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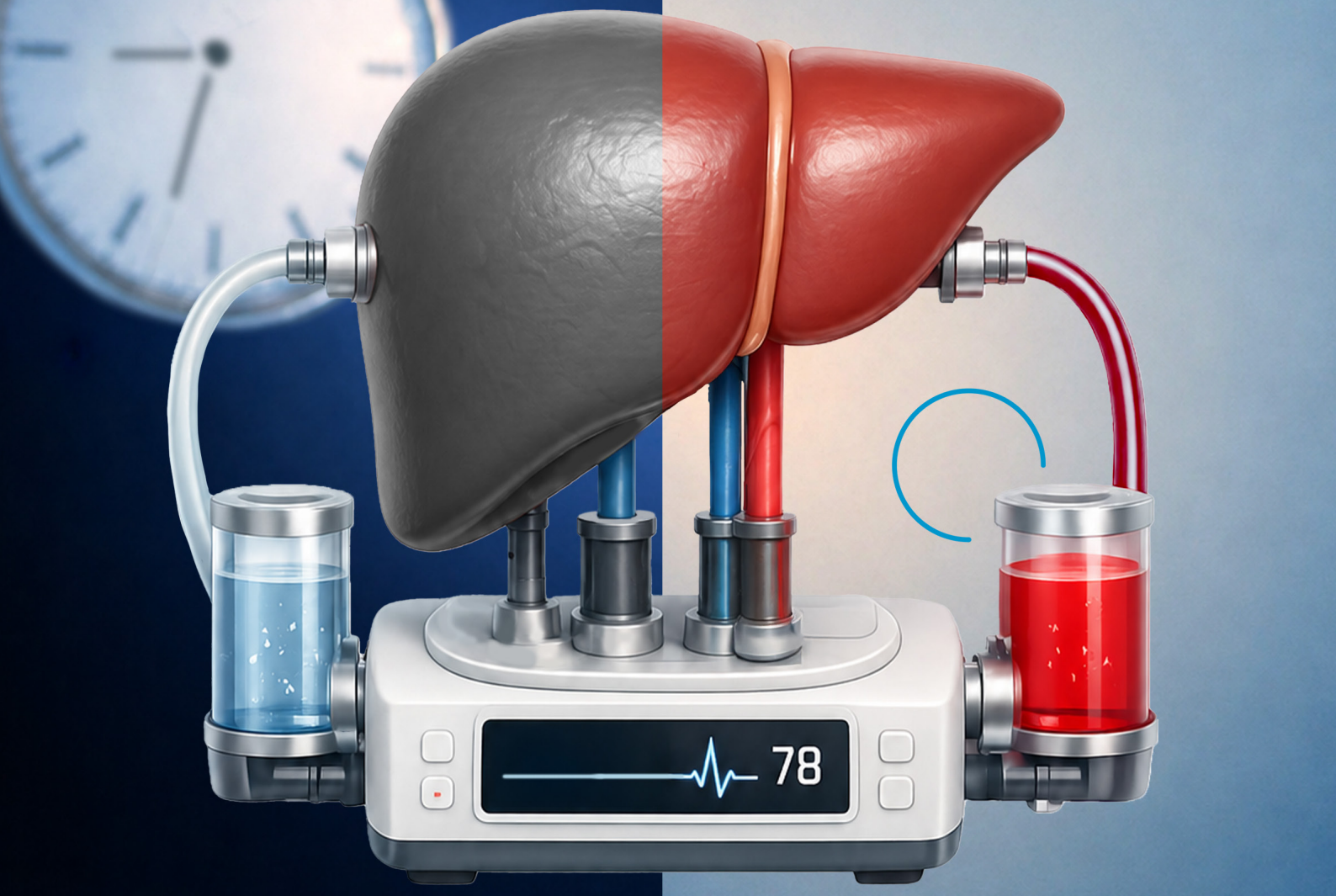

Organ Perfusion: Time As a Therapeutic Ally

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Organ Perfusion: Time as a therapeutic ally

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Table of contents

Review - Cover article

Position Paper

Review

Original Research

Brief Research Report

Letters to the Editor

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REVIEW
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Time as a Therapeutic Ally: The Promise of Long-Term Solid Organ and Tissue Perfusion

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Rapid advances in tissue preservation and the growing adoption of machine perfusion have fundamentally reshaped solid-organ and tissue transplantation in recent years. Multiple short-term perfusion devices have received regulatory approval and are increasingly used in clinical practice to preserve grafts for several hours, improving allograft assessment. The boundaries of dynamic tissue preservation have been pushed even further in research settings, where grafts have been reliably perfused for multiple days. The extended time of long-term machine perfusion opens a new therapeutic window for interventions, allowing for reconditioning and even tissue repair of injured and diseased grafts. The increasing global organ shortage makes these approaches particularly attractive to recover additional allografts for safe transplantation. In this review, we highlight current clinical practice for *ex situ* perfused allografts, multi-day perfusions in research settings, and potential therapeutic benefits of long-term perfusion with a focus on hearts, livers, lungs and vascularized composite allografts.

Keywords: heart, kidney, liver, lung, transplant, vascularized composite allografts

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INTRODUCTION

Global Organ Shortage

Solid-organ transplantation has been successfully established since the first transplantation of a human kidney in 1954 [1]. Since then, heart, lung, liver and kidney transplantation has become the only sustainable therapy for patients with end-stage organ diseases [2]. Further, transplantation of vascularized composite allografts (VCAs), including upper and lower extremities, face, abdominal wall, uterus and penile grafts, has become an available option to recover the quality of life for patients [3, 4]. However, due to increasing demands for allografts, extension of transplant indications, and increasing prevalence of organ diseases in the general population, we face a global shortage of transplantable grafts. At the beginning of 2025, 13,570 patients were awaiting an organ transplantation in the Eurotransplant-area [5], and more than 100,000 in the US [6]. Therefore, strategies are urgently needed to increase utilization of available grafts safely. To address this gap, transplant surgeons have already started extending acceptance criteria by using marginal grafts, including those from aged donors and grafts that were donated after circulatory arrest (DCD).

Because these grafts are associated with higher risk of non-anastomotic complications when they are transplanted without being evaluated or resuscitated on a perfusion device, researchers have developed dynamic preservation platforms that allow for graft assessment and reconditioning prior to transplantation [7–9].

Dawn of Dynamic Preservation

Current perfusion concepts all share the core function of supplying oxygen to the donor graft while outside of the body, but differ in terms of temperature, perfusate composition, and time of perfusion [10]. Three core concepts of dynamic preservation have gained increasing acceptance across most allografts: *ex situ* perfusion in normothermic (35 ˚C–38 ˚C) versus hypothermic (4 ˚C–12 ˚C) conditions, and *in situ* normothermic (35 ˚C–38 ˚C) regional perfusion (NRP) [11, 12].

Normothermic machine perfusion (NMP) is generally applied either immediately after procurement and during transport, which limits the period allografts are exposed to cold ischemia, or after an initial phase of cold storage, referred to as « back-to-base», at the transplant center (end-ischemic NMP) [10]. NMP typically utilizes an oxygenated blood-based perfusate, either through blood products or donor whole blood, which is pumped through afferent vessels of the allografts. The high demands of metabolically functional allografts necessitate sophisticated perfusion machines that closely mimic the physiological environment *in situ* [13]. Acellular perfusates with synthetic oxygen carriers have also been used for NMP to avoid demand for blood products and overcome issues related to hemolysis, but these approaches remain the subject of preclinical research [14, 15]. NMP has already been leveraged to perform graft assessment, evaluating parameters, such as bile production in livers [16], weight change in VCAs [17], monitoring oxygenation in lungs [18] or coronary flow and resistance in hearts [19]. While such objective assessment has increased utilization of marginal grafts [20], consensus on biomarkers and their reliability has yet to be established.

In an alternative approach, hypothermic oxygenated perfusion (HOPE) mainly relies on chilled synthetic preservation solutions, typically containing osmotic agents, electrolytes, buffering substances, metabolic substrates, and antioxidants or free radical scavengers [21, 22], that are pumped through afferent vessels at 4 ˚C–8 ˚C [23]. In livers, perfusate may further be administered through the portal vein only (HOPE) or both, the portal vein and the hepatic artery (dual or D-HOPE), with similar long-term outcomes [24]. Hypothermic conditions markedly decrease metabolic demands, allowing allografts to rely only on dissolved oxygen in the perfusate without the need for oxygen carriers in the perfusate. Therefore, hypothermic perfusion machines are inherently simpler and less costly than their normothermic counterparts [25]. Core functions include perfusate cooling and oxygenation as well as maintaining a steady perfusate flow. The main advantage of HOPE—typically applied in an end-ischemic setting—lies in restoring aerobic metabolism at lower temperatures, thus reducing toxic metabolite accumulation (i.e., succinate, NADH) and subsequent reactive oxygen species production, with the aim of dampening ischemia–reperfusion injury (IRI) [26–28]. While HOPE, like NMP, allows for the measurement of biomarkers to predict allograft viability [29–31], assessment of allograft function during preservation remains limited in clinical practice.

NRP is initiated immediately after circulatory death with the intention of avoiding prolonged ischemia in the organ procurement process prior to reperfusion—a concept particularly appealing in donation after cardiac death (DCD) and standard practice in several countries, including Italy, Spain, France, and parts of the United Kingdom [32]. Both the abdominal compartment alone and thoraco-abdominal organs combined may be included in NRP circuits [33, 34]. NRP includes clamping either the thoracic aorta or ligating the cerebral vessels to prevent cerebral blood flow [33]. Common extracorporeal membrane oxygenation (ECMO) devices are employed to generate artificial blood flow [35]. This approach offers the earliest opportunity to assess allograft viability, though reflecting a multi-organ environment [32]. While promising from an allograft utilization perspective, this technique still faces ethical and legal barriers related to the *dead donor rule* in many countries [36–38]. Hence, NRP will not be discussed in detail in this article.

CLINICAL USE OF SHORT-TERM PERFUSION

Dynamic organ preservation has transformed transplantation across multiple organs, with accumulating evidence demonstrating superiority over static cold storage. In liver transplantation, *ex situ* machine perfusion technologies—including four RCTs for NMP [39–42] and six for HOPE [43–48] —have shown improved graft survival, reduced post-operative and liver-related complications, and decreased ischemic cholangiopathy in donation after circulatory death (DCD) transplantation (**Table 1**). Machine perfusion further improved utilization rates enabling safe use of previously declined donor livers through enhanced viability assessment [9, 20, 29, 30, 67–70]. Current research explores complementary combinations including use of controlled oxygenated rewarming (COR), combinations like HOPE-COR-NMP [71–75], NRP-HOPE [76–78], and NRP-NMP [79, 80] sequences, marking the end of static cold storage as standard practice [81]. However, NRP, which is praised for improving organ utilization and its protective effect on the biliary tree [80, 82–84], has not been studied prospectively to date.

Ex situ heart perfusion (ESHP) has progressed from case reports to RCTs and national programs, with PROCEED II demonstrating non-inferiority for NMP vs. static cold storage [49] (**Table 1**). Later on, case studies [35, 85] and a clinical trial [50] have established DCD hearts as safe alternatives to donation after brain death (DBD) allografts when reanimated and assessed with perfusion platforms, substantially increasing transplant activity without compromising survival [35].

The safety of *ex vivo* lung perfusion (EVLP) was first demonstrated at Lund University Hospital, where the initial six double-lung transplants were successfully performed

TABLE 1 | Randomized controlled trials for the use of machine perfusion in transplantation of livers, hearts, lungs, and kidneys.

Graft type	Study (year)	N (total transplants)	Graft type (perfusion group)	Perfusion type	Median perfusion duration (h)	Control	Primary endpoint
Liver	Nasralla [39]	220[a]	37.1% DCD	NMP	9.1	SCS	Peak AST in 7 days
	Markmann [40]	298[a]	19% DCD	NMP	4.6	SCS	EAD
	Ghinolfi [42]	20	DBD	NMP	4.2	SCS	6-month graft/patient survival
	Chapman [41]	266[a]	14.3% DCD	NMP	5.9[b]	SCS	EAD
	Schlegel [44]	170[a]	DBD	HOPE	1.6	SCS	Patients with Clavien ≥ III
	Panayotova [45]	179	8% DCD	HOPE	2.8	SCS	EAD
	Van Rijn [43]	160	DCD	HOPE	2.25	SCS	6-month non-anastomotic biliary strictures
	Czigany [46]	46	DBD	HOPE	2.4	SCS	Peak ALT after 7 days
	Grat [48]	104	DBD	HOPE	2	SCS	Model for early allograft function
	Ravaioli [47]	110	DBD	HOPE	2.4	SCS	EAD
Heart	Ardehali [49]	130	DBD	NMP	3.5	SCS	30-day patient and graft survival
	Schröder [50]	180	DCD	NMP	Not reported	SCS, DBD grafts	6-month survival
Lung	Slama [51]	80	DBD	NMP	4.4	SCS	Pao_2/Fio_2 ratio, primary graft dysfunction after 24h
	Warnecke [52]	320	DBD	NMP	3.7	SCS	30-day survival, absence of primary graft dysfunction grade 3 after 72h
Kidney	Moers [53]	672	12.5% DCD	HMP	15[c]	SCS	Delayed graft function
	Wang [54]	48	DCD	HMP	6	SCS	Delayed graft function
	Malinoski [55]	1349	DBD	HMP	19.3[c]	SCS	Delayed graft function
	Husen [56]	262	DBD	HMP	4.7	SCS	1-year graft survival
	Alijani [57]	58	DBD	HMP	32.5[c]	SCS	Delayed graft function
	Halloran [58]	181	DBD	HMP	30.5	SCS	1-year graft survival
	Merion [59]	100	DBD	HMP	1	SCS	Delayed graft function and post-transplant serum creatinine levels
	Summers [60]	102	DCD	HMP	13.9[c]	SCS	Delayed graft function
	Tedesco-Silva [61]	160	DBD	HMP	25.05[c]	SCS	Delayed graft function
	Van der Vliet [62]	76	DCD	HMP	Not reported	SCS	Delayed graft function and primary nonfunction
	Watson [63]	80	DCD	HMP	10.1	SCS	Delayed graft function
	Zhong [64]	282	DCD	HMP	10.3[c]	SCS	Delayed graft function
	Jochmans [65]	164	DCD	HMP	15.9[c]	SCS	Delayed graft function
	Hosgood [66]	338	DCD	NMP	1	SCS	Delayed graft function (requirement for dialysis in the first 7 days after transplant)

[a]*Intention-To-Treat (ITT).*
[b]*Only mean value reported.*
[c]*Reported cold ischemia time of perfusion group. Due to absence of oxygenation during perfusion, perfusion time is considered as cold ischemia time.*
Abbreviations: N (number), SCS (Static Cold Storage), AST (Aspartate Aminotransferase), EAD (Early Allograft Dysfunction), HOPE (Hypothermic oxygenated perfusion), HMP (Hypothermic Machine Perfusion), NMP (Normothermic Machine Perfusion).

following re-evaluation on EVLP [86, 87]. Since then, the technique has been developed and implemented clinically [18, 88, 89], with two RCTs confirming safety and efficacy for standard criteria donor (SCD) grafts (**Table 1**) [51, 52]. Further, the EXPAND and DEVELOP-UK studies transplanted extended criteria donor (ECD) lungs after EVLP, which showed elevated early primary graft dysfunction grade 3 rates, but similar long-term survival compared to standard transplantation of SCD lungs [90, 91]. EVLP has been implemented in many lung transplantation centers across the world for functional assessment of donor grafts as well as increased utilization of ECD lungs [92–98]. However, implementation challenges persist, particularly in smaller-volume centers facing cost and staffing limitations [99–103].

Ex situ machine perfusion has been established as an effective strategy to mitigate the deleterious effects of cold ischemia in kidney transplantation. The landmark randomized controlled trial (RCT) by Moers et al. first demonstrated the superiority of hypothermic oxygenated perfusion (HOPE) over static cold storage (SCS), reporting a significant reduction in delayed graft function, improved creatinine clearance, and superior one-year graft survival in the HOPE group [53]. These findings were subsequently corroborated by several independent RCTs, ultimately supporting the integration of routine HOPE into national kidney transplantation programs [104] (**Table 1**). In contrast, the use of NMP for kidneys has remained mostly experimental. Early clinical studies showed feasibility of kidney transplantation following 1 h of NMP [105, 106]. The first RCT for 1 h NMP of kidneys further demonstrated feasibility and non-inferiority relative to SCS [66]. However, no statistically significant benefit was observed regarding delayed graft function, renal function, or one-year graft survival.

Consequently, the role of short-term NMP in kidney transplantation remains a subject of ongoing debate.

Use of NMP for vascularized composite allotransplantation (VCA) has not been adopted for clinical application to the same extent as for solid organs, and clinical cases were only conducted within acceptable limits for warm ischemia. However, small case series and case reports on *ex situ* preservation of amputated extremities and free flaps support *ex situ* machine perfusion as a viable strategy for VCA preservation. This includes reports from Newsome et al. who performed 2.7 h perfusions of (musculo-fascio-cutaneous) anterior thigh flaps with successful transplantation [107], Fichter et al. who perfused radial forearm flaps for 2.5 h, followed by successful transplantation [108], and Taeger et al. who perfused and successfully transplanted a latissimus dorsi flap [109]. Prolonged perfusion durations were further achieved by Taeger et al. who successfully perfused two traumatically amputated lower limbs for 12–16 h at 20 °C [110].

LONG-TERM NORMOTHERMIC MACHINE PERFUSION IN RESEARCH SETTINGS

While short-term perfusion technologies already introduced benefits upon clinical introduction, they also bring inherent limitations. For example, HOPE approaches profit from lower metabolic activity to prolong preservation by slowing down degradation and simplifying metabolic needs. While HOPE can be used to mitigate IRI and thus recondition the graft [111, 112], it is impossible to treat and repair allografts or perform functional assessment due to the reduced metabolic rate. Therefore, long-term (>24 h) perfusion approaches were developed for different organs and VCAs with the promise to create a platform to treat injured and ECD grafts, perform rigorous graft assessment, and to transition transplant surgeries from an emergency to an elective procedure [13, 15, 113–115]. Further, prolonged perfusion may be beneficial to absorb initial IRI during an acute reperfusion phase on a machine and transplant a fully functional graft once initial inflammation is decreasing again. This hypothesis was formed based on an observation after transplantation of a liver graft after more than 3 days of *ex situ* perfusion [116, 117].

Liver

In the preclinical setting, *ex situ* perfusion of multiple days was first introduced for livers in 2020 in Zurich [13], demonstrating the feasibility of long-term (i.e., >24 h) preservation. Using a custom-built NMP device, explanted porcine and discarded human livers were preserved for up to 10 days [13]. In contrast to previously known NMP systems, the Wyss–Zurich device allowed for prolonged *ex situ* perfusion in near physiologic conditions and a functional state [13]. Thanks to automation with feed-back controllers, perfusion parameters could be automatically controlled in a tight physiological range, limiting on-site interaction to a minimum. This approach was subsequently validated in the first-in-human application on compassionate use basis, preserving a liver initially declined for transplantation for 3 days followed by successful implantation [116]. The same platform was later adapted to the needs of resected partial livers, which could be used to preserve partial human livers for a week, showing normal tissue integrity and hepatic function [118]. Besides offering a platform for profound viability assessment and organ function, such advancements have since enabled *ex situ* treatment including pharmacological defatting of steatotic grafts [119–121], building on previous efforts in short-term preservation [122, 123]. The first RCT is currently ongoing and data is expected to be available soon (ISRCTN14957538).

