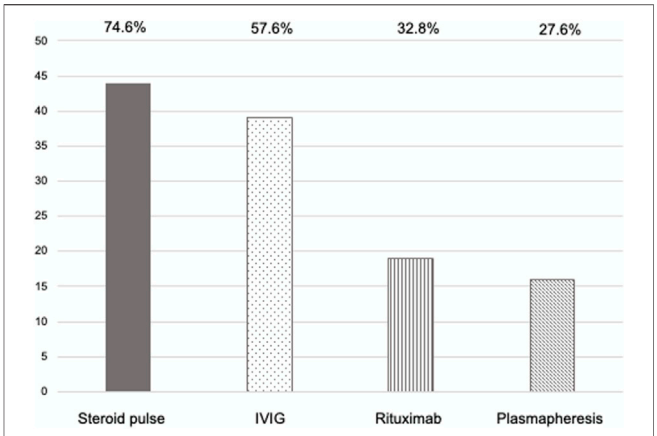


FIGURE 3 | The treatment regimen for active antibody-mediated rejection. *Y*-axis: number of patients. Steroid pulse therapy, methylprednisolone, 250–2,500 mg/body; intravenous immunoglobulin (IVIG) therapy, 0.5–5.2 g/kg; rituximab administration, 200–300 mg/body; plasmapheresis, 2–10 times including plasma exchange or double-filtration plasmapheresis with fresh frozen plasma or albumin replacement.

as per the 2019 Banff criteria. Among the remaining eight patients who were negative for both DSA and C4d staining and had MVI scores all ≥2 (suspected AABMR), the rate of developing CABMR was similar to that of patients positive for either DSA or C4d with MVI score ≥2, as shown in **Supplementary Figure S1** ($p = 0.41$).

Risk Factors for Active Antibody-Mediated Rejection Within 1 year of Kidney Transplantation

Univariable and multivariable Cox proportional hazard regression analyses were conducted to assess the hazard risk of AABMR over time after kidney transplantation within 1 year of kidney transplantation. In the univariable analysis, variables including age and sex of the recipient and donor; the relationship between the donor and recipient; ABO blood type compatibility and history of previous kidney transplantation; results of CDC-XM, FCXM, and SPI; allograft weight; and warm and total ischemia times were considered as covariables (**Table 2**). Recipient age greater than 50 years (hazard ratio [HR]: 2.72, 95% CI: 1.51–4.90, $p = 0.001$), unrelated donor (HR: 5.58, 95% CI: 2.82–11.0, $p < 0.001$), history of previous kidney transplantation (HR: 2.44, 95% CI: 1.26–4.70, $p = 0.008$), positive CDC-XM for B cells (HR: 6.84, 95% CI: 2.14–21.9, $p = 0.001$), positive FCXM (T cells, HR: 9.25, 95% CI: 5.05–16.9, $p < 0.001$; B cells, HR: 8.17, 95% CI: 3.26–20.5, $p < 0.001$), and positive SPI (HR: 9.72, 95% CI: 5.70–16.6, $p < 0.001$) were significantly associated with the incidence of AABMR within 1 year after kidney transplantation. The multivariable analysis was performed with selected variables that were statistically significant in the univariable analysis. We chose SPI for immunological risk because CDC, FCXM, and SPI tests may cause multicollinearity. Finally, unrelated donor (HR: 4.48, 95% CI: 2.05–9.79, $p < 0.001$), multiple transplantation (HR: 1.99, 95% CI: 1.01–3.91, $p = 0.05$), and positive SPI (HR: 7.05, 95% CI: 4.16–12.4, $p < 0.001$) were associated with an increased hazard risk of AABMR over time. Significant differences were shown in Kaplan-Meier curves depicting the free rate of AABMR comparing unrelated and relative donors, primary and multiple kidney transplantations, and positive and negative for SPI (**Figures 1A–C**). Conversely, ABO compatibility was not significantly different (**Supplementary Figure S2**; $p = 0.11$) although there was a nearly significant difference in the chi-square test.

Long-Term Outcomes

Figure 2 shows a comparison of the Kaplan-Meier curves of five-year death-censored graft survival between the AABMR and non-AABMR groups. Five-year death-censored graft survival rates were 96.7% and 98.0% in the AABMR and non-AABMR groups, respectively ($p = 0.23$). Collectively, these data suggest that most patients sustain long-term renal function after overcoming AABMR.

Treatments for AABMR

As shown in **Figure 3**, patients with AABMR underwent comprehensive anti-humoral immunity treatments. IVIG administration was undertaken for 74% of the patients who met the treatment criteria (18 out of 21 patients [86%] with clinical AABMR and 10 out of 17 [59%] with subclinical AABMR). Rituximab and plasmapheresis were undertaken for 32.8% and 27.6% of the AABMR patients, respectively. The dose of basic immunosuppressants was also adjusted according to the patient's physical condition.

TABLE 3 | Renal function, presence of proteinuria, and Banff classification scores at diagnosis of active antibody-mediated rejection and follow-up allograft biopsy.

	At diagnosis with AABMR[1]	At follow-up	*p*-value
Serum creatinine, mean (SD[2])	1.8 (1.6)	1.4 (1.0)	0.001
eGFR[3] (mL/min/1.73 m^2), mean (SD)	40.0 (16.5)	43.7 (13.1)	0.009
Proteinuria, *n* (%)			
None	32 (54.2)	46 (77.8)	0.003
1+	18 (30.5)	11 (18.6)	
2+	8 (13.6)	2 (3.4)	
3+	1 (1.7)	0 (0)	
Banff classification score			
i score ≥2, *n* (%)	1 of 53 (1.9)	2 of 53 (3.8)	0.56
t score ≥2, *n* (%)	2 of 53 (3.8)	3 of 53 (5.7)	0.65
g score ≥2, *n* (%)	25 of 53 (47.2)	20 of 53 (33.9)	0.01
ptc score ≥2, *n* (%)	28 of 53 (52.8)	17 of 53 (32.1)	0.03
C4d score ≥2, *n* (%)	20 of 53 (37.7)	19 of 53 (35.9)	0.76
ci score ≥2, *n* (%)	1 of 53 (1.9)	3 of 53 (5.7)	0.32
ct score ≥2, *n* (%)	1 of 53 (1.9)	3 of 53 (5.7)	0.32
cg score ≥2, *n* (%)	0 of 53 (0)	2 of 53 (3.8)	0.16
cv score ≥2, *n* (%)	0 of 53 (0)	4 of 53 (7.6)	0.04

AABMR[1], active antibody-mediated rejection; SD[2], standard deviation; eGFR[3], estimated glomerular filtration rate.

Treatment Effects for AABMR

We compared the serum creatinine levels, eGFR, proteinuria, and Banff scores before and after the treatment in 59 patients with AABMR (**Table 3**). Although two of the 59 patients with AABMR showed an immediate decrease in the eGFR and lost their graft due to hyper-AABMR that did not respond to any treatment, the serum creatinine level and eGFR were significantly improved from 1.8 ± 1.6 mg/dL and 40.0 ± 16.5 mL/min/1.73 m^2 to 1.4 ± 1.0 mg/dL and 43.7 ± 13.1 mL/min/1.73 m^2, respectively, after the treatment (p = 0.001 and 0.009, respectively). The value of proteinuria was also statistically improved from none (54.2%), 1+ (30.5%), 2+ (13.6%), and 3+ (1.7%) to none (77.8%), 1+ (18.6%), 2+ (3.4%), and 3+ (0%) (p = 0.003). Regarding Banff scores, 12 out of 59 patients had a g score of three, and 3 out of 59 patients had a ptc score of three at diagnosis of AABMR, respectively. Fifty-three of the 59 patients underwent a follow-up biopsy after treatment, with a median time of 219 days (IQR: 112–280) after AABMR diagnosis. The proportion of cases with severe g and ptc scores (≥2) significantly reduced at the follow-up biopsy (from 47.2% to 33.9%, p = 0.01; from 52.8% to 32.1%, p = 0.03, respectively), whereas no alternation was observed in scores for i, t, and C4d. In contrast, the proportion of severe cv scores (≥2) significantly increased in the follow-up biopsy (from 0% to 7.6%, p = 0.04).

Evaluation of Risk for Chronic Active Antibody-Mediated Rejection Development

Twenty-seven of 59 patients with AABMR developed CABMR at a median of 248 days (IQR: 137–295) after the initial diagnosis of AABMR. **Table 4** shows the results of the univariable and multivariable Cox proportional hazard regression analyses of the variables associated with the development of CABMR in patients with AABMR. Donor age greater than 59 years (HR: 2.68, 95% CI: 1.20–6.01, p = 0.02) and MVI (g+ ptc) score ≥4 (HR: 2.85, 95% CI: 1.33–6.10, p = 0.01) were significantly associated with the development of CABMR caused by AABMR. Multivariable analysis was conducted using significant variables of univariable analysis. As a result, donor age greater than 59 years (HR: 2.51, 95% CI: 1.12–5.64, p = 0.03) and MVI score ≥4 (HR: 2.67, 95% CI: 1.25–5.72, p = 0.01) were the independent risk factors for CABMR progression. Kaplan-Meier curves depicting the CABMR-free survival rate revealed significantly poor outcomes after AABMR in cases with older donors and those with severe MVI scores (**Figures 4A, B**).

DISCUSSION

We demonstrated favorable treatment outcomes for patients with AABMR within 1 year of kidney transplantation at our institution. The five-year death-censored graft survival rate was 96.2%, and renal function and MVI (g +ptc) were significantly improved after AABMR treatment. Those data collectively suggest the benefit of our new desensitization regimen and AABMR treatment regimen. However, approximately half of the patients with AABMR eventually developed CABMR. We found that older donor age and higher Banff classification g-scores were independent risk factors for the development of CABMR after AABMR diagnosis.

The current study involved patients with high immune risk, whereas the incidence rate of AABMR within 1 year after KTx was not high (6.1%) compared to our previous report, which showed a rate of 10.8% between 2000 and 2008 [25]; this indicates that our current desensitization protocol was successful. Consistent with the results of the current study, a previous study reported that DSAs are a predominant predictor of acute rejection [3]. In contrast, the current study showed that ABO-incompatible kidney transplantation was not related to the development of AABMR. In the reported meta-analysis, including studies from 1999 to 2016, ABO-incompatible

TABLE 4 | Results of Cox hazard regression analysis of variables associated with chronic active antibody-mediated rejection after active antibody-mediated rejection.

		Univariate				Multivariate			
		HR[1]	95% CI[2]		*p*-value	HR	95% CI		*p*-value
Recipient age	<56 years	reference							
	≥56 years	1.72	0.80	3.73	0.17				
Donor age	<59 years	reference				reference			
	≥59 years	2.68	1.20	6.01	0.02	2.51	1.12	5.64	0.03
Diabetes mellitus	Absence	reference							
	Presence	0.96	0.38	2.38	0.93				
ABO compatibility	Compatible	reference							
	Incompatible	0.47	0.19	1.18	0.11				
Number of transplantations	Primary	reference							
	Secondary	2.02	0.80	5.06	0.14				
Number of HLA[3] mismatches	<4	reference							
	≥4	0.92	0.39	2.19	0.85				
CDC[4] for B cells	Negative	reference							
	Positive	1.22	0.28	5.25	0.79				
FCXM[5] for T cells	Negative	reference							
	Positive	1.34	0.58	3.08	0.49				
FCXM for B cells	Negative	reference							
	Positive	2.17	0.63	7.47	0.22				
Solid-phase immunoassay	Negative	reference							
	Positive	1.32	0.56	3.13	0.53				
MFI[6] of preformed DSA[7]	<5,000	reference							
	≥5,000, <10,000	1.27	0.47	3.45	0.64				
	≥10,000	1.89	0.69	5.17	0.21				
MFI of *de novo* DSA	<3,000	reference							
	≥3,000	0.67	0.20	2.25	0.52				
eGFR[8] before the treatments	<40 mL/min/1.73 m^2	reference							
	≥40 mL/min/1.73 m^2	0.69	0.32	1.47	0.33				
i score at AABMR[9] diagnosis	<2	reference							
	≥2	1.73	0.23	12.9	0.59				
t score at AABMR diagnosis	<2	reference							
	≥2	3.31	0.77	14.3	0.49				
g score at AABMR diagnosis	<2	reference							
	≥2	1.96	0.91	4.26	0.09				
ptc score at AABMR diagnosis	<2	reference							
	≥2	1.43	0.66	3.09	0.36				
C4d score at AABMR diagnosis	<2	reference							
	≥2	0.97	0.43	2.16	0.94				
MVI[10] (g+ptc) at AABMR diagnosis	<4	reference				reference			
	≥4	2.85	1.33	6.10	0.007	2.67	1.25	5.72	0.01
Coexistence of TCMR[11]	Absence	reference							
	Presence	0.75	0.10	5.60	0.78				

HR[1], hazard ratio; CI[2], confidence interval; HLA[3], human leucocyte antigen; CDC[4], complement-dependent cytotoxity; FCXM[5], flow cytometry crossmatch; MFI[6], mean fluorescence intensity; DSA[7], donor-specific antibody; eGFR[8], estimated glomerular filtration rate; AABMR[9], active antibody-mediated rejection; MVI[10], microvascular inflammation; TCMR[11], T-cell mediated rejection.

transplantation was significantly associated with ABMR compared with ABO-compatible transplantation, and graft survival in ABO-incompatible kidney transplantation was also inferior to ABO-compatible [26]. Indeed, we observed more ABO-incompatible patients in the AABMR group in the current study (**Table 1**). Conversely, we previously reported that the rate of ABMR and graft survival in ABO-incompatible kidney transplantation was not significantly different from those in ABO-compatible in an era between 2005 and 2013, whereas that was inferior to ABO-compatible between 1989 and 2004 [16]. Consistent with our previous study, Cox proportional hazard regression analysis revealed that the ABO-incompatible transplantation was no longer the risk for AABMR development (**Table 2**; **Supplementary Figure S2**). We assume that the development of immunosuppressive agents and the recent desensitization protocol for ABO-blood antibodies decreased the rate of rejection and improved graft survival.

We treated AABMR with combination therapy consisting of steroid pulse therapy, IVIG, rituximab administration, and plasmapheresis. Although two patients had graft loss, most patients showed significant improvements in both renal function and microvascular inflammation. The effective treatments for AABMR were initially thought to be plasmapheresis, which removes humoral mediators from the circulation, and IVIG-inhibiting antibody synthesis [5, 6]. A previous report showed that the combination of

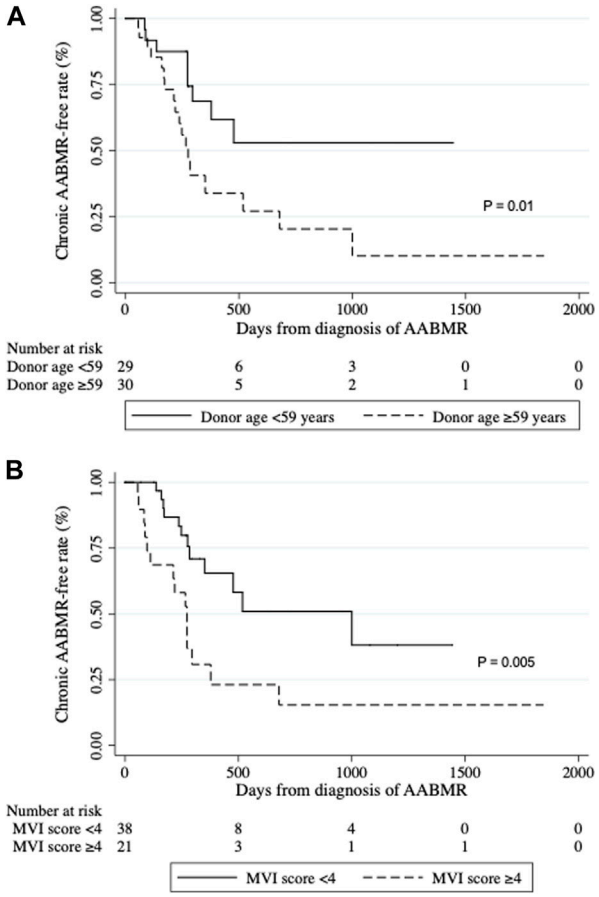


FIGURE 4 | Development of chronic active antibody-mediated rejection (CABMR). Kaplan-Meier curves of the CABMR-free rate after AABMR diagnosis comparing **(A)** the patients with donor ages of ≥59 and <59 years ($p = 0.01$); and **(B)** the patients with MVI score ≥4 and <4 at diagnosis of AABMR ($p = 0.005$). p-values calculated by the Log-rank test were shown.

plasmapheresis and IVIG significantly improved the one-year graft survival rate compared with plasmapheresis alone [7]. Another study also reported that the combination significantly decreased the graft failure rate (risk ratio: 0.26) compared with a control, with a mean follow-up of 7 years [8]. Furthermore, the addition of rituximab significantly decreased the MFI value of the DSAs and Banff classification scores, resulting in improved graft survival [9–11]. In contrast, a randomized controlled trial did not show a significant difference in one-year graft survival between rituximab and control groups based on plasmapheresis, steroid pulse, and IVIG treatment protocols, whereas microvascular inflammatory scores (glomerulitis and peritubular capillaritis) and chronic injury scores (interstitial fibrosis and tubular atrophy) significantly decreased in the rituximab group [12]. In all studies, the level of evidence for AABMR treatment was low because the data were from a small series. However, the effectiveness of new therapeutic strategies, including proteasome and complement inhibitors, remains unclear [27].

The five-year death-censored graft survival rate of AABMR was 96.2%, which was as good as that in the non-AABMR group (98.5%), indicating the potential of our ABMR treatment regimens. However, 27 of 52 patients with AABMR developed CABMR, which is a well-known risk factor for graft loss [28]. Generally, our treatment regimen effectively prevented early graft loss though it might be difficult to prevent CABMR development and future deterioration of graft function. A longer-term follow-up would be required.

Older donor age was one of the independent risk factors for the development of CABMR. In a previous study, graft survival was lower in transplants from ≥60-year-old donors compared with 18–49-year-old donors. Patient survival was also significantly lower in transplants from donors aged >50 years, compared to transplants from 18 to 49-year-old donors [29]. Similar to our result, a study reported that older donor age was significantly associated with increased susceptibility to chronic allograft damage [30]. Additionally, acute tubular necrosis detected by pretransplant biopsy results or total ischemic time is significantly associated with poor graft outcomes in elderly donors [31, 32]. Irreversible changes may occur if allografts from elderly donors are damaged.

The MVI (g + ptc) score at diagnosis of AABMR was also significantly associated with CABMR development. Several studies have also demonstrated that microvascular injury, including glomerulitis, is correlated with chronic microvascular damage and poor graft prognosis [33–37]. Moreover, graft survival with severe glomerulitis with a g score of three on the Banff classification was 70% a few years after the biopsy [38]. Consistent with these reports, we observed that the MVI score ≥4 was an independent risk factor for CABMR in the current study.

Although CABMR is one of the main causes of late graft failure, there are no approved drugs for its prevention or treatment. A multicenter randomized trial of treatment for transplant glomerulopathy with IVIG and rituximab versus placebo did not show significant differences in eGFR decline, increased proteinuria, Banff classification scores at 1 year, and MFI of immunodominant DSAs [39]. New reagents, such as proteasome inhibitors that eliminate plasma cells producing alloantibodies or anti-C5 monoclonal antibodies that inhibit the activation of C5, did not also show significant improvement in the eGFR and MFI value of DSAs, compared with the control group [40, 41]. More recently, C1 esterase inhibitors that block early complement pathways or inhibitors of the interleukin (IL)-6 and IL-6 receptor axes have been expected to be effective [28].

This study possesses certain limitations. First, it was conducted retrospectively within a singular institution, involving a relatively small cohort. However, the limited number of patients with ABMR is not unexpected, considering the diminishing incidence of acute rejection attributed to advancements in immunosuppressive medications. Second, although the Banff criteria strongly advises testing non-HLA antibodies [42] if HLA antibody testing is negative despite pathological ABMR features, we have not screened out non-HLA antibodies. However, we assume that those cases should be

included in AABMR because the rates of development to CABMR following AABMR were similar between true AABMR cases and suspected AABMR cases. Third, because of the retrospective nature of this study, treatments for AABMR were not completely consistent. When adjusting for the severity of ABMR, there was no significant difference in CABMR development between patients treated and those not treated with IVIG (data not shown); however, due to a variety of background differences, we cannot draw the exact conclusion. The clinical impact of IVIG on AABMR needs to be confirmed in future randomized clinical trials.

In conclusion, the AABMR treatment regimen resulted in good short-term graft survival and significant improvements in renal function with reduced Banff scores; however, it did not prevent the development of CABMR. Further treatment options should be considered, especially in patients with older donors and severe MVI.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

This study was approved by the Health Sciences Institutional Review Board (IRB) of Tokyo Women's Hospital (approval number: 4460-R), and the procedures followed were in accordance with the ethical standards of the local IRB and with the Helsinki Declaration of 1975, as revised in 2013. Informed consent was waived because patient data were extracted as anonymized data.

AUTHOR CONTRIBUTIONS

TB and TH contributed to the study conception and design. RO, TY, KU, TK, KO, and TS drafted and reviewed the study critically for important intellectual content. HI and TT approved the final version to be published. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.11960/full#supplementary-material

SUPPLEMENTARY FIGURE S1 | CABMR free survival rate comparing AABMR and suspected AABMR patients. AABMR, active antibody-mediated rejection; MVI score ≥2 with either DSA- or C4d-positive. suspected AABMR, MVI score ≥2 with both DSA- and C4d-negative. CABMR, chronic active antibody-mediated rejection; DSA, donor-specific antibody. p = 0.41 was calculated by the Log-rank test between the groups.

SUPPLEMENTARY FIGURE S2 | Development of active antibody-mediated rejection (AABMR) within 1 year after kidney transplantation comparing ABO-compatible and incompatible kidney transplantation. Kaplan-Meier curves for AABMR free survival comparing ABO-compatible and incompatible kidney transplantation. The rates of development of AABMR were similar between both groups (p = 0.11).

