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Beta cell replacement at a crossroads

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TRANSPLANT TRIAL WATCH
published: 15 April 2025
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Transplant Trial Watch

Simon R. Knight[1,2]* *and John Fallon*[1]*

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Keywords: randomised controlled trial, liver transplantation, kidney transplantation, antibody mediated rejection, immunosupression

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

Randomized Trial Investigating the Utility of a Liver Tissue Transcriptional Biomarker in Identifying Adult Liver Transplant Recipients Not Requiring Maintenance Immunosuppression.

by Vionnet, J., et al. American Journal of Transplantation 2024 [record in progress].

Aims

They aim to assess whether a previously derived 5-gene liver tissue transcriptional biomarker accurately identifies stable adult liver transplant (LT) recipients who can safely discontinue maintenance immunosuppression (IS) without rejection—referred to as operational tolerance.

Interventions

Divided into two arms: Arm A (Non-biomarker-based): All participants underwent a gradual IS weaning protocol, regardless of biomarker results. Arm B (Biomarker-based): Participants first underwent a liver biopsy, which was tested using the 5-gene "tolerance" biomarker: Arm B+ (biomarker-positive): Proceeded to the same IS weaning protocol as in arm A. Arm B– (biomarker-negative): Continued baseline IS with no weaning.

Participants

116 adult, stable LT recipients, at least 3 years post-transplant if age >50 or ≥6 years if age ≤50, with normal allograft function, no active viral disease/autoimmune condition, no recent rejection. Arm A: 58 patients and Arm B: 58 patients (24 biomarker-positive, 34 biomarker-negative).

Outcomes

1. Primary Outcome: Operational tolerance at 1-year post–IS withdrawal, defined by (i) successful IS discontinuation for ≥12 months, (ii) normal liver function, and (iii) absence of rejection or inflammation on protocol biopsy. 2. Secondary Outcomes: Rate, severity, and timing of acute rejection; histologic changes on protocol biopsies; formation of donor-specific antibodies; biomarker performance (sensitivity, specificity, predictive values); immune characterisation (circulating T- and B-cell subsets, RNA-seq of liver tissue, immunohistochemistry measures of immune synapses).

Transplant Trial Watch

OPEN ACCESS

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Knight SR and Fallon J (2025) Transplant Trial Watch. Transpl. Int. 38:14654. doi: 10.3389/ti.2025.14654

Follow-Up
At least 1 year after cessation of IS (with a 1-year protocol biopsy). Some participants had extended follow-up to 2 years post–IS withdrawal for additional histologic assessments.

CET Conclusion
by John Fallon

The authors present the LIFT trial, a phase IV, open-label, prospective, multi-centre, randomised controlled trial assessing transcriptional biomarkers for operational tolerance in liver transplantation. Stable livers transplant patients were randomised into one of Arm A, where IS was progressively weened regardless of biomarker status or Arm B, in which biomarker positive patients were offered IS withdrawal and those who were biomarker negative remained on baseline IS. Over all the biomarker, the 5-gene liver transcriptional test, failed to predict who would tolerate IS withdrawal (sensitivity 54%, specificity 42%, positive predictive value ~16%). They found a low prevalence of operational tolerance, at only 16% (13/80) of patients fully weaned off IS at 1-year post-withdrawal met histologic criteria for operational tolerance. They found that indicators of tolerance to be longer time since transplant and older recipient age, and circulating exhausted/senescent $CD8^+$ T cells. Whereas *De novo* donor-specific antibodies were strongly associated with failure of IS withdrawal. The trial found comparable rates of true operational tolerance (~16%) with the OPTIMAL trial, which had a near identical protocol. They ended recruitment early when interim analysis indicated the biomarker's positive predictive value was unlikely to meet pre-specified criteria, while ethically justified and common in futility analyses, it reduced the final sample size and power, especially for the low-prevalence outcome of operational tolerance. Operational tolerance was adjudicated mainly at 1 year post-IS withdrawal with a follow-up biopsy. Some participants with mild inflammation at 1 year remained off immunosuppression and eventually stabilized on subsequent histologic checks, meaning the "final" tolerance outcomes might not be fully captured in a single 12-month time point, which given the low overall event rate, could be relevant. The minor methodological concerns revolve around the open-label design, the premature closure of recruitment, and the inherent difficulty of studying an event that is both rare and histologically stringent such as operational tolerance. These factors constrain the ultimate power and precision in estimating the biomarker's predictive value. Nevertheless, the study's careful design, protocol harmonization with the OPTIMAL trial, and robust immunologic/histologic analyses make a strong case for the likely negative utility of the 5-gene liver tissue transcriptional biomarker.

Jadad Score
2.

Data Analysis
Per protocol analysis.

Allocation Concealment
Yes.

Trial Registration
ClinicalTrials.gov - NCT024989977; ISRCTN - 47808000; EudraCT - 2014-004557-14.

Funding Source
Non-industry funded.

RANDOMISED CONTROLLED TRIAL 2

Donor-Derived Cell-Free DNA Monitoring for Early Diagnosis of Antibody-Mediated Rejection After Kidney Transplantation: A Randomized Trial.

by Akifova, A., et al. Nephrology Dialysis Transplantation 2024 [record in progress].

Aims
This study aimed to determine if monitoring donor-derived cell-free DNA (dd-cfDNA) could lead to early diagnosis of antibody-mediated rejection (AMR) in kidney transplant recipients.

Interventions
Participants were randomly assigned to either dd-cfDNA-guided kidney allograft biopsy or clinician-guided biopsy.

Participants
40 adult kidney transplant recipients >180 days following kidney transplantation, with prevalent dnDSA and an estimated glomerular filtration rate (eGFR) ≥20 mL/min/1.73 m^2.

Outcomes
The primary outcome was the time from study inclusion to diagnosis of active AMR or chronic active AMR. Secondary outcomes were time from first dnDSA occurrence to the diagnosis of AMR and diagnostic test metrics.

Follow-Up
24 months after baseline.

CET Conclusion
by Simon Knight

This small, single-centre RCT investigated the potential role of donor derived cell-free DNA (cfDNA) monitoring in kidney transplant recipients with *de novo* DSA. Patients were randomised to routine cfDNA monitoring with biopsy at a threshold of >50 copies/mL, *versus* biopsy for clinical indication. The primary endpoint of time to diagnosis of antibody mediated rejection (AMR) was significantly shorter in the cfDNA group (2.8 months vs. 14.5 months). There are very few prospective RCTs of biomarkers for post-transplant

monitoring, and so the authors should be congratulated. It should be noted that the study is open-label and there is a risk of measurement bias, as biopsies in the control group were at the discretion of the clinical team. Whilst the time to diagnosis was shorter in the cfDNA group, the study is too small to demonstrate whether this improves clinical outcomes through earlier treatment, and so is unable to truly assess the benefits of routine monitoring.

Jadad Score
2.

Data Analysis
Per protocol analysis.

Allocation Concealment
Yes.

Trial Registration
ClinicalTrials.gov - NCT04897438.

Funding Source
Industry funded.

CLINICAL IMPACT SUMMARY
by Simon Knight

Antibody-mediated rejection (AMR) in renal transplant recipients is associated with poor outcomes and graft loss, and is often more resistant to treatment than cellular rejection [1]. Mainstay of diagnosis is biopsy in response to deranged graft function, along with detection of donor specific antibodies (DSA). Waiting for biochemical evidence of graft dysfunction means that diagnosis is often late. Biomarkers that can detect AMR in the earlier stages may allow earlier diagnosis and improve response to treatment. Some centres monitor for *de novo* DSA (dnDSA) routinely, but this strategy is expensive, there is no agreement on the frequency of monitoring for optimal detection, and not all dnDSA lead to rejection [2].

Alternative blood- or urine-based biomarkers may afford more sensitive, non-invasive tools for earlier detection of AMR. One promising candidate is detection of donor-derived cell-free DNA (dd-cfDNA). Graft injury results in release of donor DNA into the recipient circulation, which can be differentiated from recipient cfDNA resulting in a sensitive marker of graft injury. A number of studies have demonstrated strong performance of dd-cfDNA in detection of graft injury resulting from AMR, with high negative predictive values [3]. Despite this promise, there are very few prospective studies examining the impact of biomarker-based monitoring on clinical outcomes.

In a recent study published in Nephrology, Dialysis, Transplantation, Akifova and colleagues examined the role of targeted dd-cfDNA monitoring in kidney transplant recipients with dnDSA [4]. 40 recipients with dnDSA were randomised to 3-monthly dd-cfDNA monitoring, or standard of care, with biopsies performed in the study group where elevated cfDNA levels were detected. They demonstrated that dd-cfDNA monitoring resulted in earlier diagnosis of AMR in the study group (2.8 months vs. 14.5 months). dd-cfDNA monitoring had 77% positive predictive value and 85% negative predictive value for AMR.

There are some limitations to the study. In the control group, decision to biopsy was at the discretion of the clinician caring for the patient, which in an open-label study may result in measurement bias. There were some delays in biopsy in response to elevated cfDNA due to concurrent illness or logistical constraints, which may have reduced the true effect. Whilst rejection was detected earlier, there is no evidence that this earlier diagnosis results in improved clinical outcomes, and the lack of reliable, effective treatment for AMR may limit the value of monitoring at present. A larger sample would be required to assess the true clinical benefit of routine monitoring.

Nonetheless, this is one of the few prospective studies of prospective biomarker monitoring in the literature, showing promise for earlier diagnosis that may impact outcomes in the face of emerging new therapies for AMR.

Clinical Impact
3/5.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

SK has undertaken previous paid consultancy work for OrganOx Ltd.

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

GENERATIVE AI STATEMENT

The authors declare that no Generative AI was used in the creation of this manuscript.

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IN MEMORIAM
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Obituary Dr. David Sutherland MD, PhD Emeritus Professor of Surgery, University of Minnesota 25th December 1940–23rd March 2025

*Vassilios Papalois**

Department of Surgery, Imperial College London, London, United Kingdom

Keywords: David Sutherland, pancreas transplantation, islet transplantation, diabetes, chronic pancreatitis

The passing of Dr David Sutherland on the 23rd of March 2025 (**Figure 1**), deeply saddened the ESOT transplant community. I was truly humbled to receive the gracious invitation of the ESOT Executive to write this obituary on behalf of our Society, expressing our deepest respect and sorrow for the loss of one of the most distinguished servants of our vocation whose passing signified the end of a great transplant era.

When many colleagues around the world received by Dr. Rainer Gruessner the saddest news that our beloved teacher, mentor and friend Dr. David Sutherland passed away, within a matter of seconds, messages of sincere admiration and respect started pouring from all over the world praising the character, ethos, clinical and research achievements of David Sutherland, a truly remarkable man.

Dr. David Sutherland was a towering figure in the fields of transplant surgery and immunology. His astonishing career spanned nearly six decades, during which he made groundbreaking contributions to the treatment of diabetes, chronic pancreatitis, and organ transplantation.

Dr. Sutherland's journey began in the early 1960s when he entered the University of Minnesota Medical School, where he graduated in 1966. Even as a medical student, he demonstrated a keen interest in immunology - a field that would define much of his career.

After completing his medical degree, Dr. Sutherland served 2 years in the Army, a period that helped shape his strong sense of discipline, multitasking and leadership. In 1975, he completed his general surgery residency at the University of Minnesota and followed this with a transplant fellowship in 1976. By 1977, he had earned a Ph.D., marking the beginning of a prolific academic and clinical career that would lead him to become one of the foremost figures in the world of transplantation.

Dr. Sutherland joined the faculty at the University of Minnesota, where he would leave an indelible mark on the field of transplantation. He became Professor of Surgery in 1984, and he would go on to direct the University's prestigious Diabetes Institute for Immunology and Transplantation, a position he held from 1994 for almost three decades. Dr Sutherland was Head of the Division of Transplantation from 1995 to 2009 and was holder of a Diabetes Research Chair since 2003. He was a mentor to countless medical students, surgeons, and researchers, shaping the next-generation of transplant surgeons and scientists. His commitment to education and mentorship was one of his defining qualities. Over 100 transplant surgeons who trained under his guidance went on to lead pancreas and islet transplant programs around the world.

Dr. Sutherland's academic achievements were equally impressive. He authored over 1500 scientific publications, covering a broad range of topics with particular emphasis on beta-cell replacement therapy for diabetes mellitus, chronic pancreatitis, immunosuppression management, and the training of scientists and clinicians in translational research. He was one of the most cited authors in the history of the field of transplantation.

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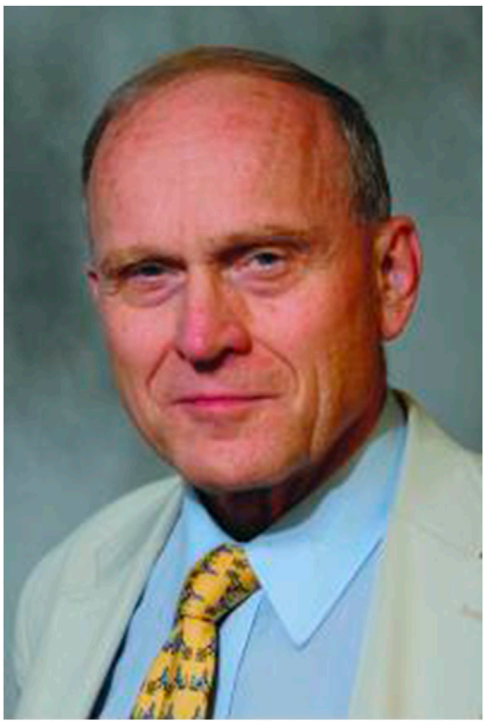

FIGURE 1 | Dr David E.R. Sutherland, 1940–2025.

His work on pancreas and islet transplantation was groundbreaking. In 1974, he conducted the world's first clinical islet transplant at the University of Minnesota. Dr. Sutherland became the Director of the University of Minnesota's pancreas transplant program in 1978, making it the oldest and largest pancreas transplant program in the world - with more than 2,400 pancreas and 1,000 islet transplants performed under his leadership. He also performed the world's first living-donor partial pancreas transplant in 1979. Furthermore, he established a very successful programme of pancreatectomy followed by islet auto-transplantation for the treatment of chronic pancreatitis. In 1980, he founded the International Pancreas Transplant Registry which remains a key resource for clinicians and researchers in the field.

Dr. Sutherland's influence extended far beyond his own research and clinical work. He was a beloved mentor and leader in the transplant community, holding prestigious leadership roles throughout his career. He served as President of several key organisations, including the American Society of Transplant Surgeons (1990–1991), the Cell Transplant Society (1995–1996), the International Society for Pancreas and Islet Transplantation (1996–1997), and The Transplantation Society (2002–2004).

Dr. Sutherland's contributions were widely recognised through numerous awards and honours including the Medawar Prize in 2012, the world's highest dedicated award for the most outstanding contributions in the field of transplantation.

Beyond his astonishing achievements, David Sutherland's intelligence, work ethic and sense of humour were legendary.

I will share some of my personal experience in working with David Sutherland and I know that it reflects similar stories by many other colleagues.

I met Dr Sutherland for the first time in August of 1988 when I was an elective medical student at the University of Minnesota. I asked his secretary for an appointment, and she told me that "Dr Sutherland will see you at 3 o'clock outside the operating rooms". I was absolutely certain that this was 3 o'clock in the afternoon but it was actually 3 o'clock in the morning! I still remember that he had three kidney-pancreas transplants going on, there was a parade of fellows waiting for him to review their abstracts, papers, and grant applications and he was also making many phone calls all over the world advising colleagues about difficult patients. A typical David Sutherland day!

Another great memory were the Friday afternoon laboratory meetings. His brain was literally a library, and he could give you all the information you needed about any published paper in the field of transplantation along with a superb critical analysis as to what you can learn from that and apply it in your research. He was challenging the research fellows by putting on the table the most provocative arguments, like "acute cellular rejection does not exist, debate me!" With his knowledge and intelligence, he could almost convince you that acute cellular rejection did not exist! However, the point of this exercise was to push to the limits our thinking and from that, all the great research ideas were coming up! Only David Sutherland could drive such a discussion!

In the clinical setting, David Sutherland has always been pushing the boundaries. I never remember him saying "This we will not do because it is difficult". If it was difficult, it was our job to do it! What was also most impressive was his amazing ability to critically analyse clinical challenges and generate the right research questions for translational research that was his great research passion. "Do not look in the libraries for ideas, look in front of you, the patients give you the ideas!" he used to say.

It was also amazing to see how the skills of David Sutherland were complementing those of John Najarian, the other transplant giant of the University of Minnesota and David Sutherland's, mentor. Between the two of them, they created one of the most successful clinical, research and training transplant programmes the world has ever seen.

Beyond the glamour of clinical and academic achievements, David Sutherland was a warm and kind-hearted human being, with a great sense of humour who was utterly loyal to his patients, colleagues and friends. He donated his kidney to his beloved wife Vanessa, and this was just one example of his genuine love and commitment to those who suffer.

Shakespeare wrote in the "Twelfth Night": "Some are born great, some achieve greatness, and some have greatness thrust upon them". David Sutherland was one of those unique human beings that had all three: he was born with great talents, he achieved great things, and he also responded to the thrust of life's challenges with creative action.

In 1989, the "Flame of Hope" was lit in London Ontario in Canada to honour those who discovered insulin and all the people who have lost their lives to diabetes. The flame will remain lit until there is a cure for diabetes. When a cure is found, the flame will be extinguished by the researchers who discover the cure. Whenever David Sutherland was asked if he believed that the flame will ever

be extinguished, he was always silent, full of emotion and his eyes were wet but full of light and hope. It is our duty to keep this hope alive until the dream is realised.

Now David Sutherland belongs to the Pantheon of transplant giants. Those of us who had the great fortune to be taught and mentored by him have the duty to pass the torch to the generations that follow. As Henry Adams said: "A teacher affects eternity. He can never tell where his influence stops."

For more than four decades, ESOT spearheaded within the European and the international transplant community the same causes and values that David Sutherland served with such distinction: commitment to patients, clinical innovation, progressive research, transformative education, equitable-diverse-inclusive spirit. Our Society will treasure and further advance this legacy in honour of David Sutherland and all the legendary figures of transplantation.

David Sutherland, 1940–1925, May He Rest in Peace.

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/s.

AUTHOR CONTRIBUTIONS

VP wrote the obituary upon the request of the Executive of the European Society of Organ Transplantation (ESOT).

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.

GENERATIVE AI STATEMENT

The authors declare that no Generative AI was used in the creation of this manuscript.

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IN MEMORIAM
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In Memoriam: David E.R. Sutherland

*Rainer W. G. Gruessner**

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Keywords: pancreas transplantation, islet transplantation, In memoriam, obituary for Dr. David Sutherland, David Sutherland

Dr. David Sutherland, considered the "father" of pancreas and islet transplantation, died peacefully in the early morning hours of 25 March 2025. As one of the most preeminent pioneers in the field of transplantation he epitomized the best of humanity, humility, empathy, integrity, and competence in all of medicine.

David Elmer Richard Sutherland ("DERS", **Figure 1**) was born on 25 December 1940, in St. Paul, MN. Upon completion of medical school, surgery residency and transplantation fellowship, all at the University Minnesota, and medical service in the Vietnam war, he stayed on the faculty of the Department of Surgery from 1976 until his retirement in 2009. He served his lifelong institution as the Chief of the Division of Transplantation and the Director of the Schulze Diabetes Institute. In his honor, an endowed chair was established at the University of Minnesota.

His scientific career began in the laboratories of Robert Alan Good (1922–2003), who performed the first successful non-twin human bone marrow transplant and Richard Carlton Lillehei (1927–1981), who performed the world's first successful pancreas transplant. Sutherland's early research work focused on the immunological role of the thymus, Peyer's patches, and appendix resulting in his first publications as a 23-year-old student in the journals *Nature* and *Lancet*. More than 1,000 peer-reviewed articles would follow over the years primarily focusing on all aspects of beta-cell replacement therapies.

Sutherland never considered pancreas and islet transplantation as competing fields, but rather as complementary treatment options in an all-inclusive, comprehensive beta-cell replacement strategy. This explains his treatment shifts from solid-organ to cellular transplantation and *vice versa*, based on the best approach for an individual patient.

His early focus on both pancreas and islet transplantation from living donors, initially with his mentor and chairman, Dr. John S. Najarian, was much more successful than from deceased donors. From the scientific perspective, and before the advent of advanced laboratory tests, Sutherland's most important immunological finding was that type 1 Diabetes Mellitus is an autoimmune disease that did recur in the twin donor pancreas graft when no immunosuppression was given; and did not recur when standard immunosuppression was administered.

During his 35-year tenure at the University of Minnesota, David Sutherland directed the world's oldest and largest pancreas and islet transplant programs. He was instrumental to a myriad of surgical "firsts" including the now-called "Sutherland" technique of spleen preservation in patients undergoing distal pancreatectomy, islet auto-transplantation after total pancreatectomy for chronic pancreatitis, and the first successful split-pancreas transplant.

For his many seminal contributions to beta-cell replacement through transplantation, Sutherland received many honors and awards during his distinguished career. He served as the President of the American Society of Transplant Surgeons (1990); of the Cell Transplantation Society (1994); of the International Pancreas and Islet Transplant Association (1995); and of The Transplantation Society (2002). He received honorary doctorates and honorary memberships from institutions around the world and, in 2012, the (Sir Peter) Medawar Prize, the world's highest dedicated award for the most outstanding contribution in the field of transplantation [1].

However, his distinguished career and unparalleled contributions do justice to only part of David Sutherland's personality. Equally important, he was an inspiring human being who cared deeply for

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FIGURE 1 | David E. R. Sutherland, MD, PhD. Reproduced with permission from "David E.R Sutherland, MD, PhD" by Rainer W.G. Gruessner and Angelika C. Gruessner, licensed under CC BY 4.0.

his patients, suffered tremendously with them in case of setbacks, and relished their successes. He performed transplants in thousands of diabetic patients, many of whom became insulin-independent and dialysis-free free for the rest of their lives. Altruistic by nature, he was a living kidney donor himself. He trained scores of transplant surgeons and physicians from all over the world who admired his humane qualities, pioneering vision, tireless passion and wonderful work ethics. He became a beloved teacher, mentor and surgeon who laid the foundation for the field of pancreas and islet transplantation as we know it. His own curiosity, ingenuity, fearlessness and willingness to think outside the box are legendary; as he once said, "true scholars don't practice evidence-based medicine, they perform evidence-gathering medicine." He set a high bar for excellence just by leading by example.

Because he was so helpful and instrumental to numerous careers, many of his trainees moved on to become directors of large pancreas transplant programs, chiefs of transplantation, or department chairs. He earned tremendous respect and admiration from his peers, patients and students alike–while remaining a truly humble, modest, compassionate, principled and easily approachable human being with a great sense of humor. His gracious personality by giving everyone a fair chance simply bred the highest esteem and loyalty. Despite becoming one of the greatest surgeons of the second half of the 20th century, he never forgot his Minnesota roots, nor his many interests in nonmedical fields such as American history and literature, classical music, baseball and horticulture.

We send our grateful sentiments and our condolences and prayers to his wife Vanesa and his family. The transplant community at large is indebted to David Sutherland.

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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The authors declare that no Generative AI was used in the creation of this manuscript.

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POINT OF VIEW
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The Last Mile in Beta-Cell Replacement Therapy for Type 1 Diabetes: Time to Grow Up

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Beta cell replacement therapy for type 1 diabetes (T1D) is undergoing a transformative shift, driven by advances in stem cell biology, gene editing, and tissue engineering. While islet transplantation has demonstrated proof-of-concept success in restoring endogenous insulin production, its clinical impact remains limited by donor scarcity, immune rejection, and procedural complexities. The emergence of stem cell-derived beta-like cells represents a paradigm shift, with initial clinical trials showing promising insulin secretion *in vivo*. However, translating these breakthroughs into scalable, widely accessible treatments poses significant challenges. Drawing parallels to space exploration, this paper argues that while scientific feasibility has been demonstrated, true accessibility remains elusive. Without a strategic shift, beta cell therapy risks becoming an elite intervention, restricted by cost and infrastructure. Lessons from gene and cell therapies for rare diseases highlight the dangers of unsustainable pricing and limited market viability. To bridge the "last mile" a Quality by Design approach is proposed, emphasizing scalability, ease of use, and economic feasibility from the outset. By emphasizing practical implementation over academic achievements, corporate interests, market economics, or patent constraints, beta cell therapy can progress from proof-of-concept to a viable, widely accessible treatment.

Keywords: diabetes type 1, beta cell replacement therapy, islet, scalability, stem cell derived beta cells

Point of View

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At the 48th Annual Conference ISPAD, held on 13–16 October 2022, in Abu Dhabi, UAE, I was invited to deliver a lecture titled *The Last Mile for Type 1 Diabetes cure* [1]. The intention behind this title was to highlight the ambivalence of the concept: while many interpreted it optimistically as signaling the imminent arrival of a definitive cure, the phrase also carries a cautionary meaning. In many fields, the "last mile" is often the most complex and challenging stage of development, requiring careful navigation to ensure successful implementation [2]. History teaches us that assuming victory just before the finish line is a surefire way to trip over our own shoelaces.

The field of beta cell replacement therapy for type 1 diabetes (T1D) is currently undergoing a remarkable transformation [3]. Over the past two decades, the well-established islet transplantation paradigm has provided compelling proof-of-concept evidence that restoring endogenous insulin production can lead to long-term glycemic control, protection from severe hypoglycemia, and improved quality of life [4–11]. However, the approach remains fundamentally constrained by the limited availability of organ donors, the need for lifelong immunosuppression, and the challenges associated with islet engraftment and survival [11–13]. In other words, we have a therapy that works beautifully—just for not enough people to make a real difference.

Recent breakthroughs in stem cell biology, tissue engineering, and gene editing are now reshaping the landscape, with the potential to overcome these intrinsic limitations [14–17]. The successful differentiation of stem cell-derived beta-like cells—whether from human embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs)—into insulin-producing cells suitable for transplantation represents a paradigm shift [18–23]. Initial clinical trials have demonstrated the feasibility of this approach, with promising preliminary data showing functional insulin secretion *in vivo* [24–30]. The possibility of encapsulating or genetically engineering these cells to evade immune rejection could eventually obviate the need for chronic immunosuppression, further expanding the therapeutic potential [3, 4]. This progress is the culmination of decades of interdisciplinary research, bringing us to an exciting and optimistic phase in the field. But before we start popping champagne, let's remember that many promising scientific advances have met their demise at the hands of real-world implementation challenges. As we celebrate these successes, it's important to recognize the complexity of what lies ahead. While the idea of the "last mile" in beta-cell replacement may suggest we are nearing a definitive solution, history shows that the final phase often brings its most rewarding challenges, offering opportunities for further breakthroughs and innovation.

To illustrate this, we can draw an analogy to space exploration. Sending the first humans to the moon was one of the most significant technological feats in modern history, showcasing the scientific ingenuity of our species. Can we say that humanity has mastered lunar travel? Certainly. Have we transitioned from exploration to colonization? Not even close. The Apollo program, which successfully landed twelve men on the moon over 12 years, cost an estimated $288 billion in today's currency and required the effort of 400,000 people. Was it worth it? Undoubtedly. The benefits of space exploration extended far beyond the moon landings themselves, driving innovations in computing, materials science, and medicine. But if our goal had been to establish a thriving lunar metropolis, we would have been woefully unprepared. The engineering required to sustain a permanent presence on the moon is vastly different from what was needed for brief exploration missions.

Similarly, while we have demonstrated that stem cell-derived beta cells can function in human recipients, scaling this intervention to treat millions of individuals with T1D presents a new set of challenges [31–34]. We've planted the flag, but we're nowhere near ready to move in. For now, we must acknowledge that only a select few will have access to this groundbreaking therapy in its early stages. Let's be realistic: sending twelve men to the moon was a tremendous achievement, but building the infrastructure to support thousands is an entirely different level of challenge—and a much greater one when scaling up to millions. If we continue to approach beta cell replacement with an "Apollo mission" mindset, we risk creating a therapy that could be limited in accessibility. This would necessitate either stringent stratification based on risk-benefit analysis or, in a more troubling scenario, allocation based on financial capacity [35, 36].

It's important to note that the biomedical field, while it shares some characteristics with space exploration in terms of complexity, is inherently different. The decentralized, iterative nature of biomedical research allows for faster and more varied innovation, often driven by global collaboration, and offers a more dynamic landscape than the singular focus of space exploration. In this regard, the biomedical field has some distinct advantages, such as flexibility and the potential for rapid progress due to the contributions of many smaller, specialized teams rather than relying on a monolithic, top-down approach. But there are also disadvantages to this fragmented approach. Without a central focus, there is a risk of research becoming too diffuse, lacking the critical mass of knowledge and resources needed to make real breakthroughs in a timely manner. The dispersed nature of the research may lead to silos of knowledge, and sometimes, these separate efforts can lack the cohesion necessary to propel the field forward efficiently. In the case of beta-cell replacement therapy, for instance, without a unified, coordinated strategy, progress may be delayed, and key challenges, such as creating scalable and affordable solutions, could remain unresolved.

Perhaps we do not fully consider the complexities of the "last mile" in scientific progress, where the challenges of scaling and ensuring widespread accessibility can be more intricate and demanding than the initial breakthroughs themselves. A recent reflection on human genome editing serves as a case in point [37]. It has been suggested that polygenic genome editing could become feasible within the next three decades, with theoretical models indicating that it could significantly reduce susceptibility to diseases such as coronary artery disease, Alzheimer's, depression, diabetes, and schizophrenia. This is an intriguing prospect with profound ethical implications, but one thing is already clear and underestimated: this approach is unlikely to be applied to a significant portion of the population within any realistic timeframe. Why? Because it would require *in vitro* fertilization for every individual undergoing genome editing [38]. Once again, we have explored the possibility, but we have not "colonized" it. For a more immediate comparison to beta cell therapy, let's assume for a moment that, starting today, we could transplant pancreatic islets without requiring immunosuppression. Would that mean we have reached the last mile in curing type 1 diabetes? Not at all. The number of donors would remain severely limited, and the procedure, still highly dependent on skilled operators, could not be automated or broadly implemented. Therefore, only a small group would have access, and this does not even take into account the cost factor.

Indeed, when considering the "last mile" in cell-based therapies, one of the major challenges is the cost, which may prevent the therapy from being automated or widely implemented. The case of gene and cell therapies for rare diseases serves as a cautionary tale [39]. Several promising therapies have been approved but later withdrawn from the market due to unsustainable pricing models and difficulties in reimbursement [40, 41]. Notable examples include Glybera, the first gene therapy approved in Europe, which was withdrawn in 2017, after being deemed commercially unviable, and Strimvelis, a gene therapy for ADA-SCID, which faced similar market challenges. News has recently emerged about the suspension of the development and commercialization of the hemophilia

B gene therapy fidanacogene elaparvovec (marketed as Beqvez in the United States and Durveqtix in Europe) by Pfizer [42]. Fidanacogene elaparvovec marks the ninth advanced therapy to be withdrawn from the European market since 2015, a significant figure considering that only 27 such therapies have reached commercialization in total [43]. Notably, no patients appear to have received the therapy after its approval in the United States. Its price tag—$3.5 million per patient—certainly does not lend itself to widespread adoption. Another shake-up in the sector came with the recent developments surrounding bluebird bio, Inc. Founded with the mission of developing gene therapies for rare diseases, the company had already sparked debate over the sustainability of advanced therapies back in 2021, following the simultaneous withdrawal of two gene therapies from the European market—one for beta-thalassemia and the other for cerebral adrenoleukodystrophy (the latter of which remained available for only 3 months). Once valued at $11 billion in 2018, bluebird bio faced mounting financial difficulties due to high development costs and limited market access. Recently, the company was acquired by U.S. funds for a mere $30 million—a staggering devaluation that underscores the economic challenges plaguing biotech firms specializing in advanced therapies.[1] The model used for rare disease therapies may not be directly applicable to widespread conditions like T1D, especially when scaling therapies like beta-cell replacement. In rare diseases, high per-patient costs are manageable, but for large populations, cost-reduction strategies are essential. A key approach is leveraging economies of scale, particularly in allogeneic therapies, where a single batch can treat multiple patients, spreading fixed costs and reducing per-patient expenses. However, autologous therapies face limitations in this regard due to the need for individualized production, which results in higher costs. Advancements in automation and bioprocessing technologies could reduce costs for both autologous and allogeneic therapies, but there are risks. Despite technological innovations, therapies may remain financially unfeasible for large populations due to high raw material costs, specialized facilities, and regulatory hurdles. Additionally, cost-reduction efforts must not compromise the therapy's quality or efficacy, as this could undermine its long-term success.

So, the real question is: how we can successfully and safely navigate the "last mile"? One option is to place unwavering faith in scientific progress, if what seems impossible today will inevitably become reality. After all, history is filled with once-fantastical ideas that have materialized into everyday technology. In *Star Trek* (1964), the crew communicated using sleek, flip-open devices—perfect for intergalactic adventures. Three decades later, Motorola's StarTAC brought that vision to life. Waiting, as Samuel Beckett illustrated in *Waiting for Godot*, carries profound human dignity. But waiting can also turn into a tragicomedy if it assumes that progress has no intrinsic limits—that it is merely a matter of time.

A second option is to take a different approach, drawing inspiration from the Quality by Design (QbD) framework [44–46]. For those unfamiliar with it, QbD is, above all, a philosophy that shifts the focus from quality control to quality by intentional design. It emphasizes that quality should not be tested into a product but rather built into it from the very beginning. At the core of QbD is the Quality Target Product Profile (QTPP), which defines the desired characteristics of a product, guiding its entire development. Fundamentally, the QbD approach marks a shift from a reactive, retrospective evaluation to a proactive, predictive model. Traditional quality control methods often rely on detecting and correcting issues after production. In contrast, QbD anticipates critical points and constraints during the design phase, allowing for a better understanding of the boundaries within which the process must operate. This shift from "test-and-fix" to "design-and-predict" enables more robust, efficient, and scalable therapeutic solutions, particularly in emerging fields like cell therapy. This concept is particularly crucial in the field of cell therapy, where the QTPP is not just about the intrinsic properties of the cellular product itself. Unlike conventional pharmaceuticals, cell therapies are living drugs, meaning their effectiveness and behavior depend on the dynamic interaction with the patient receiving the treatment. This reciprocal relationship between the therapy and the individual means that the QTPP must account for factors such as patient-specific responses, variability in the cellular product, and the evolving nature of the treatment within the body.

It is not my intention here to delve into the numerous complex aspects associated with the QbD approach in the field of beta-cell transplantation, aspects that are far from irrelevant. For example, defining Critical Quality Attributes (CQAs) for stem cell-derived beta cells (such as insulin secretion kinetics and purity) requires standardized assays, which remain underdeveloped. Even the discussion regarding the quality and potency of human pancreatic islets remains extensive [47]. To provide a sense of this complexity, in the recent FDA discussions concerning the approval of the Biologics License Application for pancreatic islets,[2] potency criteria were suggested based on parameters such as ≥70% viable islets, counting based on DTZ staining and microscopic evaluation, as well as the ratio of insulin secretion under high glucose stimulation to low glucose stimulation (≥1). Ironically, some probiotic strains, such as *Saccharomyces cerevisiae*, could potentially exhibit similar metrics under certain conditions [48], highlighting the complexity and challenges in defining appropriate potency criteria for beta cel replacement. Moreover, regulatory alignment with agencies like the FDA/EMA is also understated and warrants more attention. Regulatory agencies play a critical role in the successful development, approval, and commercialization of novel therapies. However, in the case of beta-cell replacement therapies, particularly those derived from stem cells, there is a need for more comprehensive alignment with regulatory standards and guidelines.

[1]https://www.fiercepharma.com/pharma/once-valued-10b-bluebird-bio-sells-private-equity-firms-29m

[2]https://www.fda.gov/media/170457/download

FIGURE 1 | Two beautifully designed wine glasses that perfectly match the Quality Target Product Profile (QTPP) for a wine glass, yet fail in real-world usability due to a lack of consideration for the interaction with the drinker during the design and prediction phase. Both glasses exhibit ideal material quality, clarity, durability, and aesthetic appeal, fulfilling all standard QTPP criteria. They are made of high-quality, lead-free crystal, ensuring clarity and safety. Their shape and design feature an optimized bowl size and rim thickness to enhance aroma. The capacity and volume allow for proper aeration and optimal serving. They are scratch- and shatter-resistant, suitable for repeated use. Their weight and balance make them comfortable to hold, while their design ensures stability. They are easy to clean, dishwasher-safe, and resistant to stains and odors. Finally, they are scalable for mass production while maintaining quality. However, despite excelling in these technical attributes, the glasses overlook a crucial factor: the interaction between the glass and the drinker: they have an extravagant yet impractical design, making it impossible to drink from without spilling. This serves as a metaphor for the importance of a holistic approach in Quality by Design (QbD): a product must not only meet its defined quality criteria but also be practical, user-friendly, and functional in real-world applications—a principle that applies equally to wine glasses and therapeutic innovations. The represent glasees are part of "The Uncomfortable," a collection of everyday objects that have been intentionally redesigned to be impractical by Athens-based architect Katerina Kamprani.

Instead, in this context, I propose the broader adoption of the QTPP concept. Rather than focusing solely on traditional product quality parameters, QTPP advocates for a more comprehensive, patient-centered approach—an approach especially vital in the realm of stem cell-based therapies like beta-cell transplantation. The challenge goes beyond simply creating a functional cellular product; it is also about ensuring its scalability, as a transformative therapy that remains accessible to only a few is little more than an academic achievement. Therefore, rather than concentrating exclusively on the intrinsic qualities of the cellular product, a key step should be the definition of the QTPP—not only in terms of what the therapy is, but also how it will be administered, how it will interact with the patient, and the broader context in which it will be applied (**Figure 1**). If this is the lens through which we view the problem, then as a physician-scientist, I must wonder: what kind of product would I actually want to use? Ideally, it would be cryopreserved and easily thawed at the bedside with warm water, compatible with a standard syringe, and administered much like a simple intramuscular injection—no operating room, no GMP facility for post-thaw reconstitution, no angiographic suite for infusion, etc. . . A final product of just a few milliliters, nothing more. From this endpoint, we must work backward, establishing constraints from the outset. The goal is not to design a product that functions beautifully under ideal conditions but one that remains viable when deployed at scale. The constraints should not reflect what is manageable for an expert in a specialized lab or clinic but what is operationally feasible for millions of patients worldwide. Returning to the Apollo 13 analogy, the lesson lies in NASA's approach to solving the air filter crisis [49]. They could have designed the ideal filtration system from scratch—but instead, they worked within the limits of what was already onboard, using available materials to construct a viable solution. In today's terms, this was an exercise in *design thinking*, and it is precisely the mindset we need before moving forward. Recognizing this early is critical. It informs the definition of QTPPs and *CQAs*, which, in turn, shape every aspect of development—including procedural simplicity, implant size, and invasiveness. Consider this: if we could eliminate the need for an angiographic suite, a surgical team, or anything beyond local anesthesia, would not that already be a breakthrough? Similarly, as a healthcare provider, I must consider: how much can I afford to spend on beta-cell therapy for an individual with T1D? Defining this is essential, as sustainability is a key element of scalability, and in my view, it should be incorporated into the QTPP definition. In this regard, there should be a stronger focus on academic research into the economics of Beta-Cell Replacement Therapy. Furthermore, it is crucial to recognize that the economic sustainability of therapies is not only about cost-effectiveness but also about broader financial considerations. This was demonstrated in the past with hepatitis C treatments in countries with advanced public welfare systems [50].

This phase should begin as soon as possible. Who should take responsibility for it? Undoubtedly, highly specialized academic centers with the necessary multidisciplinary expertise should lead the initial phase, overseeing both the design thinking process and, subsequently, the early-stage clinical trials to optimize the best conditions for implementation. These centers should work in close collaboration with the pharmaceutical industry, respecting each other's areas of expertise and competencies. Following this initial development, a "hub-and-spoke" model should be adopted to enable broader dissemination. From these central expertise hubs, the process can gradually expand, ensuring that the therapy becomes accessible on a larger scale while maintaining the necessary standards of quality and feasibility. Supporting initiatives in this direction is essential, and projects like ACT (Accelerate Cell Therapies;[3]) by Breakthrough T1D serve as a prime example. ACT aims to significantly accelerate the availability of cell therapy products by uniting efforts in research, development, regulation, and clinical access. A core aspect of this initiative is the establishment of Clinical Centers of Reference for Cell Therapy—expert, multidisciplinary facilities that will play a key role in the fast-tracked adoption of "off-the-shelf" cell therapies. These centers will not only provide advanced

[3]https://www.breakthrought1d.org/project-act/

treatments but also function as training hubs, helping other centers develop the expertise needed to deliver cutting-edge therapies. By prioritizing collaboration, training, and standardization of care, ACT and similar effort has the potential to ensure that life-changing therapies are accessible to millions of T1D patients worldwide.

A historical precedent for this approach can be found in bone marrow transplantation, which remains the only true cell therapy widely adopted on a global scale [51]. The foundations of this therapy were laid in the mid-20th century, with pioneering work by E. Donnall Thomas and George Mathé, who demonstrated that hematopoietic stem cells from bone marrow could be used to reconstitute the blood and immune system in patients with leukemia and other blood disorders. Their work led to the first successful human transplants in the 1960s. Initially, bone marrow transplants were highly experimental and confined to specialized centers. Over time, through continuous optimization of conditioning regimens, donor matching (HLA typing), and graft-versus-host disease management, the procedure became more standardized and scalable. Today, it is an established treatment for thousands of patients worldwide, facilitated by international donor registries and improved cryopreservation techniques that allow for broader accessibility. This evolution underscores the importance of starting with a highly controlled, expert-driven development phase, followed by a strategic expansion model to make cell therapies practical and available on a large scale.

Ultimately, if we are serious about completing the "last mile" in beta cell replacement, we must acknowledge that it is not a simple continuation of our current trajectory. It demands a fundamental shift in strategy—one that prioritizes not just scientific and technical innovation but also scalability, accessibility, and economic feasibility. Only by adopting this perspective can we transform beta cell replacement from experimental success into a truly viable treatment for millions of people with T1D. How long do we need to wait for having an exogenous insulin-free world? In all honesty, we do not know, but I am sure that the generation of individual with type 1 diabetes who will be definitively cured by beta cel replacement is already born. In the meantime, maintaining the parallel with the moon mission, we must push forward with unwavering commitment, as President John F. Kennedy said: "...We choose to go to the Moon in this decade and do the other things, not because they are easy, but because they are hard; because that goal will serve to organize and measure the best of our energies and skills, because that challenge is one that we are willing to accept, one we are unwilling to postpone, and one we intend to win, and the others, too (Rice Stadium on September 12, 1962)" This same spirit should guide our efforts in making beta-cell replacement a reality for those who need it most.

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

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POINT OF VIEW
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Allogeneic Islet Transplantation: Chronicle of a Death Foretold?

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Innovative solutions have entered the routine management of patients with type 1 diabetes or are making the headlines and this is shaking the world of beta cell replacement therapies. Above all, allogeneic islet transplantation is enthusiastically doomed to extinction by the aficionados of "closed loop" artificial insulin delivery systems or those convinced of the imminent large scale availability of stem-cell derived insulin-producing tissues. This opinion paper will propose that neither will be a universal solution in the very near future and will argue that xenogeneic islet transplantation may be a serious outsider in the race for new therapies. In the meantime, the odds are in favor of allogeneic islet (and pancreas) transplantation remaining first line options in the treatment of complicated type 1 diabetes. There is no question that "closed loop" systems have already greatly improved the management of type 1 diabetes, but, while "unlimited" sources of insulin-producing cells are jockeying for approval as standard-of-care, these improvements are more likely to drive a shift of indications -from islet transplant alone to simultaneous islet-kidney transplantation- than to herald the demise of islet transplantation.

Keywords: islet transplantation, bioengineering, stem cells, xenotransplantation, artificial insulin delivery systems

Point of View

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INTRODUCTION

Groundbreaking advancements are transforming the standard care of patients with type 1 diabetes mellitus (T1DM), sending ripples through the field of beta cell replacement therapies. Allogeneic islet transplantation, once hailed as a breakthrough, now faces existential questions amid the rise of stem cell-derived insulin-producing tissues and advanced closed-loop systems. There is a trend to believe that "closed-loop" artificial insulin delivery systems or stem cell-derived insulin-producing tissues will soon become the standard-of-care, thus limiting the remaining lifespan of islet transplantation. This opinion paper contends that allogeneic islet transplantation will persist as a key therapeutic option in the foreseeable future, not merely as a stopgap but as a complementary strategy within a diversifying armamentarium. When its decline eventually comes, if at all, the driving force behind it may not be one of the usual suspects.

Abbreviations: AHCL, advanced hybrid closed loop; ATMP, Advanced Medicinal Therapy Products; CGM, continuous glucose monitoring; ESC, embryonic stem cell; iPSC, induced pluripotent stem cell; ITA, islet transplant alone; PCMV/PRV, porcine CMV/porcine roseolovirus; PERV, porcine endogenous retrovirus; SIK, simultaneous islet-kidney transplantatio; T1DM, type 1 diabetes mellitus; TIR, time I range.

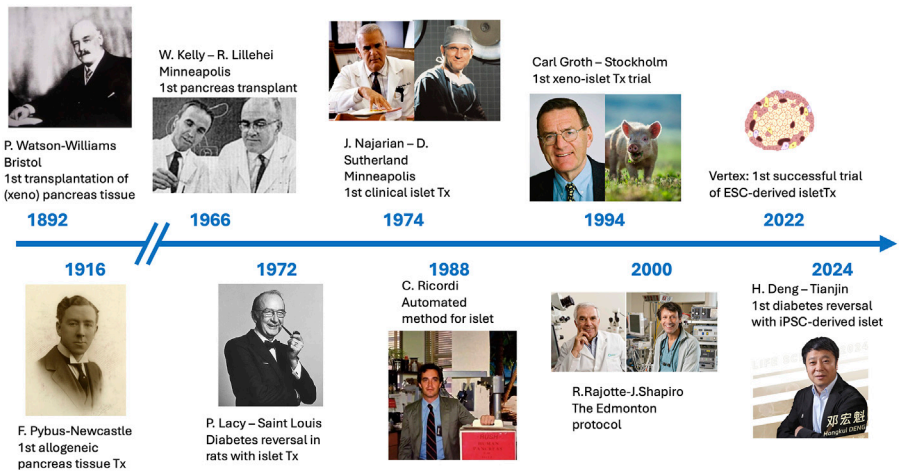

FIGURE 1 | Timelines of beta-cell replacement therapies, from the first attempt at implanting sheep pancreas fragments into a boy with keto-acidosis in 1892 by Patrick Watson-Williams to the successful transplant of iPSC-derived islets by Honghui Deng, reported in 2024.

THE CHALLENGERS (1)

A revolution is in the making in the world of beta-cell replacement (**Figure 1**). The past 2 decades have seen sustained progress in the generation of insulin producing islet-like structures, derived from embryonic (ESC) or induced pluripotent (iPSC) stem-cells, exhibiting a fully mature β-cell phenotype and able to reverse diabetes in a variety of animal models [1–7].

The first phase I/II clinical trials of ESC-derived islet cells encapsulated in a macrodevice, developed by the Viacyte company, and transplanted to T1DM patients with or without immunosuppression depending on the device structure, essentially demonstrated tolerability and safety, notably absence of off-target growth or occurrence of teratoma [8, 9]. However, only minimal amounts of C-peptide were detected in less than half the study subjects, even after optimization of the number of transplanted cells [10]. The double hurdle of assessing at the same time cells still at the progenitor stage and an immune-isolating device may have accounted for these less-than-ideal results.

Meanwhile, the Vertex company designed 2 clinical trials, in which ESC-derived islet-like cell, developed from the works of the Harvard Stem Cell Institute [3], were transplanted to patients with T1DM. Importantly, these VX-880 cells are fully mature. The first Vertex trial, in which VX-880 cells were transplanted into the portal vein -as in clinical islet transplantation- and with immunosuppression, have demonstrated impressive results. In their latest press release, Vertex announced that islet cell engraftment and glucose-responsive insulin production occurred in all subjects. Nearly all participants (11 of 12) had a reduction or elimination of exogenous insulin use at their last visit, and all three patients who had reached at least 1 year of follow-up had come off insulin [11]. These remarkable results have allowed Vertex to announce the approval to move this trial to phase III [12]. A second trial in which the same cells are transplanted inside macrodevices without immunosuppression has been launched in the meantime.

Similarly spectacular clinical observations, albeit on a smaller scale, were reported from China, using iPSC-derived islet cells as the source of insulin-producing tissue. Chemically induced iPSC-derived autologous islets [7] were transplanted in a patient with T1DM, who was already on immunosuppression for a previous liver transplant. At 1-year post-transplant, patient was off-insulin, with normal blood sugar levels (time in range 99%) and normal HbA1c [13]. It is difficult to predict whether the autologous transplanted cells would have been protected from immune rejection or prone to recurrence of autoimmunity without immunosuppression.

Another group in China, reported the outcomes of a type 2 diabetic patient, already transplanted with a kidney and therefore on immunosuppression, in whom iPSC-derived islets were transplanted intraportally. Again, with more than 2 years follow-up, the patient remained off insulin, with normal blood sugar levels (time in range 99%) and normalized HbA1c [14]. Although its breakthrough nature was acknowledged, this report was met with cautious optimism, notably regarding the immunogenic profile of autologous iPSC-derived cells and their fate in the absence of immunosuppression [15]. Indeed, in contrast with ESCs that grow into teratomas into mice of the same genetic background, autologous iPSCs, reprogrammed from fetal fibroblasts by viral or non-viral genetic approaches, elicit an unexpected immune reaction in genetically identical mice, resulting in their rejection [16].

In an opinion paper published in the same issue of Transplant international, L. Piemonti discusses why, in spite of these spectacular breakthroughs, the large-scale application of stem cell therapy as a “cure” for T1DM may still face considerable hurdles before coming into implementation [17]. Large scale application, i.e. to “all” patients with T1DM before they develop complications of the disease in the form of severe hypoglycemia or micro/macrovascular disease, will require

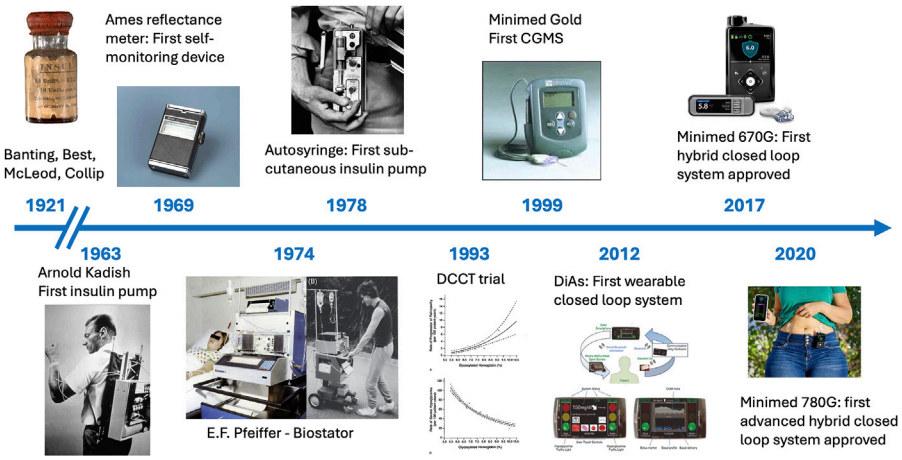

FIGURE 2 | Timelines of insulin therapy, from its "discovery" in 1921 by Frederick Banting and Charles Best to the approval of the first advanced hybrid closed loop system in 2020.

circumventing the need for lifelong immunosuppression. Solutions may include immune-isolating encapsulation systems and localized immunomodulation of the graft microenvironment or of the implanted cells themselves, rendering them "invisible" to the immune system by gene editing technologies [18–22]. However, translating these strategies into clinically viable Advanced Medicinal Therapy Products (ATMP), as they are classified in the European regulation, will demand significant technical and regulatory efforts, entail important costs, require cross-sector collaboration among all stakeholders -including academia, industry, healthcare systems, physicians, patient advocacy groups - and will take considerable time [23, 24].

A critical gap remains the lack of a "quality by design" approach, wherein diabetes-curing ATMPs are conceptualized holistically from inception—integrating cellular components, delivery systems, and immune protection—rather than retrofitting specific innovations into existing platforms *post hoc* [17]. For instance, the Vertex's VX-880 product, a leading ESC-derived islet therapy, has shown remarkable early efficacy in Phase I/II trial, but its reliance on immunosuppression and its high production costs will likely restrict access to a privileged minority in the foreseeable future.

THE CHALLENGERS (2)

The quest for a fully functional, fully autonomous "artificial pancreas" has relied on the parallel development, since the 1960s, of glucose sensors, which have evolved into continuous glucose monitoring (CGM) systems and of insulin delivering pumps [25] (**Figure 2**). The combination of these two technologies into what are known as "hybrid closed loop systems" is now part of the standard of care of patients with T1DM in industrialized countries. These systems rely on the measure (sensing) of subcutaneous glucose levels, which are entered into an algorithm that in turn determines the dose of insulin to inject subcutaneously. The "hybrid" terminology relates to the fact that, although the loops can effectively be closed, they still require input from the patient about carbohydrate intake or physical activity to complement the automated component of the system. The more recent generation, termed "advanced hybrid closed loop" systems (AHCL) have been approved by healthcare systems since 2020.

AHCL systems are extremely effective at improving glycemic control. Several studies with "real world" patients (i.e., not subject to the strict inclusion/exclusion criteria of randomized trials) have demonstrated a significant improvement of the glycemic time in range (TIR; 70–180 mg/dL), reaching 72%–74%, and HbA1c of approximately 7%, with 1-year follow-up periods [26–28].

AHCL systems have markedly improved both disease management and glycemic control of patients with T1DM. However admirable these achievements are, they should not conceal that the TIR targeted by diabetologists is not equate the normal glycemic range they have defined themselves. Investigators having looked at the time in "tight" range (70–140 mg/dL) obtained by AHCL systems, showed that it was in fact only 43%, even though a TIR of 73% was achieved [29]. The 7% HbA1c obtained, which is in line with accepted diabetologic targets, is in fact not better than the results of the DCCT/EDIC trials, which showed that intensive insulin therapy resulting in mean HbA1c of~ 7% maintained over a mean 6.5 years reduced the development and progression of early microvascular complications associated with diabetes by 34%–76% [30]. New diabetes treatment technologies have thus resulted in a progressive slowing down of the development of end-stage nephropathy in patients with T1DM; as reported in a Swedish cohort, the onset of end-stage renal failure has been postponed at least 10 years compared with that in older prospective cohort studies [31].

From the patient perspective, AHCLs are generally very favorably considered, although a recent study reported that it

did not improve diabetes treatment satisfaction, diabetes-specific quality of life, hypoglycemia awareness, or perceived frequency of unacceptably low glucose levels in study subjects [32]. Acceptability of AHCL is not universal (sensor issues, sports, . . .) and in some cohorts, the percentage of dropout from AHCLs was up to 30% [33].

In other words, and as already expressed by F. Banting in his Nobel acceptance speech, "insulin is not a cure, it is a treatment" [34]. No matter how sophisticated the AHCL device and the algorithm governing it are, the beta cell, and all the crosstalk and interactions that occur between the various cellular components of an islet of Langerhans, cannot be mimicked by a glucose sensor connected to insulin pump [35].

THE OUTSIDER

The field of xenotransplantation has recently garnered significant attention due to the breakthrough transplantation of porcine kidneys and hearts into brain-dead human subjects (the decedent model) and living patients [36–40]. Encouraging, and even spectacular, results have been largely achieved thanks to the availability of genome-edited pigs, with genetic modifications knocking-out genes related to carbohydrate antigens known to cause hyperacute rejection and human transgene insertions, designed to modulate the human immune system [41, 42].

It is quite strange to observe that islets have not yet joined this bandwagon, since it has long been considered the ideal modality for a potential first successful xenotransplantation trial [43]. The technical aspects of an islet transplant much easier than those of a vascularized organ transplant and the consequences of a failed graft are much less dramatic. Additionally, porcine insulin differs from human insulin by only one amino acid and has been the mainstay of T1DM management for decades before the arrival of synthetic insulins. Unsurprisingly, early trials using wild-type or minimally modified porcine islets, often with suboptimal encapsulation strategies, unsurprisingly yielded poor outcomes [44].

Another interesting feature of islet grafts is that they are disconnected from their own vascularization at the time of implantation, and revascularized with vessels growing from the host over the first weeks of engraftment [45, 46]. This means that there is no encounter of the donor epithelium with the host antibodies, and therefore some extent of protection from antibody-mediated rejection [46]. These experimental observations have indeed been largely verified in the clinical field, in which no correlation was seen between occurrence of *de novo* donor-specific antibodies and islet graft loss [47, 48]. Thus, the humoral component of xenorejection, which is thought to be the major immunological hurdle for graft survival is likely to be of no consequence in islet xenotransplantation.

How close are we to efficacious clinical islet xenotransplantation [49]? In recent years, several groups have reported long-term islet graft survival in pig-to-nonhuman primate experiments, mostly using wild type adult pigs as donors [44]. The government of South Korea has invested significant funding to advance the field of islet xenotransplantation, and a sponsor-initiated trial (Seoul National University), using islets from pathogen-free, wild type adult pigs was approved the authorities and should be initiated shortly [44]. The pilot study will enroll 2 patients, with an immunosuppression protocol associating induction with T-cell and B-cell depletion and TNF and IL-1 blockade, and maintenance with tacrolimus and sirolimus, the former to be switched to JAK inhibitors at about 2 months [50].

Meanwhile, the Sydney group has recently achieved long-term porcine islet graft survival, well over 1 year, in nonhuman primates, using multigene-edited pigs and less heavy immunosuppression [51]. It seems that bringing islet transplantation to the clinic with acceptable immunosuppressive regimens will depend on the availability of genetically modified pigs and a better definition of which are the genes necessary (and sufficient) to edit in or out [42, 49, 52, 53].

Bringing islet xenotransplantation to the clinic will also require the resolution of regulatory issues, notably pertaining to biosafety in general, and specifically zoonosis transmission [54]. Despite initial concerns in the pioneering times, transmission of porcine endogenous retroviruses (PERV) has in fact never been observed, is easy to monitor and can be totally prevented by the now available pig in which the 57 PERV genes have been edited out [55]. Of greater concern is the risk of porcine CMV (in fact a porcine roseolovirus, PCMV/PRV) transmission, for which no treatment is known, and which has drastically reduced survival in pig-to-non human primates [41, 56, 57]. Although it is easy to breed pigs in PCMV/PRV-free conditions and this virus can be easily detected (PCR, serologies) [57], PCMV/PRV is likely to have been involved in the death of the first recipient of a porcine heart [58].

Islet xenotransplantation stands at a crossroads. Its unique biological advantages, coupled with advancing genetic and immunosuppressive tools, position it as a promising "outsider" in the race for scalable diabetes therapies. While technical and regulatory hurdles persist, the convergence of bioengineering innovation and clinical experience may yet propel islet xenotransplantation from theoretical promise to practical reality.

ALLOGENEIC ISLET TRANSPLANTATION: QUO VADIS?

The authors of this point of view hope to have convinced the reader that despite the recent reported successes, stem cell-derived islets are unlikely to become available to a large patient population in a so near future. Although, the proof of concept was spectacularly obtained in the recent Vertex trial, incorporation of the cells into a finalized immune-protected system still has to be achieved. It should also be mentioned that, although off-target cell proliferation has not been observed so far, it remains a potential hazard that, if verified, would set the field many years back.

If investigators engaged in the field of islet xenotransplantation are careful to engage early enough in the "quality by design approach" advocated by Piemonti [17], islet xenotransplantation might find itself having an edge in the

TABLE 1 | Comparison of management technologies for T1DM.

	Allogeneic islets	Stem-cells	Xenogeneic islets	Closed loops
Status	Standard-of-care	Phase III	Phase I/II	Standard-of-care
Glycemic control	Good	Good	Uncertain	Acceptable
Availability	Limited	Theoretically infinite	Theoretically infinite	Unlimited
Limiting factor	Organ donors	Bioreactor capacity	Breeding capacity	n.a.
Costs	High	Very high	High	Acceptable
Safety risks	Donor-derived infection or malignancy	Tumorigenicity: off-target growth, teratoma	Zoonosis	none
Immunology	Allorejection	Allorejection (ESC) Immunogenicity of autologous iPSC	Xenorejection	n.a.
Immune modulation	Encapsulation strategies Gene-editing	Encapsulation strategies Gene-editing	Encapsulation strategies Gene-editing	n.a.

ESC, embryonic stem cells; iPSC, induced pluripotent stem cells; n.a., not applicable.

pursuit for an "infinite" source of insulin-producing tissue, available to all patients with T1DM without the need for lifelong immunosuppression.

In this context, the "quality by design approach" refers to a bioengineering strategy that holistically addresses the key challenges of functionality, safety, biocompatibility, immune-protection, ease of implantation and retrieval, cost efficiency, and patient acceptability [59]. These factors are essential prerequisites for designing and constructing a bioartificial pancreas, regardless of whether the insulin-producing tissue is derived from stem cells or xenogeneic sources [45, 60–63].

Meanwhile, we hope to have shown that the closed-loop systems, rhetorically referred to as an "artificial pancreas," are in fact simply a way -albeit a sophisticated one-of administering exogenous insulin, and are no more a cure for type 1 diabetes than dialysis is a cure for kidney failure. AHCLs can minimize the risk of severe hypoglycemia, and help keeping sugar levels "in range" about 70% of the time, allowing patients to maintain HbA1c levels at around 7%. This is more than bettered by islet transplantation, which keeps patients in a truly physiologic range for a higher part of the time [64], and for which follow-up data as long as 20 years are now available [65].

What then are the perspectives for allogeneic islet transplantation as a clinical activity, in the years to come, arguably for longer than predicted by some? Allogeneic islet transplantation has of course its limitations, primarily the scarcity of donors and the need for lifelong immunosuppression, carrying infectious, tumoral and nephrotoxicity risks.

Since the publication of the seminal "Edmonton protocol" paper, islet-transplant-alone (ITA) for severe hypoglycemia/hypoglycemia unawareness is the leading modality for allogeneic islet transplantation [66, 67]. As we have discussed above, AHCLs are mitigating the risks of severe hypoglycemia, and the indications for ITA are likely to drop. Some patients will still be reluctant to be on a pump or will not respond to technology adequately, and will therefore remain *bonafide* candidates for an ITA. The other impact of AHCLs is not to prevent, but to slow down the progression of diabetic nephropathy, and thus increase the age at which patients with T1DM who develop chronic kidney failure will have to face renal replacement therapy. We will have to care for an increased population of older, frailer patients, who would have been ideal candidates for simultaneous pancreas-kidney transplantation at a younger age, with a better general condition and fewer cardio-vascular issues. These patients, if they are fit to receive a kidney transplant, and most of them will, should therefore be offered simultaneous islet-kidney (SIK) transplantation rather than remain on insulin, while being immunosuppressed anyway [68]. This will amount to an ironical "return to the future," since SIK was by far the main modality of islet transplantation before the "Edmonton protocol" induced a paradigm shift in the world of beta cell replacement [69].