Other groups focused on adapting currently available NMP devices to meet the requirements of multi-day perfusion [124–127], i.e., nutrition, precise control of acid-base balance and blood gases, glucose control, as well as dialysis [128–131]. Using and modifying commercially available devices comes with the obvious advantage of easy accessibility and reproducibility [132]. The limitation to this approach is the need for constant human intervention. The longest preservation time with this strategy so far was reported by the Italian group, which successfully preserved a declined human liver over 17 days by incorporating an extracorporeal blood purification system into their NMP device [127]. Indeed, hemodialysis was shown to further improve perfusate quality in multiple organs for long-term perfusions [133]. Further, successful prevention of microbial contamination [134, 135] and hemolysis [13] were found to be crucial for long-term perfusions. Besides mere preservation of viability, the Australia group pioneered the ability to perform liver split procedures of 10 whole livers without interrupting perfusion [126, 136]. Their work is based on the seminal work of performing split procedures during short-term perfusion [137–140] and marks a milestone in *ex situ* liver research. Establishing such long-term perfusion liver models has the immense potential to revolutionize the study of liver injury, repair and regeneration [141–143].

Heart

While *ex situ* heart perfusion has not been performed for longer than 24 h to date, an increasing amount of case reports that document prolonged *ex situ* perfusions that enabled long distance transport of allografts [144–146]. Notably, a heart was successfully transported across the Atlantic ocean while being perfused *ex situ* for 16 h, illustrating that NMP can even enable world-wide organ sharing [145]. Collectively, the clinical literature agrees on three consistent conclusions: *i.* NMP is safe and non-inferior to SCS for standard donors; *ii.* NMP enables reliable functional assessment that can rescue marginal or DCD hearts; and *iii.* scaling DCD programs with perfusion platforms can substantially expand transplant activity, especially with prolonged perfusion durations that enable longer transport thus wider organ sharing.

Lung

Extending the interval between donor lung procurement and implantation has several important clinical and logistical implications. Prolonged preservation facilitates broader donor-recipient matching across larger geographic regions and allows

transplant centers to avoid nighttime surgery, which is associated with increased complication rates and inferior outcomes [147, 148]. More importantly, lengthening EVLP duration transforms the platform from a short-term assessment tool into a therapeutic environment in which injured or initially discarded lungs can be actively rehabilitated [149].

The Toronto lung transplantation program first reported long-term perfusion in porcine lungs and human discarded donor lungs only years after the advent of EVLP [150]. Ever since, the group has extensively investigated prolonged EVLP in porcine models, demonstrating that continuous EVLP for 12–24 h is feasible [151, 152]. Similarly, other institutions report successful extension of porcine EVLP durations for up to 24 h [153–156], the Hannover program transplanting the extended EVLP lungs into healthy porcine recipients with subsequent short periods of graft evaluation [157]. In Minnesota, the 24 h prolonged evaluation was extended to human discarded donor grafts [158]. A maximum preservation time of 3 days has been reported in porcine lungs using a protocol of two short (4 h) normothermic EVLP cycles alternating with cold storage at 10 °C [159].

Despite strong interest in the topic and several pre-clinical reports of long-term EVLP for up to 24 h or more, clinical evidence remains largely limited to case reports with the longest documented clinical normothermic continuous EVLPs being 11.25 h and 15.5 h, respectively [160, 161]. However, a recent report from the Netherlands group describes the first clinical experience with n-EVLP–HOPE, using hypothermic oxygenated machine perfusion (HOPE) after a period of normothermic EVLP (n-EVLP) in a small cohort of human lung transplantation patients [162]. Grafts from the n-EVLP–HOPE group did not differ significantly in early post-transplantation outcomes compared with the control group.

There are ongoing controversial discussions about what parameters are needed for long-term EVLP, such as what temperature to use, perfusate composition, whether the perfusate should be exchanged in the EVLP and in what intervals. However, despite those advances, additional comprehensive research is needed to advance clinical implementation.

Vascularized Composite Allografts

Use of machine perfusion has been reported *in vivo* to salvage free flaps after thrombosis of vascular anastomosis. Wolff et al. first reported manual rhythmic perfusion of 3 fibula flaps with heparinized red blood cells at 38 °C for 10–12 days, resulting in flap survival and neovascularization [163]. The same group reported using a closed-loop, low-flow circuit (Novalung MiniLung) at 37 °C, to perfuse thin anterolateral thigh flaps and radial forearm flaps for 4–6 days *in vivo* in five high-risk patients. Stable coverage was achieved in four of five cases. One subtotal flap was lost due to infection, two cases due to complete epithelial loss, and one case featured a venous congestion [164]. Since then, there has been a decade of incremental experimental progress, but NMP of VCAs beyond 24 h remains uncommon [114, 165–167], with most experiments failing by 30 h of perfusion [15, 166, 168]. The longest reported perfusion durations were 72 h for human upper extremities [169] and 144 h for human fasciocutaneous flaps [170].

Kidney

Similar to liver transplantation, prolonged NMP promises safe inclusion of additional kidney grafts for transplantation. Therefore, protocols and perfusion devices have been refined to extend preservation times beyond 1 day. Notably, the first clinical report was recently published by Dumbill et al. who perfused kidneys for up to 24 h before transplantation and showed correlation between NMP biomarkers and 12-month graft function [171]. Discarded human kidneys could be further perfused for 48 h, while maintaining urine excretion [172]. The longest *ex situ* kidney preservation was reported by de Haan et al. who perfused human kidneys for 4 days at sub-normothermic conditions, maintaining a metabolically active state [173].

Limitations

Despite recent advances, substantial differences persist in achievable preservation periods across graft types. While organ-specific factors contribute to these variations, maintaining perfusate quality represents a fundamental limitation that constrains perfusion duration across all graft types. The longest perfusion durations were currently achieved for liver grafts, ranging up to 2 weeks [119, 127, 132]. This achievement reflects the liver's unique capacity to actively maintain perfusate quality through its inherent metabolic and detoxification functions. While soluble waste products and toxins can be easily removed with hemodialysis [133], many metabolic byproducts rely on hepatic clearance. Thus, only perfusate exchange can effectively mitigate waste and toxin accumulation for non-liver grafts.

Maintenance of adequate oxygen delivery presents another critical challenge related to perfusate quality. Erythrocyte supplementation has proven essential for NMP of kidneys, VCAs, livers, and hearts [39, 49, 66, 114]. Even in lung perfusion, where acellular Steen solution is routinely utilized in some EVLP protocols, erythrocyte supplementation has demonstrated improved tissue preservation through enhanced oxygen transport and reduced reactive oxygen species generation [86, 157, 174]. However, hemolysis resulting from shear forces in tubing and pumps, combined with suboptimal environmental parameters [175], necessitates ongoing erythrocyte supplementation to maintain sufficient hematocrit levels.

Beyond waste products and oxygen carriers, perfusate further carries a variety of signaling molecules. Suboptimal management of IRI, inflammatory activation triggered by unphysiological environmental parameters, and endothelial injury induced by oncotic pressure fluctuations, supraphysiological shear forces, and IRI itself collectively limit perfusion duration and initiate release of pro-inflammatory molecules [176, 177]. Although cytokine filters have been employed clinically in kidney, liver, and lung, as well as experimental heart perfusion [178–181], extended perfusion periods require comprehensive pharmacological interventions to minimize IRI and attenuate cellular damage responses.

TABLE 2 | Collection of assessment parameters from literature classified by the Zurich assessment approach—based on assessment time and invasiveness [113].

Graft type	Stage 1 Perfusion parameters	Stage 2 Graft function (metabolic and mechanistic function)	Stage 3 Organ damage (tissue inflammation and damage markers, imaging modalities)	Stage 4 Invasive testing (biopsy-based analysis)
Liver	• Oxygen consumption [118, 126] • Arterial resistance [126, 183] • pH [39]	• Lactate clearance [39, 40] • Bile production [13] • Ammonia clearance [13] • Bilirubin levels [13, 126] • Glucose metabolism [13] • Albumin synthesis [183] • Coagulation factor synthesis [13]	• AST, ALT [184] • ALP [13, 126] • FMN [29–31, 185, 186] • IL-6 [13] • GGT [126] • LDH [183]	• Steatosis [122, 187] • ATP [13] • Glycogen storage [13] • H & E staining [13, 141] • Gene expression [188]
Heart	• Coronary resistance [189] • Coronary flow [189] • Oxygen consumption [190]	• Lactate [191] • Uric acid [192] • Cardiac high-energy phosphates [192] • Electrograms [191]	• Echocardiography [193] • X-ray fluoroscopy [192] • IL-6 [192] • TNF-α [192] • Heart-type fatty acid binding protein [192] • Procalcitonin [192] • Cardiac troponin [193]	• H&E staining [189] • ATP [194] • TUNEL staining [195] • Caspase-3 staining [195]
VCA	• Graft weight [17] • Vascular resistance [114] • Tissue oxygen saturation [114]	• Lactate [114] • Muscle contractility [114] • Glucose consumption [114] • Lactic acid [114]	• ICG angiography [114] • Thermal imaging [196]	• H&E staining [114] • Caspase-3 staining [196]
Lungs	• Lung oxygenation [197–201] • Pulmonary vascular resistance [197–200] • Pulmonary artery pressure [51, 201, 202] • Perfusion flow [51] • Peak airway pressure [51, 52, 201–203] • Compliance [197–201] • Perfusate loss [201]	• Base excess [201] • Glucose consumption [201] • Lactate production [201] • pH [203, 204]	• IL-6 [205] • IL-8 [205] • NETs [198, 206] • TNF-α [207] • IL-1β [207] • cfDNA [208] • Radiography [201, 209] • Bronchoscopy [201, 209] • Pulmonary artery angioscopy [210] • Ultrasound [211, 212] • Lung weight [213]	• H&E staining [197–200] • Immunohistochemistry staining [197, 198] • Immunofluorescence staining [198] • Mass spectrometry [199, 214] • RNA sequencing [215]

(*Continued*)

TABLE 2 | Continued

Graft type	Stage 1 Perfusion parameters	Stage 2 Graft function (metabolic and mechanistic function)	Stage 3 Organ damage (tissue inflammation and damage markers, imaging modalities)	Stage 4 Invasive testing (biopsy-based analysis)
Kidney	• Pressure and flow in renal artery [66, 171, 172, 216] • Macroscopic appearance [66] • Oxygenation [171, 216] • Intrarenal resistance [171, 216] • pCO2 [171, 216]	• Urine production [66, 171, 216] • Urine chloride [172] • Urine potassium [172] • Proteinuria [172] • pH [171, 172, 216] • Lactate [171, 172, 216] • Glucose [171, 172, 216] • GFR [171, 217]	• AST [172] • LDH [171, 172] • CXCL10 [172] • TFF3 [172] • NGAL [171, 172, 216] • Osteopontin [172] • Cystatin C [172] • Clusterin [172] • IP-10 [172] • KIM-1 [216] • TNF-a [172] • VEGF [172] • IL-2, IL1b,IL-4,IL-10,IL-5 [172] • IFN-gamma [172] • L-FABP [171] • Gluthathione Serum transferase [171]	• H & E Staining [171, 172, 216] • Periodic acid Schiff Staining [171] • KIM-1 antibody staining [216] • LC-MS protein quantification [217]

FUTURE IMPACT OF LONG-TERM PRESERVATION

Graft Assessment

While donor characteristics, such as age or cause of death, as well as procurement parameters, including warm ischemia time, are routinely collected it is challenging to base transplant decisions on these parameters alone [182]. Therefore, machine perfusion has been increasingly used as a platform to perform rigorous graft assessment. As there is no consensus on assessment parameters, we summarized a collection of assessment parameters across different graft types, categorized based on invasiveness and evaluation time [113] (**Table 2**). Importantly, metabolic function, and associated biomarkers are temperature dependent, leading to higher values for NMP compared to HOPE. For example, the prominent biomarker for mitochondrial injury, flavin mononucleotide (FMN), was established for HOPE in liver transplantation [29–31, 218], but requires different thresholds during NMP [30, 219]. Importantly, many assessment parameters may not be diagnostically conclusive during the first hours of reperfusion as the graft is exposed to IRI and undergoes a temporary reperfusion phase [117]. Hence, all assessment parameters must be evaluated in the context of time, which is currently not standard practice [113]. Another major limitation of current assessment strategies is the lack of normalization for perfusate-borne biomarkers, which should be normalized by perfusate volume and graft size. Given this, transplant communities ideally transition to systematic reporting that allows for comprehensive data collection and identification of indicative assessment parameters with corresponding thresholds, which can be later implemented in national guidelines. Given these, NMP is a platform that substantially improves objective graft assessment and has the potential to safely allow for transplantation of additional grafts.

Graft-Specific Repair Strategies

Liver

Multi-day preservation of livers in a functioning state ultimately opens a therapeutic window and provides a platform for therapeutic intervention. Such interventions may be of pharmacological nature, including cell-based or gene therapies, or may be based on novel tissue- and bioengineering approaches [12, 113]. While many strategies have emerged to safely increase

the donor pool, we highlight the frequently discussed concepts: defatting of steatotic grafts and regeneration of partial grafts.

Defatting of Steatotic Grafts

Grafts with more than 30% macrosteatosis are usually discarded for transplantation because of the established risk of post-transplant liver failure [220–222]. Consequently, reversing hepatic fat infiltration during *ex situ* preservation represents an attractive application of long-term perfusion platforms. Perfusion for 10 days alone can achieve complete defatting in some grafts [119]. Fat metabolization can be further enhanced through pharmacological intervention by leveraging adipose triglyceride lipase (ATGL) driven lipolysis and β-oxidation (L-carnitine, UCB9608, fenofibrate) [119, 120]. These preliminary findings strongly encourage further refinement of protocols enabling efficient and consistent long-term defatting. Other groups found a 40% fat reduction within 6–12 h of NMP with the application of a *defatting cocktail* (forskolin, scoparone, nuclear-receptor ligands, hypericin, and visfatin). Such cocktails, however, raise some concerns regarding toxicity and are not safe for human use [122, 184]. While short-term (<24 h) perfusion platforms are currently being trialed for their anti-fat effects [187], long-term perfusion uniquely opens new horizons to fully and safely reverse clinically relevant steatosis via the addition of pharmacological agents to the perfusate.

Liver Regeneration

Achieving *ex situ* regeneration to augment transplantable mass could revolutionize transplant medicine, but relevant volume increase requires sufficient time for tissue proliferation. Major liver surgery is based on the unique hepatic capacity to regenerate. The most remarkable volume increase is seen after ALPPS (Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy), demonstrating that the human liver can regain up to 80% volume within a week [223, 224]. The pathway responsible for the accelerated regeneration that is seen after ALPPS has been identified to involve paracrine JNK1–IHH signaling [225, 226] and could be a suitable target for future modulation. Various other complex pathways, such as Hippo–YAP1 [227–229] and Wnt/beta catenin [230, 231], play key roles in liver regeneration [232] and may be explored additionally as future therapeutic targets. Furthermore, the regenerative capacity of bile ducts was already explored in a recent study during long-term perfusion [141], with promising results published for cholangiocyte organoid repair [233].

Heart

For cardiac allografts, repair and modulation techniques are in the early stages of development. For example, DCD hearts were reconditioned after 30 min of warm ischemia time by reperfusing them at hypothermic conditions with histidine-tryptophane-ketoglutarate-N solution, which improved cell swelling and reduced oxidative stress, nitrosative stress and necrosis prior to normothermic reperfusion [234]. Reconditioning entails improvement of both systolic and diastolic function, which are important for transplant outcome, and come with their own challenges. Diastolic relaxation requires good coronary microvascular function as decreased microvascular circulation can lead to diastolic cross-bridge cycling [235]. This motivates research on vascular and microvascular modulation. It was already demonstrated in a porcine model that HOPE, using traditional histidine-tryptophan-ketoglutarate (HTK) solution, improved systolic function. Similar effects were shown for HOPE with HTK-N solution, which is supplemented with protective amino acids and iron chelators [234]. Compared with regular HTK solution, HTK-N was more effective in improving diastolic function and restoring coronary microvascular circulation (CMVC) [236].

Another *ex situ* treatment is senotherapy, which addresses the adverse effects of aged, senescent cells, such as the senescence-associated secretory phenotype (SASP) in tissues. Use of hearts that were donated from aged donors promises to increase the number of transplantable grafts after senotherapy. Therefore, a first implementation of senotherapy was shown in a rat model during *ex situ* NMP [237]. Here, senomorphic treatment of the donor hearts improved CMVC in grafts substantially during *ex situ* NMP, especially in grafts that were donated from old male animals [237]. As for the other organs and VCAs, only NMP allows for active metabolism, which is required for some repair strategies.

Lung

Like other solid organ and VCA perfusion approaches, machine perfusion of lungs offers a unique treatment opportunity for injured donor lungs. Because the organ is isolated during perfusion, therapies can be applied without the risk of systemic off-target effects that would occur if the same treatment were administered *in vivo* [18, 149, 238].

Several interventions have been developed and tested to enhance graft recovery during EVLP and subsequently evaluated during transplantation in pigs. Infected grafts have been successfully treated using cytokine adsorption devices to reduce inflammatory burden and improve lung physiology [149, 197, 214, 239]. Lungs affected by aspiration injury have shown functional recovery following the application of neutrophil extracellular trap (NET) removal technologies [198, 206]. Additionally, mesenchymal stromal cells (MSCs) have been administered during EVLP to stabilize the endothelial and epithelial barriers, attenuate IRI, and promote alveolar repair [149, 199].

Another strategy is gene therapy, using viral vectors to deliver transgenes for up- or downregulation of specific pathways. Moreover, genome-editing tools such as clustered regularly interspaced short palindromic repeats (CRISPR)-based systems can be used to allow for active gene editing in the tissue *in vivo* [18, 238]. For example, one group used an adenoviral vector encoding human IL-10 to enhance IL-10 expression in porcine lungs during EVLP with subsequent transplantation and demonstrated improved lung function 7 days after gene delivery [240]. They further developed this approach by using CRISPR-associated technologies to activate IL1RN and IL-10 in a rat transplantation model, showing that the gene modifications were successfully induced and retained after transplantation into healthy recipients [241]. The Hannover group instead used lentiviral vectors carrying shRNA sequences downregulating

swine leukocyte antigen (SLA) to genetically engineer miniature swine donor lungs during EVLP [242]. Remarkably, five of seven treated pigs survived for more than 4 years without immunosuppression, whereas no animals survived in the control group [243].