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ORIGINAL RESEARCH
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Pre-Transplant Hyperparathyroidism and Graft or Patient Outcomes After Kidney Transplantation

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The impact of pre-transplant parathyroid hormone (PTH) levels on early or long-term kidney function after kidney transplantation is subject of debate. We assessed whether severe hyperparathyroidism is associated with delayed graft function (DGF), death-censored graft failure (DCGF), or all-cause mortality. In this single-center cohort study, we studied the relationship between PTH and other parameters related to bone and mineral metabolism, including serum alkaline phosphatase (ALP) at time of transplantation with the subsequent risk of DGF, DCGF and all-cause mortality using multivariable logistic and Cox regression analyses. In 1,576 kidney transplant recipients (51.6 ± 14.0 years, 57.3% male), severe hyperparathyroidism characterized by pre-transplant PTH ≥771 pg/mL (>9 times the upper limit) was present in 121 patients. During 5.2 [0.2–30.0] years follow-up, 278 (15.7%) patients developed DGF, 150 (9.9%) DCGF and 432 (28.6%) died. A higher pre-transplant PTH was not associated with DGF (HR 1.06 [0.90–1.25]), DCGF (HR 0.98 [0.87–1.13]), or all-cause mortality (HR 1.02 [0.93–1.11]). Results were consistent in sensitivity analyses. The same applied to other parameters related to bone and mineral metabolism, including ALP. Severe pre-transplant hyperparathyroidism was not associated with an increased risk of DGF, DCGF or all-cause mortality, not supporting the need of correction before kidney transplantation to improve graft or patient survival.

Keywords: kidney transplantation, graft survival, delayed graft function, hyperparathyroidism, mineral metabolism

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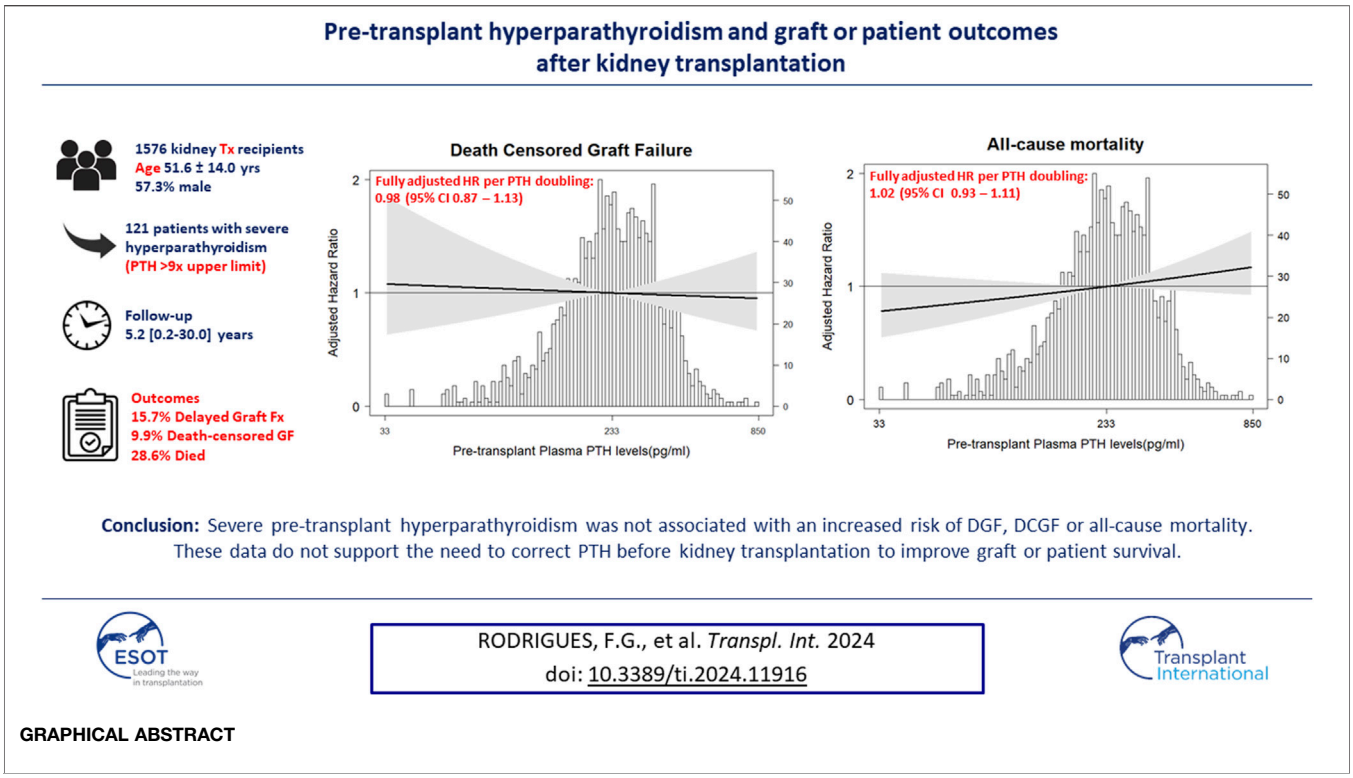
INTRODUCTION

Hyperparathyroidism is a frequent complication of advanced chronic kidney disease (CKD) [1]. While a moderate increase in plasma parathyroid hormone (PTH) may be indicative of an appropriate compensatory response to maintain normal calcium balance, very high PTH levels have been associated with reduced quality of life and an increased risk of cardiovascular and bone disease and premature mortality in patients with kidney failure [2–4].

Kidney transplantation may at least in part resolve metabolic disturbances including hyperparathyroidism [5]. However, the presence of severe hyperparathyroidism at the time of



GRAPHICAL ABSTRACT

transplantation may induce both short- and long-term adverse effects to the kidney. Early after successful kidney transplantation, persistently elevated levels of the phosphaturic hormones PTH and fibroblast growth factor-23 in the context of restored kidney function can induce high urinary concentrations of calcium and phosphate, which may lead to the deposition of calcium-phosphate and, consequently, acute tubular necrosis [6–8]. On the longer term, persistent or recurrent abnormalities in mineral metabolism have been associated with death-censored graft failure (DCGF), progression of vascular calcification and premature mortality [9, 10]. Similar to PTH, pretransplant serum total alkaline phosphatase, calcium and phosphorus levels have been associated with an increased risk of unfavorable outcomes after kidney transplantation [7, 9, 11, 12]. For these reasons, current Kidney Disease Improving Global Outcomes (KDIGO) guidelines suggest to not transplant patients with severe hyperparathyroidism until they are adequately treated, with PTH levels in the range of approximately 2–9 times the upper normal limit for the assay [13–15]. In contrast, the European Renal Association recommended in 2013 that a deceased donor allograft should not be refused only because of uncontrolled hyperparathyroidism in the recipient [15]. These conflicting recommendations urge for large cohort studies to examine the association between pre-transplant PTH level and clinically important post-transplant outcomes [13] including graft and patient outcomes.

Therefore, the aim of the present study was to assess whether patients with higher pre-transplant plasma PTH levels, and particularly those with severe hyperparathyroidism, have a higher risk of delayed graft function (DGF), death-censored graft function (DCGF), or all-cause mortality. We addressed this aim in a large contemporary cohort of kidney transplant recipients, and also studied associations of other mineral parameters (total alkaline phosphatase, calcium, and phosphate), measured before transplantation, with post-transplant outcomes.

MATERIALS AND METHODS

For the current study, all patients who underwent kidney transplantation at the University Medical Center Groningen (UMCG), Netherlands, between April/1986-December/2019 were considered eligible for inclusion. Of patients who had undergone multiple kidney transplantations, only data regarding the first kidney transplantation were used (N = 1,717). Patients with missing pre-transplant plasma PTH (N = 29) or if pre-transplant plasma PTH measurement was measured longer than 90 days before transplantation (N = 112) were excluded, leaving 1,576 patients for the DGF analysis. Furthermore, we excluded patients who developed graft failure or died within 3 months after transplantation (N = 67) [10], leaving 1,509 patients for the DCGF and all-cause mortality analysis (**Supplementary Figure S1**). The study protocol has been approved by the Institutional Review Board (METc 2014/077), was performed under the Strengthening the Reporting of Observational Studies in Epidemiology guidelines [16], adheres to the local UMCG Biobank Regulations, and is in

accordance with the WMA Declarations of Helsinki and Istanbul.

Laboratory Data

Routine laboratory measurements were extracted from the laboratory information system of the UMCG. Plasma PTH, calcium, phosphate, total alkaline phosphatase (ALP), creatinine, and albumin concentrations were measured at outpatient visits. Plasma calcium was corrected for albumin according to the following formula: corrected calcium (mg/dL) = measured calcium (md/dL)+0.025*(40–[albumin (g/dL)]). All routine measurements before March 2006 were performed on the Merck Mega Analyzer (Merck); measurements after March 2006 were performed on the Roche Modular (Roche Ltd.). Laboratory measurements prior to March 2006 were converted according to the equations [17] listed in **Supplementary Table S1**. The last PTH measurement prior to the kidney transplant procedure was used for analyses. Reference values for plasma-corrected calcium were 8.8–10.4 mg/dL (2.20–2.60 mmol/L) and for plasma phosphate 2.17–4.64 mg/dL (0.70–1.50 mmol/L) [10]. At each individual measurement, patients were classified as having hypo-, normo-, or hypercalcemia and hypo-, normo-, or hyperphosphatemia according to these definitions. Creatinine-based eGFR was calculated according to the CKD Epidemiology Collaboration Equation (EPI) equation [18, 19]. Primary cytomegalovirus (CMV) infection was defined as CMV viremia demonstrated by PCR in the absence of CMV-specific IgG antibodies. All other measurements were performed using standard laboratory techniques.

Follow-Up

All patients who received a kidney transplant underwent a standardized follow-up regime. Patients received a standardized immunosuppression protocol, comprising triple therapy with tacrolimus or cyclosporine, in combination with mycophenolate mofetil and corticosteroids, as previously reported [20]. Shortly after transplantation, patients visit the outpatient department weekly. The frequency of visits is tapered to every 4–6 weeks during the first year after transplantation, and at least four times a year after the first year. End of follow-up was December 2020. Donor and recipient characteristics were collected as part of the TransplantLines registry [21]. The primary cause of kidney failure was categorized according to the European Renal Association Registry Coding System [22]. Acute rejection was defined according to the Banff criteria. There was no loss to follow-up.

Study Endpoints

The three co-primary outcomes were DGF, defined as the need for dialysis within the first 7 days posttransplant, DCGF, defined as return to dialysis or re-transplantation, censored for death, and all-cause mortality. Up-to-date follow-up was warranted through the continuous surveillance system of the outpatient clinic.

Statistical Analyses

Statistical analyses were performed using IBM SPSS version 23.0 (SPSS Inc., Chicago, IL). In all analyses $p < 0.05$ was considered significant. Variable distribution was evaluated by Kolmogorov Smirnov test. Categorical variables are presented as n (%), normally distributed variables as mean ± standard deviation (SD) and non-normally distributed variables as median with interquartile range (IQR). Skewed variables were log-transformed where appropriate. We handled remaining missing data for key variables using multiple imputation of variables with less than 10% missing data. Data of the following variables were imputed using multiple imputation by chained equations with five imputations: total alkaline phosphatase, calcium, phosphate, cold ischemia time, warm ischemia time, number of human leucocyte antigen (HLA) mismatches, body mass index (BMI), donor status, presence of diabetes, use of cinacalcet, and use of vitamin D. Results from analyses on each imputed data set were then pooled according to Rubin's rules [23].

To analyze whether pre-transplant plasma PTH, ALP, calcium and phosphate were independently associated with DGF, DCGF and all-cause mortality, we performed logistic and Cox-proportional regression analyses, respectively. Plasma levels were analyzed as categorical variables and as (log-transformed) continuous variables. The proportional hazard assumption was tested using statistical tests and graphical diagnostics based on the scaled Schoenfeld residuals. For PTH, patients were clustered in three groups according to the KDIGO guidelines-recommended thresholds: ≤2 times (≤150 pg/mL), >2 and <9 times (>150 and <771 pg/mL), or ≥9 times (≥771 pg/mL) of the upper limit of normal for the assay [24], using the middle range as reference. For ALP, calcium and phosphate, patients were analyzed in quartiles. Since most patients had normal or elevated levels of ALP at time of transplantation, we defined the lowest quartile as reference category for this parameter. For calcium and phosphate, we used the second quartile as reference category, since both very high and very low levels may occur at transplantation and both might be associated with adverse outcomes. We performed multivariate Cox regression analyses, cumulatively adjusted for age and sex (Model 1), and further variables previously associated with outcomes after kidney transplantation and bone mineral metabolism such as primary cause of kidney failure, primary CMV infection, acute allograft rejection, dialysis vintage, preemptive transplant, number of HLA mismatches, donor age and sex, living donor status, cold and warm ischemia time, history of diabetes, body mass index, serum calcium, phosphate and albumin at transplantation time, cinacalcet, vitamin D use, history of parathyroidectomy and decade of transplantation (Model 2; **Supplementary Figure S2**). The associations of pre-transplant PTH levels with DGF, DCGF and mortality were further investigated using restricted cubic splines using fully adjusted models. We also evaluated whether pre-transplant plasma PTH and ALP contributed to prediction of mortality risk using ROC-curve analysis with determination of the area under the ROC-curve (AUC). Finally, we studied the relationship between PTH at 1 year post-transplantation and DCGF or

TABLE 1 | Baseline characteristics of the cohort.

Baseline characteristics	Total *n* = 1,576	PTH ≤150 pg/mL[a] n = 496	PTH >150 < 771 pg/mL[b] n = 959	PTH ≥771 pg/mL[c] n = 121
Age at time of kidney transplantation, years	51.6 ± 14.0	51.7 ± 13.6	51.9 ± 14.2	47.8 ± 13.9
Sex (male), *n* (%)	903 (57.3)	271 (54.6)	556 (58.0)	76 (62.8)
Decade of transplantation				
1980–1989, *n* (%)	29 (1.8)	20 (4.0)	8 (0.8)	1 (0.8)
1990–1999, *n* (%)	87 (5.5)	53 (10.7)	30 (3.1)	4 (3.3)
2000–2009, *n* (%)	411 (26.1)	130 (26.2)	233 (24.9)	48 (39.7)
2010–2019, *n* (%)	1,049 (66.6)	293 (59.1)	688 (71.7)	68 (56.2)
BMI, kg/m^2	26.2 ± 10.2	26.6 ± 4.7	27.1 ± 4.6	27.4 ± 5.0
Primary kidney disease				
Glomerulonephritis, *n* (%)	443 (28.1)	149 (30.0)	266 (27.7)	28 (23.1)
Interstitial nephritis, *n* (%)	264 (16.8)	67 (13.5)	173 (18.0)	14 (11.6)
Cystic kidney disease, *n* (%)	211 (13.4)	76 (15.3)	121 (12.6)	69 (57.0)
Diabetes Mellitus, *n* (%)	72 (4.6)	29 (5.8)	40 (4.2)	3 (2.5)
Renal vascular disease, excluding vasculitis, n (%)	82 (5.2)	32 (6.5)	48 (5.0)	2 (1.7)
Other congenital/hereditary kidney disease, *n* (%)	110 (7.0)	26 (5.2)	69 (7.2)	15 (12.4)
Other multisystem diseases, *n* (%)	78 (4.9)	24 (4.8)	50 (5.2)	4 (3.3)
Other, *n* (%)	59 (3.7)	15 (3.0)	33 (3.4)	11 (9.1)
Unknown, *n* (%)	257 (16.3)	78 (15.7)	159 (16.6)	20 (16.5)
Medication use				
Cinacalcet, *n* (%)	198 (12.6)	32 (6.4)	123 (12.8)	43 (35.5)
Vitamin D, *n* (%)	909 (57.7)	238 (48.0)	595 (62.0)	76 (62.8)
Antihypertensives, *n* (%)	1,270 (80.6)	373 (75.2)	798 (83.2)	99 (81.8)
Statins, *n* (%)	427 (27.1)	117 (23.6)	281 (29.3)	29 (24.0)
Laboratory parameters				
PTH, pg/mL	231 (122–425)	85 (49–117)	311 (215–451)	942 (838–1,236)
Calcium, mg/dL	9.6 ± 0.8	9.7 ± 0.8	9.5 ± 0.7	9.6 ± 0.8
Phosphate, mg/dL	4.8 ± 1.5	4.6 ± 1.4	4.9 ± 1.5	5.3 ± 1.4
Total alkaline phosphatase, U/L	100 (80–140)	69 (55–89)	79 (62–102)	111 (83–158)
Albumin, g/dL	4.3 ± 0.5	4.3 ± 0.5	4.3 ± 0.4	4.3 ± 0.4
Transplantation data				
Pre-emptive transplant, *n* (%)	535 (33.9)	154 (31.0)	345 (36.0)	36 (29.8)
Dialysis vintage, months	18.0 (0–40.0)	18.0 (0–38.0)	16.0 (0–39.0)	34.0 (0–63.0)
Living donor, *n* (%)	785 (49.8)	243 (49.0)	498 (51.9)	44 (36.4)
Donor age, years	51.2 ± 13.5	50.0 ± 14.4	52.5 ± 13.0	48.0 ± 13.3
Donor sex (male), *n* (%)	808 (51.2)	252 (50.8)	494 (51.5)	62 (51.2)
Number of HLA mismatches (A/B/DR)	2.0 (1.0–3.0)	3.0 (2.0–4.0)	3.0 (2.0–4.0)	3.0 (2.0–4.0)
Cold ischemia time, hours	5.0 (2.0–14.0)	7.7 (2.6–15.3)	3.7 (2.6–13.9)	11.8 (2.9–16.3)
Second warm ischemia time, minutes	41.4 ± 12.0	41.2 ± 11.9	41.5 ± 12.2	40.6 ± 10.5
Acute rejection, *n* (%)	256 (16.2)	77 (15.5)	163 (17.0)	16 (13.2)
CMV infection, *n* (%)	687 (43.5)	224 (45.2)	415 (43.3)	48 (39.7)

Categories correspond to <2x[a], 2–9x[b], and >9x[c] upper limit of normal for the assay.
Data are presented as mean ± standard deviation (SD), median (IQR) or number (%). Abbreviations: BMI, body mass index; PTH, parathyroid hormone; HLA, human leukocyte antigen; CMV, cytomegalovirus.

mortality, both in continuous analysis and using quartiles (fully adjusted models as described above). In these analyses, patients who developed graft loss or died within the first year post-transplant were excluded.

Potential effect modification for the association between pre-transplant PTH and outcomes was explored using multiplicative interaction terms followed by prespecified subgroup analyses according to age, sex, use of vitamin D, use of cinacalcet, preemptive kidney transplant, dialysis vintage, donor status (living or deceased), serum calcium, phosphate and ALP. The *p*-values of interaction terms were considered significant when <0.05.

Finally, we performed sensitivity analyses for the DGF analysis, restricted to patients who received from a postmortal donor (as DGF is much more common after postmortal donor kidney transplantation), and for the mortality analyses after exclusion of individuals who died within the first year post-transplant, and restricted to patients transplanted after 2010.

RESULTS

Baseline Characteristics

A total of 1,576 kidney transplant recipients (KTRs) (age 51.6 ± 14.0 years, 57.3% male) were included in the primary analyses. Baseline patient and transplant characteristics are presented in **Table 1**. In brief, donor age was 51.2 ± 13.5 years, 785 (49.8%) patients received a graft from a living donor, median (IQR)

TABLE 2 | Association of pre-transplant PTH plasma levels with risk of DGF, DCGF and all-cause mortality.

Pre-transplant plasma PTH (pg/mL)	Events	Model 1: Adjusted for age + sex		Model 2: Fully adjusted	
		HR (95% CI)	*p*	HR (95% CI)	*p*
DGF					
Pre-KTx PTH, per doubling	**278/1,576**	1.23 (1.07–1.42)	<0.01	1.06 (0.90–1.25)	0.48
Pre-KTx PTH (pg/mL), groups					
PTH ≤150 pg/mL[a]	69/496	1.40 (1.03–1.89)	0.09 (p-trend)	0.82 (0.50–1.35)	0.35 (p-trend)
PTH >150 < 771 pg/mL	189/959	Reference		Reference	
PTH ≥771 pg/mL[b]	20/121	1.19 (0.69–2.05)		0.56 (0.24–1.32)	
DCGF					
Pre-KTx PTH, per doubling	**150/1,509**	0.98 (0.89–1.08)	0.68	0.98 (0.87–1.13)	0.86
Pre-KTx PTH (pg/mL), groups					
PTH ≤150 pg/mL[a]	56/475	0.98 (0.70–1.39)	0.78 (p-trend)	1.03 (0.62–1.67)	0.98 (p-trend)
PTH >150 < 771 pg/mL	83/921	Reference		Reference	
PTH ≥771 pg/mL[b]	15/113	0.80 (0.43–1.50)		1.01 (0.45–2.25)	
All-cause mortality					
Pre-KTx PTH, per doubling	**432/1,509**	1.06 (0.99–1.12)	0.17	1.02 (0.93–1.11)	0.66
Pre-KTx PTH (pg/mL), groups					
PTH ≤150 pg/mL[a]	157/485	0.99 (0.66–1.51)	0.14 (p-trend)	0.84 (0.63–1.12)	0.10 (p-trend)
PTH >150 < 771 pg/mL	248/921	Reference		Reference	
PTH ≥771 pg/mL[b]	27/113	1.22 (0.82–1.81)		0.56 (0.31–1.03)	

[a]*2X of upper limit of normal for assay.*

[b]*9X of upper limit of normal for assay.*

Model 1–adjusted for age, sex; Model 2–Model 1 + Primary kidney disease, cytomegalovirus (CMV) infection, acute allograft rejection, dialysis vintage, preemptive transplant, number of HLA, mismatches, donor age and sex, living donor status, cold and warm ischemia time, history of diabetes, body mass index, serum calcium, phosphate, total alkaline phosphatase and albumin at time of transplantation, cinacalcet and vitamin D use, history of parathyroidectomy and decade of transplantation. Abbreviations: DGF, delayed graft function; DCGF, death censored graft failure; PTH, parathyroid hormone; KTx, kidney transplantation; HR, hazard ratio; CI, confidence interval.

dialysis vintage was 18.0 (0–40.0) months, and 535 (33.9%) patients underwent a pre-emptive transplantation. Median (IQR) pre-transplant plasma PTH concentration was 231 (122–425) pg/mL, with 121 (7.7%) of patients presenting with plasma PTH ≥771 pg/mL (median 942 [838–1,236]pg/mL). Calcimimetics were used at time of transplant by 198 (12.6%) patients, and vitamin D analogs by 58% of patients. There were few missing data points (**Supplementary Table S2**).