To summarize (**Table 1**), we foresee that, although they hold serious promise, regenerative medicine solutions still have a long way to go before being available to more than a lucky few patients with T1DM. Xenotransplantation of islets is a serious outsider that will face the same as yet unresolved issues as stem cells. We therefore believe that, in times where technology has measurably impacted the management of T1DM patients, but not to the point of offering a physiologic metabolic control, allogeneic islet transplantation still has several years of existence ahead. Indications for islet transplantation will undergo modifications, rather than see a decrease in activity. It is very likely that we will observe a diminution of the number of ITAs performed, but an increase in SIK activity, without a drop in overall islet transplant activity, and that "foretold death" of allogeneic islet transplantation will only be witnessed by the next-generation of diabetologists and transplant physicians.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Images presented in the article come from publicly available sources.

AUTHOR CONTRIBUTIONS

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

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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.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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REVIEW
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The Path Forward: A Review on Enhanced Recovery After Cardiothoracic Transplantation

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Enhanced Recovery After Surgery (ERAS) protocols represent a contemporary, evidence-based strategy for optimizing perioperative care to enhance patient outcomes through a standardized approach. While ERAS protocols have demonstrated significant benefits across a range of surgical specialties, specific guidelines tailored for cardiothoracic transplantation have yet to be developed. Given the unique complexity and heightened vulnerability of transplant patients, the implementation of ERAS principles in this context could potentially mitigate postoperative complications, reduce the length of hospital stays, and facilitate improved recovery trajectories. This review highlights the critical importance of adapting and applying ERAS methodologies in cardiothoracic transplantation to achieve improved surgical outcomes and elevate patient quality of life.

Keywords: ERAS in cardiothoracic transplantation, enhanced recovery after surgery (ERAS), cardiac transplantation, lung transplant, prehabilitation

INTRODUCTION

Cardiothoracic transplantation, including heart (HTx) and lung transplantation (LTx), is considered a final treatment option for patients with end-stage heart or lung disease. It provides a significant improvement in both quality of life and survival rates. However, these surgeries are very complex and biologically demanding, and they can be performed on critically ill patients, which increases the risk of complications, longer hospital stays, and extended recovery periods. Additionally, many candidates for heart and lung transplantation experience frailty and malnutrition [1, 2], leading to decreased physical resilience and increased susceptibility to worse outcomes, making their treatment and recovery more challenging [3, 4].

Enhanced Recovery After Surgery (ERAS) protocols are a modern approach to perioperative care designed to improve patient outcomes through a well-structured, evidence-based pathway. In the field of cardiothoracic surgery, ERAS protocols focus on optimizing every stage of the patient's journey [5, 6]. This involves comprehensive preoperative information and preparation to ensure the patient is in optimal condition for surgery, utilizing a minimally invasive approach whenever possible, and providing meticulous intraoperative care to reduce trauma and stress.

Despite the proven advantages of ERAS protocols in various surgical specialties, there are currently no established ERAS guidelines specifically tailored for cardiothoracic transplantation. Given the high stakes associated with these procedures, implementing such protocols is essential. One aspect that may facilitate the implementation of ERAS protocols in HTx and LTx for the frailest

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patients is the frequent delay of surgery due to the waiting list time. In this context, ERAS may turn the danger of the delay into an opportunity. Considering the complex and vulnerable state of transplant patients, ERAS can play a pivotal role in reducing postoperative complications, shortening hospital stays, and enhancing overall recovery. These protocols offer a systematic approach to care that can significantly improve patient outcomes, thereby becoming an invaluable component of cardiothoracic transplantation programs. This article explores the application of ERAS in this field, underscoring the necessity of its adoption to achieve superior surgical results and enhance patient quality of life.

CONCEPT OF FRAILTY IN HEART AND LUNG TRANSPLANT CANDIDATES

Frailty is a syndrome characterized by an increased vulnerability to stressors resulting from an accumulation of age- and health-related deficits that diminish physiological reserve [7, 8]. This accumulation includes disabilities, comorbidities, and various signs and symptoms that affect overall function and health status.

Frailty can be assessed in multiple ways, but the two primary approaches were the short physical performance battery (SPPB), which relies on phenotypic models based on physical functioning, and the frailty index, which is based on a summation of medical conditions, clinical symptoms, and laboratory data. [9]. Singer et al. [10] developed in 2023 a new index to assess the frailty in lung transplant candidates, the Lung Transplant Frailty Scale (LT-FS) had superior predictive validity over established measures.

Frailty is common in HTx patients and encompasses physical, psycho-cognitive, social, and nutritional aspects. While some components of frailty can be treated, others require supportive care. Identifying and understanding the major components of frailty is crucial for tailoring interventions after HTx. Frailty that develops while waiting for a transplant often guides rehabilitative interventions and should drive the tailoring of ERAS procedures. Research has shown that frailty within 6 months before HTx is linked to higher mortality and prolonged hospitalization post-transplant. Therefore, it is essential for congestive heart failure (CHF) specialists to establish a common method for evaluating frailty.

Recommendations from the ESC and ESOT have suggested the need for a common language to manage CHF and transplanted patients [11]. AGILE is a 10-item tool that evaluates mental, physical, socioeconomic, and nutritional domains [12].

In HTx, the prevalence of frailty varies with the New York Heart Association (NYHA) class. It affects around 10% of patients in class III and up to 40% in class IV. Frailty is an independent risk factor for mortality after HTx or after bridge-to-transplant ventricular assist device (BTT-VAD) implantation. Frail patients tend to have longer stays in the intensive care unit (ICU) and hospital, as well as lower survival rates [9]. The Heart Frailty Workgroup has reported an increased risk of mortality, readmission, disability, and adverse clinical outcomes in frail patients with systolic and diastolic heart failure. Additionally, in patients undergoing left ventricular assist device (LVAD) implantation, frailty is associated with longer times on a ventilator and extended hospital stays [10].

Frailty is prevalent among lung transplant candidates, with reported rates varying between 10% and 54%, depending on the assessment tool used [13]. This condition is associated with increased risks of delisting or death before transplantation, as well as higher early post-transplant mortality. For instance, frail patients have been observed to have a 2-fold higher risk of death within 1–3 years post-transplantation. Additionally, frailty correlates with longer hospital stays and reduced health-related quality of life after transplantation [14]. Despite these risks, frail LTx candidates can still derive significant benefits from transplantation, including improved dyspnea scores and 6-min walking distances [15]. Post-transplant frailty can be common, but it can also improve with outpatient physical therapy programs [16].

ERAS: ENHANCED RECOVERY AFTER SURGERY IN HEART AND LUNG TRANSPLANTATION

The ERAS program is a comprehensive care plan designed to improve the patient's condition before surgery, reduce the stress response during the operation, lower the risk of complications, decrease the length of hospital stays, and speed up recovery [17]. These benefits result from minimizing the physiological stress and disturbance associated with surgery, which typically lead to increased oxygen demand and catabolism. By doing so, postoperative organ dysfunction is reduced, and recovery is facilitated [2].

The protocol presents a multimodal evidence-based approach to patient care from the pre-, over the intra-to the postoperative period (**Figure 1**).

Cardiothoracic transplantation is a surgical process that can also benefit from ERAS protocols despite the lack of extensive scientific evidence in this field. The four arms implicated in the pre- and post-transplant periods are strength-conditioning and respiratory physiotherapy, nutritional support, and psychosocial support. Intraoperatively, the anesthesiologist's management, the minimally invasive approach, and correct pain management play a crucial role (**Table 1**) (**Figure 2**).

Prehabilitation

Improved nutrition and physical activity may greatly benefit patients awaiting heart or lung transplantation. Mobilizing these patients and ensuring adequate preoperative protein caloric intake represent significant improvements. Additionally, CHF patients have a high burden of chronic renal failure and impairment of iron metabolism that may lead to anemia and need of poli-transfusions, affecting the outcome of the index procedure and the risk of prolonged ICU stay.

Physical Therapy and Respiratory Physiotherapy

Sarcopenia, the reduction in muscle mass and function [37], is a relevant risk factor for waiting list mortality in patients

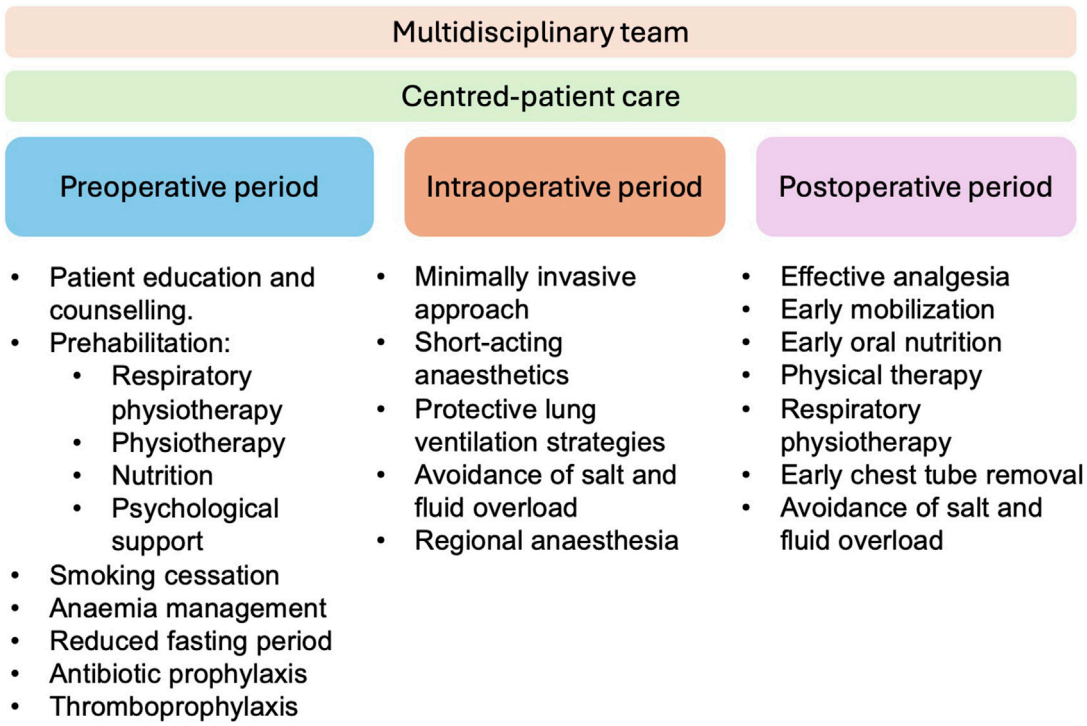

FIGURE 1 | Multimodal evidence-based approach in cardiothoracic transplantation.

undergoing HTx. Roehrich et al. [38] showed that the muscle area of the erector spinae muscle appears to be a risk factor for death in patients on the waiting list for HTx. The preoperative pectoralis muscle size and attenuation in CT scans are predictors of outcomes after the implantation of a left ventricular assist device (LVAD) [39].

The placement of a mechanical circulatory support device (MCSD) to aid physical therapy in advanced heart failure patients suggests that approximately 50% of the patients show improvement in their frailty level [40]. Chicano-Corrales et al. [41] demonstrated that patients with MSCD on the waiting list for HTx have high mobility, better 6-min walking distance (6MWD), shorter periods of invasive mechanical ventilation, and better nutritional status.

The use of an LVAD can improve frailty. Chung et al. [19] found that frailty was reversed after LVAD implantation, with 45% of patients improving their hand grip strength 6 months after implantation. Implementing LVAD in heart failure patients has been associated with decreased frailty. ECMO patients are the most challenging patients for pre-habilitation. Venous cannulation from the upper body, arterial cannulation in the axillary artery, and double-lumen cannulas for veno-venous ECMO or Oxy-RVAD should always be privileged to keep the patient active and avoid the shifting toward disability.

In selected patients, poor functional status related to end-stage pulmonary disease may be improved by adding veno-venous ECMO (VV ECMO). This approach avoids the complications associated with prolonged intubation and ventilator-associated lung injury. Several cohort studies and case series have demonstrated the feasibility and safety of a strategy that maximizes the opportunity for mobilization when active physical therapy is combined with awake, non-sedation, and non-paralytic protocols [18, 42, 43].

Several clinical trials have shown the beneficial effects of physical therapy in improving frailty by increasing muscle mass. The REHAB-HF trial [20] in a small cohort of patients demonstrated an improvement in the SPPB index and the 6MWD at three and 6 months after the intervention. The exercises included static and dynamic balance training, mobility training, functional strengthening of the lower extremities, and endurance training.

LTx candidates typically have decreased muscle mass, strength, and function, which are associated with worse outcomes [44] and a higher risk of 1-year mortality [45, 46]. The 6MWD is a suitable index for determining baseline physical functioning in various patient populations with chronic illnesses and it is associated with higher rate of mortality and worse outcomes after LTx [47]. Despite this, its role in predicting post-transplant outcomes remains uncertain. A study analyzing over 9,500 lung transplant recipients found that although 6MWT distance was significantly associated with post-transplant survival, relying on a single, dichotomous value [47] (e.g., above or below a specific distance) was limited in predicting outcomes. This suggests that 6MWT should be considered on a continuous basis rather than using arbitrary cutoffs

Lung function, as measured by VO2max, is associated with post-transplant survival and outcomes. Bakelants et al. [21]

TABLE 1 | Interventions on cardiothoracic ERAS protocol.

Period	Intervention	Results
Pre-operative period	Physical therapy and respiratory physiotherapy	ECMO-awake strategy [18] LVAD in heart failure [19] Physical therapy improves SPPB and 6WT [20] Inspiratory muscle training improves 6WT, DLCO [21]
	Nutritional support	Global nutritional assessment to [22] - correct nutritional deficiencies - support the healing process for surgical wounds - to strengthen the immune system PEG tubes play a crucial role in managing malnutrition, particularly when oral intake is insufficient [23, 24]
	Psychosocial support	cognitive-behavioral therapy to reduce psychosocial distress [23]
Intraoperative period	Anaesthesia management	Minimize premedication [25] Lung protective strategies [26] Transfusions should be minimized Fibrinogen concentrate, prothrombin complex or antifibrinolytic aprotinin can be use Control of intraoperative risks of PGD Early extubation is feasible [27, 28] Thoracic epidural anesthesia is recommended for analgesia management [26]
	Surgical technique	Minimal invasive surgery in lung transplantation showed better outcomes [29] V-A ECMO decreased rates of morbidity instead of CPB [30]
Post-operative period	MCS	Standardized protocols can significantly improve weaning success [31] Awake-ECMO should be considered in patients who cannot wean off ECMO [32]
	Post-operative pain management	Multimodal pain management strategies are recommended Thoracic epidural analgesia is considered the gold standard [33]
	Chest drain management	The duration of chest tube should be minimized promoting early mobilization
	Early mobilization	Early mobilization helps to maintain physical fitness even in the context of ECMO [34]
	Physical therapy Chest physiotherapy	Respiratory physiotherapy improves lung function, exercise tolerance, and overall quality of life
	Nutritional support	Starting enteral feeding within 48 h improves wound healing, reducing infection rates and minimize the stress response [35]
	Psychosocial support	Psychosocial support reduces stress, improving adjustment, and ensuring better clinical outcomes [36]

LVAD: left ventricular assist device; SPPB: short physical performance battery; 6WT: 6-min walking distance; DLCO: alveolar volume ratio of carbon monoxide diffusion capacity; PEG: percutaneous endoscopic gastrostomy; PGD: primary graft dysfunction; V-A ECMO: veno-arterial ECMO; CPB: cardiopulmonary bypass.

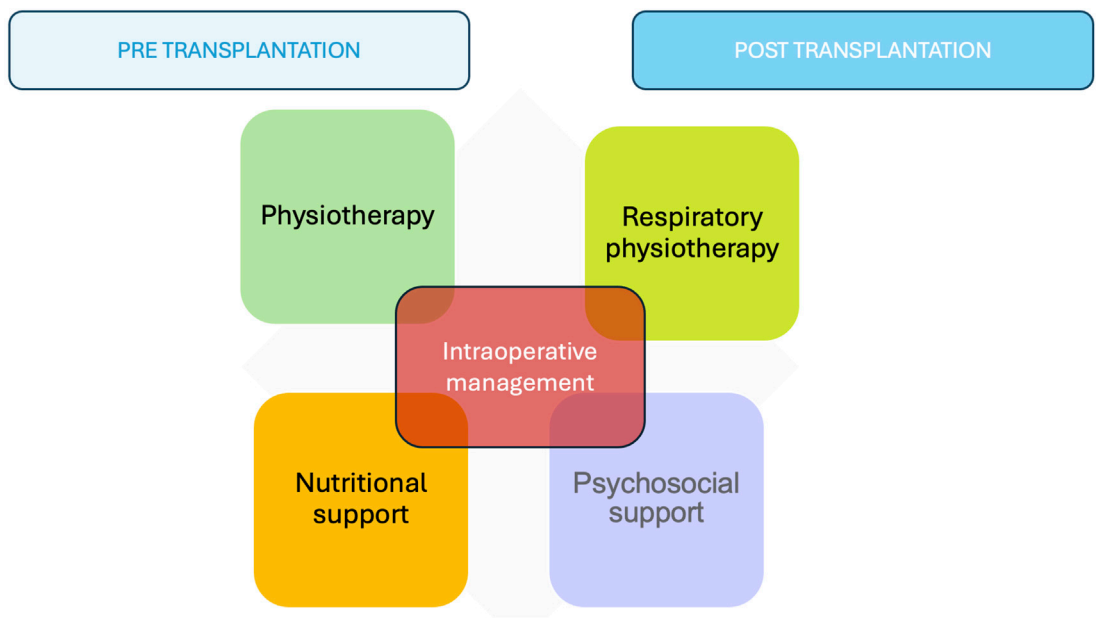

FIGURE 2 | The four arms implicated in the pre- and post-transplant periods.

showed that lower pretransplant VO2 max is associated with worse lung function and 3-year mortality after LTx.

Several studies have demonstrated the effects of physical therapy and respiratory physiotherapy [48]. The addition of the inspiratory muscle training [49] increased 6MWD by 100 m, improved the alveolar volume ratio of carbon monoxide diffusion capacity and maximum inspiratory pressure, and decreased the dyspnea score.

Nutritional Support

Malnutrition, resulting from insufficient energy and protein intake or hyper-catabolism, is frequent in patients who have been transplanted or are awaiting transplantation.

Routine nutritional screening is useful. The use of BMI as a metric of nutritional status is advantageous due to its ease of use, and correlation to outcomes based on BMI extremes. However, the use of BMI alone may lead to miscalculation of a candidate's true nutritional status [50].

The prevalence of heart failure-associated malnutrition is estimated to be up to 70%, with 15%–50% of patients globally being cachectic. Malnutrition is an independent risk factor for postoperative complications and mortality after HTx [51]. Nutritional supplementation has been reported to be beneficial for candidates for HTx. In a meta-analysis, Veronese observed that multi-nutrients significantly improved handgrip strength and chair rise time in frail/sarcopenic elderly patients [23].

The incidence of malnutrition in waitlisted LTx patients is near 40%. It is an independent risk factor for waitlist and post-transplant mortality [52, 53]. Congedi et al. [54] found a correlation between pre-transplant serum albumin values and the duration of invasive mechanical ventilation and ICU stay.

Patients with cystic fibrosis (CF) often experience malnutrition due to factors like malabsorption and increased energy expenditure. A high-calorie, high-fat, nutrient-dense diet is recommended to meet their energy and nutritional needs. Despite aggressive nutritional interventions, studies have shown limited improvements in body mass index (BMI) or fat-free mass before transplantation [55]. Systemic sclerosis (SSc) patients frequently face gastrointestinal complications leading to malnutrition, which can adversely affect transplant eligibility and outcomes. Comprehensive nutritional assessments are essential to identify deficiencies and implement appropriate interventions [56]. In both CF and SSc populations, individualized nutritional plans and close collaboration with dietitians are imperative to optimize transplant success and enhance patient outcomes.

Optimal and individualized nutritional management thus appears indispensable both pre- and post-transplant. Boura [22] applied the recommendations of the French Speaking Society of Clinical Nutrition and Metabolism to pre- and post-transplant patients and observed maintenance in BMI. Nutritional management of LTx candidates and recipients should include a global nutritional assessment to correct or prevent nutritional deficiencies, support the healing process for surgical wounds, and optimize nutrient stores to strengthen the immune system. In certain patients, such as those with CF or SSc, percutaneous endoscopic gastrostomy (PEG) tubes play a crucial role in managing malnutrition, particularly when oral intake is insufficient. Studies have demonstrated that PEG feeding is well-tolerated in CF patients, leading to significant improvements in weight, body mass index, and stabilization of pulmonary function over time [57]. Patients with SSc who underwent PEG insertion experienced substantial weight gain and enhanced nutritional parameters. Moreover, PEG feeding can be crucial in managing severe swallowing dysfunction in SSc, providing a reliable route for nutrition when oral intake is compromised [24].

Psychosocial Support

Depression, anxiety, and general distress are common among cardiothoracic transplant candidates and persist in many patients following transplantation. Psychosocial evaluation and support enable care planning and the provision of interventions to improve patients' viability as transplant candidates and facilitate post-transplantation care to support optimal psychosocial and medical outcomes.

The transplant candidate faces various events and stressors throughout the evaluation and waitlist periods. Specific stressors associated with the evaluation include uncertainty about suitability for transplantation and concerns about changes to future life plans. Smith et al. [58] found that depression and distress were associated with increased mortality.

The guidelines for ERAS in thoracic surgery [6] strongly recommend counselling and patient empowerment. Rosenberg [23] defends cognitive-behavioral therapy as a way to reduce psychosocial distress.

Intraoperative Period

Anesthesia Management

An extended, holistic, and comprehensive role for anesthesia care is needed throughout the entire perioperative period in the ERAS era for cardiothoracic transplantation. The new trend focuses on preserving allograft quality, maintaining cardiovascular stability, and preventing extrapulmonary complications [26].

Preparation for Anesthesia

Anesthesia premedication for heart and lung transplantation requires careful consideration due to the patients' compromised cardiopulmonary function and the complexity of the procedures. The consensus emphasizes minimizing sedative premedication to reduce the risk of respiratory depression and hemodynamic instability. Any necessary premedication should be administered in a controlled setting with appropriate monitoring to ensure patient safety [25]. Patients scheduled for lung transplantation typically have compromised respiratory function. To avoid exacerbating respiratory depression, sedative premedication is usually minimized or avoided. The focus is on maintaining adequate ventilation and oxygenation preoperatively [59]. In both heart and lung transplantation cases, the anesthetic plan should be tailored to the individual patient's condition.

Mechanical Ventilation

Mechanical ventilation (MV) strategies in heart and lung transplantation aim to protect lung function. Intraoperative

ventilation practice should include low tidal volume, recruitment maneuvers, and appropriate PEEP. Lung protective strategies should also consider driving pressures and stress index. The ventilation of allografts should avoid high FiO2 to reduce the potential for hyperoxia and oxidative stress [26].

Bleeding Management

Bleeding management during heart and lung transplantation within an ERAS protocol focuses on minimizing blood loss and transfusion requirements to improve patient outcomes.

Physical methods or locally active hemostatic measures may reduce bleeding and should be considered. The adverse immune effects suggests red cell transfusions should be minimized, platelet transfusion based on counts alone should be avoided and frozen plasma is not indicated unless haemorrhage is uncontrolled. Catastrophic surgical bleeding may be replaced in the 1:1:1 ratio based on the major trauma setting [26]. Other measures like fibrinogen concentrate, prothrombin complex concentrates or the antifibrinolytic aprotinin could be used. Recombinant Factor VIIa has demonstrated thrombotic events and shouldn't be used.

Minimizing Development of Primary Graft Dysfunction (PGD)

All efforts of anaesthesia management should be undertaken to control intraoperative risks of PGD.

Reduction of pulmonary hypertension and pulmonary vascular resistance remains a primary objective throughout all phases of lung transplantation to optimize right ventricular function and graft perfusion. Avoiding cardiopulmonary bypass (CPB) when feasible is one of the most effective strategies for minimizing postoperative morbidity in lung transplant recipients. However, in cases of severe and persistent cardiorespiratory instability, the timely initiation of CPB or VA-ECMO should not be delayed to prevent hemodynamic deterioration. The use of inhaled nitric oxide (iNO) as a sole agent for reperfusion therapy is not recommended. Nevertheless, it may serve as an adjunctive component of hemodynamic management, particularly for pulmonary artery pressure regulation and the mitigation of shunt circulation during reperfusion [26].

Extubation Management

The cornerstone of anaesthesia care in cardiothoracic transplantation is early extubation, which reduces postoperative complications such as pneumonia associated with MV, sarcopenia, prolonged mechanical ventilation time, and decreased cardiac performance. The early extubation period is variable, some authors consider early extubation the timeframe between 6 and 8 h after surgery or 4 h after the arrival at the ICU [60]. In any case, prolonged MV is defined as the need for mechanical ventilation for more than 24 h [61].

Totonchi [62] showed in a randomized controlled trial (RCT) the feasibility of early extubation in cardiac surgery after mechanical circulatory support (MCS) thanks to a combination of inhalational-intravenous anesthesia, maintaining an adequate anesthesia depth and reducing the total dose of anesthesia through a multiple monitoring system. Kianfar [27] demonstrated the benefits of early extubation after HTx, which included decreased ICU length of stay, (ICU LOS) fewer days on MV, and similar survival rates. Fessler [28] published findings on the effects of early extubation after LT in selected patients. They observed a lower incidence of primary graft dysfunction (PGD), shorter MV time, shorter ICU LOS, and potentially increased survival rates.

The use of short-acting drugs combined with thoracic epidural analgesia, the avoidance of excessive fluid support, the maintenance of normothermia, and the systematic application of postoperative noninvasive ventilation allows for optimal early extubation management in selected patients after cardiothoracic transplantation.

Analgesia Management

In cardiothoracic surgery, postoperative pain control is mandatory to facilitate mobilization of secretions and decrease the number of reintubations and respiratory complications such as atelectasis or pneumonia. Thoracic epidural anesthesia is recommended in LTx [26]. McLean [63] demonstrated shorter MV time, ICU LOS, less opioid consumption, and no neurological complications or epidural hematomas despite the high rate of MCS (89.5%) with a preoperative thoracic epidural.

Surgical Technique

Minimally invasive surgery (MIS) is the gold standard approach in thoracic surgery. MIS shows significantly lower morbidity rates and shorter hospital stays in patients undergoing VATS lobectomy compared with open thoracotomy [6]. Fischer [64] described in 2001 the video-assisted minimally invasive approach in bilateral LT. Marczin [65] and Thomas [29] demonstrated better outcomes, showing less blood or platelet transfusion, decreased median days of MV, shorter ICU LOS, and improved lung function after transplantation. Emerson [66] described the first eight cases of robotic lung transplantation with similar outcomes.

In cardiac surgery, minimally invasive cardiac surgery has increased in prevalence, showing less hospital mortality, lower 30-day mortality, fewer renal complications, postoperative infections, and atrial fibrillations in some minimally invasive approaches such as valve replacement. [67, 68]. However, in heart transplantation, the only significant attempt to reduce the biological impact of the surgery is to minimize the use of cardiopulmonary bypass to the strict necessary switching to ECMO as soon as it is required and reducing the blood losses to reduce the need of blood products [69].

The choice of the correct anticoagulant, the sparing of vasodilators in patients on the High Urgency List to reduce the risk of postoperative vasoplegia, and the proactive management of preoperative anemia are valuable strategies during the waitlist period. Careful separation using pre-emptive ECMO support may avoid dreadful prolonged phases of postoperative low-output states requiring fluids, vasoconstrictors, and the need for postoperative continuous renal replacement therapy (CRRT) [70, 71] From this perspective, the team managing the recipient must design the

patient's entire journey, considering the risks related to the organ allocated, its preservation, the donor-recipient matching, and the recipient's features.

The use of intraoperative extracorporeal life support (ECLS) in lung transplantation is a controversial issue. Strategies vary from center to center, ranging from off-ECLS to CPB or ECMO (V-V or V-A). The International Consensus Recommendations for Anesthetic and Intensive Care Management of Lung Transplantation [26] recommend avoiding cardiopulmonary bypass during LTx when it's possible. However, using ECLS should not be delayed in severe and ongoing cardiorespiratory instability cases.

The American Association for Thoracic Surgery expert consensus [72] suggest that the use of routine V-A ECMO should be implemented in lung transplantation in order to control the graft reperfusion and decrease PGD, however they accept the need of randomized prospective clinical-trial to confirm it [70]. Van Slambrounck et al. [73] demonstrated in a retrospective study the benefit of right-first implantation to reduce PGD grade 3 without ECLS. They defend [74] the benefit of holistic approach increasing the space thanks to ribs and diaphragm retraction, arterial clamping probe and gradual reperfusion, short clamping left atrium avoiding external compression and short implant time.

The use of V-A ECMO instead of CPB has shown improved rates of PGD and decreased rates of morbidity [30].

Post-Operative Period

Mechanical Circulatory Support

Successful weaning from ECLS after cardiothoracic transplantation is a critical process influenced by various factors. Studies have highlighted that implementing standardized protocols, such as a stepwise weaning protocol guided by echocardiography, can significantly improve weaning success rates and patient outcomes [31] Factors affecting successful weaning include daily echocardiography, circulatory support with dobutamine, longer ECLS duration, older age, female gender, low preoperative glomerular filtration rate, and hemodynamic monitoring post-extracorporeal cardiopulmonary function [75]. Integrating these findings into an ERAS process for cardiothoracic transplantation could enhance successful weaning outcomes by focusing on tailored protocols, comprehensive monitoring, and patient-specific factors.

Patients who cannot wean off ECMO may benefit from the awake ECMO strategy, allowing them to remain physically active and avoid the complications associated with invasive mechanical ventilation. Studies indicate that this approach leads to better postoperative outcomes, such as shorter ICU stays, more ventilator-free days, and improved physical condition [32], thus aligning well with ERAS goals of promoting early mobilization and recovery.

Post Operative Pain Management

Optimal pain management post-cardiothoracic transplantation is crucial for patient outcomes. Multimodal pain management strategies, including regional anesthesia and systemic analgesics, are recommended to reduce postoperative morbidity and mortality. The postoperative pain treatment is crucial for early rehabilitation. Effective treatment involves regional analgesia combined with a multimodal approach as quickly as possible orally. Thoracic epidural analgesia is often considered the gold standard due to its effectiveness and associated benefits, although some prefer less invasive techniques like chest wall blocks. The use of these regional analgesia techniques aims to minimize opioid use, enhance patient comfort, and promote faster recovery [33, 76].

Early extubation of patients may benefit from early analgesia strategies with continuous local anesthetic infusion, while those remaining ventilated may have delayed regional analgesia.

Chest Drains Management

Recent research on chest tube management after thoracic transplantation highlights the importance of standardizing protocols to optimize patient outcomes and reduce recovery time. In thoracic surgery, the key elements include minimizing the duration of chest tube placement, promoting early mobilization, and utilizing modern drainage systems. Batchelor [6] highlights the importance of early chest tube removal, no routine suction, and the use of digital drainage systems to monitor and manage air leaks and fluid outputs. This approach facilitates early mobilization and reduces the need for opioid analgesia, contributing to better postoperative outcomes [77].

Early Mobilization, Physical Therapy and Chest Physiotherapy

Early mobilization and physical therapy are critical components of postoperative care in thoracic transplantation, playing a vital role in ERAS protocols. They enhance physical and mental recovery, reduce complications, and contribute to a quicker and more efficient recovery process. Early mobilization, involving out-of-bed activities and ambulation, helps to maintain physical fitness and reduces the risk of complications such as respiratory infections and muscle atrophy.

In the context of ECMO for cardiopulmonary failure, early mobilization has been shown to be safe and feasible, even with femoral cannulation, and is associated with improved transplant outcomes [34].

Postoperative physical therapy significantly improves skeletal muscle function, exercise capacity, and quality of life. Rozenberg [15] highlighted that rehabilitation programs are beneficial in optimizing physical function and aiding recovery postoperatively. Weight gain, hypertension, diabetes, dyslipidemia, and hyperglycemia rank among the five most common morbidities after lung transplantation. Exercise training and regular physical activity may be effective in reducing the incidence of metabolic syndrome [78].

Respiratory physiotherapy plays a significant role in managing patients after thoracic transplantation, particularly lung transplantation, by improving lung function, exercise tolerance, and overall quality of life.

Kerti et al. [79] demonstrated significant improvements in chest wall expansion, lung function, and quality of life markers

with perioperative pulmonary rehabilitation in lung transplant patients.

Nutritional Support

Post-operative nutritional support can enhance recovery, reduce complications, and improve the quality of life for HTx and LTx patients.

Anbar et al. (2003) emphasized the importance of early postoperative nutritional support in improving wound healing and reducing infection rates in transplant recipients. Data from Lopez-Baamonde [80] demonstrated the effectiveness of a prehabilitation multimodal program based on an intervention designed to enhance functional capacity (with exercise training and promotion of physical activity), nutritional counseling (and supplementation), and psychological resilience Ikeda et al. [35] demonstrated that early postoperative nutritional support following LTx helps to suppress weight and muscle loss, thereby enhancing recovery. The comprehensive care outlined by Sriram [81] and Francisco José et al. (2012) further underscores the necessity of nutritional optimization in preventing malnutrition, muscle wasting, and infection, which are critical for the successful outcome of thoracic transplants. Bannister (2014) highlighted the role of nutritional support in promoting growth and energy balance in pediatric HTx recipients, showing improvements in weight-for-age and a transition from tube to oral feeding post-transplant.

Starting enteral feeding within 48 h after transplantation helps to minimize the stress response and maintain gut integrity with high-protein, caloric-dense formulas to meet the increased metabolic demands with supplementation with essential vitamins and minerals to support healing and immune function. When enteral nutrition is not feasible, a balanced mix of amino acids, lipids, glucose, vitamins, and minerals tailored to the patient's need for parenteral formula should start as soon as possible [35, 81].

Psychosocial Support

Posttransplant psychological interventions are crucial as they directly influence medical outcomes and overall recovery. Recommendations highlight the importance of addressing psychological domains during the posttransplant recovery period, as illustrated in the works of Patel and Chernyak [82], who emphasize the need for comprehensive psychological rehabilitation. Moreover Sher [36]discusses the persistent challenges of depression and anxiety post-transplant and their effects on graft survival and patients. Integrating psychosocial support into pulmonary rehabilitation programs, both pre- and post-transplant, further underscores its importance in reducing stress, improving adjustment, and ensuring better clinical outcomes.

CONCLUSION

The use of ERAS protocols in cardiothoracic surgery has demonstrated promising results in improving patient outcomes, reducing hospital stays, and minimizing opioid use. However, despite these advancements, the adoption of ERAS protocols in the field of transplantation remains limited and under-investigated. This gap in the literature requires further comprehensive research to confirm the effectiveness and safety of ERAS protocols in this patient population. Additionally, it is critical to establish evidence-based guidelines tailored to the unique perioperative challenges of cardiothoracic transplantation. Such guidelines would standardize care, improve recovery processes, and ultimately enhance the quality of life for transplant recipients.

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CONFLICT OF INTEREST

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GENERATIVE AI STATEMENT

The authors declare that no Generative AI was used in the creation of this manuscript.

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Contrast-Enhanced Ultrasound to Assess Kidney Quality During *Ex Situ* Normothermic Machine Perfusion

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Normothermic machine perfusion (NMP) provides opportunity for viability assessment of donated kidneys. Diminished microvascular perfusion, despite adequate total blood flow, is a key pathophysiology in ischaemia-mediated acute kidney injury. Contrast-enhanced ultrasound (CEUS) could allow objective assessment of microvascular perfusion during renal NMP. Blood-based NMP was performed on porcine kidneys (circulatory death model) and human kidneys declined for transplant (preclinical). CEUS was performed with a contrast bolus into the NMP circuit arterial limb. Microvascular perfusion quality was quantified and z-score normalisation allowed combination of metrics and regions into an overall "CEUS-score." In porcine kidneys, inferior microvascular perfusion of cortex and medulla correlated with increased urinary NGAL (Neutrophil gelatinase-associated lipocalin) and histological DNA-fragmentation (a hallmark of apoptosis). In human kidneys, CEUS-score at 2 h was correlated with histological DNA-fragmentation ($r = -0.937$; $P = 0.019$) and predicted urinary NGAL at 24 h of NMP ($r = -0.925$; $P = 0.024$). Total renal flow was not correlated with these outcomes. An open-source web application (stingle.shinyapps.io/Time_intensity_analysis) and R package ("tican") were developed for quantitative time-intensity curve analysis. CEUS allows objective point-of-care microvascular perfusion assessment during NMP. As 2-hour CEUS-score predicts NGAL at 24 h, CEUS warrants future clinical investigation as a potential tool to assess kidney quality in assessment and reconditioning centres.

Keywords: kidney transplantation, machine perfusion, contrast enhanced ultrasound, viability assessment, assessment and reconditioning, time intensity curve, ischaemia reperfusion, R package

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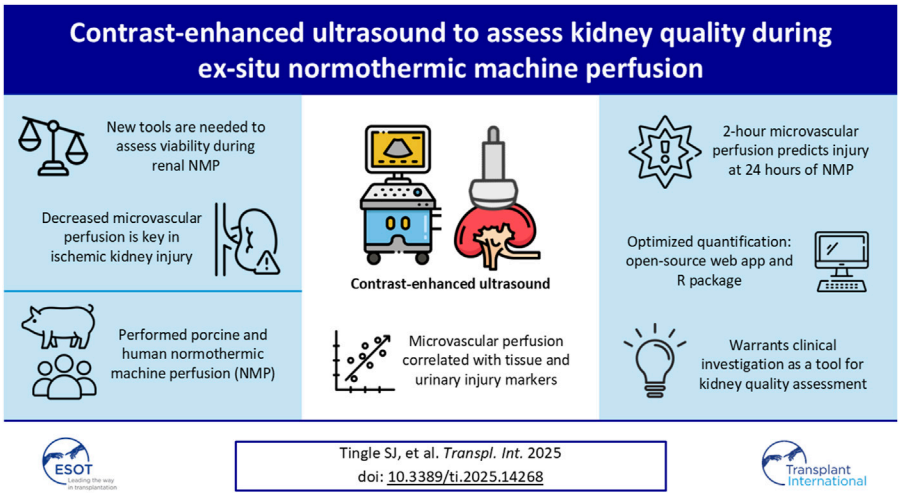
Abbreviations: AKI, Acute Kidney Injury; ARC, Assessment and Reconditioning Centre; CEUS, Contrast-Enhanced Ultrasound; CIT, Cold Ischaemic Time; IRI, Ischaemia Reperfusion Injury; MSB, Martius Scarlet Blue; NGAL, Neutrophil Gelatinase-Associated Lipocalin; NMP, Normothermic Machine Perfusion; ORC, Organ Recovery Centre; PI, Peak Intensity; ROI, Region of Interest; TTP, Time-to-Peak; TUNEL, TdT-mediated dUTP Nick-End Labelling; WIT, Warm Ischaemic Time.



GRAPHICAL ABSTRACT |

INTRODUCTION

International disparities between waiting list demands and availability of suitable donor organs drive new approaches to increase the donor pool. As such, there is an increased use of marginal organs from extended criteria donors. Technologies that can improve selection, allocation and utilisation of organs with confidence are vital to ensure we meet the demands of the waiting list. *Ex situ* normothermic machine perfusion (NMP) of isolated organs offers an optimal platform for viability assessment of marginal kidneys prior to transplantation.

The majority of the validated tools used to assess organ quality during machine perfusion have been based on biochemical read-outs, blood flow rate or urine output [1]. In renal transplantation the "Quality Assessment Score" combines urine output by 1 h, with renal blood flow and visual assessment of global perfusion at 1 h [2, 3]. However, this did not correlate with kidney transplant outcomes in a large randomised controlled trial, driving the need for improved assessment modalities [4].

Poor renal microvascular perfusion, despite adequate total renal blood flow, is increasingly seen as one of the hallmarks of renal ischaemia reperfusion injury in the setting of acute kidney injury (AKI) [4, 5]. Contrast-enhanced ultrasound (CEUS) is an imaging technique commonly used in clinical practice, including post-liver and kidney transplantation, to assess microvascular perfusion [6]. CEUS utilises the infusion of microbubbles of sulphur hexafluoride in a phospholipid shell into the circulation. These bubbles are small enough to reach the capillary bed but not small enough to pass out into the interstitium therefore giving a global picture of tissue perfusion. This avoids the radiation and nephrotoxicity of alternative imaging/contrast techniques [6].

In our group, we have experience of using contrast enhanced ultrasound (CEUS) during hypothermic machine perfusion of kidneys and we have developed this technology to apply it to normothermic machine perfusion of both livers and kidneys [7, 8]. We have also previously demonstrated that CEUS was a valuable tool in the assessment of a cellular therapy delivered during machine perfusion [9]. However, no study to date has assessed the validity of the use of CEUS as a viability tool during renal NMP. This study aimed to develop CEUS as a potential viability assessment tool for human kidneys undergoing NMP.

MATERIALS AND METHODS

Porcine Circulatory Death Model

All animals were euthanised by overdose of anaesthetic according to schedule 1 of the United Kingdom Animals (Scientific Procedures) Act 1986. Use of animals and collection of kidneys for these studies was approved after a full ethical review by Newcastle Universities Animal Welfare and Ethical Review Board and ongoing review via study plan approval (AWERB number 854, study plan 38). Porcine kidneys were retrieved from 60 kg 16-week-old white landrace pigs. These were sedated using intramuscular injection of approximately 5 mL Tiletamine and Zolazepam (Zoletil™, Virbac). Pigs were then euthanised using ear vein injection of 25 mL (Euthatal™, Dopharma Research B.V.). Approximately 500 mL of blood was collected using standard clinical blood bags depleted of CDPA-1 and filled with 10,000 IU heparin and 50 mL saline (Fresenius Kabi). Kidneys were retrieved and kept in the body cavity until 25 min after confirmation of death, then flushed with 1 L of 4°C University of Wisconsin solution with 25,000 IU sodium heparin (Panpharma). This standardised the warm ischaemic time (WIT) to 25 min. Kidneys were kept on ice for 16 h of cold ischaemic time (CIT) prior to initiation of machine perfusion. All porcine kidneys reported here came from different pigs.

We chose a WIT of 25 min followed by CIT of 16 h following a previous series of optimisation experiments which explored a range of ischaemic times (data not published). These ischaemic times provide sufficient ischaemia to mimic the injury seen during NMP of extended criteria human kidneys. This is the same protocol used in our previous studies of therapeutic delivery during porcine NMP [10].

Human Kidneys

Human kidneys retrieved for transplant but then deemed unsuitable were included. Ethical approval for accepting these kidneys was granted by the national research ethics commission in the United Kingdom, National Research Ethics System (15/SC/0161). We gained approvals for this project from the National Health Services Blood & Transplant's (NHSBT) Research Innovation and Novel Technologies Advisory Group (RINTAG), who oversee the allocation of such research organs to authorized research groups. In all cases donor families provided generic consent to approved research projects.

Machine Perfusion

NMP was performed using a customised Medtronic pediatric cardiopulmonary bypass system. The renal artery was cannulated, and oxygenated perfusate with red blood cells was perfused at 37°C (continuous flow), aiming for a mean arterial pressure of 75 mmHg. Porcine and human perfusions differed in terms of their duration (6 h and 24 h respectively), source of blood (autologous whole blood versus packed red cells) and perfusate constituents. The increased duration in human versus porcine experiments reflects the growing international research interest in prolonged kidney perfusion and the recent establishment of these extended NMP protocols in our own perfusion laboratories [11]. Full protocols and lists of perfusate constituents for the two protocols is given in **Supplementary Tables S1, S2**. Total renal blood flow was measured using the Medtronic flow sensor (TX50P flow transducer, Medtronic). Urine production rate was measured via a paediatric nasogastric tube tied into the ureter. Oxygen consumption was calculated from blood gas and flow data, as previously described [12].

CEUS

Ultrasound was performed with an eL18-4 probe of the Philips EPIQ7 Ultrasound machine with QLab 8.1 software (Philips). The probe (with sterile cover) was placed directly onto the kidney and held stationary to capture a longitudinal view of the kidney.

For CEUS imaging, 1 mL sulphur hexafluoride contrast agent (SonoVue®, Bracco) was reconstituted with 4 mL of perfusate in a syringe. The contrast agent was then administered via a three way tap to the arterial limb of the circuit as a rapid bolus. A detailed standard operating procedure for capturing these cine loop recordings is available in **Supplementary Table S3**. We analysed CEUS scans from 6 h of perfusion (end of perfusion) for porcine kidneys and 2 h of perfusion for human kidneys. Each scan represents a single recording following a single bolus injection of contrast (recording was continued for a minimum of 60 s after contrast was seen reaching the kidney).

The built-in Philips "Contrast" mode was used; this is optimised for capturing microbubble contrast whilst minimising signal from any human tissue. When using the settings detailed in our standard operating procedure (**Supplementary Table S3**) this resulted in zero or negligible background signal, eliminating the need for normalisation of peak intensity to baseline value.

CEUS Quantification Analysis

Raw CEUS DICOM files were imported into QLAB Advanced Quantification Software (Release 15.5 Philips) and analysed using the ROI QApp to get mean intensity for various regions of interest. A 5 × 5 mm square was used for the cortex. A freeform polygon was used to draw further regions of interest around the medulla. This was split equally into outer medulla (closest to the kidney surface) or inner medulla (closest to kidney hilum). **Figure 1** displays drawing the regions of interest.

All cineloops were cut so that time zero was the frame that contrast was first seen (in segmental/interlobar arteries). All clips were cut to 30 s total length (selected as this is significantly longer than the time required for all regions to reach peak intensity). The ROI QApp then calculated the mean contrast intensity in the various regions of interest, for each frame of the ultrasound loop. This raw data (mean pixel intensity in decibels on every timestamped frame) for each region of interest was then exported for downstream analysis.

Analysis of CEUS data was performed in R (R Foundation for Statistical Computing, Vienna, Austria) [13]. A curve was plotted to the raw data, using a LOESS smoother. This was performed using the loess() base R function with loess.span set to 0.06 [13]. Data was extracted from this curve to calculate the peak intensity of contrast and time-to-peak intensity.

Assays for Tissue DNA Fragmentation and Urinary NGAL

A TUNEL (TdT-mediated dUTP Nick-End Labelling) assay was performed on 4 μm FFPE sections. The DeadEnd™ Fluorometric TUNEL System (Promega) was carried out according to manufacturer's instructions. Slides were mounted using VECTASHIELD mounting medium with DAPI (Vector labs). Cells with DNA fragmentation (as a hallmark of apoptosis) and DAPI-stained nuclei were counted using Fiji ImageJ software. Persons performing TUNEL assay and image analysis were blinded to CEUS data.

Neutrophil gelatinase-associated lipocalin (NGAL) concentration in urine was analysed by ELISA (DuoSet Cat: DY1757 for human, Abcam Cat: ab207924 for porcine). This was multiplied by urine production rate to get total nanograms of urinary NGAL per minute.

Quantifying Red Cell Aggregates

Martius Scarlet Blue (MSB) staining was used to visualise erythrocytes, red cells, fibrin and collagen. Following dewaxing and rehydration, tissue was stained using a Martius Scarlet Blue

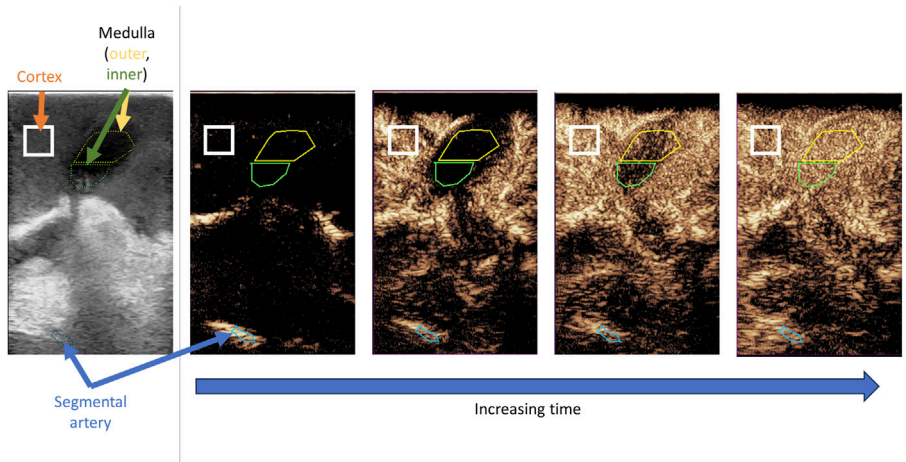


FIGURE 1 | Representative contrast enhanced ultrasound loop with regions of interest selected. Standard B mode ultrasound image (left panel) is used to ensure cortex and medulla are in view. As time progresses there is sequential perfusion of segmental artery, cortex, outer medulla and inner medulla.

Stain Kit (Atom Scientific) according to manufacturer's instructions. LABKIT, a Fiji ImageJ plugin for segmentation of microscopy images was used to create a pixel classifier [14]. This enabled automatic segmentation of fibrin rich red cell aggregates (representative images in **Supplementary Figure S1**), which could be used to calculate the percentage of each image containing such aggregates.

Statistical Analysis

To generate a single score for each region normalised values were required; z-scores for peak intensity and time-to-peak were therefore calculated [15]. The z-score for time-to-peak (TTP) could then be subtracted from the peak intensity (PI) z-score, such that the score for each region was calculated as follows:

$$Region\,CEUS\,score = \frac{\text{Sample PI} - \text{cohort mean PI}}{\text{cohort standard deviation PI}} - \frac{\text{Sample TTP} - \text{cohort mean TTP}}{\text{cohort standard deviation TTP}}$$

A table of the cohort average and standard deviation for peak and time-to-peak for each region, which were used to calculate these normalised z-scores, is given in **Supplementary Table S4**. As these are all normalised and on the z-score scale, scores for the three regions were added to generate an overall score for each kidney [15].

The correlation between CEUS metrics and NMP outcomes was assessed using the Pearson correlation coefficient. All statistical analyses were performed in R [13].

RESULTS

An annotated example CEUS cine loop can be viewed in the **Supplementary Video**. In total, 8 porcine kidneys and 5 human kidneys were included. Porcine kidneys came from 8 separate female donors. 3 kidneys were left and 5 were right.

CEUS Is Associated With Urinary NGAL and Tissue Apoptosis in Porcine Kidneys

As shown in **Figures 2A, B**, perfusion of the cortex and medulla was correlated with urinary NGAL, an important predictor of kidney quality during NMP [16, 17]. Increasing time-to-peak (indicating worse microvascular perfusion) was associated with higher levels of damage marker NGAL ($r = 0.90$, $P = 0.002$ and $r = 0.745$, $P = 0.034$ for cortex and outer medulla respectively).

Similarly, improved quality of perfusion in these two regions was associated with a lower proportion of cells with DNA fragmentation (a hallmark of apoptosis) on histology (**Figures 2C, D**). Representative TUNEL images from kidneys with relatively poor versus good microvascular perfusion are shown in **Supplementary Figure S2**. There was no significant correlation between any CEUS metric and 6-hour urine flow rate.

Negative Correlation Between Medullary Perfusion and Total Blood Flow Suggests Shunting

In the setting of AKI, shunting of blood through the kidney without parenchymal perfusion has been described as a key pathophysiological factor [4, 5]. We therefore correlated total blood flow through the kidney with parenchymal perfusion at an identical timepoint.

As shown in **Figure 3A** there was no significant correlation between cortex perfusion and total renal blood flow. However, there was a strong negative correlation between the quality of microvascular perfusion of the medulla and total renal blood flow (**Figures 3B, C**), indicating that more severe injury leads to

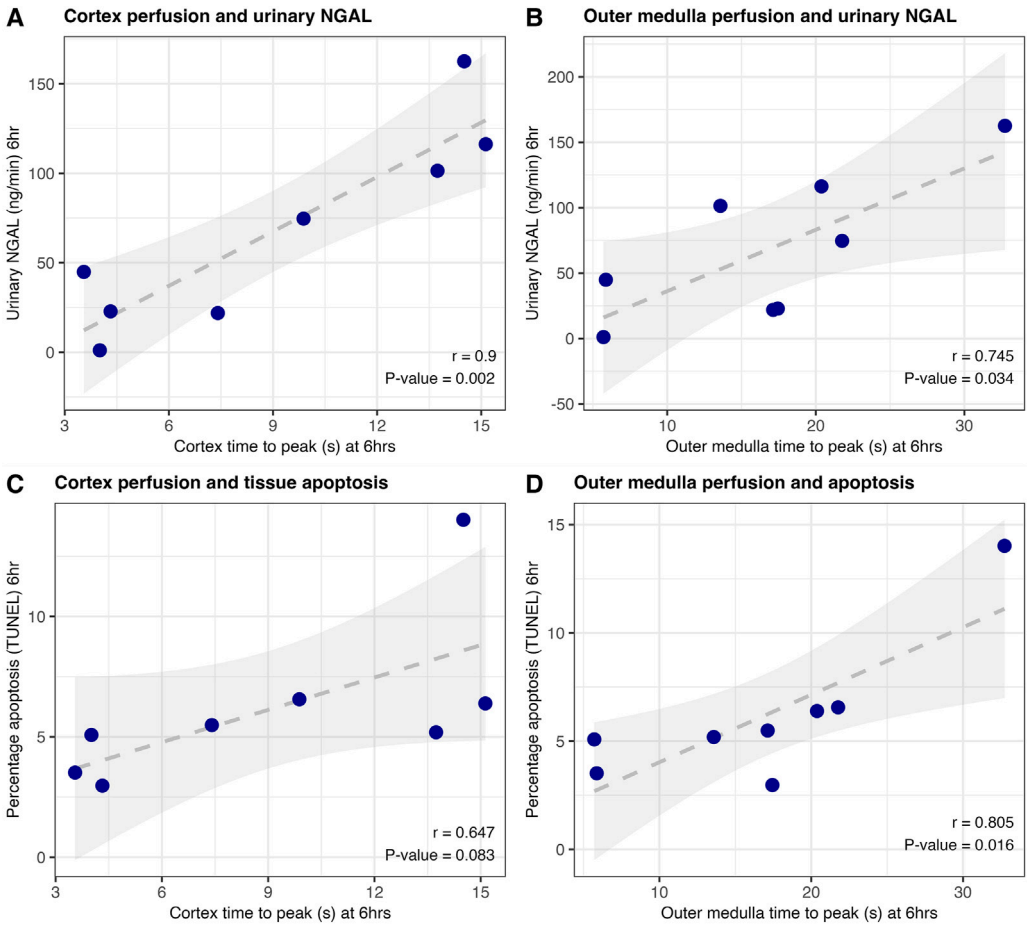


FIGURE 2 | Correlation of CEUS results and urinary NGAL **(A, B)** or tissue DNA fragmentation (a hallmark of apoptosis) on TUNEL staining **(C, D)** at end of porcine machine perfusion (6 h); n = 8. Pearson correlation coefficient and associated p-value are displayed.

shunting through low resistance vessels, with increased total flow but decreased parenchymal perfusion.

One potential contributing factor to the lack of microvascular perfusion is the presence of capillary obstruction by red cell aggregates, which have been reported during NMP previously [18, 19]. We found no correlation between burden of red blood cell microvascular occlusion and microvascular perfusion of the cortex or medulla at 6 h (**Supplementary Figures S1, S3**). Potentially indicating the role of shunting as opposed to occlusion.

Validation of CEUS in Human Kidney Cohort

Human kidneys from five deceased donors were included. Donor demographics are provided in **Table 1**. Three kidneys were rejected due to extra-renal malignancy, one due to presence of glomerulosclerosis on biopsy and CIT, and one due to a significantly calcified aortic patch. None of the donors received normothermic regional perfusion.

The association of CEUS metrics with both tissue DNA fragmentation and urinary NGAL was assessed. Mirroring the porcine results, improved microvascular perfusion was correlated with lower levels of tissue DNA fragmentation, and lower levels of the damage marker NGAL (**Supplementary Figures S4, S5** respectively). We also assessed associations between 2-hour CEUS score and oxygen consumption; those with signs of improved cortex and medullary perfusion showed increased oxygen consumption by the kidney (**Supplementary Figure S6**). There was no significant correlation between CEUS metrics and urine flow rate or renal blood flow at the time of the scan, or at 24 h.

Correlating Human Kidney CEUS Region and Overall Scores With Tissue Apoptosis and NGAL

To generate a single CEUS score for each region, which could be combined to give an overall CEUS score for each kidney, z-score normalised peak and time-to-peak values were calculated. A table of the cohort means and standard deviations which were used to calculate these normalised z-scores, is given in **Supplementary Table S4**.

As shown in **Figures 4A–C** these region scores have a negative correlation with tissue DNA fragmentation;

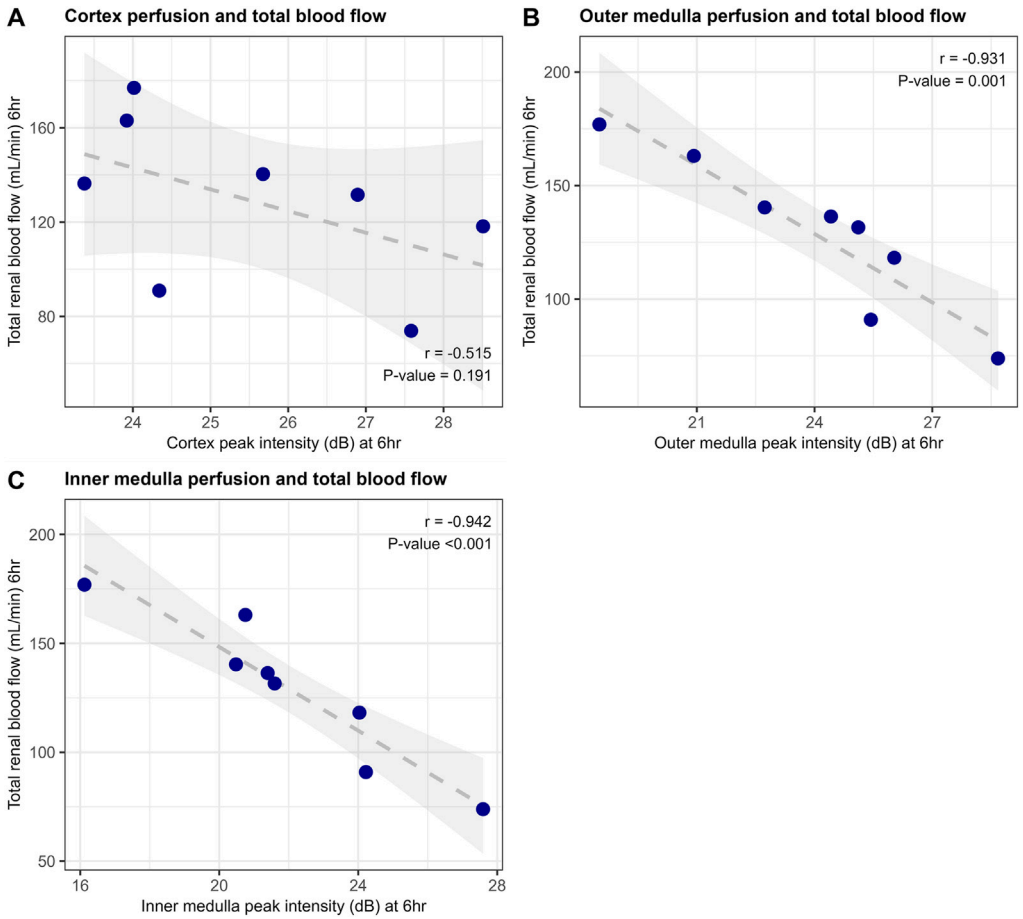


FIGURE 3 | Correlation of end of machine perfusion (6 h) total renal blood flow and CEUS microvascular perfusion of cortex **(A)**, outer medulla **(B)** and inner medulla **(C)** in porcine kidneys (n = 8).

TABLE 1 | Cohort demographics, with one column per human donor.

Variable	Demographics for each kidney				
Cold ischaemia time (hours)	15.5	18	29	29.5	13
Donor age	42	72	73	73	79
Donor sex	F	F	M	M	F
Donor type	DBD	DCD	DBD	DBD	DCD
WIT (WLST to aortic cold flush)	N/A	104	N/A	N/A	29
Donor hypertension	No	Yes	No	Yes	No
Creatinine at retrieval (μmol/L)	166	50	77	76	55
Creatinine at admission (μmol/L)	98	68	83	80	48
Cause of death	HBD	HBD	HBD	ICH	ICH
Quality of cold perfusion (retrieval surgeon)	Good	Good	Good	Good	Good
UKDRI 2019[a]	1.09 (D2)	1.95 (D4)	2.08 (D4)	1.79 (D4)	2.11 (D4)
Quality assessment score[b]	3	3	4	3	2

DBD, donation following circulatory death; DCD, donation following brainstem death; HBD, hypoxic brain death; ICH, intracranial haemorrhage.
[a]UK kidney donor risk index 2019 version; score and quartile given where D1 is the best quartile and D4 is the worst quartile [35].
[b]Quality assessment score at 1 h as described in Hosgood et al. [2].

kidneys with more DNA fragmentation at the start of perfusion displayed worse perfusion of both cortex and medulla at 2 h. The overall score combining the three regions of interest displayed the strongest correlation with the percentage cells with DNA fragmentation on histology (**Figure 4D**; Pearson r = - 0.937, P = 0.019).