Another important consideration is the need to ensure that the engineered grafts maintain their function post-transplantation by transplanting EVLP-treated lungs into a relevant animal model for evaluation. As the Lund group and others have shown, lungs treated with, for example, cytokine adsorption or stem cells during EVLP, can deteriorate after transplantation and require additional treatment beyond the EVLP period [197, 199].

As genome-editing and gene-delivery strategies become more complex, longer perfusion times will likely be required. Extended perfusion would allow sufficient time for cellular uptake of the vectors, expression or editing of the target genes, and verification of successful modification before transplantation.

Vascularized Composite Allografts

There is currently no evidence supporting specific interventions that can actively promote repair in VCAs. Nevertheless, *ex situ* perfusion appears to exert an intrinsic reconditioning effect, providing the muscle with a physiologic environment that supports cellular repair and functional recovery. The Cleveland group showed that NMP of human upper extremities improved limb condition. At the time of procurement, most (8/10) upper extremities were harvested edematous and cold. However, during the first hours of perfusion, electrolytes, muscle, and surface temperature normalized, and, most importantly, muscle contraction was restored and maintained for 30.5 h [114]. Similarly, the Michigan group demonstrated sustained muscle contractility (grade 4/5) in human limbs during 24 h of perfusion [165].

The Cleveland group performed genomic analysis and identified 2,283 differentially expressed genes in perfused limbs compared to SCS. The perfusion group exhibited upregulation of genes associated with wound healing and inflammation, alongside downregulation of genes involved in apoptosis. These findings suggest that *ex situ* perfusion induces a state of preconditioning that preserves the metabolic viability of VCAs and promotes intrinsic tissue repair mechanisms. Metabolic profiling of perfused human limbs further demonstrated that the tissues remained metabolically active throughout the duration of perfusion over multiple days. Notably, there was a depletion of taurine, an amino sulfonic acid essential for maintaining mitochondrial respiratory chain function. These findings suggest that taurine supplementation during perfusion may mitigate oxidative stress and help preserve mitochondrial integrity [244]. Given these findings, we see substantial potential for future research to establish strategies to enhance muscle repair and regeneration during NMP.

CONCLUSION

Short-term machine perfusion has already improved the utilization of donated allografts and is increasingly being adopted in transplant centers worldwide. Beyond enabling DCD heart transplantation and reducing non-anastomotic complications from IRI in liver grafts, perfusion systems have proven valuable for assessing the viability and function of extended-criteria donor organs. However, the maturity of evidence varies considerably across graft types. For VCAs in particular, consistent outcomes have not been achieved yet, even for short-term perfusions with durations between 12 and 24 h, requiring establishment of robust protocols and harmonized outcome reporting as the direct next step.

Beyond short-term use of NMP, growing evidence further suggests that prolonged, multi-day perfusion may support the safe recovery of injured or diseased grafts across a wide range of organ types. With the exception of hearts, multi-day perfusions have now been successfully demonstrated for livers, kidneys, lungs, and vascularized composite allografts, underscoring a strong forward trajectory in the field. Given these predominantly pre-clinical advancements, the clinical utility of long-term perfusion must now be established for livers, lungs, kidneys and hearts through prospective, controlled trials that extend beyond case reports. Such trials are essential not only to develop and validate standardized, organ-specific viability criteria, but also to determine whether prolonged *ex situ* perfusion can effectively mitigate and absorb IRI on the device. Importantly, these investigations must proceed in close collaboration with device manufacturers as part of rigorous regulatory certification processes, as currently no perfusion platform is approved for extended use beyond 24 h. Translation of this technology from experimental application to routine clinical practice further requires logistical frameworks, which may include centralization of national perfusion and repair centers, and legal frameworks for policy development and routine implementation.

Long-term systems also provide a powerful research platform for the discovery and testing of new therapeutic strategies which can be explored in research settings while long-term perfusion is clinically established. Although many of these innovations remain in early development, they hold tremendous promise for enhancing graft utilization and improving transplant safety through rigorous *ex situ* assessment.

Taken together, long-term perfusion represents a promising technological advancement that may translate into broader clinical practice in the near future as the evidence base is continuously maturing. With this transition, we will see a transformation of transplantation logistics and procedures, improved safety for patients, and better utilization of available grafts, ultimately resulting in lower waitlist mortality.

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CONFLICT OF INTEREST

P-AC and MT are co-founders of Apersys AG, which aims to commercialize long-term perfusion technologies.

The remaining author(s) declared that this work 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) declared that generative AI was not used in the creation of this manuscript.

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POSITION PAPER
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Shaping the Future of AI in Organ Transplantation: Position Paper of the European Society for Organ Transplantation

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Advances in AI hold considerable promise for organ transplantation. While every transformation brings change, not all change is transformative. Despite the rapid growth of AI in medicine, most applications remain in developmental or experimental stages, with relatively few having been successfully integrated into routine clinical practice. As a professional society, ESOT recognises that achieving meaningful impact will require more than technical progress. This position paper outlines five critical domains for successful implementation. (1) High-quality development: Coordinated collaboration and methodological rigour are prerequisites for trust; AI is only as robust as the data used to train it. (2) Ethical considerations: We must address risks to equity and access to care, and move from generic ethical principles to transplantation-specific ethical guidance. (3) Regulatory landscape: AI in transplantation is regulated under both EU medical device and AI legislation; compliance is central to stakeholder trust. (4) Responsible adoption: AI should augment, not replace, human expertise. Strengthening AI literacy is essential for meaningful adoption. (5) Participatory design: Active involvement of transplant

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Abbreviations: AI, Artificial Intelligence; ESOT, European Society of Transplantation; EU, European Union; GDPR, General Data Protection Regulation; IEEE, Institute of Electrical and Electronics Engineers; IVDR, In Vitro Diagnosis Devices Regulation; MDR, Medical Device Regulation; MDSW, Medical Device Software; NIST, National Institute of Standards and Technology; OECD, Organisation for Economic Co-operation and Development; SHAP, Shapley Additive Values; UNESCO, United Nations Educational, Scientific and Cultural Organization; WHO, World Health Organization.

professionals and patients is essential to address real clinical needs. These statements serve as a strategic framework to guide clinicians, researchers, and policymakers in making AI a genuine force multiplier for the transplant community.

Keywords: artificial intelligence (AI), ESOT, machine learning, organ transplantation (OT), position paper

INTRODUCTION

Artificial intelligence (AI) is increasingly recognised as a highly disruptive technology with the capacity to address challenges across multiple domains of medicine. Broadly defined, AI refers to computational systems that perform tasks traditionally requiring human intelligence. These include learning from data, recognising patterns, making predictions, and supporting complex decisions. In healthcare, AI encompasses a diverse set of tools, including supervised and unsupervised machine learning, natural language processing, computer vision, and generative models. These technologies process large datasets, support clinical judgement, and enhance precision in diagnosis, treatment, and follow-up.

The development of AI in medicine has gained considerable momentum in recent years. Improvements in model performance, the availability of large-scale data, and increased computational power have accelerated both academic and industry-driven research [1, 2]. In transplantation, emerging AI applications span donor–recipient matching [3–5], outcome prediction [6–10], computer vision tools [11–16], and generative AI [17]. Despite rapid progress, relatively few of these applications have so far been integrated into routine clinical practice.

As the European Society for Organ Transplantation (ESOT), we recognise both the considerable promise and the set of challenges that accompany the advancement of AI in our field. AI could contribute along the entire transplant pathway by supporting donor–recipient matching and organ allocation, guiding organ acceptance decisions, and enhancing risk stratification and follow-up after transplantation, effectively moving from an office-based model of care delivery to continuous monitoring, placing the patient at the centre of post-transplant care [18]. While AI offers clear opportunities to improve patient outcomes, streamline clinical workflows, and advance healthcare equity, the path to meaningful adoption must be balanced against complex technical, regulatory, and ethical considerations. Similar themes are emerging across other medical disciplines, although transplantation presents distinct challenges related to organ scarcity, allocation, and longitudinal outcome assessment [19, 20]. Ensuring that AI adoption serves the interests of patients and clinicians is paramount.

ESOT is committed to taking an active role in shaping the future of AI in transplantation. Achieving positive and impactful progress in transplantation will require strategic alignment among all stakeholders, including patients, clinicians, researchers, policymakers, regulators, and industry partners. Building a future where AI can be a true force multiplier in transplantation will depend on collaboration, transparency, and a shared commitment to ethical, patient-centred innovation.

The position statements aim to identify how current opportunities can be realised most effectively while navigating the technical, ethical, and regulatory challenges that may hinder meaningful implementation. By outlining these considerations, the paper seeks to provide a clear and pragmatic framework to support responsible, patient-centred AI adoption across the transplant pathway.

DEVELOPMENT OF THE ESOT AI POSITION STATEMENTS

The ESOT AI Working Group was convened to produce position statements to guide the adoption of AI in transplantation. The multidisciplinary team comprised physicians and surgeons involved in transplantation, AI and data science experts, bioethicists, transplant recipients and patient representatives, as well as policy and regulatory experts in digital health. The recommendations and position statements were developed iteratively through a series of structured virtual and in-person discussions, including a focused in-person meeting at the ESOT 2025 Congress in London. The process was designed to identify key domains and produce a framework for responsible AI adoption in transplantation. This was not a formal Delphi process, and no predefined voting threshold was used. Instead, statements were refined through repeated group discussion until agreement was reached on their wording and scope.

In parallel with the expert consensus process, ESOT conducted a survey of its membership to explore familiarity with AI, current use of AI tools, and perceived opportunities and concerns regarding AI in transplantation. The survey findings were used to contextualise the consensus statements and to assess their alignment with the views of the wider ESOT membership. Overall, survey responses were consistent with expert opinion. The survey questionnaire and summary of the results are provided in the **Supplementary Material SA1, 2**.

To structure the recommendations and position statements, five domains were chosen reflecting the key requirements and challenges relevant to AI in transplantation: high-quality AI development and validation, ethical considerations, the regulatory landscape, responsible adoption, and participatory design (**Figure 1**).

HIGH-QUALITY AI DEVELOPMENT AND VALIDATION

Recommendation 1: Clinicians collecting or providing data for use by AI algorithms should ensure that the data are accurate, valid, correctly labelled, comprehensive, unaffected by selection bias, and stored securely.

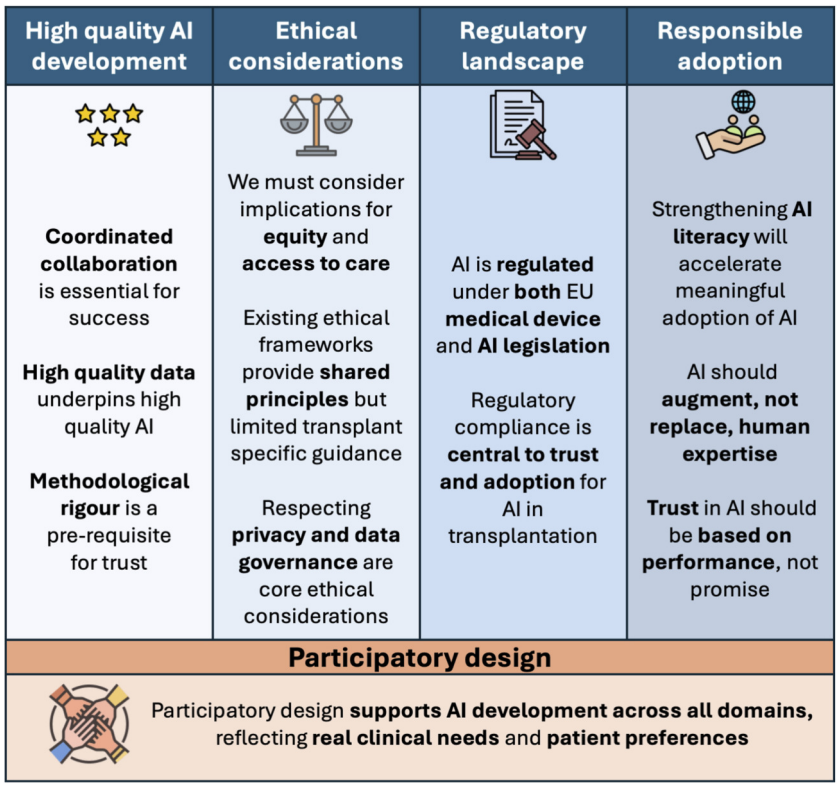

FIGURE 1 | Overview of ESOT position statements on artificial intelligence in transplantation. Four domains—high-quality AI development, ethical considerations, the regulatory landscape, and responsible adoption—summarise key considerations for implementation of AI in transplantation. The fifth domain, participatory design, underpins all domains, ensuring that AI development reflects real clinical needs and patient preferences.

Coordinated Collaboration Is Essential for Developing Robust AI

AI innovation will not succeed in isolation. High-quality AI development in transplantation depends on coordinated collaboration across clinical, scientific, and institutional boundaries. Given the complexity and high-stakes nature of donation and transplantation, AI systems developed in isolation are unlikely to generate sufficiently robust or generalisable evidence to support clinical use [21]. Although single-centre studies may offer early insights, multi-centre collaborations are necessary to enable external validation and, where feasible, provide prospective evaluation across populations and practice settings [22, 23].

Collaboration is also critical for curating datasets of sufficient scale and representativeness. Although several data-sharing initiatives and open-science portals now support AI research, few currently include transplantation-specific data [24, 25]. ESOT has already taken steps to address this by creating a pan-European registry platform, supported by the European Commission, that will host data on both transplant recipients and living donors [26]. Finally, parallel research in synthetic data generation offers another potential route to mitigating data scarcity without compromising patient privacy [27, 28].

High-Quality AI Relies on High-Quality Data

AI tools are only as robust as the datasets used to train them. Although advanced algorithms can identify complex patterns within large datasets, they cannot compensate for poor data quality, inconsistency, or systematic bias [29]. In transplantation, these challenges are pronounced due to variations in clinical practice, donor and recipient characteristics, and allocation processes across centres, regions, and health systems [21]. Without careful curation and transparent reporting, such heterogeneity risks producing AI models that perform well in development settings but fail to generalise in practice [30].

Data governance requirements introduce additional and unavoidable complexity. Strong safeguards are essential to protect patient privacy and maintain public trust. Yet restrictive data-sharing frameworks can unintentionally reinforce fragmented, single-centre datasets and limit opportunities for validation and collaboration. The twin challenges of heterogeneity and restricted access must be addressed in parallel. Well-assembled data that is inaccessible cannot drive progress, just as open, poor-quality data cannot yield trustworthy insights.

Reliable Evidence Is a Prerequisite for AI to Be Trusted in Transplantation

Methodological rigour is essential at all stages of healthcare AI development, as these tools will support decisions that are high-stakes, time-sensitive, and often irreversible. Evidence must therefore demonstrate not only technical performance but also reliability, generalisability, and clinical relevance across diverse populations and practice settings.

Addressing the diversity of research requirements will require the use of multiple study designs. Registry-based analyses offer scale, longitudinal follow-up, and real-world relevance. Still, they are frequently affected by missing data, heterogeneity, and unmeasured confounding, which can limit model validity if not appropriately addressed [31]. Prospective studies and trials provide more controlled evaluation but are resource-intensive and may not fully reflect real-world complexity. Across study designs, transplantation AI studies should report not only discrimination, but also calibration, external validation, subgroup performance, and the handling of missing data [32, 33]. Where relevant, analyses should also account for variation between centres which may substantially influence model performance and transportability.

Transparent reporting of data provenance, handling of "missingness," and clarity about sources of bias are therefore critical, regardless of study design. To support consistent evaluation and informed clinical decision-making, AI research in transplantation should adhere to established, evidence-based frameworks for development and reporting, including TRIPOD + AI, DECIDE-AI, and CONSORT-AI [32, 34, 35]. Alignment with such standards is necessary to enable critical appraisal and to distinguish exploratory research from tools sufficiently mature for clinical use. Without this level of methodological transparency and rigour, AI systems risk undermining trust, hindering responsible adoption in practice.

ETHICAL CONSIDERATIONS

Recommendation 2: To use AI tools responsibly, transplant clinicians should be familiar with the international guidelines on the ethical use of AI and understand how their patients' data will be protected.

AI in Transplantation Introduces Ethical Considerations for Equity and Access to Care

The design, development, and deployment of AI in transplantation raise profound ethical considerations. Central among these is the risk of bias. Where an inadequately diverse dataset is used to train an AI algorithm, the system's outputs may be negatively affected, leading to inaccurate outcomes and/or systematic discrimination when applied in particular populations. Examples include predictive modelling tools that provide inaccurate information for specific socio-economic and racial groups [36, 37]. Bias may arise at multiple stages of the AI lifecycle, including data collection, labelling, feature selection, and deployment, and its effects often disproportionately impact populations that are already marginalised or underserved [37, 38].

In transplantation, these risks are significant given the scarcity of deceased donor organs, the high stakes of allocation decisions, and the potential for AI outputs to influence access to life-saving treatments. Ethical concerns also arise in decisions about where and how AI systems are deployed, which populations benefit from their use, and how their impacts are monitored over time. Together, these issues underscore the need for explicit ethical scrutiny of AI systems used in transplantation.

Existing Ethical AI Frameworks Provide Shared Principles but Limited Transplantation-Specific Guidance

The landscape of ethical AI guidelines is characterised by a convergence of international, regional, and organisational frameworks that share core principles while emphasising distinct implementation approaches. Despite the large number of guidelines, there is striking overlap in the principles and themes they address, namely 1) transparency and explainability, 2) accountability, 3) fairness and non-discrimination, 4) privacy and data protection, 5) safety and reliability, and 6) human oversight, which is often considered a prerequisite for the ethically justified use of AI in healthcare. An overview of published ethical AI guidelines relevant to healthcare and transplantation is summarised in **Table 1**.

Most guidance remains high-level, lacking detail and specificity, perhaps reflecting the nascent stage of AI development as various technologies evolve from the innovation stage and become more widely implemented. High-level guidance stating the need to ensure distributive justice by avoiding bias, for example, does not help identify the practical steps to be taken by any sector, profession, or particular technology. The objectives of AI ethics must shift from provision of generic guidance to development of context-specific and implementable guidance to address real problems/ethical concerns impacting clinical practice and patient outcomes.

This is particularly important in the context of organ donation and transplantation systems, since their unique ethical complexity may be exacerbated by the introduction of AI. Here, an essential first step will be to identify and characterise the challenges. A non-exhaustive list of examples includes: bias in algorithmic organ allocation; the potential effect of AI use on public willingness to donate; and how to mitigate the known underrepresentation of specific populations, such as ethnic minorities, in transplantation databases [48–50].

Respecting Privacy and Data Rights Are Core Ethical Considerations

Since AI development relies on the collection and processing of large datasets, data security and privacy are core ethical concerns.

TABLE 1 | Overview of international, regional, and organisational ethical frameworks relevant to the development and use of artificial intelligence in healthcare.