Pre-Transplant Plasma PTH Levels and Post-transplant Outcomes

As shown in **Table 2**, 278 (17.6%) patients developed DGF. Upon fully adjusted logistic regression analysis with pre-transplant PTH as a continuous variable, no significant association was found with DGF. When analyzing patients in three groups (≤2 times, >2 and <9 times, ≥9 times the upper limit of normal for the assay of plasma PTH, corresponding with ≤150 pg/mL, >150 and <771 pg/mL, and ≥771 pg/mL, respectively), patients with pre-transplant PTH >771 pg/mL had a risk of DGF that was comparable to the reference group (**Table 2**). Interaction analysis revealed significant effect modification by sex (P-interaction = 0.03), as shown in **Figure 1**. The incidence of DGF was similar among men (18.6%) and women (16.3%, p = 0.15 vs. men). The fully adjusted association between plasma PTH and DGF was significant among women (HR 1.37 [95% CI 1.05–1.78], p = 0.02), but not among men. In a sensitivity analysis restricted to women who received a graft from a postmortal donor, the association between low PTH levels and DGF did not persist (HR 1.14 [95% CI 0.89–1.46], p = 0.30).

During median follow-up of 5.0 (range 0.2–29.5) years, 150 (9.9%) patients developed DCGF. In fully adjusted Cox regression analyses, pre-transplant plasma PTH levels were not associated with DCGF (**Figure 2A**). As shown in **Table 2**, patients with pre-transplant PTH ≤150 pg/mL or PTH >771 pg/mL had a risk that was comparable to the reference group (HR 0.98 [95% CI 0.87–1.67], p = 0.85; HR 1.01 [95% CI 0.45–2.25], p = 0.59, respectively). No significant effect modification was observed (**Figure 3**). Sensitivity analyses after exclusion of 39 individuals who developed DCGF within the first year after transplantation (N = 111, fully adjusted HR 0.94 [95% CI 0.83–1.08], p = 0.45) yielded similar results. At 1 year post-transplantation, the median PTH level was 110.5 (72.4–168.3) pg/mL. A higher PTH level at 1 year after transplantation was associated with an increased risk of DCGF (fully adjusted HR 1.31 [95% CI 1.03–1.69], p = 0.03) in continuous analysis (**Supplementary Table S3**). Patients in the highest quartile of PTH levels at 1 year post-transplant also had an increased risk of DCGF when compared with the lowest quartile (HR 2.64 [95% CI 1.21–5.80], p = 0.02).

During median follow-up of 5.2 (range 0.2–29.5) years, 432 (28.6%) patients died. In fully adjusted Cox regression analyses, pre-transplant plasma PTH levels were not associated with all-cause mortality (**Table 2**; **Figure 2B**). There was no significant effect modification in interaction analyses (**Supplementary Figure S3**). Sensitivity analyses after exclusion of 30 individuals who died within the first year post-transplant (N = 402, fully adjusted HR 1.03 [95% CI 0.95–1.12], p = 0.50), restricted to patients who were transplanted after 2010 (N = 197, HR 1.11 [95% CI 0.97–1.27], p = 0.12), or after exclusion of preemptive transplantation (N = 94, HR 0.99 [95% CI 0.82–1.19],

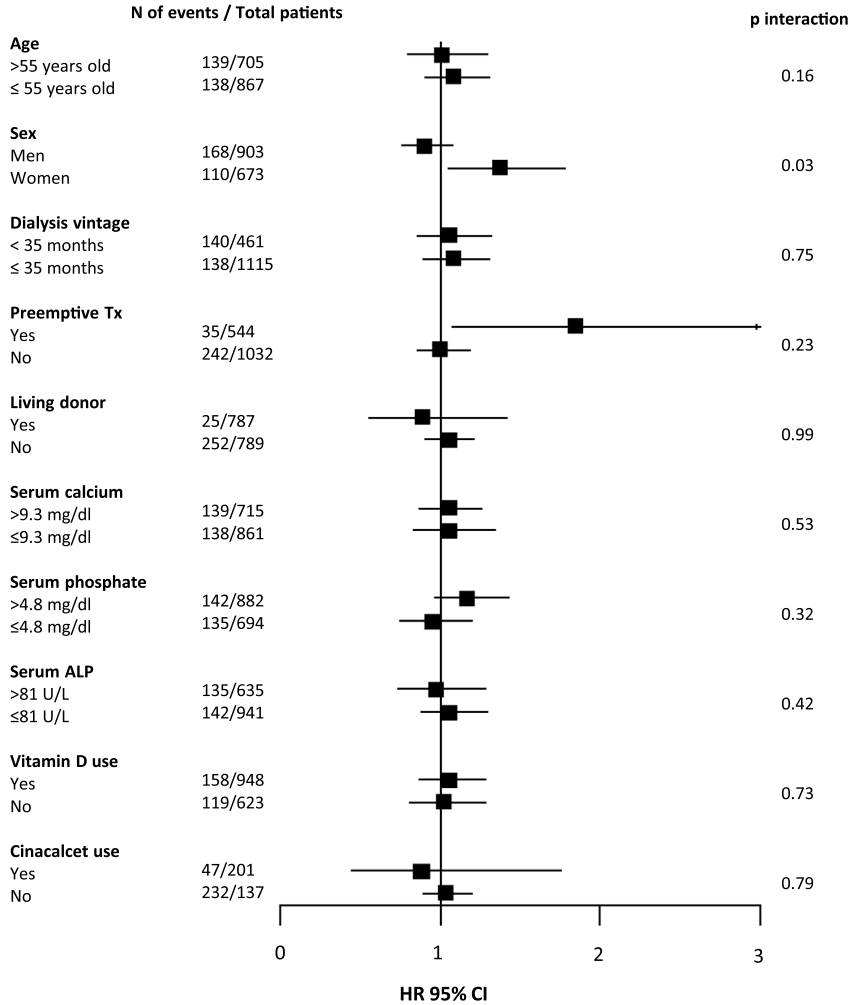


FIGURE 1 | Forest plot showing associations between pre-transplant plasma PTH level (per doubling) and delayed graft function (DGF) according to parameters at time of transplantation. In total, 278 (16.7%) kidney transplant recipients developed DGF. Abbreviations: N, number; Tx, transplant; ALP, alkaline phosphatase; HR, hazard ratio.

p = 0.91) yielded similar results. An additional analysis in a limited number of patients with available PTH data from 6 to 12 months prior to transplantation (N = 187) showed no significant association with all-cause mortality (HR 0.78 [95% CI 0.55–1.16], p = 0.23). Finally, PTH at 1 year after transplantation showed a trend towards association with all-cause mortality (fully adjusted HR 1.14 [95% CI 0.99–1.32], p = 0.05) upon continuous analysis; patients in the highest PTH quartile (at 1 year post-transplant) had a higher risk of all-cause mortality than those in the lowest quartile (HR 2.64 [95% CI 2.20–5.81], p = 0.02).

Pre-Transplant ALP, Calcium, and Phosphate and Post-transplant Outcomes

Subsequently, we analyzed the associations of pre-transplant serum ALP levels with risk of DGF, DCGF and all-cause mortality. There were no significant associations between serum ALP and risk of DGF, or DCGF (**Supplementary Table S4**). In age- and sex-adjusted analyses, higher serum ALP was associated with an increased risk of all-cause mortality, but this association lost significance upon multivariable adjustment. Compared with patients in the first quartile of pre-transplant serum ALP (52.0 [46.0–57.0]U/L), patients in fourth quartile with median pre-transplant serum ALP of 126.0 [110.8–158.0]U/L had a higher risk of all-cause mortality in fully adjusted model (HR 1.27 [95% CI 1.00–1.87]). There was no effect modification by pre-transplant dialysis status (preemptive or not) in interaction analysis (HR 1.30 [95% CI 0.72–2.32], p = 0.39). ALP, either alone or combined with PTH, did not change risk prediction for mortality when added to a model with established risk factors (**Table 3**). Plasma calcium was not significantly associated with the risk of DGF, DCGF, or all-cause mortality (**Supplementary Table S5**). Compared with patients in the second quartile of pre-transplant serum phosphate (4.2 [3.8–4.7]mg/dL), those in

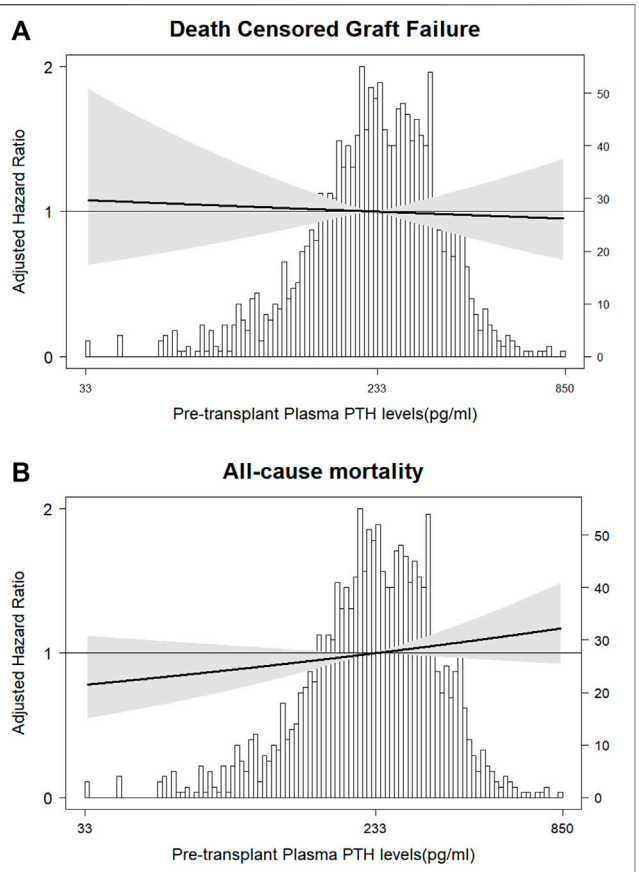


FIGURE 2 | Association of pre-transplant plasma PTH with risk of **(A)** DCGF and **(B)** all-cause mortality. The solid lines represent the fully adjusted hazard ratios (HRs) for DCGF (Cox regression Model 2) and all-cause mortality (Cox regression Model 2). The grey areas represent the 95% confidence intervals of the HRs.

the fourth quartile with median pre-transplant serum phosphate of 6.5 [5.7–12.9]mg/dL had an increased risk of DGF in the fully adjusted model 2.21 [95% CI 1.21–4.03, $p < 0.01$] (**Supplementary Table S6**). Interaction analysis revealed significant effect modification by donor status (P-interaction<0.001). The fully adjusted association between plasma phosphate and DGF was significant among recipients of post-mortal draft (HR 1.5 [95% CI 1.03–2.33], $p = 0.03$), but not among recipients from a living donor. Plasma phosphate at transplantation was not associated with DCGF or mortality.

Post-Transplant Course of Plasma PTH, Calcium and Phosphate

From 121 patients with severe hyperparathyroidism before transplantation, only 12 (10%) remained with elevated PTH (≥771 pg/mL) at 1 year post-transplant (**Figure 4A**). At baseline, almost one-third of patients had a calcium value outside the reference (13% hypocalcemia and 12% hypercalcemia). At 1 year after transplantation, 4.2% of patients presented with hypocalcemia, while 14.4% presented with hypercalcemia (**Figure 4B**). At time of transplantation more than half of patients had hyperphosphatemia (873 [55.6%] patients). At 1 year after transplantation, the prevalence of hyperphosphatemia had decreased to 3%. On the other hand, hypophosphatemia had a prevalence of 2% at baseline and of 10% at 1 year after transplantation (**Figure 4C**).

DISCUSSION

In this cohort of 1,576 primary stable kidney transplant recipients, we observed no associations between pre-transplant serum PTH levels and risk of DCGF, DGF and all-cause mortality in the primary analyses. Further, one-year post-transplant PTH levels were associated with DCGF and all-cause mortality. Interestingly, pre-transplant serum ALP levels higher than 126.0 (110.8–158.0) U/L were associated with higher risk of all-cause mortality, while serum phosphate levels higher than 6.5 (5.7–12.9) mg/dL were associated with higher risk of DGF; serum calcium levels at transplantation were not associated with post-transplant outcomes.

Previous studies regarding the potential risk of (severe) hyperparathyroidism at time of transplantation have shown conflicting results. In line with our findings, a previous large study found no associations with graft failure or mortality [9]. While this study only included patients that had been on chronic dialysis before transplantation, our study extends their findings by also including pre-emptive transplantations. A prior study from the same group also did not find a significant relationship between PTH and allograft loss and death [25]. In contrast, another study with a smaller sample size did find that a higher pre-transplant plasma PTH level was associated with a higher risk of death-censored graft failure (DCGF), but not with DGF or premature mortality [26]. Our finding that PTH at 1 year post-transplant was associated with DCGF and mortality is in line with prior studies [27–29], underscoring the importance of closely monitoring PTH levels after kidney transplantation so that patients with persistent or new-onset HPT post-transplant can be treated appropriately. Furthermore, it is important to consider other outcomes beyond graft and patient outcomes. While, to the best of our knowledge, no prior studies addressed the association between PTH at transplantation and fractures after kidney transplantation, on the other hand, post-transplant hyperparathyroidism has been associated with an increased risk of fractures [30], and correction of hyperparathyroidism by parathyroidectomy improved bone mineral density (BMD) [31]. Unfortunately, we could not address these associations in the current study as data on fractures or BMD were unavailable.

Interestingly, we found a significant association between higher PTH levels and DGF in women, but not in men. It has been described in experimental studies that female mice display greater tolerance for ischemia-reperfusion injury in multiple organs, including the kidney, and that estrogen may play a

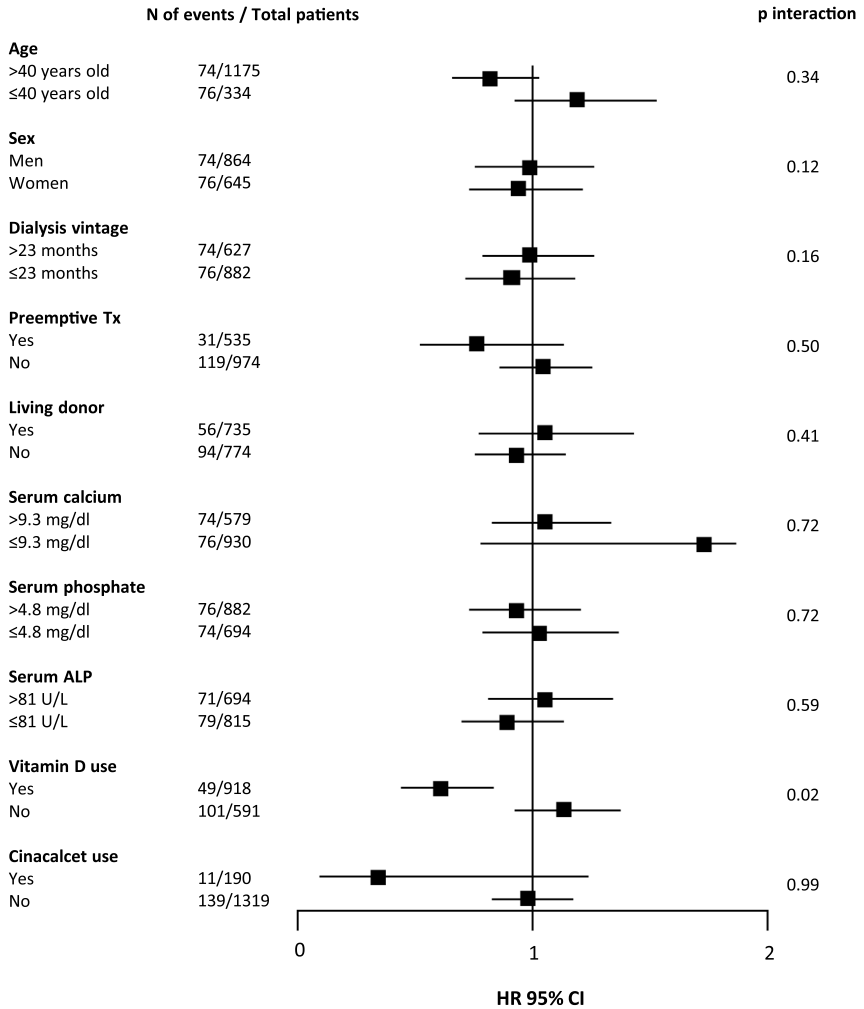


FIGURE 3 | Forest plot showing associations between pre-transplant plasma PTH levels (per doubling) and death censored graft function (DCGF) according to parameters at time of transplantation. In total, 150 (9.9%) kidney transplant recipients developed DCGF. Abbreviations: N, number; Tx, transplant; ALP, alkaline phosphatase; HR, hazard ratio.

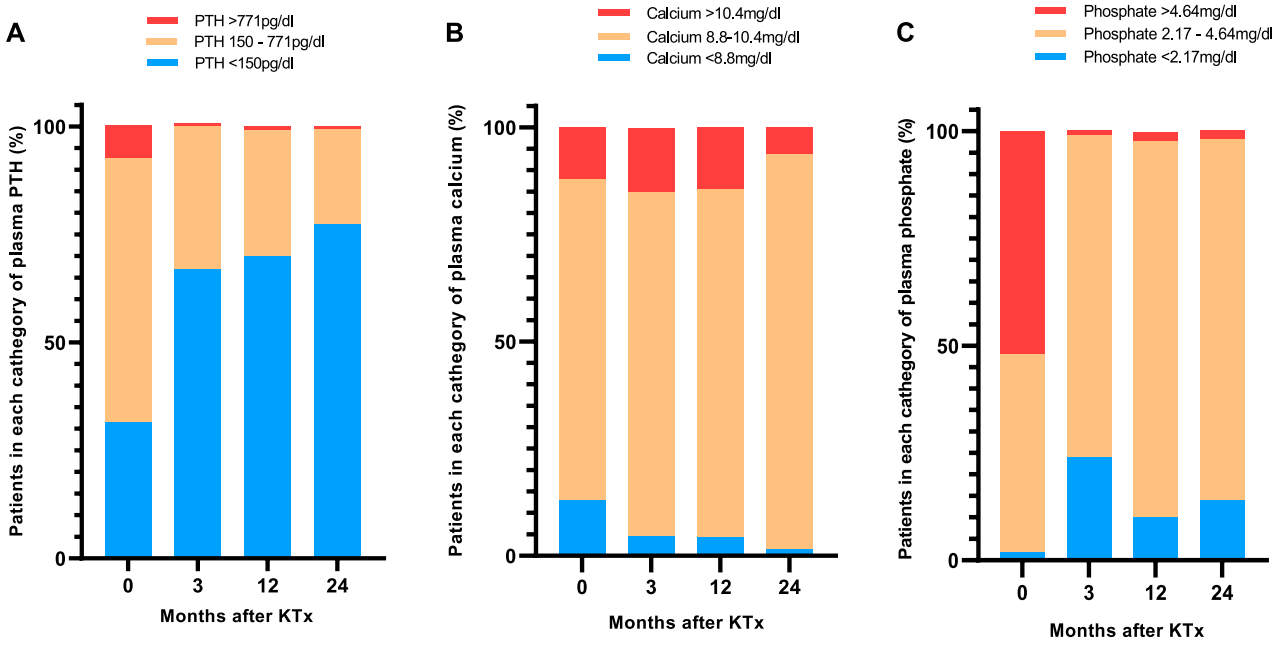
TABLE 3 | Receiver operating characteristic (ROC) curve analysis for all-cause mortality.

	AUC	SE	95% CI	*p*-value (for change)[a]
Model 1	0.752	0.015	0.723–0.780	—
Model 2	0.752	0.015	0.723–0.780	0.61
Model 3	0.763	0.015	0.732–0.793	0.31
Model 4	0.763	0.015	0.733–0.793	0.45

AUC, area under curve; SE, standardized error; CI, confidence interval.
[a]*Versus Model 1.*
Model 1: recipient age, corrected serum calcium, serum phosphate, serum albumin, preemptive transplantation, donor age, total cold ischemia, vitamin D use. Model 2: Model 1 + plasma PTH, at transplantation time. Model 3: Model 1 + serum ALP, at transplantation time. Model 4: Model 1 + plasma PTH, and ALP, at transplantation time.

role in this protective mechanism [32–34]. In humans, a large cohort study suggested that this sex-dependent response to injury may have clinical implications for DGF after kidney transplantation [35]. In that study, the risk of DGF was significantly higher in male recipients, even after adjusting for potential confounders [35]. In the present study, we only observed a small and non-significant difference in incidence of DGF in men vs. women (18.3% vs. 16.1%, $p = 0.15$). At the same time, the apparent sex-specific association of PTH with DGF did not translate into a sex-specific (or overall) association with DCGF, and it lost significance in a sensitivity analysis restricted to patients with a postmortal donor. Therefore, our results on a potential sex-specific association between PTH and DGF require confirmation by an independent study, and the implications for clinical practice could well be limited.