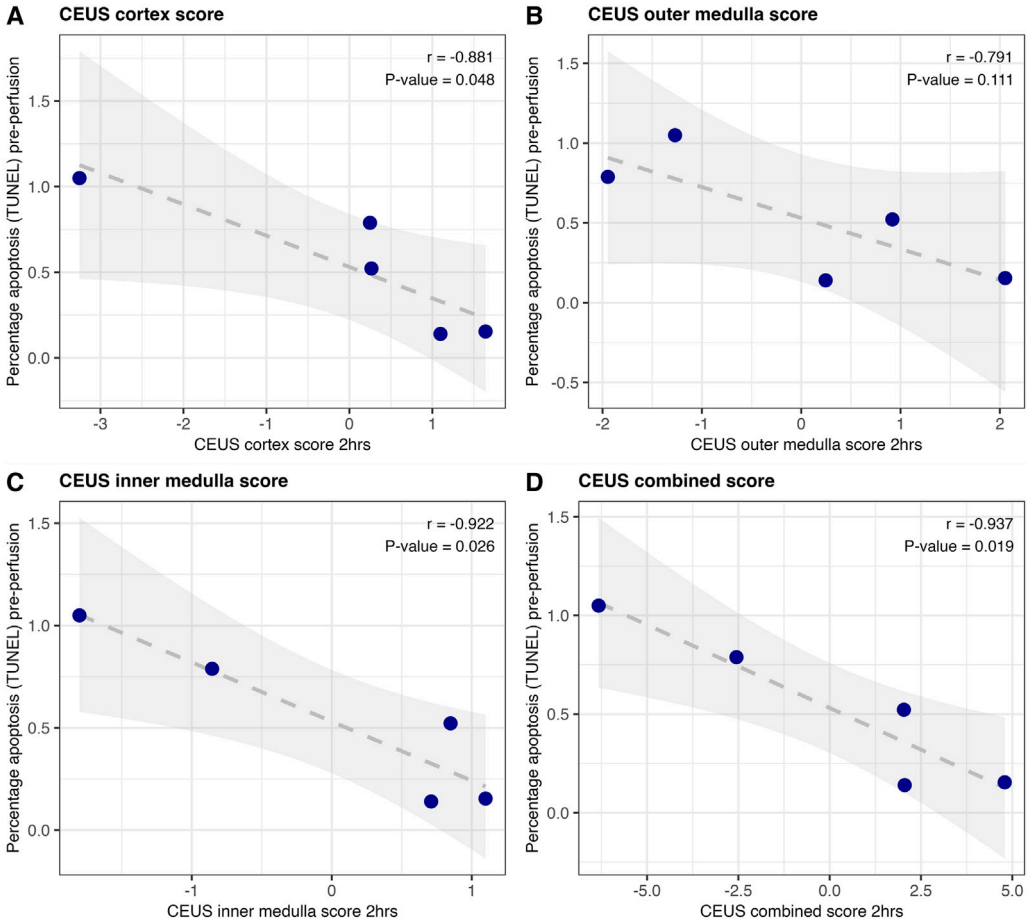


FIGURE 4 | Correlation of region scores with the percentage of cells with DNA fragmentation (a hallmark of apoptosis) on TUNEL at the beginning of human kidney perfusion (n = 5). Region scores for cortex **(A)**, outer medulla **(B)** and inner medulla **(C)** were calculated as peak intensity z-score minus time-to-peak z-score. The three were summed to generate an overall score for the kidney **(D)**. Pearson correlation coefficient and associated p-value are displayed.

CEUS scores at 2 h were also correlated against 24-h urinary NGAL. Improved CEUS scores at 2 h of NMP were corelated with lower urinary NGAL at the end of perfusion (24 h); **Figure 5**. This was statistically significant for both outer and inner medulla regions (Pearson r = −0.902, P = 0.036; Pearson r = −0.968, P = 0.007 respectively; **Figures 5B, C**), and for the overall CEUS score (Pearson r = −0.925, P = 0.024; **Figure 5D**). Neither CEUS region scores, nor CEUS combined score, showed a significant association with renal blood flow at the time of the scan (**Supplementary Figure S7**), or the 1-hour "quality assessment score" [2].

Creation of Web Application and R Package

Following the analyses described above, we wanted to make these techniques accessible to other groups. We have used the shinyapp framework [20] to create a freely available web application [21]. This allows any time-intensity data to be uploaded, and calculates peak and time-to-peak, with additional options for calculating area under the curve and time-to-peak proportion (**Figure 6A**). A report is also generated to visually confirm the results (**Figure 6B**). We have also created an R package "tican," which is freely available to import from CRAN [22], for those who wish to perform these time-intensity curve analyses using R code.

DISCUSSION

This preclinical study has demonstrated that the ability of contrast-enhanced ultrasound to assess microvascular perfusion can be applied to kidneys during NMP. The quality of microvascular perfusion was associated with tissue DNA fragmentation (TUNEL positive cells as a marker of apoptosis), as well as the damage marker urinary NGAL [17], in both porcine and preclinical human perfusions. Normalisation allows the combination of time-to-peak and peak intensity values into region and overall scores; in human perfusions these scores showed higher correlation with injury markers than any

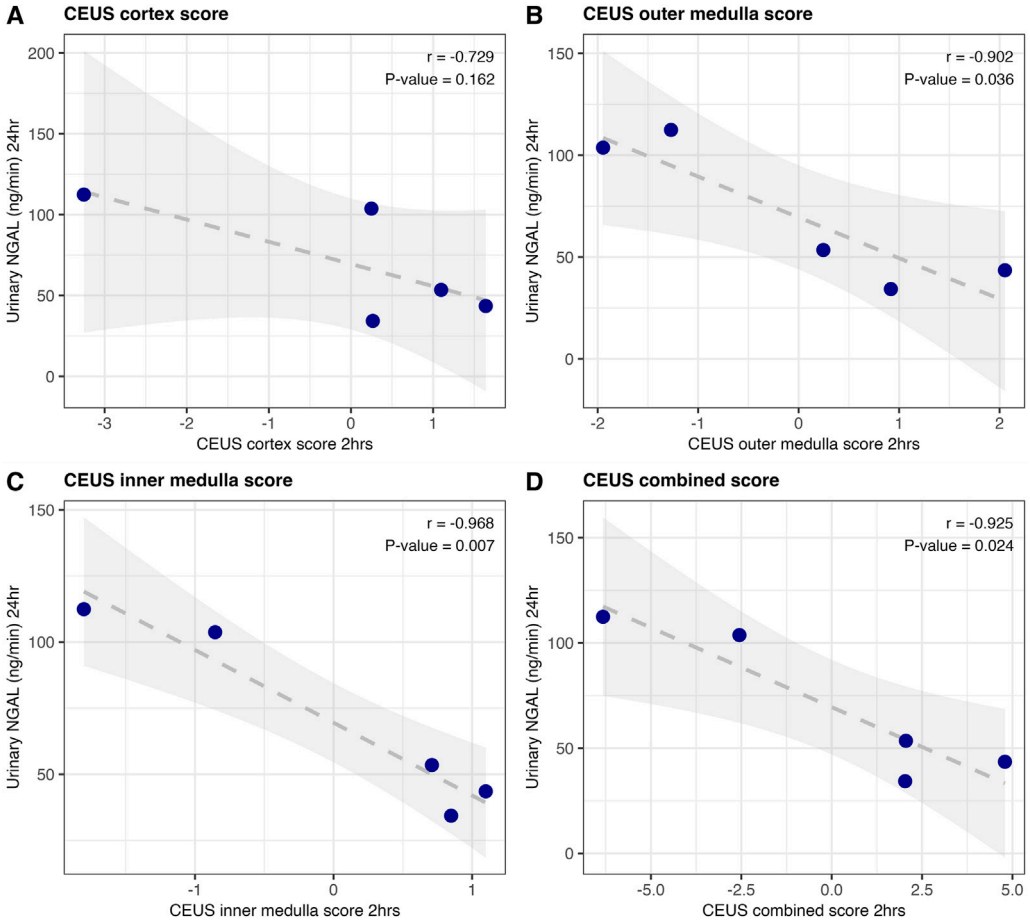


FIGURE 5 | Correlation of CEUS scores at 2 h, with urinary NGAL at 24 h of human NMP (n = 5). Region scores for cortex **(A)**, outer medulla **(B)** and inner medulla **(C)** were calculated as peak intensity z-score minus time-to-peak z-score. The three were summed to generate an overall score for the kidney **(D)**. Pearson correlation coefficient and associated p-value are displayed.

individual metric (**Figures 4**, **5** versus **Supplementary Figures S4, S5**). In human kidneys 2-hour CEUS-score was associated with the key biomarker urinary NGAL at 24 h [3, 17]. This is of particular interest as CEUS is a bedside, immediate, non-invasive test with a non-toxic contrast agent [6].

In recent years there has been increasing interest in assessment and reconditioning centres (ARCs) and organ recovery centres (ORCs) to deliver NMP, as a key strategy to assess and recondition marginal organs and increase the donor pool [23–26]. For the ARC concept to be successful, we require validated, real-time assessments of organ quality. These novel techniques are needed both for viability assessment alone, and for assessing potential improvements in organ quality after delivery of advanced therapies. We have previously shown that CEUS could be a powerful tool for assessing response to therapy [9].

Previous work on renal ischaemia reperfusion injury (IRI) provides the biological basis for microvascular perfusion assessment in this setting. Renal IRI is the core pathophysiology damaging organs during retrieval/preservation but is also the core pathophysiology in pre-renal AKI. Previous studies have described intra-renal shunting of blood away from the cortex/medulla to be a hallmark of renal IRI in AKI [4, 5, 27]. A recent study performed microvascular perfusion assessment on patients with AKI versus healthy controls, and found significant decreases in microvascular parenchymal perfusion, despite no change in total renal blood flow [5]. Our data support this; in the porcine setting improved total blood flow is associated with worse microvascular perfusion, which we hypothesise may be due to low resistance shunting. In the human kidneys we found no association between total blood flow and microvascular perfusion; as kidneys were perfused at a fixed pressure this confirms that inferior microvascular perfusion of cortex and medulla seen with CEUS is not related to global or large vessel resistance increases.

Several other viability assessment methods have been developed for use in renal NMP. The best-known is the "quality assessment score" [2, 3]. However, a recent large randomised controlled trial found no association between this score and transplant outcome [28]. Others have suggested potential perfusate or tissue biomarkers which correlate with

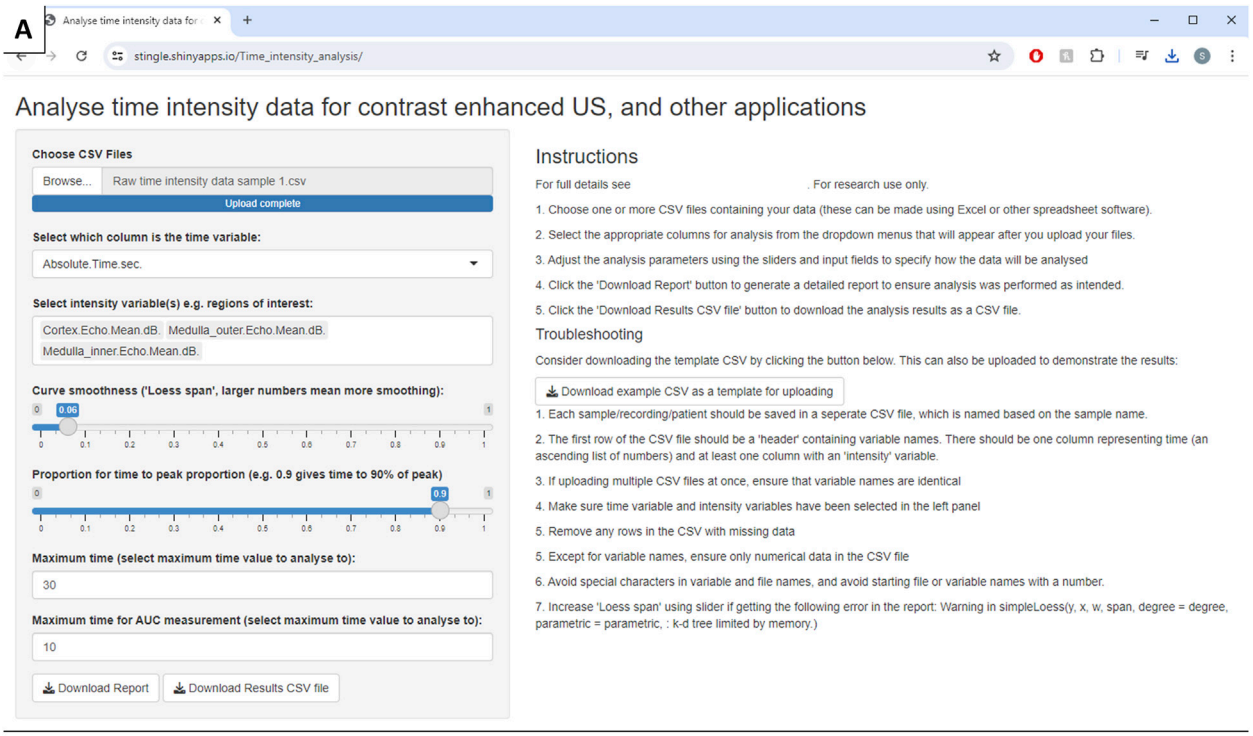


FIGURE 6 | Developed web application for analysis of time-intensity data. **(A)** Web application interface. **(B)** Example intensity time plots from CEUS report. Black dots represent raw data (mean contrast intensity in the given region of interest for that frame). The red line is a LOESS smoother through the raw data. Blue dotted line represents the peak intensity, and time-to-peak intensity, and green dotted line shows 90% of the peak intensity and time until 90% peak intensity.

outcome, such as markers of cell death or inflammation [1, 29]. However, these markers generally cannot be assessed in real time, do not measure function, have shown relatively weak correlation with outcome, and have been selected after screening of multiple potential markers without external validation [29].

This has driven recent interest in utilising imaging modalities to assess organs during NMP [30]. Methods such as MRI and CT have shown some promise, however these would be very challenging to deliver clinically during NMP, as well as introducing risks of nephrotoxic contrast and ionising radiation [30, 31]. In comparison, CEUS is simple to deliver as a point-of-care "bedside" test, gives immediate quantifiable results, and is entirely non-invasive and non-nephrotoxic. It is also relatively easy to perform and there is potential for this to be done by surgeons in the operating theatre prior to transplant. Compared to other modalities CEUS is also relatively inexpensive; whilst costs will vary internationally, in the UK setting the national tariff is less than $100 (USD) per CEUS scan.

To our knowledge this is the first study to focus on CEUS for renal NMP viability assessment, and the first study to assess CEUS for the potential viability assessment of any human organ [30]. Our group has previously applied CEUS during liver NMP in a pilot study, reporting arterial microcirculation improves over the duration of liver NMP [8]. The ability of CEUS to assess the viability of liver, and other perfused organs, is an interesting topic for future research. Novel techniques such as ultrasound localization microscopy offer far higher spatial resolution, which may further delineate microvascular changes during NMP [32]. However, this technique is not in routine clinical practice, as a result of significant disadvantages in terms of device and computing costs [33].

The analysis and quantification of CEUS data, or more broadly any time-intensity data, is relevant outside of the setting of transplantation [5, 31]. Our freely available open source web application (stingle.shinyapps.io/Time_intensity_analysis) and R package ("tican") could therefore be used in a wide range of research settings, to quantify time-intensity curve data for subsequent analysis [21, 22]. When analysing CEUS recordings, the region of interest assessment could be performed using the ImageJ/Fiji "ROI manager" [34], followed by the use of our tools, to make the entire analysis pipeline free and open-source.

The core limitation of this work is its preclinical nature and the lack of post-transplant data. Clinical validation of this technique is required to translate this preliminary work and assess the ability of CEUS to predict post-transplant outcome. We did not explore trends in CEUS scores over different timepoints in this study; we would be keen to explore CEUS trends in future clinical studies, as these may offer additional information capable of improving our ability to predict post-transplant outcome.

Due to small sample sizes, we focussed on correlation with a relatively small number of NMP outcomes to avoid type 1 error. We attempted to focus on the machine perfusion outcomes which currently have the best evidence for correlating with post-transplant outcome in human; urinary NGAL, urine flow and total blood flow [2, 3, 17]. However, there exists no accurate marker during NMP to act as a "ground-truth" to compare against [28]. Another limitation is the fact that CEUS scores generated here with z-score normalisation are likely specific to the perfusion protocol and imaging system used, and may require adaptation for use in alternative perfusion protocols or ultrasound machines.

In conclusion, CEUS allows point-of-care real-time assessment of microvascular perfusion during renal NMP, which is non-invasive and non-toxic. Techniques for quantification of CEUS were developed and disseminated via open-source software. CEUS-score at 2 h showed correlation with key biomarker urinary NGAL at 24 h of NMP in preclinical human kidneys. This warrants clinical research to assess the ability of CEUS during renal NMP to predict post-transplant outcome.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

All animals were euthanised by overdose of anaesthetic according to schedule 1 of the United Kingdom Animals (Scientific Procedures) Act 1986. Use of animals and collection of kidneys for these studies was approved after a full ethical review by Newcastle Universities Animal Welfare and Ethical Review Board and ongoing review via study plan approval (AWERB number 854, study plan 38). Human kidneys retrieved for transplant but then deemed unsuitable were included. Ethical approval for accepting these kidneys was granted by the national research ethics commission in the United Kingdom, National Research Ethics System (15/SC/0161). We gained approvals for this project from the National Health Services Blood & Transplant's (NHSBT) Research Innovation and Novel Technologies Advisory Group (RINTAG), who oversee the allocation of such research organs to authorized research groups. In all cases donor families provided generic consent to approved research projects.

AUTHOR CONTRIBUTIONS

ET, RF, and BS were responsible for initial study conception. CC, BG, EG, and ST were lead perfusionists for the included experiments. ST was responsible for analysis, developing methods for quantifying the scans, and development of the web application and R package. ST drafted the initial manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.14268/full#supplementary-material

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ORIGINAL RESEARCH
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T_1 Relaxation Time for the Prediction of Renal Transplant Dysfunction

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Quantitative magnetic resonance imaging (MRI) is emerging as a non-invasive tool to measure tissue scarring in renal allografts. However, whether prolonged T_1 relaxation time results in lower transplant survival rates is unknown. This retrospective cohort study analyzed the capability to predict renal allograft dysfunction based on median T_1 time. Forty-six transplant recipients with non-contrast 1.5T MRI and allograft biopsy were included. The primary endpoint was the eGFR slope over 24 months. T_1 relaxation time correlated significantly with eGFR levels at all follow-up stages. Patients with T_1 relaxation time above the median (T_1^{high}) had a consistent decline in kidney function as compared to the patient group below the median (T_1^{low}): overall eGFR slope: 11.3 vs. 1.4 mL/min/1.73 m^2 over 24 months, p = 0.016. Graft survival rates at 24 months were 52% in the T_1^{high} vs. 87% in the T_1^{low} group, p = 0.0015. ROC analysis discovered a positive predictive value of 52% and a negative predictive value of 91% for graft loss. T_1 mapping identified patients with a persistent decline of allograft function and an increased risk of allograft loss. MRI could significantly influence monitoring strategies in transplant surveillance, offering a safe, non-invasive alternative to traditional diagnostic methods.

Keywords: kidney transplantation, T1 relaxation time, allograft dysfunction, non-invasive, biomarkers

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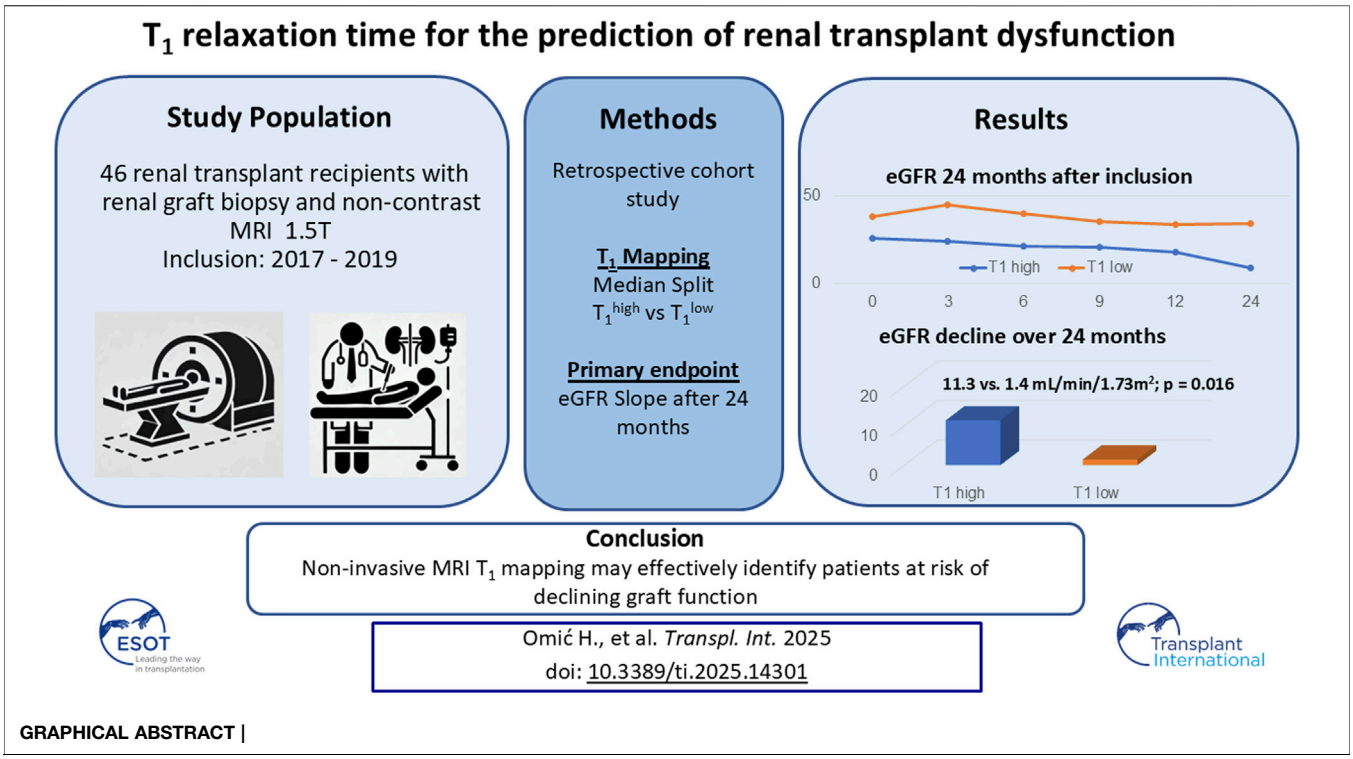
INTRODUCTION

Kidney transplantation is the preferred treatment for end-stage renal disease [1]. One cornerstone of mitigating renal allograft dysfunction lies in the early, accurate diagnosis of graft pathologies and prompt initiation of treatment. Ideally, a diagnostic tool should detect allograft dysfunction, differentiate between its etiologies, and monitor graft function throughout therapeutic interventions, all while minimizing patient risk.

Currently, percutaneous biopsies are the gold standard for diagnosing kidney allograft pathologies. However, the procedure is not without risks, including a significant complication

Abbreviations: AMR, antibody-mediated rejection; ADC, apparent diffusion coefficient; BKPyVAN, BK Polyomavirus associated nephropathy; ci, Banff score for interstitial fibrosis; ct, Banff score for tubular atrophy; cg, Banff score for double contours; cv, Banff score for vascular fibrous intimal thickening; DWI, diffusion-weighted imaging; HR, hazard ratio; GFR, glomerular filtration rate; IQR, interquartile range; LOCF, last observation carried forward; mo/mos, month/months; MRI, magnetic resonance imaging; ms, milliseconds; NPV, negative predictive value; PPV, positive predictive value; SD, standard deviation; TCMR, T cell-mediated rejection; TMA, thrombotic microangiopathy; Tx, transplantation.



GRAPHICAL ABSTRACT |

rate of up to 2% in transplanted kidneys [2, 3]. Biopsies are also susceptible to interobserver variability and sampling errors, which can compromise diagnostic accuracy [4, 5]. Furthermore, practical limitations such as anticoagulation therapy, hypertension, urinary infections, or simply the patient's subjective refusal may delay a biopsy and, consequently treatment initiation. Especially in the field of renal transplantation, where sequential biopsies are common, there is an emerging interest in exploring the potential of magnetic resonance imaging (MRI) as a complementary non-invasive diagnostic tool [6–11]. MRI is distinguished by its exceptional soft tissue contrast. Its evolution, particularly in enhancing temporal and spatial resolution, has broadened its application and allows assessing functional aspects of the kidney, including renal perfusion and tissue oxygenation [12–15].

In a recent study from our center, we demonstrated a significant correlation between advanced interstitial fibrosis (Banff ci) and high cortical T_1 [8]. T_1 was also significantly associated with other chronic lesion markers such as tubular atrophy (Banff ct), glomerular basement membrane double contours (Banff cg), and vascular intimal thickening (Banff cv). This implies that histological scarring leads to local microstructural magneto-chemical alterations, quantifiable by MRI [15, 16]. Similar findings were also reported by other studies exploring the relationship between apparent diffusion coefficient (ADC), T_1 and T_2 in various kidney allograft pathologies [17–19].

However, previous publications mostly focused on correlations between MRI and biopsy findings measured at one-time point cross-sectionally. The longitudinal assessment of allograft function in relation to T_1 values was studied to a much smaller extent. Due to less risk of sampling error in MRI assessments, it may be hypothesized that T_1 mapping could even exceed the prognostic value of histologically-quantified lesion markers.

A study from Berchtold et al. showed that ADC was able to predict the progression of interstitial fibrosis more reliably than serum creatinine alone [20]. Yet, to our knowledge, it is unexplored whether high T_1 subsequently precedes reduced allograft survival. To test this hypothesis, we analyzed the course of graft function in a group of 46 patients who underwent transplant biopsies and cortical T_1 mapping.

MATERIALS AND METHODS

Study Design and Patient Cohort

The aim of this retrospective cohort study was to analyze the course of renal allograft function in a group of 46 transplant recipients who underwent both MRI and transplant biopsy simultaneously. Thirty-two of those patients were included in our previous prospective study, which focused on assessing correlations between T_1 mapping, Banff lesion scores, and conventional graft function parameters [8]. The other fourteen patients underwent MRI before the initial study due to clinical indications and as part of a quality assurance protocol to test its basic feasibility.

Patients were screened for study inclusion at our outpatient clinic. Detailed inclusion criteria are provided in the study from Beck-Tölly et al. [8]. All suitable renal transplant patients scheduled for protocol or indication biopsies were actively asked for study participation. The main inclusion criteria were:

age over 18 years and an estimated glomerular filtration rate (eGFR) of more than 10 mL/min/1.73 m^2 (calculated using the Modification of Diet in Renal Disease formula). Exclusion criteria included MRI-incompatible metallic implants or pacemakers, claustrophobia, and pregnancy. Recruitment took place from December 2017 to January 2019. Non-contrast MRI scans were performed shortly before or after the biopsy, using a whole-body 1.5 T MR system (MAGNETOM Avanto Fit; Siemens Healthineers; Erlangen, Germany).

The primary endpoint was the course of graft function after assessment of baseline MRI T_1. Longitudinal graft function was calculated based on serum creatinine levels measured in a three-month interval over the period of 24 months after the MRI. To further quantify changes in kidney function, the eGFR delta (ΔeGFR) and eGFR slopes were calculated for each observation period.

The secondary endpoint was the frequency of death-censored graft loss in relation to baseline T_1. Graft loss was defined as the resumption of dialysis. All participants provided informed consent. Ethical approval for the study was granted by the institutional ethics committee (Approval No. 1893/2017). The study adhered to Good Clinical Practice guidelines, the principles of the Declaration of Helsinki, and the Declaration of Istanbul.

MRI

MRI protocols and methods used in this study have been described in detail elsewhere [8]. In short, we extracted T_1 measurements from our multiparametric MRI images, measured across three paraxial (cranial, middle, caudal) and three paracoronal (anterior, middle, posterior) planes, involving six independent regions of interest per plane. The median of those 36 measurements was defined as the overall median T_1 cortical relaxation time. The choice to focus this current analysis on T_1 was based on results from preceding research, which estimated kidney function based on T_1 in patients with glomerulonephritis [21], as well as one study quantitatively evaluating renal function and renal fibrosis in patients with chronic kidney disease [22].

Biopsy

Morphologic lesions were assessed on formalin-fixed paraffin-embedded sections using standard methodology [8]. Banff single lesions and rejection phenotypes were scored based on the Banff 2017 scheme [23]. In addition to Banff criteria, chronic structural damage in kidney grafts was assessed using the chronicity index as described by Haas et al. [24]. This index combines four key histological features: interstitial fibrosis (ci), tubular atrophy (ct), vascular fibrous intimal thickening (cv), and chronic glomerulopathy (cg). Each feature was scored on a scale from 0 (no changes) to 3 (severe changes), with the chronic glomerulopathy score being doubled. The total chronicity index ranged from 0 to 15, with higher scores indicating more significant chronic injury.

Statistical Analysis

Continuous variables were reported as means with standard deviations (SD) or medians with interquartile ranges (IQR). Categorical variables were summarized as counts and percentages. The median split method was employed to divide patients into two groups of equal size based on the overall T_1. Hence, the "T_1^{high}" group referred to patients with T_1 values above and the "T_1^{low}" group for patients with T_1 values below the median. Spearman´s correlation coefficients were used to analyze the associations between T_1 and baseline variables, including transplant age, baseline eGFR, and the histological parameters ci, ct—as well as the chronicity index. To compare the predictive validity of Banff ci scores with T_1, Fisher's Z transformation was performed.

The linear mixed-effects model was performed to analyze the changes in the estimated glomerular filtration rate (eGFR slope) over time between the groups.

To compare graft survival, the Kaplan-Meier survival curve and log-rank test were calculated. To address the loss of graft function and the subsequent missing data points in our longitudinal follow-up, we implemented the "last observation carried forward" (LOCF) imputation method. Additionally, the Receiver Operating Characteristic (ROC) analysis was performed to evaluate the ability of T_1 to predict the occurrence of allograft loss. The p-value of <0.05 was considered statistically significant.

Statistical computations and analyses were conducted using SPSS for Mac Version 20 (SPSS Inc., Chicago, IL), GraphPad Prism (GraphPad Prism 10.0.3 (217) Macintosh Version by Software MacKiev © 1994–2023 GraphPad Software, LLC), R (R Core Team, 2023) and RStudio (2022 by Posit Software, PBC).

RESULTS

Study Population

Forty-six patients were included, 30 (65%) were male; the mean age at transplantation was 54.3 ± 14.8 years (mean ± SD). Baseline parameters of the total group and the subgroups (T_1^{high} and T_1^{low}) are displayed in **Table 1**. The majority of patients (80.4%) received deceased donor kidneys. The median time from transplantation to study inclusion was 3 years (IQR 0.7–11.2). Six (13%) participants underwent magnetic resonance imaging before [4 ± 2.5 days, (mean ± SD)] and 38 (82.6%) after (7.9 ± 9 days) the biopsy. Two (4.4%) patients had the MRI on the day of the biopsy. The median cortical T1 was 1,369 ms (IQR 1,279–1,511). The median eGFR at the time of biopsy was 30.8 mL/min/1.73 m^2 (IQR 20.1–49.6). Fourteen (30.4%) patients reached the endpoint graft loss. Four patients (8.6%) were lost to follow-up before the end of our observation period of 24 months.

Biopsy Findings

Thirty-seven biopsies (80.4%) were performed based on clinical indications, primarily due to the deterioration of graft function, while the other nine biopsies (19.6%) were protocol biopsies. In 14 (30.4%) biopsies, graft rejection was diagnosed (see **Table 1**). The overall rate of rejections was equally distributed between the T_1^{high} and T_1^{low} groups (30.4% each, $p > 0.99$). Antibody-mediated rejection (AMR) was numerically but not significantly higher in the T_1^{high} group (26.1% vs. 13%, p =

TABLE 1 | Baseline parameters of the study population.

Variable	Total n = 46	T_1^{high} n = 23	T_1^{low} n = 23	P-value
Male sex, n (%)	30 (65.2)	21 (91.3)	9 (56.2)	**<0.01**
BMI, mean ± SD	25.5 ± 3.7	25.8 ± 3.9	25.3 ± 3.7	0.72
Recipient age (years), mean ± SD	54.3 ± 14.8	54.2 ± 17.3	54.4 ± 12.3	0.95
Deceased donor, n (%)	37 (80.4)	19 (82.6)	18 (78.3)	0.50
First transplantation, n (%)	34 (73.9)	18 (78.2)	16 (69.6)	0.43
Biopsy after Tx (years), median (IQR)	3 (0.7 to 11.2)	3 (1 to 12)	1 (0 to 9)	0.26
Protocol biopsy n (%)	9 (19.6)	1 (4.3)	8 (34.8)	0.02
HLA mismatch, median (IQR)	3 (2 to 4)	3 (2 to 4)	2 (2 to 3)	0.21
Rejection diagnosed in biopsy, n (%)	14 (30.1)	7 (30.4)	7 (30.4)	>0.99
AMR	9 (19.6)	6 (26.1)	3 (13.0)	0.45
TCMR	5 (10.9)	1 (4.3)	4 (17.4)	0.34
Borderline TCMR	3 (6.5)	0 (0.0)	3 (13.0)	0.23
Banff1A	1 (2.2)	0 (0.0)	1 (4.3)	>0.99
Banff2A	1 (2.2)	1 (4.3)	0 (0.0)	>0.99
BKPyVAN	3 (6.5)	0 (0.0)	3 (13.0)	0.23
TMA	1 (2.2)	1 (4.3)	0 (0.0)	>0.99
eGFR 3 m before biopsy, (mL/min/1.73 m^2), median (IQR)	31.7 (22.1 to 54.0)	28.6 (22.1 to 60.8)	34.9 (22.6 to 50.3)	0.92
eGFR 1 m before biopsy, (mL/min/1.73 m^2), median (IQR)	32.3 (23.5 to 49.0)	27.3 (17.9 to 43.6)	42.0 (26.9 to 51.9)	0.08
eGFR at biopsy, (mL/min/1.73 m^2), median (IQR)	30.8 (20.1 to 49.6)	25.56 (19.5 to 43.3)	37.9 (22.1 to 53.2)	0.20
Proteinuria (mg/g), median (IQR)	484.5 (130.5 to 1,750.25)	1717 (365 to 2,914)	193 (101 to 665)	**<0.01**
Albuminuria (mg/g), median (IQR)	209 (32.5 to 1,256.5)	1,200 (164–2,710)	68 (14.8 to 229)	**<0.01**
ΔeGFR 3 m (mL/min/1.73 m^2), median (IQR)	−1.9 (−7.1 to 3.4)	−6.3 (−11.4 to 0.0)	1.6 (−2.8 to 5.6)	**<0.01**
ΔeGFR 6 m (mL/min/1.73 m^2), (Median [IQR])	−3.9 (−8.7 to 2.2)	−7.2 (−14.4 to −5.1)	0.5 (−1.6 to 2.5)	**<0.01**
ΔeGFR 12 m (mL/min/1.73 m^2), (Median [IQR])	−6.3 (−12.4 to −0.4)	−8.2 (−15.7 to −6.1)	−1.8 (−8.4 to 8.4)	**<0.01**
ΔeGFR 24 m (mL/min/1.73 m^2), (Median [IQR])	−9.3 (−16.6 - 1.9)	−13.1 (−25.3 to −7.5)	0.6 (−11.8 to 6.7)	**<0.01**
Graft loss after 24 m, n (%)	14 (30.1)	12 (52.17)	2 (8.70)	**<0.01**

Abbreviations: AMR, Antibody-mediated Rejection; BMI, Body Mass Index; BKPyVAN, BK Polyomavirus-Associated Nephropathy; m, months; eGFR, CKD-EPI-estimated glomerular filtration rate; HLA, Human Leukocyte Antigen; IQR, interquartile range; mL, milliliter; MRI, Magnetic Resonance Imaging; TCMR, T-cell-mediated Rejection; TMA, Thrombotic microangiopathy.

Bold values indicate significant differences.

0.45). The T cell-mediated rejection (TCMR) frequency also did not differ significantly between both groups (4.3% vs. 17.4%, p = 0.34). Twenty-six allografts (56.6%) exhibited high-grade interstitial fibrosis (ci 2 or 3), and in 18 kidneys (39.1%), high-grade tubular atrophy (ct 2 or 3) was found (**Supplementary Table S1**). Allografts in the T_1^{high} group had more severe interstitial fibrosis: 47.8% with ci 3 compared to 21.7% in the T_1^{low} group (p = 0.044). Tubular atrophy was also more advanced in the T_1^{high} group (ct 3: 30.4% versus 8.7% in the T_1^{low} group, p = 0.031). Although not statistically significant, arterial intimal thickening showed higher severity in the T_1^{high} group (52.2% at cv 2 compared to 34.8% in the T_1^{low} group, p = 0.059). The severity of glomerular basement membrane double contours (cg), did not differ between the groups; cg grades 2 or 3: 22.7% in the T_1^{high} group vs. 14.2% in the T_1^{low} group (p = 0.14). Chronicity index differed significantly between the groups: T_1^{high} 8.5 (5–11) vs. 3 (IQR 2.5–6.5) in the T_1^{low} group, p < 0.01.

Correlation of T_1 With Histology and Baseline Variables

There was a significant positive correlation between median T_1 and interstitial fibrosis (ρ = 0.36, p = 0.01) as well as tubular atrophy (ρ = 0.45, p < 0.01). Further on, the chronicity index correlated positively with T_1 (ρ = 0.46, p < 0.01). No significant correlation was found between median T_1 and the time since transplantation (ρ = 0.20, p = 0.16). T_1 did not correlate with median eGFR at baseline (ρ = −0.25, p = 0.09, see **Figure 1**).

Analysis of Graft Function in Relation to T_1

In the T_1^{high} group, eGFR levels consistently declined over time. At baseline, the T_1^{high} group had a median eGFR of 25.6 [19.6–43.3 (median, IQR)], compared to 37.9 (22.1–53.2) mL/min/1.73 m^2 in the T_1^{low} group (p = 0.21) in the T_1^{low} group (p = 0.20). Across all other time points, the T_1^{high} group experienced a significant and steady decrease in eGFR (**Figure 2**). The ΔeGFR between various time points (0–3, 0–6, 0–12, and 0–24 months) indicated a significant decline in graft function in the T_1^{high} group over all time points. At 3 months, the ΔeGFR was −6.3 (−11.4 to 0.0) mL/min/1.73 m^2 in the T_1^{high} group and 1.6 (−2.84 to 5.62) mL/min/1.73 m^2 in the T_1^{low} group (p < 0.01). At 24 months, the T_1^{high} group had a ΔeGFR of −13.0 (−25.3 to −7.48) mL/min/1.73 m^2 compared to the T_1^{low} group with 0.6 (−11.80 to 6.68) mL/min/1.73 m^2 (p < 0.01, see **Table 1**).

Correlation of Graft Function and T_1

We analyzed the correlation between median T_1 and eGFR values over time. A significant inverse relationship was found between T_1 and eGFR at different time points. At 3 months, the correlation between T_1 and eGFR was moderate (ρ = −0.42, p < 0.01). This negative correlation continued at 6 months (ρ = −0.38, p < 0.01), 12 months (ρ = −0.43, p < 0.01), and remained stable at 24 months (ρ = −0.41, p < 0.01). Fisher's Z transformation

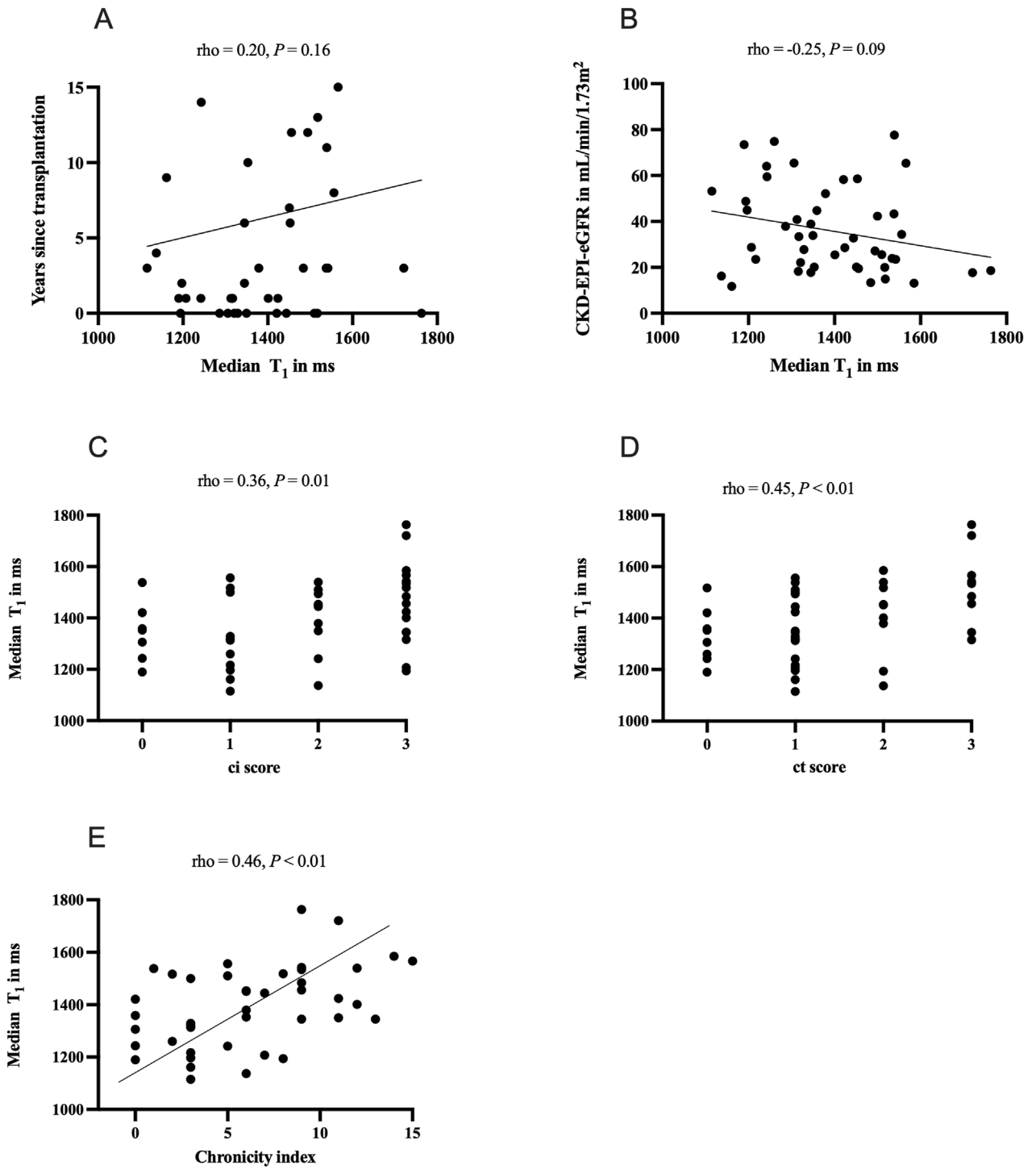


FIGURE 1 | Correlations of clinical and histological parameters and T_1 relaxation times: panel **(A)** correlation of time since transplantation and median T_1 in ms; panel **(B)** correlation of baseline estimated glomerular filtration rate (CKD-EPI-eGFR) and median T_1 in ms; panel **(C)** correlation of interstitial fibrosis (Banff ci score) and median T_1 in ms; panel **(D)** correlation of tubular atrophy (Banff ct score) and median T_1 in ms, panel **(E)** correlation of chronicity index and T_1 median in ms. The chronicity index described by Haas et al. [24] combines interstitial fibrosis (ci), tubular atrophy (ct), vascular fibrous intimal thickening (cv), and chronic glomerulopathy (cg).

analysis between T_1 and ci association with graft function revealed no significant differences, showing that T_1 is similarly correlated with kidney function as the established ci score (details see **Supplementary Table S2**). In the subgroup, including only patients who underwent protocol biopsies, we also found significant correlations between T_1 and eGFR at months 3 ($\rho = -0.71$, p =

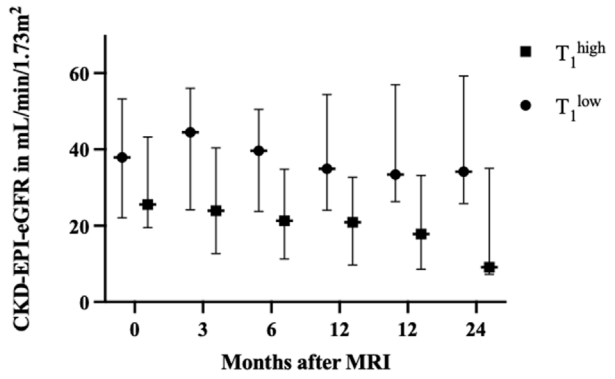


FIGURE 2 | Renal graft function during the follow-up period, compared between the T_1^{high} and T_1^{low} groups. No differences were observed at baseline. By 3 months, the T_1^{high} group's median estimated glomerular filtration rate (eGFR) was 23.9 (12.7–40.4) mL/min/1.73 m^2, compared to 44.5 (24.2–56.01) mL/min/1.73 m^2 in the T_1^{low} group (p = 0.011). At 6 months, the T_1^{high} group's median eGFR was 21.34 (11.3–34.8) mL/min/1.73 m^2 compared to 39.6 (23.8–50.5) mL/min/1.73 m^2 in the T_1^{low} group (p = 0.007). This trend continued, with the T_1^{high} group having a significantly lower median eGFR at 9 months (20.9 [9.7–32.7] mL/min/1.73 m^2) than the T_1^{low} group (34.9 [24.1–54.4] mL/min/1.73 m^2, p = 0.007). By 12 months, the T_1^{high} group's median eGFR had decreased to 17.8 [8.6–33.1] mL/min/1.73 m^2, compared to 33.4 [26.3–56.9] mL/min/1.73 m^2 in the T_1^{low} group (p = 0.006). This significant decline persisted at 24 months, where the T_1^{high} group had a median eGFR of 9.1 (7.3–35.0) mL/min/1.73 m^2, whereas the T_1^{low} group maintained a median of 34.1 (25.8–59.2) mL/min/1.73 m^2 (p = 0.005). Values of eGFR are shown as median with whiskers indicating the interquartile range. Abbreviations: MRI: magnetic resonance imaging, CKD-EPI-eGFR: estimated glomerular filtration rate calculated with CKD-EPI equation, in mL/min/1.73 m^2.

0.047), 9 (ρ = −0.81, p = 0.015), 15 (ρ = −0.81, p = 0.015), 18 (ρ = −0.74, p = 0.037), 21 (ρ = −0.81, p = 0.015), and 24 (ρ = −0.83, p = 0.010) (see **Supplementary Tables S3, S4** for details).

eGFR Slope

The baseline (month 0) eGFR intercept for the T_1^{low} cohort was 39.9 mL/min/1.73 m^2, while the T_1^{high} group had a baseline eGFR intercept that was 9.20 units lower (p = 0.096). Over time, the T_1^{low} group showed a slight, non-significant decline in eGFR at a rate of 0.06 mL/min/1.73 m^2 per month (p = 0.63). In contrast, the T_1^{high} group experienced a significantly steeper decline, with an additional 0.41 units per month (p = 0.016) compared to the T_1^{low} group. This resulted in a total eGFR decline of 11.31 mL/min/1.73 m^2 for the T_1^{high} group and 1.40 mL/min/1.73 m^2 for the T_1^{low} group over 24 months (**Figure 3**).

ROC Analysis

We used ROC analysis to assess if T_1 can be used as a predictive marker for renal allograft loss (**Figure 4**). T_1 above the median resulted in a PPV for predicting graft loss of 52.2% with an AUC of 0.75, p = 0.007. Conversely, the NPV was 91.3%. T_1 demonstrated a sensitivity of 100% across the lower cutoff values, specifically from ">1,126 ms" to ">1,317 ms". At the cutoff of ">1,317 ms", the sensitivity slightly decreased to 92.9%, while the specificity saw a substantial increase, indicative of fewer false-positive results. At ">1,337 ms", sensitivity is still 92.86%, but specificity has increased to 53.1%. At ">1,352 ms", the sensitivity remained at 92.86%, and the specificity increased further to 62.5%. The analysis identifies T_1 ">1,352" ms as an optimal cutoff point in our patient cohort for balancing sensitivity and specificity in a clinical setting.

Survival Analysis and Kaplan-Meier Curve

The Kaplan-Meier survival analysis revealed significant differences in graft survival between the groups (**Figure 5**). After 12 months, all kidney transplants in the T_1^{low} group were still functioning, compared to 91.3% in the T_1^{high} group. This difference became more pronounced over time, with survival rates of 91.3% versus 60.9% at 21 months and 87.0% versus 52.2% after 24 months (Log-rank test, p = 0.0015, **Figure 5**). A T_1 above the median was a significant risk factor for graft loss (HR 7.3, 95% CI: 2.6–21.0). The cortico-medullary difference of the T_1 (ΔT_1) was available in 32 patients. Patients without graft loss had a mean ΔT_1 of −337.13 ms, while those with graft loss had a mean of −251.81 ms, with no significant differences (p = 0.417).

DISCUSSION

We had hypothesized that T_1, as measured by MRI, could serve as a reliable non-invasive biomarker for predicting kidney allograft dysfunction. T_1 mapping is an emerging tool to quantify high-grade interstitial fibrosis in renal allografts [11, 15, 25]. Yet, little is known about the prognostic relevance of T_1, a prerequisite for broader use as a non-invasive surveillance tool.

As a major finding of our study, we were able to show that elevated cortical T_1 not only correlates with histological markers for chronic lesions but can also predict worsening allograft function. Patients with T_1 above the median had eGFR levels comparable to the T_1^{low} group at baseline but significantly worse graft function across all follow-up intervals. We further compared the predictive power with established markers of chronic allograft injury, such as interstitial fibrosis. The Z scores, ranging from −0.08 to 1.03, indicate that the correlation of T_1 with eGFR levels is slightly lower than that of Banff ci across all time points. Yet, the magnitude of the Z scores suggests that these differences are small and not significant, highlighting the potential utility of T_1 mapping as an accurate, non-invasive alternative to quantify chronic allograft injuries.

Similar results were previously published by Bane et al., where, as part of a multiparametric MRI, T_1 and diffusion-weighted imaging (cortical ADC values) allowed good prediction of eGFR decline after 18 months [17]. Yet, in comparison to our study, only 12 patients with allograft dysfunction underwent biopsies, and those were performed at more variable time intervals. With the higher sample size and a longer follow-up period of our study, we were not only able to confirm the findings from Bane et al. but showed that also cortical T_1 alone allows a decent prediction of graft function during midterm follow-ups. As the measurement of cortical T_1 times alone is less time-demanding as a

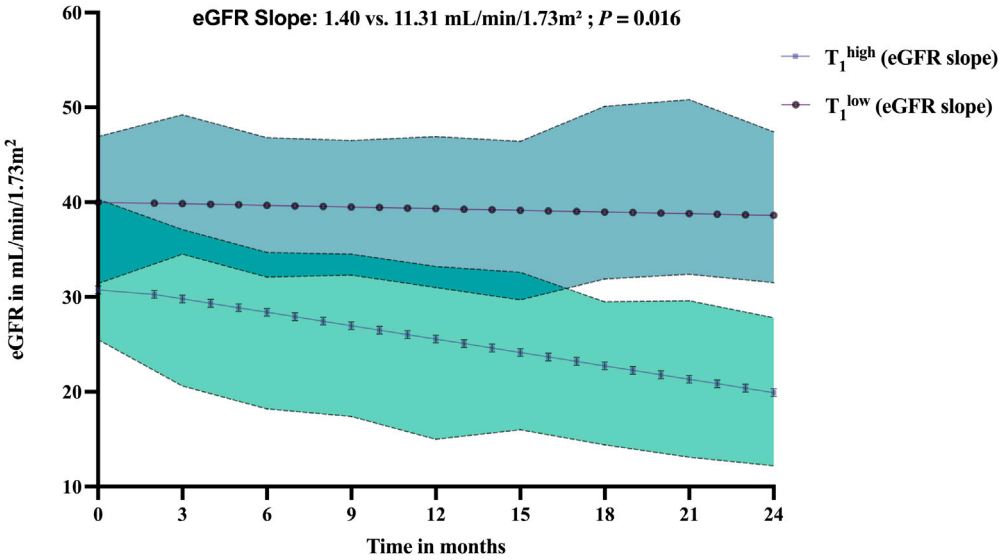


FIGURE 3 | Comparative analysis of estimated glomerular filtration rate (eGFR) values (presented with 95% confidence intervals as shaded areas) over 24 months in kidney transplant recipients compared between the T_1^{high} and T_1^{low} groups. Abbreviations: eGFR, estimated glomerular filtration rate calculated with CKD-EPI equation, in mL/min/1.73 m^2.

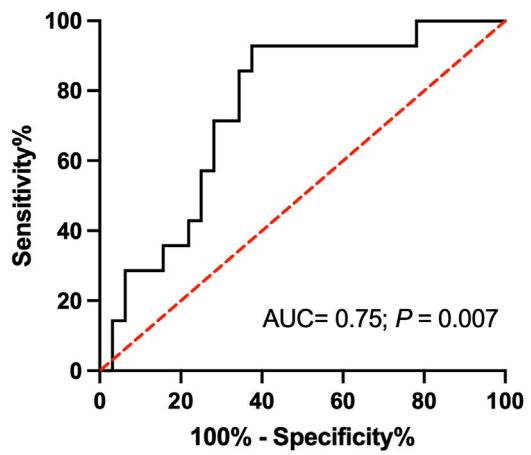


FIGURE 4 | Receiver operating characteristic (ROC) analysis for T_1 relaxation time and graft loss after 24 months of follow-up. Higher T_1 values indicate a higher likelihood of graft loss. The optimal cutoff of "> 1,352 ms" provides the best balance for accurately identifying patients with an increased risk of graft loss. Abbreviation: AUC, area under the curve, ms: millisecond.

multiparametric protocol, it may further facilitate the implementation of MRI in post-transplant surveillance programs.

A previous study from Shi et al. reported that cortical T_1 was associated with higher fibrosis and worse renal outcomes in native kidneys [26]. Interestingly, similar to the study from Shi et al., we observed that in some patients with the lowest Banff ci score (ci 0), cortical T_1 was above our median split value. Whether this was due to sampling error in the biopsy or based on other factors influencing MRI results remains speculative [15, 25, 27]. In a previous study from Berchtold et al., it was shown that altered T_1 might even precede the development of histological signs of chronic injury [28]. Besides chronic fibrosis, animal studies with ischemia-induced acute kidney injury showed that T_1 also correlates with the degree of capillary leakage and both cellular and interstitial edema, essential components of acute local inflammation. Unfortunately, our subgroup of patients with ci 0 was too small to study this finding in more detail.

Moreover, our research gave insight into the prognostic implications of T_1 through ROC analysis and Kaplan-Meier survival curves. The high NPV of T_1 suggests that magneto-chemical alterations caused by morphological changes associated with deterioration of graft function are absent, and probability of short-term graft loss is low. Concurrently, the Kaplan-Meier analysis demonstrated a significant survival advantage for allografts with lower T_1, further cementing the potential prognostic relevance of renal MRI in post-transplant care. A new aspect of our study was the exploration of eGFR slopes over time, the currently most endorsed method to quantify renal function declines [29].

Certain limitations in our study need to be addressed. We focused our analysis on T_1 and did not include other MRI methods. On the other hand, we were able to show that even with one single MRI parameter, meaningful prognostic estimates are possible. The study's sample size, while adequate for preliminary analysis, necessitates larger, multicenter trials to validate our findings across diverse populations and clinical settings. The use of the last observation carried forward (LOCF) method to address data

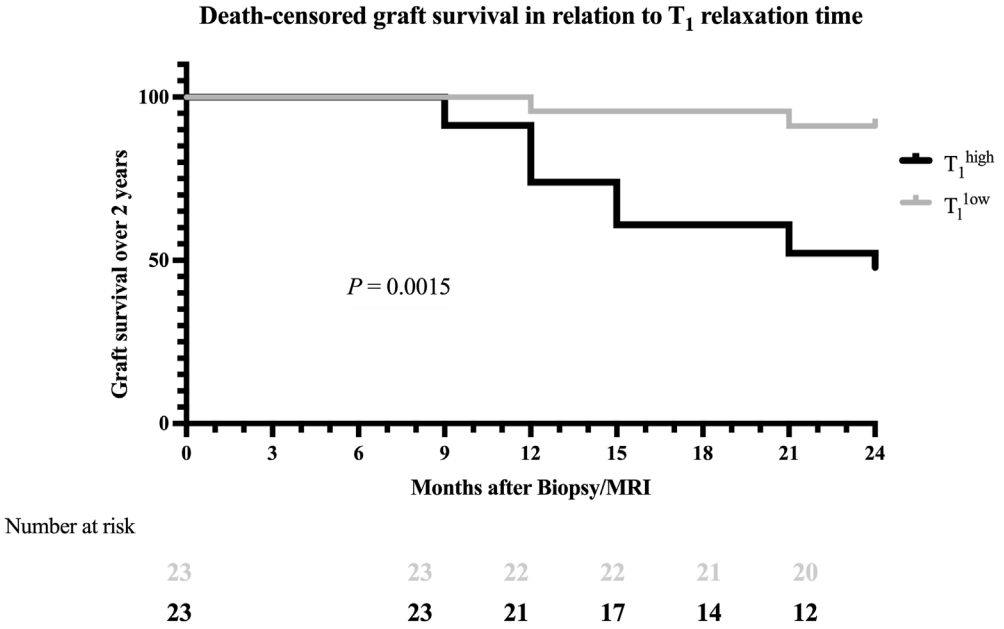


FIGURE 5 | Kaplan-Meier survival curve for the death-censored graft survival analysis 24 months after study enrollment. The black line illustrates graft survival for T_1^{high} patients, and the grey line illustrates graft survival for T_1^{low} patients. Within the timeframe of 24 months, a significant difference in graft survival was found (Log-rank test: p = 0.0015).

discontinuity due to graft loss, while methodologically sound, may introduce a conservative bias, potentially underestimating the predictive power of T_1. Additionally, the study's reliance on a single MRI parameter, despite its advantageous application capabilities, might not capture the entirety of the post-transplant complexities. It is also noteworthy that a number of patients in the T_1^{high} group were diagnosed with antibody-mediated rejections in their biopsies, possibly indicating a more aggressive underlying disease. Whereas in the T_1^{low} group, pathologies with potentially benign outcomes such as BKPyVAN were found, our MRIs were performed between 2017 and 2019, a time before the emerging AMR treatments were available [30]. Results from our ROC analysis are based on a relatively high graft loss rate, especially in the T_1^{high} in our patient population. Yet, to apply our reported PPV and NPV values in an overall renal transplant cohort, further studies including more stable renal grafts (e.g., only protocol biopsies) may be necessary.

In conclusion, our study contributes to the growing field of renal transplant diagnostics by highlighting the prognostic value of T_1. Yet, the adoption of MRI in routine post-transplant monitoring still hinges on considerations of cost, accessibility, and the standardization of imaging protocols [11, 31]. By demonstrating the potential to identify patients at high risk for midterm graft failure, we further add to the growing data, highlighting the potential utility of this non-invasive marker. Future research, encompassing larger cohorts and longitudinal studies, will be instrumental in integrating MRI into kidney transplant surveillance.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, upon reasonable request.

ETHICS STATEMENT

The studies involving humans were approved by Medical University of Vienna Institutional Review Board Nr. 1893/2017. 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

HO, ME, and FE have participated in the research design, research performance, and the writing of the manuscript. DB, MW, NK, GB, and AB-T. have contributed to data analysis and the writing of the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

GENERATIVE AI STATEMENT

The author(s) declare that Generative AI was used in the creation of this manuscript. Two pictograms used in the graphical abstract were generated with the assistance of OpenAI's ChatGPT, based on the GPT-4 model (version as provided by OpenAI at the time of use). These images were created to visually summarize key concepts in the manuscript. The AI-generated graphics were subsequently reviewed, refined, and adapted by the authors to ensure they accurately reflect the study's content.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.14301/full#supplementary-material

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ORIGINAL RESEARCH
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Lapdoctor: Multicentre Validation of a Scoring System for Preoperative Evaluation of Difficulty of Laparoscopic Donor Nephrectomy

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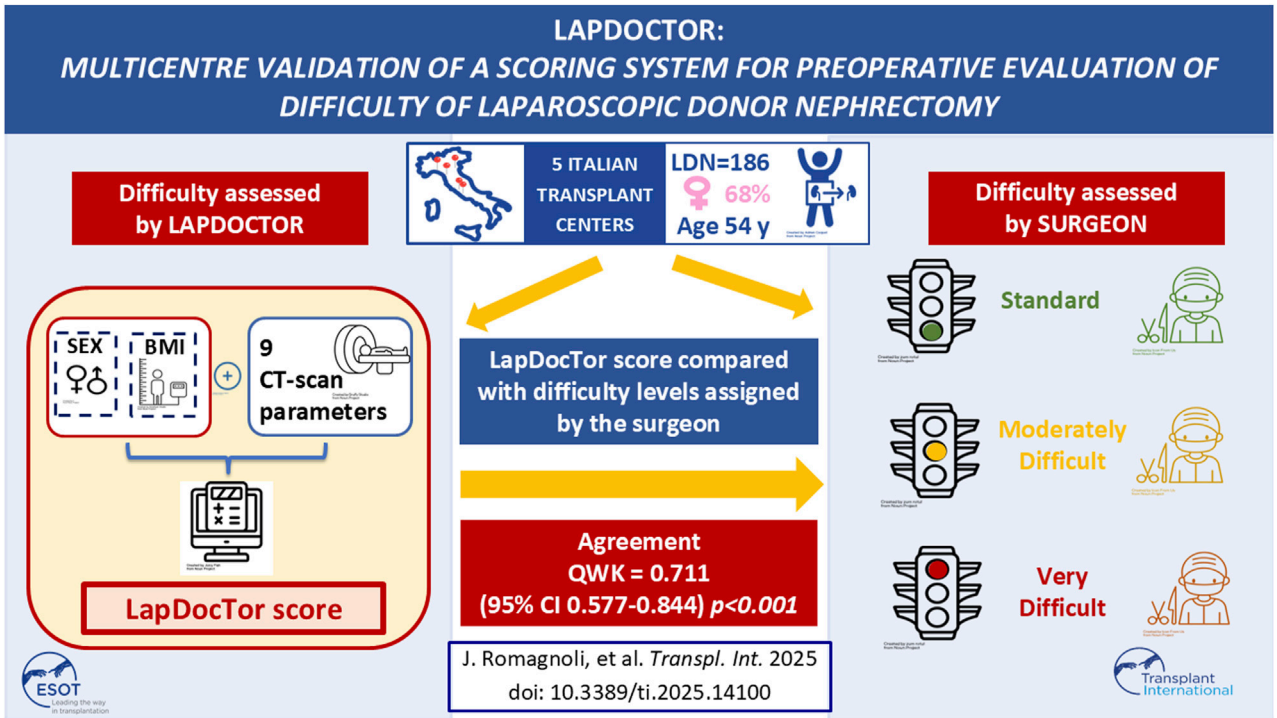
We previously developed and validated LAPDOCTOR (LAParoscopic-Donor-nephreCTomy-scORe), a novel scoring system for the preoperative assessment of the difficulty of living donor nephrectomy (LDN). To prove its significance, we extended our investigation to a prospective, multicenter, national study. Difficulty was assessed by the operating surgeon using a scale from 1 to 3 (1-standard, 2-moderately difficult, 3-very difficult) based on eight parameters: availability of laparoscopic space, mobilization of the colon, kidney, gonadal, adrenal and renal vein, renal artery, and ureter. Donor CT-scans were blindly reviewed by a radiologist, and the LAPDOCTOR scores were compared with the difficulty levels assigned by the surgeon to investigate the match rates. One hundred eighty-five donors were enrolled, with a mean age of 54 years (range 24–77), BMI 25 kg/m2 (range 17–35), and male/female 59/126. LDN was blindly scored as standard in 45% of the cases, moderately-difficult in 52%, and very-difficult in 3%. The agreement between the LAPDOCTOR and expert donor surgeons' rate in categorizing LDN into risk groups had a QWK of 0.711 (95% CI 0.577–0.844) with $p < 0.001$. The LAPDOCTOR enables precise preoperative determination of the difficulty of LDN, particularly in very difficult cases, and assessment of surgical risk in living kidney donors.