Issuing body	Guideline
United Nations Educational, Scientific and Cultural Organisation (UNESCO)	AI Ethics Recommendation [39]
World Health Organisation (WHO)	Guidance on Ethics and Governance of Artificial Intelligence for Health [40]
European Union (EU)	Ethics Guidelines for Trustworthy AI [41]
European Union (EU)	EU AI Act Regulation (EU) [30]
Organisation for Economic Co-operation and Development (OECD)	AI Principles [42]
Institute of Electrical and Electronics Engineers (IEEE)	IEEE Ethically Aligned Design [43]
Microsoft	Responsible AI Standard [44]
Google	AI Principles [45]
National ethical framework – Australia	AI Ethics Principles [46]
National ethical framework – USA	National Institute of Standards and Technology (NIST) AI Risk Management Framework [47]

When large amounts of personal data are collected and processed their secure storage, appropriate anonymisation for confidentiality, and alignment with data protection standards present difficulties [51]. The growing volume of data collection and secondary data use can challenge individuals' ability to understand, control, and consent to how their information is shared [52]. In transplantation, where public trust underpins both donation and long-term engagement with care, perceived failures in data stewardship risk undermining confidence in AI-enabled practices.

Ethical data governance therefore extends beyond legal compliance to include transparency about data use, proportionality in data collection, and accountability for downstream impacts. The challenge is to balance the societal value of data-driven innovation with respect for individual rights and expectations, particularly in a field where data originates with vulnerable patients and incapacitated donors. Failure to address these ethical dimensions risks eroding trust and limiting the potential of AI applications in transplantation.

REGULATORY LANDSCAPE

Recommendation 3: Clinicians should ensure that AI tools used in routine clinical practice have undergone appropriate regulatory approval.

AI in Transplantation Is Regulated Under Both EU Medical Device and AI Legislation

In the European Union (EU), most AI applications relevant to organ donation and transplantation will be regulated as medical device software and, with rare exceptions, as high-risk AI systems. Although the EU has long had a regulatory framework for the medical applications of AI, it is rapidly evolving. This is in part due to the planned introduction of new AI legislation and standards, and in part to the landscape which develops behind technologies submitted for approval.

The EU's AI regulatory framework is now both multi-layered and intersecting. It combines the "vertical", product-specific Medical Device Regulation (MDR), (Regulation (EU) 2017/745) [53] with the recent "horizontal" EU AI Regulation (Regulation (EU) 2024/1689) [54]. The latter is the world's first comprehensive AI law, establishing a risk-based classification system that spans all product domains, including AI-enabled tools classified as medical device software (MDSW). AI systems used in healthcare are classified as 'high-risk' if they require third-party assessment under the MDR (or the related *In Vitro* Diagnosis Devices Regulation (IVDR), Regulation (EU) 2017/746) [55].

In practice, most AI-enabled medical devices require such third-party assessment, including all medical software used for individual patient diagnosis and therapy, and all software used for tissue typing of blood, tissues, or organs intended for transfusion or transplantation. The MDR applies to all new medical devices entering the market and to all MDSW from no later than 31 December 2028, while the AI Act applies from no later than 2 August 2027.

An unresolved challenge is regulating adaptive AI systems that learn from new data after deployment. Current regulatory frameworks are built around "locked" models whose performance is fixed at the point of release. These models are controlled via version releases and formal change management. Consequently, AI-enabled medical devices are authorised as static algorithms with periodic, regulator-reviewed updates rather than continuously learning systems operating in real time. Moving forward, we require clearer approaches to post-deployment monitoring and real-world performance evaluation, including the use of registry-based data where appropriate. Such mechanisms may help detect performance drift, assess real-world safety and effectiveness, and provide ongoing assurance after deployment. However, regulatory expectations for these lifecycle issues, particularly for adaptive models, remain incompletely defined and continue to evolve. Given the rapid pace of AI development, we need pathways that accommodate adaptive AI tools so that governance keeps pace with real-world innovation.

Taken together, the EU AI Regulation and the MDR require compliance with strict requirements for both the developers and deployers (i.e., the health system that makes the AI system available to end users). These obligations cover core principles such as the *General Safety and Performance Requirements* (MDR), which mandate a highly prescriptive medical device quality management system and detailed technical documentation. Furthermore, the AI Regulation introduces requirements for *Data Governance* (ensuring high-quality, representative datasets to avoid bias) and mandates *Transparency and Human Oversight* (designing systems to be explainable and subject to human control). To demonstrate compliance with both regulations, most AI-enabled transplant-related devices must undergo a joint formal conformity assessment by a notified body before they can be placed on the market or put into service. This dual approach ensures that AI applications in medicine are transparent, ethical, accountable, safe, and effective.

Regulatory Compliance Is Central to Trust and Adoption of AI Systems in Transplantation

The need for robust regulatory compliance cannot be overstated. For any AI technologies to be successfully adopted across any medical field, they must first gain the trust of all stakeholders: decision-makers, physicians, surgeons, nurses, patients, and the public. For transplant teams, the knowledge that an AI system has successfully navigated the regulatory pathway provides assurance that it was developed using appropriate quality and safety management processes. These processes are designed to ensure that data used for training and validation are of sufficient quality and that foreseeable risks, including those related to bias and error, are identified and mitigated.

Safe implementation and knowledge exchange across centres will also require greater consistency in terminology, evaluation criteria, and monitoring strategies. Depending on the intended use-case, this may include clearly defined inputs and outputs, structured thresholds for action, prospective monitoring of performance after deployment, and processes for review, recalibration, or suspension if performance drift or safety concerns emerge [56].

Compliance with the AI Act also requires that high-risk AI systems incorporate meaningful human oversight and are both tested and documented in ways that support traceability and audit. This is especially important for AI tools whose internal workings may be difficult to interpret. While regulation does not eliminate all concerns about opacity, it does require manufacturers to provide sufficient information about system behaviour, limitations, and conditions of use to enable informed clinical deployment and to support investigation of adverse events. In effect, the regulatory framework provides a structured basis for accountability when AI systems are integrated into complex decision-making processes such as transplantation.

RESPONSIBLE ADOPTION

Recommendation 4: Transplantation professionals need a role-appropriate understanding of what an AI system is designed to do, the data sources and cohort groups used to develop it, and its strengths and limitations.

Strengthening AI Literacy Will Accelerate Meaningful Adoption of AI

Improved digital and AI literacy is a fundamental prerequisite for the responsible implementation of AI in transplantation. Transplant clinicians and trainees must be equipped to appraise AI-based tools critically and to recognise when use of "AI" adds clinical value. However not everyone needs to become a technical AI expert. The accepted standard should be that transplantation professionals understand how a tool was developed and its expected strengths and limitations when applied to their patients [57].

This level of literacy will enable clinicians to better interpret and implement AI-generated outputs within their specific clinical contexts. It will also enable clinicians to act as informed intermediaries between AI systems and patients, explaining in clear terms the role of AI in their care without overstating its capabilities. In transplantation, where trust underpins both donation and recipient care, AI literacy should become a practical requirement for transplantation professionals rather than a special interest for enthusiasts.

AI in Transplantation Should Augment, Not Replace, Human Expertise

The increasing availability of decision-support tools raises important concerns about how clinicians interact with AI systems. Automation bias, in which clinicians defer to algorithmic outputs over other available evidence, can occur when an AI-generated recommendation is perceived as more objective or defensible than personal clinical judgment, even when the latter may be superior [44]. This risk is heightened for less experienced clinicians and in time-pressured or complex situations, where there may be a stronger temptation to follow the system rather than question it [58].

In parallel, the growing reliance on algorithmic assistance raises new concerns about deskilling—the potential erosion of clinical ability as tasks get increasingly delegated to AI [59]. Patterns of "deskilling," "mis-skilling," or even "never-skilling" may emerge if clinicians rely on AI before core competencies are fully developed. Safeguarding against this will require deliberate strategies, such as structured training that maintains hands-on clinical judgment, critical appraisal of AI outputs, and periodic "AI-off" practice to preserve independent reasoning [60]. Ensuring that AI augments rather than replaces human expertise will be essential for sustaining confidence, competence, and quality of care.

Trust in AI Systems Should Be Based on Performance, Not Promise

AI is increasingly touted in healthcare, yet the term "AI" is often overused and poorly defined, with many systems branded as "AI" offering little advantage over traditional analytics [61–63]. Tools should demonstrate strong performance for their intended task, with results reported across clinically relevant subgroups, including those often underrepresented in training data. Developers should actively look for performance gaps, communicate where predictions are less reliable, and, where possible, mitigate these limitations. These expectations are consistent with the ethical and regulatory principles outlined above, including attention to bias, transparency about limitations, and appropriate evaluation before clinical deployment. In the mid- to long-term real-world evaluations showing improved outcomes or decision quality compared with current practice will provide the most convincing basis for clinician and patient trust [64].

Alongside performance, the way AI systems present and justify their outputs also shapes trust. Explainability is frequently proposed as a solution to the 'black box' nature of many AI systems [65]. Post-hoc methods such as Shapley Additive Values (SHAP) can help reveal which features contributed most to a prediction and have shown promise in transplantation outcome models [9]. However,

explainability is not without its challenges. Emerging evidence suggests that, when AI systems are wrong, accompanying explanations can sometimes increase clinician overconfidence and worsen decisions, an "explainability paradox" that needs to be acknowledged and considered [66]. In this context, explainability should be seen as a tool that requires evaluation and thoughtful design, rather than a substitute for robust validation and clinically meaningful evidence of benefit.

PARTICIPATORY DESIGN

Recommendation 5: Active involvement of transplant professionals, recipients, and the public is essential at every stage of AI development, from initial concept to routine clinical implementation and evaluation.

Participatory Design With Clinicians, Patients, and Scientists Is Essential to Ensure AI in Transplantation Addresses Real Clinical Needs

For AI systems to provide value in transplantation, they must be built around clearly defined clinical needs rather than driven by technical novelty alone. Participatory design involves clinicians, patients, scientists, and industry partners throughout the design, implementation, and evaluation of AI tools [67, 68]. Such collaboration helps identify priority use cases, specify how tools should fit into existing workflows, and anticipate unintended consequences. By avoiding siloed development and treating end users as active contributors rather than passive recipients, participatory design increases the likelihood that AI systems will be clinically relevant, usable in practice, and aligned with the priorities of the transplant community.

Decisions About How AI Is Used in Transplantation Should Align With Patient and Public Preferences

Patient and public involvement is equally important in deciding where AI should and should not be used. Studies of transplant recipients in the UK suggest that patients are generally open to clinical use of AI and recognise its potential benefits, but are wary of unsupervised use for high-stakes tasks, favouring "human-in-the-loop" approaches in which clinicians remain clearly responsible for decisions [69]. Similar work from the wider health system indicates that patients accept AI for administrative functions such as correspondence and scheduling, but prefer its use in a decision-support role rather than as an autonomous decision-maker for diagnosis and treatment [70]. These reports underline the need to engage patient organisations in shaping AI adoption. This is also relevant to how clinical utility is defined, as the outcomes considered most meaningful may differ between stakeholders and may extend beyond traditional clinical endpoints to include equity, transparency, burden of care, and other patient-centred priorities. Aligning implementation with expressed patient and public preferences is likely to strengthen trust, improve transparency about the role of AI in care, and reduce the risk of deploying systems in ways that conflict with the values of those most affected.

SCOPE AND FUTURE DIRECTIONS

This position paper was intentionally developed as a broad, practical framework for the responsible adoption of AI across the transplant pathway. In prioritising breadth, multidisciplinary input, and accessibility to the wider transplant community, we did not aim to provide an exhaustive review of individual AI applications. As a result, some application areas and clinical examples could not be explored in depth, and the recommendations were developed through an iterative expert consensus process rather than a formal methodology such as Delphi. We recognise these as limitations, but also as pragmatic trade-offs that enabled the development of a clear and implementable set of recommendations spanning the major technical, ethical, regulatory, and clinical issues relevant to AI adoption in transplantation.

This paper is a starting point rather than a final or exhaustive consensus instrument. Parallel ESOT initiatives are already underway to address more focused questions relating to AI in transplantation, and these will provide opportunities to explore individual topics with greater depth and specificity.

CONCLUSION/ CALL TO ACTION

The true potential of AI in transplantation will be realised through coordinated action grounded in sound scientific and ethical foundations that reflect the needs and values of our community. The scope of this work has been broad, with input from experts across multiple disciplines, and it should be viewed as a starting reference point for those wishing to explore these domains in greater depth. We urge all stakeholders to engage with these recommendations and work together to deliver on the promise of AI for transplantation.

Given the exponential development of AI technologies, these recommendations provide a basis for future refinement, keeping pace with the technology advancements while maintaining the focus on the core principles.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design of the study. GO and CW coordinated the establishment of the

ESOT AI Working Group. All authors drafted sections of the manuscript within their respective areas of expertise. GK compiled the contributions, wrote the overall manuscript, and generated the figures. All authors critically reviewed and edited the manuscript and approved the final submitted version.

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CONFLICT OF INTEREST

The author(s) declared that this work 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) declared that generative AI was used in the creation of this manuscript. AI tools were not used to generate or author the manuscript text. They were used only to support proofreading and language editing. Any changes were reviewed and approved by the authors.

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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.2026.16316/full#supplementary-material

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REVIEW
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New Antibiotics Against Multidrug-Resistant Gram-Negative Bacteria in Lung Transplantation: Clinical Evidence, Safety, and PK/PD Properties

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Infections caused by multidrug-resistant Gram-negative bacteria (MDR-GNB) and *Pseudomonas aeruginosa* are leading causes of morbidity and mortality after lung transplantation (LuTx). We reviewed the pharmacology, clinical evidence, and safety of five agents potentially active against MDR-GNB in LuTx recipients (LUTR): ceftolozane/tazobactam, ceftazidime/avibactam, meropenem/vaborbactam, imipenem/relebactam, and cefiderocol. Literature from the last 10 years was reviewed for data on activity spectrum, efficacy in LUTR and adverse events. Ceftolozane/tazobactam and ceftazidime/avibactam were the most studied, providing high cure rates for difficult-to-treat *Pseudomonas* (DTR-PA) and *Klebsiella pneumoniae* carbapenemase (KPC)-producing Enterobacterales, respectively. Meropenem/vaborbactam offers reliable coverage of KPC strains, while imipenem/relebactam is an interesting option for imipenem-non-susceptible *Pseudomonas* spp. Cefiderocol exhibits the broadest *in vitro* spectrum, including metallo-β-lactamase producers. Across agents, pharmacokinetic variability, augmented renal clearance, and extracorporeal support can compromise target attainment; prolonged or continuous infusion is preferred. Collectively, these antibiotics expand the therapeutic armamentarium against MDR-GNB in LUTR, allowing pathogen-directed, toxicity-sparing regimens. Nonetheless, prospective LuTx-focused studies are needed to optimise their use in such a peculiar setting.

Keywords: immunocompromised host, lung, MDRGNB, SOT, therapeutic drug monitoring

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INTRODUCTION

Infections due to Gram-negative bacteria represent most clinically relevant infections among lung transplant (LuTx) recipients (LUTR) in the first year after transplantation and involve primarily the respiratory tract [1]. A growing proportion of these infections is caused by multidrug-resistant Gram-negative bacteria (MDRGNB). Infections due to MDRGNB have been associated with poorer clinical outcomes [2]. This has also been verified in the LuTx setting, with in-hospital mortality rates six times higher in LUTR with infections due to MDRGNB compared to non-MDRGNB [3].

However, evidence suggests that employing an antibiotic effective against the MDRGNB bacteria, especially in *Klebsiella pneumoniae* carbapenemase (KPC)-producing strains or Difficult-to-treat *Pseudomonas aeruginosa* (DTR-PA), can counterbalance this excess mortality [4]. Therefore, it appears clear that the new molecules against MDRGNB that have become available in the last few years could improve the outcomes of MDRGNB infections in LUTR.

We consider in this review ceftolozane/tazobactam (C/T), ceftazidime/avibactam (CZA), meropenem/vaborbactam (MVB), imipenem/cilastatin/relebactam (I-R) and cefiderocol (FDC). Their arrival has provided therapeutic opportunities for difficult-to-treat infections, and scientific societies have endorsed their use for several conditions in which MDRGNB are the culprit [5–7].

In this review, we analyse these new molecules from the perspective of LuTx, focusing on their activity spectrum, safety profile and pharmacokinetic/pharmacodynamic (PK/PD) properties, including therapeutic drug monitoring (TDM). **Table 1** provides an overview of common MDRGNB resistance mechanisms and profiles, along with the corresponding activity of the molecules discussed in this review.

CEFTOLOZANE/TAZOBACTAM

Activity Spectrum

Ceftolozane/tazobactam (C/T) is the combination of ceftolozane, a fifth-generation cephalosporin, with the β-lactamase inhibitor tazobactam. Due to its ability to evade key resistance mechanisms of *P. aeruginosa*, C/T exhibits potent activity against multidrug-resistant (MDR) and extensively drug-resistant (XDR) *P. aeruginosa,* including carbapenem-resistant *P. aeruginosa* (CRPA) [8]. Additionally, it is partially effective against extended-spectrum β-lactamase (ESBL)-producing Enterobacterales, showing a preserved susceptibility in ~85% of isolates [9, 10]. However, it lacks efficacy against carbapenem-resistant Enterobacterales (CRE). C/T present no efficacy against MDRGNB producing carbapenemases, however, it could retains some sensibility against CRE with other resistance mechanism such as porin-mutation or increase membrane efflux [11, 12]. C/T is approved for the treatment of complicated urinary tract infections (cUTI), complicated intra-abdominal infections (cIAI), and ventilator-associated bacterial pneumonia (VABP). According to both IDSA and ESCMID guidelines, C/T is considered the agent of choice for treating infections caused by difficult-to-treat (DTR) *P. aeruginosa* [5, 7]. While the IDSA guidelines place C/T on par with CZA and I-R as first-line options, the ESCMID guidelines identify it as the preferred first-line agent. This indication was set as a consequence of the CACTUS study, which has shown a C/T superiority in the treatment of DTR- *P. aeruginosa* pneumonia, compared to CZA (63% versus 51% in clinical success) [13]. Moreover, CZA has been reported to be associated with higher rates of resistance development; thus, in DTR-*P. aeruginosa* isolates susceptible to both C/T and CZA, C/T may represent a more appropriate therapeutic option in order to reduce the antibiotic selective pressure [14].

For patients with normal renal function, the approved dosage is 1.5 g administered in 1 h every 8 h for cUTI and cIAI, and 3 g administered in 1 h every 8 h for VABP.

Evidence in the Clinical and LuTx Setting

A 2023 study on 163 *P. aeruginosa* isolates obtained from patients with cystic fibrosis (CF) and LuTx, reported that 81.6% of them were susceptible to C/T [15]. Among MDR and XDR isolates, 88.3% and 28.1%, respectively, were susceptible to C/T. Similarly, Pfaller et al. analysed the susceptibility of 17,315 MDRGNB isolates [16]. They found that *P. aeruginosa* susceptibility was similar between patients >65 years and immunocompromised hosts (ICH), but notably lower in Intensive care unit (ICU) patients: 96.5% vs. 99.1%/99.2% (ICH/>65 years, respectively) in samples from the US, and 80.1% vs. 93.4%/92.5% in samples from Europe.