Our study revealed an association between higher pre-transplant serum ALP and an increased risk of mortality after kidney transplantation. This observation is in line with two prior studies, one from the United States [9] and one from Korea [11]. In the study of Molnar et al. [9], it was suggested that the



Time (months)	0	3	12	24
PTx, n (%)	32 (2.2)	34 (2.2)	42 (2.7)	45 (2.9)
Patients with data				
Plasma PTH, n	1576	1045	1124	486
Serum calcium, n	1576	1496	1394	485
Serum phosphate, n	1576	1496	1281	485

FIGURE 4 | Distribution of proportion of patients with median plasma **(A)** PTH **(B)** calcium and **(C)** phosphate within, below or above the reference range for the first 24 months after kidney transplantation. Values are expressed as percentages. Abbreviations: PTH, parathyroid hormone; PTx, parathyroidectomy.

association between ALP and mortality could be driven by high-bone turnover during the dialysis period, which may influence mortality risk after transplantation. However, in our study, more than one-third of the patients received a transplant before requiring dialysis, and we found no effect modification of the association between ALP and mortality by pre-transplant dialysis status (HR 0.94 [95% CI 0.87–1.01], p = 0.08; data not shown in tables).

Whether pre-transplant plasma calcium is associated with adverse post-transplant outcomes is controversial. In the present study, we found no association between pre-transplant plasma calcium and DGF, DCGF or all-cause mortality. These results are in line with one previous study [36], while another study did show an independent association between serum calcium and DGF [7]. Interestingly, Molnar et al [9] found that high pre-transplant serum calcium levels (>9.5 mg/dL) were associated with a lower risk of graft loss, and hypothesized that this protective effect could be related to the vitamin D use. However, interaction analysis in our study did not reveal any effect modification by vitamin D use for the association between calcium and DCGF, which in itself also did not reach statistical significance (HR 0.98 [95% CI 0.33–2.90], p = 0.98).

Our findings show that a higher plasma phosphate level was associated with an increased risk of DGF. Although this result is in contrast with two prior studies that had a null outcome [37, 38], hyperphosphatemia could increase the risk of DGF through tubular deposition of calcium-phosphate crystals, leading to tubular obstruction and subsequent tubular injury, inflammation, and endothelial cell damage [39].

In our cohort, severe hyperparathyroidism at transplantation was present in only a small fraction of the population (7.7%). Furthermore, we found persistent hypercalcemia in 14.4% and hypophosphatemia in 10% of kidney transplant recipients at 12 months following transplantation. Although previous data reported the persistence of hyperparathyroidism ranging between 17% and 90% of transplanted patients, it is important to mention the use of different approaches to classify hyperparathyroidism [37–40]. Persistent hypercalcemia after kidney transplantation is relatively common with a prevalence ranging between 10% and 12% [10, 40, 41]. Although high levels of serum calcium could be the result of a persistent hyperparathyroidism, adynamic bone disease in combination with tubular reabsorption of calcium could be another cause of hypercalcemia after transplantation, and so may the use of

calcium or vitamin D supplements [42]. The occurrence of hypophosphatemia following kidney transplantation is well described in the literature since during the initial post-transplant period, the accumulated plasma levels of PTH and FGF-23, together with the restored renal excretory capacity, stimulate phosphate excretion [43, 44]. Clearly, the generally improved abnormalities in mineral metabolism may be partly driven by the fact that affected patients received treatment with calcimimetics or underwent parathyroidectomy [45].

Our study has several limitations and strengths. The observational nature of this study leaves the possibility of residual confounding. PTH measurements after 2006 were converted using an in-house established conversion formula, which could be considered a limitation even though adjustment for transplant era did not influence the results and a sensitivity analysis did not suggest that the change in assays affected our findings. The repeated measures could have led to selection bias since patients with abnormal values may have been more frequently tested; on the other hand, these patients were also at higher risk to die within the first 2 years after transplantation. The lack of data on fractures, bone density measurements or bone biopsies, which would have allowed us to investigate specific bone outcomes are another limitation. The small number of patients with very high PTH levels (>9x the upper limit of normal assay) can be also considered as a limitation, although it likely does reflect practice in our center similar to many centers elsewhere in the world. The population was predominantly Caucasian, which calls for prudence when extrapolating these results to different populations. On the other hand, strengths include the large sample size, good characterization allowing for adequate adjustment and sensitivity analyses, external validity [46], complete follow-up which was longer than previous studies [9, 26], and clinically relevant endpoints.

In conclusion, in this large contemporary cohort of kidney transplant recipients, we found no association between severe hyperparathyroidism at the time of transplantation and the risk of DCGF or all-cause mortality. The observation that higher PTH levels are associated with an increased risk of DGF in women, but not in men, requires further investigation. Our finding that PTH levels at 1 year post-transplant were associated with DCGF and mortality underscores the importance of closely monitoring patients after transplantation to provide adequate treatment for persistent or new-onset hyperparathyroidism. Overall, although further studies are needed to address the impact on bone outcomes, our findings do not support the requirement of a pre-transplant parathyroidectomy to improve graft or patient survival in transplant candidates with severe hyperparathyroidism.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available upon reasonable request.

ETHICS STATEMENT

The study protocol was approved by the Institutional Review Board of Groningen UMC (METc 2014/077)]. The study was performed under the Strengthening the Reporting of Observational Studies in Epidemiology guidelines [17], adheres to the local UMCG Biobank Regulations, and is in accordance with the WMA Declarations of Helsinki and Istanbul. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FR and MB designed the study; FR conducted data analyses and wrote the manuscript. WV and DK conducted the study and data collection. CS and AV conducted data analysis. RP and SK discussed and enriched the manuscript. IH, SB, and MD supervised and reviewed 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.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.11916/full#supplementary-material

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ORIGINAL RESEARCH
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Impact of Transplantation Timing on Renal Graft Survival Outcomes and Perioperative Complications

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Nighttime organ transplantation aims to decrease cold ischemia duration, yet conflicting data exists on its impact on graft function and perioperative complications. This multicenter TRANSPLANT'AFUF study including 2,854 patients, transplanted between 1 January 2011, and 31 December 2022, investigated nighttime kidney transplantation's impact (8:00 p.m.–8:00 a.m.) versus daytime (8:00 a.m.–8:00 p.m.) on surgical complications and graft survival. Overall, 2043 patients (71.6%) underwent daytime graft, while 811 (28.4%) underwent nighttime graft. No impact was observed of timing of graft surgery on graft survival with a median survival of 98 months and 132 months for daytime and nightime grafting, respectively ($p = 0.1749$). Moreover, no impact was observed on early surgical complications (Clavien I-II = 20.95% for DG and 20.10% for NG; Clavien III-IV-V = 15.42% for DG and 12.94% for NG; $p = 0.0889$) and late complications (>30 days) (Clavien I-II = 6.80% for DG and 5.67% for NG; Clavien III-IV-V = 12.78% for DG and 12.82% for NG; $p = 0.2444$). Noteworthy, we found a significant increase in Maastricht 3 donors' rates in nighttime transplantation (5.53% DG vs. 21.45% NG; $p < 0.0001$). In conclusion, nighttime kidney transplantation did not impact early/late surgical complications nor graft survival.

Keywords: renal transplantation, graft survival, nighttime, surgery, surgical complication

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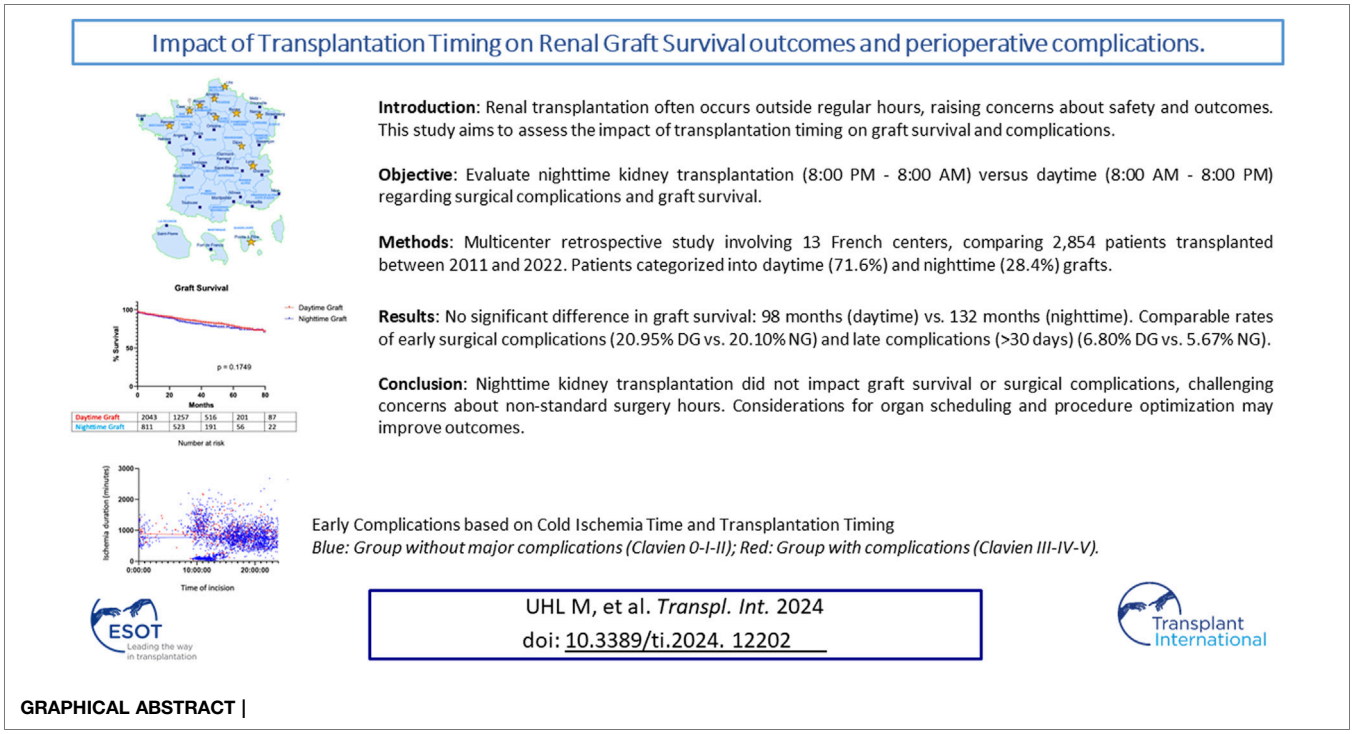
Abbreviations: BMI, Body Mass Index; CTIN, chronic tubulo-interstitial nephropathy; DCD, Deceased after Cardiac Death; DG, Daytime Graft; DGF, Delayed Graft Function; ESRD, end stage renal disease; LAT, Limitation of Active Therapies; LG, Lich-Gregoir; LRD, Living-Related Donor; M2, Maastricht Category 2; M3, Maastricht Category 3; NG, Nighttime Graft; PU, pyelo-ureteral.



GRAPHICAL ABSTRACT |

INTRODUCTION

Renal transplantation is an unplanned activity, heavily dependent on organ retrieval and the need to minimize cold ischemia time. Consequently, it is often performed outside of regular working hours. However, the safety of surgery during non-standard hours has raised concerns over the past several years, as highlighted in various studies [1], demonstrating increased morbidity and mortality among patients operated on at night [2–6], particularly attributed to practitioner fatigue [7].

Concerning renal transplantation, fewer than ten studies have been conducted in the last two decades to evaluate the impact of nighttime interventions on short- and long-term graft functionality, as well as early and late complications [8–14]. These studies were often outdated and do not account for recent transplantation data, including the use of perfusion machines, the rise of extended criteria kidneys, and Maastricht 3 donors.

The objective of this study was to evaluate the impact of nighttime renal transplantation on graft survival and early and late surgical complications.

MATERIALS AND METHODS

Study Design

As part of the TRANSPLANT'AFUF group led by the AFUF (Association Française des Urologues en Formation), a multicenter French retrospective database involving 13 centers was established. Inclusions were carried out successively from 2022 until 2011 for some centers. In all these healthcare facilities, kidney transplant surgeries are exclusively performed by urologists. Nevertheless, there is a lack of consensus regarding the operational protocols for managing renal transplantation.

Patients were divided into two groups based on the timing of the graft incision: daytime grafts (8:00 a.m.–8:00 p.m.) (DG) and nighttime grafts (8:00 p.m.–8:00 a.m.) (NG). 8:00 a.m. was chosen as the cut-off time because it corresponds to the change of anesthetists and OR nurses. All time points (including the moment of skin incision, the duration of the anastomosis, and the overall procedure duration) were electronically recorded during surgery and available on operative schedule. To perform a secondary analysis, a deep night graft (DNG) (11:00 p.m.–4:00) subgroup was also established.

Parameters and Outcome Measures

Evaluated parameters included donor, graft, and recipient characteristics, graft incision and closure times, and vascular anastomosis times (time between the beginning of the first anastomosis to the end of the last anastomosis). Cold ischemia time was calculated between organ retrieval clamp and transplantation. According to the "Agence de Biomédecine" protocol, all extended criteria and Maastricht 3 kidneys must be put on hypothermic Perfusion Machine. All Maastricht 3 protocols in France are made using a normothermic extracorporeal circulation between the cardiac arrest and the retrievals of the organs [15].

The primary outcome measure was graft survival, the graft was considered non-functional in case of patient death, return to dialysis, or removal of the graft. Secondary outcome measures

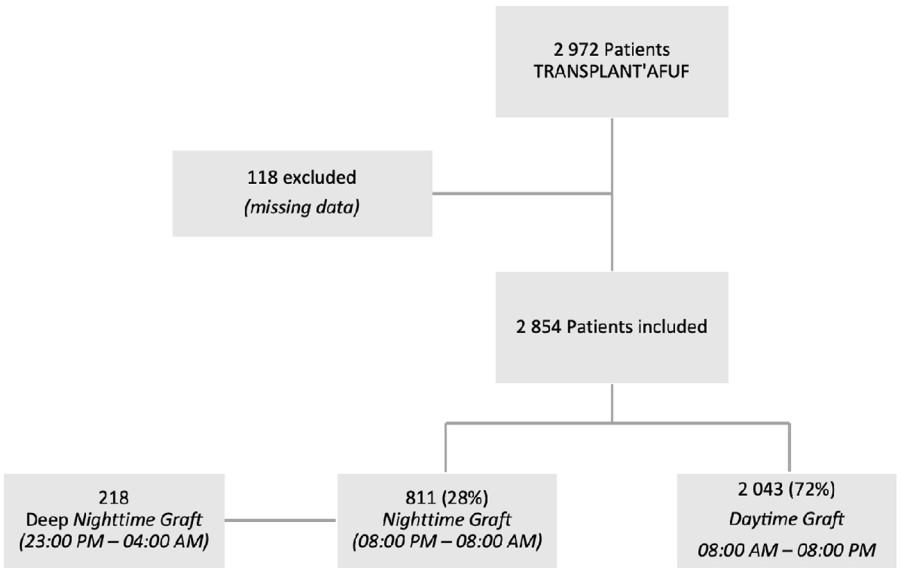


FIGURE 1 | Flow chart.

included graft function assessed by creatinine levels, delayed graft function (DGF, defined by the necessity of dialysis during the seven first day after transplantation), early complications (within the first postoperative month), and late complications assessed using the Clavien-Dindo classification. All these data were available on patient files.

We have distinguished between junior and senior surgeons for the analysis of complications. In our French centers, a senior surgeon has completed his or her post-internship formation.

Statistical Analyses

Statistical analyses were performed using SPSS software. Univariate comparison was made using Chi-squared test and Fisher's exact test for categorical variables. Continuous variables were compared using Student's t-test or Mann–Whitney U-test (when assumptions of Gaussian distribution were not met). Graft survival was estimated using Kaplan-Meier method. A significance level of $p < 0.05$ was considered for all statistical data.

Ethics

The study was a retrospective analysis and involved already available data on human participants and followed the 1964 Declaration of Helsinki and its later amendments. Data collection followed the French legislation concerning prospective non-interventional studies to evaluate routine care (Article Art.L1121-1-2 of French Public Health Code).

RESULTS

Population Description

Out of the 2,972 patients in the TRANSPLANT'AFUF database, 118 patients were excluded due to missing graft timing data, resulting in the inclusion of 2,854 patients between 01 January 2011 and 31 December 2022. Of these, 2,043 were daytime grafts (DG—8:00 a.m.–8:00 p.m.) and 811 were nighttime grafts (NG—8:00 p.m.–8:00 a.m.). 218 grafts were realized in deep night (DNG—11:00 p.m.–4:00 a.m.) (**Figure 1**).

Table 1 displays the characteristics of donors, showing significant differences in donor type with Maastricht 3 kidneys being more frequently transplanted at night (21.45% NG vs. 5.53% DG, $p < 0.0001$). Additionally, 20.80% of DG were from living donors. No differences were observed in preoperative creatinine, expanded criteria kidneys, or donor age. Regarding transplanted kidney characteristics (**Table 2**), NG kidneys were more often placed on machines (54.38% NG vs. 37.98% DG, $p < 0.0001$) and exhibited more arterial calcifications (27.37% NG vs. 22.47% DG, $p < 0.017$). However, no differences were found in the number of arteries or veins.

Regarding recipients, only preemptive transplantation was statistically significant (14.05% for DG vs. 6.29% for NG, $p < 0.0001$). There were no differences in terms of gender, age, BMI, cause of renal insufficiency, type of dialysis, residual diuresis, or the number of transplants. There was no difference between the mean follow up between DG and NG (30 months for DG; 28 months for NG; $p = 0.2064$) (**Table 3**).

Regarding surgery, there were more Lich-Gregoir (LG) ureteric anastomoses performed at night and more pyelo-ureteral (PU) anastomoses during the day (LG 78.56% for DG and 92.11% for NG; PU 14.93% for DG and 3.70% for NG; $p < 0.0001$). The duration of vascular anastomosis was significantly shorter during the day (50.5 min for DG vs. 55 min for NG, $p < 0.005$). The duration of cold ischemia was not statistically different between the two groups [777 min for DG

TABLE 1 | Donor characteristics.

	Daytime graft	Nighttime graft	*p-value*
Sex (Male/Female)	1,065/978	503/308	<**0.0001**[a]
Age (years)[b]	55 [45–56–67]	54 [47–56–65]	0.4862
Donor Type			<**0.0001**[a]
Deceased after Brain Death (DBD)	*1,492 (73.03%)*	*619 (76.33%)*	
Maastricht Category 3 (M3)	*113 (5.53%)*	*174 (21.45%)*	
Maastricht Category 2 (M2)	*9 (0.44%)*	*10 (1.23%)*	
Living-Related Donor (LRD)	*425 (20.80%)*	*0 (0%)*	
Expanded Criteria Donors	809 (39.60%)	330 (40.69%)	0.8548
Preoperative Creatinine (μmol/L)	75 (54–86)	74 (50–86)	0.4823

[a]*Statistically significant.*
[b]*Mean [first interquartile—median—third interquartile].*
Italic values means number of patients.

TABLE 2 | Renal graft characteristics.

	Daytime graft	Nighttime graft	*p-value*
Perfusion Machine	776 (37.98%)	441 (54.38%)	<**0.0001**[a]
Multiple Arteries (>1)	481 (23.54%)	211 (26.88%)	0.1164
Arterial Calcification	459 (22.47%)	211 (27.37%)	**0.0217**[a]
Multiple Veins (>1)	1915 (93.73%)	773 (95.31%)	0.4993

[a]*Statistically significant.*

(493–1,052) vs. 810 min for NG (570–1,005); p = 0.0541] (**Table 4**).

In a sub-group analysis, we found that, compared with DG, seniors realized significantly more DNG (40.72% DG vs. 49.08% DNG; p = 0.0172).

Primary Outcome Measure

Graft survival was observed, considering the graft non-functional in case of patient death, return to dialysis, or removal of the graft. There was no significant difference in graft survival between DG and NG. The time of 75% survival was 67.5 months for DG and 63.5 for NG [p = 0.1749; HR (day/night): 0.8695; 95% CI (0.7049; 1.073)] (**Figure 2**).

Secondary Outcome Measures

Creatinine levels at 1-year post-transplant did not significantly differ between the two groups [DG: 137 μmol/L (101–157); NG: 140 μmol/L (103–158); p = 0.3019].

Regarding DGF, there was no statistically significant difference observed between the daytime and nighttime groups [410 DGF for DG (20.21%); 173 DGF for NG (21.54%); p = 0.4276] (**Table 5**). The occurrence of DGF was found to be correlated with prolonged anastomosis timing, irrespective of whether it occurred during the daytime (no DGF 52 min vs. DGF 64 min; p < 0.0001) or nighttime (no DGF 55 min vs. DGF 63 min; p = 0.0024).

No significant difference was observed in surgical complications based on graft timing, either for early

TABLE 3 | Recipient characteristics.

	Daytime graft	Nighttime graft	*p-value*
Male	1,289 (63.09%)	525 (64.73%)	0.4112
Age (years)[b]	53 [42–54–65]	54 [44–55–65]	0.3618
BMI	25.48 (22.08–28.41)	25.74 (22.65–28.69)	0.1879
Cause of ESRD			0.3296
Glomerular	*737 (36.07%)*	*309 (38.10%)*	
Vascular	*227 (11.11%)*	*96 (11.84%)*	
CTIN	*159 (7.78%)*	*56 (6.91%)*	
Polycystic	*365 (17.87%)*	*120 (14.80%)*	
Uropathy	*166 (8.13%)*	*61 (7.52%)*	
Undetermined	*214 (10.47%)*	*90 (11.10%)*	
Other	*139 (6.80%)*	*67 (8.26%)*	
Preemptive Graft	287 (14.05%)	51 (6.29%)	<**0.0001**[a]
Dialysis Type	(*N* = 1,643)	(*N* = 731)	0.1608
Peritoneal	*199 (12.11%)*	*74 (10.12%)*	
Hemodialysis	*1,444 (87.89%)*	*657 (89.88%)*	
Residual Diuresis >50 mL/day	1,217 (59.57%)	488 (60.17%)	0.7314
≥3rd Transplant	31 (1.52%)	11 (1.36%)	0.6116
Mean follow up (months)[b]	30 [13–25–40]	28 [13–25–39]	0.2064

[a]*Statistically significant. BMI, body mass index; ESRD, end stage renal disease; CTIN, chronic tubulo-interstitial nephropathy.*
[b]*Mean [first interquartile–median–third interquartile].*
Italic values means number of patients.