Clinical Trial Notation: https://ClinicalTrials.gov, Identifier NCT05769686.

Keywords: laparoscopic donor nephrectomy score LDKT, living donor nephrectomy, minimal invasive, risk assessment, precision medicine

Abbreviations: BMI, body mass index; HALDN, hand assistance laparoscopic donor nephrectomy; LDN, living donor nephrectomy; LAPDOCTOR, LAParoscopic DOnor nephreCTomy score; LDKT, kidney transplantation from living donors; LOS, length of stay.



GRAPHICAL ABSTRACT |

INTRODUCTION

The superior results achieved with kidney transplantation from living donors (LDKT) have led to an increase in this method of transplantation [1]. Laparoscopic donor nephrectomy (LDN) has been spreading rapidly since it was first described in 1995 by Ratner et al. [2] introduced the principles of minimally invasive surgery in the transplantation world [3]. A part of the increase in the number of LDKT cases worldwide can be attributed to the advent of this technique [4]. LDN [5] has progressively replaced open nephrectomy owing to favorable short-term outcomes, such as less pain, reduced blood loss, and improved recovery time, and is currently the standard procedure for the procurement of kidneys from living donors [6].

It is a technically complex operation, and many surgeons prefer to select the least challenging cases, especially in the initial phase of their learning curve [7]. To make it easier, hand assistance (HALDN) has been proposed in 1998 for the first time [8], and today is widely used in many transplant centers. However, using an easier technique does not prevent unexpected difficulties, particularly in complex cases. Donors that appeared *"easy"* even after the most accurate preoperative evaluation, may inexplicably turn into difficult cases, regardless of the surgical technique or of a completely normal preoperative CT-scan. Difficulty may depend on different factors such as operator experience, donor BMI, donor anatomy, renal vascular anomalies, laparoscopic working space, quality of tissue planes, retractability of the colon and mesocolon, and sticky perinephric fat [7–9]. Unfortunately, there are no comprehensive and reliable methods to predict this type of unpredictable operative scenario.

Several attempts have been made to develop a scoring system to predict the potential difficulty of laparoscopic surgery [10–13]. However, none of them produced a real reference standard.

We previously developed the LAParoscopic Donor nephreCTomy scORe (LAPDOCTOR) [14], a calculator that showed accuracy in detecting the preoperative difficulty level of LDN in 87 patients undergoing HALDN, by combining preoperative CT-scan parameters with demographic variables. The present study was designed for prospective multicentric validation of the LAPDOCTOR.

MATERIALS AND METHODS

This prospective multicenter observational study was approved by the Ethics Committee of the Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy (FPG- 2020-2939), and conducted in accordance with the tenets of the Declaration of Helsinki. The study was registered at Clinical Trials: NCT05769686 [15]. The patients signed an informed consent form at the time of enrolment.

Five Italian transplant centers were included in this prospective multicenter national study: Fondazione Policlinico Universitario A. Gemelli-Rome, Azienda Ospedaliera Universitaria - Padova, AAST Grande Ospedale Metropolitano Niguarda-Milano, Ospedale Universitario - Parma, and Ospedale Pediatrico Bambino Gesù IRCCS - Roma.

Data were collected prospectively at the participating centers and shared with the coordinating center. Radiological analysis of the preoperative CT-scans was conducted at the coordinating center.

Donors were considered eligible for the process if they met the KDIGO criteria for living kidney donation [16].

Inclusion Criteria

Donors aged ≥18 years were deemed suitable at the end of the workup for living kidney donation.

Exclusion Criteria

The main contraindications to kidney donation for transplantation were as follows: age less than 18 years, inability to provide consent for donation, evidence of coercion, drug abuse, evidence of malignant neoplasia, pregnancy, major respiratory or cardiovascular complications, diabetes mellitus, kidney diseases, systemic diseases with renal involvement, thrombophilia, obesity, BMI greater than 35 kg/m^2, active infections, infections with hepatitis B, hepatitis C, and HIV, and hypertension under treatment with organ damage.

Collected Data

The following donor data were collected: age, sex, BMI, relationship between donor and recipient, technique of LDN (pure laparoscopic, hand-assisted, or robotic), side of LDN (right or left kidney), operative time, blood loss (need for transfusion support), conversion rate, number of renal arteries, number of renal veins, incidence of postoperative major complications (Clavien-Dindo grade ≥ III), and post-operative length of stay (LOS).

Primary Endpoint

The objective of this multicenter observational study was to validate the LAPDOCTOR, a new scoring system for preoperative prediction of the difficulty of LDN for living kidney donation in the context of transplantation.

The LAPDOCTOR is based on the analysis of 11 demographic and anatomo-radiological donor parameters, which showed a statistically significant correlation with the surgical difficulty reported by the operator in a previously conducted univariate analysis [14]. For each parameter, a progressive score was assigned based on the observed increase in difficulty. The sum of the scores assigned to each parameter produces a final score (min 11–max 33), which allocates the donor to one of three classes of progressive risk: low = 11–18, medium = 19–25, high = 26–33. The calculations were performed using a program created in Microsoft Excel (*LapDocTor calculator,* **Supplementary Material S1, S2**).

The validity of the objective score was evaluated by studying its correlation with the subjective judgment of the operator. This judgment was formulated based on a score (from 1 to 3) assigned by the donor surgeon to each of the following eight phases of the operation: mobilization of the colon, kidney, gonadal vein, adrenal vein, renal vein, renal artery, and ureter. The obtained score (range 8–24) allocates the donor into one of three difficulty classes (*standard*, *moderately difficult, very-difficult*).

All preoperative unenhanced and contrast-enhanced CT-scans were blindly reviewed by a radiologist, recording the following parameters: renal artery and vein number and anatomical variants, abdominal circumference (measured at the 12th rib, umbilicus, and iliac bone), pre- and post-renal visceral fat thickness and density on the side of the procured kidney, periumbilical subcutaneous fat tissue thickness, and oblique muscle density. Density was measured in Hounsfield Units (HU) on unenhanced CT-scans using a circular region of interest (ROI) with a radius of 5 mm to evaluate the median measured value [14] (**Figure 1**).

The CT-scans have been collected and evaluated retrospectively in order to keep the blindness of the surgeons at the time of the intervention.

In the present study, we explored the correlation between LAPDOCTOR scores and difficulty levels assigned by the operating surgeon in a multicenter setting. All surgeries were performed by one surgeon per center.

Statistical Analysis

Statistical analysis was based on examining the inter-rater reliability or agreement between the two scores (preoperative objective and postoperative subjective scores obtained from the operator) using quadratically weighted (QWK) Cohen's Kappa and corresponding 95% confidence intervals (CIs). A kappa of <0.00 is considered poor agreement, 0.00–0.20 slight agreement, 0.21–0.40 fair agreement, 0.41–0.60 moderate agreement, 0.61–0.80 substantial agreement, and 0.81–1.00 almost perfect agreement [17]. Moreover, according to Fleiss interpretation [18] values, a Kappa greater than 0.75 may be taken to represent excellent agreement beyond chance. Continuous and normally distributed variables are expressed as mean ± standard deviation, and categorical data are expressed as proportions. Data were recorded using Excel 2016 (Microsoft Corporation, Redmond, Washington, DC, United States) and analyzed using SPSS 25.0 (IBM Corporation, Armonk, New York, NY, United States).

RESULTS

During the study period, 185 donors from five italian transplant centers were enrolled. The patient demographics are shown in **Table 1**.

The mean age of donors was 54 years (range 24–77 years), 126/185 donors (68%) were female, and 111/185 (60%) were related to the recipient. Twenty-nine donors (16%) were ABO incompatible. The mean BMI was 25 kg/m^2 (range, 17–35).

The technical approach varied among centers: in 75/185 cases (41%), LDN was performed using a hand-assisted approach; in 69 cases (37%), using a pure laparoscopic approach; and in 41 cases (22%), using a robotic approach.

The left kidney was preferred in 166/185 cases (90%), whereas the right kidney was retrieved in only 19/185 cases (10%). Among the right kidney procedures (19, 10%), the majority were performed using a hand-assisted approach (11/19, 57%), which

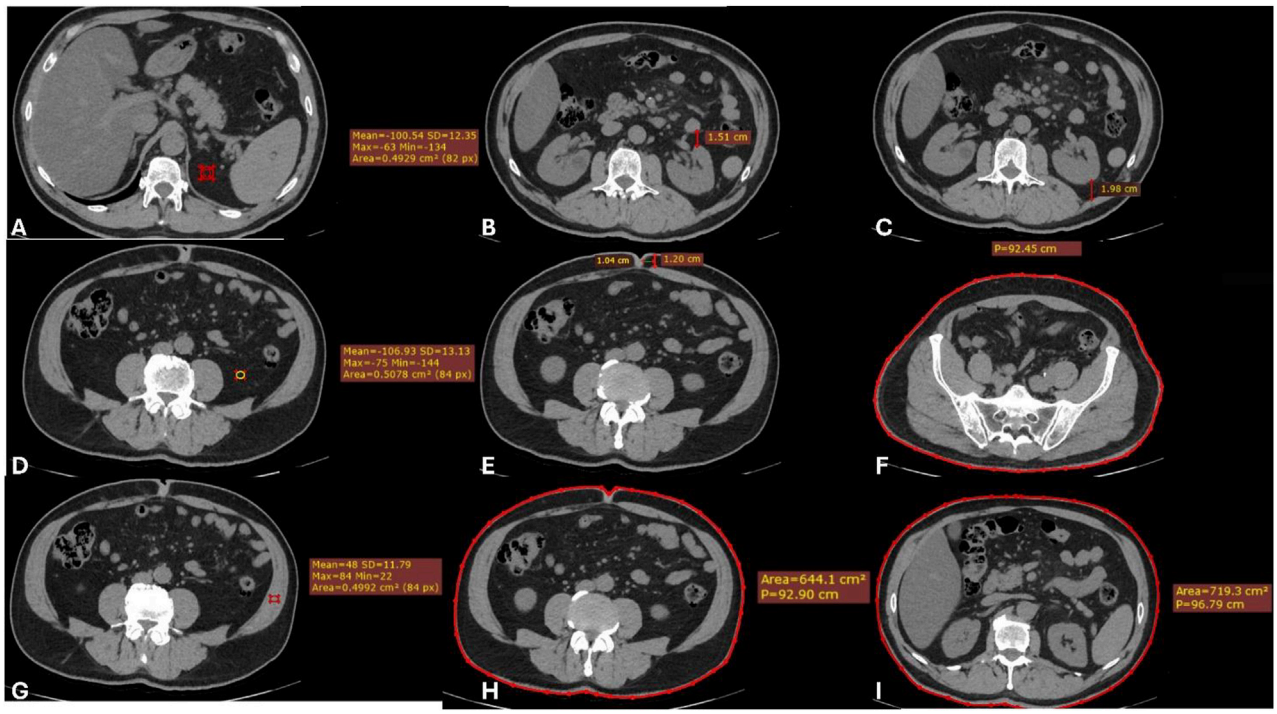


FIGURE 1 | Axial CT images showing the radiological parameters considered. **(A)** Upper renal fat tissue density (just above the kidney, ROI of 0.5 cm2). **(B)** Pre-renal fat tissue thickness (at the middle third of the kidney, from the kidney to the bowel). **(C)** Retro-renal fat tissue thickness (at the middle third of the kidney, from the kidney to the muscle). **(D)** Lower renal fat tissue density (just below the kidney, ROI of 0.5 cm2). **(E)** Abdominal wall fat tissue thickness (at 1 cm from the navel). **(F)** Abdominal circumference (at the antero-superior iliac spine). **(G)** Oblique muscles density (ROI of 0.5 cm2). **(H)** Abdominal circumference (at the navel). **(I)** Abdominal circumference (at the 12th rib).

TABLE 1 | Characteristics of participants.

Donors, n	185
Age, years	53.5 (10.6)
Male	59 (32%)
Female	126 (68%)
BMI, kg/m^2	25.1 (3.6)
Related	111 (60%)
ABO incompatible	29 (16%)
Nephrectomy Side [Left/Right]	166/19 (90%–10%)
Renal vascular Anomalies	33 (18%)
Multiple arteries	30 (16%)
Surgical Technique	
Hand-assisted	75 (41%)
Pure Laparoscopic	69 (37%)
Robotic	41 (22%)

Data are mean (SD) or n (%).

TABLE 2 | Results of LDN.

Number of procedures	185
Operative Time, minutes	267 (79)
Hand-assisted	289 (58)
Pure Laparoscopic	245 (87)
Robotic	266 (89)
Laparoscopic Time (minutes, mean ± standard deviation)	209 (86)
Hand-assisted	232 (56)
Pure Laparoscopic	213 (104)
Robotic	162 (96)
Conversion, n	1 (0.5%)
Complications according to Clavien-Dindo, n	19 (10.2%)
Grade I	6 (3.2%)
Grade II	9 (4.9%)
Grade III a-b	3 (1.6%)
Length of stay	5 (2)

Data are mean (SD) or n (%).

seems to make transplant surgeons feel more confident in recovering the right kidneys [19] and a robotic approach in approximately one-third of the cases (6/19, 31%). This approach was chosen because it is the routine technique used for both the right and left kidneys in one of the five participating centers.

Regarding anatomical variations, 33 kidneys (18%) had vascular anatomical variants, with the majority (30 cases, 16%) presenting with multiple arteries.

The mean operative time (from skin incision to skin closure) was 267 ± 79 min, with a mean laparoscopic time of 209 ± 86 min. The operative time was longer for hand-assisted procedures than for laparoscopic or robotic procedures (data shown in **Table 2**).

All procedures were performed transperitoneally. There was one case (0.5%) of conversion of a left pure LDN to an open nephrectomy, which resulted in a successful operation, preserving both patient and graft survival.

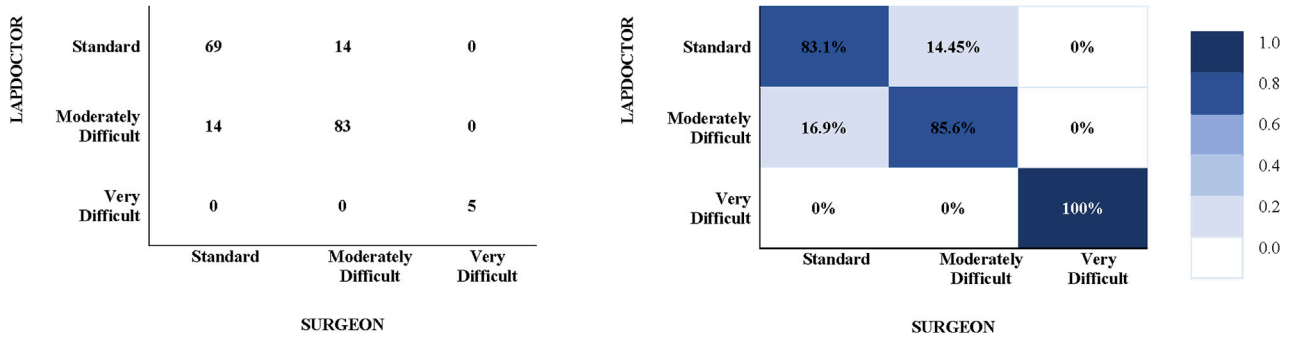


FIGURE 2 | Agreement rate for level of difficulty between LAPDOCTOR score and Donor Surgeon's score: excellent concordance in risk group classification by a QWK of 0.711 (95% CI 0.577–0.844), p < 001.

The overall incidence of complications was 10.2%, which is consistent with the literature (8%–18%) [5]. According to the Clavien-Dindo classification, only 1.6% were grade III (a-b) and 4.9% were grade II (**Table 2**).

After all procedures, the first operator collected a survey, grading each of the eight steps from 1 to 3 based on the level of perceived difficulty. The procedures were classified as *standard* in 83/185 cases (45%), *moderately difficult* in 97/185 (52%), and *very difficult* in 5/185 (3%).

In **Supplementary Table S4**, we reported values of cases stratified as standard, moderately difficult, and very difficult, further categorized by surgical phase for each surgeon.

A single radiologist blindly reviewed all pre-operative CT-Scan images and collected anatomical and radiological donor parameters. Based on these parameters, BMI and sex were added (**Supplementary Table S3**). The LAPDOCTOR classified 83/185 procedures (45%) as *standard*, 97/185 (52%) as *moderately difficult*, and 5/185 (3%) as *very difficult*.

All data were centrally resumed in the dataset. The agreement between LAPDOCTOR and the donor surgeons' rate in categorizing LDN into *standards*, *moderately difficult*, and *very difficult* risk groups had a QWK of 0.711 (95% CI 0.577–0.844) with $p < 0.001$ (**Figure 2**). Considering the individual QWK, "*standard*" cases had a QWK of 0.831 (95% CI, 0.550–0.838, $p < 0.001$), *moderately difficult* 0.856 (95% CI, 0.552–0.841, $p < 0.001$), and *very difficult* 1.00 (95% CI, 0.856–1.144, $p < 0.001$).

We performed a sub-analysis of cases with observed discrepancy between the surgeon's judgment and the LAPDOCTOR prediction and found that in cases deemed *standard* by the surgeon but *moderately difficult* by LAPDOCTOR, the average values of most parameters tended to align more closely with those of the *moderately difficult* LAPDOCTOR cases. We speculate that the greater confidence of an experienced surgeon may have resulted in an easier perception of *moderately difficult* cases.

A similar consideration applies to cases where the surgeon's experience of a *moderately difficult* operation did not match the LAPDOCTOR's "*standard*" rating.

DISCUSSION

Our study introduces a novel difficulty scoring system for LDN that enables preoperative identification of technically challenging cases based on readily available donor parameters. By analyzing 185 living donors within the context of a multicenter prospective clinical trial, we demonstrated that this grading system can accurately identify potentially difficult donors and define the expected level of difficulty, regardless of the type of laparoscopic approach used.

The implications of this study are significant. In the presence of multiple potential donors, the LAPDOCTOR can assist in selecting the least challenging donor. Conversely, if only one donor is available, it can help the surgeon plan a safer operation by being aware of potential difficulties. From a training perspective, it allows for the selection of easier cases for junior fellows, thereby reducing unnecessary risks to the donor, surgeon, and trainee.

This study was conducted in response to the strong need for tools that help donor surgeons plan safer living donor operations. Several difficulty scoring systems have been proposed for laparoscopic surgery [10–13], with models based on preoperative donor characteristics or preoperative imaging, however, we did not find comparable methods to comprehensively and reliably assess difficulty of LDN. Surgeons have also developed renal morphometry scoring systems, such as the R.E.N.A.L. nephrometry score, PADUA prediction score, and centrality index (C-index), to analyze anatomical findings that can predict the complexity of nephrectomy and the likelihood of complications [20–22]. The Mayo group proposed the Mayo Adhesive Probability Score (MAP) [23] to predict the presence of adherent perinephric fat. Other scoring systems have used various variables, particularly radiographic variables, to correlate the operative difficulty and postoperative outcomes [24].

Most studies have used factors such as sex, body mass index (BMI), perirenal fat, and number of renal arteries and veins as measures of difficulty. Ratner et al. [7] attempted to create a scoring system to determine whether anatomical or radiologic parameters could accurately assess the technical difficulty of LDN

preoperatively. They reviewed CT scans and graded the different phases of the operation on a scale of 1–4 but found that technical difficulty could not be predicted by body habitus from the variables examined in their study.

However, none of these scoring systems have considered a multiparametric approach or combined objective preoperative data with an intraoperative surgeon's score based on perceived difficulty. To overcome the bias of subjectivity, we designed a multicenter study involving five experienced transplant surgeons from five major Italian transplant centers. In three centers, LDN was performed using different laparoscopic approaches (pure laparoscopy or robotic) based on the surgeon's experience. In the remaining two centers HALDN was the standard.

This could be a limitation of our study; however, LAPDOCTOR compared the difficulty of different donors using the same set of parameters, regardless of the approach. The term of comparison used to validate the scoring system is the experience of the operating surgeon with the technique with which they are most confident. The LAPDOCTOR has shown no statistical differences in its ability to identify difficult cases in donors operated with either hand-assisted, pure laparoscopic, or robotic techniques. Notably, there was a full match for the very difficult cases. Nonetheless, right now, whether there is a more favorable technique cannot be drawn neither from our data, nor from literature's data.

LAPDOCTOR proved helpful in our practice for the preoperative surgical evaluation of living donors. With a simple excel sheet saved on the PC desktop of the transplant clinic, ready to be filled with a set of easy to obtain parameters, even a junior surgeon can objectively categorize the surgical risk of LDN, instead of relying on subjective judgment "by eye," based only on personal experience of a senior surgeon. Moreover, in the setting of an academic training center, the utility of LAPDOCTOR resides in its ability to sort out the most adequate cases to train transplant fellows in this very delicate operation. In many centers part of this operation is entrusted to senior trainees, under consultant's supervision and LAPDOCTOR facilitates the choice of the proportion of risk one can decide to allocate them, depending on the individual skills and experience of each trainee. Of note, the longer operation times observed in donors operated with HALDN are indeed easily explained by the training needs, one of the main reasons for the choice of this technique being the possibility to allow trainees to make experience and progress with this operation, while preserving donor safety and senior surgeon's coronaries. The dissemination of LAPDOCTOR, by standardizing the scoring system, would also help in the mutual exchange and interpretation of collected data coming from different centers, thus promoting further progress in our knowledge of such a sensitive topic.

Limitation

The present study has some limitations that need to be acknowledged. The study is multi-centric, but all participating centers were from a single country (Italy); we included different surgical techniques, and the sample is relatively small, so that a sub-group analysis is not feasible and does not allow for the individual validation of LapDocTor. Since our main purpose was to challenge the ability of the scoring system to predict difficulty, we did not assess long-term outcomes. Despite the excellent agreement between our score and the surgeon's judgment, the latter remains inherently subjective and may explain the discordance found for some cases, likely due to individual surgeon's experience.

For these reasons, our findings will require external validation in a larger, specifically designed, possibly multi-ethnic, international cohort study.

Conclusion

The LAPDOCTOR is a very simple scoring system that accurately determines the expected level of difficulty for laparoscopic donor nephrectomy by utilizing donor demographics and CT scan parameters. It is particularly effective in identifying the most challenging cases, enabling surgeons to plan operations more safely by being aware of the potential risks. Additionally, it is valuable for training purposes as it assists in selecting easier cases for surgical training, thereby minimizing unnecessary risks for the donor, surgeon, and trainee.

Further studies are warranted to investigate the correlation between the LAPDOCTOR scores and long-term patient and graft outcomes.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy. 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

JR, GS, FR, LC, and CP: involved in draft conception and design, data acquisition, drafting of the manuscript, critical revision, and approval of the final version. RI, AP, MS, PS, and BF: involved in data acquisition, manuscript drafting, critical revision, and approval of the final version. AR: involved in draft conception and design, data acquisition, drafting of the manuscript, statistical support, critical revision, and approval of the final version. CS: involved in data acquisition, critical revision and approval of the final version. AG, MP, MI, and LF: involved in the draft conception and design, drafting the manuscript, critical revision, and approval of the final version. 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.

GENERATIVE AI STATEMENT

The authors declare that no Generative AI was used in the creation of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.14100/full#supplementary-material

SUPPLEMENTARY FIGURE S1 | Examples of results calculated with LapDocTor.

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ORIGINAL RESEARCH
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Donor-Recipient Mismatch in Lung Transplantation: The Role of Graft Sizing in Clinical Outcomes

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Lung transplantation is a life-saving procedure for end-stage lung diseases. Size matching is critical in the donor-recipient selection process. This retrospective study analyzed 146 patients who underwent lung transplantation between 2013 and 2023. Patients who required graft resizing were assigned to the sizing group (S), non-resizing cases to the non-sizing group (NS). The primary goal was to identify predictive factors for graft resizing. Secondary endpoints included ischemia time, ventilation time, primary graft dysfunction (PGD) and hospital stay. The S group was further stratified on baseline parameters to assess differences in outcomes. Recipient height and single transplants were higher in the NS group. Donor-recipient height ratio was the only predictor for resizing ($p = 0.02$). Postoperative outcomes and overall survival were similar between the groups. In Group S, male patients showed higher rates of acute kidney injury (AKI) and chronic rejection, the former being associated also with anatomical resections; patients older than 50 experienced higher rates of PGD. Graft resizing is a feasible strategy for addressing size mismatch, but it is associated with increased risks of PGD and AKI, particularly in older male recipients and those undergoing anatomical resections. These findings highlight the importance of careful preoperative donor-recipient size matching.

Keywords: lung transplantation, lung resection, donor selection, size matching, allograft

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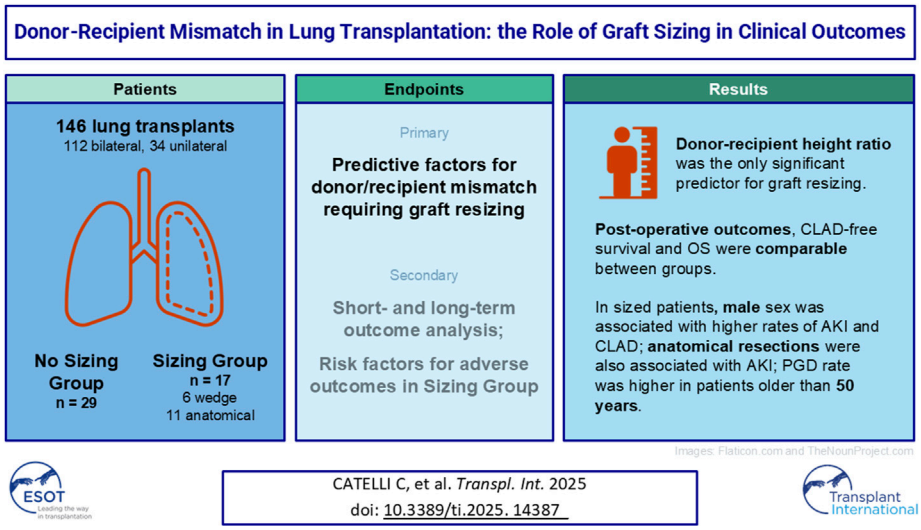
INTRODUCTION

Despite its success in prolonging survival, lung transplantation faces several challenges, one of the most significant being the mismatch between the donor's and recipient's lung size and physiological characteristics. Such mismatches can contribute to a range of postoperative complications, including primary graft dysfunction, bronchiolitis obliterans syndrome, and overall reduced graft survival [1, 2]. Several factors—both anatomical and physiological—contribute to this mismatch, including the recipient's chest wall mechanics, lung compliance, and the size of the donor's lungs relative to the recipient's thoracic cavity [3].

In particular, the recipient's lung capacity and thoracic dimensions can vary significantly, creating potential challenges when selecting an appropriate donor lung [4, 5]. Over- and under-sizing of the lung graft are associated with various complications, ranging from impaired gas exchange to increased risk of rejection and graft dysfunction [6]. Conversely, an undersized graft may fail to



GRAPHICAL ABSTRACT

meet the recipient's functional needs, compromising postoperative outcomes and leading to complications such as early graft failure.

In response to these challenges, there has been a growing focus on developing strategies to optimize the donor-recipient match [7, 8]. Graft sizing techniques have emerged as a potential solution, utilizing advanced imaging methods, such as three-dimensional computed tomography (CT) volumetry [9, 10], to better assess the donor lung's size and its compatibility with the recipient. However, in some cases, particularly when the recipient is in poor general condition or has a rare blood type, oversized organs may be necessary.

In cases where there is a small size discrepancy between the donor and recipient, limited non-anatomic or sublobar graft resections are often effective. However, for more significant mismatches, lobar reduction is typically the preferred surgical approach [11]. Due to the technical challenges, the available case series on this technique are few [12], and the outcomes reported across studies have been inconsistent.

This study aims to investigate the key predictive factors that contribute to mismatch between donor and recipient in lung transplantation and the subsequent need for graft sizing. Furthermore, it will evaluate the clinical outcomes of patients undergoing graft sizing procedures, analyzing potential risk factors for poorer outcomes in certain patient categories.

MATERIALS AND METHODS

Patients

This is a retrospective study involving all patients who underwent single or double lung transplantation between 1 January 2013, and 31 December 2023, at the Lung Transplant Unit of the University Hospital of Siena. The study was approved by the Institutional Review Board (IRB). Patients who underwent graft reduction for reasons other than mismatch (such as pulmonary contusions, lobar edema, or parenchymal consolidation) were excluded from the study, as these lungs may have been compromised before transplantation, thus increasing the risk of complications regardless the sizing procedure. The type of surgical resection performed in our study was operator-dependent and based primarily on four assessments. These included [1]: a reduction in systemic pressure due to heart compression observed during chest closure [2]; an evident mismatch identified either before the graft implantation or during chest closure [3]; atelectasis of part of the lung parenchyma during recruitment due to insufficient thoracic cavity size and [4] an increase in registered ventilatory pressures observed during chest closure.

Patients were divided into two groups based on the need for sizing at the time of implantation due to dimensional mismatch between the donor and recipient. The sizing group (Group S) included patients who underwent atypical and/or anatomical lung resections (segmentectomy, lobectomy), while Group NS included all patients who did not require graft resizing. At our center, donor lungs are allocated based on blood group, Lung Allocation Score (LAS), height, and age.

In the two groups, discrepancies between the donor and recipient were analyzed in terms of sex, race, BMI ratio (donor/recipient), height ratio (donor/recipient), weight ratio (donor/recipient), and age ratio (donor/recipient). The comorbidities of both the recipient and donor, as well as the type of transplant performed (single or double), were also analyzed and compared. The primary endpoint of the study was to evaluate which characteristics of the donor and recipient were predictive factors for D/R mismatch requiring lung resection on the graft. The secondary endpoint was to

analyze primary outcomes, such as overall survival, and secondary outcomes, such as the occurrence of Primary Graft Dysfunction (PGD), Chronic Lung Allograft Dysfunction (CLAD), ischemia time, and duration of mechanical ventilation in the two groups. In patients who received sized grafts, outcomes were then stratified based on the following patient characteristics: BMI (greater or less than 25); sex (male or female); age (greater or less than 50 years); type of end-stage pulmonary disease (restrictive or obstructive); type of sizing (lobectomy/segmentectomy or atypical resection). Ethical approval was not required for the study, in accordance to the local legislation, because of its retrospective nature.

Surgical Procedure

The lung transplant procedure was standard, performed through a clamshell incision for bilateral transplants or a posterolateral thoracotomy for single transplants. In the case of graft sizing, anatomical resections were performed at the back table or after lung implantation, before hemostasis and chest closure, using mechanical staplers for bronchial and vascular structures, while atypical resections were always performed after lung implantation with the use of mechanical staplers. The decision to perform graft reduction and determine the type of resection is based on visual inspection and clinical parameters. Specifically, the final decision on the need for sizing is made after a thorough inspection of the recipient's thoracic cavity. If the mismatch is immediately apparent, sizing is performed through anatomical resection before graft implantation. If, however, the mismatch is not evident during clinical inspection but hemodynamic instability occurs during chest closure due to compression of the overinflated lung on the cardiac cavities, resection is performed at the end of the procedure. The choice of which part of the lung to sacrifice was based on the recipient's thoracic configuration, with middle lobectomy or lingulectomy being preferred in cases of antero-posterior mismatch, while the sacrifice of the lower lobes was preferred in cases of diaphragmatic elevation [13]. The decision was also influenced by the appearance of the lung, such as sacrificing the most difficult-to-recruit or edematous portion after implantation. Postoperatively, patients received appropriate antibiotic prophylaxis (Vancomycin, Cefepime, Ganciclovir, and Ig-CMV) and immunosuppressive therapy (Basiliximab, methylprednisolone, tacrolimus, and mycophenolate mofetil).

Statistical Analysis

The results data were expressed as mean ± standard deviation and median (interquartile range), as appropriate. Non-parametric tests were adopted for data analysis: comparisons between two groups were determined by Mann-Whitney U test; ANOVA test (Kruskal–Wallis and Dunn's multiple tests) were performed to compare more than two groups. Contingency analysis was performed to evaluate the association and the independence between the parameters as well as to calculate various association measures. Correlations between variables were determined by Spearman correlation coefficient. Survival distribution in the two groups was evaluated using a weighted Kaplan–Meier approach.

TABLE 1 | Recipients' characteristics.

Variable	Group NS N = 129	Group S N = 17	P
Age (y)	55.5 (17–66)	55.0 (23–64)	0.462
Sex (M; F)	84 (65%); 45 (35%)	8 (47%); 9 (53%)	0.147
BMI	24.0 (14.8–34.2)	26.4 (17.0–34.9)	0.357
Height (cm)	169 (150–196)	164 (150–178)	**0.004**
Weight (kg)	68 (38–120)	70 (40–101)	0.852
LAS	20 (2–63)	31 (4–88)	0.668
FEV1%	38 (9–125)	35 (12–90)	0.975
FVC%	49 (20–168)	51 (15–87)	0.741
DLCO%	29 (2–62)	30 (5–85)	0.475
Pattern of lung disease			0.944
Restrictive	52 (40.3%)	8 (47.1%)	
Obstructive	8 (6.2%)	3 (17.6%)	
Cystic Fibrosis	23 (17.8%)	3 (17.6%)	
Mixed	25 (19.4%)	3 (17.6%)	
Transplant type			**0.020**
SLTX	31 (24.0%)	3 (17.7%)	
BLTX	98 (76.0%)	14 (82.3%)	
Comorbidities			
Arterial hypertension	37 (29%)	4 (24%)	0.657
Pulmonary hypertension	19 (15%)	3 (18%)	0.762
Dyslipidemia	18 (14%)	2 (12%)	0.796
Diabetes mellitus	22 (17%)	4 (24%)	0.522
Obesity	8 (6%)	1 (6%)	0.953
Osteopenia/osteoporosis	47 (36%)	7 (41%)	0.721

The categorical variables are presented as percentages; the continuous variables are expressed as the median and interquartile range (IQR). BLTX, Bilateral Lung Transplant; BMI, Body Mass Index; DLCO, Diffusing Capacity of the Lung for Carbon Monoxide; FEV1, Forced Expiratory Volume in one second; FVC, Forced Vital Capacity; LAS, Lung Allocation Score; SLTX, Single Lung Transplant. Significant p values are reported in bold type.

Statistical analysis was performed by GraphPad Prism 9.10.3, XLSTAT 2021 and Jamovi software.

RESULTS

The study included 146 patients who underwent lung transplantation at our center. 17 patients (11.6%) underwent lung resection due to graft mismatch (Sizing Group or Group S), while the remaining 129 did not require lung resection (Non-Sizing Group or Group NS). One patient was excluded from the study as they underwent graft resection due to pulmonary consolidation in the right lower lobe, which developed during graft reperfusion via *ex vivo* lung perfusion (EVLP). **Table 1** summarizes the recipient characteristics, stratified by group. In the Sizing Group, the average age of the recipients was 55 years (range 23–64), with 53% (9 patients) being female and an average BMI of 26.4, indicating mild overweight. The majority of patients in Group S had a restrictive type of end-stage lung disease (8 patients, 47%).

No statistically significant differences were observed between the groups in terms of age at transplant, sex, or BMI. However, a statistically significant difference in recipient height was observed (164 cm in Group S vs. 169 cm in Group NS, p = 0.004), with shorter recipients in the Sizing Group. No significant differences were found in preoperative forced expiratory volume in one

TABLE 2 | Donor characteristics and donor/recipient ratio.

Variable	Group NS N = 129	Group S N = 17	P
Comorbidities			
Arterial Hypertension	14 (11%)	2 (12%)	1.000
Diabetes Mellitus	5 (4%)	0 (0%)	0.962
Asthma	3 (2%)	0 (0%)	0.853
Smokers	28 (22%)	7 (41%)	0.121
Age ratio D/R	0.845 (0.25–2.37)	0.990 (0.50–2.04)	**0.041**
Weight Ratio D/R	1.00 (0.63–1.70)	0.995 (0.76–2.00)	0.527
Height Ratio D/R	1.01 (0.91–1.11)	1.04 (0.98–1.17)	**0.004**
BMI Ratio D/R	1.00 (0.57–1.73)	0.935 (0.67–1.73)	0.560
Sex mismatch	31 (24.0%)	5 (29.4%)	0.734
Race mismatch	17 (13.2%)	5 (29.4%)	0.111

The categorical variables are presented as percentages; the continuous variables are expressed as the median and interquartile range (IQR). D/R, Donor/Recipient; BMI, Body Mass Index. Significant p values are reported in bold type.

second (FEV1%), forced vital capacity (FVC%), or diffusing capacity of the lung for carbon monoxide (DLCO%), nor in the type of end-stage lung disease between the two groups. Bilateral lung transplants were more frequent in Group S (82.3%) compared to Group NS (76.0%, p = 0.02). No significant differences were observed between the two groups in terms of patient comorbidities. Despite this, it was noted that the Lung Allocation Score (LAS) was higher in Group S (31 vs. 20), which is clinically relevant, although not statistically significant (p = 0.668).

Table 2 presents the characteristics of the donors and the donor-recipient discrepancies. No differences were observed in the comorbidities of the donors between the two study groups, nor in the frequency of smoking habits. The ratio between the donor's and recipient's age was lower in Group NS (0.845) compared to Group S (0.990, p = 0.041). A statistically significant difference was also observed in the ratio between the donor's and recipient's height (1.01 in Group NS vs. 1.04 in Group S, p = 0.004). No significant differences were found between the two groups in terms of sex and race mismatch between donor and recipient, nor in the donor-recipient ratio for weight or BMI.

The correlation analysis showed that only the ratio between the donor's and recipient's height was considered a predictive factor for the need for graft sizing (p = 0.02, OR 2.70e + 10, 95% CI 41.4–1.87e + 19).

Table 3 illustrates the types of lung resections performed on the graft following the diagnosis of mismatch. The majority of patients (n = 11, 64.7%) underwent anatomical lung resections, most commonly involving the removal of two lobes/segments (n = 7, 41.2%). The most common anatomical resections performed were middle lobectomy (n = 9, 52.9%) and left lingular segmentectomy (n = 5, 29.4%).

Postoperative outcomes are summarized in **Table 4**. Although not statistically significant, a shorter ischemia time was observed in lungs that underwent sizing, both for the first lung (238 vs. 294 min in Group S and NS, respectively, p = 0.053) and the second lung (396 vs. 333 min in Groups S and NS, respectively, p = 0.108). Similarly, although not statistically significant,

TABLE 3 | Types of graft resections performed for donor-recipient mismatch (every resection is reported separately, even multiple resection cases).

Type of resection	N	%
Atypical (wedges)	6	35.3%
Anatomical	11	64.7%
Segmentectomy		
Lingula	5	29.4%
Apex	2	11.8%
Lobectomy		
ML	9	52.9%
RUL	1	5.9%
RLL	1	5.9%
LUL	2	11.8%
LLL	1	5.9%
Bilobectomy	2	11.8%
Type of resection		
<1lobe/segment	6	35.3%
= 1 lobe/segment	2	11.8%
= 2 lobes/segments	7	41.2%
= 3 lobes/segments	2	11.8%

LLL, left lower lobectomy; LUL, left upper lobectomy; RML, right middle lobectomy; RLL, right lower lobectomy; RUL, right upper lobe.

TABLE 4 | Patients' clinical outcomes after lung transplantation.

Outcomes	Group NS N = 128	Group S N = 17	P
First lung ischemia (min)	294 (108)	238 (86)	0.053
Second lung ischemia (min)	396 (144)	333 (141)	0.108
FEV1 1 month (mL)	2.18 (0.57–3.47)	2.10 (1.46–2.38)	0.487
FEV1 2 months (mL)	2.18 (1.13–3.36)	1.77 (1.08–2.86)	0.080
FEV1 3 months (mL)	2.14 (0.93–427)	1.75 (1.33–2.46)	0.077
PGD			0.130
Grade 1	23 (18.0%)	4 (23.5%)	
Grade 2	36 (28.1%)	3 (17.6%)	
Grade 3	38 (29.7%)	9 (53.0%)	
Acute Kidney Injury	27 (21.1%)	5 (29.4%)	0.196
Prolonged MV (>5 days)	39 (30.5%)	7 (41.1%)	0.361
Post-operative ECMO	21 (16.4%)	5 (29.4%)	0.189
CLAD	44 (34.4%)	4 (23.5%)	0.382
In-hospital stay (days)	37 (0–403)	34 (9–109)	0.583
OS (months)	36.8 (39.4)	25.2 (34.8)	0.098

Data are shown as medians with interquartile range (IQR) or absolute numbers with percentage when adequate. PGD: primary graft dysfunction; MV: mechanical ventilation; ECMO: extracorporeal membrane oxygenation; CLAD: chronic lung allograft dysfunction; OS: overall survival.

PGD3 was more frequent in Group S (53% vs. 29.7% in Group NS, p = 0.13). Although not statistically significant, a higher rate of prolonged mechanical ventilation (41.1% vs. 30.5% in Groups S and NS, respectively, p = 0.361), postoperative ECMO requirement (29.4% vs. 16.4% in Groups S and NS, respectively, p = 0.189), and acute kidney injury (29.4% vs. 21.1% in Groups S and NS, respectively, p = 0.196) was observed in patients who underwent lung resection. Additionally, it was noted that, starting from 3 months after the procedure, FEV1 decreased in patients who underwent graft resection, a phenomenon not observed in patients who did not undergo sizing, where FEV1 remained stable at 3 months post-operation, although this finding did not reach statistical

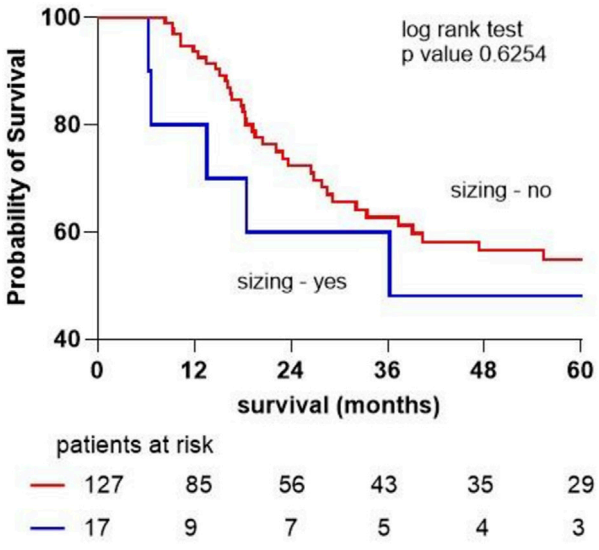


FIGURE 1 | Kaplan-Meier survival curve for patients not undergoing lung resection and those undergoing graft sizing due to mismatch.

significance (reduction of 0.04 mL and 0.35 mL in Groups NS and S, respectively, from 1st to 3rd month post-surgery, p = 0.077). No differences were observed in the length of hospital stay. The development of CLAD was lower, though not statistically significant, in Group S (23.5%, p = 0.382).

Survival analysis using Kaplan-Meier demonstrated no statistically significant difference in overall survival between the two groups (p = 0.625) (**Figure 1**).

To further investigate potential differences in outcomes within the group of patients undergoing lung graft sizing, stratification was performed based on age, sex, BMI, type of end-stage lung disease, and type of lung resection performed. The results of this stratification are presented in **Table 5**.

Within the Sizing Group, there were no differences in outcomes based on the recipient's BMI or the underlying type of lung disease. In the male subgroup undergoing sizing, a higher rate of CLAD onset was observed (4 patients, 50% in the male group, none in the female group, p = 0.015) as well as AKI (4 patients, 50% in the male group, none in the female group, p = 0.016). In patients over 50 years old, a higher rate of PGD was observed (100% in the > 50 years group vs. 86% in the < 50 years group). In the group undergoing anatomical lung resection, the onset of AKI was statistically significant (45% in the anatomical resection group vs. 0% in the non-anatomical resection group, p = 0.018). Although not statistically significant, PGD development was observed in all patients with a BMI < 25, all male patients, all patients over 50 years old, those with obstructive lung disease, and all patients undergoing anatomical resection.

DISCUSSION

Lung transplantation is a life-saving intervention for patients with end-stage lung disease, but donor-recipient mismatch, particularly in terms of lung size, can contribute to significant postoperative complications. These complications, including PGD, bronchiolitis obliterans syndrome, and overall reduced graft survival, highlight the importance of optimizing donor-recipient matching.

Our results indicate that donor-recipient mismatch, particularly in terms of donor-recipient height, plays a crucial role in determining the necessity for graft sizing. Specifically, a height discrepancy between donor and recipient was significant between the two groups, with shorter recipients in the Sizing Group (p = 0.004). This is consistent with previous studies [5] suggesting that lung size and thoracic dimensions are critical factors in ensuring a functional match between donor lungs and recipients.

TABLE 5 | Outcomes in the group of patients undergoing graft reduction, stratified by clinical characteristics and type of resection performed.

Subgroups	N	OS (months)	Degenza (days)	PGD	PGD grade 1	PGD grade 2	PGD grade 3	CLAD	AKI
BMI > 25	8	30 (35)	51 (33)	7 (84%)	1 (12%)	3 (36%)	3 (36%)	1 (12%)	3 (36%)
BMI < 25	9	19 (35)	31 (13)	9 (100%)	3 (33%)	0 (0%)	6 (67%)	3 (33%)	2 (22%)
p		0.423	0.289	0.114				0.312	0.293
Females	9	13 (16)	38 (30)	8 (89%)	1 (11%)	2 (22%)	5 (55%)	0 (0%)	0 (0%)
Males	8	39 (45)	46 (25)	8 (100%)	3 (36%)	1 (13%)	4 (50%)	4 (50%)	4 (50%)
p		0.277	0.413	0.494				**0.015**	**0.016**
Age < 50years	7	22 (37)	30 (14)	6 (86%)	1 (14%)	3 (42%)	2 (28%)	1 (14%)	4 (25%)
Age > 50years	10	27 (35)	50 (32)	10 (100%)	3 (30%)	0 (0%)	7 (70%)	3 (30%)	3 (60%)
p		0.601	0.241	**0.05**				0.452	0.293
Restrictive disease	8	16 (17)	48 (37)	7 (84%)	2 (25%)	0 (0%)	5 (75%)	1 (17%)	2 (12%)
Obstructive disease	7	23 (43)	39 (16)	7 (100%)	1 (28%)	1 (28%)	4 (66%)	1 (13%)	2 (12%)
p		0.754	0.846	0.391				0.825	1.000
Atypical resection	6	41 (38)	34 (15)	5 (83%)	2 (33%)	0 (0%)	3 (50%)	3 (50%)	0 (0%)
Anatomical resection	11	16 (31)	46 (32)	11 (100%)	2 (18%)	3 (27%)	6 (54%)	1 (9%)	5 (45%)
p		0.098	0.615	0.277				0.057	**0.018**

Data are shown as medians with interquartile range (IQR) or percentage when adequate. AKI, Acute Kindney Injury; CLAD, Chronic Lung Allograft Dysfunction; PGD, Primary Graft Dysfunction; OS, Overall Survival. Significant p values are reported in bold type.

Another significant indicator of mismatch was the donor-recipient age ratio. The results show that the ratio was 0.99 in the Sizing Group, compared to a lower ratio (0.845) in the Non-Sizing Group. This result may indicate a bias in organ allocation based on donor age, where, for ethical reasons, younger organs are preferentially allocated to younger recipients, and older organs to older recipients. This ethical factor may sometimes take precedence over D/R size matching, which could contribute to mismatches and the need for sizing.

The only predictive factor for the need for graft sizing was the ratio between donor and recipient height ($p = 0.02$). Interestingly, factors such as BMI, weight, and sex did not appear to predict the necessity for graft resizing, further emphasizing the importance of anatomical dimensions, such as height, over overall body mass in determining compatibility, in contrast to what has been observed in cardiac transplantation [14, 15].

A statistically significant difference was observed in the type of transplant performed, with a greater need for sizing in bilateral transplants ($p = 0.02$), as previously noted in other studies [16]. Single lung transplantation, especially in cases of pulmonary fibrosis or COPD, can lead to adaptation of the intrathoracic structures, with the graft tending to overinflate and occupy more space, creating an intrathoracic asymmetry between the native lung and the transplanted lung [17]. Due to the possible deviation of the structures toward the native lung, a larger donor lung can be used in a single transplant without the need for sizing. This is not the case in bilateral transplants, where, in the event of oversizing, graft reduction is necessary.

Although not statistically significant, it is evident that the restrictive pattern is the most frequently represented among patients who underwent sizing. This suggests that the thoracic dimensions of patients with restrictive lung disease tend to overestimate the actual intrathoracic size, which more frequently leads to the need for graft trimming.

Another important observation is that patients who underwent graft sizing had higher Lung Allocation Scores (LAS) compared to those in the non-sizing group (31 vs. 20). This difference, although not statistically significant, likely reflects the urgency under which these transplants were conducted. In many cases, organs that were less dimensionally compatible were allocated to patients with more critical conditions and higher LAS. This emphasizes the complex decision-making process in organ allocation, particularly in urgent transplant situations, where matching is often secondary to the need for a life-saving procedure [18]. This observation may also explain the lower overall survival (OS) in the Sizing Group compared to the NS Group, although not statistically significant (36.8 vs. 25.2 months in Group NS and Group S, respectively, $p = 0.625$), as patients in the Sizing Group had a higher mortality risk for their critical conditions. The numerical trend toward lower survival in the Sizing Group (25.2 vs. 36.8 months) suggests a need for longer follow-up studies to assess the long-term impact of graft resizing on survival and CLAD.

In our study, the most common surgical approach to address dimensional mismatch was anatomical resections, most commonly middle lobectomy and lingulectomy, similar to other studies [19]. This targeted approach suggests that the most common mismatch is related to the lung's shape and volume in the antero-posterior direction, which necessitates the removal of portions of the middle or lingular lobes. Furthermore, the majority of graft sizings involved the removal of two segments/lobes, indicating a bilateral dimensional mismatch.

In the study, there were no statistically significant differences in postoperative outcomes between the sizing and non-sizing groups, suggesting that lung resection remains a viable option in cases of donor-recipient size mismatch, especially in situations of donor scarcity. As observed, a reduced recipient height can contribute to increased waiting times and a higher risk of mortality on the waiting list [20]. Nevertheless, a trend toward worse outcomes, such as higher rates of PGD3 (29.7% vs. 53.0% in Groups NS and S, respectively) and extended mechanical ventilation (30.5% vs. 41.1% in Groups NS and S, respectively), was observed in the sizing group. This suggests that graft resizing may be associated with more complex procedures and potentially poorer short-term outcomes. Therefore, although it is a procedure that expands the donor pool, a careful clinical assessment is needed to ensure the best treatment for each individual recipient.

The correlation between graft reduction and a higher rate of PGD3 is likely due to three factors. First, when a graft is resized, particularly through anatomical resections, the vascular bed of the donor lung is reduced. This reduction alters the distribution of blood flow to the remaining lung tissue. After resection, blood flow to the remaining lung segments may increase to compensate for the reduced surface area, potentially leading to capillary-alveolar damage and the leakage of fluid into the alveolar spaces. This vascular redistribution can exacerbate PGD, as impaired gas exchange occurs due to the accumulation of fluid and damage to the alveolar-capillary membrane. Secondly, undersized grafts, particularly when they are overinflated to fit within the recipient's thoracic cavity, pose a significant risk for mechanical ventilation injury. Overinflation leads to ventilator-induced lung injury, as excessive tidal volumes and pressures can damage the alveolar walls and exacerbate PGD. This is a known phenomenon in mechanical ventilation, particularly when the lung is artificially expanded beyond its optimal volume. Additionally, hyperinflation in undersized grafts increases the risk of barotrauma, contributing to ventilator-induced damage, which may lead to prolonged mechanical ventilation and poorer overall outcomes. [21–23]. Finally, another mechanism resulting from graft resizing is the increased risk of pulmonary edema. In cases where significant lung tissue is removed to match the donor and recipient size, the remaining lung tissue may be more susceptible to fluid buildup. The reduction in lung volume can lead to impaired lymphatic drainage and increased capillary permeability, particularly in the post-operative period. This results in pulmonary edema,

further impairing gas exchange and contributing to the development of PGD and CLAD over time.

While the study found no significant differences in survival between the groups, it is worth noting the potential long-term impact of graft sizing on overall graft function. The FEV1 values, although not statistically significant, were lower in the graft-sizing group after 3 months, suggesting that the initial postoperative challenges may extend into longer-term pulmonary function. These findings align with previous studies, which have shown that lung size mismatch can negatively impact graft function, especially in bilateral lung transplantation [24].

One notable aspect of our study is the stratification analysis, which revealed that factors such as male sex, age over 50 years, and anatomical sizing (such as segmentectomy or lobectomy) may influence the occurrence of specific complications like PGD, acute kidney injury (AKI), and CLAD. The relevance of this observation lies in the fact that older patients typically have diminished physiological reserves, which may exacerbate the effects of graft sizing. Advanced age is a recognized risk factor for increased mortality and complications after lung transplantation, possibly due to age-related changes in pulmonary and systemic vascular function, immune response, and wound healing. Older males, in particular, may face compounded risks due to gender-specific differences in immune response, which can influence both graft rejection and long-term survival. Given these factors, it would be necessary to consider tailored monitoring strategies for high-risk subgroups, including adjusting immunosuppressive therapy, managing fluid balance carefully to avoid AKI, and using advanced ventilation strategies to minimize mechanical damage to the lung. By identifying these at-risk populations early and adjusting perioperative management accordingly, it may be possible to reduce complications and improve overall outcomes.

Additionally, special attention should be paid to the development of PGD in all patients, particularly those who are male, over 50 years old, have obstructive lung disease, and undergo anatomical resections. As observed in our study and supported by the literature, anatomical resections (lobectomy or segmentectomy) are more commonly associated with complications such as AKI and PGD. These resections involve removing larger portions of lung tissue, which leads to more significant changes in the vascular bed and mechanical function of the graft. In contrast, preserving more lung tissue, atypical resections may mitigate the extent of vascular disruption, reducing the likelihood of pulmonary edema and mechanical ventilation injury [6, 25].

The higher incidence of CLAD in male patients may be related to the development of PGD, which is now recognized as a risk factor for the development of CLAD [26]. The reduction in the vascular bed, combined with an increased risk of pulmonary edema, also necessitates maintaining a more negative electrolyte balance and the use of vasoconstrictors in patients undergoing graft sizing. This can lead, especially in older patients, to the development of AKI in the postoperative period due to renal hypoperfusion. This complication was found to be significant not only in male patients but also in those who underwent anatomical resections.

These results highlight the importance of anticipating the need for graft sizing and carefully assessing the individual recipient's risk of requiring sizing. Based on the observations, graft volume reduction is likely preferable in female recipients, those under 50 years old, and those with restrictive lung disease. Furthermore, the role of atypical resections in cases of mismatch should certainly be reevaluated in comparison to anatomical resections.

Limitations of the Study

This study has several limitations that should be considered. Firstly, it is a retrospective analysis, which inherently limits the ability to establish causal relationships and may introduce selection bias. The relatively small sample size, especially in the Sizing Group (n = 17), may limit the statistical power to detect significant differences in complications. Multi-center studies are needed to validate these findings and refine clinical guidelines for graft resizing. Another limitation is the absence of a standardized protocol for graft resizing, as surgical decisions were based on clinical judgment, which may introduce variability in the outcomes. Moreover, a potential bias of the study lies in the fact that the immunosuppressive therapy of the patients and whether the donor lungs were standard or marginal were not considered, which could clearly influence the outcomes observed. In summary, while the study provides valuable insights, further research with a larger, prospective cohort and longer follow-up is needed to validate these findings and refine the criteria for graft resizing in lung transplantation.

CONCLUSION

Height discrepancy between donor and recipient is a key predictor for resizing, aligning with previous research emphasizing the importance of anatomical dimensions over other factors like BMI or sex. Although graft resizing is a viable solution for size mismatches, it may be associated with worse short-term outcomes, such as higher rates of PGD and prolonged mechanical ventilation, especially in patients with obstructive pulmonary disease, older males, and those undergoing anatomical resection. These findings emphasize the importance of preoperative donor-recipient size matching, particularly in male recipients over 50 and those with obstructive lung disease. When resizing is unavoidable, non-anatomical resections may be preferred to minimize postoperative complications.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical approval was not required for the study involving humans in accordance with the local legislation and

institutional requirements. 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

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript. CC contributed to data curation and writing; MD'A conducted data analysis; AL, DB, and FF contributed to data collection and review; AL contributed to writing and review; PP, EB, and LL conceptualized, reviewed and supervised the research process. 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.

GENERATIVE AI STATEMENT

The authors declare that no Generative AI was used in the creation of this manuscript.

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Tremors and Health-Related Quality of Life in Liver Transplant Recipients: Post-hoc Analysis of a Multicenter, Randomized, Controlled Trial Comparing a Life Cycle Pharma-Tacrolimus Regimen and Extended-Release Tacrolimus Regimen

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We investigated whether life cycle pharma (LCP)-tacrolimus compared to extended-release (ER)-tacrolimus results in a difference in severity of tremors and HRQoL. In this multi-center, open-label, randomized, controlled trial, 108 patients were randomized in a 1:1 ratio to either LCP-tacrolimus regimen or ER-tacrolimus regimen after transplantation. HRQoL was assessed with the EQ-5D-5L and SF-36 questionnaire (two generic HRQoL instruments) and the quality of life in essential tremor (QUEST) questionnaire (domain specific HRQoL instrument). The EQ-5D-5L scores were translated to the societal values. We examined the HRQoL over the course of the study by fitting generalized mixed effect models. In total, 105 patients were included, 53 to the LCP- and 52 to the ER-tacrolimus regimen. Baseline questionnaires were available for every LT recipient. At 12 months 25% [10/40], 95% confidence interval (CI) 14.2%–40.2% of the LT recipients in the LCP-tacrolimus regimen group experienced tremors compared to 30.4% [14/46], 95%-CI

Abbreviations: CNI, Calcineurin inhibitors; eGFR, Estimated glomerular filtration rate; ER, Extended-release; HCC, Hepatocellular carcinoma; HRQoL, Health-related quality of life; IQR, Interquartile range; LCP, Life cycle pharma; LT, Liver transplantation; SAE, Serious adverse event; SD, Standard deviation; QUEST, Quality of life in essential tremor; VAS, Visual analogue scale.

19.1%–44.8% of the LT recipients in the ER-tacrolimus regimen group; risk difference: 0.054; 95%-CI –0.151–0.249; p = 0.63. No statistically significant differences in HRQoL were seen between the two regimens. We could not demonstrate differences in the HRQoL or occurrence of tremors between LCP-tacrolimus and ER-tacrolimus regimens

Keywords: liver transplantation, immunosuppressive therapy, tacrolimus, tremors, healthrelated quality of life

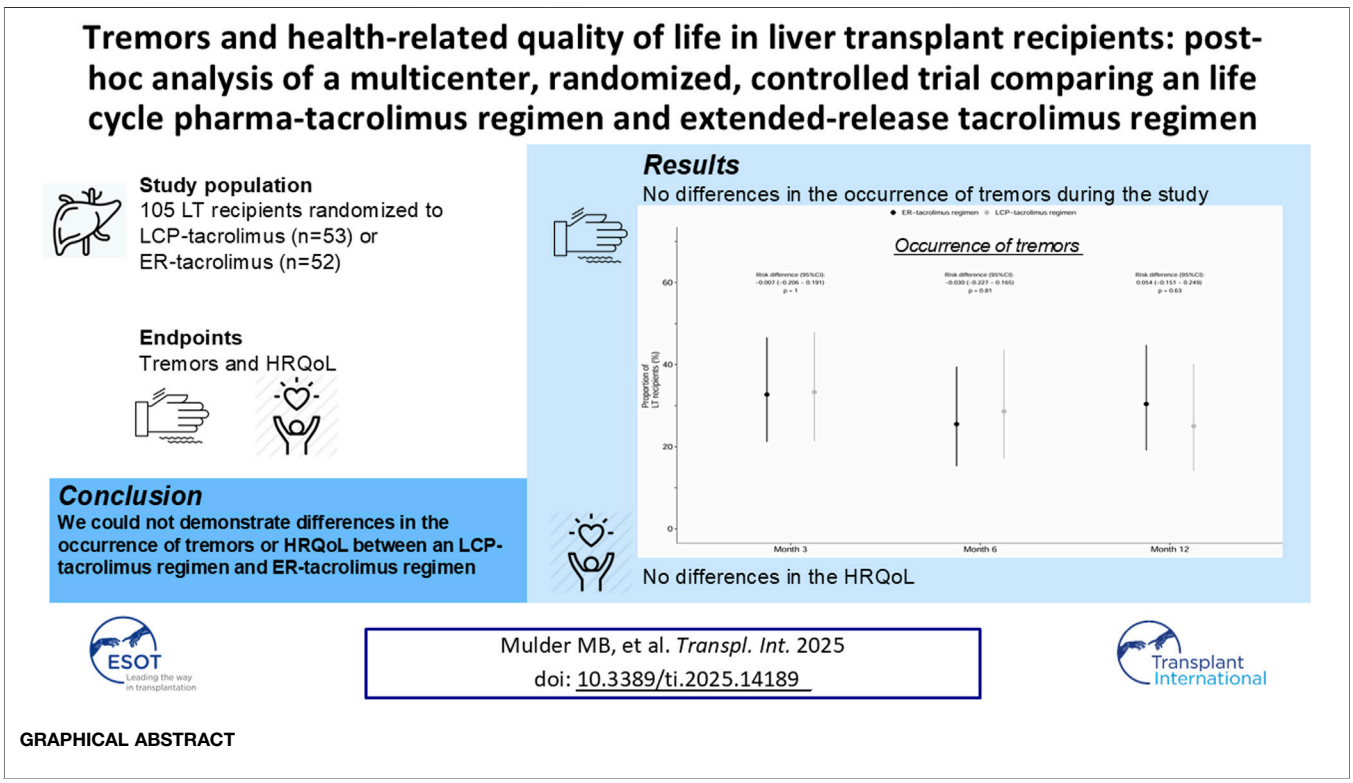
INTRODUCTION

Liver transplantation (LT) is the preferred treatment for patients with end-stage liver disease and unresectable hepatocellular carcinoma (HCC). After LT, health-related quality of life (HRQoL) generally reaches a level like the general population, except for the aspect of physical functioning [1, 2]. In general, transplant recipients need to take lifelong immunosuppressive agents. These agents are not free from side effects with everyday challenges to the quality of life [3]. Therefore, the choice of immunosuppressive agents may impact the HRQoL of LT recipients.