Clinical evidence on the use of C/T in LUTR remains limited, summarised in **Table 2**. In a recent French prospective cohort study involving 63 CF patients, of whom 19% (12/63) were LUTR, with *P. aeruginosa* lower respiratory tract infections (LRTI) treated with C/T, 89.3% of the strains were susceptible to C/T. The median treatment duration with C/T was 15 days, and clinical improvement was observed in 88.9% of patients [17].

Another multicentre retrospective cohort study of 69 ICH patients, of whom 68% with a history of solid organ transplant (SOT), assessed the outcomes of C/T use in different infections due to MDR *P. aeruginosa*. The most frequent infection sites were the LRTI (57%). The mean length of C/T therapy was 13 ± 10.8 days. The all-cause 30-day mortality rate among the entire cohort was 19% (13/69), while clinical cure was achieved in 68% (47/69) of patients. This rate was higher in patients with LRTI infections who received 3-g regimens compared to those who received 1.5-g regimens (75% vs. 30%) [18].

Haidal et al. conducted a retrospective study of 21 patients, among whom 7 were LUTR, treated with C/T for MDR *P. aeruginosa* infections. Most patients (18/21, 86%) had LRTI. The 30-day all-cause and attributable mortality were 10% (2/21) and 5% (1/21), respectively, and the C/T failure rate was 29% (6/21). Resistance to C/T emerged in three patients (14%), primarily associated with *de novo* mutations. Overexpression and mutations of AmpC were identified as potential mechanisms underlying this resistance [19].

Adverse Events and Limitations

Data regarding the concomitant use of C/T and immunosuppressive agents in SOT recipients remain scarce. Ceftolozane is unlikely to cause clinically relevant drug–drug interactions. Conversely, tazobactam is a substrate of organic anion transporters 1 and 3, and coadministration of inhibitors of these transporters may elevate tazobactam plasma concentrations, warranting cautious monitoring. However, as shown by real-world data, C/T is generally well-tolerated, and the most frequently reported AEs are nausea, vomiting, and diarrhoea.

TABLE 1 | Activity spectrum of recently approved antibiotics against multidrug-resistant Gram-negative bacteria.

Antibiotic (year of approval by EMA)	ESBL	KPC	MBL	Amp-C	Oxa-48	P.aer-DTR[a]	CRAb	*S. maltophilia*
Ceftolozane/tazobactam (2015)	✓/✗	✗	✗	✓	✗	✓	✗	✗
Ceftazidime/avibactam (2016)	✓	✓	✗	✓	✓	✓/✗	✗	✗
Meropenem/vaborbactam (2018)	✓	✓	✗	✓	✗	✗	✗	✗
Imipenem/relebactam (2020)	✓	✓	✗	✓	✗	✓	✗	✗
Cefiderocol (2020)	✓	✓	✓	✓	✓	✓	✓	✓

ESBL: extended-spectrum β-lactamases; KPC: Klebsiella pneumoniae *carbapenemase; MBL: metallo-β-lactamase; Amp-C: AmpC β-lactamases; OXA-48: OXA-48, carbapenemase; P.aer-DTR: difficult-to-treat* Pseudomonas aeruginosa*; CRAb: carbapenem-resistant* Acinetobacter baumannii.
[a]*Non-MBLs producing.*

TABLE 2 | Overview of real-life studies describing ceftolozane/tazobactam use among LuTx recipients.

Author, year	Country	Study design	Pathogen	Infection type	Main results	AE
Burgel, [17]	France	Prospective cohort study on 63 patients with CF of whom 12 (19%) where LuTx recipients	*Pseudomonas aeruginosa* (97.6%) Escherichia *coli*, *Citrobacter koseri*, *Proteus mirabilis*, and *Serratia marcescens*	LRTI	- C/T susceptibility: 89.3% - Clinical improvement: 88.9% - Mean FEV_1 improved from 1.33 L to 1.47 L before and after C/T treatment, respectively (p = 0.057)	Two (3.2%) leading to therapy discontinuation (pruritus, skin rash), but no new safety concerns identified
Hart, [18]	United States	Retrospective cohort study of 69 immunocompromised hosts of whom 47 (68%) were SOT recipients	MDR *Pseudomonas aeruginosa*	LRTI (57%) and wound infection (12%)	- 30-day all all-cause mortality: 19% (13/69) - Clinical cure: 68% (47/69) [higher in patients with respiratory tract infections who received 3-g regimens vs. 1.5-g regimens (75% vs. 30%).]	No data provided
Haidal, [19]	United States	Retrospective cohort study of 21 patients whom 7 (33%) were LuTx	MDR *Pseudomonas aeruginosa*	86% LRTI, less common BSI, cIAI, or cUTI	- 30-day all-cause mortality: 10% (2/21) - 30-day attributable mortality 5% (1/21) - C/T failure rate: 29% (6/21) - Resistance to C/T: 14% (3%)	One leading to therapy discontinuation (skin rash); two patients developed thrombocytopenia while on linezolid and linezolid + valganciclovir
Amore [20]	Italy	Case series of 7 LuTx recipients, of whom 4 (57%) were treated with C/T	MDR *Pseudomonas aeruginosa*	LRTI	- Mortality: 1/7 (primary graft dysfunction)	No severe AE occurred
Stokem [21]	United States	Case report of one LuTx recipients with CF	MDR *Pseudomonas aeruginosa*	Pulmonary exacerbation	- Clinical and laboratory improvement - Adequate pharmacokinetic levels	No AE occurred
Escolà-Vergè [22]	Spain	Retrospective cohort study of 38 patients treated with C/T of whom 10 (26.3%) were LuTx	XDR *Pseudomonas aeruginosa*	LRTI, ABSSSI, UTI (not clear the site of infection in LuTx)	- Clinical and laboratory improvement - Adequate pharmacokinetic levels - C/T resistence - All-cause mortality 5/38	No severe AE; CD enteritis (1/38)

AE: adverse event; CF: cystic fibrosis; LRTI: lower respiratory tract infection; C/T: ceftolozane/tazobactam; FEV_1: forced expiratory volume in 1 s; SOT: solid organ transplant; MDR: multi-drug-resistant; LuTx: lung-transplant recipient; BSI: bloodstream infection; cIAI: complicated intrabdominal infection; cUTI: complicated urinary tract infection.

Key Messages

Clinical data regarding the use of C/T in the LuTx setting are still scarce. C/T is considered the first-line agent for MDR *P. aeruginosa*, which, in the LuTx setting, is among the most frequently isolated pathogens [23, 24]. In this context, the use of C/T may represent a valuable therapeutic option.

CEFTAZIDIME/AVIBACTAM

Activity Spectrum

Ceftazidime/avibactam (CZA) combines the third-generation anti-pseudomonal cephalosporin ceftazidime with the novel non-β-lactam BLI avibactam, restoring ceftazidime's *in vitro*

TABLE 3 | Overview of real-life studies describing ceftazidime/avibactam use among LuTx recipients.

Author, year	Country	Study design	Pathogen	Infection type	Main results	AE
Chen, [26]	China	Retrospective study on 15 LUTR	CRPA	Not reported	- 14-day mortality: 6.7% - 30-day mortality: 13.3% - Clinical cure: 53.3% - Microbiological cure: 60.0% - Recurrence: 3/15 (20%)	Not reported
Chen, [27]	China-Japan	Retrospective study on 10 LUTR	XDR-GNB (CRKP, CRPA)	PN, BSI, cIAI,	- 30-day mortality: 100% - 90-day mortality: 90% - Relapse of CRKP or CRPA: 5/10 patients (50%) - Microbiological cure: 90.0% - Clinical response: WBC and PCT at 7 and 14 days significantly dropped ($p < 0.05$) PaO2/FiO2 ratio significantly dropped ($p < 0.05$)	No severe AE occurred (2 patients experienced increase of urea and creatinine levels)
Amore, [20]	Italy	Case series of 7 LuTx recipients, of whom 4 (57%) were treated with C/T	*K. pneumoniae* MDR (1 coinfection with *P. aeruginosa*)	LRTI	- Mortality: 1/7 (primary graft dysfunction)	No severe AE occurred
Peres-Nadales, [28]	Spain	Retrospective cohort study of 149 SOT, of whom 6 (4%) were LUTR	CPKP	BSI	- CZA treated patients had higher 14-day clinical success: 80.7% vs. 60.6% of BAT ($p = 0.011$) - CZA treated patients had higher 30-day clinical success: 83.1% vs. 60.6% of BAT ($p = 0.004$) - CZA treated patients had lower 30-day mortality: 13.3% vs. 27.3% of BAT ($p = 0.053$)	Not reported
Daccò, [29]	Italy	Case report	*Burkholderia multivorans*	BSI and brain abscesses	- Successful treatment	Not reported
Canton Bulnes, [30]	Spain	Case report	*Burkholderia cepacia* complex	BSI, LRTI	- Successful treatment	Not reported

AE: adverse event; LUTR: lung transplant recipient; CRPA: Carbapenem-resistant Pseudomonas *aeruginosa; XDR-GNB: Extensively drug-resistant gram-negative bacilli; CPKP: carbapenemase producing* klebsiella pneumoniae*; BSI: bloodstream infection; LRTI: lower respiratory tract infection; cIAI: Complicated intra-abdominal infection; CZA: ceftazidime-avibactam; MDR: multi-drug resistant; BAT: best available therapy; SOT: solid organ transplant; CRE: Carbapenem-resistant Enterobacterales.*

activity against Ambler class A, class C, and specific class D β-lactamases [25]. However, it remains ineffective against metallo-β-lactamase (MBLs). The primary function of this agent is to treat CRE. To address infections caused by MBL-producing bacteria, CZA is co-administered with aztreonam, taking advantage of their synergistic activity [26]. CZA is currently approved for the treatment of cIAI, UTI, and HBAP/VBAP.

For patients with normal renal function, the recommended dosage is 2.5 g administered in 2 h every 8 h.

Evidence in the Clinical and LuTx Setting

The most significant data regarding the use of CZA in LuTx are described in **Table 3**.

A Chinese retrospective observational study on 15 LUTR, investigating the use of CZA in infections caused by CRPA, reported 14-day and 30-day mortality rates of 6.7% and 13.3%, respectively. Moreover, clinical and microbiological cure rates after CZA therapy were 53.3% and 60% [27].

A similar retrospective study conducted on 10 LUTR treated with CZA for carbapenem-resistant *K. pneumoniae* (CRKP) and CRPA infections showed 30-day and 90-day survival rates of 100% and 90%, respectively. However, recurrent CRKP and CRPA infection did occur in 50% of patients [31].

An international, retrospective cohort study evaluated the efficacy of CZA compared with best available therapy (BAT) in a cohort of 149 SOT recipients with BSI caused by CRKP. LUTR accounted for 4% of the overall SOT population, and among the 83 patients treated with CZA, two were LUTR. Treatment with CZA was associated with a significantly higher rate of clinical success at day 14 compared to BAT (80.7% vs. 60.6%). A similar pattern was observed for clinical success at day 30, with statistically significant differences favouring CZA [27].

Notably, CZA treatment was associated with improved survival outcomes in the CAVICOR study, which represents the largest cohort to date investigating the impact of CZA on mortality in infections caused by CRE. However, only 45 out of 339 patients (13.2%) included in the analysis were SOT recipients, and no stratification by type of transplant was provided [32].

Adverse Events and Limitations

CZA has demonstrated a favourable tolerability profile, with no severe AEs reported in the studies reviewed herein. Only mild

AEs were observed. Furthermore, no significant interactions with immunosuppressive therapy were reported.

Key Messages

Real-world clinical experience with CZA in LUTR, particularly in CRE infections, remains limited. While available data support the efficacy and safety of CZA in treating *P. aeruginosa* infections, evidence specifically about LUTR remains scarce. Further studies are warranted to evaluate the use of CZA in this population. Additionally, close monitoring is advised during treatment, especially for the potential emergence of CZA resistance in *K. pneumoniae* producing KPC-2 and KPC-33 [33, 34].

MEROPENEM/VABORBACTAM

Activity Spectrum

Meropenem/Vaborbactam (MVB) combines meropenem with vaborbactam, a novel non-β-lactam BLI.

The primary function of this agent is to treat Enterobacterales that produce KPC enzymes, including those harbouring KPC genes that confer resistance to CZA [35, 36]. In a comparative analysis involving clinical isolates of KPC-positive Enterobacterales, MVB showed more potent *in vitro* activity compared to other drugs alone [37]. Moreover, MVB demonstrated the highest susceptibility rates against the majority of MDRGNB in a surveillance study that included patients with HBAP [38].

However, vaborbactam does not inhibit Ambler classes B or D carbapenemases. MVB's activity against other DTR-Gram-negative varies and its activity against *P. aeruginosa*, *Acinetobacter* spp., is generally comparable to that of MEM alone [39].

For patients with normal renal function, the recommended dosage is 2/2 g administered in 3 h every 8 h.

Evidence in the Clinical and LuTx Setting

Two phase 3 clinical trials have evaluated the efficacy and safety of MVB: the TANGO I trial and the TANGO II trial [40, 41].

The latter is an RCT evaluating the efficacy and safety of MVB versus BAT in adults with CRE infections. Bacteraemia was the more relevant infection (46.8%) while HABAP/VABP was found in 10.6% of patients. ICH, including two SOT recipients, represented 32% of the total cohort. Considering the characteristics of the infections in the population with microbiological confirmation, the trial showed similar mortality rate after 28 days for patients treated with MVB for HABAP/VABP and bacteraemia compared to with BAT (22% vs. 44%, p = NS).

In another retrospective multicentre study, describing clinical characteristics and outcomes of 126 patients treated with MVB for MDRGNB infections, the most common infections were LRTI (38.1%), and the most common isolated pathogens were CRE (78.6%). Thirty-day mortality occurred in 18.3% of patients (n = 23), but only half of these patients received an appropriate dose of medication based on their renal function. Outcomes were similar between patients with CRE and *Pseudomonas* spp. isolates [42].

Lastly, in a retrospective study comparing the efficacy of MVB (n = 26) with CZA (n = 105) in patients with CRE infections, the clinical success rate was similar in both groups, with around half of the patients treated with MVB having an LRTI (n = 12) [41].

Adverse Events and Limitations

In both the TANGO I and TANGO II trials, patients receiving MVB experienced fewer side effects than those receiving other treatments. In the TANGO II trial, AEs associated with MVB included diarrhoea, anaemia and hypokalaemia. Interestingly, patients receiving MVB treatment experienced a lower incidence of renal failure than those receiving BAT [40, 41].

In a study comparing the efficacy of MVB and CZA, rates of AEs were similar between the CZA group and the MVB group (34.3% versus 23.1%, respectively; p = 0.27). Nephrotoxicity was the most frequent AE, with rates of 29.2% and 14.3% in the CZA and MVB groups, respectively (p = 0.16).

Key Messages

Considering its broad spectrum of activity and good lung penetration, MVB could be a promising option for LUTR with infections caused by KPC-producing CRE. The use of an adequate dose adjusted to renal function will be a future challenge in using this molecule, to ensure correct drug exposure and minimise AEs and the development of resistance.

IMIPENEM/RELEBACTAM

Activity Spectrum

Imipenem/Relebactam (I-R) combines imipenem with relebactam, a novel BLI without direct antimicrobial activity, but providing reliable inhibition of many Ambler class A and class C [43] β-lactamases, as well as *Pseudomonas*-derived cephalosporinase [43]. Relebactam is not active against MBLs or class D oxacillinases [44].

The activity of I-R is similar to CZA against CRPA. In addition, in a small percentage of cases, I-R also showed activity against those bacteria that had developed resistance to C/T and CZA [45]. It remains ineffective against *A. baumannii* and *Stenotrophomonas maltophilia* and exhibits limited activity against OXA-48-like enzymes [46]. Some data have highlighted the emergence of I-R resistance during this treatment in patients with *P. aeruginosa* HABAP/HAVAP previously exposed to other cephalosporins. This mechanism could be due to increased expression or structural changes in the MexAB-OprM and MexEF-OprN efflux pumps [47, 48].

I-R against KPC-producing Enterobacterales demonstrated good *in vitro* activity. Different *in vitro* strain analyses reported a 98% susceptibility rate for *K. pneumoniae* producing KPC. However, a pooled estimation of around 280,000 isolates revealed an I-R resistance prevalence rate of approximately 14.6% (95% CI, 0.116%–0.182%), with rates exceeding 50% observed in many countries worldwide [49].

For patients with normal renal function, the recommended dosage is 1.25 g administered in 30′ every 6 h.

Evidence in the Clinical and LuTx Setting

Clinical data on I-R use among LuTx patients is lacking. Safety and efficacy of I-R among patients with VAP or HAP are studied in two Phase 3 non-inferiority trials (RESTORE-IMI-1 and RESTORE-IMI-2).

In the first trial, I-R (n = 21) was compared to colistin plus IMP treatment (n = 10) in patients with IMP-susceptible bacterial infections. Patients were treated for HABAP/HAVAP (n = 11, 35%), cUTI (n = 16, 52%) or cIAI (n = 4, 13%). Favourable overall responses were achieved in both arms (I-R 71%; colistin + IMP, 70%) [50].

The second trial evaluated I-R (n = 246) versus TZP (n = 267) in patients with HABAP/HAVAP. In this trial, critically ill patients were studied, but ICH were excluded. The most common pathogens were *K. pneumoniae* (25.6%), *P. aeruginosa* (18.9%), *Acinetobacter calcoaceticus-baumannii* complex (15.7%), and *Escherichia coli* (15.5%). I-R was non-inferior to TZP, considering 28-day all-cause mortality (15.9% and 21.3% respectively). Instead, on day 28, all-cause mortality in microbiologically modified intent-to-treat patients with a primary diagnosis of HABAP/HAVAP was lower in the I-R arm than the TZP arm (18.6% vs. 30.8%), and the incidence of relapse/clinical failure was comparable between I-R and TZP (14% vs. 12%). Patients with *P. aeruginosa* infections had a lower clinical response and a higher 28-day mortality rate in the I-R arm. Nevertheless, both treatment arms had comparable microbiological eradication rates at the end of treatment (67% for I-R versus 72% for TZP). This result requires further interpretation as it may be due to differences between the treatment groups that are unrelated to the causative pathogen, given the limited sample size in the I-R group [51].

Another randomised non-inferiority trial compared I-R and TZP for the treatment of HABAP/HAVAP. Again, ICH were excluded from this cohort. The study confirmed that I-R was non-inferior to TZP in terms of 28-day all-cause mortality (11% vs. 5.9%; non-inferiority p = 0.024). It should be noted that mortality was numerically higher in the I-R treatment group, despite non-inferiority being reached [52].

Adverse Events and Limitations

I-R treatment is generally well tolerated. The most common AEs in registration studies were anaemia (10%), nausea and diarrhoea (8%), and elevated liver enzymes (12%) [50, 51].