TABLE 4 | Perioperative data.

	Daytime graft	Nighttime graft	*p-value*
Implantation Site Calcification	317 (15.52%)	112 (13.81%)	0.2783
Operation Duration (min)	180 (145–210)	178 (146–204)	0.1911
Bleeding (mL)	205 (50–300)	221 (50–300)	0.1391
Intraoperative Transfusion	98 (4.80%)	31 (3.82%)	0.3163
Senior Surgeon	829 (40.58%)	361 (44.51%)	0.0503
Urinary Anastomosis			**<0.0001**[a]
Lich Gregoir	*1,605 (78.56%)*	*747 (92.11%)*	
Pyeloureteral	*305 (14.93%)*	*30 (3.70%)*	
Leadbetter	*12 (0.59%)*	*30 (0.37%)*	
Other	*1 (0.05%)*	*0 (0%)*	
Vascular Anastomosis Duration (min)	50.5 (35–61)	55 (39–67)	**0.0097**[a]
Cold Ischemia Duration (min)	777 (493–1,052)	810 (570–1,005)	0.0541

[a]*Statistically significant.*
Italic values means number of patients.

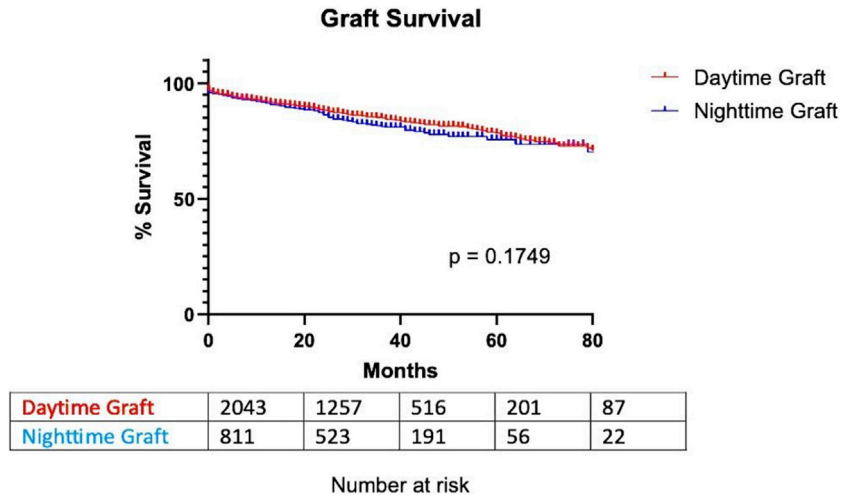


FIGURE 2 | Graft survival based on transplantation timing.

TABLE 5 | Postoperative data.

	Daytime graft	Nighttime graft	*p-value*
Delayed Graft Function	410 (20.21%)	173 (21.54%)	0.4276
Complication < 30 Days			0.0889
Clavien I-II	428 (20.95%)	163 (20.10%)	
Immediate postoperative transfusion	344 (16.84%)	134 (16.52%)	0.8388
Clavien III-IV-V	315 (15.42%)	105 (12.94%)	
Transplantectomy	75 (3.67%)	26 (3.21%)	
Thrombosis	80 (3.92%)	35 (4.32%)	
Surgical reintervention	241 (11.80%)	87 (10.73%)	
Urinoma	68 (3.33%)	23 (2.84%)	
Complication > 30 Days			0.2444
Clavien I-II	139 (6.80%)	46 (5.67%)	
Clavien III-IV-V	261 (12.78%)	104 (12.82%)	
Ureteric stenosis	69 (3.38%)	35 (4.32%)	0.2276

complications <30 days (Clavien I-II DG 20.95% vs. NG 20.10%; Clavien III-IV-V DG 15.42% vs. NG 12.94%; p = 0.0889) or late >30 days (Clavien I-II DG 6.80% vs. NG 5.67%; Clavien III-IV-V DG 12.78% vs. NG 12.82%; p = 0.2444). Looking in detail at early and late Clavien III complications (IIIa vs. IIIb), we also found no difference between DG and NG (<30 days: DG Clavien

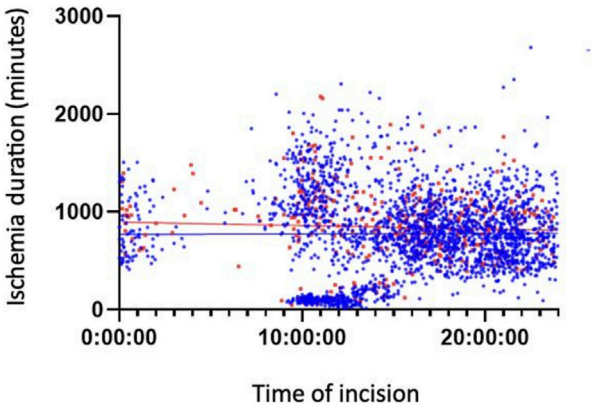


FIGURE 3 | Early Complications based on cold ischemia time and transplantation timing.

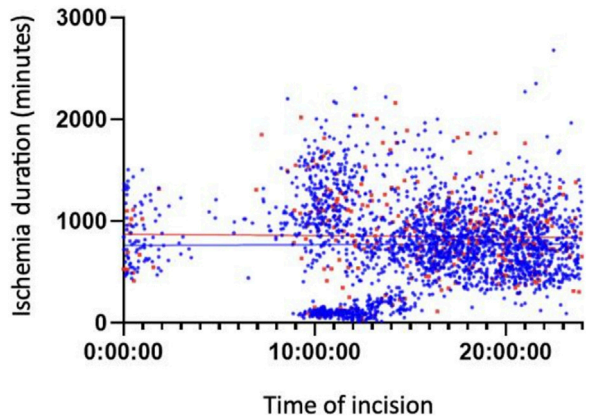


FIGURE 4 | Late Complications based on Cold ischemia time and transplantation timing.

IIIa 1.57%—Clavien IIIb 9.83% vs. NG Clavien IIIa 0.97%—Clavien IIIb 8.75%; $p = 0.4072$. >30 day: DG Clavien IIIa 1.81%—Clavien IIIb 9.15% vs. NG Clavien IIIa 0.97%—Clavien IIIb 9.73%; $p = 0.1217$) (**Table 5**).

There was also no difference in surgical complications: immediate postoperative transfusion, graft removal, thrombosis, surgical reintervention, urinoma or ureteric stenosis (**Table 5**). In subgroup analysis, no significant difference was observed in surgical complications Clavien III-V for early complication <30 days (16.06% DNG vs. 12.78% DG; $p = 0.1724$).

Figures 3, **4** show the association between surgical complications with cold ischemia time and transplantation timing, which revealed no difference between the two groups.

DISCUSSION

This French multicenter study is one of the most comprehensive analyses to date regarding the impact of transplantation timing on renal graft survival and early and late surgical complications. Organ transplantation outcomes are inconsistent, with liver grafts showing negative effects of nocturnal interventions [16], while cardiac and lung grafts remain unaffected [17]. Cold ischemia time significantly influences long-term renal graft survival [18–21]. However, surgical complications necessitating subsequent interventions have been linked to reduced graft survival [8, 22, 23], particularly vascular complications [24] and increased operative time [25]. Despite multiple retrospective analyses, these studies are often limited by small cohorts, leading to non-significant results [11–14]. Only three retrospective studies have yielded significant outcomes concerning complication rates and graft survival for nighttime kidney transplants.

Brunschot et al. [9] analyzed 4,519 kidney transplants performed between 2000 and 2013 in the Netherlands, of which 1,480 occurred at night. The results showed significantly lower technical failure rates for nighttime grafts (1%) compared to daytime grafts (2.6%). Another single-center study by Shaw TM et al. [10], involving 633 kidney transplants from 2000 to 2008, revealed increased urinary complications for grafts between 3:00 a.m. and 6:00 a.m., without a significant difference in 1-year graft survival. Fechner et al. [8] conducted a third study in 2008, comparing 260 daytime and nighttime kidney transplants between 1994 and 2004, demonstrating an elevated risk of delayed graft function recovery and vascular complications for nighttime grafts, without differences in cold ischemia time. In our study, the observed rate of surgical complications aligns with literature findings [26], showing no significant increase in surgical complications based on graft timing, despite a significant extension in vascular anastomosis time during the night.

Kidney transplants from Maastricht 3 donors were more frequently performed at night. This trend is largely attributed to the need to minimize cold ischemia time for expanded criteria grafts. However, no national protocol exists for the optimal timing of Limitation of Active Therapies (LAT) in France. The reduction of nighttime transplants could be considered by scheduling LAT for these donors earlier in the morning.

Additionally, the significant use of perfusion machines for nighttime grafts might be explained by the proportion of living-related donors (LRDs) during the day, who do not require perfusion machines, as well as the increased proportion of Maastricht 3 donors. The utilization of these machines could contribute to improved graft function [27, 28]. Similarly, the higher proportion of Maastricht 3 donors at night probably explains the gender difference, since these donors are mainly men [29].

Finally, **Figures 2**, **3** illustrate a cluster of morning grafts characterized by a cold ischemia window of 16–30 h, which could correspond to grafts rescheduled for the following morning to avoid procedures during the deep nighttime hours (12:00 p.m. to 08:00 a.m.). This observed behavior may be attributed to concerns regarding potential surgical complications and hesitancy within surgical teams. Our study indicates that, despite a somewhat slower pace, as evidenced by

prolonged anastomosis time, potentially linked to fatigue, the initial concerns may not be substantiated. It is established that, beyond a 6-h threshold, each additional hour of cold ischemia time does impact graft survival [18–21]. Unfortunately, the limitations of our retrospective study design and the extended follow-up duration hinder our ability to conclusively demonstrate the enduring effects of cold ischemia. Facilitating easier and more direct access to the operating room holds promise for enhancing long-term graft survival, all the while maintaining organizational efficiency and ensuring the quality of work performed by surgical teams.

In conclusion, this study demonstrates that nighttime transplantations do not result in delayed graft function recovery, despite an extended vascular anastomosis time. Furthermore, these nocturnal interventions do not lead to an increased risk of early or late surgical complications. However, it's important to note that most grafts are already scheduled for the early morning to avoid procedures during the deep nighttime period (12:00 p.m. to 08:00 a.m.), and this choice does not negatively impact graft functional recovery. However, our study could not conclude on the impact of this choice on very long-term graft survival. To reduce the number of early-night grafts, earlier LAT for Maastricht 3 donors could be considered. Additionally, the significant use of perfusion machines for nighttime grafts could contribute to the favorable graft functional recovery. We also believe that by prioritizing access to the operating room for kidney transplants, we could reduce the number of transplants delayed until the morning, and thus their cold ischemia.

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 study was a retrospective analysis and involved already available data on human participants and followed the 1964 Declaration of Helsinki and its later amendments. Data collection followed the French legislation concerning prospective non-interventional studies to evaluate routine care (Article Art.L1121-1-2 of French Public Health Code).

AUTHOR CONTRIBUTIONS

MU: redactor. PD: senior of MU for the article, head of department. TW: contributor of the database, statistics, corrections. ES: project leader of TRANSPLANT'AFUF. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

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BRIEF RESEARCH REPORT
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Aortobifemoral Bypass in Kidney Transplant Candidates: A Ten-Year Experience

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In patients with severe aorto-iliac calcifications, vascular reconstructions can be performed in order to allow kidney transplantation. The aim of this study was to analyze the outcomes of kidney transplant candidates who underwent an aortobifemoral bypass (ABFB) for aorto-iliac calcifications. A retrospective study including all kidney transplant candidates who underwent an ABFB between 2012 and 2022 was performed. Primary outcome was 30-day morbidity-mortality after ABFB. Secondary outcome was accessibility to kidney transplant waiting list. Twenty-two ABFBs were performed: 10 ABFBs in asymptomatic patients presenting severe aorto-iliac circumferential calcifications without hemodynamic consequences, and 12 ABFBs in symptomatic patients in whom aorto-iliac calcifications were responsible for claudication or critical limb threatening ischemia. Overall 30-day mortality was 0%. Overall 30-day morbidity was 22.7%: 1 femoral hematoma and 1 retroperitoneal hematoma requiring surgical drainage in the asymptomatic group, and 2 digestive ischemia requiring bowel resection and 1 femoral hematoma requiring surgical drainage in the symptomatic group. Among the 22 patients, 20 patients could access to kidney waiting list and 8 patients underwent a kidney transplantation, including 3 living-donor transplantations. Aorto-iliac revascularization can be an option to overcome severe calcifications contraindicating kidney transplantation.

Keywords: kidney transplantation, vascular calcification, vascular surgical procedures, blood vessel prosthesis, kidney

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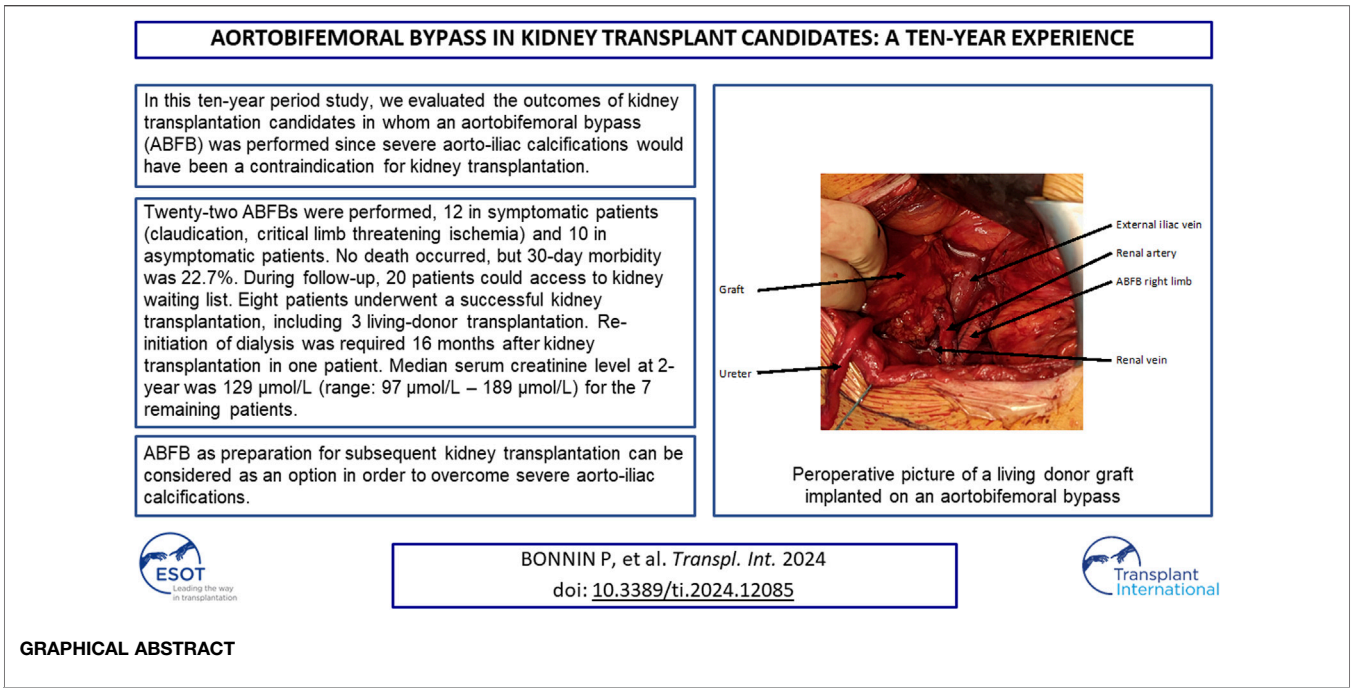
INTRODUCTION

Aorto-iliac vascular disease is frequently found during the work-up for kidney transplantation due to increasing age and comorbidities accompanying end-stage renal disease. Moreover, it is well known that a positive association between calcification and age as well as time on dialysis exists [1, 2]. Accordingly, the number of kidney transplantation candidates presenting with aorto-iliac calcifications is increasing, due to the ageing of the population and the increasing time on dialysis before transplantation given organ shortage.

Severe calcification can cause a hemodynamically significant stenosis which needs repair in case its location is in the inflow tract of the kidney graft. On another hand, also in case of non-stenotic calcification, kidney transplantation can be contraindicated if there is no soft artery left for the

Abbreviations: ABFB, aortobifemoral bypass.



GRAPHICAL ABSTRACT

clamping and arterial anastomosis. Accordingly, aorto-iliac calcifications are a common barrier to listing for kidney transplantation and among the patients on dialysis, around 33% of them are not on kidney transplant waiting list due to a vascular contraindication [3].

On another hand, kidney transplantation remains the best treatment modality for most patients with kidney failure to reduce all-cause mortality, but also regarding quality of life aspects and economic perspectives [4]. However, clear recommendations on the management of these patients with severe aorto-iliac calcifications are lacking. The Kidney Disease Improving Global Outcomes (KDIGO) guidelines state that aorto-iliac vascular disease is a relative contraindication for kidney transplantation but that selected patients can be considered for revascularization procedure to facilitate transplantation [5, 6].

Revascularization can include endovascular procedures such as iliac stenting, or perioperative iliac endarteriectomies in order to allow adequate clamping and arterial anastomosis. However, in some cases with severe and circumferential bilateral aorto-iliac calcification, a complete arterial reconstruction in order to prepare subsequent kidney transplantation is needed [7–9]. The aim of this study was to analyze the outcomes of kidney transplantation candidates who underwent aortobifemoral bypass (ABFB) for aorto-iliac calcifications over a 10-year period.

MATERIAL AND METHODS

Design of the Study

A retrospective review of a prospective database including kidney transplantation candidates who underwent an ABFB due to severe aorto-iliac calcifications between January 2012 and December 2022 was performed. The study was approved by the institutional review board. Severe aorto-iliac calcifications were defined as circumferential calcifications on both right and left iliac arteries not allowing arterial clamping and subsequent arterial anastomosis. Indications for ABFBs were discussed in multidisciplinary meetings including nephrologists, radiologists and vascular surgeons.

Patient's Data

The following preoperative parameters were recorded: demographic data (age, sex), cardiovascular risk factors (hypertension, diabetes mellitus, dyslipidemia, smoking, obesity), comorbidities (cardiac or pulmonary comorbidities), time on dialysis, and clinical presentation (asymptomatic, or symptomatic: claudication or critical limb threatening ischemia).

Surgical Procedures

Revascularization data were recorded: operative time, blood loss, and need for transfusion. Length of intensive care unit was also recorded.

Outcomes

Primary outcomes was defined as 30-day mortality and morbidity. Morbidity was defined as any digestive, ischemic or hemorrhagic complications.

Secondary outcomes was accessibility to kidney transplant waiting list. Post-transplant follow-up was recorded for patients who underwent kidney transplantation.

Statistical Analysis

Not normally distributed data are presented with median value with data range (minimum to maximum).

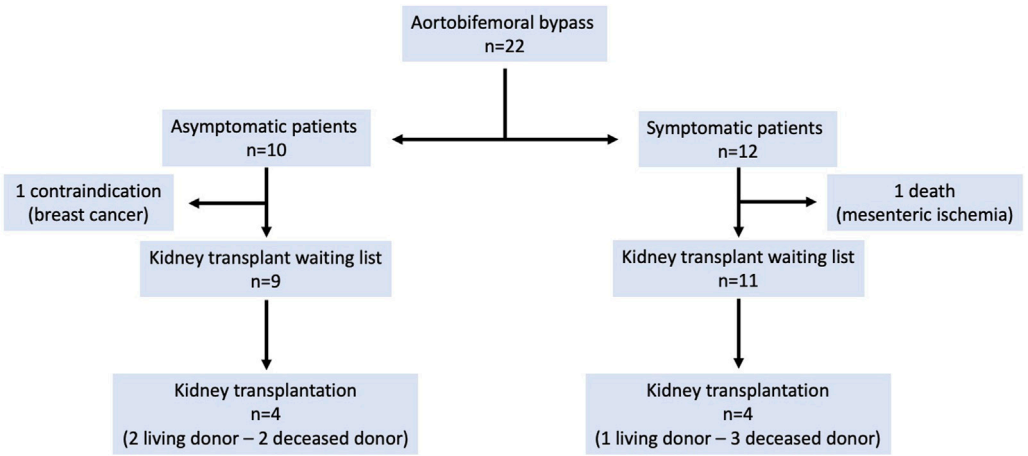


FIGURE 1 | Outcomes of patients.

TABLE 1 | Kidney transplantated patients.

Pedant	Age	Group	Time between ABFB and kidney transplantation	Donor	Donor age	Graft characteristics	Operative time (minutes)	Warm ischamia (minutes)	Serum aeatinine level at discharge (1 mol/L)	Serum creatinine level at 2-year (lnol/L)
1	63	Asymptomatic	10 months	Deceased donor	78	1 artery, 1 vein, 1 ureter	150	20	181	173
2	73	Asymptomatic	45 months	Dece donorased	83	1 artery, 1 vein, 1 ureter	150		189	307
3	53	Asymptomatic	6 months	Living donor	52	2 arteries, 2 veins, 1 ureter	240	44	132	107
4	70	Asymptomatic	10 months	Living donor	70	1 artery, 1 vein, 1 ureter	170	19	136	121
5	74	Symptomatic	18 months	Deceased donor	73	1 artery, 1 vein, 1 ureter	140	24	101	97
6	57	Symptomatic	5 months	Living donor	41	1 artery, 1 vein, 1 ureter	160	25	158	144
7	74	Symptomatic	63 months	Deceased donor	82	1 artery, 1 vein, 1 ureter	150	22	229	Hemodialysis
8	46	Symptomatic	48 months	Deceased donor	50	1 artery, 1 vein, 1 ureter	140	23	162	129

RESULTS

During the study period, 22 ABFBs were performed for severe aorto-iliac in kidney transplantation candidates. Patients were 20 men and 2 women. Median age was 64 years (range 46–78 years).