Tacrolimus is the cornerstone of the immunosuppressive regimen after LT and belongs to the class of calcineurin inhibitors (CNIs) [4]. CNIs are associated with neurotoxicity and affect the central and peripheral nervous systems [5, 6]. Peripheral tremors are the most frequently occurring neurological side effect and affect 30%–55% of solid organ transplant recipients [7]. Tacrolimus exposure (whole blood trough concentrations) are associated with the severity of tremors [7].

Life cycle pharma (LCP)-tacrolimus, (Envarsus®; Chiesi Farmaceutici S.p.A.) is a prolonged-release tacrolimus formulation utilizing a new drug delivery technology (MeltDose) [8, 9]. This formulation has lower peak-through blood level fluctuations and a higher bioavailability compared to the other tacrolimus formulations, resulting in a lower dose requirement to reach the intended tacrolimus exposure [8, 10]. Therefore, it is hypothesized that LCP-tacrolimus could reduce the frequency and severity of peripheral tremors.

Several studies investigated the change in tremor severity after switching from tacrolimus twice-daily capsules (Prograf®, Astellas Pharma) or extended-release (ER)-tacrolimus (Advagraf®, Astellas Pharma) to LCP-tacrolimus once-daily tablets in kidney transplant recipients [11, 12]. These studies found that patients on LCP-tacrolimus experienced significant improvement of tremor and QoL post-switch to LCP-tacrolimus irrespective of the previous tacrolimus formulation administered. However, a major limitation of these non-randomized, uncontrolled post-switch studies is the fact that only kidney transplant patients were included who already experienced a clinically significant tremor observed by a healthcare provider or by patient complaint. Up until now no head-to-head comparison between the two once-daily tacrolimus formulations has been performed.



GRAPHICAL ABSTRACT

The aim of this post-hoc analysis of our multicenter, randomized, controlled trial (MOTTO study) was to investigate whether an LCP-tacrolimus regimen compared to an ER-tacrolimus regimen results in a difference in the severity of tremors and HRQoL.

MATERIALS AND METHODS

Study Design and Participants

An extensive description of the MOTTO study design has been published previously [13]. In brief, from day 5 after LT patients received twice-daily, immediate-release (IR) tacrolimus. After achieving stable tacrolimus trough levels between 8–10 μg/L, patients were randomized in a 1:1 ratio to either a LCP-tacrolimus regimen or an ER-tacrolimus regimen. To prevent for to toxicity (renal insufficiency or tremors), rejection or to prevent recurrence of hepatocellular carcinoma: in the LCP-tacrolimus group 19 patients used a combination regimen of LCP-tacrolimus with mycophenolic acid and 2 LT recipients to LCP-tacrolimus and sirolimus. In the ER-tacrolimus group 22 LT recipients used to a regimen of ER-tacrolimus and mycophenolic acid. The study was performed at two centers in the Netherlands: The Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands and Leiden University Medical Center, Leiden, Netherlands. The study was approved by the institutional Ethical Committees of these institutions, registered in the EudraCT database (EudraCT: 2018-002856-34) and conducted in accordance with the latest version of the declaration of Helsinki. The inclusion period ran from April 2019 until October 2021.

Patient-Reported Outcomes

The evaluation of the severity of tremors and the HRQoL comprised a pre-defined secondary objective of the MOTTO study. The MOTTO study was initially designed to investigate whether LCP-tacrolimus compared to ER-tacrolimus results in a difference in the prevalence of post-transplant diabetes mellitus, new onset hypertension and chronic kidney disease at 12 months after transplantation.

HRQoL and Severity of Tremor Assessments

HRQoL was assessed with the validated Dutch version of the EQ-5D-5L questionnaire and the SF-36 questionnaire (two generic HRQoL instruments) and the quality of life in essential tremor (QUEST) questionnaire (a domain specific HRQoL instrument). The questionnaires were distributed at the day of randomization, month 3, 6, and 12.

The EQ-5D-5L questionnaire is based on a descriptive system that defines health in terms of 5 states: Mobility, Self-Care, Usual Activities, Pain/Discomfort, and Anxiety/Depression [14]. Each dimension has 5 response categories corresponding to no problems, slight problems, moderate problems, severe problems, and extreme problems. EQ-5D-5L scores were transformed to societal values based on the Dutch tariff for the EQ-5D-5L established by Versteegh *et al.* [15].

In the EQ-5D-5L questionnaire, the respondents' overall health on the day of the interview (patient's self-rated HRQoL scores) was rated on a 0–100 hash-marked, vertical visual analogue scale (EQ-VAS). The threshold for the minimally important difference (MID), indicating a clinical meaningful improvement, in the EQ-VAS score was defined as ≥7 points [16].

The SF-36 questionnaire contains 36 items grouped in eight domains: physical functioning, role limitation-physical, pain, general health, energy/fatigue, social functioning, emotional wellbeing, role limitation-emotional. Each domain is scored between 0 and 100 points, with higher scores indicating better HRQoL.

The QUEST questionnaire is a self-administered questionnaire with 30 items on a five-point scale (0–4), corresponding to the frequency (never, rarely, sometimes, frequently, always) with which tremor is perceived to currently impact five domains: physical, psychosocial, communication, hobbies/leisure and work/finance [17, 18]. The score on each domain is expressed as a percentage of the total score possible on that domain, with a higher score indicating greater dissatisfaction with that domain of QoL. A total score was computed by calculating the mean of the five domain scores.

Given that the QUEST is "domain specific" for "patients with essential tremors," this questionnaire is most likely more sensitive than the generic EQ-5D-5L and SF-36. The value of those two questionnaires is the ability to formulate "values" of quality of life for cost effectiveness analysis, and these generic questionnaires can measure side effects outside the measuring domain of the QUEST.

Management of Tremors

In this study treating physicians were allowed to apply the current common practice in order to manage the severity of tremors. This includes either reduce the dose of tacrolimus while maintaining the LT recipient on monotherapy tacrolimus or start combination therapy of tacrolimus and another immunosuppressive agent. No comedication to actively treat tremors such as beta-blockers or anticonvulsants were allowed to start for the treatment of tremors.

Data Collection

Variables collected included recipient socio-demographic, clinical and transplantation parameters, the HRQoL and tremor severity and trough levels tacrolimus.

Statistical Analysis

The HRQoL analysis included all patients within the MOTTO study who responded to at least one questionnaire. The EQ-5D-5L, SF-36 and QUEST questionnaire included in the analysis missed <5% based on the total number of measurements across all patients and questions. The missing data were considered as missing completely at random.

Two generalized linear mixed effect models were fitted to examine the HRQoL (EQ-VAS and the societal values of the EQ-5D-5L) over the course of the study. The models included

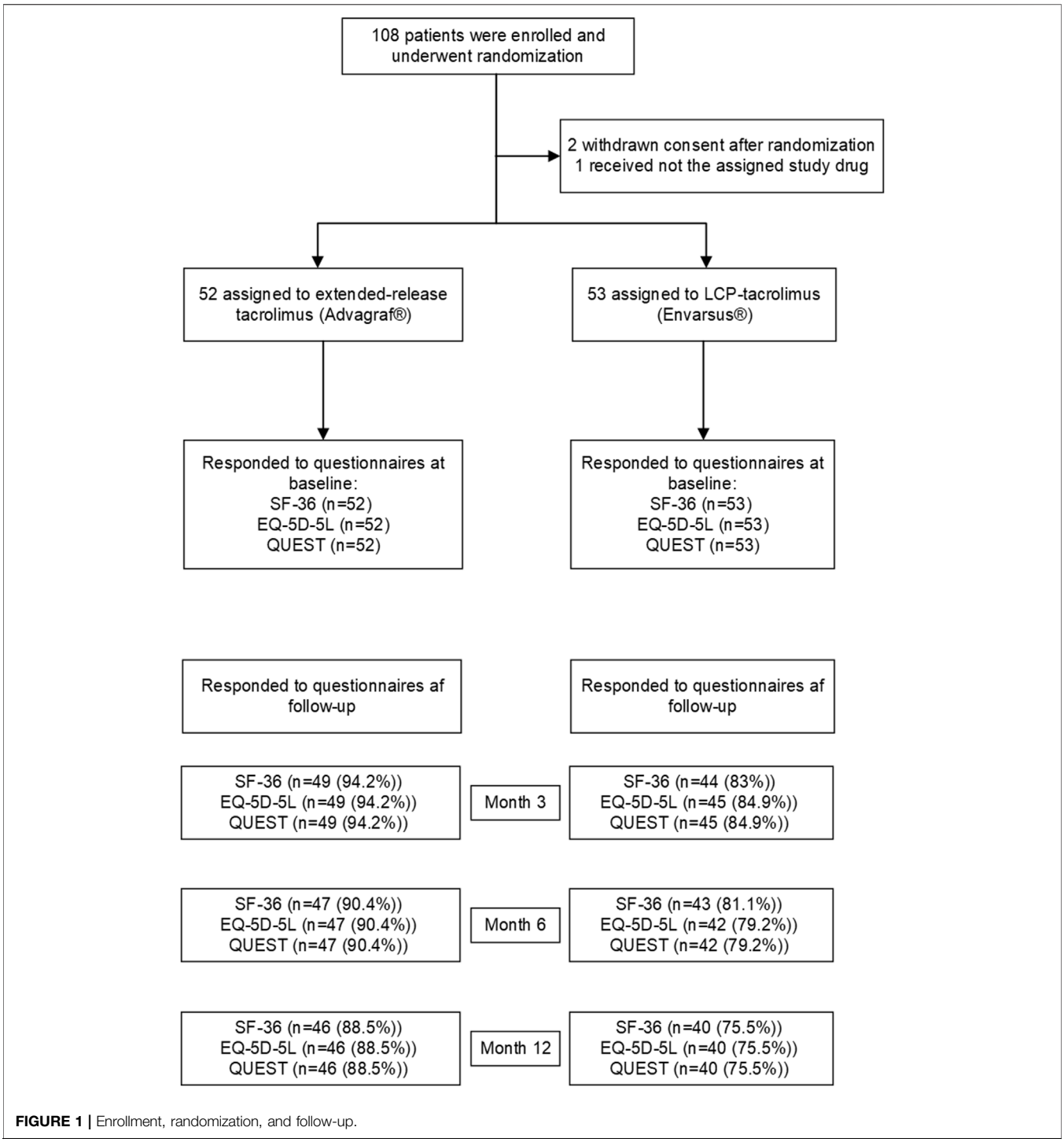


FIGURE 1 | Enrollment, randomization, and follow-up.

covariates shown or suggested to be relevant: time since transplantation, study group, tacrolimus trough concentrations, kidney function, hemoglobin, recipient age and sex, primary disease, diabetes mellitus and hypertension pre-transplantation as well as the interaction between visit and the study group. Participant specific random intercepts were included to account for correlation among repeated measurement nested within each participant. Natural cubic splines were used to model the potentially nonlinear trajectories of the EQ-VAS and societal values of the EQ-5D-5L over time. The need for these splines was evaluated using likelihood-ratio tests. Splines provide a convenient non-parametric way to flexibly model (potentially) non-linear associations in regression models. Instead of using one

TABLE 1 | Baseline characteristics.

	Extended-release tacrolimus (n = 52)	LCP-tacrolimus (n = 53)
Recipient demographics at randomization		
Age, year (median, IQR)	58.50 (46.75–65.25)	56.50 (46.25–63)
Gender, male (n, %)	41 (78.8%)	35 (66%)
Primary Disease (n, %)		
Hepatocellular carcinoma	19 (36.5%)	12 (22.6%)
(Non)alcoholic steatohepatitis	7 (13.5%)	10 (18.9%)
Primary sclerosing cholangitis	10 (19.2%)	8 (15.1%)
Acute liver failure	3 (5.8%)	3 (5.7%)
Cryptogenic cirrhosis	3 (5.8%)	3 (5.7%)
Metabolic diseases	-	4 (7.5%)
Viral Hepatitis	3 (5.8%)	3 (5.7%)
Other[a]	7 (13.5%)	11 (20.8%)
Lab		
Hemoglobin, mmol/L (mean ± SD)	6.25 ± 0.90	6.13 ± 0.84
eGFR, mL/min/1.73m^2 (mean ± SD)	82.08 ± 17.83	79.44 ± 20.43
Tacrolimus trough blood level, μg/L (mean ± SD)	6.94 ± 3.05	7.46 ± 3.28
Smoking (n, %)	11 (21.2%)	8 (14.8%)
Recipient demographics pre-transplantation		
Pre-existing Diabetes, Yes (n, %)	11 (21.2%)	13 (24.5%)
Pre-existing Hypertension, Yes (n, %)	17 (32.7%)	11 (20.8%)
EQ-5D-5L questionnaire		
VAS (mean ± SD) [ref: 0–100]	65 ± 15	58 ± 17
Societal values of the EQ-5D-5L based on the Dutch tariff for the EQ-5D-5L (median, IQR) [ref: –0.466–1]	0.53 (0.35–0.62)	0.56 (0.37–0.67)
QUEST questionnaire		
LT recipients and tremors before the start of study drug, Yes (n, %)	10 (19.2%)	16 (30.2%)
Hours of tremors per day (median, IQR)	1.0 (1.0–3.5)	4.0 (1.0–7.0)
Total score QUEST (median, IQR)	1.15 (0.28–3.33)	12.29 (1.25–23.96)

Abbreviations: eGFR, estimated glomerular filtration rate based on the CKD-EPI, formula; INR, international normalized ratio; SD, standard deviation; IQR, interquartile range; QUEST, quality of life in essential tremor.

[a]*Other includes: primary biliary cirrhosis, secondary biliary cirrhosis, autoimmune cirrhosis, cholangiocarcinoma, Caroli disease, polycystic liver disease, neuroendocrine tumor liver metastases.*

polynomial (e.g., a quadratic or cubic function) that spreads over the whole range of the covariate, splines use a set of several polynomial functions that are defined over smaller intervals. This allows the resulting fit to be more flexible and less influenced by outliers than when using a single polynomial. To visualize the estimated associations, the expected HRQoL across the course of the study was calculated while fixing the values of all other covariates to the median or reference category.

Secondary endpoints were analyzed using the Pearson's Chi-square test or Mann-Whitney U Test. Confidence intervals for binomial proportions were calculated using the binconf package for R software. For all statistical tests, a (two-sided) p-value of <0.05 was considered to indicate statistical significance.

All data were collected in CastorEDC and analysis was conducted with R software (version 4.2.1) [19, 20].

RESULTS

Patient and Treatment Characteristics

A total of 108 LT recipients was included and randomized. No LT recipient included was diagnosed with a neurological movement disorder pre-transplantation. At baseline, 100% of the LT recipients responded to the EQ-5D-5L, SF-36 and QUEST questionnaires. The response rate decreased during follow up to a minimum of 75.5% at the end of the study (**Figure 1**).

Table 1 shows the baseline characteristics. No relevant differences in the baseline characteristics for the EQ-5D-5L questionnaire existed between the two regimens. However, more LT recipients randomized to the LCP-tacrolimus regimen experienced tremors compared to the LT recipients randomized to the ER-tacrolimus regimen [30.2% (16/53), 95%-confidence interval (CI) 19.9%–44.3% *versus* 19.2% (10/52), 95%-CI 10.8%–31.9%]. The mean tacrolimus trough level at the day of randomization to the LCP-tacrolimus regimen was 7.5 ± 3.3 μg/L and in the ER-tacrolimus regimen 6.9 ± 3.1 μg/L, p = 0.38. LT recipients in the LCP-tacrolimus regimen were converted to that formulation after 11 days (IQR: 9.25–15.25 days) and in the ER-tacrolimus regimen, LT recipients were converted to that formulation after 13.5 days (IQR: 9–15.75 days).

Tremors

Figure 2 shows the proportion of LT recipients experiencing tremors during study follow up. **Supplementary Table S1** shows the QUEST questionnaire results and the tacrolimus levels of the LT recipients during the study. No statistically significant differences

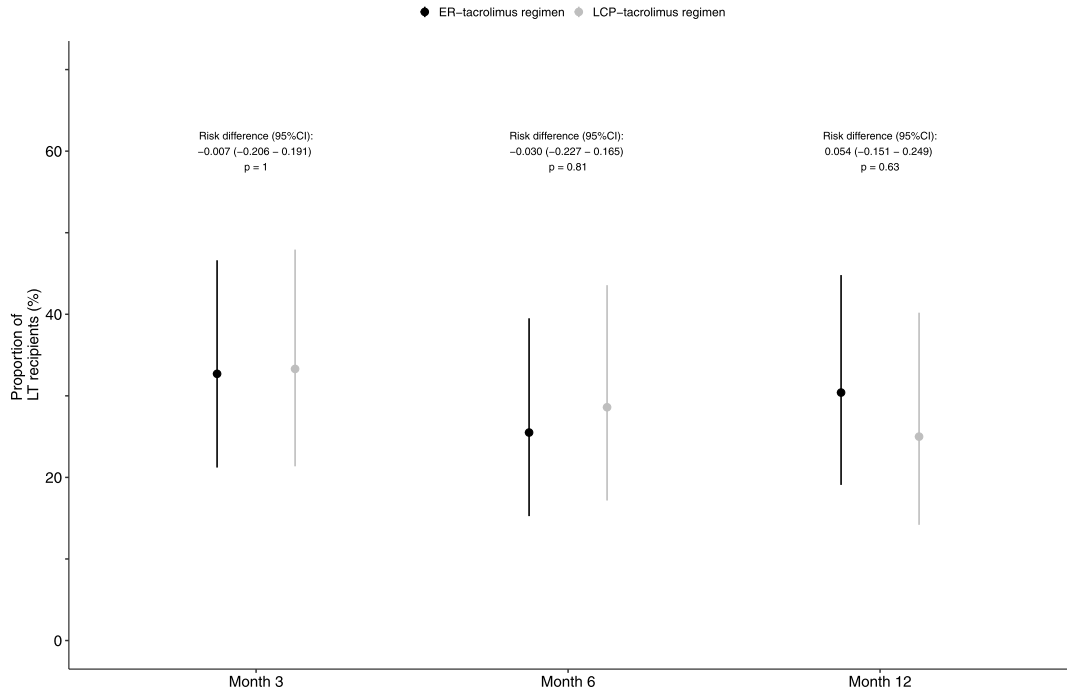


FIGURE 2 | Proportion of LT recipients experiencing tremors during follow-up. The proportion of LT recipients with 95%-CI experiencing tremors during follow-up. At 12 months 25% [10/40], 95% confidence interval (CI) 14.2%–40.2% of the LT recipients in the LCP-tacrolimus group *versus* 30.4% [14/46], 95%CI 19.1%–44.8% of the LT recipients in the ER-tacrolimus group experienced tremors; risk difference: 0.054; 95%CI -0.151–0.249; p = 0.63.

between the two regimens were found at 3, 6 and 12 months in the frequency and severity of tremors. At 12 months 25% [10/40], 95%-CI 14.2%–40.2% of the LT recipients in the LCP-tacrolimus regimen *versus* 30.4% [14/46], 95%-CI 19.1%–44.8% of the LT recipients in the ER-tacrolimus regimen experienced tremors; risk difference: 0.054; 95%CI -0.151–0.249; p = 0.63.

The mean tacrolimus trough level at 12 months in the LCP-tacrolimus regimen was statistically significantly higher compared to the ER-tacrolimus regimen: 7.6 ± 3.1 μg/L *versus* 6.3 ± 2.2 μg/L, p = 0.026. No statistically significant differences were observed in any of the five domains and the total score of the QUEST (**Supplementary Figure S1**). No relevant differences were found for tacrolimus levels versus regimen and the presence of tremors (**Supplementary Figure S5**).

Interestingly we did see effects of switching and dose reduction of tacrolimus. During the study period, some patients changed the immunosuppressive therapy because of tremors: one patient switched from ER-tacrolimus to LCP-tacrolimus, two patients switched from monotherapy LCP-tacrolimus to combination therapy of low-exposure LCP-tacrolimus with mycophenolic acid and one patient switched from monotherapy ER-tacrolimus to combination therapy of low-exposure ER-tacrolimus with mycophenolic acid. In all four LT recipients a reduction in the severity of tremors and an improved QUEST score after the switch was observed. The other patients experiencing tremors were managed by reducing the dose of the tacrolimus formulation while maintaining these patients on monotherapy with tacrolimus.

Health-Related Quality of Life Outcomes

Supplementary Figure S2 shows the proportion of responses by level of severity for the EQ-5D-5L dimensions during the study period. Overall, patients reported the least issues in the states of Self-Care and Anxiety/Depression and the most problems in the states of Usual Activities and Pain/Discomfort. No evidence for differences between the study groups in any of the five domains was found.

The likelihood-ratio tests indicated non-linear patient specific trajectories of HRQoL scores and the societal values of the EQ-5D-5L. No evidence was found for between-group differences over the course of the study based on the mixed effect models. The hemoglobin level was statistically significantly associated with a higher EQ-VAS and EQ-5D-5L score, whereas tacrolimus trough levels were statistically significantly associated with a lower EQ-VAS and EQ-5D-5L score (**Supplementary Table S2**). **Figure 3** visualize the expected HRQoL scores and societal values of the EQ-5D-5L together with the corresponding observed values per time point and study group. At the end of the study, the patient's self-rated HRQoL scores as expressed with the EQ-VAS approximate the mean self-reported EQ-VAS score by the general Dutch population. For both arms, the societal values of the EQ-5D-5L were below those of the general Dutch population.

LT recipients in both study groups achieved a clinically meaningful improvement (>7 points) in the EQ-VAS score at 12 months (LCP-tacrolimus: 20.8 points and ER-tacrolimus: 14.3 points difference with the moment of randomization).

Supplementary Figure S3 shows the results from the SF-36 questionnaire. Every domain of the SF-36 questionnaire

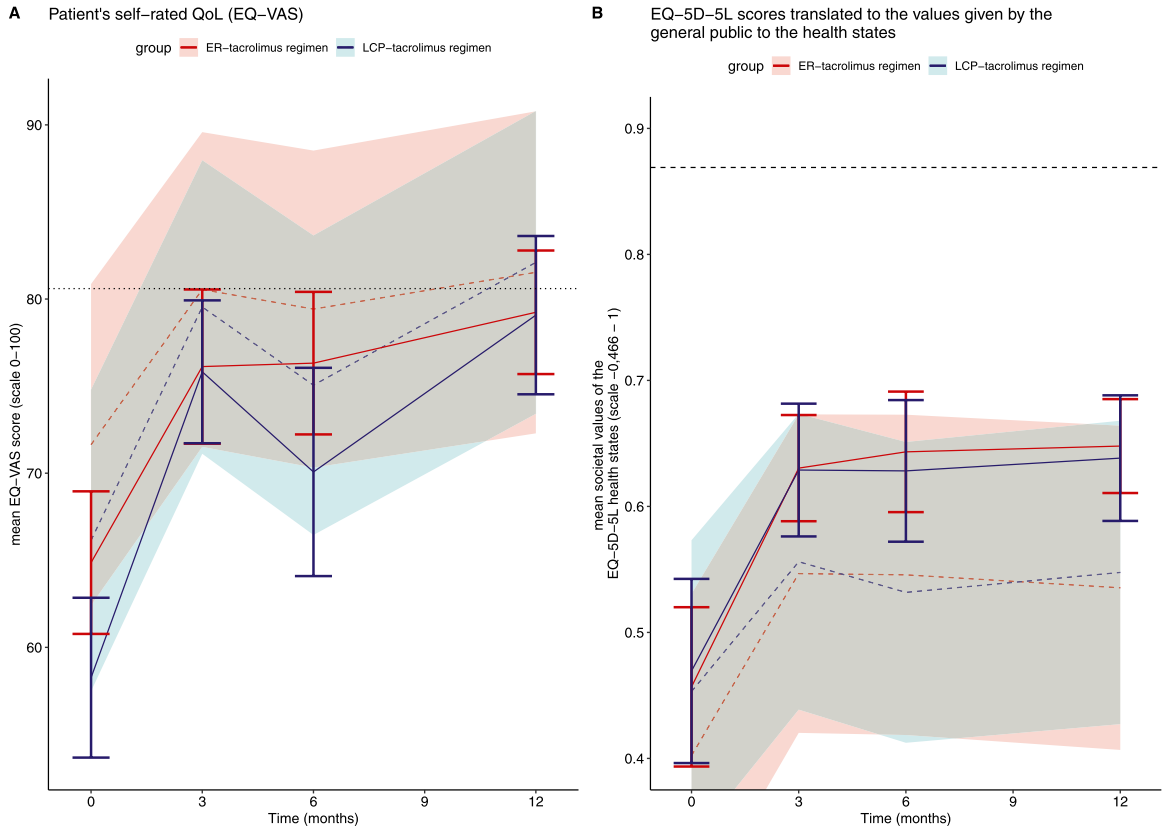


FIGURE 3 | EQ-VAS score and EQ-5D-5L scores on the dimensions translated to the societal values. **(A)** Patient's self-rated QoL (EQ-VAS) Group-wise mean EQ-VAS with 95%-confidence interval (CI) during the course of the study represented as solid lines. The dashed lines and shaded areas indicate the expected values and corresponding 95%-CI from the generalized mixed effect model (assuming the median or reference value for the continuous or categorical covariates, respectively: tacrolimus trough concentrations, kidney function, hemoglobin, recipient age and sex, primary disease, diabetes mellitus and hypertension pretransplantation as well as the interaction between visit and the study group). Dotted black line indicates the mean self-reported EQ-VAS score by the general Dutch population [15]. **(B)** EQ-5D-5L scores translated to the values given by the general public to the health states. Group-wise mean of the societal values of the EQ-5D-5L health states with 95%-confidence interval (CI) during the course of the study represented as solid lines. The dashed lines and shaded areas indicate the expected values and corresponding 95%-CI from the generalized mixed effect model (assuming the median or reference value for the continuous or categorical covariates, respectively tacrolimus trough concentrations, kidney function, hemoglobin, recipient age and sex, primary disease, diabetes mellitus and hypertension pretransplantation as well as the interaction between visit and the study group). Dotted black line indicates the mean EQ-5D-5L score given by the general Dutch population to the health states. [15]. Abbreviations: QoL, quality of life; VAS, visual analogue scale.

improved during the follow-up. Most improvement was shown in the domains: physical functioning, social functioning and pain. No statistically significant differences were found between both study groups on any of the eight domains.

An analysis of the EQ-VAS score in relation to tremors did not show statistically significant differences between LT recipients with and without tremor as indicated by the QUEST questionnaire (**Supplementary Figure S4**).

DISCUSSION

This is the first head-to-head comparison of two once-daily tacrolimus formulation regimens (i.e., LCP- and ER-tacrolimus) evaluating tremor and HRQoL in the first year after liver transplantation. In this randomized controlled study, we found no significant differences in terms of both the frequency and severity of tremors and HRQoL in LT recipients using an LCP-tacrolimus regimen compared to an ER-tacrolimus regimen. HRQoL improved over the first 12 months after liver transplantation equally in both regimens.

The findings of our study on HRQoL are in line with several other studies showing that the HRQoL of LT recipients rapidly improves after LT [1, 21]. However, conflicting results regarding the use of different immunosuppressive agents and their impact on the HRQoL of LT recipients are reported [2, 22]. We did not find evidence for differences in the HRQoL between both once-daily formulations of tacrolimus, despite a different pharmacokinetic profile and assumed lower peak levels [8]. In addition, in a previous study by our research group we also did not find a difference in the HRQoL between two regimens with different immunosuppressive agents, namely, normal dose tacrolimus *versus* a combination of low dose tacrolimus and sirolimus [2].

During the current study follow-up, the EQ-VAS approximated the mean self-reported EQ-VAS score by the general Dutch population, whereas the societal values of the EQ-5D-5L were below those of the general Dutch population. Based on the limited available evidence, it remains to be determined whether different immunosuppressive agents and different formulations of immunosuppressive agents have a clinically relevant impact on the HRQoL of LT recipients.

We did not find a difference in frequency and severity of tremors between both once daily tacrolimus regimens. This in contrast with two clinical studies in kidney transplant recipients with pre-existing tremor showing less tremors after switching to LCP-tacrolimus [11, 23]. However, these two studies evaluated the conversion from different formulations of tacrolimus, i.e., twice daily immediate-released tacrolimus and extended-released tacrolimus to once-daily LCP-tacrolimus. To emphasize, we performed a head-to-head comparison between LCP- and ER-tacrolimus and not a conversion study, which could explain our different findings.

Based on our results, we cannot conclude whether LCP-tacrolimus or ER-tacrolimus is the best treatment option to reduce tremors. In daily clinical practice, when using tacrolimus, up to 50% of the solid organ transplant recipients experience tremors [6, 11]. In this study up to 34% of the LT recipients experienced tremors while using tacrolimus. A recent study showed that high tacrolimus trough concentrations were the main determinant of tremor [7]. Interestingly, in our study, the mean tacrolimus trough levels in the LCP-tacrolimus group were statistically significantly higher at the end of the study follow-up, while no differences in frequency and severity of tremor were found. This finding suggests that higher trough levels and a more stable pharmacokinetic profile of LCP-tacrolimus seems not to be related to the occurrence of tremors. Hypothetically more equal tacrolimus trough levels in both study groups might have resulted in less tremors in the LCP-tacrolimus group. Furthermore, previously we showed that the use of LCP-tacrolimus was associated with significantly lower rates of kidney dysfunction and hypertension [13].

Multiple factors have an influence on the appearance and severity of tremors such as the height of the tacrolimus trough levels, smoking, medical conditions (e.g., hypothyroidism and hypoglycemia) or the use of certain medications (e.g., beta-blockers, bronchodilators, anticonvulsants, antidepressants) [7, 24]. The number of LT recipients smoking was equally divided over both study groups. Unfortunately, adequate information regarding the use of concomitant drugs influencing tremors was not available. Since beta-blockers are occasionally prescribed to treat post-transplant hypertension the frequency and severity of tremors in this study might be underestimated.

Another study showed that severe tremor in solid organ transplant recipients was strongly and independently associated with lower physical and mental HRQoL [7]. We could not find lower HRQoL scores for LT recipients experiencing tremors compared to LT recipients without tremors.

A major strength of this study is the fact that this is a randomized controlled trial and not a conversion or switch study with a high response rate and longitudinal assessment of the HRQoL and severity of tremors. A limitation is the lack of statistical power in this post-hoc analysis. At 12 months, we found no statistical significant differences between the two treatment groups. However, we did find less tremors in the LCP-tacrolimus group. Potentially with more power and, as described above, more equal tacrolimus trough levels in both study groups we might have found a statistical significant and clinical relevant difference in the frequency of tremors in LT recipients on tacrolimus. An other limitation is the fact that the tremors reported by LT recipients were not evaluated by a physician using the Fahn-Tolosa-Marin tremor reporting scale. This tremor reporting scale was developed to quantify essential tremor severity and has been used in large trials for essential tremor. The QUEST questionnaire is a self-assessment and therefore the results regarding the severity of the tremor are not objectified.

We believe that reducing the dose of tacrolimus with or without adding another immunosuppressive agent is the way to go to reduce neurotoxicity in LT recipients.

In conclusion, based on this clinical study, an once-daily LCP-tacrolimus regimen is not associated with an improvement in the HRQoL or a reduction in the occurrence of tremors compared to an ER-tacrolimus regimen. Aiming for lower tacrolimus trough levels or exposure seems a better strategy to reduce the severity and frequency of tremors.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by The Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands and Leiden University Medical Center, Leiden, Netherlands. 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

HM designed the study. MM, BH, WP, IA, BW, SM, EV-H, LE, DH, CH, and HM were involved in the execution of the study. MM and HM had access to and verified the underlying data. MM and NE analyzed the data. MM wrote the manuscript with input from all other authors. All authors participated in data interpretation, manuscript writing, review, and approval of the final version of the manuscript for submission. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

MM has received lecture fees and consulting fees from Chiesi Pharma and grant support from Astellas Pharma. MM does not have employment or stock ownership at any of these companies, and neither does he have patents or patent applications. DH has received lecture fees and consulting fees from Astellas Pharma, Astra Zeneca, Chiesi Pharma, Medincell, Novartis Pharma, Sangamo Therapeutics and Vifor Pharma. He has received grant support from Astellas Pharma, Bristol-Myers Squibb and Chiesi Pharma (paid to his institution). DH does not have employment or stock ownership at any of these companies, and neither does he have patents or patent applications. CH has received lecture fees and consulting fees from Chiesi Pharma, Takeda, Novartis Pharma, Abacus medical and travel grants from Orphalan. CH does not have employment or stock ownership at any of these companies, and neither does she have patents or patent applications. HM has received lecture fees from Astellas Pharma and received grant support from Astellas Pharma, Novartis Pharma and Chiesi Pharma.

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The authors declare that no Generative AI was used in the creation of this manuscript.

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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.2025.14189/full#supplementary-material

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ORIGINAL RESEARCH
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Clinical and Radiological Fusion: A New Frontier in Predicting Post-Transplant Diabetes Mellitus

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This study developed a predictive model for Post-Transplant Diabetes Mellitus (PTDM) by integrating clinical and radiological data to identify at-risk kidney transplant recipients. In a retrospective analysis across three Mayo Clinic sites, clinical metrics were combined with deep learning analysis of pre-transplant CT images, focusing on body composition parameters like adipose tissue and muscle mass instead of BMI or other biomarkers. Among 2,005 nondiabetic kidney recipients, 335 (16.7%) developed PTDM within the first year. PTDM patients were older, had higher BMIs, elevated triglycerides, and were more likely to be male and non-White. They exhibited lower skeletal muscle area, greater visceral adipose tissue (VAT), more intermuscular fat, and higher subcutaneous fat (all $p < 0.001$). Multivariable analysis identified age (OR: 1.05, 95% CI: 1.03–1.08, $p < 0.0001$), family diabetes history (OR: 1.55, CI: 1.14–2.09, $p = 0.0061$), White race (OR: 0.43, CI: 0.28–0.66, $p < 0.0001$), and VAT area (OR: 1.37, CI: 1.14–1.64, $p = 0.0009$) as predictors. The combined model achieved C-statistic of 0.724 (CI: 0.692–0.757), outperforming the clinical-only model (C-statistic 0.68). Patients with PTDM in the first year had higher mortality than those without PTDM. This model improves predictive precision, enabling accurate identification and intervention for at risk patients.

Keywords: post transplant diabetes, kidney transplant, obesity, adiposity, visceral diposity

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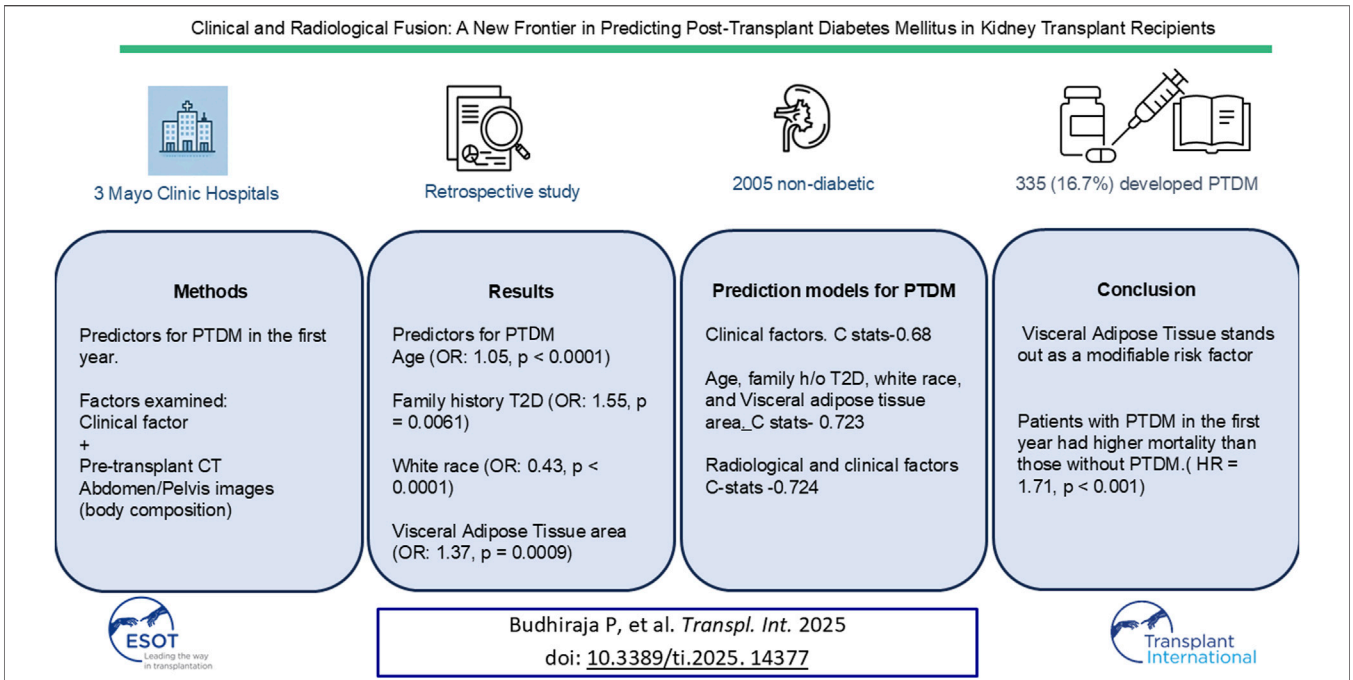
INTRODUCTION

Post-transplant diabetes mellitus (PTDM) refers to the onset of diabetes in previously nondiabetic individuals following organ transplantation. The incidence of PTDM varies depending on the type of organ transplanted and the post-transplant period. Studies estimate that, at 12 months post-transplant, the incidence ranges from 10% to 30% for kidney transplant recipients [1–6]. This

Abbreviations: AUC, Area under the curve; BMI, Body Mass Index; CI, Confidence intervals; CT, Computed Tomography; DM, Diabetes Mellitus; DXA, Dual-Energy X-ray Absorptiometry; GLP-1 RAs, Glucagon-Like Peptide-1 receptor agonists; IMAT, Intermuscular adipose tissue; PTDM, Post Transplant Diabetes Mellitus; SAT, Subcutaneous adipose tissue; T2D, Type 2 diabetes; VAT, Visceral adipose tissue.



GRAPHICAL ABSTRACT

variation may be attributed to differences in diagnostic criteria for type 2 diabetes (T2D), diverse study populations, varying immunosuppression protocols, and the timeframes of the studies.

PTDM has a significant impact on transplant outcomes, being associated with an increased risk of graft rejection [7], infections [7], graft loss [8], cardiovascular mortality, and overall mortality [8–10]. In a United States Renal Data System study of 11,659 patients who received a kidney transplant between 1996 and 2000, PTDM was associated with a more than 60% increase in the incidence of graft failure and a 90% increase in mortality [10]. Additionally, PTDM negatively affects quality of life and substantially raises annual healthcare costs [11].

There is a nine-fold increased risk of diabetes in solid organ transplant recipients compared to their age-matched controls [12]. While the pathophysiology of PTDM mirrors that of T2D, it is further complicated by both transplantation-specific and non-transplantation-related risk factors [13]. The incidence of PTDM is rising, driven by the increasing number of kidney transplants, an aging recipient population, growing obesity trends, and the widespread use of tacrolimus [1, 9, 10, 12, 14].

Obesity is on the rise, leading to an increased risk of PTDM. Obesity is often assessed using body mass index (BMI), a widely used but limited measure [15–17].

BMI overlooks important variations in body composition and fat distribution across different ethnic groups, ages, and genders. It does not differentiate between muscle and fat mass, nor does it distinguish between subcutaneous and visceral adipose tissue (VAT)—the latter being more strongly associated with insulin resistance, metabolic syndrome, and elevated mortality [18, 19].

Given these limitations, there is increasing interest in using body composition analysis to provide deeper insights into metabolic health and improve the accuracy of PTDM risk prediction. Unlike BMI, a single axial computed tomography (CT) slice of the abdomen can visualize and quantify subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), intermuscular adipose tissue (IMAT), and skeletal muscle areas. These more detailed measurements provide a clearer understanding of PTDM risk factors and open new avenues for targeted interventions.

We propose a prediction model that incorporates body composition vs. BMI as a surrogate marker for obesity [20, 21]. Our team has developed a deep learning analysis of cross-sectional imaging to quantify body composition [22]. This algorithm automatically segments the following compartments: SAT, VAT, muscle, bone, and visceral organs. In the present study, we integrated clinical data with information from this deep learning model to predict PTDM.

Given the complexity and burden of PTDM, developing a comprehensive predictive tool incorporating body composition using deep learning can significantly enhance precision-based medicine for transplant recipients.

MATERIALS AND METHODS

Study Design and Setting: This is a retrospective study of the three Mayo Clinic sites. (Arizona, Florida, and Rochester). The Mayo Clinic Institutional Review Board approved this study.

Participants: The subjects were from three Mayo sites. The Mayo Clinic Arizona cohort was selected from 1/2007 to 1/2022, and the other two sites were included from 1/2014 to 1/2022 due to changes in the pre-transplant candidate imaging testing protocol. The cohort included both living and deceased donor transplants. The last follow-up was at the end of 1/2023.

Preoperative CT scans were primarily performed for vascular assessment, which has become the standard of care for evaluating kidney transplant candidates. Initially, CT imaging was selectively used in patients with peripheral vascular disease, diminished distal pulses, polycystic kidney disease, or a history of previous transplants. The primary purpose of these scans was to assess vascular anatomy and identify potential complications that could impact the surgical approach. Over time, as the clinical benefits of comprehensive vascular imaging became evident, preoperative CT scans were expanded to include all transplant candidates to ensure thorough pre-surgical planning and risk assessment. The study was approved by the Mayo institutional review board and was conducted in compliance with the Declaration of Helsinki.

Inclusion criteria:

- Kidney transplant recipients who have had:
  - Pre-operative CT abdomen and pelvis within 1 year before transplant or 1 month after kidney transplant
  - At least 1 year of follow-up at Mayo Clinic
  - Patient and graft surviving at 1 year.

Exclusion criteria:

- Patients with pre-existing Diabetes Mellitus (DM)
- Multivisceral organ transplants.
- Previous kidney transplant

Immunosuppression Protocol

All patients received induction immunosuppression. Before 2011, patients received induction with rabbit-anti thymocyte globulin. After 2011, induction was with Alemtuzumab. Patients over 65 received Basiliximab, which did not change during the study period. Patients receiving induction with the depleting agents had a complete withdrawal of corticosteroids by post-transplant day 5, while those receiving Basiliximab inductions continued maintenance corticosteroids. Steroids were maintained if they had panel reactive antibody >80%, donor-specific antibody, or end-stage renal disease from glomerulonephritis. Maintenance immunosuppression was with tacrolimus and mycophenolate mofetil. The trough tacrolimus levels were 8–10 ng/mL for the first month and then 6–8 ng/mL.

Diagnosis of PTDM

In this study, we diagnosed PTDM using the American Diabetes Association definition based on Hba1c ≥6.5%, or fasting blood sugar ≥126 mg/dL, or random glucose ≥200 mg/dL or medications for diabetes management [23].

Clinical Model for PTDM Prediction

In our previous work, we examined PTDM risk using the clinical factors [17] through two multivariable approaches [1]: a standard model that included continuous and discrete variables without categorization and [2] a dichotomized model, where variables were assigned binary values based on clinically relevant cut points. In the standard model, continuous variables (such as recipient age, baseline BMI, steroid use, triglycerides, pretransplant fasting glucose, and family history of type 2 diabetes) were included and weighted according to their β-coefficients in the multivariable logistic model. In the dichotomous model, continuous variables were dichotomized based on clinically relevant cut points (values below and above the cut point assigned 0 and 1, respectively) and weighted according to the β-coefficients. This approach included age ≥50 years, BMI ≥30 kg/m^2, steroid use post-transplant, triglycerides ≥200 mg/dL, pretransplant fasting glucose ≥100 mg/dL, and family history of T2D.

Building on this foundation, we aimed to develop a more advanced and comprehensive predictive model for PTDM by integrating clinical and radiological data. This new model includes body composition measures derived from automated CT analysis, which provide a more precise assessment than BMI. We compared the performance of our previously established clinical model with this enhanced radiological approach, enabling a detailed assessment of PTDM risk linked to specific body composition profiles.

Automated Body Composition Analysis

Mayo Clinic has previously developed deep learning models that automatically calculate highly accurate body composition measurements from CT images to inform individual care. These models use a fully automated abdominal segmentation deep neural network [22]. Furthermore, our model can segment SAT and VAT, muscle, abdominal organs, and bone; most fully automated algorithms are demonstrated on adipose tissue and muscle alone. We obtained the following measures: skeletal muscle, SAT, VAT, and IMAT.

Examinations were segmented into four compartments—subcutaneous adipose tissue, muscle, viscera, and bone—and pixels external to the body. The visceral compartment was further separated into VAT-free tissue and VAT using thresholding. Visceral adipose tissue-free tissue is primarily composed of abdominal organs, vessels, and the contents of the digestive tract. Further details of the model are available in the manuscript by Weston et al. [22].

To determine whether a model trained on a 2D section at the level of the Lumbar 3 transverse processes could generalize across the entire abdomen, L2 complete examinations of the abdomen from the inferior endplate of the L1 vertebra to the superior endplate of the L5 vertebra were used. Each section in this range was segmented. This is an example of a three-dimensional model using the deep learning algorithm developed by the group. The image variables were scaled by their standard deviation (using standardization).

Statistical Analysis

Descriptive statistics were reported as mean (standard deviation) for continuous variables and frequency (percentage) for categorical variables. We compared continuous variables in 2 groups using a Student's t-test and dichotomous outcomes using chi-square. Nonparametric tests compared heavily skewed data. A p-value <0.05 was considered statistically significant. Missing data was not imputed; models were only fit on complete datasets.

We analyzed factors associated with the development of PTDM using univariate analysis. The factors significant in univariate analysis were included in the Multivariable analysis.

These models included:

1. The previously established clinical continuous model (Age, BMI at baseline, steroid maintenance, pretransplant fasting glucose, pre-transplant fasting triglycerides (log-transformed), family history of T2DM [17].
2. Clinical discrete model (Age ≥50 years, BMI ≥30 kg/m^2, steroid maintenance, fasting triglycerides ≥200 mg/dL, fasting glucose ≥100 mg/dL and family history of T2D) [17].
3. Baseline clinical factors that were significant in our model on univariate analysis
4. Model with radiology morphometric features (skeletal muscle area, SAT area, VAT area, IMAT area)
5. The model combining model 3 (Baseline factors that were significant in our model on the univariate analysis) and model 4 (radiology morphometric features)
6. Model with baseline factors that were significant on a multivariable analysis of model 5.

We evaluated the performance of various predictive models for diabetes mellitus post-transplant using the C-statistic. The comparisons were conducted on the same population to ensure consistency. The C-statistics and their corresponding 95% confidence intervals (CIs) were calculated, and cross-validation was performed to obtain mean C-statistics. Additionally, a C-statistic comparison was executed using the infinitesimal jackknife method.

We also examined the impact of the development of PTDM within the first year on patient and graft survival.

All statistical analyses were performed using the R Statistical Program, Version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

In a cohort of 2,005 nondiabetic kidney transplant recipients, PTDM occurred in 335 patients (16.7%) within the first year post-transplant. The mean age of recipients was 52.6 years (SD = 14.2), and 56.9% were male (**Table 1**).

The average age at transplant was significantly higher for those who developed PTDM, at 58.6 years (SD = 12.4), compared to 51.3 years (SD = 14.2) for those who did not develop PTDM (p < 0.001). In the post-transplant diabetes mellitus (PTDM) group, 34.7% of patients received a living donor kidney transplant, compared to 39.4% in the non-PTDM group (p = 0.105). The proportion of male recipients was significantly higher among those who developed PTDM (63.8%) compared to those who did not (55.5%) (p = 0.006). The racial distribution also differed significantly between the two groups. Among patients who developed PTDM, 58.8% were White, 17.3% African American, 6.9% Asian, 12.5% Hispanic, 1.2% Native American, and 3.3% Other. In contrast, among those who did not develop PTDM, the distribution was 67.7% White, 12.2% African American, 4.0% Asian, 11.5% Hispanic, 1.9% Native American, and 2.7% Other (p = 0.008). This suggests that the proportion of White recipients was lower among those who developed PTDM (58.8% vs. 67.7%), while the proportions of African American, Asian, and Hispanic recipients were higher among those who developed PTDM.

The difference between the two groups in preemptive transplant versus dialysis status before transplant was not statistically significant (28.7% vs. 27.1%, p = 0.565). However, at baseline, PTDM patients had a significantly higher BMI (27.0 kg/m^2 vs. 25.9 kg/m^2, p < 0.001). C-peptide levels were similar between the groups.

Radiological Characteristics

In this study, several key differences in body composition were observed between individuals who developed PTDM and those who did not (**Table 2**).

PTDM patients had a lower skeletal muscle area (165.3 cm^2 vs. 171.5 cm^2, p = 0.001) and lower skeletal muscle mean Hounsfield Units (HU) (32.6 vs. 33.0, p = 0.001), indicating reduced muscle mass and poorer muscle quality compared to non-PTDM patients. HU values measure tissue density, and lower values indicate less healthy muscle.

Moreover, PTDM patients exhibited larger areas of both SAT (285.1 cm^2 vs. 275.8 cm^2, p = 0.001) and VAT (121.7 cm^2 vs. 111.8 cm^2, p = 0.001). Additionally, a higher proportion of PTDM patients (57.6%) were in the highest quartile (Q4) of VAT compared to non-PTDM patients (73.5% in Q1-Q3, p = 0.001).

There was also a significant increase in IMAT in PTDM patients (2.4 cm^2 vs. 1.7 cm^2, p = 0.001). This increase in IMAT is associated with reduced muscle function and metabolic health. No significant differences were found in the quality (HU values) of SAT (p = 0.084) or IMAT (p = 0.318). However, PTDM patients had slightly lower VAT HU values (−6.6 vs. −6.4, p = 0.001), suggesting that the visceral fat in PTDM patients was denser, which could be metabolically more harmful, as denser fat is associated with worse metabolic outcomes.

Risk Factors for PTDM

In multivariable analysis, key predictors of PTDM included recipient age (OR: 1.05, 95% CI: 1.03–1.08, p < 0.0001), family history of type 2 diabetes (OR: 1.55, 95% CI: 1.14–2.09, p = 0.0061), White race (OR: 0.43, 95% CI: 0.28–0.66, p < 0.0001), visceral adipose tissue (VAT) area (OR: 1.37, 95% CI: 1.14–1.64, p = 0.0009), and weight change (OR: 1.02, 95% CI: 1.00–1.03, p = 0.013). Other factors, such as BMI, steroid use, and various adipose tissue measures, showed associations in univariate

TABLE 1 | Demographics and clinical characteristics.

Variable	No PTDM (N = 1,670)	Developed PTDM (N = 335)	Total (N = 2005)	p-value
Sex				0.006
Female	733 (44.5%)	119 (36.2%)	852 (43.1%)	
Male	916 (55.5%)	210 (63.8%)	1,126 (56.9%)	
Age at Transplant	51.3 (14.2)	58.6 (12.4)	52.6 (14.2)	<0.001
RACE				0.008
African American	203 (12.2%)	58 (17.3%)	261 (13.0%)	
Asian	67 (4.0%)	23 (6.9%)	90 (4.5%)	
Hispanic	192 (11.5%)	42 (12.5%)	234 (11.7%)	
Native American	32 (1.9%)	4 (1.2%)	36 (1.8%)	
Other	45 (2.7%)	11 (3.3%)	56 (2.8%)	
White	1,131 (67.7%)	197 (58.8%)	1,328 (66.2%)	
Age of donor	40.3 (14.9)	42.6 (15.4)	40.7 (15.0)	0.011
Dialysis before transplant				0.565
No	447 (27.1%)	94 (28.7%)	541 (27.4%)	
Yes	1,202 (72.9%)	234 (71.3%)	1,436 (72.6%)	
Weight (kilogram)	75.9 (18.4)	80.1 (19.2)	76.6 (18.6)	<0.0001
Body mass index (kg/m^2)	25.9 (5.1)	27.0 (5.4)	26.1 (5.2)	<0.0001
C-peptide before transplant Median (Interquartile range) (ng/mL)	7.200 (4.425, 11.175)	8.200 (4.900, 14.300)	7.300 (4.600, 11.700)	0.942

TABLE 2 | Radiological factors.

Variable	No PTDM (N = 1,670)	Developed PTDM (N = 335)	Total (N = 2005)	Values-value
Skeletal muscle area				
Mean (SD)	3.7 (1.0)	3.9 (1.0)	3.7 (1.0)	0.001
Median (Q1, Q3):	3.6 (2.9, 4.4)	3.9 (3.2, 4.5)	3.6 (2.9, 4.4)	
Range	0.5–7.4	1.8–7.5	0.5–7.5	
Skeletal muscle mean HU				
Mean (SD)	3.3 (1.0)	3.0 (1.0)	3.2 (1.0)	<0.001
Median (Q1, Q3):	3.3 (2.6, 3.9)	2.9 (2.3, 3.7)	3.2 (2.6, 3.9)	
Range	−0.5–8.6	0.5–5.6	−0.5–8.6	
Subcutaneous adipose tissue area				
Mean (SD)	1.7 (1.0)	2.0 (1.0)	1.8 (1.0)	<0.001
Median (Q1, Q3):	1.6 (1.0, 2.3)	1.8 (1.2, 2.6)	1.6 (1.1, 2.3)	
Range	0.1–5.8	0.3–5.2	0.1–5.8	
Subcutaneous adipose tissue mean HU				
Mean (SD)	−4.0 (1.0)	−4.1 (0.9)	−4.0 (1.0)	0.084
Median (Q1, Q3):	−4.3 (−4.7, −3.7)	−4.3 (−4.7, −3.9)	−4.3 (−4.7, −3.7)	
Range	−5.6–7.2	−5.4–0.3	−5.6–7.2	
Visceral adipose tissue area				
Mean (SD)	1.3 (1.0)	1.8 (1.1)	1.3 (1.0)	<0.001
Median (Q1, Q3):	1.1 (0.5, 1.8)	1.7 (1.0, 2.5)	1.2 (0.5, 1.9)	
Range	0.0–6.0	0.0–6.0	0.0–6.0	
Visceral adipose tissue area quartile				<0.001
Q1-3:	1,104 (78.6%)	170 (57.8%)	1,274 (75.0%)	
Q4:	301 (21.4%)	124 (42.2%)	425 (25.0%)	
Visceral adipose tissue mean HU				
Mean (SD)	−6.3 (1.0)	−6.6 (1.0)	−6.3 (1.0)	<0.001
Median (Q1, Q3):	−6.4 (−7.0, −5.7)	−6.8 (−7.3, −6.0)	−6.5 (−7.0, −5.7)	
Range	−17.0–3.1	−17.0–3.1	−17.0–3.1	
Intermuscular adipose tissue area				
Mean (SD)	1.7 (1.0)	2.1 (1.0)	1.8 (1.0)	<0.001
Median (Q1, Q3):	1.6 (1.1, 2.2)	1.9 (1.4, 2.4)	1.7 (1.1, 2.3)	
Range	0.0–11.3	0.3–8.9	0.0–11.3	
Intermuscular adipose tissue mean HU				
Mean (SD)	−13.8 (1.0)	−13.9 (0.9)	−13.8 (1.0)	0.318
Median (Q1, Q3):	−13.8 (−14.4, −13.2)	−13.9 (−14.3, −13.3)	−13.8 (−14.4, −13.2)	
Range	−17.3–9.3	−16.7–10.6	−17.3–9.3	

HU, Hounsfield units; SD, standard deviation; Q1, Quartile 1; Q3, Quartile 3.

TABLE 3 | Factors associated with the development of Post Transplant Diabetes Mellitus.

Variables	N univariate	Odds ratio (CI) univariate	P value univariate	N multivariable	Odds ratio (CI) multivariable	P value multivariable
Recipient Age	2005	1.04 (1.03, 1.05)	<0.0001	1,603	1.05 (1.03, 1.08)	<0.0001
BMI (Baseline)	1670	1.04 (1.02, 1.06)	0.001	1,603	1.03 (0.98, 1.07)	0.2110
Dialysis Duration	1977	0.93 (0.71, 1.21)	0.565	NA	NA	NA
Steroid maintenance	1968	1.38 (1.06, 1.82)	0.0180	1,603	1.27 (0.91, 1.80)	0.1669
Family h/o type 2 diabetes mellitus	1947	1.55 (1.20, 1.99)	0.0007	1,603	1.55 (1.14, 2.09)	0.0061
Triglyceride pre transplant	1213	1.00 (1.00, 1.00)	0.0382		1.00 (1.00, 1.00)	0.3640
Fasting glucose pre transplant	1115	1.01 (0.99,1.03)	0.4010	NA		
Sex Male	1978	1.41 (1.11, 1.81)	0.0058	1,603	1.05 (0.68, 1.64)	0.8137
Fasting glucose	1644	0.98 (0.86, 1.12)	0.8041	NA	NA	NA
Asian	2005	1.20 (0.68, 2.08)	0.5178	1,603	1.01 (0.49, 2.01)	0.9753
Hispanic	2005	0.77 (0.49, 1.19)	0.2378	1,603	0.63 (0.35, 1.12)	0.1168
Native American	2005	0.44 (0.13, 1.16)	0.1334	1,603	0.29 (0.06, 0.94)	0.0629
Other Race	2005	0.86 (0.40, 1.71)	0.6715	1,603	0.73 (0.22, 2.02)	0.5696
White	2005	0.61 (0.44, 0.85)	0.0032	1,603	0.43 (0.28, 0.66)	<0.0001
Skeletal Muscle Area	1699	1.23 (1.09, 1.39)	0.0011	1,603	1.06 (0.84, 1.33)	0.6311
Subcutaneous Adipose Tissue Area	1699	1.26 (1.12, 1.42)	<0.0001	1,603	1.02 (0.81, 1.29)	0.8390
Intermuscular Adipose Tissue Area	1699	1.35 (1.20, 1.51)	<0.0001	1,603	0.97 (0.81, 1.15)	0.7578
Visceral Adipose Tissue Area	1699	1.63 (1.45, 1.84)	<0.0001	1,603	1.37 (1.14, 1.64)	0.0009

BMI, Body mass index; CI, Confidence Interval.

TABLE 4 | Models for post-transplant diabetes mellitus prediction.

Model	Variables	C-statistic	Mean Cross-validated C-statistic
Continuous model	Recipient Age, baseline Body mass index, steroid maintenance, pretransplant fasting glucose, pre-transplant fasting triglycerides (log-transformed), family history of diabetes mellitus	0.68 (0.636, 0.724)	0.676
Discrete model	Age ≥50, baseline Body mass index ≥30 kg/m^2, steroid maintenance, pretransplant fasting glucose ≥100 mg/dL fasting triglycerides ≥200 mg/dL, family history of diabetes mellitus	0.656 (0.612, 0.699)	0.651
Baseline clinical factors significant on univariate analysis	Sex, age, race, baseline Body mass index, family history of diabetes	0.701 (0.668, 0.734)	0.686
Radiology only	Skeletal muscle area, subcutaneous adipose tissue area, visceral adipose tissue area, intermuscular adipose tissue area	0.658 (0.625, 0.692)	0.656
Baseline factors significant on univariate analysis with radiology (baseline + radiology)	Sex, age, race, baseline Body mass index, family history of diabetes mellitus, skeletal muscle area, subcutaneous adipose tissue area, visceral tissue area, intermuscular adipose tissue area	0.724 (0.692, 0.757)	0.705
Baseline Variables significant on multivariable analysis	Age, family history of diabetes mellitus, race, visceral adipose tissue area	0.723 (0.691, 0.754	0.714

analysis but did not retain significance in the multivariable model (**Table 3**).

Models for PTDM Prediction

Table 4 summarizes the results of various predictive models for PTDM. The previously established clinical continuous model in this study achieved a C-statistic of 0.68 (95% CI: 0.636, 0.724) with a mean cross-validated C-statistic of 0.676. The clinical discrete model, which used binary cut-points for key variables, had a C-statistic of 0.656 (95% CI: 0.612, 0.699) and a mean cross-validated C-statistic of 0.651 (**Table 4**).

Baseline clinical factors that were significant on univariate analysis (sex, recipient age, race, baseline BMI, and family history of type 2 diabetes) achieved a C-statistic of 0.701 (95% CI: 0.668, 0.734) with a mean cross-validated C-statistic of 0.686. When these baseline clinical factors were combined with radiological measures (skeletal muscle area, SAT area, VAT area, and IMAT area), the "Baseline + radiology" model achieved the highest C-statistic of 0.724 (95% CI: 0.692, 0.757) and a mean cross-validated C-statistic of 0.705. This finding suggests that integrating radiological factors with clinical data yields the most accurate prediction of PTDM risk in this study.

The multivariable significant variables model, which included only age, family history of diabetes, race, and VAT, demonstrated nearly equivalent predictive performance with a mean cross-validated C-statistic of 0.714. This streamlined model provides a strong balance of predictive accuracy and simplicity, making it potentially more practical for clinical application.

Survival Analysis

Patients who developed PTDM within the first year showed lower patient survival rates compared to those

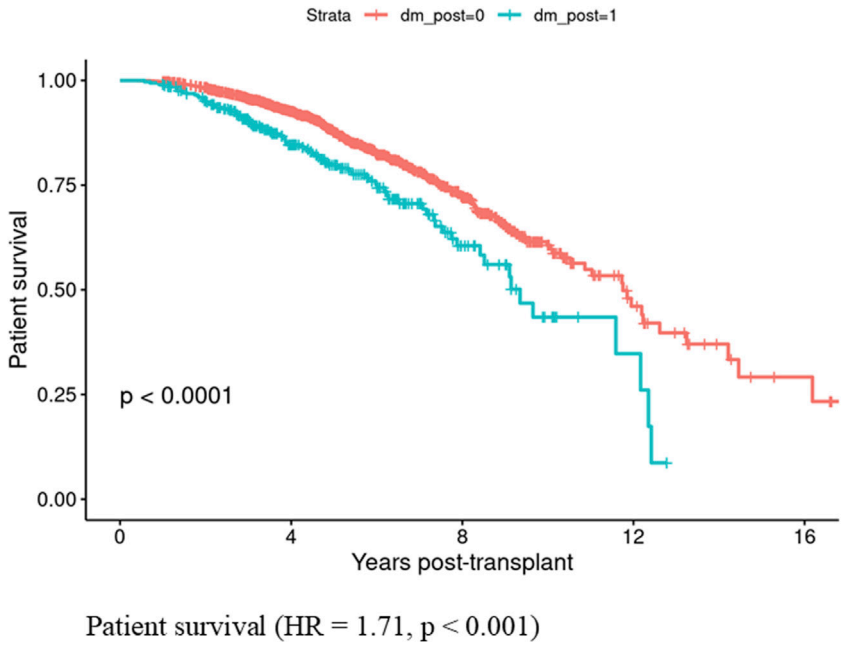


FIGURE 1 | Patient survival in those who developed Posttransplant diabetes mellitus vs., those who did not did not develop posttransplant diabetes mellitus in the first year if kidney transplant.