Regarding renal toxicity, I-R was associated with a more favourable renal safety profile than colistin-based therapy in RESTORE IMI-1. So far, some drug-drug interactions have been described [53]. Carbapenems (imipenem as well as meropenem) have been linked with an increased risk of seizures, especially with the concomitant administration of certain antiepileptic drugs (e.g., valproic acid), due to a marked decrease in those drugs' levels. Additionally, concomitant use of ganciclovir requires monitoring due to an increased risk of central nervous system toxicity [54]. These interactions are not associated with the new beta-lactamase inhibitor but are inherent to carbapenems.

Key Messages

The role of I-R among ICH, particularly LUTR, requires further investigation. However, considering the data on patients with pneumonia and its anti-pseudomonal spectrum, I-R could play a promising role in this setting. Caution must be exercised regarding DDI and the potential for resistance to emerge.

CEFIDEROCOL

Activity Spectrum

Cefiderocol (FDC) is a novel catechol-substituted siderophore cephalosporin. FDC can bind extracellular iron and use iron-regulated outer membrane proteins to gain access to bacteria. FDC can overcome resistance mechanisms due to efflux pumps, ubiquitous in MDRGNB such as *P. aeruginosa* [55]. Moreover, FDC's potent activity against MDRGNB is also related to its high stability against various ESBLs and carbapenemases (IMP-1, VIM-2, NDM-1, KPC-2/3, L1, OXA-23) [56]. Moreover, FDC demonstrated *in vitro* activity against AmpC-overproducing strains, a low affinity for chromosomal AmpC β-lactamases, and a low propensity for temporal induction of AmpC β-lactamases [57].

This translates into a potentially vast activity spectrum against MDRGNB, with data from the SIDERO-WT study showing susceptibility rates to FDC of 99.8%, 99.9% and 96% for clinical isolates of Enterobacterales, *P. aeruginosa* and *A. baumannii*, respectively [58]. A recent systematic review reported slightly less favourable proportions, with susceptibility rates to FDC of 97%, 91.2%, and 96% for Enterobacterales, *P. aeruginosa,* and *A. baumannii*, respectively. Of note, FDC-resistance was significant in NDM-producing Enterobacterales (38.8%, 95% CI 22.6%–58.0%), NDM-producing *A. baumannii* (44.7%, 95% CI 34.5%–55.4%), and CZA-resistant Enterobacterales (36.6%, 95% CI 22.7%–53.1%), suggesting a cautious use against these microorganisms [59].

The recommended dosage is 2 g administered in 3 h every 8 h for patients with normal renal function.

Evidence in the Clinical and LuTx Setting

A growing bulk of evidence is accumulating regarding the use of FDC in IC hosts, including LUTR (**Table 4**).

Persaud et al. presented a single-centre, retrospective description of FDC among 15 LUTR. FDC was initiated at a median of 105 days post-transplant, with treatment courses ranging from 1 to 93 days. MDR *P. aeruginosa* was the target pathogen in 13 cases. Of the 15 patients, 14 underwent FDC susceptibility testing, with three yielding an intermediate result, despite no prior exposure to the agent. Overall, 30-day mortality was 26% [60].

In the CEFI-ID study, an analysis of FDC use in 114 ICH adults treated for MDRGNB infections, LUTR constituted 15% (17/114) of the study population. LRTI were the most common infection (55/114, 48.2%), and *P. aeruginosa* (51/114, 56%) was the most common pathogen. At day 28, clinical success was achieved in 53.3% of cases, and overall mortality was 37.7%.

The PERSEUS study, an analysis of the 261 Spanish patients with severe infections due to MDRGNB (excluding *Acinetobacter*

TABLE 4 | Overview of real-life studies describing cefiderocol use among LuTx recipients.

Author, year	Country	Study design	Pathogen	Infection type	Main results	AE
Persaud, [60]	USA	Case series of 15 LUTR	MDR *P. aeruginosa* (14/15)	LRTI	- 30-day all-cause mortality: 26%; - Microbiological clearance: 9/13	Not reported
Soueges, [61]	France	Multicentre retrospective study including 114 ICH (LUTR 14.9%)	*P. aeruginosa* (56%) of whom VIM producers (11.7%)	LRTI (48.2%), cUTI (14%), cIAI (9.6%)	- 28-day clinical success: 53.3% - 28-day mortality: 37.7% - 28-day relaps: 17.5%	Not reported
Torre-Cisneros, [60]	Spain	Early access program analysis including 261 patients (SOT 12.6%)	*P. aeruginosa* (67%), many CZA/C/T resistant	LRTI (47.9%), cIAI (14.6%), cUTI (14.6%)	- 28-day mortality: 21.5% - Clinical cure: 76% (LRTI)	2.2%: one rash, one leukopenia, one fatal toxic epidermal necrolysis
Lombardi, [62]	Italy	Post-hoc multicentre national analysis including 185 patiens (ICH 45.4%, SOT 6.5%)	*P. aeruginosa,* Enterobacterales*, A. baumannii*	Empirical therapy: sepsis (36/54, 66.7%) Targeted therapy: LRTI (56/131, 42.8%)	- 28-day clinical cure: 81% (*P. aeruginosa*), 77.3% (Enterobacterales), 42% (*Acinetobacter baumannii*); - 30-day all-cause mortality: 40.8%	2%: two rash, one increase liver enzyme values, one status epilepticus

AE: adverse event; LUTR: lung transplant recipient; ICH: immunocompromised host; SOT: solid organ transplant; BSI: bloodstream infection; LRTI: lower respiratory tract infection; cIAI: Complicated intra-abdominal infection; cUTI: complicate urinary tract infection; CZA: ceftazidime-avibactam; C/T: ceftolozane/tazobactam; MDR: multidrug-resistant.

spp.) enrolled in the FDC early access program, included a relevant proportion of SOT recipients (34/261, 13%). The most represented pathogen was *P. aeruginosa* (174/261, 66.7%), with 99 (76.7%) isolates resistant to both CZA and C/T. Interestingly, the highest 28-day mortality (27.2%) and the second lowest clinical cure rate (76%) were reported among patients with LRTI, the most common infection site (47.9%), suggesting how infections of this compartment are particularly relevant in shaping the clinical course of patients [61].

Finally, in a post-hoc analysis focused on ICH of the first collected data from the prospective, multicenter national CEFI-SITA study, 84 ICH cases were compared to non-ICH cases. Thirty-day mortality was comparable between ICH and non-ICH (40.8%, 95%CI 27.9%–56.8% vs. 33.3%, 95% CI 22.9–46.9; p = 0.5430). In the multivariable analysis, ICH status and its groups were not associated with higher mortality [63].

Adverse Events and Limitations

FDC is primarily eliminated unchanged in the urine and is not extensively metabolised by the liver [64]. FDC may cause renal impairment [65]. Therefore, it is necessary to closely monitor renal function [64]. Finally, therapeutic and supratherapeutic doses of FDC had no apparent clinically significant effect on the QTc [66].

In the real-life experiences mentioned above, AEs related to FDC were rare or not reported. In the PERSEUS study, 7/314 patients (2.2%) experienced a suspected drug-related AE during FDC administration, and three patients discontinued the drug [60]. In the CEFI-SITA study, 4/200 patients (2.0%) experienced a suspected AE during FDC administration, and FDC was discontinued in two patients [63].

Key Messages

FDC is a solid addition to the therapeutic armamentarium against MDRGNB in LUTR, and scientific societies have endorsed its use for some conditions. In the field of LuTx, FDC is likely a valid solution for treating LRTI due to MDR *P. aeruginosa* and an alternative for infections caused by MBL-producing Enterobacterales. Further studies are required to understand the need for combination therapy when used in difficult-to-reach sites, such as the lung or the abdomen, and the emergence of resistance.

PK/PD OF NEW MOLECULES

All the antibiotics described in this review belong to the β-lactams class. β-lactams are defined as 'time-dependent' antibiotics, indicating that their bactericidal activity is dependent on the proportion of time (T) their unbound concentration (*f*) remains above the minimal inhibitory concentration (MIC) of the bacterial pathogen. The PK/PD index is expressed as $fT > MIC$ [67]. β-lactams are hydrophilic molecules with a relatively small volume of distribution and are eliminated by renal clearance. Hence, physiopathological changes that frequently occur in critically-ill patients can greatly affect the PK of β-lactams [67, 68]. For these reasons, while 50% $fT > MIC$ is likely enough to obtain standard efficacy of β-lactam antibiotics, in critically ill IC individuals up to 100% fT >4-6 x MIC should be ensured for optimal drug exposure and suppression of resistance development [67, 69, 70].

Rando et al. recently published a systematic review specifically focused on pulmonary PK/PD data of novel β-lactams. Overall, probabilities of target attainment rates were reported above 90% using current licensed dosing regimens, although significant heterogeneity was reported between studies, both in terms of clinical population and PK models [71].

When dealing with LUTR with severe infections, major strategies to overcome the PK/PD challenges and optimise β-lactam efficacy include prolonged infusion (PI) and TDM. The duration of β-lactams infusion has been shown to influence their $fT > MIC$, increasing the chances of target attainment. Several experimental and clinical studies support PI (either continuous or

extended infusion) of β-lactams in the setting of severe infections [69, 70, 72].

In 2024, Abdul-Aziz et al. conducted a meta-analysis of 18 randomised controlled trials comparing PI versus intermittent infusion of β-lactams in critically ill adults with sepsis/septic shock, involving over 9,000 patients. PI was associated with reduced all-cause 90-day mortality (risk ratio 0.86, 95%CrI 0.72–0.98), reduced risk of ICU-mortality (risk ratio 0.84, 95%CrI 0.70–0.97) and an increase in clinical cure (risk ratio 1.16, 95%CrI 1.07–1.31) [72].

In 2022, the first consensus guidance on the use of β-lactams as PI was published. The panel was unanimously in favour of PI over standard infusion in severely ill adult patients, particularly those with MDRGNB infections [70].

TDM consists of measuring a drug concentration in a specific biological sample to help clinicians achieve the PK/PD target. Yet, implementation of β-lactams TDM into a hospital system requires resources, practical workflow considerations and expertise that need to be considered thoroughly [67, 73]. Patients that would benefit the most from β-lactams TDM are those at risk of sub-therapeutic concentrations due to PK variability (e.g., augmented renal clearance, ECMO or renal replacement therapy) or PD characteristics (e.g., MDR bacteria with high MIC values or deep-seated infection with high bacterial inoculum) [67].

In 2022, Pai Mangalore et al. conducted a systematic review and meta-analysis on TDM-guided dosing in over 1,400 critically ill patients. The TDM group was associated with increased target attainment (risk ratio 1.85, 95%CI 1.08–3.16) and improved clinical cure (risk ratio 1.17, 95% CI 1.04–1.31), microbiological cure (risk ratio 1.14, 95%CI 1.03–1.27), and reduced treatment failure (risk ratio 0.79, 95%CI 0.66–0.94) [74].

On the other hand, the clinical benefit of beta-lactam TDM have been questioned. Evidence, including recent meta-analyses, shows no clear impact of TDM on mortality or clinical cure. In general, most beta-lactams are well tolerated, supporting high-dose regimens, even with mild renal impairment, usually guaranteeing high serum concentrations [75].

In the management of LUTR patients, a unique condition is represented by the perioperative period. Candidates often arrive at transplant with respiratory tract colonisation by MDR bacteria and could require novel β-lactams as antibiotic prophylaxis [62]. Antibiotic underdosing in prophylactic regimens may increase the risk of infection and ultimately, graft failure.

In 2020, Taccone et al. published a single-centre retrospective study on 70 LUTR that received prophylactic therapy with a β-lactam antibiotic and underwent TDM in the early postoperative period. Insufficient drug concentrations were found in 28/70 (40%) patients, significantly associated with CF, younger age and increased creatinine clearance. Interestingly, patients with inadequate drug concentrations during postoperative antibiotic prophylaxis developed MDR acquisition and/or early infection more frequently than those with adequate drug concentrations (22/28, 79% vs. 20/42, 48%; $p = 0.01$) [76].

Studies assessing the PK/PD target attainment of new β-lactams when employed in transplant prophylaxis are required to evaluate the need for routine TDM in this crucial phase of the patients' management.

CONCLUSION

Infections caused by MDRGNB remain a critical challenge in LUTR. The availability of new antibiotics, supported by growing evidence on their PK/PD profiles, safety, and efficacy, offers valuable therapeutic options. However, clinical experience in the LuTx setting is still limited for several agents, and optimal use often requires individualised dosing strategies, TDM, and a deep understanding of local resistance patterns. Future research should prioritise prospective, SOT-specific trials to define the most effective and safe use of these agents and to guide stewardship in this vulnerable population.

AUTHOR CONTRIBUTIONS

AL, DM, and AB conceived the study. AL, DM, GV, LA and PS wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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GENERATIVE AI STATEMENT

The author(s) declared that generative AI was not used in the creation of this manuscript.

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GLOSSARY

ABC *Acinetobacter baumannii-calcoaceticus* complex

AE Adverse event

BAT Best available therapy

BL β-lactam

BLI β-lactamase inhibitor

BSI Bloodstream infection

CF Cystic fibrosis

CI Continuous infusion

cIAI Complicated intra-abdominal infection

CNS Central nervous system

CRE Carbapenem-resistant Enterobacterales

CRKP Carbapenem-resistant *Klebsiella pneumoniae*

CRPA Carbapenem-resistant *Pseudomonas aeruginosa*

C/T Ceftolozane/tazobactam

cUTI Complicated urinary tract infection

CZA Ceftazidime/avibactam

DTR Difficult-to-treat

ECMO Extracorporeal membrane oxygenation

ESBL Extended-spectrum β-lactamase

FDC Cefiderocol

HABAP/HAVAP Hospital-acquired/ventilator-associated bacterial pneumonia

ICH Immunocompromised host

ICU Intensive care unit

IPM Imipenem

I-R Imipenem-relebactam

KPC *Klebsiella pneumoniae* carbapenemase

LRTI Lower respiratory tract infection

LUTR Lung transplant recipient

MBL Metallo-β lactamase

MDR Multidrug-resistant

MDRGNB Multidrug-resistant Gram-negative bacteria

MIC Minimal inhibitory concentration

MVB Meropenem/vaborbactam

NDM New Delhi metallo-β-lactamase

OXA Oxacillinase

PI Prolonged infusion

PK/PD Pharmacokinetic/pharmacodynamic

SOT Solid organ transplant

TDM Therapeutic drug monitoring

TZP Piperacillin/tazobactam

VIM Verona Integron-encoded Metallo-β-lactamase

XDR Extensively drug-resistant

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Exploring Disease-Specific Waitlist Outcomes in Simultaneous Liver-Kidney Transplantation

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The current allocation system does not account for liver etiology in simultaneous liver-kidney transplantation (SLKT). This study aims to assess differences in waitlist outcomes among major liver disease groups (alcohol-related liver disease [ALD], metabolic dysfunction-associated steatohepatitis [MASH], hepatitis C virus infection, and biliary diseases) in SLKT using Organ Procurement and Transplantation Network (OPTN) registry. In total, 4,846 adult SLKT candidates listed between January 2018 and March 2024 were enrolled. Patients with MASH had worse waitlist 1-year mortality compared to ALD adjusted for patient characteristics at listing (HR 1.300, 95% CI 1.059–1.597, p = 0.012), whereas the 1-year SLKT probability was comparable. When patients were categorized by MELD score at listing (6–20, 21–29, and ≥30), patients with MASH had significantly higher 1-year waitlist mortality compared to those with ALD in the middle MELD score group (HR 1.365, 95% CI 1.008–1.834, p = 0.044). Prior to the allocation policy change in 2020, patients with MASH experienced higher waitlist mortality compared to ALD, however, this disparity was not observed following the policy change. Waitlist outcomes varied significantly depending on the etiology in SLKT. The revised 2020 allocation policy may be temporally associated with changes in mortality disparities across different liver etiologies.

Keywords: allocation policy change, liver etiology, metabolic dysfunction-associated steatohepatitis, simultaneous liver-kidney transplantation, waitlist outcomes

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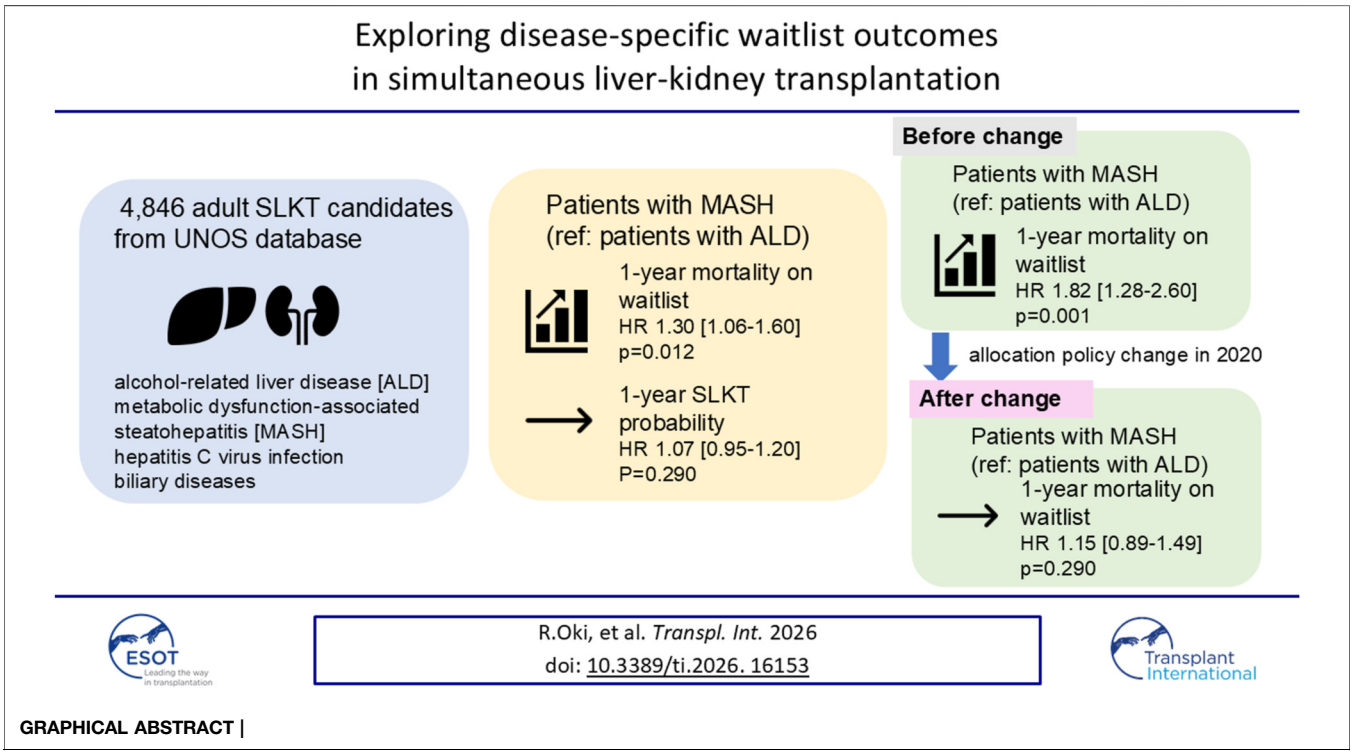
INTRODUCTION

Simultaneous liver and kidney transplantation (SLKT) is an appropriate option for those suffering from end-stage liver disease and critical renal insufficiency. Following the introduction of the Model for End-Stage Liver Disease (MELD) scoring system in 2002, the number of SLKT has increased because serum creatinine substantially impacts MELD score prioritization [1]. Due to concern about the potentially disproportionate use of renal allografts in SLKT, standardized medical criteria were established in 2017 to promote equitable allocation between kidney and liver transplant candidates [2, 3]. We previously reported that following the 2017 policy change, the number of SLKT declined despite increased registrations, while LT (liver transplantation) alone increased and were associated with worse 1-year graft survival (GS), highlighting the need for ongoing evaluation of transplant policy [4].