All patients presented with hypertension, 7 were diabetics, 19 had dyslipidemia, 16 were former smoker, and 2 were obese. Median body mass index was 24.6 kg/m^2 (range 18.9–33 kg/m^2). Cardiac comorbidity (coronary surgery or stenting) was noticed in 8 patients and pulmonary comorbidity (chronic obstructive pulmonary disease) in 5 patients. Twenty patients were on hemodialysis (radiocephalic fistula in 9 patients, ulnar basilic fistula in 1 patient, brachiocephalic fistula in 8 patients, central venous catheter in 2 patients). Median time on dialysis was 2 years (range: 11 months–13 years).

Ten patients were asymptomatic with severe aorto-iliac circumferential calcifications without hemodynamic consequences, and 12 patients were symptomatic since aorto-iliac calcifications were responsible for claudication in 11 patients, and critical limb threatening ischemia with tissue loss in 1 patient. Among the 12 symptomatic patients, 1 patient also presented chronic mesenteric ischemia and a concomitant revascularization of the superior mesenteric artery was planned.

Surgical Procedures

Median operating time was in average 3.5 h (range 2–6.2 h). Median blood loss was 690 mL (range 150 mL–2.1 L).

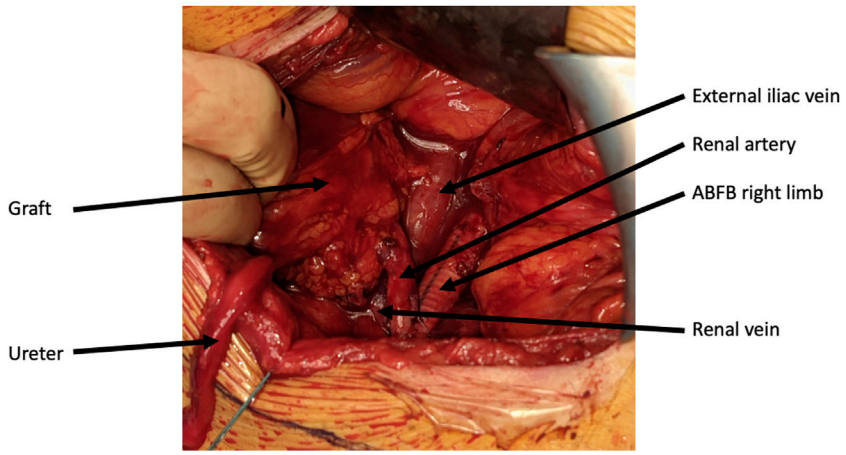


FIGURE 2 | Per-operative picture of a living donor kidney graft implanted on an aorto-bi-femoral bypass.

Postoperative transfusion was required in six patients. Median length of Intensive Care Unit stay was 2 days (range 2–8 days).

Primary Outcome

Thirty-day mortality was 0%. Overall 30-day morbidity was 22.7%: 1 femoral hematoma and 1 retroperitoneal hematoma requiring surgical drainage in the asymptomatic group, and 2 digestive ischemia requiring bowel resection and 1 femoral hematoma requiring surgical drainage in the symptomatic group.

Secondary Outcome

Follow-up of patients is presented in **Figure 1**. Median follow-up was 25 months (range: 2 months–8 years). The patient who underwent a concomitant revascularization of the superior mesenteric artery during ABFB died 15 months after the surgical procedure, from mesenteric ischemia. One patient was contraindicated since she was diagnosed a breast cancer. The 20 remaining patients could access to kidney waiting list. Median time between ABFB and registration (without contraindication) in kidney waiting list was 6 months (range 1–14 months).

Kidney transplantations were performed in 8 patients (**Table 1**): 5 deceased donor transplantations and 3 living donor transplantations (**Figure 2**). Median time between ABFB and kidney transplantation was 14 months (range: 5–63 months). Median operative time for kidney transplantation was 2.5 h (range 140–240 min) and median warm ischemia time was 23 min (range 24–44 min). Delayed graft function was noticed in 1 patient (patient 2), requiring dialysis for 1 week. Median length of hospital stay was 9 days (range: 6–13 days). Median serum creatinine level at discharge was 160 µmol/L (range: 101–307 µmol/L).

During follow-up, re-initiation of dialysis was required 16 months after kidney transplantation in one patient (patient 7). The patient presented polyomavirus associated nephropathy and acute cellular and humoral rejection. Median serum creatinine level at 2-year was 129 µmol/L (range: 97–189 µmol/L) for the 7 remaining patients.

DISCUSSION

Over a ten-year period, 22 ABFBs were performed in patients in whom severe aorto-iliac calcifications would have been a contraindication for kidney transplantation. Thirty-day morbidity was 22.7%. During follow-up, 20 patients could access to kidney waiting list and 8 patients underwent a successful kidney transplantation, including 3 living donor transplantation. Accordingly, ABFB as preparation for subsequent kidney transplantation can be considered as an option in order to overcome severe aorto-iliac calcifications.

However, an ABFP remains a high-risk procedure. Bredhal et al. reported the outcomes of 3,623 patients who underwent aortic surgeries for occlusive disease over a 20-year period: 30-day mortality was 3.6% and 30-day major complications rate was 20% [10]. In this study, renal insufficiency appeared as risk factor for 30-day mortality. Performing an ABFP in kidney transplant candidates is therefore risky, and it is mandatory to carefully select the patients susceptible to benefit from such a high-risk procedure. Moreover, the outcome of such transplantation is unpredictable, the expected patient survival can be low, such as the lifetime of the transplanted kidney. Undoubtedly, careful selection of patients is mandatory.

Performing an ABFB in symptomatic patients presenting with lower limb ischemia is less questionable, since revascularization is required in order to improve arterial insufficiency and therefore vascular-related symptoms, even in order to avoid major amputation. Revascularization in asymptomatic patients in whom aorto-iliac calcifications are not responsible for haemodynamic changes and vascular-related symptoms is however more questionable. Organ scarcity is global and the cost of transplantation including interventions for wait-listing is high. Accordingly, performing demanding surgery such as ABFBs in asymptomatic patients requires a careful selection and the decision whether or not to operate the patient must be based on a multidisciplinary discussion. Franquet et al. investigated the outcomes of 21 patients that underwent vascular bypass surgery

prior to kidney transplantation without any vascular-related symptoms [11]. The authors reported that 2 patients (9.5%) died related to the bypass surgery and that early post-operative morbidity involved 11 patients (52.4%). Among the 21 patients, 11 (52.4%) were transplanted. Transplanted patients were significantly younger at the time of bypass and were less frequently treated for coronary heart disease. The authors concluded that aortic bypass surgery performed prior to kidney transplantation among asymptomatic patients has significant mortality and morbidity rates, but when transplantation is possible, results are satisfying. In our study, 10 ABFBs were performed in asymptomatic patients. No death occurred, but 30-day morbidity in this subgroup of patients was 20%. Four of the 10 asymptomatic patients were transplanted, the remaining patients are still on waiting list. In our experience, mortality and morbidity were lower than those reported by Franquet et al., but patients in our study might be younger with less comorbidities. Larger studies are therefore mandatory, in order to identify and better select patients in whom revascularization would benefit.

Open surgery has been the gold standard for revascularization procedures. With further advances in tools and techniques, endovascular procedures are increasing. It is obvious that percutaneous endovascular procedures should be the therapy of choice in kidney transplant recipients since they are less invasive, are associated with less morbidity and lower mortality, can be repeated if necessary and allow more rapid recovery of patients. However, in some patients with severe and circumferential bilateral aorto-iliac calcification, a complete arterial reconstruction in order to prepare subsequent kidney transplantation is required [8, 9]. Nevertheless, the timing of kidney transplantation is unpredictable and transplantation is not guaranteed even if revascularization is performed before. One might assume that revascularization, especially in asymptomatic patients, could be performed concomitantly to kidney transplantation. Gouny et al. reported the outcomes of five patients who underwent vascular procedures concomitant to kidney transplantation [7]. All patients had occlusive disease. An ABFB was performed in two symptomatic patients complaining from claudication. In two patients, aorto-iliac lesions were discovered intraoperatively and treated by iliac endarteriectomy. In the last patient, iliac lesions were initially neglected but an iliac endarteriectomy was necessary since the graft remained hypoperfused. One patient (patient with an ABFB) died at day 4 from septic shock and kidney rupture. The authors concluded that kidney transplantation is possible without major difficulties when ABFB is performed before surgery, but that severe complications are observed when kidney transplantation is performed concomitantly to revascularization procedures. Even if the advantages of simultaneous procedures are obvious, this strategy carries a significant risk of morbidity and mortality when a major surgery such as an ABFP is required. Accordingly, the authors recommended a two-stage procedure, with a minimal delay of 6 weeks between both procedures [12]. In our experience, a living donor kidney transplantation was planned in three patients. This could help selecting patients that could be considered for revascularization procedure to facilitate subsequent transplantation.

CONCLUSION

ABFB as preparation for subsequent kidney transplantation can be considered as an option in order to overcome severe aorto-iliac calcifications. However, patients should be carefully selected and clear information should be given concerning morbidity and mortality. Further larger studies are however required in order to better identify the patients in whom such major revascularization procedures would benefit.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by the University Hospital of Strasbourg. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from all patients in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

PB: data collection, writing. SK: data collection, writing. SC: study design, writing. NC: study design, writing. AL: data analysis, writing. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

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CORRECTION
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Corrigendum: Real-World Treatment Patterns of Antiviral Prophylaxis for Cytomegalovirus Among Adult Kidney Transplant Recipients: A Linked USRDS-Medicare Database Study

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Keywords: kidney transplantation, antiviral, cytomegalovirus, prophylaxis, pharmacoepidemiology

A Corrigendum on

Real-World Treatment Patterns of Antiviral Prophylaxis for Cytomegalovirus Among Adult Kidney Transplant Recipients: A Linked USRDS-Medicare Database Study
by Raval AD, Ganz ML, Fraeman K, Lorden AL, Saravanan S, Tang Y and Santos CAQ (2022). Transpl Int. 35:10528. doi: 10.3389/ti.2022.10528

Expression of Concern: Real-World Treatment Patterns of Antiviral Prophylaxis for Cytomegalovirus Among Adult Kidney Transplant Recipients: A Linked USRDS-Medicare Database Study
by Transplant International Editorial Office (2023) Transpl Int. 36:12367. doi: 10.3389/ti.2023.12367

In the original article, there was a mistake in the **Graphical Abstract** as published. The number of participants in the study sample has changed, along with minor changes to the proportion of KTRs receiving CMV prophylaxis by risk strata, time to discontinuation of CMV prophylaxis, and the factors influencing discontinuation of CMV prophylaxis. The corrected **Graphical Abstract** appears below.

In the original article, there was a mistake in **Figure 1** as published. Programing errors in the cohort selection led to differences in the cohort selected along with minor changes to the patient attrition related to inclusion and exclusion criteria. The corrected **Figure 1** appears below.

In the original article, there was a mistake in **Figure 2** as published. Changes in the composition of the study cohort due to programming changes resulted in slightly different KM curves. The corrected **Figure 2** appears below.

In the original article, there was a mistake in **Table 1** as published. Changes in the composition of the study cohort due to programming errors that were corrected resulted in different numbers of patients reported throughout the table and slight differences in the proportions of patients in the various subgroups reported. The corrected **Table 1** appears below.

In the original article, there was a mistake in **Table 2** as published. Changes in the composition of the study cohort due to programming errors that were corrected resulted in different numbers of patients reported throughout the table and slight differences in the proportions of patients in the various subgroups reported. The corrected **Table 2** appears below.

In the original article, there was a mistake in **Table 3** as published. Changes in the composition of the study cohort due to programming errors that were corrected resulted in different coefficients and confidence intervals for the variables included in the regression. While a few relationships changed,

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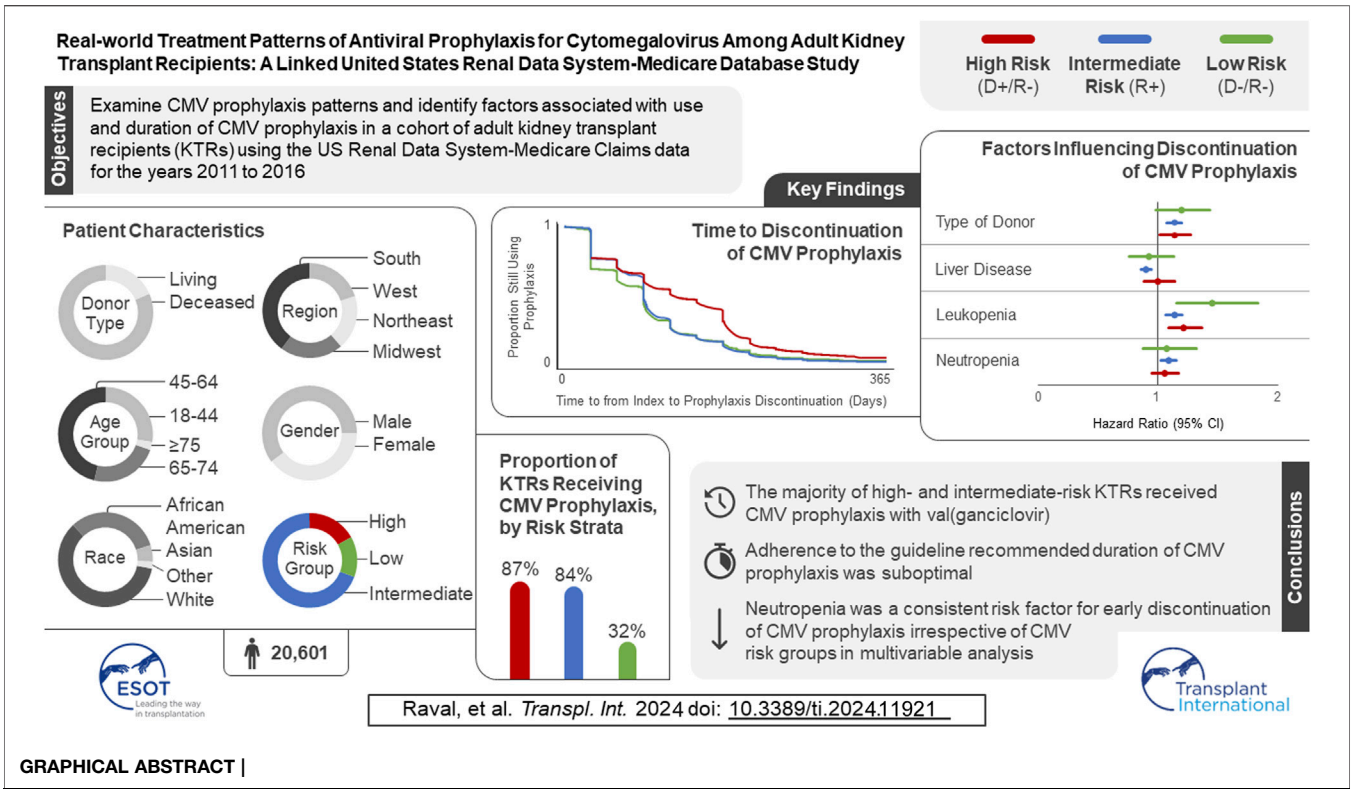

GRAPHICAL ABSTRACT |

those that did change did not influence or create a need to revise the conclusions of the study. The corrected **Table 3** appears below.

In the original article, there was a mistake in **Table 4** as published. Changes in the composition of the study cohort due to programming errors that were corrected resulted in different coefficients and confidence intervals for the variables included in the regression. While a few relationships changed, those that did change did not influence or create a need to revise the conclusions of the study. The corrected **Table 4** appears below.

In the original article, there was a mistake in **Supplementary Table 1** as published. Changes in the composition of the study cohort due to programming errors that were corrected resulted in different numbers of patients reported throughout the table and slight differences in the proportions of patients in the various subgroups reported. The corrected **Supplementary Table 1** is available at the **Supplementary Material** link of the original paper.

In the original article, there were several errors. Changes in the composition of the study cohort due to programming errors that were corrected resulted in changes to all numeric results. However, conclusions drawn from the corrected analysis have not changed from those originally presented. Below are the changes necessary to correct all paragraphs reporting numeric values from the analysis.

A correction has been made to the **Abstract**:

"Using United States Renal Database System registry data and Medicare claims (1 January 2011–31 December 2017), we examined CMV antiviral use in 20,601 KTRs who received their first KT from 2011 to 2016. Three-quarters of KTRs started CMV prophylaxis (86.9% of high-, 83.6% of intermediate-, and 31.7% of low-risk KTRs). Median time to prophylaxis discontinuation was 121, 90, and 90 days for high-, intermediate-, and low-risk KTRs, respectively. Factors associated with receiving CMV prophylaxis were high-risk status, diabetes, receipt of a well-functioning kidney graft, greater time on dialysis before KT, panel reactive antibodies ≥80%, and use of antithymocyte globulin, alemtuzumab, and tacrolimus. KTRs were more likely to discontinue CMV prophylaxis if they developed leukopenia/neutropenia, had liver disease, or had a deceased donor."

A correction has been made to **Results**, *Baseline Characteristics*, paragraph 1:

"We identified 67,838 individuals who received their first KT from 2011 to 2016, of whom 20,601 satisfied all inclusion and exclusion criteria (**Figure 1**). **Table 1** summarizes the characteristics of our sample. Most (69.2%) KTRs were at intermediate risk of CMV infection, while 17.0% and 13.8% were at high and low risk, respectively. KTRs were, on average, 53.2 years of age at their initial KT. Most KTRs were male (60.1%) and White (60.0%); one-third were African American. Diabetes (28.4%), hypertensive nephrosclerosis (27.8%), polycystic kidney disease (6.3%), focal glomerular sclerosis (5.6%), and systemic lupus erythematosus (3.6%) were the five most frequent primary diseases leading to ESRD. More than one-third (37.9%) of the KTRs had a CCI score ≥5,

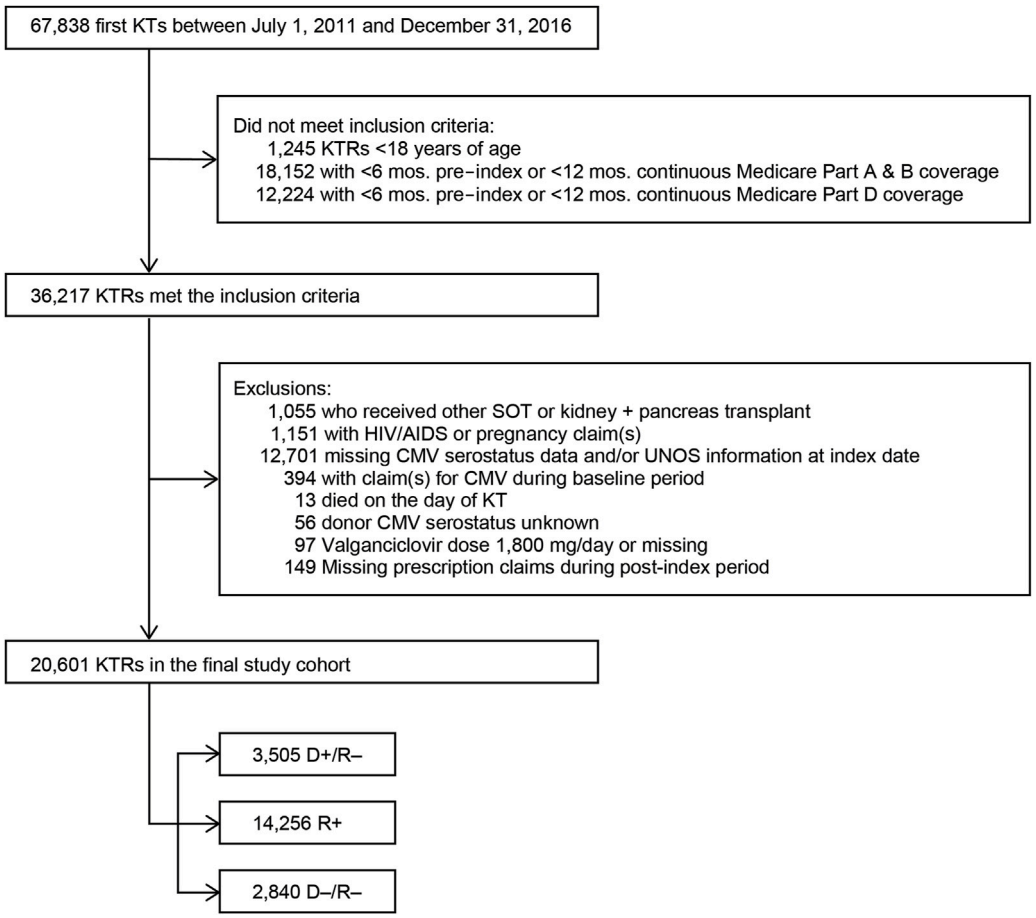

FIGURE 1 | Study sample selection. Abbreviations: AIDS, acquired immunodeficiency syndrome; CMV, cytomegalovirus; D+, seropositive donor; D–, seronegative donor; HIV, human immunodeficiency virus; KT, kidney transplant; KTRs, kidney transplant recipients; mos., months; R+, seropositive recipient; R–, seronegative recipient; SOT, solid organ transplant.

and nearly one-quarter of KTRs also had congestive heart failure (23.8%). KTRs spent, on average, 4.8 years on dialysis prior to their KT and 2.6 years on the transplant waiting list. Large proportions of KTRs received their kidney grafts from a deceased donor (81.5%) and were positive for Epstein-Barr virus (82.0%). Most donor kidneys experienced <24 h of cold ischemia time (81.6%) and were well-functioning (donor creatinine clearance ≤1.5 mg/dL). Approximately 22% had HLA A B donor-recipient match scores ≥3, and 9.2% of KTRs had PRA ≥80%. ATG was the most used induction immunosuppressive agent (54.7%), followed by basiliximab (22.2%) and alemtuzumab (16.5%). Almost all KTRs used prednisone and/or methylprednisolone (96.3%), MMF (96.3%), and tacrolimus (94.9%) as maintenance immunosuppressive agents. High-risk KTRs were more likely to have had PRA equal to zero, and high- and intermediate-risk KTRs were less likely to have had three or more HLA A B matches than other KTRs. Intermediate-risk KTRs were slightly older and more likely to be female, African American or Asian, Hispanic, reside in the South or West regions, have diabetes or hypertensive nephrosclerosis as the primary cause of ESRD, have a CCI score ≥5, and PRA ≥80% than KTRs in the other groups. Low-risk KTRs were more likely to reside in the Northeast or Midwest, and they were less likely to have had comorbid diabetes and to have used basiliximab as an induction immunosuppressive agent than other KTRs."