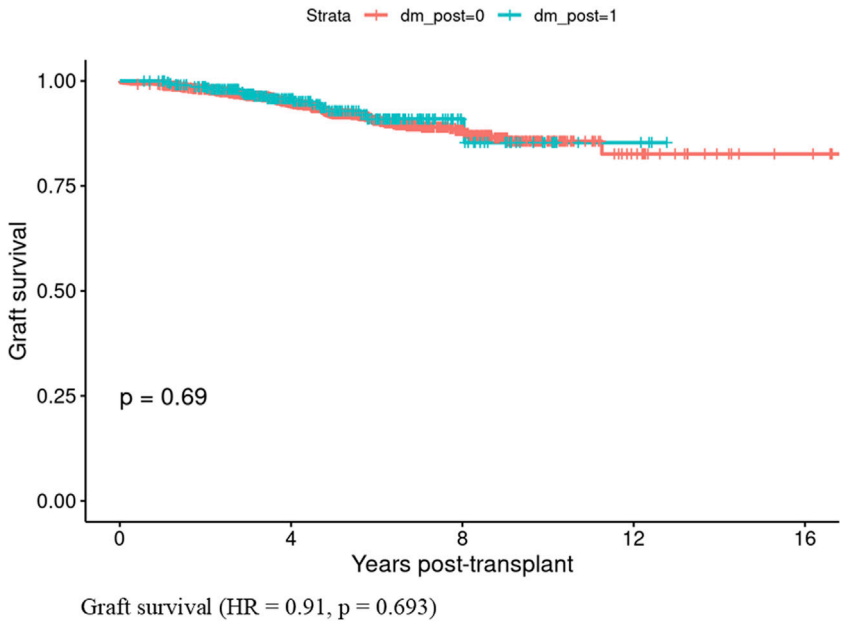


FIGURE 2 | Graft survival in those who developed Posttransplant diabetes mellitus vs., those who did not develop posttransplant diabetes mellitus in the first year of kidney transplant.

who did not develop PTDM (HR = 1.71, CI: 1.33–2.21, p < 0.001) (**Figure 1**). In contrast, graft survival in the first year was comparable between patients with and without PTDM (HR = 0.91, CI 0.56–1.47, p = 0.693) (**Figure 2**).

DISCUSSION

This study presents a comprehensive model for predicting PTDM in kidney transplant recipients, utilizing both clinical and advanced radiological data. Our model is innovative in

incorporating body composition, moving beyond the conventional BMI-based obesity assessment, and providing a higher precision to identify high-risk PTDM patients preemptively. In a large, diverse cohort of 2,005 nondiabetic kidney transplant recipients, 335 (16.7%) developed PTDM within the first year. Older age, family history of diabetes, nonwhite race, and increased VAT are significant predictors of PTDM. Importantly, patients with PTDM within the first year post-transplant demonstrated significantly higher mortality (HR = 1.71, $p < 0.001$) compared to those without PTDM, highlighting the adverse impact of PTDM on patient longevity.

Our model achieved high predictive performance, with the combination of baseline clinical factors and radiological measures ("Baseline + radiology" model) reaching a C-statistic of 0.724 (95% CI: 0.692, 0.757), surpassing traditional clinical models with a C-statistic of 0.68. This improvement highlights the value of integrating radiological factors, particularly VAT, with clinical data to enhance PTDM prediction accuracy. A simplified model with variables from multivariable analysis (age, family history of diabetes, race, and VAT) achieved similar predictive value with a C-statistic of 0.723 (95% CI: 0.691, 0.754) and a cross-validated C-statistic of 0.714, suggesting this precise model offers clinical practicality without compromising accuracy.

Among the predictors identified, VAT stands out as a modifiable risk factor, while age, family history of T2D, and race is nonmodifiable. Our findings underscore VAT's role as a stronger predictor of PTDM than BMI. Patients who developed PTDM had significantly larger VAT areas (121.7 cm^2 vs. 111.8 cm^2), more intramuscular fat (2.4 cm^2 vs. 1.7 cm^2), and lower skeletal muscle mass (165.3 cm^2 vs. 171.5 cm^2), indicating the critical impact of fat distribution and muscle quality on PTDM risk. Increased VAT intramuscular fat and reduced muscle mass may impair glucose metabolism, promoting insulin resistance and PTDM development [18, 19].

PTDM patients exhibited higher subcutaneous and visceral fat levels, particularly a significantly larger VAT area. VAT is strongly associated with metabolic risks, including diabetes, and is considered more harmful than SAT due to its location, secretions, and contribution to insulin resistance. While previous models relied on BMI, triglycerides, HDL, uric acid, and fasting glucose markers to assess metabolic risk, these markers do not fully capture the underlying metabolic dysfunction. In contrast, VAT, as a metabolically active tissue, plays a key role in insulin resistance and metabolic dysregulation, which was strongly supported by our findings where BMI was not significant, but VAT emerged as a robust predictor. VAT contains more immune cells than SAT, secreting higher levels of pro-inflammatory mediators and cytokines that exacerbate insulin resistance, contributing to diabetes [18, 19]. This unique secretome of VAT has a distinct and negative impact on hepatocyte and muscle insulin action, highlighting the depot-specific differences in adipose tissue secretome composition and their effects on metabolic syndrome and diabetes. By incorporating VAT as a central feature, our model provides a more precise reflection of metabolic risks compared to the previous reliance on traditional markers.

While Ji Eun Kim et al. [24] used deep learning-based quantification of 3D visceral fat volume, their study focused solely on body composition analysis for PTDM without integrating clinical risk factors into a predictive model. Their approach was based on volumetric analysis of total visceral fat. In contrast, our study incorporates CT-derived VAT area measurements combined with clinical parameters to develop a comprehensive predictive model for PTDM. This distinction enhances the practical applicability of our model in transplant decision-making, allowing for better risk stratification and clinical translation than a body composition-only approach.

Furthermore, Feng et al. [25] identified intermuscular adipose tissue (IMAT) as the primary driver of PTDM, while our study found VAT to be the strongest predictor. These discrepancies likely arise from differences in study populations, imaging methodologies, and statistical models. Importantly, IMAT was significant in univariate analysis but did not remain significant in the multivariable model. In contrast, VAT remained an independent predictor of PTDM along with age, race, and family history of diabetes. This suggests that IMAT's effect was confounded by stronger predictors, particularly VAT, which has a well-established role in insulin resistance and metabolic dysfunction. Given VAT's pro-inflammatory profile, direct portal exposure, and stronger association with metabolic syndrome, its predictive value surpassed that of IMAT.

Unlike prior studies, which were often constrained by small sample sizes and a lack of diverse populations, our study stands out due to its large, multicenter design and incorporation of advanced radiological analysis [24–26]. Future research should evaluate how different fat depots contribute to PTDM risk in various transplant populations and explore whether a combined VAT + IMAT model could further enhance predictive accuracy. Additionally, we acknowledge the potential value of impedance-based techniques, such as multi-frequency BIA and phase angle analysis, as alternative tools for assessing metabolic risk when CT imaging is unavailable.

Our findings highlight the VAT's predictive value for PTDM. Unlike prior studies, which were often constrained by small sample sizes and a lack of diverse populations, our study stands out due to its large, multicenter design and the incorporation of advanced radiological analysis [24, 26]. By integrating age, family history of diabetes, race, and VAT area into our model, we achieved a high AUC for PTDM prediction, demonstrating the model's robustness and clinical utility. Furthermore, deep learning-based body composition analysis provided precise and detailed insights into the relationship between VAT and PTDM risk, offering a more nuanced understanding than traditional approaches.

Given VAT's modifiable nature, targeted interventions focused on VAT reduction—such as diet, exercise, and Glucagon-Like Peptide-1 receptor agonists (GLP-1 RAs)—could be promising. Studies have shown that GLP-1 RAs significantly decrease VAT content compared to other medications, placebos, and lifestyle interventions [27, 28]. Real-time Polymerase Chain Reaction and

immunofluorescence studies show that GLP-1 receptors are more abundant in VAT and epicardial adipose tissue than in SAT [29]. Animal studies with liraglutide, a GLP-1RA, demonstrated a reduction in VAT and an increase in SAT, likely due to altered lipid metabolism [30]. Additionally, rodent models suggest that GLP-1 receptor activation enhances sympathetic activity, promoting VAT lipolysis over SAT. These medications effectively reduce VAT compared to other treatments [31, 32].

Metabolic risk factors pre-transplant may worsen after transplant. Our analysis also reveals that patients who developed PTDM exhibit worsened metabolic parameters, including elevated triglycerides, reduced HDL, and increased BMI post-transplant (**Supplementary Table S1**). Therefore reinforcing the need for early intervention pretransplant.

Advancing the research in the field of our study with a large cohort of over 2,000 diverse participants from three different sites provides greater reliability and generalizability compared to other smaller studies. Using deep learning to analyze CT scans, we achieved precise measurements of VAT, SAT, and muscle mass, offering better insights into PTDM risk than BMI and other clinic laboratory factors as surrogates for obesity. The identification of VAT as a key predictor of PTDM underscores the need for CT-based body composition analysis in pre-transplant evaluations, as BMI may miss high-risk individuals. Targeting VAT reduction through lifestyle changes, GLP-1 receptor agonists, or metabolic surgery could lower PTDM risk.

While our model is comprehensive, it has limitations. It is a retrospective study, and though we adjusted for key confounders, unmeasured variables could still impact results, and CT scans may not be widely done. Although CT-based VAT quantification offers a superior metabolic risk assessment compared to BMI and traditional clinical markers, CT imaging is not universally performed for all kidney transplant candidates. However, given that many centers already conduct preoperative CT scans for vascular and anatomical evaluation, leveraging these existing images for VAT analysis adds clinical value without additional radiation exposure or cost. In settings where CT is not routinely available, alternative methods such as Dual-Energy X-ray Absorptiometry (DXA) or bioelectrical impedance analysis (BIA) could be explored in future studies as potential surrogates for VAT estimation.

Second, while BMI, bioelectrical impedance analysis (BIA), and DXA are widely used for body composition assessment, they lack the ability to precisely differentiate visceral adipose tissue (VAT) from subcutaneous fat (SAT), which is crucial since VAT is the primary driver of insulin resistance and PTDM. Unlike BMI, which does not account for fat distribution, and BIA, which is influenced by hydration status, CT directly quantifies VAT, allowing for a more accurate assessment of metabolic risk. Skinfold calipers, though simple and inexpensive, only estimate subcutaneous fat and are operator-dependent, making them unreliable for deep fat compartments such as VAT.

Future research should assess whether emerging impedance-based technologies, such as multi-frequency BIA and phase angle analysis, can enhance metabolic risk prediction in transplant candidates. Third, our study excluded patients with previous kidney transplants to maintain a homogeneous study population and improve model validity. However, this exclusion may limit the generalizability of our findings to patients undergoing repeat transplantation, who often have different metabolic profiles and long-term immunosuppression exposure. Lastly, while the combined model incorporating clinical and radiological data improved predictive performance (C-statistic of 0.724 vs. 0.686 for clinical-only models), the absolute increase is moderate. However, even small gains in predictive accuracy are clinically relevant as they allow for earlier identification of high-risk patients, targeted lifestyle interventions, and personalized metabolic management strategies to mitigate PTDM risk. The most parsimonious model—incorporating only age, family history of diabetes, race, and VAT area—achieved a C-statistic of 0.723, reinforcing VAT's independent predictive value. Unlike BMI, which does not account for fat distribution, VAT directly contributes to insulin resistance and systemic inflammation. Given that VAT is modifiable, identifying high-VAT patients early enables targeted lifestyle interventions, glucose monitoring, and adjustments to immunosuppressive regimens.

Thus, while the numerical increase in C-statistic may seem moderate, its clinical implications are substantial, reinforcing VAT's value in pre-transplant metabolic risk assessment. Future research should explore machine learning-based models and additional metabolic biomarkers to further refine PTDM prediction.

This precise model provides a valuable conceptual framework for stratifying risk, continuing efforts to adopt it into mainstream practice, and guiding targeted therapies for high-risk patients.

This study highlights the importance of body composition, particularly VAT and muscle mass, in predicting PTDM risk among kidney transplant recipients. By integrating clinical factors with radiological metrics, our model demonstrated greater predictive accuracy than traditional BMI-based assessments, emphasizing the need for CT-based body composition analysis in pre-transplant evaluations. While factors like age, family history of diabetes, and race are nonmodifiable, VAT represents a valuable modifiable target for intervention. Our findings also indicate that metabolic risks often worsen post-transplant, suggesting that transplantation alone does not fully address these challenges.

Our model provides a more sensitive identification of high-risk patients identified before or shortly after transplantation. Future research is needed to validate this model across different populations and healthcare settings to ensure broader applicability. Additionally, studies should explore timely interventions aimed at VAT reduction and muscle preservation—such as lifestyle modifications, pharmacologic agents like GLP-1 receptor agonists, or bariatric surgery—to maximize the benefits of these strategies. Early, tailored interventions could reduce PTDM incidence, improve patient survival, and enhance graft outcomes. This comprehensive model lays the framework for precision medicine, enabling early identification of at-risk individuals for PTDM and optimizing post-transplant care.

DATA AVAILABILITY STATEMENT

Data is available on request due to privacy/ethical restrictions. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to containing research participant information.

ETHICS STATEMENT

The studies involving humans were approved by mayo clinic institutional review board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

PB, AK, and HC conceived the data. Radiological analysis was done by TK and PK. BS did statistical analysis. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.14377/full#supplementary-material

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A Retrospective Test-Negative Case-Control Study to Evaluate Influenza Vaccine Effectiveness in Preventing Influenza Among Immunocompromised Adults With a Solid Organ Transplant

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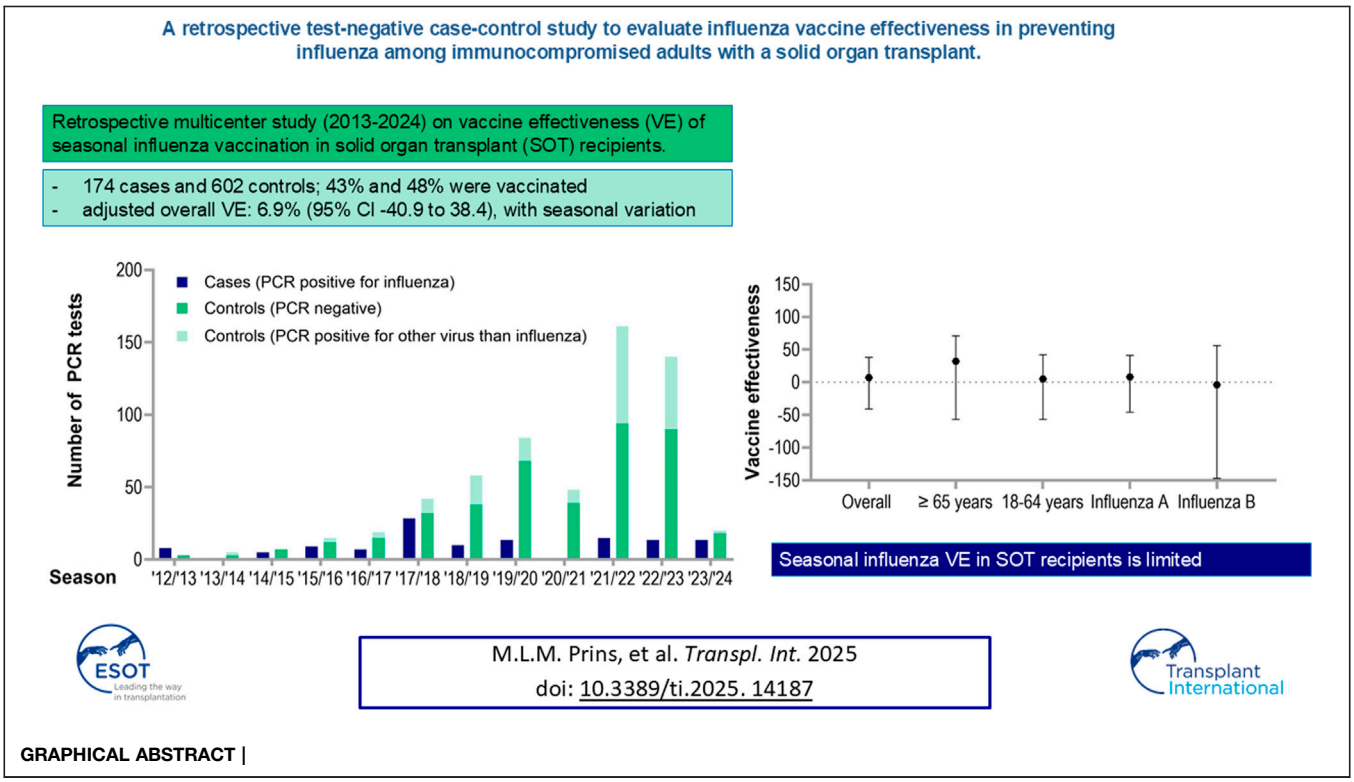
Vaccination may prevent influenza in solid organ transplant (SOT) recipients. This study evaluates the influenza vaccine effectiveness (VE) in this high-risk population in the Netherlands. We also compared disease progression and 30-day mortality between vaccinated and unvaccinated influenza patients. In this multicenter, test-negative case-control study, SOT recipients with respiratory symptoms were included when tested for viral respiratory infections during the respiratory seasons between 1 January 2013 and 1 July 2024. Cases had a positive influenza PCR, while controls tested negative. Influenza vaccination in cases (74/174) and controls (291/602) were compared after adjusting for potential confounders. VE was calculated as (1-adjusted odds ratio) x 100. The overall VE was 6.9% (95% CI –40.9 to 38.4), with considerable variation across seasons. For those aged ≥65 years, VE was higher (32.4%, 95% CI –56.5–70.8) compared to those aged 18–64 years (4.8%, 95% CI –56.5 to 42.1). The adjusted VE against influenza A [7.5% (–46.0 to 41.3)] was higher than against influenza B (–3.8% (–146.7 to 56.3)). No differences in influenza-related complications were observed between the vaccinated and unvaccinated cases. The observed seasonal influenza vaccine effectiveness in adult SOT recipients is limited; further investigation for improvement is warranted.

Keywords: influenza, influenza vaccine effectiveness, influenza vaccination, Netherlands, solid organ transplant patients



GRAPHICAL ABSTRACT |

INTRODUCTION

Influenza viruses are globally among the most common causes of respiratory infections in both immunocompetent and immunocompromised individuals, like recipients of a solid organ transplant (SOT) [1]. The prevalence of seasonal influenza among viral pathogens in SOT recipients may vary annually, depending on the types and intensity of circulating viruses, vaccine coverage (i.e., the percentage of a specific population that has received the vaccine), vaccine efficacy related to vaccine-match and dosage of influenza vaccines, type of transplant, and adherence to non-pharmacological interventions [2]. National data from Finland suggests a substantial increased likelihood of detecting laboratory-confirmed influenza and hospitalization due to influenza in kidney transplant recipients compared to the general population [3].

While infection in healthy, immunocompetent individuals may present as a mild and self-limiting condition [4], SOT recipients have an increased risk of influenza-related complications, including secondary bacterial pneumonia, acute graft rejection and mortality [2, 5–8]. Moreover, SOT patients with influenza have a significantly elevated risk of hospitalization, up to 70% [3, 7, 9].

Annual seasonal vaccination is the primary measure for preventing influenza [2] and is universally recommended for SOT recipients [10]. Nevertheless, vaccination rates among SOT recipients are reported to be low in both US and European settings and nearly half of SOT recipients were unvaccinated in registries from the US and Denmark [11, 12].

Lifelong use of immunosuppressive medication affects the lymphocyte function of SOT recipients, thereby leading to an immunocompromised status. Several mechanisms are known, depending on the specific immunosuppressive drug used: reduced T-cell activity, direct suppression of B-cells or antibody production, suppression of cytokine production or inhibition of immune cell proliferation and differentiation. The amount of impairment depends on several factors, such as type of transplant, type of immunosuppression such as mycophenolate or co-stimulation blockers, use of T-cell depleting agents in the year before vaccination and time since transplantation [2, 13]. Consequently, the immunogenicity of the influenza vaccine in SOT recipients is reduced compared to immunocompetent persons, reported as reduced serologic immune responses to influenza vaccines and lower seroprotection rates, based on hemagglutination-inhibition (HI) titers [6, 13–21]. In addition to the immunological (surrogate) marker, two other clinical outcome measures are commonly used for the protective effects of vaccines: vaccine efficacy and vaccine effectiveness (VE). Vaccine efficacy refers to how well a vaccine performs in controlled settings (e.g., clinical trials), while VE describe its performance in real-world conditions. Ultimately the VE is the most relevant outcome. The immune response does not always correlate with the clinical effectiveness of a vaccine. In addition, the VE of the influenza vaccine varies yearly, with mismatches negatively

affecting its effectiveness [22]. In the general population, influenza VE ranged from 19% to 59%, with lower percentages among people above 65 years [23–31]. However, studies on the VE of the influenza vaccine in SOT recipients are lacking and therefore its effectiveness remains controversial. In several epidemiological studies, the benefit of influenza vaccination in SOT recipients is only reported in relation to disease progression and the occurrence of complications, such as pneumonia, graft outcomes, intensive care unit (ICU) admission and mortality [9, 12, 19, 32, 33].

The aim of this study is to determine the influenza VE among immunocompromised adult SOT recipients in the Leiden transplantation region in the Netherlands.

MATERIALS AND METHODS

Study Design

We performed a multicenter, retrospective test-negative case-control study [34] to estimate VE of seasonal influenza vaccination in SOT recipients. Patients in the Leiden University Medical Center (LUMC), one of seven transplantation centers in the Netherlands, and its seven affiliated shared-care hospitals (Alrijne Hospital, Amphia Hospital, Groene Hart Hospital, Haga Hospital, Haaglanden Medical Center, Reinier de Graaf Hospital, Spaarne Hospital), were eligible. The study period was between 1st January 2013, and 1st July 2024.

Study Participants

All adult patients (≥18 years) who received a SOT (kidney, liver, pancreas, islet cells of Langerhans, or a combination of these), and underwent diagnostic testing for influenza in an outpatient setting or within 24 h after hospital admission, were included. Other types of SOT, such as heart or lung transplants, were not included, as these are not performed at the LUMC. The standard protocol in our center mandates SOT recipients to contact the hospital (academic hospital or the nearest affiliated hospital, depending on the duration post-transplantation and the hospital were the patient is monitored) if they experience fever or respiratory symptoms. Influenza diagnostics via polymerase chain reaction (PCR) are readily available during the respiratory virus season in the emergency departments or outpatient clinics. We included only symptomatic patients. The indication for PCR test was determined by the treating physician and hospital.

The respiratory virus season in the Netherlands spans from week 40 in 1 year to week 20 in the following year (early October to mid-May) [35]. Subjects enrolled outside this season were excluded from analysis to avoid bias by calendar time [22]. Patients could be included only once a season, but could be included multiple times if they were tested for influenza during multiple seasons. They were classified as cases if there was at least one positive test during the respiratory virus season; otherwise they were controls. For cases, outcomes up to 30 days following the first positive test were studied, for controls outcomes after the first negative test.

Patients were defined as vaccinated if they had received the seasonal influenza vaccine (standard dose) in the ongoing respiratory virus season, prior to PCR testing. Patients were defined as unvaccinated if no influenza vaccine was received in the current season prior to PCR testing.

Data Collection

In the Netherlands, the seasonal influenza vaccine, standard-dose trivalent (season 2013/14–2018/19) or quadrivalent (since 2019/20) vaccine, is administered to risk groups by general practitioners (GP), primarily in the months October and November. Influenza vaccination is free of charge. After receiving a standard-dose influenza vaccination, the GP documents the type and date/month of this vaccination in their GP electronic information system. Therefore, data regarding influenza vaccination history was obtained by contacting the patient's GP, either through a letter/email or by phone. In cases where the vaccination history was not accurately recorded at the GP, the patient was contacted directly. Patients were excluded from analysis if no information was available regarding their vaccination status.

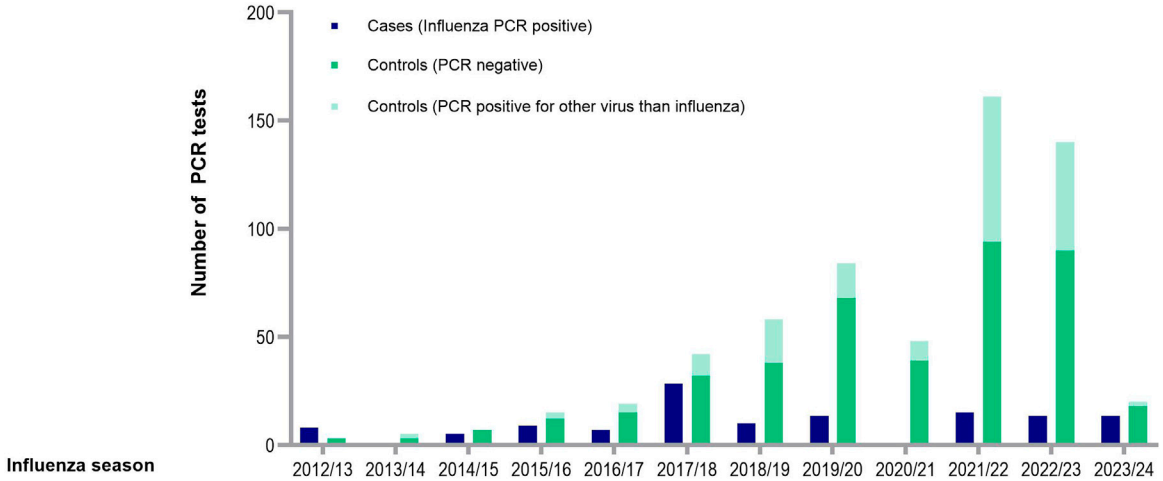
In addition, we retrieved detailed clinical information from the electronic healthcare records, including baseline demographics, test results for (other) respiratory pathogens, comorbidities, and use of immunosuppressive agents. Comorbidity was categorized into cardiovascular disease (CVD), chronic pulmonary disease and diabetes mellitus (DM). The degree of immunosuppression was determined by the type of induction, maintenance and/or rejection therapy. Patients were considered to be highly immunosuppressed if they were treated with triple therapy and/or had received lymphocyte depleting agents (anti-thymocyte globulin and/or alemtuzumab) in the preceding 6 months.

Outcome Measures

The primary outcome is the adjusted influenza VE over the whole period in preventing the occurrence of laboratory-confirmed influenza in patients with a SOT. Adjusted VE by season, age group and by influenza subtype were also determined. Secondary to this, we compared course of disease (hospital length of stay, ICU-admission, need for mechanical ventilation) and 30-day mortality between vaccinated and unvaccinated lab-confirmed influenza patients.

Sample Size

The influenza vaccination rate for the entire target population has varied from 50% to 57% in the Netherlands in recent years [36]. The VE in the overall vaccinated population in the Netherlands ranged from 31% to 57% [23, 24]. Based on that data, our hypothesis is that the VE in SOT recipients is around 40%, and the vaccination rate in this group is 50%. This VE corresponds to an odds ratio (OR) of 0.6 and a vaccination rate of 0.375 in the influenza-positive group. Based on an expected case/control ratio of 1/3, the required sample size is 165 cases and 495 controls to detect a VE of 40% with a power of 80% and an alpha of 0.5.



Influenza season	2012/13	2013/14	2014/15	2015/16	2016/17	2017/18	2018/19	2019/20	2020/21	2021/22	2022/23	2023/24
VE in SOT recipients	NA*	NA*	99	95	-517	-16	-35	-30	NA*	-32	38	30
VE in general population in the Netherlands	62[&]	65[&]	-11[&]	44[&]	48[&]	42[&]	27[&]	48[^]	NA*	34[^]	52[#]	50[#]
Incidence influenza in SOT recipients (%)	0.80	0.07	0.53	0.79	0.45	2.08	0.62	0.85	0	0.823	0.74	0.73

FIGURE 1 | Number of cases and controls, incidence of influenza in SOT recipients and influenza vaccine effectiveness each respiratory season. Presented in the figure are the amount of cases and controls each respiratory season. Below the figure, the adjusted VE in SOT recipients is presented each respiratory year, compared to the yearly influenza VE in the general population in the Netherlands, reported by the National Institute for Public Health and the Environment. In addition, incidence of influenza cases is calculated among all SOT recipients still alive during a respiratory season at January 1 of that season. *NA because no cases were detected (2020/2021) or the sample size was too small (2012/2013, 2013/2014). [&]Reported by the National Institute for Public Health and the Environment. [^]Adjusted for the confounders age, history of chronic pulmonary disease, history of rejection therapy, hospital of inclusion, season, use of cell division inhibitors, highly immunosuppressed status. Abbreviations: NA, not applicable; VE, vaccine effectiveness; PCR, polymerase chain reaction; SOT, solid organ transplant.

Statistical Analysis

Continuous variables were reported as means and standard deviations (SD) or as median and interquartile range (IQR), depending on distribution. Categorical variables were reported as numbers and percentages. Baseline differences between groups were evaluated using the independent T-test, Mann-Whitney U test and Chi-squared test, with significance set at $p < 0.05$. VE was calculated as (1-adjusted OR) x 100% and reported as percentages. The OR is the ratio of the odds of being vaccinated versus not vaccinated with a standard vaccine dosage against influenza among cases and controls. Adjusted ORs and 95% confidence intervals (95% CI) were calculated using multiple logistic regression, with influenza PCR results as the outcome and vaccination status as the primary variable at interest. A univariate logistic regression analysis identified factors independently associated with influenza status, with variables showing $p < 0.10$ included in the multivariable model (age, history of chronic pulmonary disease, history of rejection therapy, hospital of inclusion, season), alongside clinically relevant factors (use of mycophenolic acid [cell division inhibitors] or highly immunosuppressed status). Incidences were calculated by dividing the number of new influenza cases during a respiratory season by the total number of individuals who underwent organ transplantation at the LUMC and were still alive on January 1 during that season, multiplied by 100. All calculation were made using SPSS statistics 25.0 for Windows.

Reporting and Ethics

The study was done in accordance with Good Clinical Practice Guidelines. The study was approved by the Institutional Review Board of the LUMC (nWMODIV2_2022034) and the need for informed consent was waived. The study was described according to the STROBE checklist for observational studies.

RESULTS

After excluding 30 patients due to missing vaccination data, 776 participants were included in the analysis: 174 cases and 602 controls. Of all the participants, 207 were included more than once, including 29 cases and 178 controls. Among the controls, 183 had a positive PCR result for another viral pathogen, while 419 patients had a negative result (**Figure 1**). Of the patients with positive PCR, SARS-CoV-2 (59%), respiratory syncytial virus (16%) and rhinovirus (13%) infections were most common. Most controls underwent PCR testing in 2022 (28.7%), followed by 2023 (15.1%),

TABLE 1 | Characteristics of patients included in the analysis.

	Overall (n = 776)	Influenza negative/ controls (n = 602)	Influenza positive/ cases (n = 174)	p[a]
Male sex	459 (59.1)	360 (59.8)	99 (56.9)	0.49
Age, mean (SD)	59.7 (13.4)	60.8 (13.3)	56.2 (13.3)	<0.001
BMI, mean (SD)	25.9 (5.1)	25.8 (5.0)	26.2 (5.6)	0.42
Type of influenza				
A	129 (16.6)	-	129 (74.1)	-
B	45 (5.8)		45 (25.9)	
Month of testing				<0.001
January	149 (19.2)	99 (16.4)	50 (28.7)	
February	133 (17.1)	92 (15.3)	41 (23.6)	
March	141 (18.2)	103 (17.1)	38 (21.8)	
April	89 (11.5)	77 (12.8)	12 (6.9)	
May	34 (4.4)	32 (5.3)	2 (1.1)	
October	61 (7.9)	60 (10.0)	1 (0.6)	
November	66 (8.5)	65 (10.8)	1 (0.6)	
December	103 (13.3)	74 (12.3)	29 (16.7)	
Pre-existing cardiovascular disease	649 (83.6)	506 (84.1)	143 (82.2)	0.56
Pre-existing lung disease	227 (29.3)	186 (30.9)	41 (23.6)	0.06
	119 (15.3)	99 (16.4)	20 (11.5)	0.11
Asthma/COPD	142 (18.3)	119 (19.8)	23 (13.2)	0.05
Other[b]				
Pre-existing diabetes	309 (39.8)	241 (40.0)	68 (39.1)	0.82
Empiric antibiotics	189 (24.4)	155 (25.7)	34 (19.5)	0.09
Time between transplantation and PCR in years, median (IQR)	7 (3–13)	7 (3–13)	6 (2–12)	0.01
Type transplantation				0.41
Kidney	642 (82.7)	503 (83.6)	139 (79.9)	
Pancreas	2 (0.3)	2 (0.3)	-	
Islets of Langerhans	2 (0.3)	1 (0.2)	1 (0.6)	
Liver	105 (13.5)	77 (12.8)	28 (16.1)	
Kidney & pancreas	13 (1.7)	8 (1.3)	5 (2.9)	
Kidney & liver	11 (1.4)	10 (1.7)	1 (0.6)	
Kidney & islets of Langerhans	1 (0.1)	1 (0.2)	-	
Type induction[c]				0.88
IL-2 inhibitor	440 (87.8)	336 (88.0)	103 (86.6)	
Alemtuzumab	47 (6.1)	36 (9.4)	12 (10.1)	
No. of				0.32
Immunosuppressive	68 (8.8)	50 (8.3)	18 (10.3)	
agents	402 (51.8)	322 (53.5)	80 (46.0)	
1	305 (39.2)	229 (38.0)	76 (43.7)	
2				
3				
Type of				
immunosuppressive	675 (87.0)	522 (86.7)	153 (87.9)	0.67
agents	612 (78.9)	479 (79.6)	133 (76.4)	0.37
Corticosteroids	449 (57.9)	343 (57.0)	106 (60.9)	0.35
Calcineurin inhibitors	52 (6.7)	41 (5.9)	11 (6.8)	0.82
Cell division inhibitors	48 (6.2)	37 (6.1)	12 (7.9)	0.93
MTOR inhibitors				
Lymphocyte depleting agents				
Rejection therapy	151 (19.5)	108 (17.9)	43 (24.7)	0.047
<6 months ago	12 (1.5)	10 (1.7)	2 (1.1)	
Once	139 (17.9)	98 (16.3)	41 (23.6)	
Never	625 (80.5)	494 (82.1)	131 (75.3)	
Type of rejection therapy				
Solumedrol	124 (16.0)	88 (14.6)	36 (20.7)	0.10
Alemtuzumab	36 (4.6)	25 (4.2)	11 (6.3)	0.26
ATG	31 (4.0)	21 (3.5)	10 (5.7)	0.22
Other[d]	39 (5.0)	28 (4.7)	11 (6.3)	0.37
Time between rejection therapy and PCR in years, median (IQR)	6 (2–16)	2 (6–15)	6 (3–18)	0.07
Hospital of inclusion				<0.001
Hospital 1	26 (3.4)	21 (3.5)	5 (2.9)	
Hospital 2	88 (11.3)	78 (13.0)	10 (5.7)	
Hospital 3	43 (5.5)	41 (6.8)	2 (1.1)	
Hospital 4	171 (22.0)	147 (24.4)	24 (13.8)	
Hospital 5	54 (7.0)	47 (7.8)	7 (4.0)	
Hospital 6	249 (32.1)	143 (23.8)	106 (60.9)	
Hospital 7	45 (5.8)	41 (6.8)	4 (2.3)	
Hospital 8	100 (12.9)	84 (14.0)	16 (9.2)	
Vaccinated	365 (47.0)	291 (48.3)	74 (42.5)	0.18
Time between vaccination and PCR in months, mean (SD)	2.8 (1.8)	2.8 (1.8)	2.6 (1.5)	0.53

(Continued in next column)

(*Continued*)

Data are presented per episode. In total, 207/776 (26.7%) patients were included more than one time. Data are presented as no. (%) unless otherwise indicated.
Abbreviations: IL-2, interleukine-2; SD, standard deviations; IQR, interquartile range; BMI, body mass index; MTOR, mammalian target of rapamycin; ATG, anti-thymocyte globulin.
[a]Independent T-test, Chi-squared test or Mann-Whitney U test.
[b]Other types of lung diseases are active lung cancer, bronchiectasis, cystic fibrosis, pulmonal hypertension, sarcoidosis, tuberculosis, obstructive sleep apnea syndrome (OSAS).
[c]Valid percentages are presented (numbers do not always add up to 776 as there are some missing data).
[d]Other types of rejection therapy are OKT3 (muromonab), plasmapheresis, IVIG, rituximab, switch to tacrolimus, addition of third agent).

2021 (14%) and 2020 (12.6%). Among the cases, 74% tested positive for influenza A and 26% tested positive for influenza B. The influenza A subtype was not determined. Estimated yearly incidence of influenza among transplant recipients is presented in **Figure 1** and ranged between 0% (2020/21) and 2.08% (2017/2018).

The demographic characteristics of the participants are presented in **Table 1**. Cases were slightly younger than controls and the percentage of cases varies by month. Overall, 47% of the participants were vaccinated: 43% of cases (74/174) and 48% of controls (291/602). Among patients aged 65 years and older, 168 out of 365 (46%) were vaccinated, compared to 147/411 (36%) individuals under the age of 65.

Overall Vaccine Effectiveness and for Each Individual Season

After adjusting for the previously mentioned confounders, the adjusted VE over the whole period was 6.9% (95% CI -40.9 to 38.4). VE for individual seasons varied widely (**Figure 1**). Nonetheless, this study was not powered to analyze these yearly VE's, leading to wide confidence intervals. In the 2020/2021 season, no VE could be determined as no individuals tested positive for influenza. Similarly, VE could not be calculated for

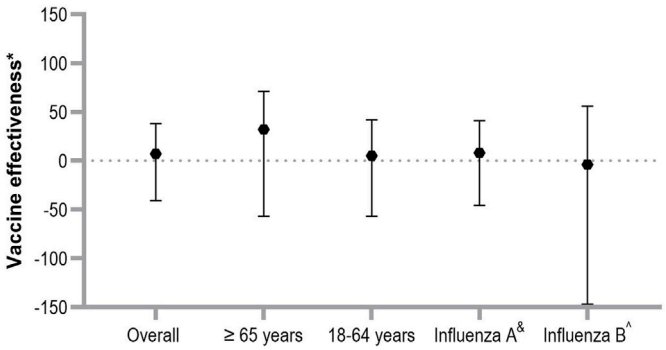


FIGURE 2 | Estimation of vaccine effectiveness against laboratory confirmed influenza. Overall VE in SOT recipients, VE by age group and by influenza virus subtype. Errors bars represent 95% CI. *Corrected for age, history of chronic pulmonary disease, history of rejection therapy, hospital of inclusion, season, use of cell division inhibitors, highly immunosuppressed status. [&]Only cases with influenza A subtypes were included; cases with influenza B virus subtypes were excluded. ^Only cases with influenza B virus subtypes were included; patients with influenza A virus subtype were excluded. Abbreviations: OR, odds ratio; VE, vaccine effectiveness; CI, confidence interval.

TABLE 2 | Course of disease in patients who tested positive for influenza.

	Overall (n = 174)	Vaccinated (n = 74)	Unvaccinated (n = 100)	p[a]
Admission in the hospital	112 (64.4)	51 (68.9)	61 (61.0)	0.28
Hospital length of stay, median (IQR)	3.0 (2.0–5.0)	3.0 (2.0–4.0)	3.0 (2.0–7.0)	0.25
ICU-admission	6 (3.4)	2 (2.7)	4 (4.0)	0.61
Need for mechanical ventilation	5 (2.9)	2 (2.7)	3 (3.0)	0.92
30-day mortality	3 (1.7)	1 (1.4)	2 (2.0)	0.75
Rejection	2 (1.1)	0	2 (2.0)	0.22

Data are presented as no. (%) unless otherwise indicated.
[a]*Chi-squared test, Fisher's exact test or Mann-Whitney U test.*

the 2012/2013, 2013/2014 and 2014/2015 seasons due to small sample sizes. After excluding this three seasons, the adjusted VE was 4.3% (95% CI −46.6 to 37.5).

Vaccine Effectiveness by Age Group and by Influenza Virus Type

Among individuals aged 18–64 years, the adjusted VE from 2013 to 2024 was 4.8% (95% CI −56.5 to 42.1), compared to a VE of 32.4% (95% CI −56.5–70.8) among those aged 65 years and older (**Figure 2**). The total adjusted VE against influenza A was 7.5% (95% CI −46.0 to 41.3), while the total adjusted VE against influenza B was −3.8% (95% CI −146.7 to 56.3).

Course of Disease in Patients Who Tested Positive for Influenza

Overall, 112 influenza-positive patients (64.4%) were hospitalized, with a median stay of 3 days (IQR 2–5 days) (**Table 2**). Six patients (3.4%) required ICU admission, five of whom needed mechanical ventilation. Overall, the all-cause 30-day mortality among lab-confirmed influenza cases was 1.7%. The course of disease for vaccinated SOT recipients was similar to that of unvaccinated patients. ICU admission, mechanical ventilation, 30-day mortality and treatment for rejection after influenza illness (1.7%) did not differ between vaccinated and unvaccinated patients (**Table 2**).

DISCUSSION

In this retrospective test-negative case-control study, the observed adjusted VE against influenza infection of the standard-dose seasonal influenza vaccine in SOT recipients was low over the years 2013–2024 in the Netherlands, with a most optimal adjusted VE of 6.9%. Compared with VE in people below 65 years, the adjusted VE in patients above 65 years was higher (4.8% versus 32.4%, respectively). The VE against influenza B was lower than against influenza A (−3.8% versus 7.5%, respectively). We also showed that influenza-related complications did not differ between the vaccinated and unvaccinated influenza cases.

Data on vaccine effectiveness for preventing influenza infection in adults with immunocompromised status are scarce. Most research has concentrated on assessing the humoral antibody responses by measuring influenza-specific antibody levels, associated with protection in healthy adults, using standard HI assays [37–40]. However, these antibody concentrations are surrogate markers of vaccine efficacy and if these are also protective in SOT recipients is unknown. Therefore, it remains important to determine VE as the primary outcome measure, rather than relying on the immunological response.

Previous immunogenicity studies have reported a lower humoral response to influenza vaccination in SOT recipients compared with healthy controls [15, 18, 21]. Our study is among the first to demonstrate and quantify the clinical impact of this

known reduced immunological vaccine response in SOT recipients.

In the Netherlands, the effectiveness of the (inactivated) influenza vaccine ranged from −11% to 65% in the past decade in the general population [23–27]. Our findings suggest that VE against influenza in SOT recipients is low compared to the general healthy population. Similarly, a study by Hughes et al reported an adjusted VE of 5% against influenza-associated hospitalizations among eight categories of immunocompromised adults during the 2017–2018 season, compared to 41% among non-immunocompromised adults [41].

Numerous studies have shown that the estimates of VE in the general population are higher in subjects under the age of 65 years than in those aged 65 years or older [30, 31]. In contrast, we found a higher VE in those aged 65 years or older compared to those aged 18–64 years. This finding aligns with data from the Dutch National Institute for Public Health and the Environment, which also reported higher VE in the older population compared to the younger population [23–27, 42, 43]. A possible explanation could be differences in exposure, healthcare-seeking behavior or disease severity between these age groups. Younger patients with (mild) symptoms may be less likely to seek hospital care than older individuals. This could lead to undocumented mild infections, which might attenuate VE estimates. The low annual incidences of influenza observed in our population, compared to the general Dutch population, supports the idea that there may be more mild cases among vaccinated individuals or high levels of vaccination in household contacts of SOT recipients that may prevent secondary transmission. However, the incidence rates in the general population reflects influenza-like illness (ILI) reported by GP's, rather than laboratory-confirmed influenza reported by hospitals. Since not everyone with ILI seek hospital care, this may account for the lower incidences of influenza observed in our population.

In earlier influenza seasons, PCR was less widely used than in the (post-) COVID-19 seasons, where PCR on RSV/SARS-CoV-2/influenza was likely done more routinely to all patients with equal severity of disease (who where not tested before COVID pandemic). However, this would not have had an impact on the VE. Lower threshold for PCR testing may result in testing less severely ill patients, resulting in more influenza negative patients (controls). However, the ratio of vaccinated to unvaccinated individuals in a population with fewer cases does not change (as doctors are unaware of the vaccination status of the patient), and the OR and consequently the VE remains unaffected (OR= ((a/b)/(c/d)), where "a" represents the number of vaccinated cases, "b" the number of unvaccinated cases, "c" the number of vaccinated controls, and "d" the number of unvaccinated controls).

Our results showed that influenza-related outcomes -such as hospital length of stay, need for ICU admission and/or mechanical ventilation, 30-day mortality and rejection- did not differ between the vaccinated and unvaccinated influenza cases. However, this only applies to those who presented at the hospital. Due to the retrospective design of the study, we cannot accurately quantify the extend of illness prevented by the influenza vaccine. However, we do instruct SOT recipients to contact the hospital in case of respiratory infection symptoms. Studies evaluating the impact of antecedent influenza vaccination in SOT recipients with influenza disease are scarce. One study that assessed the impact of the 2010–2011 seasonal influenza vaccination on illness severity among SOT recipients with influenza disease reported similar results [19]. The study indicated that receiving the influenza vaccine was not associated with a decreased risk of hospitalization, ICU admission, mortality or severe disease. In contrast to our study, it did find an association with shorter hospital stay. In addition, Kumar et al reported that receiving the influenza vaccine in the current season was associated with a lower incidence of ICU admission in a multivariate model among 616 patients with a SOT or hematopoietic stem cell transplantation [9].

The observed reduced influenza VE in SOT patients in comparison to the healthy population warrants further investigation aimed at improving the VE or investigation to explore alternative strategies to protect this vulnerable group. Various methods had been previously evaluated to improve vaccine immunogenicity in immunocompromised patients, including adjuvanted vaccines [44], the use of high-dose (HD) influenza vaccines [45–48], administration of a booster-dose (BD) [21, 49], intradermal vaccination [50–52] and adjusting immunosuppression to target [53]. Most of these measures have not resulted in clinically significant increases in immunogenicity compared with single standard-dose intramuscular strategies [54]. Of these strategies, HD (especially those four times the standard dose) and BD vaccines seem to be the most promising for enhancing immunogenicity and are generally well tolerated [54].

Several limitations should be considered when interpreting these results. First, the wide confidence intervals surrounding the VE estimate limit the strength of our conclusion. However, the upper bound of the confidence interval still remains below the VE observed in the healthy population. Second, VE fluctuate annually, depending on the degree of antigenic match between vaccine strains and circulating strains [22]. Our study focused on the adjusted VE over 11 respiratory seasons, as yearly sample sizes were insufficient for reliable calculating, introducing some heterogeneity. Third, the observational design of the study also introduces potential confounding. Although we adjusted for all known confounding variables, residual confounding still exist. The test-negative design required that cases seek medical attention, which might not occur for mild symptoms. However, SOT recipients are more likely to contact the hospital for mild symptoms compared to the general population, as they are advised to do so in the presence of fever or symptoms of a viral respiratory infection. Moreover, during the COVID-19 pandemic and the subsequent years, patients were more inclined to seed medical care and get tested for respiratory viruses more readily, which likely mitigates the risk of underestimating VE. Next, the timing of vaccination was not accounted for due to the often unknown exact dates of vaccine administration at many GP offices. Lastly, our criteria for being considered vaccinated were fairly stringent, requiring individuals to have received the seasonal influenza vaccine in current respiratory season before PCR testing.

Those vaccinated in the previous season were considered unvaccinated. Less stringent criteria would likely lower the VE estimate, as studies indicate a progressive decline in antibody titers within a year after vaccination [37, 49, 55, 56]. Additionally, VE tends to drop during the season, beginning around 100 days post-vaccination [30]. Thus, vaccinated patients receiving their influenza vaccination longer ago (e.g., those who present to the hospital between May and October) were less protected against influenza disease, which consequently should influence the VE estimate. However, since individuals between week 20 and week 40 were excluded, we believe that the impact of waning immunity on our estimates limited.

The test-negative design represents a strength of our study. By ensuring that all laboratory-confirmed cases and test-negative controls sought care in the same healthcare settings for similar sets of symptoms, we reduce bias related to community-level variations in vaccine coverage. In addition, cases and non-cases will typically originate from the same communities. Another advantage of this design is the reduction in disease misclassification, as cases are confirmed through laboratory testing. Furthermore, we assessed vaccination history by contacting GP's, who were unaware of their patients' respiratory infections when verifying vaccination status, thereby reducing misclassification of vaccine history as a potential source of bias. Selection bias, which could arise from physicians' clinical decision-making regarding testing for influenza, is also mitigated. Since patients' vaccine history is generally unknown to treating physicians in hospitals- who typically rely on GPs for such records- we further limit potential biases in vaccine status that could affect outcomes.

In conclusion, the results of our study demonstrate that seasonal effectiveness of the standard-dose influenza vaccine against laboratory confirmed influenza in adult SOT recipients is limited. Despite the low precision and limitations of a retrospective analysis, our findings prompt further investigations aimed at improving VE in SOT recipients. New vaccine formulations or a different vaccination strategy may increase VE. In addition, more prospective data with larger sample size on such regional VE estimates are needed, as it could help convince both doctors and patients of the benefits of vaccination. This data collection should not only focus on influenza VE, but also on burden of disease and VE of other vaccine-preventable infections in SOT recipients, such as COVID-19 and RSV. If the low VE and low burden of disease due to influenza were to be confirmed, annual vaccination campaigns focusing on single pathogens may be questioned and use of combination-vaccines including influenza, COVID-19 and RSV would be preferred to limit the number of vaccinations and healthcare consultations.

DATA AVAILABILITY STATEMENT

The data will be made available on reasonable request.

ETHICS STATEMENT

The requirement of ethical approval was waived by Institutional Review Board of the LUMC for the studies involving humans because It concerns a study not subject to the WMO as the individuals are not subjected to procedures or were imposed with behavioral rules. It is an observational study. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board also waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/ next of kin. In addition, it concerns such large groups that the research is reasonably not feasible through obtaining individual consent. Additionally, it was expected that some patients will have already passed away, making it impossible to obtain consent from them.

AUTHOR CONTRIBUTIONS

MP, GG, AdV, SC, LV, AV, and MT participated in research design. MP, ED, DH, ES, IM, and JG participated in the performance of research. MP, SC, and ED participated in data analysis. MP and ED participated in writing the article. GG, AdV, LV, SC, AV, MT, ES, IM, and JG participated in revision of the article. 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.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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ORIGINAL RESEARCH
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Brain-Death in Rats Increases Neutrophil Extracellular Trap Formation in Donor Organs

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During brain-death, increased numbers of neutrophils are recruited to organs as part of the inflammatory response. In the organ microenvironment, the recruited neutrophils may release neutrophil extracellular traps (NETs) through interaction with various pro-inflammatory stimuli, contributing to brain-death-induced endothelial activation, microthrombus formation and ultimately a decline in organ quality. To investigate whether NETs form in organs from brain-dead donors; kidneys, hearts, livers, and plasma samples were collected from brain-dead or sham-operated rats. The presence of NET-specific components, neutrophils and macrophages were analyzed through immunofluorescent microscopy. Endothelial activation and platelet infiltration were analyzed through immunohistochemistry and qRT-PCR analysis. Plasma free thiol levels were used to evaluate systemic oxidative stress. Increased neutrophils, NETs and NET/neutrophil ratios were observed in kidneys, hearts and livers of brain-dead rats compared to sham-operated rats. Numbers of NETs positively correlated with the extent of endothelial cell activation. Brain-dead animals also had increased kidney and liver macrophages, increased infiltrated platelets in the liver, and elevated systemic oxidative stress, compared to sham-operated animals. Our findings established the presence of NETs in organs from a brain-dead donor model and suggest that NETs, alongside increased inflammation and a redox imbalance, might prime organs for microvascular endothelial dysfunction and increased injury during brain-death.

Keywords: donor, brain-death, neutrophils, neutrophil extracellular traps, endothelial activation

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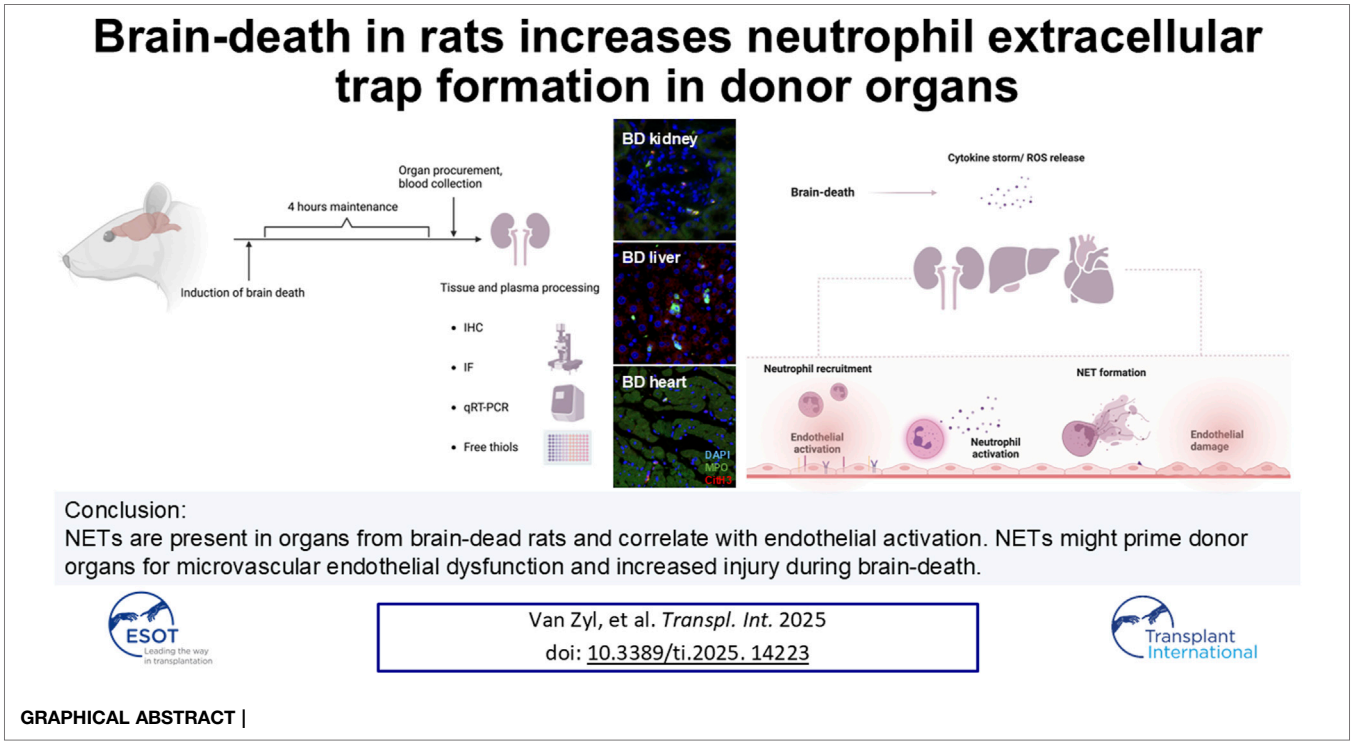
Abbreviations: AKI, acute kidney injury; BD, brain-dead; CitH3, citrullinated histone 3; DAB, 3,3′-Diaminobenzidine; DAMPs, danger associated molecular patterns; FFPE, formalin fixed paraffin embedded; *g*, gravity; ICAM-1, intercellular adhesion molecule-1; IHC, immunohistochemistry; IF, immunofluorescence; I/R, ischemia/reperfusion; MPO, myeloperoxidase; NADPH, nicotinamide-adenine dinucleotide phosphate; NE, neutrophil elastase; NETs, neutrophil extracellular traps; PAD4, peptidyl arginine deiminase; ROS, reactive oxygen species; VCAM-1, vascular cell adhesion molecule.



GRAPHICAL ABSTRACT |

INTRODUCTION

Organ transplantation remains the best treatment option for patients facing end-stage organ failure [1]. Globally, the rise in high disease burden results in an increase in the demand for donor organs, contributing to long waiting lists at transplantation centers [2]. A significant proportion of transplants are performed using grafts from brain-dead (BD) donors [3], however, the quality of these organs may be compromised due to various systemic changes occurring during brain-death [4]. Hemodynamic instability and a build-up in intracranial pressure results in a reduced cardiac output which is overcompensated for by a catecholamine storm, ultimately resulting in the release of a cascade of pro-inflammatory cytokines, organ hypoperfusion [4], activation of complement [5] and coagulation [6], the release of reactive oxygen species (ROS) [7], and increased organ leukocyte infiltration [8]. Increased immune activation thus already starts in the BD donor, which exacerbates ischemia/reperfusion injury (I/R) [9] and potentially also leads to inferior transplant outcome in the recipient compared to living donor transplantation [10, 11]. Finding strategies to modulate immune cell infiltration and activation prior to transplantation might therefore not only enlarge the pool of transplantable organs, but also improve graft survival and transplant outcome.

The pro-inflammatory microenvironment during BD might stimulate neutrophil extracellular trap (NET) formation both within the graft and in the systemic circulation [12, 13]. NETs are characterized by the release of the neutrophil's nuclear contents to the extracellular space in web-like structures [14]. NET formation may be initiated in response to pathogens [15] or, as is relevant in the context of BD, during sterile inflammation, through stimulation with danger associated molecular patterns (DAMPs) [16], pro-inflammatory cytokines [17], and activated platelets [18]. Following neutrophil stimulation, histones undergo decondensation, mediated by the activity of cytoplasmic enzymes, myeloperoxidase (MPO) or neutrophil elastase (NE), or citrullination through peptidyl arginine deiminase 4 (PAD4) [19]. NET formation culminates in nuclear disintegration or blebbing, and the release of webbed structures containing histones, DNA, MPO and NE [15]. The components of NETs are cytotoxic and often cause extensive tissue damage [16, 20], endothelial cell activation, death [21, 22] and increased vascular permeability [23], the activation of coagulation and complement, and the recruitment of additional immune cells [24]. A mounting body of evidence suggests a role of NET formation in transplantation-related complications in various organs [25, 26]. NETs have been associated with acute antibody-mediated rejection in transplanted kidneys [25], primary graft dysfunction in lungs [27], and acute liver rejection [28]. However, the presence of NETs in organs from BD donors remains unexplored.

By using a rat BD model, we aimed to explore NET formation in organs during BD, hypothesizing that NET formation already starts in the donor. This might cause damage prior to transplantation and potentially exacerbate I/R injury following implantation, contributing to delayed graft function and graft failure since NETs have been shown to be associated with acute kidney injury [16].

Furthermore, it has been established that the endothelium undergoes various changes during brain-death, including the

increased expression of adhesion molecules involved in neutrophil recruitment, and transformation to a pro-inflammatory, pro-coagulant state resulting in increased platelet activation and adhesion [6, 7]. Brain-death is also accompanied by progressive systemic oxidative stress through gradual, cumulative release of ROS by stressed, hypoxic cells [7, 29].

During brain-death, endothelial activation, already observed early during the onset of BD [7, 30], might increase neutrophil recruitment and consequently also increase NET formation, enhancing organ injury through endothelial damage [31] and thrombus formation [32]. Neutrophils might not only be primed for increased NET formation through ROS release by other cells, but NETs might also contribute to the gradual onset of the systemic oxidative burden during brain-death [33].

We therefore also aimed to investigate the association between NET formation and endothelial activation, platelet infiltration, and ROS production during brain-death in our model.

MATERIALS AND METHODS

Animal Maintenance

In this post-hoc study, eight-week-old, male and female Wistar F344/IcoCrl rats (Charles River, Italy) were used for all experiments. Prior to experiments, the rats were allowed free access to water and food and maintained at ambient room temperature with a 12 h light/dark cycle. During care of the animals, *The Principles of Laboratory Animal Care* were followed. The local animal ethics committee approved the protocol in accordance with the Experiments on Animals Act and ARRIVE guidelines (IvD 171245-01-002).

Brain-Death (BD) Rat Model and Sample Collection

Rats in the BD group (n = 18, 10 males and 8 females) were subjected to slow-induced BD by simulating cerebral hemorrhage through intracranial balloon catheterization and inflation, which was maintained for 4 h as previously described [34], visually depicted in **Supplementary Figure S1** and detailed in **Supplementary Digital Content**. Sham-operated animals (n = 16, 8 males and 8 females) received the same cranial perforation but without BD induction. Organs were either flash frozen, or formalin-fixed and paraffin-embedded (FFPE) for analysis. Plasma samples were obtained by centrifuging heparinized whole blood, collected from the abdominal aorta, at 1,200 x *g* for 10 min at 4°C. Frozen tissue or plasma samples were stored at −80°C until use.

Immunofluorescence

The co-localization of DNA, MPO and citrullinated histone 3 (CitH3) was used to identify NETs, while an antibody against CD68 was used to identify monocytes and macrophages to correct for MPO expressing macrophages. FFPE rat kidneys, hearts and livers were sectioned into 3 μm sections and mounted onto slides. The slides were deparaffinized in xylene, rehydrated in gradient steps of alcohol, submitted to 180 min of heat-induced antigen retrieval at 60°C in a citrate buffer (pH 6.0) and blocked for 1 h with 5% bovine serum albumin (BSA). The tissue was incubated with primary antibodies against CitH3 (1: 100, ab5103, Abcam, UK), MPO (1:200, AF3667, Novus Biologicals, United States) and CD68, clone ED-1 (1:100, MCA341GA, BIO-RAD) for 90 min at room temperature followed by incubation with donkey anti-rabbit Alexa-Fluor® 647 (1:500), donkey anti-goat Alexa-Fluor® 488 (1:500), and donkey anti-mouse Alexa-Fluor® 586 (1:500) from Abcam (UK). The slides were mounted with Vectashield antifade mounting medium (Vector Laboratories, United States) containing DAPI (ThermoFisher Scientific, United States) and digitized with an Olympus VS200 Fluorescent Slide Scanner (Olympus, Japan). NETs were quantified with Qupath (v. 0.4.1.) and expressed as the number of MPO^+CitH3^+ double positive but CD68 negative cells per area, or the number of $CD68^+$ cells (macrophages) per area. To compare different organs, NETs were expressed as fold increase from sham. Technical details on reagents, antibodies and methods used during immunofluorescence can be found in the **Supplementary Digital Content**, **Supplementary Tables S1, S2**.

Immunohistochemistry

Endothelial activation in kidney, heart and liver sections was analyzed by quantifying protein expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). Frozen kidney, liver and heart sections were cut in 3 μm sections, dried and fixed with acetone. The sections were blocked with 3% H_2O_2 and 5% BSA followed by incubation with mouse anti-ICAM-1 (1:100) from BD Pharmingen and mouse anti-VCAM1 (1:100) from Biogen. The primary antibodies were detected through horseradish peroxidase (HRP)-conjugated secondary and tertiary antibodies [goat anti-mouse-HRP and rabbit anti-goat-HRP [(both 1:100) from DAKO, Denmark] and visualized with 3,3′-Diaminobenzidine (DAB). The sections were counterstained with hematoxylin and dehydrated before mounting with Dibutylphthalate Polystyrene Xylene mounting medium.