GRAPHICAL ABSTRACT |

As the MELD score does not incorporate the etiology of liver disease, the current allocation system does not account for liver etiology. We previously evaluated disease-specific differences in waitlist outcomes among LT alone candidates with different underlying etiologies, including alcohol-related liver disease (ALD), nonalcoholic steatohepatitis (NASH), hepatitis C virus infection (HCV), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis [5]. Patients with NASH had a significantly higher risk of 1-year waitlist mortality than those with ALD at comparable MELD scores [5]. The recent retrospective studies also showed that patients with metabolic dysfunction-associated steatohepatitis (MASH) had higher LT waitlist mortality [6, 7]. An Italian registry study showed that patients with MASH were associated with higher LT waitlist mortality, while these candidates demonstrated the greatest 5-year LT survival benefit at the same MELD score [6]. However, few studies have evaluated potential differences in disparities in waitlist outcomes among major liver disease etiologies in the context of SLKT.

This study aims to assess differences in waitlist outcomes among major liver disease groups, including ALD, HCV, MASH and biliary diseases by using Organ Procurement and Transplantation Network (OPTN) dataset in the contemporary SLKT policy era established in 2017. On February 4, 2020, the liver allocation system in the United States underwent a major revision, replacing donation service areas with acuity circles as the geographic basis for organ distribution, and shifting from a predominantly local allocation approach to a broader sharing model [8]. Therefore, we also examined whether disease-specific waitlist outcomes changed before and after the allocation system change in 2020.

MATERIALS AND METHODS

Patients and Data Collection

This was a retrospective cohort study using OPTN database. We included all adult (age at listing >18 years) SLKT candidates who were listed between January 2018 and March 2024 with last follow-up as of March 2025, and whose primary liver disease etiologies were ALD, MASH, HCV, and biliary diseases. Biliary diseases included primary biliary cholangitis, primary sclerosing cholangitis, and other biliary diseases. Following the consensus policy enacted by UNOS in 2017, candidates for SLKT must meet specific criteria, including having chronic kidney disease (CKD), sustained acute kidney injury (AKI), or a metabolic disease, as diagnosed by a nephrologist [9]. For CKD, eligibility requires an estimated glomerular filtration rate (eGFR) ≤60 mL/min for at least 90 days, an eGFR ≤30 mL/min at the time of listing, or ongoing chronic dialysis [9]. For sustained AKI, candidates must have received dialysis for a minimum of 6 weeks or have had documented eGFR values ≤25 mL/min during that period [9]. Patient characteristics at listing included age, sex, race, liver disease etiology, frailty level, body mass index (BMI), dialysis, creatinine (Cr), diabetes, ascites, encephalopathy, life support use, serum sodium level, total bilirubin, international normalized ratio (INR) and MELD score. Race was classified into five categories; White, Black, Hispanic, Asian, and Others. The frailty level was based on the Karnofsky Performance Status, with scores of 80 or higher classified as normal, scores of 50–70 classified as mild frailty, and scores of 10–40 classified as severe frailty [10]. Exclusion criteria included the following: (1) LT combined with thoracic organ(s), pancreas, and/or intestine,

(2) patients with MELD exception for HCC and other reasons (non-HCC condition), (3) patients whose liver etiology was not classified into ALD, MASH, HCV, and biliary diseases, (4) patients with overlapping liver disease etiologies (e.g., HCV and ALD).

This study used the OPTN dataset provided by the United Network for Organ Sharing (UNOS), in which all individually identifiable information is encrypted. Henry Ford Institutional Review Board (IRB) exempted this study from IRB approval. All methods of research procedures were performed in accordance with the Declaration of Helsinki.

Waitlist Outcomes

The waitlist outcomes included mortality and SLKT. Removal from the waitlist due to clinical deterioration (too sick for transplantation) was included in mortality. Mortality, SLKT, LT alone (not undergoing KT (kidney transplantation) due to recovery of kidney function or other reasons), recovery of liver function (too well for transplantation), and removal from the waitlist for other reasons were considered competing risk events. Differences in waitlist outcomes between liver etiologies were evaluated. ALD was selected as the reference etiology for the following reasons: (1) it represents one of the major underlying causes of SLKT, (2) it was used as the reference group in our prior study of LT waitlist outcomes [5], and (3) it has been shown to have a lower risk of mortality on the LT waitlist compared with other liver diseases [11].

First, 90-day and 1-year waitlist outcomes were compared across the four liver disease etiologies. Then, patients were stratified into three categories based on their MELD score at listing (6–20, 21–29, and ≥30), and 90-day and 1-year waitlist outcomes were subsequently compared across the disease etiologies.

To assess whether waitlist outcomes among liver disease etiologies differed before and after the February 4, 2020 allocation policy change, we defined two periods: Period 1 (listed from January 1, 2018 to February 3, 2020) and Period 2 (listed from February 4, 2020 to March 31, 2024). Patients were assigned to Period 1 if their listing initiation date was before February 4, 2020, and to Period 2 if their initiation date was on or after February 4, 2020. For those listed in Period 1 whose removal date extended beyond February 3, 2020, follow-up was administratively censored at February 3, 2020. Waitlist outcomes were then compared across liver etiologies in each period separately.

Post-Transplantation Outcomes

Among patients who underwent SLKT, we also examined the association between etiology and patient death within 1 year as well as patient death within 3 years. We further assessed whether cause-specific mortality varied across etiologies.

Statistical Analyses

All statistical analyses were conducted using the following software (SPSS®, Version <28.0.1>; IBM Corp., Armonk, NY, USA), EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan, Version <1.70>), and a graphical user interface for R (R Foundation for Statistical Computing, Vienna, Austria). Continuous data were expressed as mean ± standard deviation or median (interquartile range). Student's t-tests or Mann–Whitney U-tests were used to compare continuous variables. The chi-square test or Fisher's exact test was used to compare the categorical variables. To assess differences in 90-day and 1-year waitlist outcomes, the cumulative incidence of competing events was compared using Gray's test, and multivariable Fine-Gray proportional hazards regression was performed to identify risk factors. Among patients who underwent SLKT, we evaluated the association between etiology and cause-specific mortality using multivariable Fine–Gray proportional hazards regression. Multivariable Cox regression analysis was performed to examine the association between liver etiology and patient death among patients who received SLKT. The risks were adjusted for patient characteristics at listing, including age [12], ascites [13], BMI [12], diabetes [12], encephalopathy [14], frailty [15], dialysis [13], life support measures [13], sex [16], MELD score [16], and race [17], which have been reported to be factors associated with waitlist outcomes for LT. *P-values* less than 0.05 were considered as significant.

RESULTS

Characteristics of Study Participants

In total, 8,026 adult SLKT candidates were listed during the study period. Excluding 110 candidates listed with other organs at the same time and 328 patients with MELD exception, a total of 7,588 patients were identified. Among them, only those with ALD (n = 1,751), HCV (n = 628), MASH (n = 2,183), or biliary diseases (n = 285) were included in the study cohort (Total n = 4,846). **Table 1** presents the baseline characteristics of all patients. The median patient age was 60.0 years and females accounted for 43.5%. The median MELD score at listing was 24, and approximately half of the recipients were already on dialysis. Patient backgrounds differed significantly across all etiologies of liver dysfunction. Patients with MASH had the highest age, BMI, and prevalence of diabetes mellitus. In patients with ALD, the rate of dialysis and moderate to severe hepatic encephalopathy was higher than in other groups, and their MELD scores were the highest among all etiologies.

Comparison of Waitlist Outcomes Among Liver Disease Etiologies

Figure 1 shows the unadjusted cumulative incidence of waitlist outcomes among patients with the four liver disease etiologies. There was no difference in 90-day waitlist mortality according to liver etiology (p = 0.062). ALD patients demonstrated the highest 90-day SLKT probability (p < 0.001). In contrast, patients with

TABLE 1 | Background characteristics of patients on waitlist.

Variables	All (N = 4,846)	ALD (N = 1,751)	HCV (N = 628)	MASH (N = 2,183)	Biliary disease (N = 285)	P value
Age (y.o.)	60.0 [53.0, 66.0]	55.0 [45.0, 62.0]	61.0 [57.0, 65.0]	63.0 [57.0, 67.0]	61.0 [52.0, 66.0]	<0.001
Female (%)	2,107 (43.5)	521 (29.8)	198 (31.5)	1,220 (55.9)	169 (59.3)	<0.001
BMI	28.4 [24.7, 33.1]	26.9 [23.4, 30.9]	27.1 [23.9, 31.1]	30.8 [26.9, 35.4]	25.8 [22.6, 29.9]	<0.001
Diabetes (%)	2,527 (52.2)	439 (25.1)	371 (59.1)	1,609 (73.8)	108 (36.9)	<0.001
Race (%)						<0.001
White	3,106 (64.1)	1,201 (68.6)	266 (42.4)	1,474 (67.5)	165 (57.9)	
Black	418 (8.6)	104 (5.9)	174 (27.7)	74 (3.4)	66 (23.2)	
Hispanic	1,062 (21.9)	362 (20.7)	154 (24.5)	509 (23.3)	38 (13.3)	
Asian	164 (3.4)	48 (2.7)	26 (4.1)	83 (3.8)	7 (2.5)	
Others	96 (2.0)	36 (2.1)	8 (1.3)	43 (2.0)	9 (3.2)	
Albumin (g/dL)	3.30 [2.80, 3.70]	3.30 [2.80, 3.70]	3.40 [2.90, 3.90]	3.20 [2.80, 3.70]	3.20 [2.70, 3.70]	<0.001
Bilirubin (mg/dL)	1.70 [0.90, 3.80]	2.20 [1.10, 6.15]	0.90 [0.50, 1.90]	1.60 [0.90, 3.10]	2.10 [0.90, 7.70]	<0.001
Creatinine (mg/dL)	2.94 [1.95, 4.61]	3.11 [2.04, 4.70]	3.92 [2.33, 6.12]	2.68 [1.85, 4.11]	2.90 [1.96, 4.42]	<0.001
INR	1.40 [1.20, 1.76]	1.50 [1.20, 1.90]	1.20 [1.10, 1.50]	1.40 [1.20, 1.70]	1.30 [1.10, 1.60]	<0.001
Sodium	136 [133, 139]	136 [133, 138]	138 [135, 140]	137 [134, 139]	137 [134, 140]	<0.001
MELD score	24 [21, 31]	27 [22, 34]	22 [20, 26]	23 [20, 29]	23 [20, 31]	<0.001
Dialysis (%)	2,389 (49.3)	1,039 (59.3)	359 (57.3)	872 (39.9)	119 (41.8)	<0.001
Life support measure (%)	345 (7.1)	166 (9.5)	39 (6.2)	122 (5.6)	18 (6.3)	<0.001
Ascites (%)	2,165 (44.7)	902 (51.5)	208 (33.1)	1,027 (47.0)	82 (28.2)	<0.001
Encephalopathy						<0.001
None/mild (%)	4,320 (89.1)	1,523 (87.0)	583 (92.8)	1945 (89.1)	270 (94.7)	
Moderate/Severe (%)	526 (10.9)	228 (13.0)	45 (7.2)	238 (10.9)	15 (5.3)	
Frailty						<0.001
Normal (%)	624 (13.1)	181 (10.5)	99 (15.9)	301 (14.1)	43 (15.4)	
Mild (%)	2,248 (47.3)	722 (42.1)	321 (51.6)	1,065 (49.9)	140 (50.0)	
Severe (%)	1883 (39.6)	814 (47.4)	202 (32.5)	770 (36.0)	97 (34.6)	

ALD, alcohol-related liver disease; HCV, hepatitis C virus infection; MASH, metabolic dysfunction-associated steatohepatitis; BMI, body mass index; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease.

MASH experienced significantly higher 1-year waitlist mortality ($p < 0.001$), whereas patients with ALD had the highest 1-year SLKT probability ($p < 0.001$, **Figure 2**). We next evaluated the probability of LT alone (not undergoing KT due to recovery of kidney function or other reasons). ALD patients demonstrated the highest 90-day and 1-year LT alone probability ($p < 0.001$, $p = 0.001$, respectively). The 90-day cumulative incidence was 4.6% (95% CI 3.7–5.6) in patients with ALD, 1.8% (0.9–3.0) in HCV, 2.6% (2.0–3.3) in MASH, and 2.1% (0.9–4.3) in biliary disease. The 1-year cumulative incidence was 6.0% (95% CI 5.0–7.2) in patients with ALD, 2.7% (1.6–4.2) in HCV, 4.1% (3.3–5.0) in MASH, and 3.2% (1.6–5.7) in biliary disease (**Supplementary Figure S1**).

Adjusted Risks for Waitlist Outcomes in Each Liver Disease Etiology vs. ALD

Adjusted hazards of 90-day and 1-year waitlist mortality and SLKT probability in HCV, MASH and biliary diseases were estimated and compared to the ALD group. No significant difference in 90-day waitlist mortality or SLKT probability was observed among the liver disease etiologies (**Table 2**). The overall risk of 1-year waitlist mortality was significantly higher in MASH compared to ALD (HR 1.300, 95% CI 1.059–1.597, $p = 0.012$), whereas the 1-year SLKT probability was comparable between MASH and ALD (**Table 3**). Regarding the probability of LT alone (not undergoing KT due to recovery of kidney function or other reasons), there was no significant difference between the liver etiologies (**Supplementary Table S1**).

Adjusted Risks for Waitlist mortality in Each Liver Disease Etiology vs. ALD According to MELD Score Category at Listing

When evaluating outcomes according to MELD score categories at listing, 90-day waitlist mortality did not significantly differ across liver disease etiologies in any MELD score group. In the higher MELD score subgroup, patients with MASH demonstrated a higher 90-day SLKT probability compared to those with ALD. (HR 1.239, 95% CI 1.038–1.478, $p = 0.018$, **Table 4**).

In contrast, the 1-year waitlist mortality in the middle MELD score subgroup was significantly higher (HR 1.365, 95% CI 1.009–1.834, $p = 0.044$), although the 1-year SLKT probability did not differ across the etiologies (**Table 5**). In the higher MELD score subgroup, patients with MASH demonstrated a higher 1-year SLKT probability compared to those with ALD. (HR 1.188, 95% CI 1.001–1.410, $p = 0.048$).

Change of Waitlist Outcomes Before and After Allocation Policy Change

To assess differences in waitlist outcomes among liver disease etiologies before and after the February 4, 2020 allocation policy change, we compared these outcomes across etiologies within each period. There were no significant differences in 90-day waitlist mortality across liver etiologies before the allocation

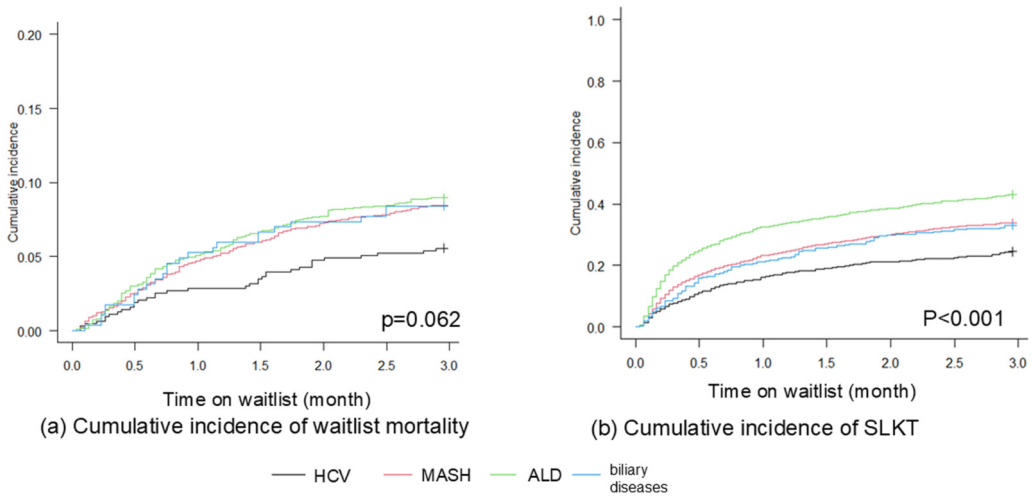


FIGURE 1 | Cumulative incidence curves showing 90-day **(a)** waitlist mortality and **(b)** SLKT probability, among HCV, MASH, ALD, and biliary diseases (Gray test). **(a)** There were no significant differences among liver etiologies for 90-day mortality (p = 0.062). **(b)** Patients with ALD demonstrated the highest 90-day SLKT probability (p < 0.001). ALD, alcohol-related liver disease; HCV, hepatitis C virus infection; MASH, metabolic dysfunction-associated steatohepatitis.

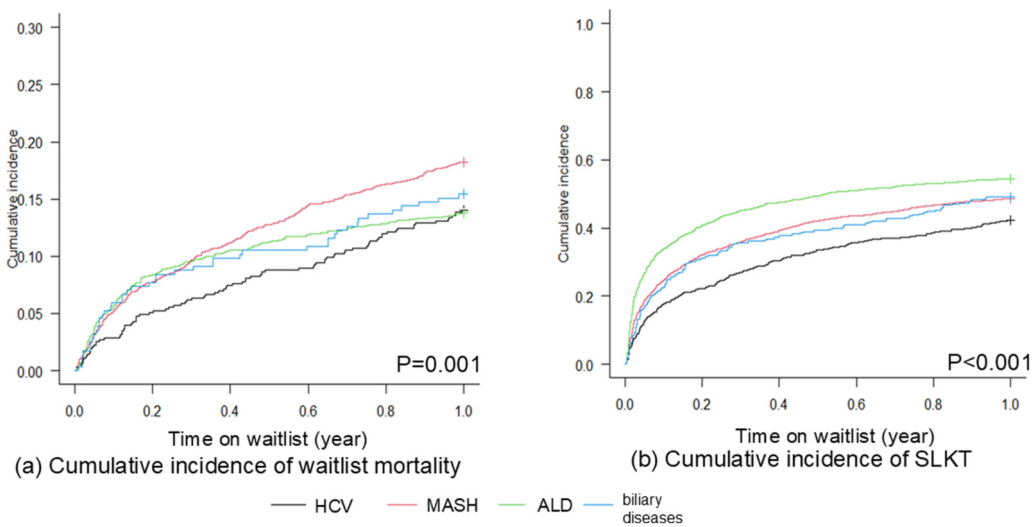


FIGURE 2 | Cumulative incidence curves showing 1-year **(a)** waitlist mortality and **(b)** SLKT probability among HCV, MASH, ALD, and biliary diseases (Gray test). **(a)** Patients with MASH demonstrated the highest 1-year waitlist mortality (p = 0.001). **(b)** Patients with ALD demonstrated the highest 1-year SLKT probability (p < 0.001). ALD, alcohol-related liver disease; HCV, hepatitis C virus infection; MASH, metabolic dysfunction-associated steatohepatitis.

policy change (**Figure 3**). There were no significant differences in 90-day SLKT probability across liver etiologies in both periods (**Figure 4**).