A correction has been made to **Results**, *Use and Factors Associated with the Use of CMV Antiviral Prophylaxis*, paragraph 1:

"**Table 2** displays, and compares across risk groups, the CMV prophylaxis characteristics of KTRs who started CMV prophylaxis. Slightly over three-quarters (77.0%) of KTRs started CMV prophylaxis (86.9% of high-, 83.6% of intermediate-, and 31.7% of low-risk KTRs). Overall, 59.7% and 32.5% of KTRs who started CMV prophylaxis used valganciclovir 450 mg and 900 mg, respectively, while 7.8% used other doses of valganciclovir; no patients used ganciclovir. Overall, KTRs who started prophylaxis did so, on average, 4.2 days after receiving their KTs; time to starting

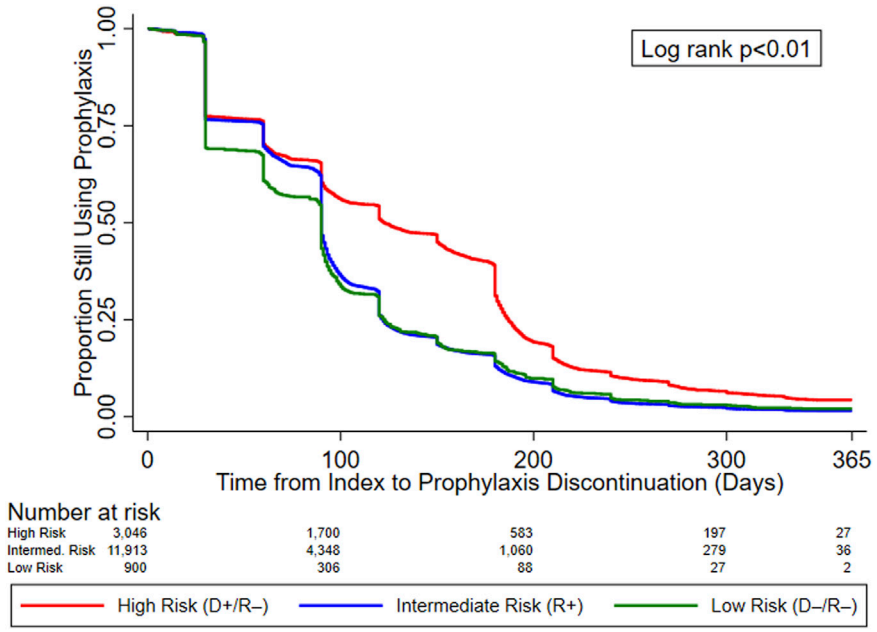

FIGURE 2 | KM curves for time to prophylaxis discontinuation, by serostatus (CMV Risk Group). Abbreviations: D+, seropositive donor; D–, seronegative donor; R+, seropositive recipient; R–, seronegative recipient.

prophylaxis did not vary substantially across risk groups (4.1–4.5 days)."

A correction has been made to **Results**, *Use and Factors Associated with the Use of CMV Antiviral Prophylaxis*, paragraph 2:

"**Table 3** displays the results of the logistic regression models for use of CMV prophylaxis (descriptive statistics stratified by CMV prophylaxis status within risk group are available in **Supplementary Table 1**). In general, CMV risk status was the factor most strongly associated with the use of CMV prophylaxis. KTRs who were younger, female, African American or of other races, resided in the Northeast, as well as those whose donor creatinine levels were >1.5 mg/dL, who spent more time on dialysis prior to KT, had PRA ≥80%, and who used ATG, and alemtuzumab were more likely to receive CMV prophylaxis (all and intermediate-risk KTRs). KTRs whose kidney graft experienced cold ischemia time <24 h, used basiliximab, AZA, everolimus, or cyclosporine, or prednisone and/or methylprednisolone were less likely to receive CMV prophylaxis (all and intermediate-risk KTRs). Additionally, high-risk KTRs who had PRA ≥80% were more likely to receive CMV prophylaxis; whereas those with comorbid diabetes, and who used AZA, everolimus, or cyclosporine, MMF or other maintenance immunosuppressive agents were less likely to receive CMV prophylaxis. Low-risk KTRs who were female, resided in the South, and used ATG and alemtuzumab or other immunosuppression as induction immunosuppressive agents were more likely to receive CMV prophylaxis."

A correction has been made to **Results**, *Duration of Prophylaxis and Factors Associated with Risk of CMV Prophylaxis Discontinuation*, Paragraph 1:

"**Figure 2** displays the KM curves for time to prophylaxis discontinuation. The median time to prophylaxis discontinuation (i.e., prophylaxis duration), derived from the KM curves, for the high-risk group of KTRs was longer (121 days) than for intermediate- (90 days) and low-risk (90 days) KTRs. Regardless of type of antiviral agent used, 10.9% of KTRs who used CMV prophylaxis did so for ≥200 days (23.4% and 12.7% of high-risk KTRs who used valganciclovir 450 mg and 900 mg, respectively, did so for ≥200 days) and more than half (55.8%) of high-risk KTRs used CMV prophylaxis for ≥100 days (64.0% and 44.8% of high-risk KTRs who used valganciclovir 450 mg and 900 mg, respectively, did so for ≥100 days). Over one-third (36.5%) of intermediate-risk KTRs used CMV prophylaxis for ≥100 days (39.4% and 23.3% of intermediate-risk KTRs who used valganciclovir 450 mg and 900 mg, respectively, did so for ≥100 days)."

A correction has been made to **Results**, *Duration of Prophylaxis and Factors Associated with Risk of CMV Prophylaxis Discontinuation*, Paragraph 2:

"**Table 4** displays the results of the PH Cox regression models for time to CMV prophylaxis discontinuation. We found that, regardless of risk group, KTRs who resided in the South and who developed leukopenia were more likely to discontinue CMV prophylaxis; all KTRs, as well as intermediate-risk group KTRs who developed neutropenia were also more likely to discontinue. Additionally, overall and intermediate-risk KTRs with comorbid liver disease, who experienced a longer wait time, lived in the

TABLE 1 | Baseline demographic, clinical, and medication-related characteristics of adult KTRs.

Characteristic	Overall (*N* = 20,601)	High risk (D+/R–) (*N* = 3,505)	Intermediate risk (R+) (*N* = 14,256)	Low risk (D–/R–) (*N* = 2,840)	*p*-value[a]
Mean age in years (SD)	53.2 (14.0)	51.7 (14.6)	53.9 (13.6)	51.4 (15.0)	<0.01
Age category in years, *N* (%)					
18–44	5,670 (27.5%)	1,102 (31.4%)	3,601 (25.3%)	967 (34.0%)	<0.01
45–64	9,545 (46.3%)	1,538 (43.9%)	6,862 (48.1%)	1,145 (40.3%)	
65–74	4,837 (23.5%)	779 (22.2%)	3,400 (23.8%)	658 (23.2%)	
≥75	549 (2.7%)	86 (2.5%)	393 (2.8%)	70 (2.5%)	
Gender, *N* (%)					
Male	12,383 (60.1%)	2,467 (70.4%)	7,951 (55.8%)	1,965 (69.2%)	<0.01
Female	8,218 (39.9%)	1,038 (29.6%)	6,305 (44.2%)	875 (30.8%)	
Race, *N* (%)					
White	12,366 (60.0%)	2,479 (70.7%)	7,757 (54.4%)	2,130 (75.0%)	<0.01
African American	6,600 (32.0%)	932 (26.6%)	5,029 (35.3%)	639 (22.5%)	
Asian	1,147 (5.6%)	50 (1.4%)	1,060 (7.4%)	37 (1.3%)	
Otherb					
Hispanic ethnicity, *N* (%)					
Yes	4,346 (21.1%)	435 (12.4%)	3,642 (25.5%)	269 (9.5%)	<0.01
No	16,093 (78.1%)	3,037 (86.6%)	10,504 (73.7%)	2,552 (89.9%)	
Unknown	162 (0.8%)	33 (0.9%)	110 (0.8%)	19 (0.7%)	
Geographic region, *N* (%)					
Northeast	3,830 (18.6%)	720 (20.5%)	2,406 (16.9%)	704 (24.8%)	<0.01
Midwest	4,424 (21.5%)	815 (23.3%)	2,822 (19.8%)	787 (27.7%)	
South	8,156 (39.6%)	1,377 (39.3%)	5,869 (41.2%)	910 (32.0%)	
West	4,137 (20.1%)	589 (16.8%)	3,123 (21.9%)	425 (15.0%)	
Other US territories	54 (0.3%)	<11	36 (0.3%)	14 (0.5%)	
Primary diagnosis leading to ESRD, *N* (%)					
Diabetes mellitus, Type 2	5,843 (28.4%)	873 (24.9%)	4,343 (30.5%)	627 (22.1%)	<0.01
Hypertensive nephrosclerosis	5,724 (27.8%)	863 (24.6%)	4,130 (29.0%)	731 (25.7%)	
Polycystic kidney disease	1,289 (6.3%)	255 (7.3%)	826 (5.8%)	208 (7.3%)	
Focal glomerular sclerosis	1,157 (5.6%)	221 (6.3%)	761 (5.3%)	175 (6.2%)	
Systemic lupus erythematosus	751 (3.6%)	108 (3.1%)	561 (3.9%)	82 (2.9%)	
Diabetes mellitus - Type I	720 (3.5%)	146 (4.2%)	427 (3.0%)	147 (5.2%)	
IGA nephropathy	669 (3.2%)	125 (3.6%)	421 (3.0%)	123 (4.3%)	
Chronic glomerulonephritis unspecified	502 (2.4%)	88 (2.5%)	343 (2.4%)	71 (2.5%)	
Malignant hypertension	250 (1.2%)	46 (1.3%)	174 (1.2%)	30 (1.1%)	
Membranous glomerulonephritis	199 (1.0%)	47 (1.3%)	126 (0.9%)	26 (0.9%)	
Other Disease	3,497 (17.0%)	733 (20.9%)	2,144 (15.0%)	620 (21.8%)	
Charlson Comorbidity Index, *N* (%)					
0	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	<0.01
1–2	4,683 (22.7%)	839 (23.9%)	3,114 (21.8%)	730 (25.7%)	
3–4	8,110 (39.4%)	1,438 (41.0%)	5,525 (38.8%)	1,147 (40.4%)	
≥5	7,808 (37.9%)	1,228 (35.0%)	5,617 (39.4%)	963 (33.9%)	
Comorbid health conditions, *N* (%)					
Congestive heart failure	4,912 (23.8%)	782 (22.3%)	3,483 (24.4%)	647 (22.8%)	0.01
Diabetes	9,091 (44.1%)	1,441 (41.1%)	6,565 (46.1%)	1,085 (38.2%)	<0.01
Diabetes without chronic complication	3,948 (19.2%)	635 (18.1%)	2,802 (19.7%)	511 (18.0%)	0.03
Diabetes with chronic complication	8,586 (41.7%)	1,358 (38.7%)	6,220 (43.6%)	1,008 (35.5%)	<0.01
Chronic pulmonary disease	3,345 (16.2%)	587 (16.7%)	2,288 (16.0%)	470 (16.5%)	0.54
Peripheral vascular disease	5,025 (24.4%)	849 (24.2%)	3,575 (25.1%)	601 (21.2%)	<0.01
Rheumatologic disease	1,389 (6.7%)	208 (5.9%)	1,016 (7.1%)	165 (5.8%)	<0.01
Mild to moderate liver disease	3,016 (14.6%)	482 (13.8%)	2,147 (15.1%)	387 (13.6%)	0.04
Sever liver disease	91 (0.4%)	<11	75 (0.5%)	<11	0.02
Myocardial infarction	1,843 (8.9%)	307 (8.8%)	1,275 (8.9%)	261 (9.2%)	0.84
Dementia	164 (0.8%)	38 (1.1%)	96 (0.7%)	30 (1.1%)	0.01
Mean time on dialysis prior to KT (SD), years	4.8 (3.2)	4.6 (3.1)	4.9 (3.3)	4.1 (3.1)	<0.01
Mean wait time (SD), years	2.6 (2.1)	2.5 (2.1)	2.6 (2.2)	2.2 (1.9)	<0.01
PRA, *N* (%)					
0%	13,565 (65.8%)	2,498 (71.3%)	9,066 (63.6%)	2,001 (70.5%)	<0.01
1%–19%	1,791 (8.7%)	308 (8.8%)	1,240 (8.7%)	243 (8.6%)	

(Continued on following page)

TABLE 1 | (*Continued*) Baseline demographic, clinical, and medication-related characteristics of adult KTRs.

Characteristic	Overall (*N* = 20,601)	High risk (D+/R–) (*N* = 3,505)	Intermediate risk (R+) (*N* = 14,256)	Low risk (D–/R–) (*N* = 2,840)	*p*-value[a]
20%–79%	3,100 (15.0%)	464 (13.2%)	2,255 (15.8%)	381 (13.4%)	
80%–100%	1,898 (9.2%)	196 (5.6%)	1,555 (10.9%)	147 (5.2%)	
Missing	247 (1.2%)	39 (1.1%)	140 (1.0%)	68 (2.4%)	
HLA A B donor-recipient match, *N* (%)					
0	4,338 (21.1%)	701 (20.0%)	3,086 (21.6%)	551 (19.4%)	<0.01
1	6,872 (33.4%)	1,217 (34.7%)	4,778 (33.5%)	877 (30.9%)	
2	4,553 (22.1%)	777 (22.2%)	3,127 (21.9%)	649 (22.9%)	
≥3	4,601 (22.3%)	766 (21.9%)	3,104 (21.8%)	731 (25.7%)	
Missing	237 (1.2%)	44 (1.3%)	161 (1.1%)	32 (1.1%)	
Hepatitis C seropositive, *N* (%)	849 (4.1%)	108 (3.1%)	648 (4.5%)	93 (3.3%)	<0.01
Epstein-Barr virus antibody positive, *N* (%)	16,887 (82.0%)	2,737 (78.1%)	11,864 (83.2%)	2,286 (80.5%)	<0.01
Calendar year of transplant, *N* (%)					
2011	1,857 (9.0%)	338 (9.6%)	1,287 (9.0%)	232 (8.2%)	<0.01
2012	3,613 (17.5%)	607 (17.3%)	2,523 (17.7%)	483 (17.0%)	
2013	3,552 (17.2%)	598 (17.1%)	2,515 (17.6%)	439 (15.5%)	
2014	3,516 (17.1%)	609 (17.4%)	2,441 (17.1%)	466 (16.4%)	
2015	3,950 (19.2%)	659 (18.8%)	2,679 (18.8%)	612 (21.5%)	
2016	4,113 (20.0%)	694 (19.8%)	2,811 (19.7%)	608 (21.4%)	
Used immunosuppressive agents, *N* (%)	20,376 (98.9%)	3,466 (98.9%)	14,092 (98.8%)	2,818 (99.2%)	0.21
Induction immunosuppressive therapy, *N* (%)					
ATG	11,148 (54.7%)	1,801 (52.0%)	7,808 (55.4%)	1,539 (54.6%)	<0.01
Basiliximab	4,518 (22.2%)	805 (23.2%)	3,114 (22.1%)	599 (21.3%)	0.16
Alemtuzumab	3,369 (16.5%)	600 (17.3%)	2,316 (16.4%)	453 (16.1%)	0.36
Rituximab	142 (0.7%)	12 (0.3%)	117 (0.8%)	13 (0.5%)	<0.01
Muromonab-CD3	20 (0.10%)	<11	<11	<11	0.02
Daclizumab	<11	0 (0.0%)	<11	0 (0.0%)	NA
Cyclophosphamide					
Maintenance immunosuppressive therapy, *N* (%)					
Prednisone or methylprednisolone	19,623 (96.3%)	3,320 (95.8%)	13,595 (96.5%)	2,708 (96.1%)	0.13
MMF	19,624 (96.3%)	3,328 (96.0%)	13,613 (96.6%)	2,683 (95.2%)	<0.01
Tacrolimus	19,327 (94.9%)	3,272 (94.4%)	13,383 (95.0%)	2,672 (94.8%)	0.40
Belatacept	530 (2.6%)	89 (2.6%)	381 (2.7%)	60 (2.1%)	0.21
Cyclosporine	399 (2.0%)	70 (2.0%)	275 (2.0%)	54 (1.9%)	0.95
Sirolimus	239 (1.2%)	45 (1.3%)	144 (1.0%)	50 (1.8%)	<0.01
Everolimus	207 (1.0%)	44 (1.3%)	125 (0.9%)	38 (1.3%)	0.02
Leflunomide	11 (0.05%)	<11	<11	<11	0.72
AZA	65 (0.3%)	12 (0.3%)	42 (0.3%)	11 (0.4%)	0.70
Other	338 (1.7%)	53 (1.5%)	248 (1.8%)	37 (1.3%)	0.19
Donor type, *N* (%)					
Deceased	16,789 (81.5%)	2,907 (82.9%)	11,866 (83.2%)	2,016 (71.0%)	<0.01
Living	3,812 (18.5%)	598 (17.1%)	2,390 (16.8%)	824 (29.0%)	
Mean cold ischemia time in hours (SD)	14.9 (10.0)	14.7 (9.6)	15.4 (10.0)	12.9 (9.9)	<0.01
Cold ischemia time in hours category, *N* (%)					
<24 h	16,807 (81.6%)	2,896 (82.6%)	11,514 (80.8%)	2,397 (84.4%)	<0.01
≥24 h	3,443 (16.7%)	551 (15.7%)	2,537 (17.8%)	355 (12.5%)	
Missing	351 (1.7%)	58 (1.7%)	205 (1.4%)	88 (3.1%)	
Mean donor creatinine in mg/dL (SD)	1.1 (1.0)	1.1 (1.1)	1.1 (0.9)	1.1 (0.9)	0.03
Donor creatinine in mg/dL category, *N* (%)					
≤1.5 mg/dL	17,187 (83.4%)	2,935 (83.7%)	11,817 (82.9%)	2,435 (85.7%)	<0.01
>1.5 mg/dL	3,399 (16.5%)	568 (16.2%)	2,429 (17.0%)	402 (14.2%)	
Missing	15 (0.07%)	<11	<11	<11	

Abbreviations: ATG, antithymocyte globulin; AZA, azathioprine; D, donor; D+, seropositive donor; D–, seronegative donor; ESRD, end-stage renal disease; HLA, human leukocyte antigen; IGA, immunoglobulin A; KT, kidney transplant; MMF, mycophenolate mofetil; NA, not applicable; PRA, panel-reactive antibody; R, recipient; R+, seropositive recipient; R–, seronegative recipient; SD, standard deviation; US, United States.

[a]p-values are compared across patients by donor/recipient serostatus group using t-tests or analysis of variance (ANOVA) for continuous variables or chi-square tests for categorical variables.

[b]*Other includes American Indian, Alaska Native, Native Hawaiian, Pacific Islander, multiracial, other, and unknown.*

TABLE 2 | Characteristics of CMV prophylaxis among adults undergoing first kidney transplant by serostatus.