For quantification of platelets, rat kidney, liver and heart FFPE sections were deparaffined, rehydrated, submitted to 15 min of heat-induced antigen retrieval at 95°C in a citrate buffer (pH 6) and blocked as described for ICAM-1 and VCAM-1. The tissue was incubated with a rabbit CD41 antibody (1:500, Proteintech, United States) for 1 h. Primary antibody binding was detected with goat anti-rabbit-HRP (1:100) and with rabbit anti-goat-HRP secondary antibodies from DAKO (Denmark) followed by incubation with DAB, a counterstain with hematoxylin and mounting. All tissues were digitized with a Hamamatsu NanoZoomer Digital slide scanner (Hamamatsu, Japan). The expression of ICAM-1, VCAM-1, and platelets were analyzed as the percentage positive area with Fiji/ImageJ (v. 3). Antibody and reagent details are specified in **Supplementary Digital Content**, **Supplementary Tables S1, S3**.

TABLE 1 | Primer sequences for qRT-PCR.

Gene	Sequence
B-actin	5′-GGAAATCGTGCGTGACATTAAA-3′
	5′- GCGGCAGTGGCCATCTC -3′
ICAM1	5′-CCTGGAGATGGAGAAGACCTA -3′
	5′-GGGAAGTACCCTGTGAGGTG -3′
VCAM1	5′-GCTCCTCTCGGGAAATGCCA -3′
	5′-ACAACGGAATCCCCAACCTGT -3′
E-selectin	5′-ACTTGTGAAGCCCCAGCCAA -3′
	5′-TGGCAGCTACTAGCAGGAACG -3′

Abbreviations: ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1.

mRNA Analyses

The mRNA expression levels of endothelial adhesion molecules (ICAM-1, VCAM-1, and E-selectin), were analyzed through SYBR™ Green qRT-PCRs. Intron-spanning primers for the target genes were designed through Primer-Blast (NIH, United States) and checked for potential hairpins or dimers with an Oligonucleotide properties calculator. Forward and reverse primers used for genes of interest are summarized in **Table 1**. RNA was isolated from frozen sections of rat kidneys, hearts and livers using TRIzol® and stored at −20°C until use. The purity, quantity and quality of the isolated RNA were evaluated with a NanoDrop spectrophotometer and agarose gel electrophoresis. Complementary DNA (cDNA) was synthesized using Random primer hexamers and Superscript II (ThermoFisher Scientific, United States). The samples were loaded onto PCR microplates with a master mix containing SYBR™ Green (ThermoFisher Scientific) and gene-specific primers. Amplification of the targets was done with a Roche LightCycler® 480 System (Roche, Switzerland). Melt-curves and Ct values were obtained through Roche LightCycler 480 software (v 1.2.9.11). The relative expression of target genes was normalized against ß-actin expression. Technical details of qRT-PCRs are summarized in **Supplementary Digital Content**, **Supplementary Table S4**.

Plasma Free Thiol Levels

Free thiol levels were determined in rat plasma to measure systemic oxidative stress as previously described [35]. Rat plasma samples were diluted 20-fold with a 0.1 M Tris buffer (pH 8.2). The standard curve consisted of increasing concentrations of L-Cysteine in a 0.1 M Tris, 10 mM EDTA buffer (pH 8.2). A color reaction in the sample and standard wells was developed through the addition of 1.9 mM DTNB in 0.1 M phosphate buffer (pH 7.0) for 20 min at room temperature. The absorbance was measured at 412 nm with a Clariostar plus microplate reader (BMG Labtech, Germany). Technical details of the assay are specified in **Supplementary Digital Content**, **Supplementary Table S5**.

Statistics

Sample datasets were examined for normality using the Shapiro-Wilk normality test. Subsequently, Welch t-tests were conducted, allowing for unequal standard deviations, for populations exhibiting normal distribution, whereas Mann-Whitney U tests were employed for data displaying non-normal distribution. For analyses involving comparison of more than one group, an ANOVA with Tukey post-hoc analyses and Bonferroni correction, or Kruskal Wallis with Duns post-hoc test and Bonferroni correction was used. Associations between variables were evaluated using either Pearson or Spearman correlation tests based on normality. Results are presented as either mean and standard error of the mean (SEM) or median and interquartile range (IQR). A significance threshold of $p < 0.05$ was applied for all analyses. Statistical analyses and data visualization were performed using GraphPad Prism (v. 10.0.0).

RESULTS

Increased CitH3-Positive Neutrophils in Brain-Dead Kidneys

Compared to sham-operated animals, (**Figure 1A**), BD rat kidneys (**Figure 1B**) had increased neutrophils (MPO^+) (**Figure 1C**, $p < 0.0001$). In BD, significantly more neutrophils were CitH3 positive compared to sham-operated animals (**Figure 1D**, $p < 0.0001$), indicative of NET formation. BD rat kidneys also had an increased proportion of CitH3 positive neutrophils (NETs) compared to sham (**Figure 1E**, $p < 0.01$). In the BD animals, most NETs were observed in the renal cortex (**Figure 1B**), located predominantly in the glomeruli, but also in the peritubular capillaries/interstitial spaces.

Endothelial Activation in Brain-Dead Rat Kidneys Correlates With NET Formation

BD rat kidneys had increased endothelial activation associated gene (**Figures 2A–C**) and protein expression (**Figures 2D, E**) compared to sham-operated animals. ICAM-1 (**Figure 2A**), VCAM-1 (**Figure 2B**) and E-selectin (**Figure 2C**) mRNA expression levels were elevated compared to sham-operated animals. This was also reflected on the protein level, with increased ICAM-1 (**Figure 2D**) and VCAM-1 (**Figure 2E**) protein expression levels in the glomerular capillaries, peritubular capillaries, veins, and arteries of BD rat kidneys. ICAM-1 (**Figure 3A**), VCAM-1 (**Figure 3B**) and E-selectin (**Figure 3C**) mRNA expression, and ICAM-1 (**Figure 3D**) and VCAM-1 (**Figure 3E**) protein expression significantly correlated with NET formation in BD rat kidneys. MPO positivity was observed near ICAM-1 (**Figure 4A**) and VCAM-1 (**Figure 4B**) protein expression in the glomeruli of BD kidneys.

Increased CitH3-Positive Neutrophils in Brain-Dead Rat Hearts

Similar to observations in BD rat kidneys, an increased number of neutrophils (**Figure 5C**, $p < 0.0001$) and increased NET formation (CitH3 positive neutrophils, **Figure 5D**, $p < 0.0001$) were also observed in BD rat hearts (**Figure 5B**), compared to hearts from sham-operated animals (**Figure 5A**). BD rat hearts

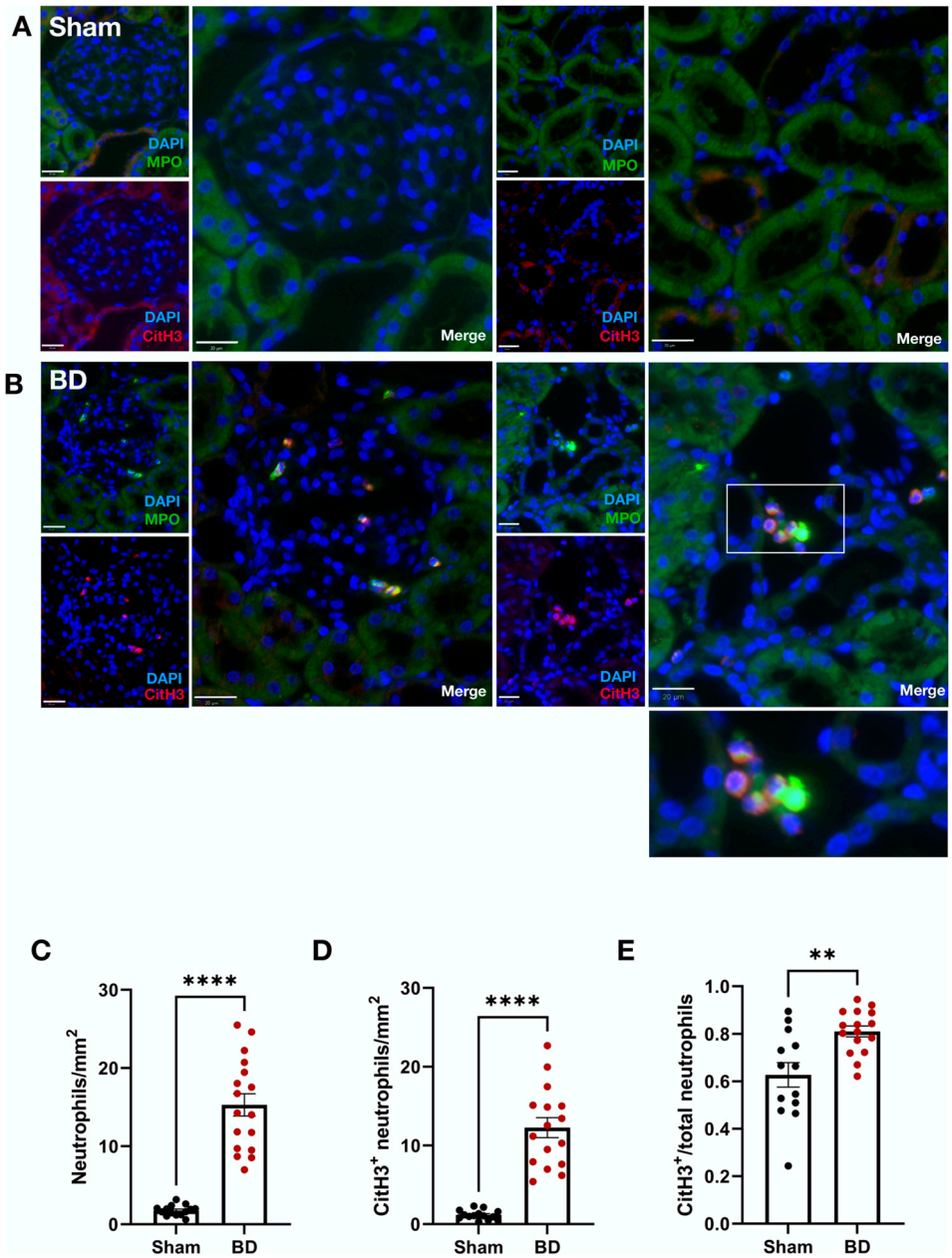


FIGURE 1 | Neutrophil infiltration and NET formation in brain-dead rat kidneys. NET formation was observed in the renal cortex of brain-dead rat kidneys **(B)**, while very few/almost no NETs were observed in kidneys from sham operated animals **(A)**. The brain-dead group had significantly more neutrophil infiltration **(C)**, CitH3 positive neutrophils **(D)**, and CitH3 positive/total neutrophil ratios **(E)** compared to sham. Scale bar equals 20 μm. ****$p < 0.0001$, **$p < 0.01$. Data expressed as means (±SEM). Abbreviations: BD, brain-dead; CitH3, citrullinated histone 3; MPO, myeloperoxidase.

also had increased NET/neutrophil ratios (**Figure 5E**, $p < 0.05$) compared to sham. NET forming neutrophils were observed between myocytes of the myocardium.

Endothelial Activation Protein in Brain-Dead Hearts Correlates With NET Formation

ICAM-1 (**Figure 6A**), VCAM-1 (**Figure 6B**) and E-selectin (**Figure 6C**) mRNA expression in BD hearts were increased compared to hearts from sham-operated animals. In line with this, BD rat hearts also had increased ICAM-1 (**Figure 6D**) and VCAM-1 (**Figure 6E**) protein expression compared to sham. In the BD hearts, both ICAM-1 and VCAM-1 were observed in capillaries and arteries (**Figures 6D, E**). Protein levels of ICAM-1, positively correlated to NET formation in the BD group ($r = 0.7$; $p = 0.002$). Significant correlation between VCAM-1 protein/endothelial activation mRNA and NET formation were not observed (not shown).

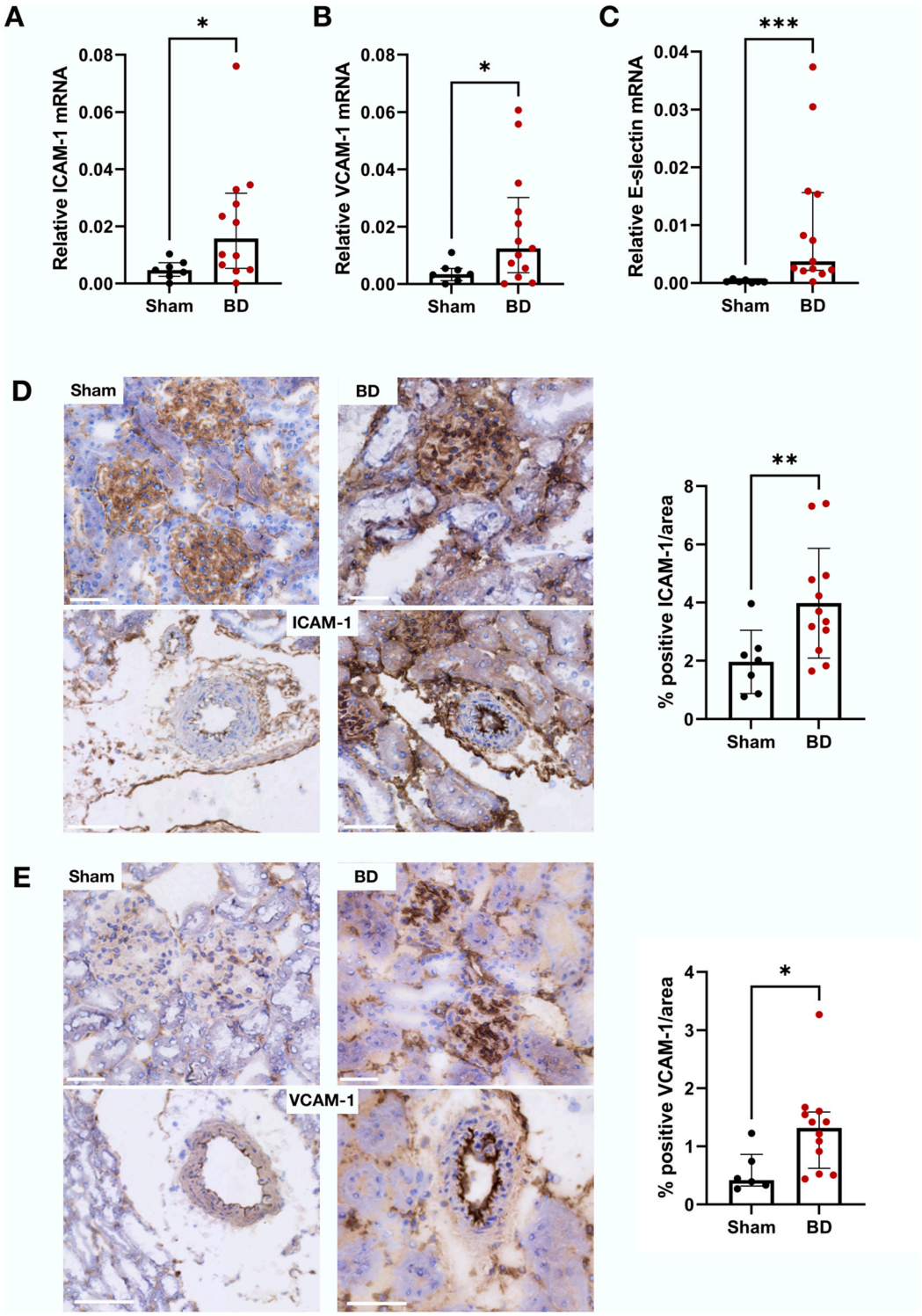


FIGURE 2 | Endothelial activation in brain-dead rat kidneys. Brain-dead rat kidneys had increased ICAM-1 mRNA expression **(A)**, VCAM-1 mRNA expression **(B)** and E-selectin mRNA expression **(C)**. ICAM-1 **(D)** and VCAM-1 **(E)** protein was observed in the glomeruli and vessels and were increased in brain dead animals compared to sham. Scale bar equals 50 μm ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. Data expressed as median (IQR). Abbreviations: BD, brain-dead, ICAM-1 intercellular cell adhesion molecule 1, VCAM-1, vascular cell adhesion molecule 1.

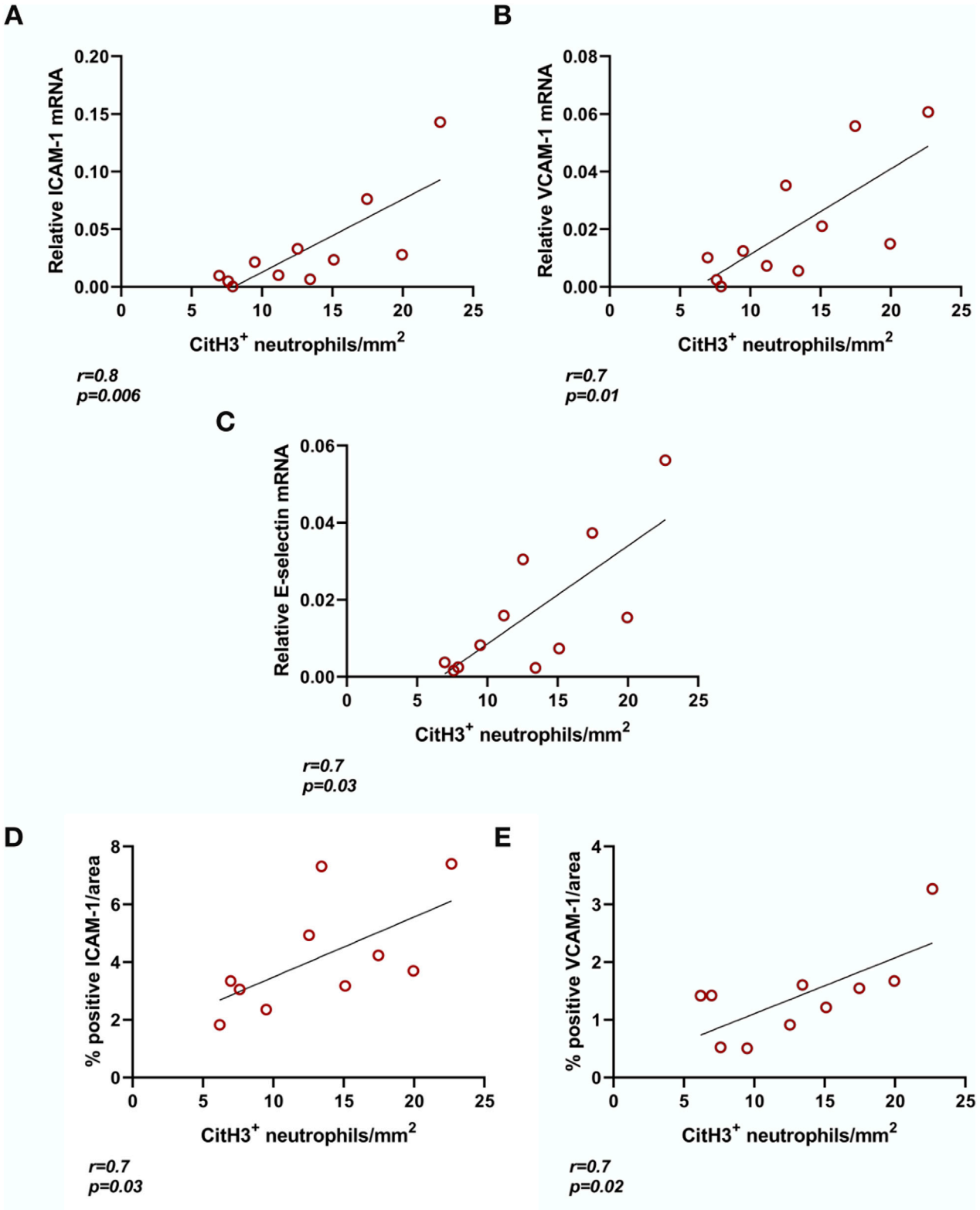


FIGURE 3 | Endothelial activation correlates with NET formation in brain-dead rat kidneys. ICAM-1 **(A)**, VCAM-1 **(B)**, and E-selectin **(C)** mRNA expression as well as ICAM-1 **(D)** and VCAM-1 **(E)** protein in brain-dead rat kidneys positively correlated to NET formation (CitH3 positive neutrophils). Abbreviations: CitH3, citrullinated histone 3; ICAM-1, intercellular cell adhesion molecule 1, MPO, myeloperoxidase, VCAM-1, vascular cell adhesion molecule 1.

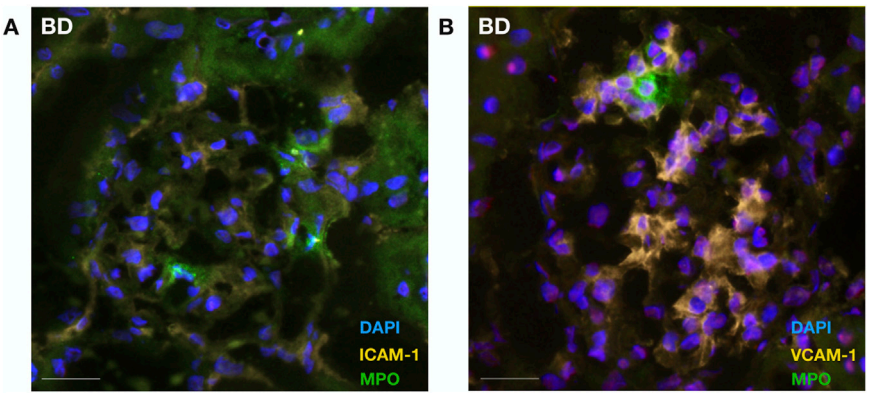


FIGURE 4 | Neutrophil colocalizes with endothelial cellular adhesion molecules in brain-dead rat kidneys. Neutrophil infiltration (MPO) was observed in proximity to ICAM-1 **(A)** and VCAM-1 **(B)** protein expression in the glomeruli of brain-dead rat kidneys. Abbreviations: ICAM-1, intercellular cell adhesion molecule 1; MPO, myeloperoxidase, VCAM-1, vascular cell adhesion molecule 1.

Increased CitH3-Positive Neutrophils in Brain-Dead Rat Livers

Livers in the BD group (**Figure 7B**) had significantly more neutrophils compared to sham (**Figures 7A, C**, $p < 0.0001$). The total number of CitH3-positive neutrophils (**Figure 7D**, $p < 0.0001$) as well as the NET/neutrophil ratios (**Figure 7E**, $p < 0.0001$) were both increased in livers from BD rats compared to sham-operated animals. In contrast to BD kidneys, only a small percentage of total neutrophils (3%-4%) were CitH3 positive in BD livers (**Figure 7E**). CitH3 positive neutrophils were mostly observed between hepatocytes in sinusoidal spaces (**Figure 7B**).

Increased Endothelial Activation in Brain-Dead Rat Livers

The mRNA levels of ICAM-1 (**Figure 8A**), VCAM-1 (**Figure 8B**) and E-selectin (**Figure 8C**) in BD rat livers were significantly higher compared to sham. BD livers also had increased ICAM-1 (**Figure 8D**) and VCAM-1 (**Figure 8E**) protein expression in the sinusoids and veins compared to sham. No significant correlation between endothelial activation markers and NET formation was observed in the livers (not shown).

Brain-Dead Rat Kidneys and Livers Have Increased Macrophage Content

Since proinflammatory macrophages may be positive for MPO, the sections were also stained for macrophages (ED1) (**Supplementary Figure S2**) to distinguish NETs (NETs were $CitH3^+$, MPO^+, $CD68^-$) from MPO-positive macrophages. BD kidneys (**Supplementary Figure S2A**) and livers (**Supplementary Figure S2B**) had increased macrophage content compared to sham-operated animals. Hearts, however, did not have significant infiltrated macrophages in the BD or sham-operated group (not shown). A comparison between kidneys and livers of BD rats (**Supplementary Figure S2C**) revealed significantly more CD68 (ED-1) positive macrophages in livers ($p < 0.0001$).

Livers From Brain-Dead Rats Have Increased Platelet Infiltration

No platelet infiltration in kidneys and hearts from BD or sham-operated animals was observed (not shown). Liver sections from BD animals (**Figure 9B**) however, had increased numbers of platelets compared to sham-operated animals (**Figures 9A, C**).

Male Brain-Dead Rat Hearts Have Increased NET Formation and Endothelial Activation

A comparison between males and females revealed no significant differences in NET formation (**Supplementary Figures S3A–F**) or endothelial activation (not shown) between sexes in BD kidneys (A-C) and livers (D-F). Sham male livers, however, had elevated neutrophils compared to females (**Supplementary Figure S3D**). BD male hearts had elevated numbers of neutrophils (**Supplementary Figure S3G**, $p < 0.01$), $CitH3^+$ neutrophils (NET formation) (**Supplementary Figure S3H**, $p < 0.0001$), and ICAM-1 protein expression ($p < 0.05$) (not shown) compared to BD females, in contrast to the sham group which had no differences in NET formation (**Supplementary Figures S3G–I**) or endothelial activation (not shown) between sexes.

Brain-Dead Rat Organs Have Differences in Cell Influx, NET Formation and Endothelial Activation

Compared to the kidneys and hearts, BD rat livers had significantly increased neutrophils, while BD rat kidneys had increased neutrophils compared to hearts (**Figure 10A**). However, when correcting for the numbers of neutrophils

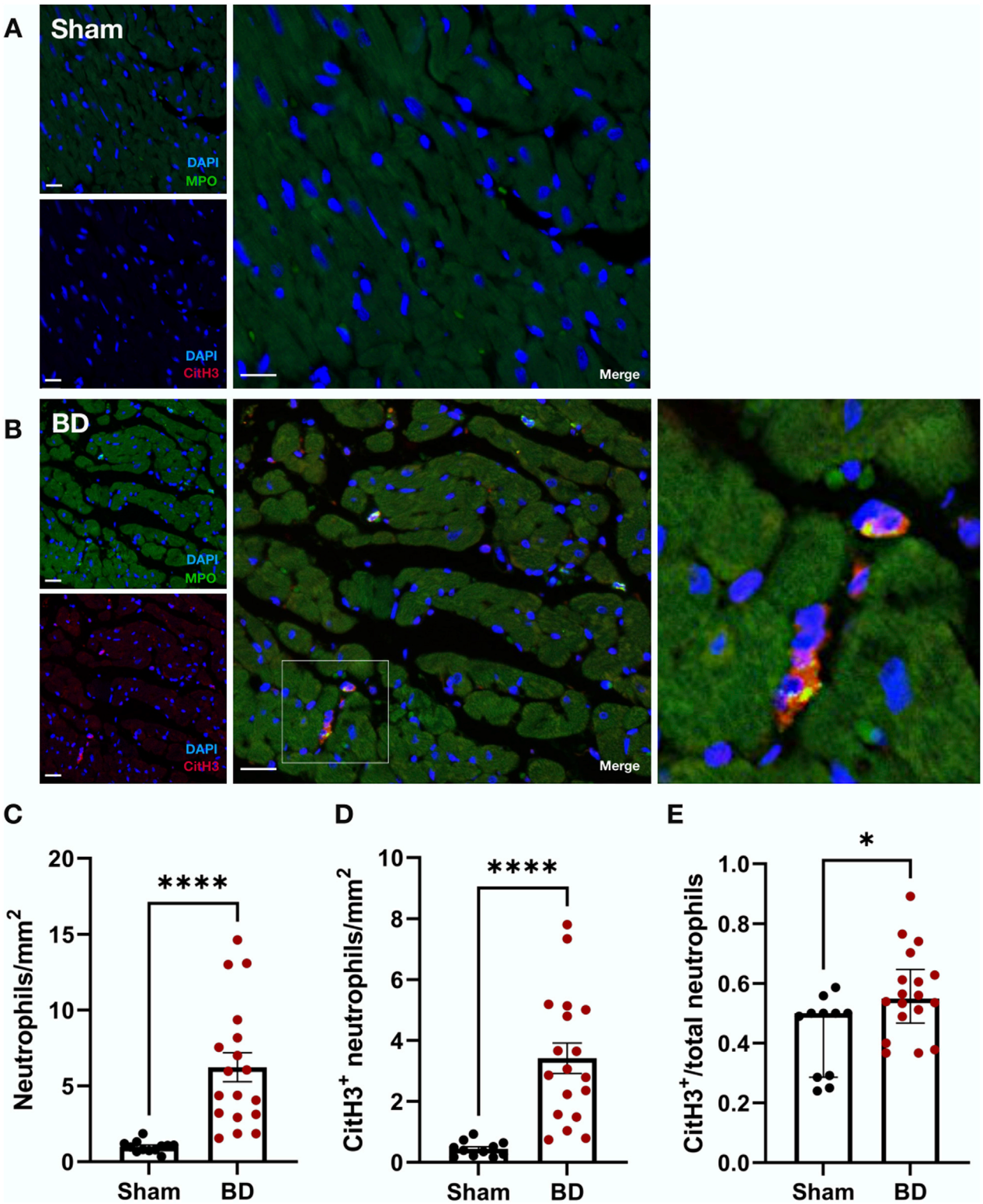


FIGURE 5 | Neutrophil infiltration and NET formation in brain-dead rat hearts. NET formation was observed between myocytes of brain-dead rat hearts **(B)** with very few to almost no neutrophil or NET formation in the hearts of sham operated animals **(A)**. Hearts from brain-dead rats had significantly increased neutrophils **(C)**, CitH3 positive neutrophils **(D)**, and CitH3 positive/total neutrophil ratios **(E)** compared to sham. Scale bar equals 50 μm. ****$p < 0.0001$,*$p < 0.05$. Data expressed as means (±SEM), C, D; or median (IQR), E. Abbreviations: BD, brain-death; CitH3, citrullinated histone 3; MPO, myeloperoxidase.

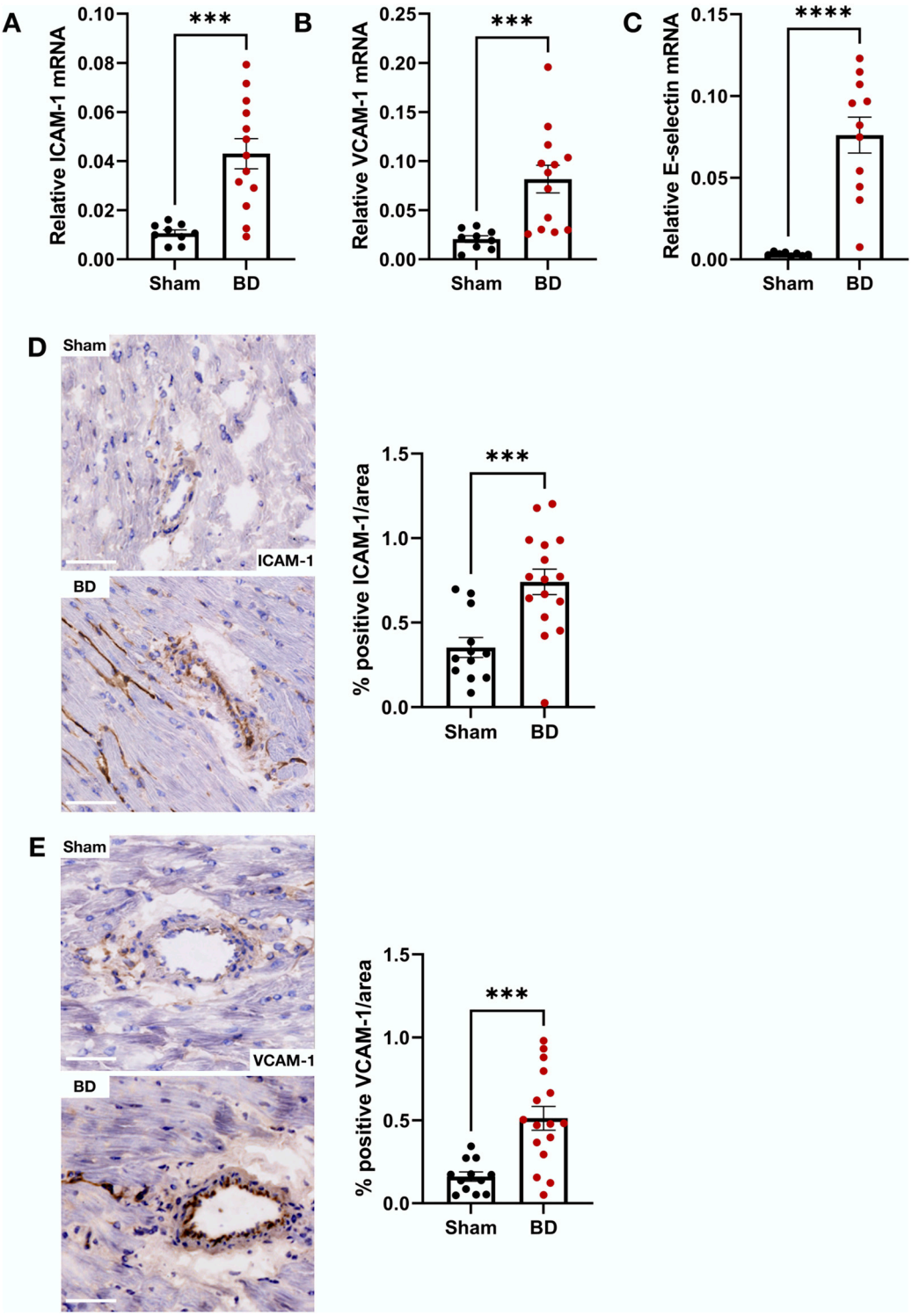


FIGURE 6 | Endothelial activation in brain-dead rat hearts. Brain-dead rats had increased ICAM-1 mRNA **(A)**, VCAM-1 mRNA **(B)** and E-selectin mRNA **(C)** as well as increased ICAM-1 **(D)** and VCAM-1 **(E)** protein levels. ICAM-1 and VCAM-1 was observed both the capillaries and larger vessels **(D, E)**. Scale bar equals 50 μm ****$p < 0.0001$, ***$p < 0.001$. Data expressed as means (±SEM). Abbreviations: BD, brain-death; ICAM-1, intercellular cell adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1.

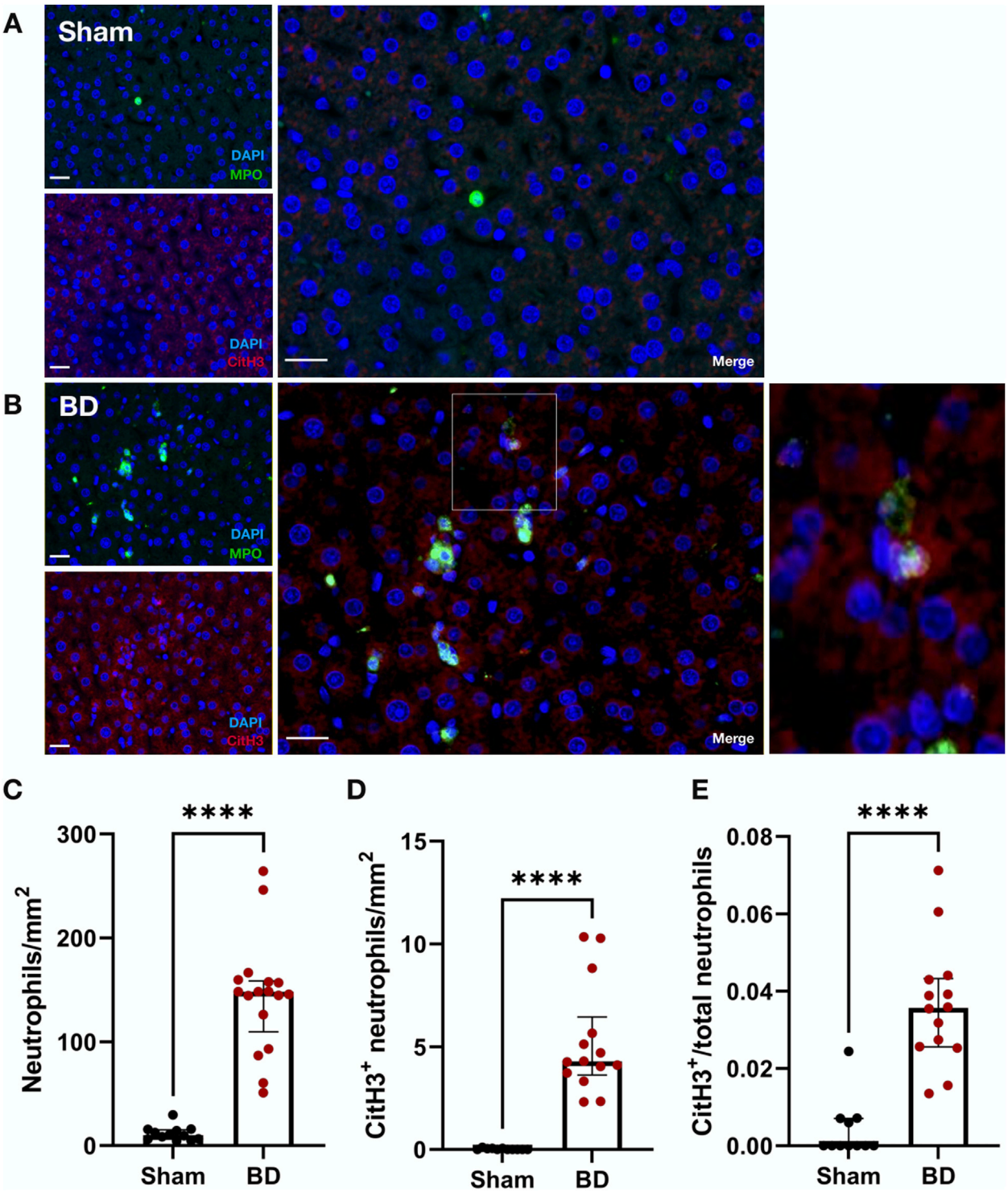


FIGURE 7 | Neutrophil infiltration and NET formation in brain-dead rat livers. NET formation was observed in brain-dead rat livers **(B)**, while sham operated livers had little to almost no NET formation **(A)**. Brain-dead livers had significantly increased neutrophil infiltration **(C)**, CitH3 positive neutrophils **(D)** and CitH3 positive/total neutrophils ratios **(E)** compared to sham. Scale bar equals 20 μm. ****$p < 0.0001$. Data expressed as median (IQR). Abbreviations: BD, brain-death; CitH3, citrullinated histone 3; MPO, myeloperoxidase.

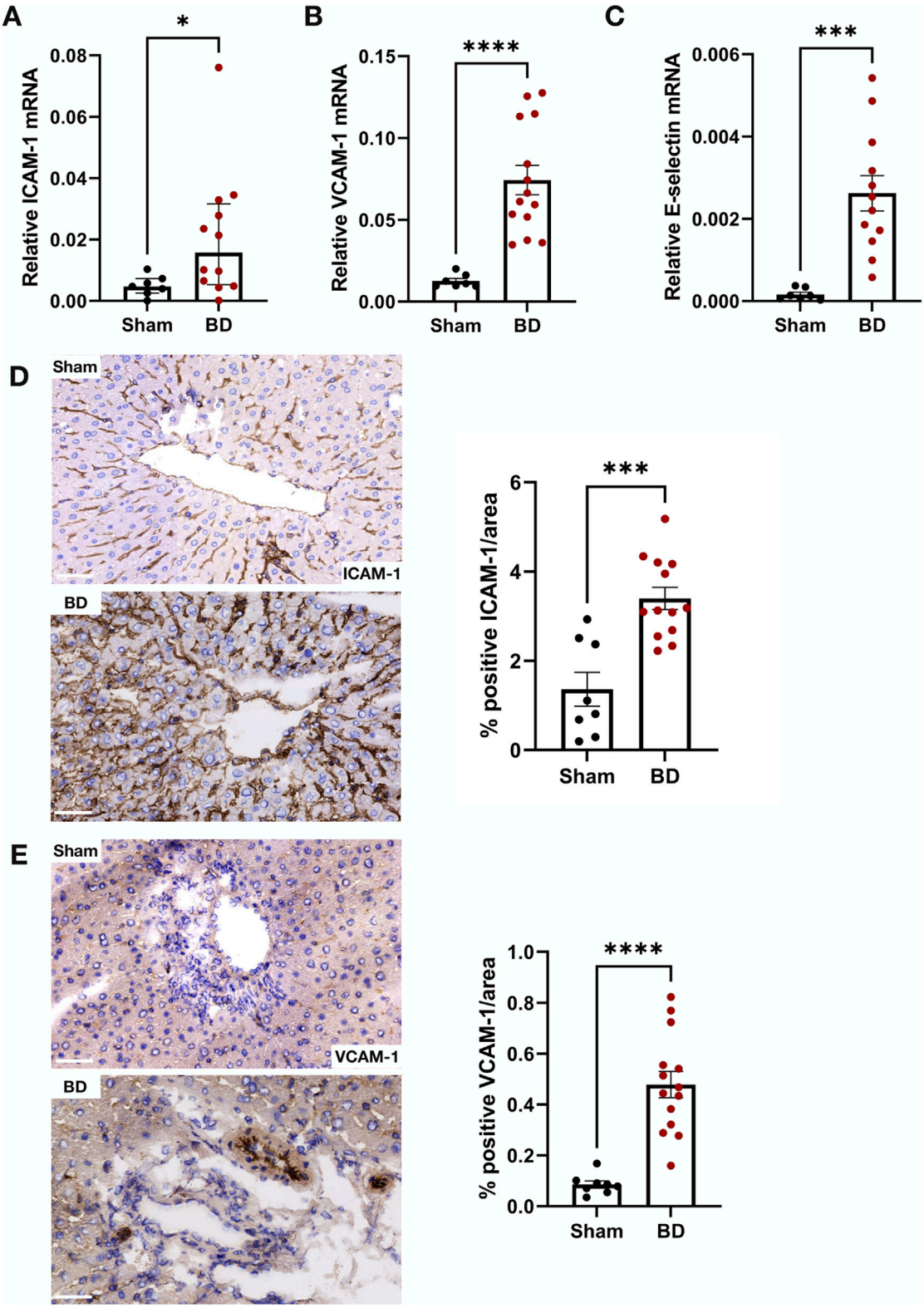


FIGURE 8 | Endothelial activation in brain-dead rat livers. Brain-dead livers had increased ICAM-1 mRNA **(A)**, VCAM-1 mRNA **(B)** and E-selectin mRNA **(C)** expression and increased ICAM-1 **(D)** and VCAM-1 **(E)** protein levels. Scale bar equals 50 µm. *p < 0.05, ***p < 0.001 ****p < 0.0001. Data expressed as median (IQR), A; or mean (±SEM), B,C,D,E. Abbreviations: BD, brain-death; ICAM-1, intercellular cell adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1.

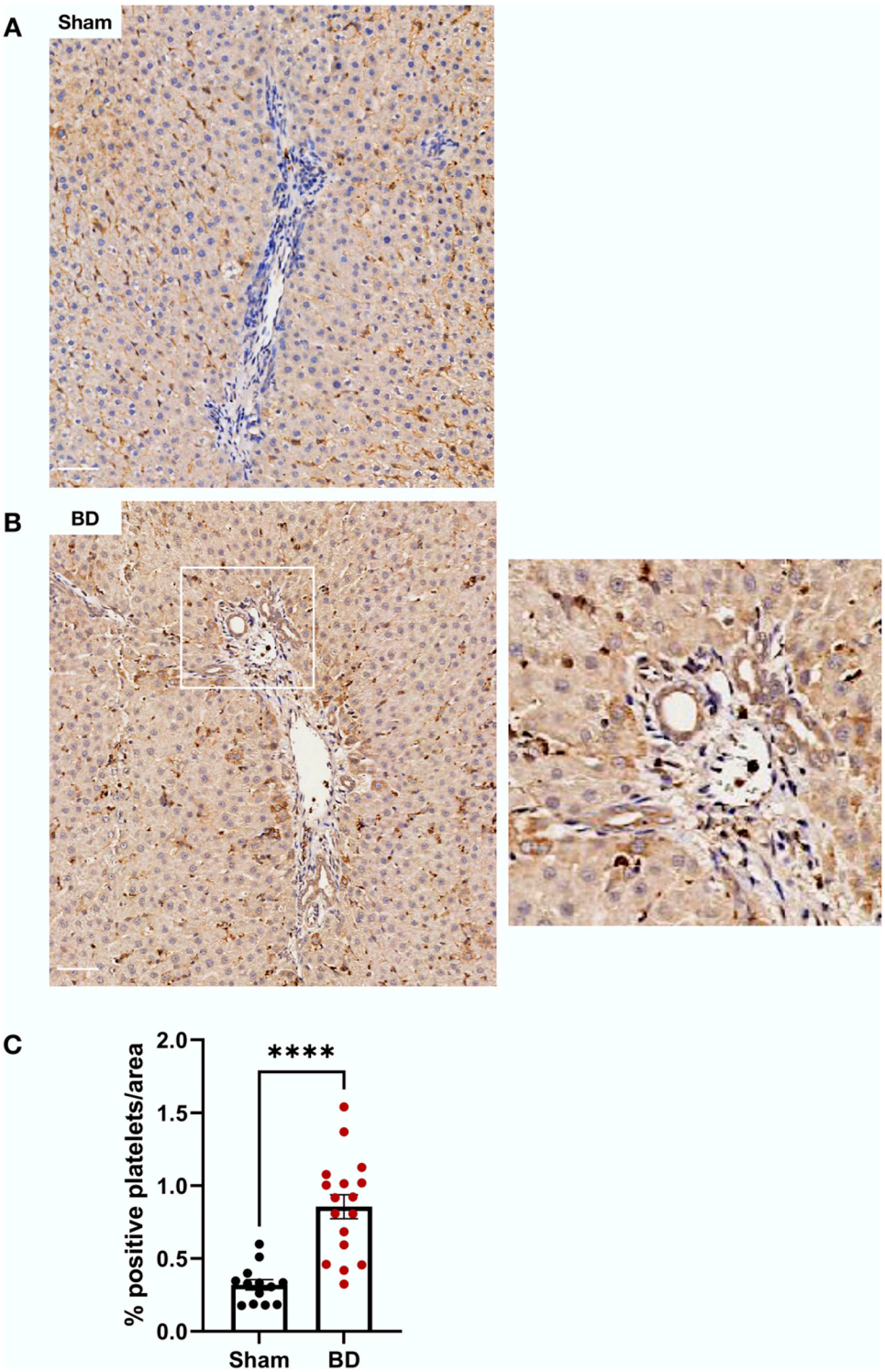


FIGURE 9 | Platelet infiltration in brain-dead rat livers. Brain-dead rat livers **(B)** had increased platelet infiltration (CD41) compared to sham **(A)** quantified as percentage positivity per area analysed **(C)**. Scale bar equals 50 μm. ****p < 0.0001. Data expressed as mean (±SEM). Abbreviations: BD: Brain-death.

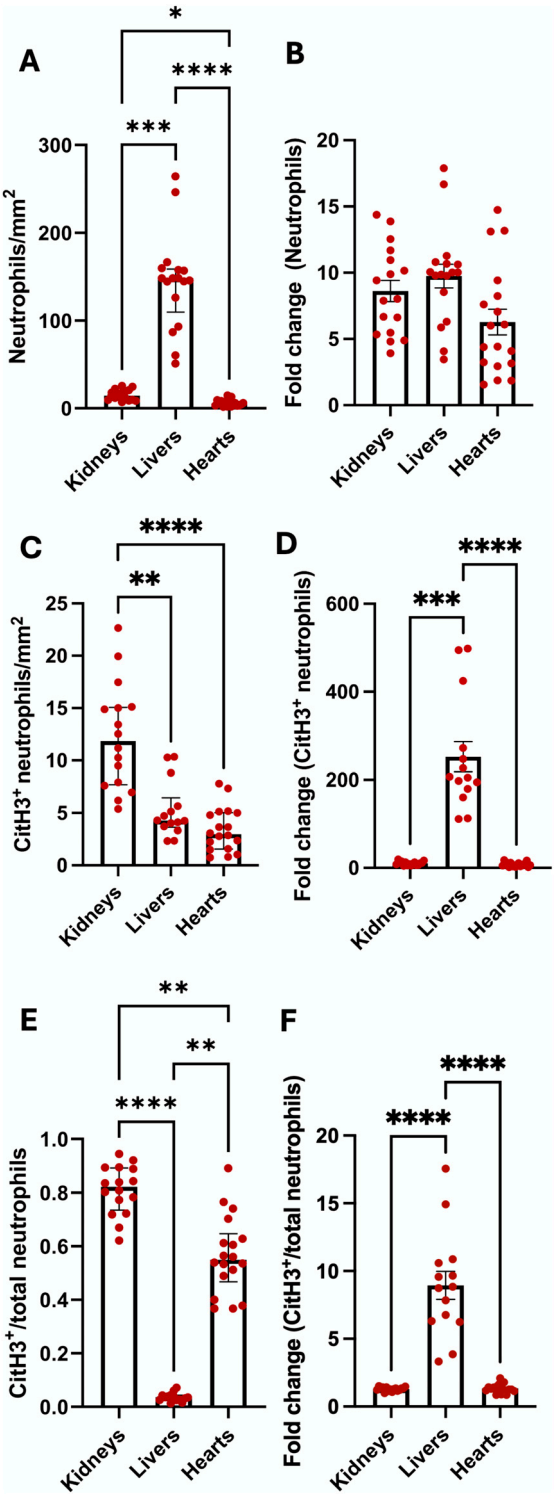


FIGURE 10 | Differences in neutrophil infiltration and NET formation between organs. Brain-dead rat livers had the most infiltrating neutrophils, while kidneys had more neutrophils compared to hearts **(A)**. No differences in neutrophil fold change from sham operated animals were observed between the organs **(B)**. Compared to livers and hearts, kidneys had the highest level of NET formation **(C)**, however, livers had an increase in NET fold change, compared to kidneys **(D)**. NET/neutrophil ratios were higher in kidneys and hearts compared to livers, while kidneys had increased ratios compared to hearts **(E)**. Livers had increased fold change ratios compared to the other organs **(F)**. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Data expressed as median (IQR), **(A**, **C** and **E)** or mean ± SEM, **(B**, **D** and **F)**. Abbreviations: CitH3, citrullinated histone 3.

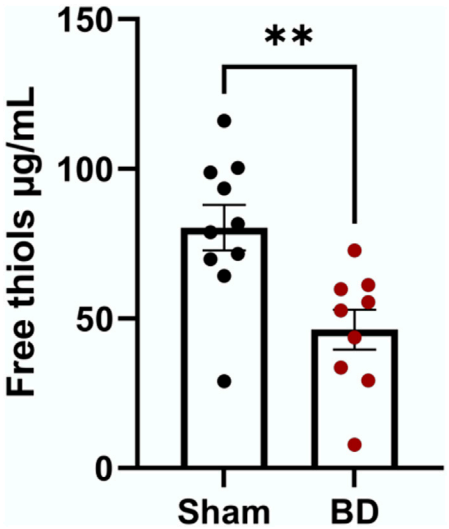


FIGURE 11 | Reactive oxygen species in brain-dead rats. Brain-dead rats had increased circulating reactive oxygen species indicated by lower free thiol levels. **p < 0.01. Data expressed as mean (±SEM). Abbreviations: Brain-death.

present in sham-operated rats (by expressing as fold-change in brain dead rats), no differences were observed between different organs (**Figure 10B**). BD kidneys had increased NET formation compared to both livers and hearts when expressed as absolute numbers of CitH3$^+$ neutrophils (NETs, **Figure 10C**) as well as increased NET/neutrophil ratios (**Figure 10E**). Fold change of NETs and NET/neutrophil ratios was, however, higher in livers (**Figures 10D, F**). Distinct differences in endothelial activation in terms of mRNA and protein levels amongst kidneys, livers and hearts (**Supplementary Figure S4**) were also observed. BD rat kidneys and hearts had decreased ICAM-1 mRNA expression and fold change, compared to livers (**Supplementary Figure S4A**). BD hearts and livers had increased VCAM-1 mRNA compared to kidneys. No significant difference in VCAM-1 mRNA fold change was observed (**Supplementary Figure S4B**). Hearts had increased relative E-selectin mRNA expression, while both hearts and kidneys had increased fold change in E-selectin mRNA compared to livers (**Supplementary Figure S4C**). On the protein level, kidneys and livers had increased ICAM-1 compared to hearts, which was not reflected in fold change from sham (**Supplementary Figure S4D**). Kidneys also expressed increased VCAM-1 compared to livers and hearts, however,

livers had increased fold change in VCAM-1 expression, compared to other organs (**Supplementary Figure S4E**).

Brain-Dead Rats Have Increased Oxidative Stress

Free thiol levels in plasma were lower in BD animals compared to sham-operated animals (**Figure 11**) reflecting increased oxidative stress in BD rats. Although free thiol levels in rat plasma tended to negatively correlate with NETs and endothelial activation in the organs analyzed (not shown), this association was not significant.

DISCUSSION

Neutrophils are the first responders of the innate immune system during inflammation and have a prominent role during different phases of transplantation [36]. This study demonstrated for the first time that NETs are already present in donor organs from a rat BD model. In this setting, the pro-inflammatory milieu during BD might prime neutrophils infiltrating the renal, cardiac, and hepatic tissues for NET formation. Given a previously established association between NET formation and organ injury [16, 21], this implicates a potential contribution of NETs towards compromised graft quality prior to reperfusion/transplantation. Moreover, associations with endothelial adhesion molecules and E-selectin might indicate a potential connection between NET formation and microvascular endothelial activation, particularly in the kidney, suggesting a role of NETs as contributing factor to organ microvascular endothelial dysfunction during brain-death.

The relation between NETs and organ injury has been explored by multiple groups, both through animal models [13, 16] and patient studies [27]. NETs are catalysts for heightened inflammation [13], coagulation, complement activation [24], the recruitment of additional immune cells and the augmentation of I/R injury [13]. Consequently, NETs have been implicated in lung [27], kidney [16], liver [13] and cardiac [37] injury in various diseases. NET components may enhance acute kidney injury (AKI) [16] and I/R-mediated liver injury [13]. In our study, the abundance of NET-forming neutrophils and accompanying increased endothelial activation suggest heightened injury, already in the donor, which is likely to worsen following reperfusion after transplantation.

Our model sheds light on differences between organs during brain-death. While the livers in brain death had increased neutrophil influx compared to kidneys and hearts, kidneys were found to have significantly increased absolute numbers of NETs and NET/neutrophil ratios compared to livers and hearts. Hearts also had increased NET/neutrophil ratios compared to livers. Increased NET and NET/neutrophil ratio fold change from sham was observed for livers compared to hearts and kidneys, but it should be considered that the liver had near zero NET formation in sham animals. Even though livers display the largest change in NET formation when comparing sham and BD, we believe the absolute number of NETs (as observed in kidneys) is most important in determining the potential effect of NET formation on graft outcome. Previous studies have shown differences in metabolic dynamics during brain-death between kidneys, hearts and livers [38, 39]. Kidneys and hearts were revealed to be more vulnerable to ischemic damage, compared to the liver, which is more resilient [38, 40]. Increased ischemic injury in kidneys and hearts might result in increased NET formation by the infiltrated neutrophils (reflected by NET/neutrophil ratios), making kidneys and hearts more vulnerable to cellular-mediated injury during brain-death. However, the impact of these differences between organs during brain-death needs to be further explored.

In the kidneys, the specific localization of cell infiltration and microvascular endothelial dysfunction (interstitium/peritubular capillaries and glomeruli) are often considered in the classification of rejection in the recipient. Recently, the profiling of innate immune cells in kidney grafts with rejection revealed an important role of innate immune cells in rejection, specifically in vasculature and glomeruli [41]. In our model, the abundance of NETs specifically located in the glomeruli might suggest a potential contribution towards rejection in the recipient by enhancing attraction of recipient immune cells to these compartments.

Endothelial activation in organs is an important indicator of graft quality as endothelial damage in deceased donors has been linked to early graft rejection [42]. During brain-death, the endothelium is activated through evolving inflammation and accordingly, inflammatory cytokine release and ROS production which leads to the expression of cell adhesion molecules and selectins on the endothelial cell surface [29, 43, 44]. In our model, an increase in the expression of kidney, heart and liver associated ICAM-1, VCAM-1 and E-selectin mRNA and protein, and increased ROS in the BD animals indicate a progression towards microvascular endothelial dysfunction during brain-death [7]. A positive correlation between ICAM-1, VCAM-1 and E-selectin, and NET formation suggest a potential relationship between increased NET formation and endothelial cell activation, especially in the kidneys, although a causal relationship needs to be explored in future experiments. An increase in the expression of cell adhesion molecules possibly contributed to increased neutrophil recruitment to organs in our model [45]. Infiltrated neutrophils, already primed for activation through inflammatory cytokines, complement, oxidative stress and coagulation parameters during brain-death might then be committed to NET formation through stimulation with DAMPs [16], inflammatory cytokines produced by other immune cells [17], existing NETs or activated endothelium [42]. NETs themselves also cause endothelial activation and damage [22, 31], therefore, during brain-death the NETs observed potentially also contributed to endothelial injury. The widespread detection of endothelial activation however likely suggests that brain death first inflicts endothelial activation/injury, before NET induced injury. It has previously been shown that endothelium from different organs are highly heterogeneous in terms of gene expression, protein and functional behavior [46]. This might shed light on the differences observed in endothelial activation between different organs and the relation with NETs during brain-death. Endothelial activation might occur earlier in the

kidneys and hearts during brain-death, compared to the livers, particularly E-selectin expression which is associated with acute inflammation [47], or the kidney and heart endothelium might be more vulnerable to NET-related injury.

Given the established role of sex differences in the inflammatory response during BD [30, 48, 49] and previous published reports on differences in NET formation in other contexts [50], we also evaluated the effect of sex dimorphism in our model. In line with previous findings, BD kidneys and livers had no differences in neutrophil infiltration and NET formation between sexes [48]. However, in contrast to reports of increased leukocytes in female BD hearts and lungs [48], the male BD hearts in our study had significantly elevated neutrophils, NETs and ICAM-1 expression compared to female BD hearts. In previous studies, it has been shown that females have a greater inflammatory response compared to males during brain death [49]. This has been ascribed to a rapid fall in estradiol levels during BD, which is protective against heightened inflammation in healthy females [30]. A discrepancy between our results and previous findings could potentially be attributed to a difference in the BD model used. It has been demonstrated that fast induction of BD, used in previous reports [48], leads to greater hemodynamic instability and inflammation compared to slow induction of BD, used in our study, potentially implicating greater hormonal fluctuations, i.e., a more rapid fall in estradiol levels [51]. Interestingly, in other disease contexts such as multiple sclerosis, male patients had increased circulating NETs compared to females [50]. In contrast, *in-vitro* studies demonstrated increased lipopolysaccharide mediated NET release by neutrophils from females compared to males. 17β-estradiol had an inhibitory effect on NET formation in male derived neutrophils, but not in females [52]. These findings highlight the complexity of sex dimorphism on immune responses, suggesting that various other factors are also at play such as enzyme activity and sex differences in neutrophil biology. Whether sex dimorphism has a role in NET formation during BD, needs to be evaluated with follow up experiments. These findings however, do corroborate that sex might be an important consideration in the evaluation of donor hearts.

The lack of platelet deposition in organs, except for the livers, which had increased platelet deposition in the BD group, might suggest that the platelet-neutrophil interaction is not central to NET formation in our BD model or that platelet-neutrophil interactions occurred earlier during brain-death/microthrombi were dissolved through heparin administration [30].

A decrease in free thiol levels in the BD animals indicates increased oxidative stress in BD animals compared to sham-operated animals. Free thiols are a vital category of antioxidants found in plasma and protect cells and organs from oxidative stress by neutralizing ROS, therefore, a reduction in free thiol levels indicates an increased oxidative burden [53, 54]. Decreased free thiols in the BD group in this study are in line with previous work which identified a decrease in free thiol levels as a biomarker of oxidative stress following traumatic brain injury [54]. Decreased free thiol levels have also been associated with worse graft function in recipients following transplantation with deceased donor grafts [53]. Despite this, oxidative stress in the form of free thiol levels has not yet been characterized in the brain-dead donor. Our study is therefore the first to describe a drop in free thiol levels/oxidative stress during brain death. It has been established that both NET formation and endothelial dysfunction are associated with the production of ROS [33]. In the current study, although some animals with increased NETs also had decreased free thiols and therefore a higher oxidative burden (negative correlation) the association between free thiols and endothelial activation/NETs in the organs was not significant. This might suggest that other pro-inflammatory processes during the onset of brain-death (also) contributed to the formation of ROS [29].

A limitation of the study is that the relationship between endothelial activation and NET formation was indicated only through correlation and not through experiments in which mechanism could be established, i.e., *in-vivo* neutrophil depletion or *in-vitro* co-culture. To establish a causal relationship and mechanism, follow-up studies need to be performed, possibly including more *in-vitro* and *in-vivo* experiments to study the interaction between the endothelium, neutrophils and subsequent NET formation at different time points. Application of treatment in the BD animals against NET formation/neutrophil depletion can also shed light on the dynamics of endothelial activation and ROS formation and whether NETs are involved. The organs were also not transplanted. Future experiments should include a transplant group to evaluate the effect of NETs on functional parameters following transplantation.

In this proof-of-concept study, we have demonstrated for the first time that brain-death in a rat model induces NET formation and is associated with increased endothelial activation. NETs have already been shown to play a role in various stages of transplantation, but only hypothesized to be relevant already in the donor [12]. This study provides a basis for future research on clinical samples to establish whether the NETs might contribute to inferior graft quality observed to be associated with BD organs such as I/R injury, delayed graft function and early graft loss.

Currently, several therapeutics against NET formation already exist. In other disease contexts, PAD4 [55] or MPO inhibitors [56], various immunomodulatory drugs [57], and DNase-1 [58] have been proven successful to inhibit NET formation or resolve existing NETs. Testing these therapeutics either in the donor or during organ preservation should be considered as potential intervention strategies to attenuate organ injury.

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 was approved by the Institutional Animal Care and Use Committee of the University of Groningen (IACUC-RUG) (IvD 171245-01-002). All experiments were performed in accordance with the Experiments on Animals Act and ARRIVE guidelines.

AUTHOR CONTRIBUTIONS

MvZ performed analyses on the samples and drafted the manuscript. RA and PO performed the animal experiments and sample collection. MvR and HvG edited and reviewed the manuscript. TL, HL, and JLH assisted in experimental design and conceptualisation, edited and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

HL is part-time CSO of 34Lives PBC, United States.