Prophylaxis Information	Overall (*N* = 20,601)	High risk (D+/R–) (*N* = 3,505)	Intermediate risk (R+) (*N* = 14,256)	Low risk (D–/R–) (*N* = 2,840)	*p*-value
All prophylaxis agents					
CMV prophylaxis					
No prophylaxis	4,742 (23.0%)	459 (13.1%)	2,343 (16.4%)	1,940 (68.3%)	<0.01
Prophylaxis	15,859 (77.0%)	3,046 (86.9%)	11,913 (83.6%)	900 (31.7%)	
Type of prophylaxis, *N* (%)					
Valganciclovir	15,859 (100.0%)	3,046 (100.0%)	11,913 (100.0%)	900 (100.0%)	NA
Index dose 450 mg	9,462 (59.7%)	1,450 (47.6%)	7,518 (63.1%)	494 (54.9%)	<0.01
Index dose 900 mg	5,153 (32.5%)	1,371 (45.0%)	3,461 (29.1%)	321 (35.7%)	
Other index dose	1,244 (7.8%)	225 (7.4%)	934 (7.8%)	85 (9.4%)	
Ganciclovir					
Mean time to initiate any CMV prophylaxis in days (SD)	4.2 (4.4)	4.5 (4.7)	4.1 (4.3)	4.4 (4.9)	<0.01
Mean duration of CMV prophylaxis in days (SD)	107.5 (74.4)	134.1 (90.5)	101.5 (68.1)	97.6 (74.1)	<0.01
Duration of CMV prophylaxis, *N* (%)					
≥72 days	10,297 (64.9%)	2,034 (66.8%)	7,752 (65.1%)	511 (56.8%)	<0.01
≥90 days	9,912 (62.5%)	1,986 (65.2%)	7,433 (62.4%)	493 (54.8%)	<0.01
≥100 days	6,359 (40.1%)	1,700 (55.8%)	4,352 (36.5%)	307 (34.1%)	<0.01
≥180 days	3,201 (20.2%)	1,187 (39.0%)	1,868 (15.7%)	146 (16.2%)	<0.01
≥200 days	1,733 (10.9%)	583 (19.1%)	1,062 (8.9%)	88 (9.8%)	<0.01
Valganciclovir 450 mg					
Mean time to initiate valganciclovir 450 mg prophylaxis in days (SD)	4.0 (4.2)	4.4 (4.8)	3.9 (4.1)	4.4 (4.8)	<0.01
Mean duration of valganciclovir 450 mg prophylaxis in days (SD)	115.2 (75.4)	151.0 (91.0)	108.7 (69.7)	109.6 (79.8)	<0.01
Duration of valganciclovir 450 mg prophylaxis, *N* (%)					
≥72 days	6,786 (71.7%)	1,093 (75.4%)	5,376 (71.5%)	317 (64.2%)	<0.01
≥90 days	6,587 (69.6%)	1,072 (73.9%)	5,206 (69.2%)	309 (62.6%)	<0.01
≥100 days	4,123 (43.6%)	928 (64.0%)	2,998 (39.9%)	197 (39.9%)	<0.01
≥180 days	2,151 (22.7%)	691 (47.7%)	1,357 (18.1%)	103 (20.9%)	<0.01
≥200 days	1,151 (12.2%)	340 (23.4%)	749 (10.0%)	62 (12.6%)	<0.01
Valganciclovir 900 mg					
Mean time to initiate valganciclovir 900 mg prophylaxis in days (SD)	3.9 (4.3)	4.2 (4.5)	3.8 (4.2)	3.7 (4.4)	0.02
Mean duration of valganciclovir 900 mg prophylaxis in days (SD)	87.7 (67.8)	111.8 (84.5)	79.6 (58.5)	72.3 (55.6)	<0.01
Duration of valganciclovir 900 mg prophylaxis, *N* (%)					
≥72 days	2,513 (48.8%)	760 (55.4%)	1,622 (46.9%)	131 (40.8%)	<0.01
≥90 days	2,413 (46.8%)	739 (53.9%)	1,544 (44.6%)	130 (40.5%)	<0.01
≥100 days	1,484 (28.8%)	614 (44.8%)	807 (23.3%)	63 (19.6%)	<0.01
≥180 days	716 (13.9%)	392 (28.6%)	305 (8.8%)	19 (5.9%)	<0.01
≥200 days	361 (7.0%)	174 (12.7%)	177 (5.1%)	<11	<0.01
Valganciclovir other dose					
Mean time to initiate valganciclovir other dose in days (SD)	6.6 (5.3)	6.9 (5.3)	6.5 (5.2)	6.8 (6.4)	0.57
Mean duration of valganciclovir other dose prophylaxis in days (SD)	131.5 (74.9)	160.9 (92.0)	125.1 (68.4)	123.7 (74.5)	<0.01
Duration of valganciclovir other dose prophylaxis, *N* (%)					
≥72 days	998 (80.2%)	181 (80.4%)	754 (80.7%)	63 (74.1%)	0.34
≥90 days	912 (73.3%)	175 (77.8%)	683 (73.1%)	54 (63.5%)	0.04
≥100 days	752 (60.5%)	158 (70.2%)	547 (58.6%)	47 (55.3%)	<0.01
≥180 days	334 (26.8%)	104 (46.2%)	206 (22.1%)	24 (28.2%)	<0.01
≥200 days	221 (17.8%)	69 (30.7%)	136 (14.6%)	16 (18.8%)	<0.01

Abbreviations: CMV, cytomegalovirus; D, donor; D+, seropositive donor; D–, seronegative donor; R, recipient; R+, seropositive recipient; R–, seronegative recipient; SD, standard deviation.

Midwest, or received MMF or tacrolimus were more likely to discontinue CMV prophylaxis. Among the overall, high-, and intermediate-risk KTRs, those who were younger, received kidney grafts from deceased donors, or lived in the West or other US territories were more likely to discontinue prophylaxis. Finally, overall, intermediate-, and low-risk KTRs who identified as African American were more likely to discontinue CMV prophylaxis, as were overall and intermediate-risk KTRs of other races."

A correction has been made to **Discussion**, paragraph 1:

"CMV prophylaxis was more common among high- (86.9%) than intermediate- (83.6%) and low-risk (31.7%) KTRs, with all those KTRs using valganciclovir and almost 60% of valganciclovir users using 450 mg per day."

A correction has been made to **Discussion**, paragraph 2:

"Furthermore, we found that the mean duration of CMV prophylaxis was also longer in our study; however, still only approximately one in five high-risk KTRs completed 200 days of CMV prophylaxis and just over one in three intermediate-risk KTRs completed 100 days of CMV prophylaxis."

In the original article, there was a mistake in the **Data Availability Statement** as published. The original statement incorrectly stated that the USRDS-Medicare data was publicly available. The corrected Data Availability statement is as follows:

TABLE 3 | Logistic regression for probability of starting CMV prophylaxis among adults undergoing a first kidney transplant.

Predictors	Overall		High risk (D+/R–)		Intermediate risk (R+)		Low risk (D–/R–)	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
CMV serostatus (vs. D–/R–)								
D+/R–	17.16 (15.04–19.59)	<0.01						
R+	11.49 (10.42–12.67)	<0.01						
Age 18–64 years (vs. age ≥65)	1.61 (1.48–1.76)	<0.01	1.91 (1.52–2.40)	<0.01	1.64 (1.48–1.82)	<0.01	1.36 (1.10–1.69)	<0.01
Female gender (vs. male)	1.15 (1.06–1.25)	<0.01	1.01 (0.80–1.28)	0.90	1.19 (1.08–1.32)	<0.01	1.22 (1.00–1.47)	0.05
Race (vs. White)								
African American	1.15 (1.05–1.26)	<0.01	1.36 (1.04–1.78)	0.08	1.11 (0.99–1.24)	0.08	1.12 (0.90–1.39)	0.29
Other[a]	1.55 (1.32–1.82)	<0.01	2.25 (0.97–5.25)	0.06	1.42 (1.20–1.69)	<0.01	1.95 (1.18–3.22)	<0.01
Region (vs. Northeast)								
Midwest	0.55 (0.49–0.62)	<0.01	0.71 (0.53–0.96)	0.03	0.41 (0.35–0.48)	<0.01	0.66 (0.52–0.84)	<0.01
South	0.85 (0.76–0.95)	<0.01	0.85 (0.64–1.14)	0.28	0.60 (0.51–0.70)	<0.01	1.63 (1.30–2.03)	<0.01
West and Other US territories	0.85 (0.75–0.97)	0.02	1.10 (0.77–1.56)	0.61	0.69 (0.58–0.82)	<0.01	0.85 (0.65–1.13)	0.27
Primary disease leading to ESRD (vs. diabetes of any type)								
Hypertensive nephrosclerosis	1.18 (1.03–1.34)	0.02	0.99 (0.70–1.39)	0.94	1.32 (1.12–1.56)	<0.01	0.98 (0.72–1.34)	0.92
Polycystic kidney disease	1.06 (0.88–1.28)	0.54	0.95 (0.59–1.52)	0.82	1.17 (0.92–1.49)	0.19	0.86 (0.57–1.31)	0.49
Focal glomerular sclerosis	1.15 (0.95–1.40)	0.16	1.34 (0.76–2.37)	0.30	1.26 (0.98–1.62)	0.07	0.82 (0.53–1.26)	0.37
Other	1.09 (0.95–1.25)	0.23	0.90 (0.64–1.27)	0.56	1.27 (1.07–1.51)	<0.01	0.81 (0.59–1.10)	0.18
CCI ≥5 (vs. <5)	1.10 (0.98–1.23)	0.12	1.04 (0.76–1.41)	0.82	1.13 (0.98–1.31)	0.10	1.03 (0.79–1.33)	0.82
Comorbid health conditions								
Cardiovascular disease	0.94 (0.86–1.03)	0.18	0.89 (0.70–1.14)	0.35	0.90 (0.81–1.01)	0.07	1.11 (0.91–1.35)	0.32
Chronic pulmonary disease	0.91 (0.82–1.01)	0.07	1.02 (0.77–1.35)	0.89	0.83 (0.73–0.95)	<0.01	1.04 (0.83–1.31)	0.72
Diabetes	1.12 (0.99–1.27)	0.08	0.75 (0.54–1.04)	0.09	1.27 (1.08–1.50)	<0.01	1.05 (0.79–1.40)	0.73
Liver disease	1.05 (0.94–1.18)	0.37	1.09 (0.80–1.49)	0.59	1.04 (0.91–1.20)	0.54	1.02 (0.80–1.31)	0.85
Rheumatologic disease	0.88 (0.75–1.03)	0.10	0.92 (0.58–1.45)	0.71	0.87 (0.72–1.05)	0.15	0.88 (0.60–1.27)	0.48
Donor type deceased (vs. living)	0.91 (0.81–1.01)	0.07	0.82 (0.61–1.11)	0.20	0.93 (0.81–1.06)	0.28	0.93 (0.75–1.16)	0.54
Cold ischemia time <24 h (vs. ≥24 h)	0.95 (0.86–1.06)	0.37	1.18 (0.89–1.55)	0.26	0.92 (0.81–1.05)	0.23	0.89 (0.69–1.15)	0.38
Donor creatinine >1.5 mg/dL (vs. ≤1.5 mg/dL)	1.21 (1.09–1.36)	<0.01	1.22 (0.91–1.64)	0.19	1.21 (1.06–1.40)	<0.01	1.13 (0.88–1.45)	0.34
Time on dialysis prior to KT in years	1.02 (1.01–1.04)	<0.01	1.01 (0.97–1.06)	0.53	1.02 (1.01–1.04)	0.01	1.02 (0.99–1.06)	0.16
Wait time in years	0.99 (0.97–1.02)	0.62	1.00 (0.95–1.06)	0.98	0.99 (0.97–1.02)	0.47	1.01 (0.96–1.06)	0.77
PRAs ≥80% (vs. <80%)	1.36 (1.17–1.59)	<0.01	1.73 (1.00–2.98)	0.05	1.29 (1.08–1.55)	<0.01	1.33 (0.92–1.94)	0.13
HLA A B donor-recipient match ≥3 (vs. <3)	0.95 (0.87–1.04)	0.28	1.07 (0.83–1.37)	0.60	0.91 (0.81–1.02)	0.11	0.98 (0.80–1.20)	0.86
Calendar year of KT 2011–2013 (vs. 2014–2016)	1.12 (1.04–1.20)	<0.01	1.10 (0.90–1.35)	0.37	1.28 (1.17–1.41)	<0.01	0.73 (0.62–0.87)	<0.01
Induction immunosuppressive therapy[b] (vs. absence of therapy)								
ATG	1.77 (1.59–1.97)	<0.01	1.18 (0.89–1.57)	0.26	2.04 (1.79–2.32)	<0.01	1.62 (1.26–2.07)	<0.01
Alemtuzumab	1.55 (1.36–1.78)	<0.01	1.06 (0.74–1.52)	0.73	1.67 (1.41–1.97)	<0.01	1.60 (1.19–2.16)	<0.01
Basiliximab	0.79 (0.70–0.88)	<0.01	0.97 (0.71–1.32)	0.84	0.71 (0.62–0.81)	<0.01	1.02 (0.78–1.35)	0.87
Other immunosuppression	1.69 (1.03–2.77)	0.04	0.69 (0.19–2.49)	0.57	1.39 (0.78–2.46)	0.27	4.48 (1.67–12.05)	<0.01
Maintenance immunosuppressive therapy[c] (vs. absence of therapy)								
MMF	1.07 (0.88–1.30)	0.5	0.73 (0.42–1.25)	0.25	1.39 (1.10–1.76)	<0.01	0.81 (0.53–1.25)	0.34
Tacrolimus	1.16 (0.96–1.40)	0.11	0.96 (0.59–1.57)	0.87	1.50 (1.20–1.88)	<0.01	0.56 (0.36–0.87)	0.01
AZA, everolimus, and/or cyclosporine	0.37 (0.29–0.46)	<0.01	0.77 (0.41–1.47)	0.43	0.34 (0.26–0.44)	<0.01	0.56 (0.30–1.04)	0.07
Other immunosuppression	1.14 (0.95–1.37)	0.15	0.74 (0.46–1.17)	0.20	1.53 (1.22–1.92)	<0.01	0.71 (0.46–1.09)	0.12
Prednisone or methylprednisolone	0.54 (0.44–0.66)	<0.01	1.28 (0.82–1.98)	0.28	0.38 (0.28–0.51)	<0.01	0.52 (0.35–0.76)	<0.01

Abbreviations: ATG, antithymocyte globulin; AZA, azathioprine; CCI, Charlson comorbidity index; CI, confidence interval; CMV, cytomegalovirus; D, donor; D+, seropositive donor; D–, seronegative donor; ESRD, end-stage renal disease; HLA, human leukocyte antigen; KT, kidney transplant; MMF, mycophenolate mofetil; OR, odds ratio; PRA, panel-reactive antibody; R, recipient; R+, seropositive recipient; R–, seronegative recipient; US, United States.

[a]*Other includes Asian, American Indian, Alaska Native, Native Hawaiian, Pacific Islander, multiracial, other, and unknown.*

[b]*Other immunosuppression therapies included daclizumab, muromonab-CD3, rituximab, and cyclophosphamide.*

[c]*Other immunosuppression maintenance therapies included sirolimus, leflunomide, belatacept, or any other.*

TABLE 4 | Cox proportional hazard regression for time to CMV prophylaxis discontinuation among adults undergoing a first kidney transplant.

Predictors	Overall		High risk (D+/R–)		Intermediate risk (R+)		Low risk (D–/R–)	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
CMV serostatus (vs. D–/R–)								
D+/R–	0.60 (0.56–0.65)	<0.01						
R+	0.96 (0.89–1.03)	0.24						
Time-varying covariates (vs. no condition)								
Neutropenia	1.08 (1.03–1.14)	<0.01	1.06 (0.95–1.18)	0.28	1.09 (1.03–1.16)	<0.01	1.08 (0.88–1.33)	0.47
Leukopenia	1.17 (1.11–1.24)	<0.01	1.22 (1.09–1.37)	<0.01	1.14 (1.07–1.21)	<0.01	1.46 (1.16–1.85)	<0.01
Age 18–64 (vs. age ≥65)	0.84 (0.80–0.87)	<0.01	0.77 (0.70–0.84)	<0.01	0.84 (0.81–0.88)	<0.01	0.86 (0.71–1.03)	0.11
Female (vs. Male)	0.97 (0.93–1.00)	0.05	0.95 (0.87–1.04)	0.25	0.97 (0.93–1.01)	0.11	0.98 (0.84–1.15)	0.83
Race (vs. White)								
African American	1.08 (1.04–1.12)	<0.01	1.02 (0.93–1.12)	0.62	1.07 (1.03–1.12)	<0.01	1.26 (1.06–1.49)	<0.01
Other[a]	0.91 (0.86–0.97)	<0.01	1.17 (0.94–1.46)	0.16	0.89 (0.84–0.95)	<0.01	1.12 (0.77–1.62)	0.56
Region (vs. Northeast)								
Midwest	1.19 (1.13–1.26)	<0.01	1.07 (0.95–1.19)	0.28	1.22 (1.15–1.30)	<0.01	1.12 (0.91–1.39)	0.27
South	1.45 (1.38–1.52)	<0.01	1.16 (1.05–1.29)	<0.01	1.51 (1.43–1.59)	<0.01	1.60 (1.33–1.91)	<0.01
West and Other US territories	1.33 (1.26–1.40)	<0.01	1.15 (1.02–1.30)	0.03	1.38 (1.30–1.47)	<0.01	1.21 (0.95–1.55)	0.12
Primary disease leading to ESRD (vs. diabetes of any type)								
Hypertensive nephrosclerosis	0.99 (0.94–1.05)	0.73	0.96 (0.84–1.10)	0.56	0.99 (0.93–1.05)	0.67	1.10 (0.85–1.43)	0.45
Polycystic kidney disease	1.03 (0.95–1.12)	0.42	0.99 (0.83–1.19)	0.94	1.04 (0.95–1.15)	0.37	1.03 (0.72–1.47)	0.86
Focal glomerular sclerosis	0.99 (0.91–1.07)	0.75	0.90 (0.75–1.09)	0.29	1.04 (0.94–1.14)	0.44	0.76 (0.53–1.09)	0.13
Other	1.00 (0.94–1.06)	0.95	0.98 (0.86–1.12)	0.77	1.00 (0.94–1.07)	0.92	0.98 (0.76–1.26)	0.87
CCI ≥5 (vs. <5)	0.99 (0.94–1.04)	0.58	0.99 (0.88–1.11)	0.85	0.99 (0.94–1.05)	0.76	0.92 (0.75–1.14)	0.44
Comorbid health conditions (vs. absence of condition)								
Cardiovascular disease	1.01 (0.97–1.05)	0.73	0.95 (0.87–1.05)	0.33	1.02 (0.97–1.06)	0.42	1.02 (0.86–1.20)	0.82
Chronic pulmonary disease	1.01 (0.97–1.06)	0.66	1.09 (0.98–1.21)	0.1	0.99 (0.94–1.04)	0.68	1.03 (0.85–1.25)	0.74
Diabetes	0.98 (0.93–1.03)	0.39	1.05 (0.92–1.19)	0.47	0.96 (0.90–1.02)	0.18	1.04 (0.82–1.32)	0.75
Liver disease	0.92 (0.88–0.96)	<0.01	1.00 (0.90–1.12)	0.93	0.90 (0.86–0.95)	<0.01	0.93 (0.76–1.14)	0.49
Rheumatologic disease	1.03 (0.97–1.11)	0.33	1.11 (0.94–1.32)	0.20	1.03 (0.96–1.11)	0.43	0.88 (0.64–1.20)	0.41
Donor type deceased (vs. living)	1.15 (1.09–1.20)	<0.01	1.14 (1.02–1.28)	0.02	1.14 (1.07–1.20)	<0.01	1.20 (0.99–1.45)	0.06
Cold ischemia time <24 h (vs. ≥24 h)	0.97 (0.93–1.01)	0.19	0.93 (0.84–1.03)	0.17	0.97 (0.93–1.02)	0.29	1.01 (0.82–1.24)	0.92
Donor creatinine >1.5 mg/dL (vs. ≤1.5 mg/dL)	0.99 (0.95–1.03)	0.61	0.94 (0.84–1.04)	0.23	1.01 (0.96–1.06)	0.76	0.85 (0.69–1.04)	0.12
Time on dialysis prior to KT in years	0.99 (0.99–1.00)	0.06	1.01 (1.00–1.03)	0.05	0.99 (0.98–1.00)	<0.01	0.99 (0.97–1.02)	0.67
Wait time in years	0.99 (0.98–1.00)	<0.01	0.98 (0.96–1.00)	0.06	0.99 (0.98–1.00)	0.02	1.00 (0.96–1.04)	0.88
PRAs ≥80% (vs. <80%)	0.96 (0.91–1.02)	0.16	1.12 (0.95–1.32)	0.18	0.95 (0.89–1.01)	0.09	0.89 (0.67–1.18)	0.42
HLA A B donor-recipient match ≥3 (vs. <3)	0.97 (0.93–1.01)	0.1	1.00 (0.92–1.10)	0.92	0.95 (0.90–0.99)	0.02	1.10 (0.93–1.30)	0.26
Calendar year of transplant 2011–2013 (vs. 2014–2016)	0.51 (0.46–0.57)							
Induction immunosuppressive therapy[b] (vs. absence of therapy)								
ATG	0.95 (0.91–1.00)	0.04	0.86 (0.77–0.95)	<0.01	0.96 (0.91–1.01)	0.13	1.15 (0.94–1.40)	0.18
Alemtuzumab	0.95 (0.89–1.00)	0.07	0.92 (0.81–1.06)	0.25	0.96 (0.90–1.03)	0.23	0.88 (0.70–1.12)	0.29
Basiliximab	0.97 (0.92–1.03)	0.31	0.99 (0.88–1.11)	0.82	0.95 (0.90–1.01)	0.12	1.11 (0.89–1.39)	0.36
Other immunosuppression	0.88 (0.74–1.04)	0.13	0.81 (0.48–1.35)	0.41	0.86 (0.72–1.04)	0.12	1.15 (0.63–2.09)	0.66
Maintenance immunosuppressive therapy[c] (vs. absence of therapy)								
MMF	0.90 (0.82–0.98)	0.01	0.86 (0.72–1.04)	0.13	0.88 (0.79–0.97)	0.01	1.11 (0.78–1.56)	0.56
Tacrolimus	0.80 (0.73–0.87)	<0.01	0.85 (0.71–1.01)	0.07	0.77 (0.70–0.85)	<0.01	0.99 (0.68–1.44)	0.94
AZA, everolimus, and/or cyclosporine	0.89 (0.79–1.01)	0.07	0.86 (0.67–1.10)	0.22	0.88 (0.75–1.02)	0.09	1.37 (0.83–2.26)	0.22
Other immunosuppression	0.98 (0.90–1.06)	0.58	1.04 (0.87–1.24)	0.70	1.00 (0.91–1.10)	0.95	0.79 (0.54–1.16)	0.23
Prednisone or methylprednisolone	0.97 (0.90–1.05)	0.49	1.02 (0.86–1.22)	0.80	1.00 (0.92–1.10)	0.94	0.60 (0.45–0.81)	<0.01

Abbreviations: ATG, antithymocyte globulin; AZA, azathioprine; CCI, Charlson comorbidity index; CI, confidence interval; CMV, cytomegalovirus; D, donor; D+, seropositive donor; D–, seronegative donor; ESRD, end-stage renal disease; HLA, human leukocyte antigen; HR, hazard ratio; KT, kidney transplant; MMF, mycophenolate mofetil; PRA, panel-reactive antibody; R, recipient; R+, seropositive recipient; R–, seronegative recipient; US, United States.

[a]*Other includes Asian, American Indian, Alaska Native, Native Hawaiian, Pacific Islander, multiracial, other, and unknown.*

[b]*Other immunosuppression therapies included daclizumab, muromonab-CD3, rituximab, and cyclophosphamide.*

[c]*Other immunosuppression maintenance therapies included sirolimus, leflunomide, belatacept, or any other.*

"This study used data from the USRDS-Medicare database, which was provided to the study team subject to the terms of data use agreement (DUA) 2020-41f. The data are not publicly available due to privacy laws and cannot be shared by the authors. However, data obtained from the USRDS-Medicare database for this study may be accessed by applying to USRDS/NIDDK/CMS at usrds@niddk.nih.gov. Upon request, the corresponding author will provide the original data request and the programs used to derive this study's analytic cohort."

The authors apologize for these errors and state that this does not change the scientific conclusions of the article. The original article has been updated.

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