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.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.14223/full#supplementary-material

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Thermal Rejection Assessment: New Strategies for Early Detection

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Skin pigmentation can pose challenges for physicians to diagnose pathologies. In Vascularized Composite Allotransplantation (VCA), this increases the difficulty of diagnosing rejection by clinical observation, which could be improved by noninvasive monitoring, thereby completely avoiding or aiding in guiding location for invasive diagnostics. In this study, pigmented and non-pigmented allogeneic and non-pigmented syngeneic control transplant recipients underwent daily thermal assessment using infrared (IR) gun and forward-looking IR (FLIR) imaging of VCAs using a rodent partial hindlimb transplant model. Daily clinical assessment was performed, and biopsies were taken on postoperative day (POD) 1, 3, and 7. Clinical and histological assessments indicated signs of rejection on POD 3. In contrast, thermal assessment using the IR gun detected significant differences as early as POD 1, notably a decrease in temperature, when comp ared to syngeneic control transplants. This demonstrates the capability of thermal assessments to identify early signs of rejection before clinical symptoms become apparent. The findings suggest that thermal assessments can serve as a non-contact, objective adjunct tool for early detection of graft rejection, with consideration of skin pigmentation. This approach may reduce the need for invasive biopsies, thereby improving patient comfort and reducing potential complications associated with current diagnostic methods.

Keywords: rejection, transplantation, infrared, FLIR, vascularized composite allografts

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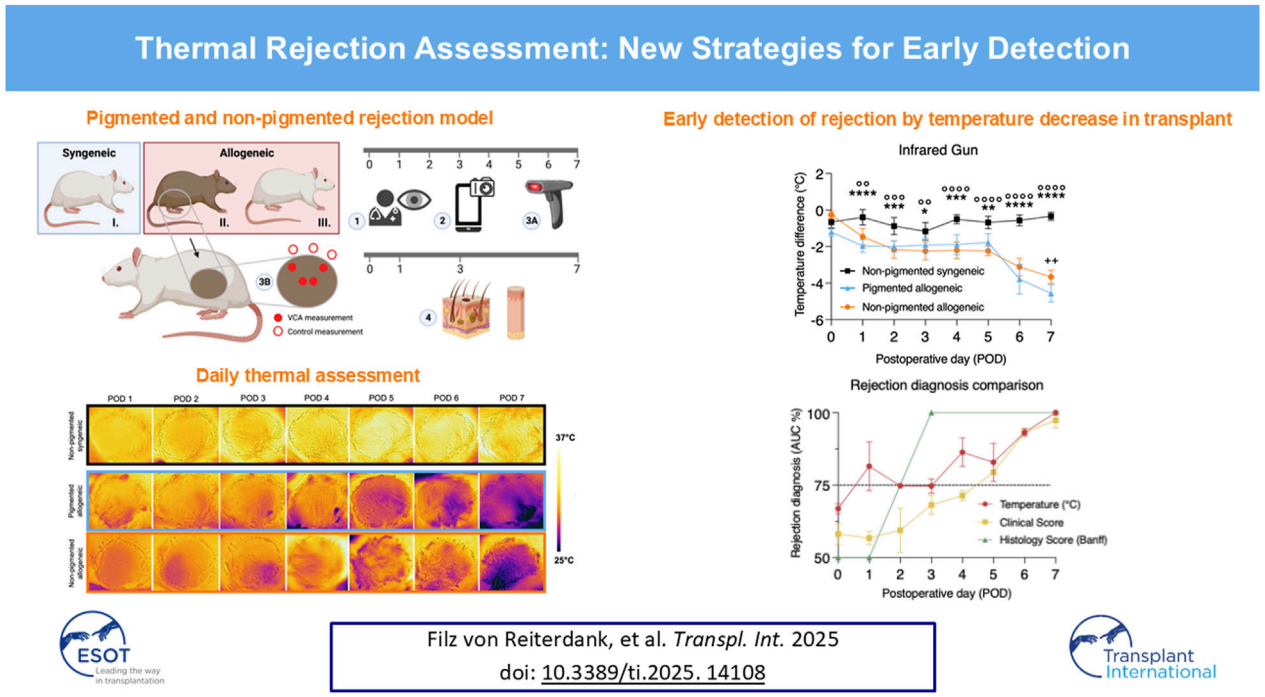
INTRODUCTION

Early diagnosis of acute rejection is essential for the immunological management of transplant patients, affecting comorbidity, chronic rejection, and risk of complete graft failure [1]. Transplants involving skin are especially high-risk due to the immunogenic nature of skin tissue, and acute rejection episodes occur in 89% of patients [2]. Traditionally, diagnosis relies on serial biopsies and clinical observation [3]. Biopsies are risky and painful, while visual assessment of the skin can be imprecise and subjective, especially in pigmented skin where early signs of rejection, such as

Abbreviations: AUC, Area Under the Curve; FLIR, Forward-Looking Infra-Red; H&E, Hematoxylin and Eosin; IR, Infra-Red; POD, Post Operative Day; ROC, Receiver Operating Characteristic; VCA, Vascularized Composite Allografts.



GRAPHICAL ABSTRACT

erythema, are less apparent [4–6]. Early detection of changes in graft health can lead to prompt treatment, reducing the severity of rejection episodes and potentially avoiding complete graft failure [1]. By developing additional non-invasive and objective methods, VCA's surface-level accessibility can be leveraged for more effective early detection and monitoring.

This study introduces an innovative engineering solution that uses thermal imaging to non-invasively and diagnose acute rejection in a rat model of VCA transplantation in a few seconds using affordable commercial devices. Infrared (IR) gun for point measurements, and forward-looking IR (FLIR) imaging technologies are used to offer a reliable adjunct tool to use across two distinct skin pigmentation levels. Both technologies record the graft surface temperature by analyzing the emitted IR from the graft in the 8 to 14 microns wavelength range. Predicated on the thermodynamic principles of heat transfer from the blood circulation to the graft, these measurements may serve as an indirect measure of skin perfusion and, consequently, graft viability with correlation to early stages of graft rejection. Non-invasive imaging has been suggested in the past to determine rejection and avoid serial biopsies, often involving blood flow assessment, visual markers after intravenous injection, or stiffness measurements using ultrasound and MRI techniques [7]. In comparison, the IR approach is fast, portable, quantitative, and particularly valuable in resource-limited settings due to its straightforward application and cost-effectiveness, thereby addressing a critical gap in skin diagnostics and reconstructive transplant surgery. However, the majority of existing studies have not studied skin pigmentation as a variable, thus potentially limiting the applicability of the technology and excluding the needs of all affected patients.

Skin-containing transplantations, which play a crucial role in reconstructive surgery, exemplify the challenges at the intersection of skin pathology and transplant medicine. Vascularized Composite Allotransplantations (VCAs), auto-transplantations, free flap transfers, and sentinel skin flaps, while innovative, are often hindered by the difficulty in early detection of complications when using subjective clinical observations, especially in pigmented skin [8]. Far from being a challenge unique to VCAs [3], such disparities are representative of a broader issue in the field of transplantation and medical diagnostics in general. Amongst others, race and ethnicity greatly determine the chance of referral for transplant evaluation, being added to the waiting list, and receiving a transplant [9]. Recent attempts to address challenges with pigmented patients have sometimes included adding more invasive procedures, placing a greater burden on the patient. For example, the first Black patient to receive a face transplant underwent additional mucosal biopsies, which were not typically required for other patients [8]. Considering these observations, inadequate diagnostic tools and sluggish technological development contribute to discriminatory practices [10] and non-invasive alternatives may be found to prevent unnecessary procedures in all patients.

By focusing on thermal parameters, this study aims to develop a method that is effective in transplant surgery. In doing so, this study investigates temperature assessment as an effective, non-invasive early detection tool for graft rejection using a rodent VCA transplantation model, suitable across different skin types.

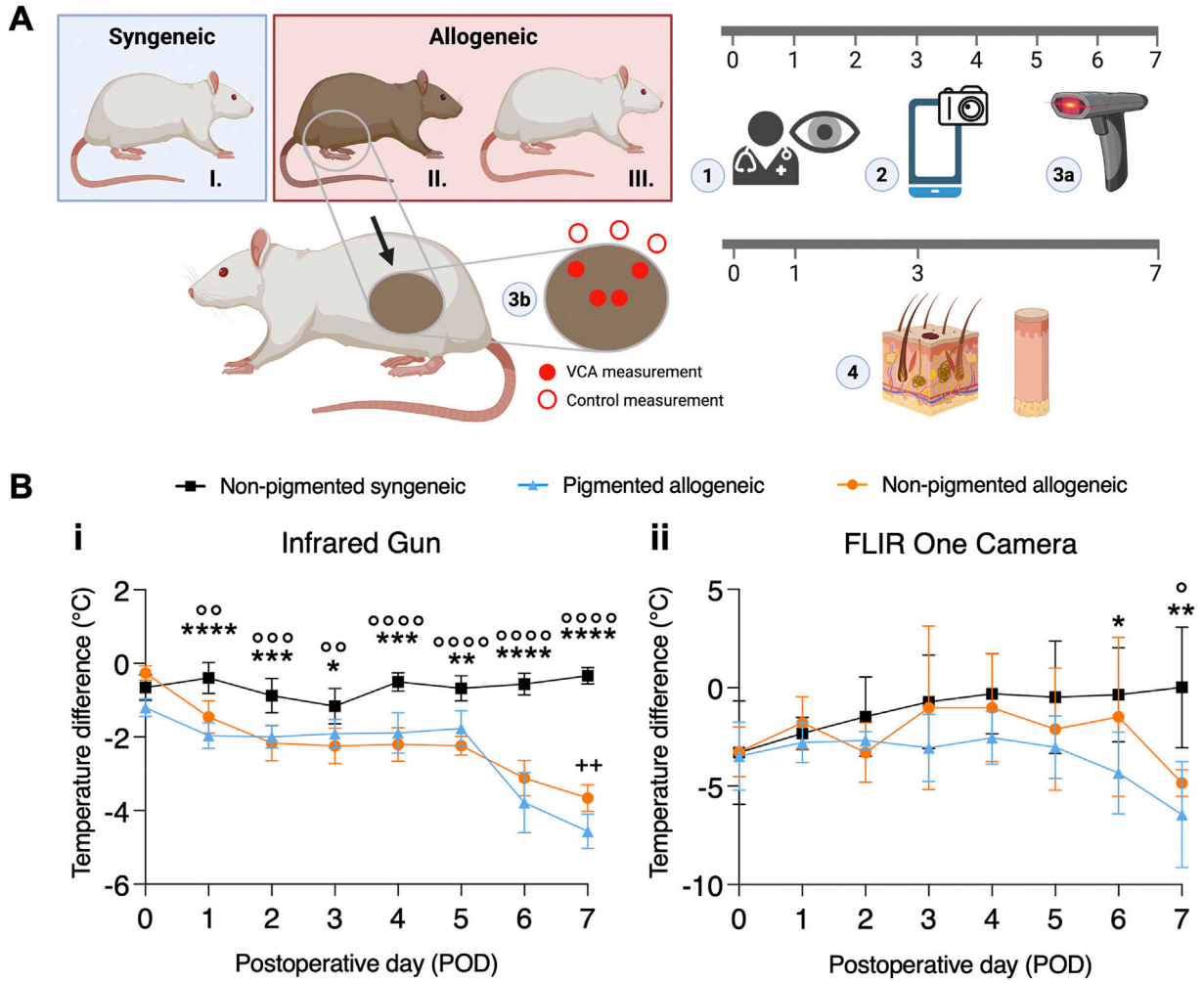


FIGURE 1 | Experimental design and temperature difference between transplant and native skin over time. **(A)** Partial hindlimb transplant model for the following three groups: I. non-pigmented syngeneic, II. pigmented allogeneic, and III. non-pigmented allogeneic transplants. Blue and red boxes at the top represent the donors in each group, while the non-pigmented animal at the bottom represents the recipients used for all groups. On the timeline, the modality and frequency of assessments are indicated. Daily (1) clinical assessment was performed by experienced surgeons, (2) Smartphone-based FLIR One images, and (3a) gun-style infra-red (IR) thermometer measurements were taken. (3b) IR gun measurements were performed daily, while (4) histology was obtained on postoperative day (POD) 0, 1, 3, and 7 biopsies were taken and assessed by a blinded pathologist. IR gun measurements were obtained from the center and periphery of the graft for adequate sampling, in addition to control measurements outside of the graft. **(B)** Temporal variation in temperature difference between VCA and native skin are displayed as mean and error ((95% CI). (i) Shows variation in temperature difference measured using IR gun, with a statistically significant difference between pigmented allogeneic (n = 8) and non-pigmented syngeneic control (n = 12, denoted using *), as well as between the non-pigmented allogeneic (n = 7) and syngeneic control groups (n = 12, denoted using °). Apart from POD7, no significant difference is found between the pigmented and non-pigemented allogeneic groups (denoted using $^{+}$) (ii) Temperature assessment using FLIR shows a similar trend despite the lack of sensitivity. */° $p \leq 0.0332$; **/°°/$^{++}$ $p \leq 0.0021$; ***/°°° $p \leq 0.0002$; ****/°°°° $p \leq 0.0001$.

The aim is to facilitate early, accessible, and straightforward intervention irrespective of skin pigmentation, leading to improved clinical outcomes and more equitable healthcare.

MATERIALS AND METHODS

Animals

60 rats (male, 250 ± 50 g) were used for all experiments, of which 42 were inbred Lewis rats, 11 Brown Norway rats, and 7 Buffalo rats (Charles River Laboratories, Wilmington, MA). The animals received humane care in accordance with the National Research Council guidelines and the experimental protocols were approved by the IACUC of Massachusetts General Hospital (Boston, MA).

Study Design

Partial hindlimb transplants were performed in three different surgical groups (**Figure 1A**) [1]: pigmented allogeneic (rejection) group (n = 11) in which Brown Norway rats were donors [2]; non-pigmented allogeneic (rejection) group (n = 7) in which

Buffalo rats were donors [3]; non-pigmented syngeneic control (no rejection) group (n = 12) in which Lewis rats were donors. In all transplants Lewis rats were recipients. Buffalo and Lewis rats are considered albino animals therefore would be considered Fitzpatrick skin type I. Brown Norway rats have a non-Agouti brown coat meaning they are solid-colored. To our knowledge, no equivalent scale to the Fitzpatrick skin types exists for rats, however, we would consider Brown Norway rats to be closest to a Fitzpatrick skin type IV-V. Use of pigmented animal models with similar immunological compatibility allows for cross-pigmentation measurements on the same timeline providing positive and negative control groups.

VCA Transplantation

After induction using isoflurane (5%) inhalation with 100% O_2, general anesthesia was sustained with inhaled isoflurane (1%–3%) and anesthesia depth was confirmed with a toe pinch test. Partial hindlimbs were procured as described earlier [11]. Briefly, grafts include the knee joint with 10 mm distal femur and 10 mm proximal femur and tibia, along with thigh muscle groups with the inguinal fat pad and calf muscles as well as the surrounding skin paddle. Femoral vessels were skeletonized and ligated 5 min after IV administration of 100 IU/mL/kg heparin in the penile dorsal vein. The femoral artery was cannulated with a 24G angio catheter and secured with a 6/0 nylon suture. The femoral vein was cut after ligation. Immediately after procurement, a pressure-controlled manual flush with 3 mL (200IU) of heparin saline at room temperature was performed. Next, the VCA was transplanted into a Lewis rat. Recipient vessels were prepared on the contralateral side in a similar fashion to the donor. Vessels were ligated distally and prepared for anastomosis. A longitudinal incision in the flank was made with subsequent tunneling to the groin area for VCA insertion. Femoral arteries and veins were anastomosed using a self-developed adjusted cuffing technique to allow for application to partial hindlimb transplant. Skin on the donor VCA was excised to create an oval flap in the flank which was secured with interrupted 5-0 sutures. Inguinal fat pad and groin skin incision were similarly closed with interrupted 5-0 vicryl sutures.

Postoperative Assessments

Postoperatively, daily flap images were taken for blinded clinical assessment by six blinded clinicians using a clinical VCA rejection score. Briefly, grade 0 constitutes no difference between graft and native skin. Grade 1 shows mild erythema, grade 2 moderate erythema with beginning of scaling and scabbing, grade 3 severe erythema and scabbing with areas of epidermolysis, and grade 4 constituting full-thickness graft epidermolysis with areas of necrosis. Temperature measurements were taken daily as displayed in **Figure 1A** using a temperature IR gun (Digisense, Cat. N° 20250-07) and FLIR thermal images (FLIR ONE® Pro – iOS). Both devices were held at approximately 20 cm distance to the region of interest. Gun measurements were taken of the center and periphery of the flap, control measurements were taken of the skin immediately dorsal to the flap. FLIR images were taken of the entire flank area. For analysis, the mean temperature of the flap area and the mean of an area immediately dorsal of the flap was taken in a blinded fashion. Diurnal variations in body temperature were accounted for by control measurements of surrounding native skin in the same animal, ensuring the reliability of the results by reducing environmental influences on the temperature.

Histology

On postoperative day (POD) 1, 3, and 7 skin and muscle biopsies were taken (**Figure 1**). On POD 7 additional muscle biopsies were taken. Biopsies were fixed in formalin and processed for histopathological examination. Slides were stained with hematoxylin and eosin (H&E). A blinded evaluation by a pathologist was performed for all biopsy samples and using the Banff criteria score to assess acute cell-mediated rejection [12, 13]. Briefly, grade 0 is considered no rejection, grade I mild (mild perivascular infiltration, no involvement of epidermis), grade II moderate (moderate perivascular infiltration, possible mild epidermal involvement), grade III severe (dense inflammation and epidermal involvement) and grade IV necrotizing acute rejection (frank necrosis of the epidermis and other skin structures). For the skin samples, a mean Banff score was calculated for comparison. Muscle tissues were evaluated and scored using the histology injury scoring system (HISS) for hypoxia-induced muscle injury [14].

Statistical Analysis

Temperature data is analyzed using a linear mixed effects model with the type of transplant (3 levels; pigmented allogeneic, non-pigmented allogeneic, non-pigmented syngeneic) and POD (8 levels; POD 0-7) as fixed effects while also accounting for their interaction. Locations on the flap (3-4 per subject) and subjects (7–12 per condition) were treated as random variables for the temperature gun data. For FLIR data, average temperature for the whole flap is used for analysis, thereby only subject is treated as the random variable. Multiple comparisons were performed using Tukey's corrected multiple comparisons test with 8 families (one for each time point). The appropriateness of the model was confirmed with a residual plot that showed no correlation of the residuals with the predicted values, and the normality assumption was confirmed with a QQ plot that showed high coincidence between the predicted and actual residual values (**Supplementary Figure S1**).

Discriminative performance of thermal assessment for detecting graft rejection in the early PODs (POD 1 and 2) was evaluated using two separate methods. Firstly, a linear mixed effects model with type of transplant (3 levels as described above) and only early PODs (2 levels; POD 1, and POD 2) as the fixed effects are used while accounting for their interaction. For the discriminatory analysis, post-hoc analysis using multiple comparisons with Tukey's correction is performed under the assumption of one family for the entire transplant type. Secondly, a binary classification system is applied, and corresponding receiver operating characteristic (ROC) curves are generated, that independently compare two pairings: pigmented rejection with the non-rejection group, and the non-pigmented rejection group with the non-rejection group. The binary classifiers also utilize temperature values from POD 1 and 2 for each pairing

type. Furthermore, the effectiveness of each pairing is compared for each individual POD.

Clinical rejection score differences between groups were analyzed using a mixed-effects model with multiple comparisons. The time-series plots are represented as mean with 95 Confidence Interval (CI), bar charts are represented as mean with Standard Deviations (SD). All statistical analyses were performed using Prism 9 for Mac OSX (GraphPad Software, La Jolla, CA). p-Values less than 0.0332 were considered to be significant.

RESULTS

Transplants in all three groups were successful until end of study as defined by visual assessment using the vascular patency test.

Postoperative Thermal Trend Analysis Indicates Rejection Can Be Detected as Early as Day 1

Representations of daily clinical images are shown in **Supplementary Figure S2A** and corresponding FLIR images are shown in **Supplementary Figure S2B**, which readily reveal visual indicators of graft rejection in a pigment-agnostic manner as early as POD1. Temperature difference between VCA and surrounding native skin using the IR gun (**Figures 1B-i**) was assessed using a mixed effects model as described in the methods section and found significant effect of both the fixed effects and their interaction ($p < 0.0001$). The standard deviation for the random effects (subject x location) is 0.51. The model was also found to have highly effective matching, indicating that the mixed effects model was the appropriate choice for analysis ($p < 0.0001$). Furthermore, post-hoc analysis to compare means for each POD shows a significant difference between the pigmented ($p < 0.0001$) and non-pigmented ($p = 0.0068$) rejection groups compared to the non-rejection group from POD 1 onwards. While the level of significance fluctuates and shows a decrease on POD3 in both groups, it remains significant until the end of study. FLIR temperature assessment (**Figures 1B-ii**) shows a similar trend in mixed effects analysis (fixed effects and interaction significant with $p < 0.05$, matching effective at $p < 0.0001$, SD of random effect: 1.17) as well as post-hoc multiple comparisons, even though statistically significant differences are not observed until POD 6.

Infrared Gun Shows Superior Sensitivity and Specificity Compared to FLIR

Figure 2A shows that thermal assessment indicated significant differences between rejection and non-rejection groups as early as POD 1 and 2, however only in the case of IR gun the average temperature difference reached statistical significance. Fitting of the mixed effects model on the data from the IR gun showed a statistically significant effect of the fixed effects, i.e., POD and type of transplant ($p < 0.005$), however, no effect of interaction between POD and transplant type was found ($p > 0.05$), allowing for grouping POD 1 and 2 data for post-hoc comparison. Tukey's corrected multiple comparison for temperatures showed statistically significant difference between each of the rejection groups with the non-rejection group ($p < 0.0001$). Neither the mixed effects model, nor the post-hoc comparison for the data from the FLIR measurements reached statistical significance ($p > 0.05$). Correspondingly, AUC analysis reflects a higher sensitivity and specificity of IR gun measurements than FLIR measurements with an AUC of 83.54% in the pigmented group and 74.32% in the non-pigmented group using combined IR gun temperature data from POD 1-2 (**Figures 2B**, **C**). AUC analysis of all other PODs is shown in **Supplementary Figure S3, S4**. Similar to the daily thermal trend analysis, daily AUC curves show some fluctuation. To minimize data dependence on daily fluctuations in **Figure 2A** temporal component was integrated by using the average of POD 1 and 2.

Clinical Assessment Does Not Diagnose Rejection Before Day 3

Representations of daily clinical images are shown in **Figure 3A**, and corresponding histological images in **Figure 3B**. In both rejection groups at POD 1, the mean clinical assessment score was 0.25 (±0.21), indicating minimal observable changes at this early stage. In the non-pigmented group the mean score was 0.35 (±0.03), while the pigmented group was only scored at a mean of 0.15 (±0.33). At POD 3, the mean score increased to 1.65 (±0.14), suggesting grafts show mild to moderate erythema with some showing the beginning of scaling and scabbing. Similarly, the non-pigmented group was scored lower at 1.22 (±0.28). By POD 7, the mean score of both rejection groups further increased to 3.47 (±0.04), reflecting pronounced clinical signs of severe erythema with areas of epidermolysis and necrosis or crust, consistent with graft rejection. This far into the rejection process, mean scores between the rejection groups were more similar with 3.57 (±0.27) in the non-pigmented group and 3.2 in the pigmented group. The mean day on which rejection was clinically diagnosed was at 2.71 ± 0.44) and 2.96 (±0.35) in the non-pigmented and pigmented grafts respectively, highlighting slightly earlier diagnosis in the non-pigmented group compared to the pigmented group. In the non-rejection group, grafts showed normal postoperative recovery signs which could be confused with early stages of rejection, however, none of the grafts showed high clinical rejection scores, as expected.

Histological Assessment Does Not Detect Rejection Before Day 3

Histology at POD 1, 3, and 7 is shown in **Figure 3C-ii** and its analysis in **Figure 3C-iii**. In both experimental groups, on POD 1, no pathological findings related to rejection were detected in skin tissue. Muscle tissue showed mild to moderate ischemic changes as displayed in **Supplementary Figure S5**. At POD 3, skin samples showed focal epidermal necrosis resulting in a Banff score of III in both experimental groups. Muscle tissue showed moderate edema and inflammation. By POD 7, a Banff score of

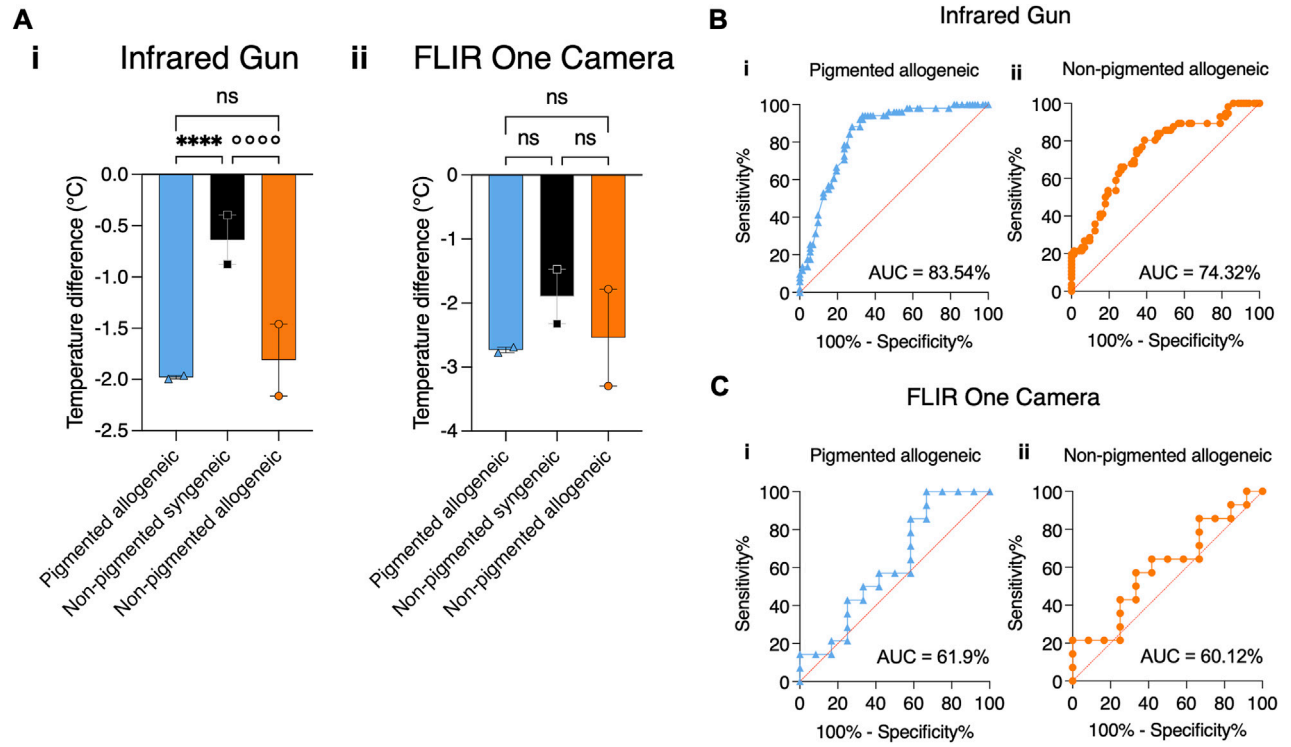


FIGURE 2 | Comparing the effectiveness of Infrared gun versus FLIR One Camera to detect temperature differences in transplanted graft using statistical tests. **(A)** Average temperature differences between VCA and native skin are shown for all groups by combining measurements from POD1 and 2. (i) Temperature difference measured by IR gun shows statistical significance between allogeneic and syngeneic groups regardless of pigmentation. (ii) Temperature measured by FLIR One Camera does not show statistical significance between allogeneic and syngeneic groups for all pigmentation levels, despite showing a similar trend as IR gun. **(B)** For the same time points, a binary classifier analysis shows a high Area Under the Curve (AUC) for a receiver operating characteristic (ROC) curve using the IR gun in detecting rejection in both the (i) pigmented (83.54%) and (ii) non-pigmented (74.32%) groups. **(C)** A similar binary classifier analysis using the FLIR One Camera shows a lower AUC of (i) 61.9% in the pigmented and of (ii) 60.12% in the non-pigmented rejection group. ****/^{oooo} $p \leq 0.0001$.

IV was found in both experimental groups based on severe ischemic changes with early necrosis of muscle tissue and full-thickness skin necrosis, indicative of advanced histological rejection, as shown in more detail in **Supplementary Figure S6**. In the non-rejection group, no pathological signs were found in skin nor muscle tissue. No significant differences were found between the non-pigmented and pigmented rejection groups.

Comparison Between Thermal, Clinical, and Histological Assessment

As shown in **Figures 3C-iii**, for the experimental groups combined it was observed that the daily AUC for temperature assessment (72.22–91.22) was consistently higher than the AUC for clinical scoring (54.46–73.08) from POD 1 until POD 4. This aligns well with our hypothesis that temperature-based assessment can provide an early measurement of the comorbidities associated with rejection. Further, the AUC of the rejection groups for temperature assessment and clinical scores are high and align well for POD 5 to POD 7 (76.29–100 and 78.57 to 100, respectively), with both techniques predicting rejection with very high confidence and accuracy.

DISCUSSION

This study presents a comprehensive examination of the utility and sensitivity of thermal assessment techniques (IR gun and FLIR imaging) in the early detection of acute rejection in a rodent VCA model in a pigmentation-agnostic manner. The presented findings may indicate a potential role of thermal assessment is more effective in early detection than clinical assessments, which often fail to detect rejection in pigmented skin until POD 3. In contrast, thermal assessment shows significant differences between rejection and non-rejection groups as early as POD 1, irrespective of skin pigmentation.

Technical Requirements of IR Technology

The use of IR technology for temperature measurement, while straightforward, has surprisingly not played a larger role in clinical practice, nor have temperature profiles of transplant organs been extensively studied. One reason for this may be that it is only in recent years that this technology has achieved affordability, accuracy, and compactness for medical use. Both IR gun and FLIR camera offer significant advantages in the <$500 price range, where the gun provides higher

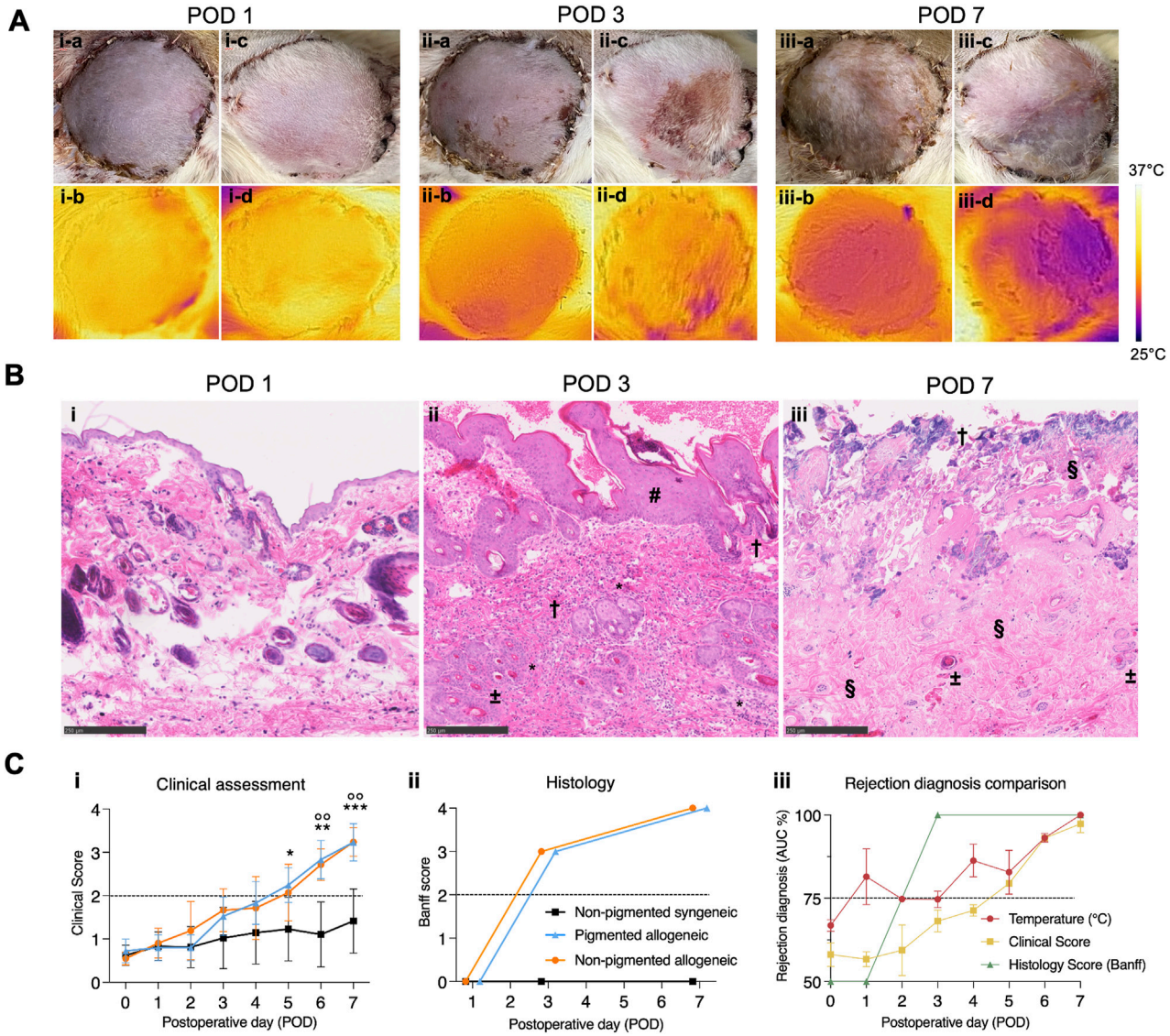


FIGURE 3 | Comparison of time to diagnosis between visual, thermal, and histological assessment methods for pigmented and non-pigmented allogeneic groups. **(A)** (i) Representative images in visible spectrum for clinical evaluation and IR spectrum show indistinguishable differences with both methods on POD1. (ii) A drop in temperature of the VCA compared to native skin is seen on POD3 in both groups. Simultaneous clinical evaluation shows subtle, erythema and epidermolysis which is clearly distinguishable in the non-pigmented group. (iii) By POD7, rejection is pronounced in both pigmented and non-pigmented allogeneic groups, as observed clinically by features of epidermolysis, necrosis, and lymphatic fluid oozing. Temperature difference of the VCA is also more pronounced in both pigmented and non-pigemented allogeneic groups. **(B)** Microscopic analysis of histology with H&E staining and bright field microscopy (scale bar 250 μm) shows (i) no abnormal features on POD1. (ii) At POD3, focal epidermal necrosis is observed in both rejection groups with epidermal thickening (#), infiltration (*), microthrombi (±) and apoptotic bodies (†). (iii) By POD7, full-thickness skin necrosis (†) with severe loss of architecture (§) and thrombi (±) is seen in the rejection groups. **(C)** Analysis of clinical assessment scores and histology grading shows (i) rejection is identifiable at a slightly earlier time in the non-pigmented group (ii) Conversely, blinded microscopic Banff evaluation shows no significant differences between groups and shows more severe rejection than clinical assessment suggests. Above the dotted black line indicates moderate to severe rejection. (iii) Association between temperature assessment, clinical rejection score, and histological Banff score (daily ROC curve based AUC of individual data points for each type of assessment versus POD curve) shows that temperature assessment has an earlier association with rejection than both other scores (dotted line at 75%). * $p \leq 0.0332$; **/°° $p \leq 0.0021$; *** $p \leq 0.0002$.

accuracy for point measurements, whereas the FLIR camera allows spatial coverage of the graft at some loss in accuracy. Additionally, FLIR cameras also require significant post-processing to obtain an average temperature of the whole graft. In this study, comparative analysis between similarly priced IR gun ($350) and FLIR imaging camera ($400) revealed that both IR gun and FLIR imaging follow a similar trend of changes in temperature for POD 0–7. However, the FLIR image-based analysis does not reach significance in early graft rejection analysis. It is likely because the resolution of the FLIR

One camera (±3°C) that was used was insufficient to capture the small-scale differences between allogeneic and syngeneic grafts. For instance, the multiple comparisons test showed a mean temperature difference of at least 0.27°C between the allogeneic groups compared to the syngeneic group on all the PODs. The necessary precision of temperature measurement is likely pathology-dependent [15, 16]. The FLIR One smartphone thermography has been used successfully in clinic [17], however, some applications, such as presented in this study and others [18], will require higher precision.

Research to Practice: Sensitivity and Specificity

When we started the study, our original hypothesis was, that a temperature increase would be found in the VCAs in the early stages of rejection, followed by a temperature decrease in the later stages of rejection. This hypothesis was based on the knowledge that endothelial activation during acute rejection can lead to vasodilation (e.g., bradykinin, prostacyclin, nitric oxide), while the activated complement system and pre-formed DSAs can trigger intravascular coagulation [19, 20]. However, our study demonstrated that rejection leads to a significant temperature decrease in VCAs as early as POD 1. The decrease in VCA temperature during rejection found may be a result of impaired microcapillary perfusion and, therefore, disrupted heat distribution. This observation is similar to the only other study that examined temperature changes during rejection in a kidney transplantation model using an implantable bioelectric device [21]. Here, continuous temperature monitoring showed a temperature increase, followed by a sharp decrease in temperature, which worsened until graft loss. It is possible that due to the full mismatch model used in our study, and the use of daily measurements rather than continuous measurements, an early rise in temperature within the first 24 h was not recorded. Mechanistic studies are required to differentiate between confounding pathologies for a drop in graft temperature, similar temperature profiles for rejection across disparate organ systems (kidney and VCA) point to the potential utility of thermal assessment of organ transplantation in general. A large animal model may be more appropriate for such work, which would also allow sequential tissue biopsies for time series analyses; this is not feasible in a small animal model since the graft size does not lend itself for multiple biopsies. For VCAs this is especially relevant in the acute phase during which high rejection rates remain a challenge [2, 22].

In our controlled laboratory setting, thermal assessment demonstrated high sensitivity and specificity compared to current subjective diagnostic methods [23]. However, additional confounding factors such as patient-to-patient variability, environmental conditions, and surgical complications may need to be accounted for in a clinical trial with patients.

Emerging Applications

Thermal assessment of skin has been suggested as a diagnostic and monitoring tool for various conditions characterized by altered skin perfusion, such as assessing burn wounds [24, 25], evaluating vessel patency in peripheral arterial disease [26], monitoring surgical flap viability [27–30], and detecting perfusion anomalies associated with tumor growth [31]. Cherchi et al. [32] even proposed a potential intra-operative role for thermography for the detection of signs of early graft dysfunction. Furthermore, the use of sentinel skin transplants has been suggested as a rejection detection tool in solid organ transplantation [33, 34], with recent reports of first clinical case results [35]. The non-contact nature of the technique is highly suitable for immediate clinical translation, as a supportive approach to enhance prediction of rejection. For future studies, we recommend to assessing long-term follow-up and evaluating the effects of immunotherapy and its withdrawal in larger animal models or by immediately incorporating this diagnostic into a clinical trial. A potential clinical plan would involve several key steps: first, measure temperature profiles in autologous skin, VCA, and free flap transfer transplants to establish standard temperature benchmarks for all patients, ideally involving a cohort of different pigmentation levels. This would effectively be a control group for non-rejection graft monitoring. The next phase would involve testing temperature profiles in allogeneic VCA patients and sentinel flap clinical trials (currently ongoing) [36] to further validate its effectiveness. Moreover, for application to research, thermal assessment has been mentioned as a technique to increase standardization and reproducibility in burn wound models and the effect of treatment in these models [37].

Limitations

Several limitations, such as moderate sample size, possible differences in skin architecture, and immunological behavior between rat and human VCA tissues remain [38]. Acute rejection has a heterogeneous distribution, as FLIR images reveal temperature variations and injury in specific areas of the flap. Despite the limitations of a small-size rodent model, the proportionate graft area is significant relative to the total body size. Refining FLIR techniques could better guide biopsies than IR gun measurements. In the FLIR images of rejection at POD 3–5, we are able to see hotspots of temperature variations within the flap. Variations across the flap could become more pronounced in larger animals or bigger flaps, which accentuates the complex and potentially localized nature of rejection, offering opportunities for more precise and targeted interventions. Depending on the application and chosen various approaches for thermal assessments can be usedm such as monitoring absolute temperature [21], identifying hot spots [39], or comparing the temperature of region of interest with surrounding native skin [25, 40].

Conclusion

This study demonstrates that a temperature decrease is found in rejecting grafts in rodents, which can be detected early, non-invasively, and objectively, independent of the presence of skin pigmentation. The results suggest that there may be a role for thermal assessment in improving patient outcomes and postoperative care as well attempting to contribute to a

reduction of health disparities. For clinical trials involving thermal imaging, future studies should address the lack of skin color variation in rodent animal models by considering the wide range of pigment differences across various racial and ethnic groups to ensure representative and inclusive recruitment. Additionally, translation to other skin pathologies can provide a more general diagnostic tool for pigmented skin. This way, physicians can be guided in the clinical decision-making process and minimize invasive, costly, and time-consuming diagnostic tools for patients.

An early detection capability is critical in the context of transplant surgery, where early intervention can significantly impact patient outcomes. Assessment techniques independent of skin pigmentation, such as shown in this study, offer a more inclusive approach to clinical care. To our knowledge, this study represents the first thermal analysis of allogeneic VCAs including analysis of pigment-dependence. It is shown that significant differences in graft temperature are found as early as POD 1 and 2, while clinical and histological assessment is delayed until POD 3, especially in pigmented grafts. Furthermore, a minimum sensitivity is needed to detect significant changes. The detection is low-cost and does not require extensive training. The results show promise for thermal assessment as an objective, quantifiable, non-invasive, easy-to-use, and quick adjunct tool for early rejection detection in a pigment-agnostic manner.

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 animal study was approved by IACUC of Massachusetts General Hospital. The study was conducted in accordance with the local legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

IFR, RJ, ST, and KU conceptualized the experiments; IFR and EC-T performed the experiments; IFR and RJ performed the data analysis; IFR drafted the manuscript. RJ, AT, AM, ST, and KU substantially revised the manuscript to reach the final version. KU supervised the study. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

The authors of this manuscript have conflicts of interest to disclose as described by the American Journal of Transplantation. IFR, RJ, ST, and KU have patent applications relevant to this field. Competing interests for Massachusetts General Hospital investigators are managed by the MGH and MGB in accordance with their conflict-of-interest policies.

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.

GENERATIVE AI STATEMENT

The authors declare that no Generative AI was used in the creation of this manuscript.

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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.2025.14108/full#supplementary-material

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LETTER TO THE EDITOR
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The Feasibility of a Beating-Heart Transplant From Brain-Dead Donors

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Keywords: graft preservation, heart transplantation, organ care system, beating heart, donor after brain death

Dear Editors,

Prolonged donor graft ischemia during retrieval and transportation may be responsible for allograft dysfunction after heart transplantation (HTx) [1]. Therefore, limiting the ischemic period and the deleterious effects of ischemia-reperfusion on the donor graft may have a favorable impact on the outcome of HTx; this would also allow longer distance procurements and acceptance of marginal grafts. This has been demonstrated using special graft preservation modalities such as those obtained when employing the Organ Care System (OCS™; *TransMedics Inc., Andover, MA, USA*) [2, 3].

Using beating grafts from donors after brainstem death (DBD) further reduces the ischemic periods in HTx by avoiding a second cardioplegic arrest; this is of the utmost importance in HTx with donors after circulatory death (DCD). This has been achieved in two patients and is described in the following report, an experience which may be considered a prelude to an ischemia-free HTx.

Both recipients gave their informed consent and the Institutional Review Board approved the procedures. Most of the relevant data of the recipients and donors are summarized in **Table 1**.

The donor hearts were arrested with cold antegrade cardioplegia, and the longest possible segment of ascending aorta was retrieved during cardiectomy. Hearts were placed on OCS after 35 and 47 min of ischemia, respectively, and then the graft was perfused with warm oxygenated donor blood through an aortic line and vented through a pulmonary arterial line. Technical details of OCS implant in our center have been previously described [3]. During transportation, constant monitoring of heart rate, aortic pressure, coronary flow, and lactate profile revealed no anomalies.

Prior to donor heart arrival, the recipients were prepared and placed on a cardiopulmonary bypass (CPB), the left ventricles were vented through the right superior pulmonary vein, and cardiectomy was carried out. Once the donor grafts reached the operative room the setup was modified so that they could be perfused through the CPB circuit. An additional arterial perfusion line, long enough to reach the OCS device, was connected, under sterile conditions, to the CPB circuit (**Figure 1A**). After the aortic cannula connector was loosened, the donor heart was manually rotated 180°, exposing its anterior aspect. The donor aorta was cannulated near the aortic sinotubular junction with a cardioplegia needle and connected to an additional arterial line of the recipient CPB circuit (**Figure 1B**). A bolus of 1g of metilprednisolone was administered and the donor heart rate was maintained at 80 beats/minute through ventricular bipolar pacing wires. Perfusion from the OCS was stopped, the aorta was cross-clamped immediately distal to the CBP perfusion line, and antegrade flow was initiated from the CPB circuit with a separate roller pump through the cardioplegia needle (**Figure 2A**); a target

Abbreviations: DBD, Donation after Brainstem Death; DCD, Donor after circulatory death; HTx, Heart transplantation; OCS, Organ Care System; CPB, Cardiopulmonary bypass; AV ECMO, Artero-venous extracorporeal membrane oxygenation.

OPEN ACCESS

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TABLE 1 | Donor and recipient data.

	Recipient 1	Recipient 2
Age (years), sex	69, male	63, male
Indication for HTx	Post-op LV failure	Ischemic CM
Pre-HTx status	Impella, VA-ECMO	IABP
Donor age (years), sex	51, male	63, male
List priority	Emergent	Urgent
Time on OCS (minutes)	256	229
Total ischemic time (minutes)	35	47
Cardiopulmonary bypass (minutes)	177	161
Recipient aorta cross clamp (minutes)	88	71
Post-HTx course	Moderate rejection, resolved	Uncomplicated

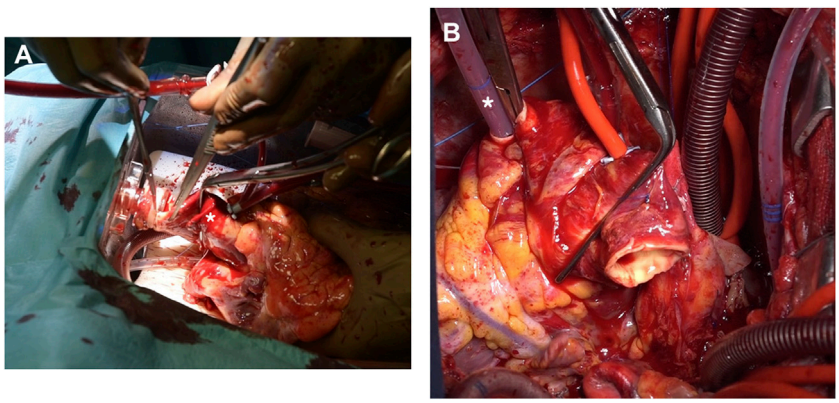

FIGURE 1 | (A) The OCS platform is kept close to the operative table and a separate arterial perfusion line (arrow), long enough to reach the donor graft, is primed. **(B)** A standard cardioplegia cannula is inserted proximally in the donor aorta (arrow) and connected to the perfusion line (asterisk).

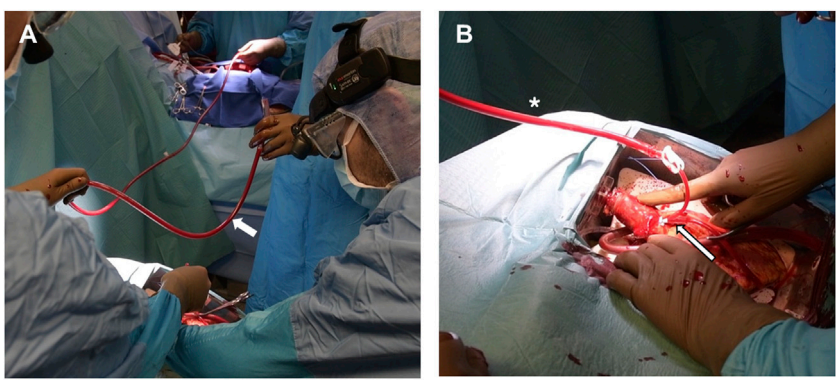

FIGURE 2 | (A) The donor aorta is cross-clamped (asterisk) and the donor graft is detached from the OCS by cutting its distal ascending aorta immediately under the connector. **(B)** During implant, the OCS pulmonary cannula is removed and replaced by a smaller and more pliable tube to vent the right ventricle (asterisk).

flow of 300–500 cc/min and a pressure of about 250–300 mmHg to achieve an aortic root pressure of 100 mmHg were maintained [4]. While moving the graft into the operative field, collection of blood loss was assured by placing the heart into a basin.

Graft implantation started with the left atrial anastomosis. Of paramount importance during this phase was avoiding any twisting or kinking of the perfusion line and of the graft aorta, which was frequently palpated to verify adequate pressure was maintained. The left ventricular vent was left in place while sewing the left atrial cuff and the OCS pulmonary artery cannula was replaced with a soft vent positioned inside the right ventricle and secured through a purse-string suture to the pulmonary artery (**Figure 2B**). The aortic anastomosis was then carried out between the two clamps, which

were released upon completion of the suture. The remainder of the operation was concluded, as in a standard HTx, by sequentially anastomosing the pulmonary arteries and the inferior and superior vena cava.

Procedural details are reported in **Table 1**. At the end of the HTx, Recipient 1 required moderate inotropic support with epinephrine, while Recipient 2 was weaned from CPB without difficulty. Recipient 1 presented moderate rejection after two days, which resolved after treatment. In Recipient 2, the postoperative course was uncomplicated.

In cases where prolonged ischemic times are required for donor graft procurement, myocardial stunning leads to a higher need for mechanical and inotropic support post-HTx [5]. Therefore, limiting warm and cold ischemic periods is a prerequisite to minimize myocardial injury in the donor heart and potentially achieve a smoother postoperative course.

The conventional static cold storage prevents safe graft preservation when long-distance procurement with extended ischemia times is required [6]. For this reason, we recently shifted to the OCS technique as the preferred method for donor graft protection during transportation; with this technique, we have obtained promising results when HTx was performed in high-risk recipients employing donor marginal grafts or in patients bridged to HTx on mechanical support [2, 3]. The lesson learned from this favorable experience indicates that, when possible, greater reduction of ischemic times or even an ischemia-free procedure should be aimed for in HTx.

With this in mind, we have recently performed two HTx using beating grafts from DBD with the advantage of eliminating the initial period of warm ischemia when using a graft from DCD [4].

This preliminary experience demonstrates that HTx with a beating heart obtained by DBD is feasible, and the brief warm ischemia required to institute OCS support does not adversely influence patient outcome. However, it is more cumbersome compared to a standard HTx, due to difficulty in manipulating a moving graft while performing the left atrial anastomosis; there are also concerns regarding longer operative times and additional costs. In fact, compared to traditional ice-cold storage, the OCS platform is rather expensive and requires a trained staff able to manage *ex-situ* perfusion and possible device troubles. Therefore, the widespread use of this procedure may be limited by financial and logistical reasons.

Nevertheless, although eliminating even a brief period of ischemia might not significantly influence HTx results in low-risk recipients, the surgical complexity should be counterbalanced by beneficial effects, especially when using marginal donors, DCD, or long-distance procurements. These expectations will have to be confirmed by studies conducted on larger populations.

With this technique, only one cardioplegia infusion is required, but retrieving the beating donor heart without any cardioplegic arrest, as recently reported in a DBD [5], may provide additional benefit by avoiding any ischemia-reperfusion injury, ideally leading to an ischemia-free HTx in all cases.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by Institutional Review Board (IRB-DMED) - UNIUD. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

Study design: AL and SN. Data collection: SS. Draft writing: AL and UB. Revision: UL and IV. Final approval: IV. All authors contributed to the article and approved the submitted version.

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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.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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LETTER TO THE EDITOR
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Intraparenchymal Enzyme Injections in Islet Isolations With Incomplete Ductal Perfusion of Enzymes

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Keywords: intraparenchymal injection, pancreatic islet, islet isolation, digestion, collagenase

Dear Editors,

Pancreatic islet isolation relies on the complete perfusion of digestive enzymes throughout the pancreas for dissociation of the extracellular matrix to digest pancreatic tissue and maximize the islet yield [1–3]. Enzymes are perfused in the pancreatic duct by retrograde cannulation (RC) or through combined ante- and retrograde cannulation (ARC) by dissecting the pancreas at the neck [4, 5]. Incomplete enzyme perfusion is often observed in pancreases of patients with chronic pancreatitis undergoing total pancreatectomy with islet autotransplantation (TPIAT) and at the dissection surface during ARC procedures. Here we describe intraparenchymal injections (IPI) of digestive enzymes as a potential solution to overcome incomplete perfusion.

All data were collected on consecutive human pancreatic islet isolations for clinical use between December 2014 and February 2024 in the Leiden University Medical Center. Pancreases for allogeneic islet transplantation were allocated by Eurotransplant. Pancreases for autologous islet transplantation were obtained after total pancreatectomy. Islet isolations were performed using an adapted version of the semi-automated method [4, 6]. RC was the standard method of cannulation, ARC was used if RC proved challenging. Experienced members of the islet isolation team examined the pancreas for hypoperfused tissue areas and performed intraparenchymal injections of digestive enzymes using 25–30 gauge needles until those areas were distended. IPI is demonstrated in **Supplementary Video S1**. Further details are provided in the **Supplementary Methods**.

Data from 253 consecutive islet isolations from donor pancreases intended for allogeneic islet transplantation, and 26 islet isolations from pancreases intended for autologous islet transplantation were included. Allogeneic organ donors had a mean age of 47.9 ± 12.7 years, 45.5% were female, and the body mass index was 27.3 ± 5.1 kg/m^2. In procedures involving donor pancreases, RC was performed in 218 (86.2%) and ARC in 35 (13.8%) of the isolations (**Supplementary Table S1**). Patients with an indication for total pancreatectomy and islet autotransplantation had a mean age of 45.5 ± 14.9 years, 65.4% were female, the body mass index was 24.0 ± 4.1 kg/m^2, and 84.6% had a history of chronic pancreatitis (**Supplementary Table S2**)

In islet isolations for autologous transplantation, digestion with IPI was higher (IPI 81.4% ± 15.5% vs without IPI 55.0% ± 27.4%, 95% CI of change: 7.82–45.02, p = 0.01, **Figure 1D**). Median islet yield was 5,540 (IQR 3,100–7,330) IEQ/g with IPI and 2,570 (IQR 1,870–3,230) IEQ/g without IPI (p = 0.05, **Figure 1E**) (**Supplementary Table S3**). We found that ductal cannulation with enzyme perfusion was not possible in 6 of these islet isolations. In these 6 isolations, we performed intraparenchymal enzyme injections only and isolated between 190.000 and 705.000 IEQ (range 2972–9503 IEQ/kg, **Figures 1F, G**; **Supplementary Table S4**). Five out of 6 islet preparations were

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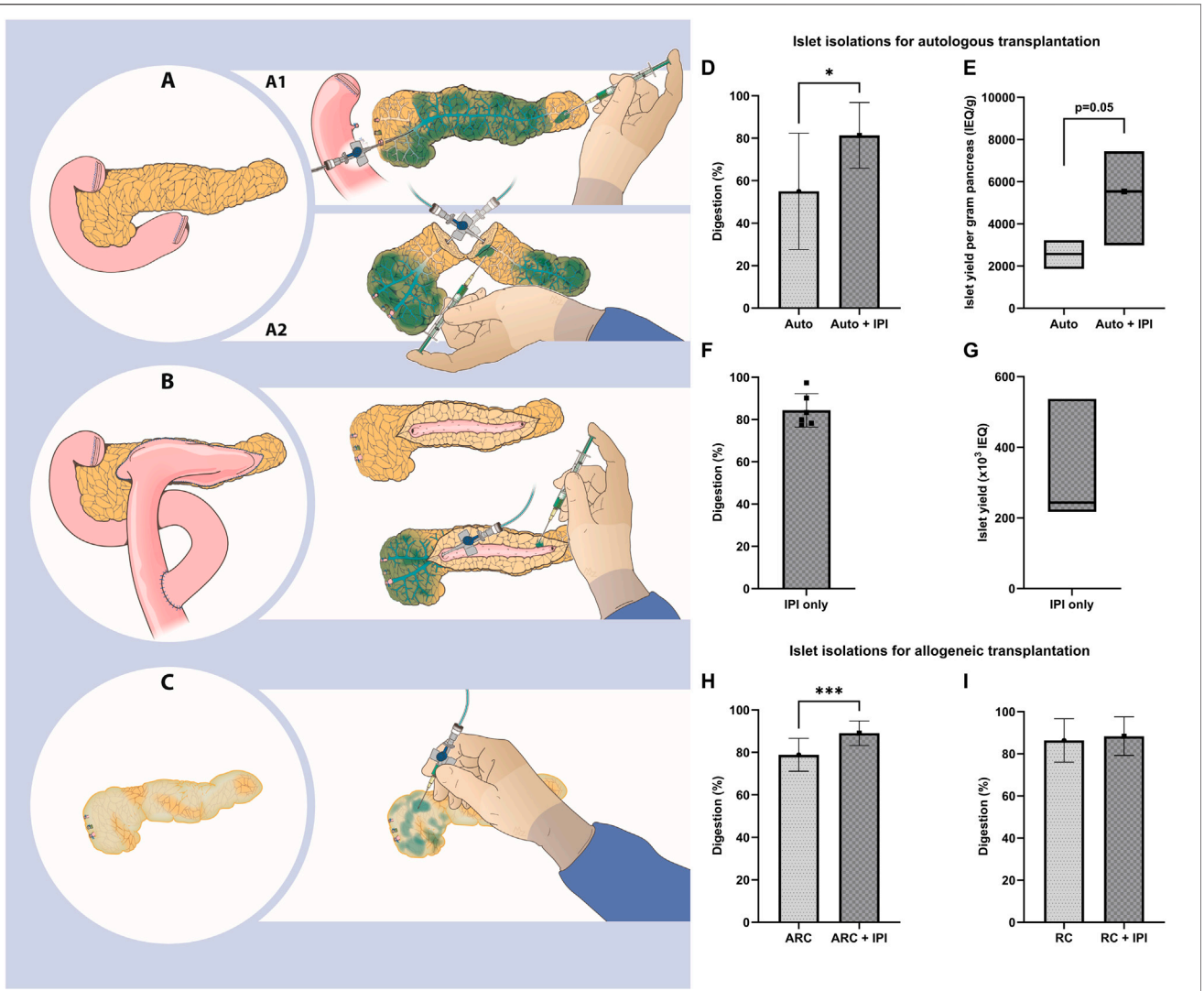

FIGURE 1 | (A) The starting point for islet isolation with the pancreas connected to the duodenum. A1) The pancreas is separated from the duodenum and perfused by retrograde cannulation (RC) of the pancreatic duct. Certain tissue areas are poorly perfused and intraparenchymal injections (IPI) are performed to deliver enzymes (depicted in blue). A2) The pancreas is cut at the neck and enzymes are perfused by ante- and retrograde cannulation (ARC) of the pancreatic duct. Parenchyma around the cut surface is poorly perfused and IPI is performed. **(B)** A pancreas after pancreaticojejunostomy. The jejunum is removed and the pancreatic duct exposed. Enzymes are perfused by ARC and IPI. **(C)** A smaller, fibrotic pancreas. Ductal cannulation is not possible and IPI is performed. **(D-G)** Islet isolations for autologous transplantation. **(D)** Percentage digestion (mean ± standard deviation). **(E)** islet yield per gram pancreas (median and interquartile range). **(F)** Percentage digestion (mean ± standard deviation) and **(G)** islet yield (median and interquartile range) using only IPI. **(H, I)** Islet isolations for allogeneic transplantation showing percentage digestion (mean ± standard deviation) for ARC **(H)** and RC **(I)**. IEQ, islet equivalent.

transplanted. One of these islet products could not be transplanted because of a high endotoxin concentration.

In islet isolations for allogeneic transplantation, we found a higher digestion in ARC isolations with IPI of 10.0%pt. (95% CI: 5.99–14.08, $p < 0.001$, **Figure 1H**), without a difference in islet yield per gram pancreas. For RC islet isolations, digestion and islet yield per gram pancreas were similar between the isolations with and without IPI (**Figure 1I**).

Generation of a maximal number of viable and functional islets is the most important goal for islet isolation. In this observational study we show that intraparenchymal injection is unlikely to have a negative effect on islet yield. The potential contribution of intraparenchymal enzyme injections was demonstrated in 6 isolations for autologous islet transplantation with a sufficient islet yield for autotransplantation. Digestion rate and islet yield of isolations using ARC in donor pancreas and of isolations for TPIAT were higher when IPI was performed based on the presence of hypoperfused pancreas parenchyma. In RC isolations, similar digestion and islet yield were present.

IPI could be considered in pancreases with an altered anatomy, such as after dissection of the neck for ARC (**Figure 1A**) and after previous pancreatic surgery. Damage to

the pancreas due to dissection, which is inherent to ARC, leads to hypoperfusion and subsequent incomplete digestion. Fibrosis, calcification (**Supplementary Figures S1A, C**) and previous surgery (e.g., Frey, Beger, Puestow procedures; **Figures 1B, C**) are often present when pancreases are presented for isolation in the context of autologous islet transplantation [7]. These surgical procedures may render classical perfusion methods inadequate and negatively affect islet yield [8]. In these instances, intraparenchymal injections may facilitate more complete perfusion of the parenchyma with digestive enzymes, potentially supporting digestion and islet yield.

There are no previous studies on how to deal with hypoperfused pancreatic parenchyma during isolation. A strength of this study is the inclusion of consecutive islet isolations of pancreases for both allogeneic and autologous islet transplantation. Study limitations include its retrospective, observational nature and judgement of hypoperfusion by experienced members of the islet isolation team. In order to obtain more robust information of the contribution of IPI on islet isolation outcome, randomized studies with or without IPI, and more objective assessment of hypoperfusion should be performed.

In conclusion, intraparenchymal injections may improve digestion and islet yield, representing a potential addition to current islet isolation practice.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because instutition-guided restrictions may be applicable. Requests to access the datasets should be directed to ME, m.a.engelse@lumc.nl.

ETHICS STATEMENT

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. 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. Oral informed consent was obtained from the individuals for the publication of any potentially identifiable images or data included in this article as per institution guidelines.

AUTHOR CONTRIBUTIONS

MT, DJC, EK, and ME participated in the design of the research. MT, DJC, YC, EW, JS, JD, CV, MH, ER, SM, BB, VH, and ME participated in performing the research. MT participated in data analysis. MT, DJC, EK, and ME participated in the preparation of the article. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.13507/full#supplementary-material

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