Regarding 1-year waitlist mortality, patients with MASH had significantly worse mortality prior to the allocation policy change (HR 1.899, 95% CI 1.310–2.753, p < 0.001); however, this difference was no longer significant in the post-policy era. (HR 1.122, 95% CI 0.865–1.455, p = 0.390) (**Figure 5**) The 1-year SLKT probability in patients with MASH was comparable to that in patients with ALD in both periods (**Figure 6**).

The Probability of Patient Death Among Patients Who Received SLKT

Among patients who underwent SLKT, those with MASH or biliary disease had a significantly higher risk of patient death at 1 year and 3 years post-transplantation compared with those with ALD, after adjustment for clinical factors at listing (**Table 6**). There was no significant difference in distribution of causes of death across liver etiologies (p = 0.858). Cardiovascular disease (CVD) and infection were the leading causes of death in all groups (**Supplementary Table S2**). In cause-specific analyses, patients with MASH or biliary disease

TABLE 2 | Multivariable Fine-Gray proportional hazards regression for 90-day waitlist outcomes.

Variables	Death		SLKT	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age	1.018 (1.006–1.030)	0.003	1.002 (0.997–1.008)	0.440
Female	1.239 (1.003–1.529)	0.047	0.814 (0.730–0.907)	<0.001
BMI	0.992 (0.974–1.010)	0.360	0.994 (0.986–1.003)	0.200
Diabetes	1.155 (0.907–1.472)	0.240	1.032 (0.916–1.162)	0.610
Etiology of liver disease (ref; ALD)				
HCV	0.949 (0.620–1.453)	0.810	0.887 (0.733–1.073)	0.220
MASH	1.167 (0.882–1.544)	0.280	1.080 (0.963–1.275)	0.150
Biliary disease	1.201 (0.763–1.890)	0.430	0.967 (0.761–1.228)	0.780
Race (ref: White)				
Black	1.065 (0.728–1.559)	0.740	0.821 (0.656–1.029)	0.086
Hispanic	1.028 (0.801–1.320)	0.830	0.726 (0.634–0.831)	<0.001
Asian	0.491 (0.217–1.112)	0.088	0.788 (0.579–1.072)	0.130
Others	0.581 (0.243–1.386)	0.220	0.912 (0.648–1.284)	0.600
MELD score	1.103 (1.086–1.120)	<0.001	1.100 (1.090–1.110)	<0.001
Dialysis	1.138 (0.908–1.427)	0.260	0.929 (0.829–1.039)	0.200
Life support measures	1.070 (0.781–1.463)	0.680	0.774 (0.623–0.962)	0.021
Ascites	1.182 (0.954–1.465)	0.130	1.136 (1.020–1.265)	0.020
Encephalopathy (ref; none or mild)				
Severe	1.037 (0.784–1.372)	0.800	1.044 (0.883–1.235)	0.610
Frailty (ref; normal)				
Severe	2.257 (1.358–3.750)	0.002	1.627 (1.333–1.985)	<0.001

ALD, alcohol-related liver disease; HCV, hepatitis C virus infection; MASH, metabolic dysfunction-associated steatohepatitis; BMI, body mass index; MELD, Model for End-Stage Liver Disease; SLKT, simultaneous liver-kidney transplantation.

TABLE 3 | Multivariable Fine-Gray proportional hazards regression for 1-year waitlist outcomes.

Variables	Death		SLKT	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age	1.023 (1.014–1.032)	<0.001	1.002 (0.997–1.007)	0.480
Female	1.170 (1.005–1.362)	0.043	0.835 (0.761–0.915)	<0.001
BMI	0.984 (0.971–0.997)	0.017	1.001 (0.993–1.008)	0.840
Diabetes	1.062 (0.895–1.259)	0.490	1.033 (0.936–1.141)	0.510
Etiology of liver disease (ref; ALD)				
HCV	1.093 (0.829–1.442)	0.530	0.953 (0.819–1.108)	0.530
MASH	1.300 (1.059–1.597)	0.012	1.066 (0.947–1.200)	0.290
Biliary disease	1.200 (0.857–1.680)	0.290	1.040 (0.856–1.269)	0.680
Race (ref: White)				
Black	0.923 (0.688–1.239)	0.590	0.864 (0.724–1.031)	0.110
Hispanic	1.020 (0.853–1.220)	0.830	0.795 (0.711–0.890)	<0.001
Asian	0.797 (0.511–1.245)	0.320	0.837 (0.654–1.072)	0.160
Others	0.642 (0.341–1.212)	0.170	1.088 (0.827–1.430)	0.550
MELD score	1.042 (1.030–1.055)	<0.001	1.069 (1.061–1.077)	<0.001
Dialysis	1.076 (0.916–1.263)	0.370	0.903 (0.819–0.994)	0.037
Life support measures	1.112 (0.850–1.456)	0.440	0.827 (0.681–1.006)	0.057
Ascites	1.173 (1.005–1.369)	0.043	1.102 (1.006–1.208)	0.037
Encephalopathy (ref; none or mild)				
Severe	1.040 (0.829–1.307)	0.730	1.043 (0.895–1.215)	0.590
Frailty (ref; normal)				
Severe	1.250 (0.956–1.635)	0.100	1.465 (1.258–1.705)	<0.001

ALD, alcohol-related liver disease; HCV, hepatitis C virus infection; MASH, metabolic dysfunction-associated steatohepatitis; BMI, body mass index; MELD, Model for End-Stage Liver Disease; SLKT, simultaneous liver-kidney transplantation.

TABLE 4 | Multivariable Fine-Gray proportional hazards regression for 90-day waitlist outcomes categorized by initial MELD score. Adjusted for baseline characteristics at listing, including age, ascites, BMI, diabetes, encephalopathy, dialysis, life support measures, frailty, sex and race.

Variables	MELD 6–20		MELD 21–29		MELD>30	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Etiology of liver disease (ref; ALD)						
(a) Death						
HCV	2.293 (0.663–7.925)	0.190	0.697 (0.347–1.399)	0.310	1.260 (0.707–2.241)	0.430
MASH	0.998 (0.323–3.081)	1.000	1.393 (0.867–2.238)	0.170	1.041 (0.726–1.493)	0.830
Biliary disease	Not calculable		1.391 (0.611–3.167)	0.430	1.277 (0.748–2.181)	0.370
(b) SLKT						
HCV	1.437 (0.741–2.787)	0.280	0.809 (0.623–1.049)	0.110	0.934 (0.696–1.252)	0.650
MASH	1.717 (0.959–3.076)	0.069	0.934 (0.768–1.135)	0.490	1.239 (1.038–1.478)	0.018
Biliary disease	1.571 (0.687–3.594)	0.280	0.840 (0.589–1.198)	0.340	0.956 (0.709–1.287)	0.760

ALD, alcohol-related liver disease; HCV, hepatitis C virus infection; MASH, metabolic dysfunction-associated steatohepatitis; BMI, body mass index; MELD, Model for End-Stage Liver Disease; SLKT, simultaneous liver-kidney transplantation.

TABLE 5 | Multivariable Fine-Gray proportional hazards regression for 1-year waitlist outcomes categorized by initial MELD score at listing. Adjusted for baseline characteristics at listing, including age, ascites, BMI, diabetes, encephalopathy, dialysis, dialysis duration, life support measures, frailty, sex and race.

Variables	MELD 6–20		MELD 21–29		MELD>30	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Etiology of liver disease (ref; ALD)						
(a) Death						
HCV	1.916 (1.018–3.606)	0.044	0.881 (0.601–1.292)	0.520	1.530 (0.928–2.524)	0.095
MASH	1.667 (0.962–2.891)	0.069	1.365 (1.008–1.834)	0.044	1.121 (0.797–1.576)	0.510
Biliary disease	0.722 (0.253–2.062)	0.540	1.386 (0.854–2.250)	0.190	1.203 (0.712–2.034)	0.490
(b) SLKT						
HCV	0.883 (0.595–1.310)	0.540	0.999 (0.817–1.222)	0.990	0.934 (0.708–1.233)	0.630
MASH	1.115 (0.820–1.515)	0.490	0.989 (0.840–1.164)	0.890	1.188 (1.001–1.410)	0.048
Biliary disease	1.207 (0.766–1.900)	0.420	1.041 (0.786–1.379)	0.780	0.960 (0.724–1.272)	0.780

ALD, alcohol-related liver disease; HCV, hepatitis C virus infection; MASH, metabolic dysfunction-associated steatohepatitis; BMI, body mass index; MELD, Model for End-Stage Liver Disease; SLKT, simultaneous liver-kidney transplantation.

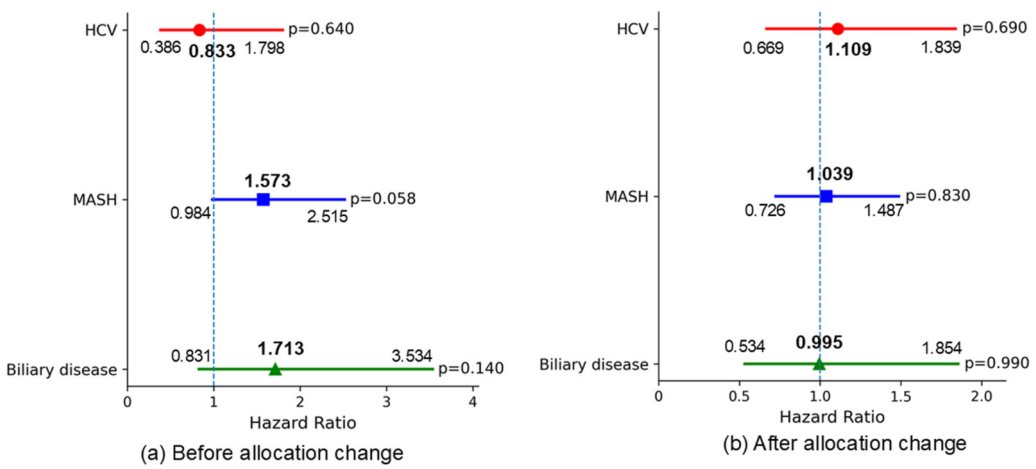


FIGURE 3 | Multivariable Fine-Gray proportional hazards regression for 90-day waitlist mortality (reference: ALD). Adjusted for baseline characteristics at listing, including age, ascites, BMI, diabetes, encephalopathy, dialysis, life support measures, sex, MELD score, frailty, and race. **(a)** No significant differences in 90-day waitlist mortality were observed among the liver etiologies before the allocation policy change. **(b)** No significant differences in 90-day waitlist mortality were observed among the liver etiologies after the allocation policy change. ALD, alcohol-related liver disease; HCV, hepatitis C virus infection; MASH, metabolic dysfunction-associated steatohepatitis; BMI, body mass index; MELD, Model for End-Stage Liver Disease.

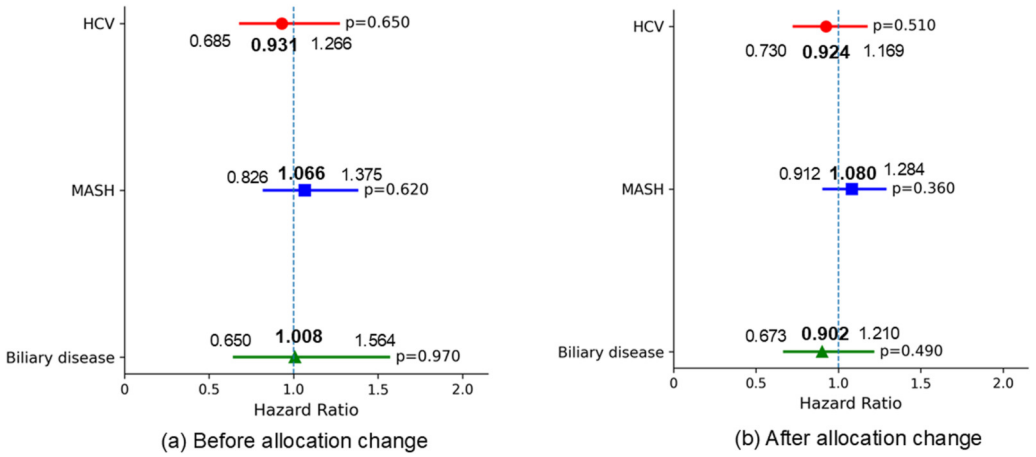


FIGURE 4 | Multivariable Fine-Gray proportional hazards regression for 90-day SLKT probability (reference: ALD). Adjusted for baseline characteristics at listing, including age, ascites, BMI, diabetes, encephalopathy, dialysis, life support measures, sex, MELD score, frailty, and race. **(a)** No significant differences in 90-day SLKT probability were observed among the liver etiologies before the allocation policy change. **(b)** No significant differences in 90-day SLKT probability were observed among the liver etiologies after the allocation policy change. ALD, alcohol-related liver disease; HCV, hepatitis C virus infection; MASH, metabolic dysfunction-associated steatohepatitis; BMI, body mass index; MELD, Model for End-Stage Liver Disease.

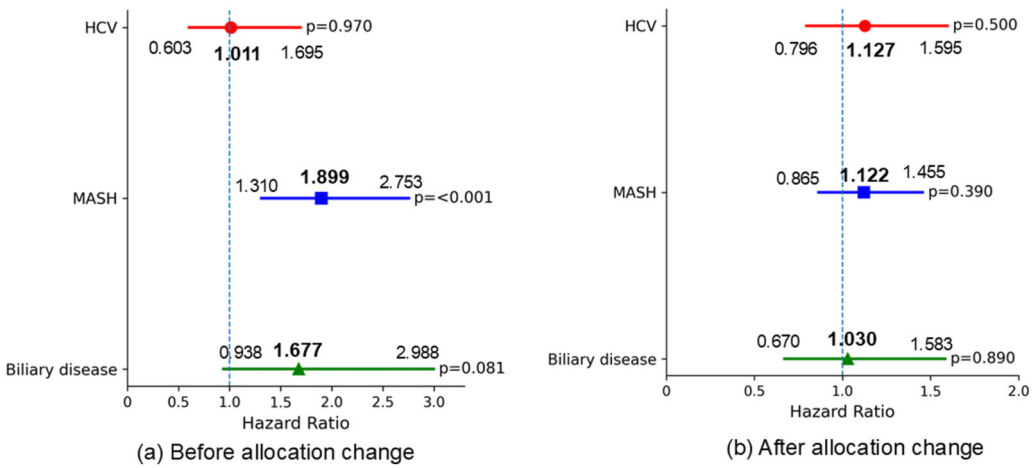


FIGURE 5 | Multivariable Fine-Gray proportional hazards regression for 1-year waitlist mortality (reference: ALD). Adjusted for baseline characteristics at listing, including age, ascites, BMI, diabetes, encephalopathy, dialysis, life support measures, sex, MELD score, frailty, and race. **(a)** Patients with MASH had significantly worse 1-year waitlist mortality before the allocation policy change, compared to those with ALD. **(b)** No significant differences in 1-year waitlist mortality were observed among the liver etiologies after the allocation policy change. ALD, alcohol-related liver disease; HCV, hepatitis C virus infection; MASH, metabolic dysfunction-associated steatohepatitis; BMI, body mass index; MELD, Model for End-Stage Liver Disease.

were significantly associated with a higher risk of CVD-related death (**Supplementary Table S3**).

DISCUSSION

In this study, we evaluated waitlist outcome variations across major liver disease etiologies, including ALD, HCV, MASH, and biliary diseases in SLKT. Waitlist outcomes varied significantly depending on the liver etiology in SLKT candidates. The Fine-Gray model revealed that patients with MASH had worse 1-year mortality compared to patients with ALD, after adjustment for patient characteristics at listing, whereas the 1-year SLKT probability was comparable between patients with MASH and patients with ALD. When patients were categorized by MELD score at listing (6–20, 21–29, and ≥30), in the middle MELD score group, patients with MASH had a significantly higher 1-year mortality compared to those with ALD. In addition, prior to the allocation policy change in 2020, patients with MASH experienced significantly higher 1-year waitlist mortality compared to those with ALD; however, this disparity was not observed following the policy change. During

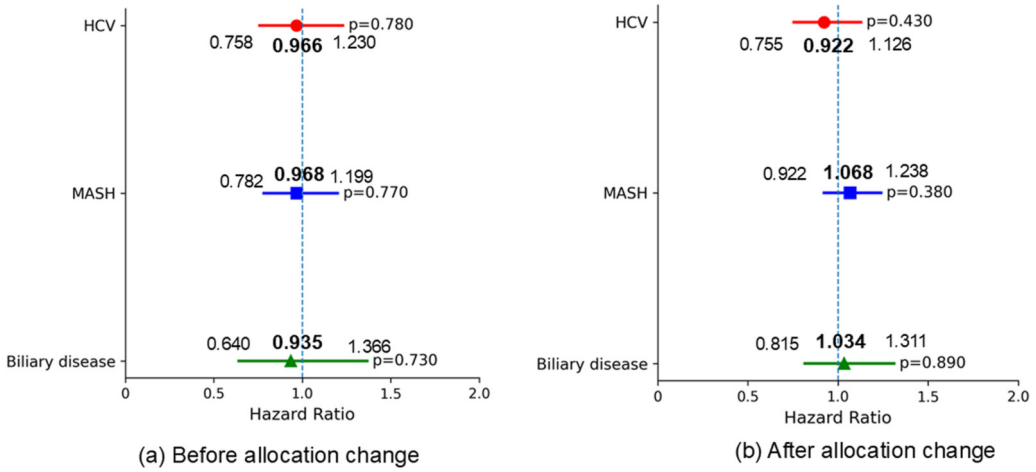


FIGURE 6 | Multivariable Fine-Gray proportional hazards regression for 1-year SLKT probability (reference: ALD). Adjusted for baseline characteristics at listing, including age, ascites, BMI, diabetes, encephalopathy, dialysis, life support measures, sex, MELD score, frailty, and race. **(a)** No significant differences in 1-year SLKT probability were observed among the liver etiologies before the allocation policy change. **(b)** No significant differences in 1-year SLKT probability were observed among the liver etiologies after the allocation policy change. ALD, alcohol-related liver disease; HCV, hepatitis C virus infection; MASH, metabolic dysfunction-associated steatohepatitis; BMI, body mass index; MELD, Model for End-Stage Liver Disease.

TABLE 6 | Multivariable logistic regression for patient death among patients who received SLKT. Adjusted for baseline characteristics at listing, including age, ascites, BMI, diabetes, encephalopathy, dialysis, life support measures, frailty, sex, MELD score, and race.

Variables	Patient death within 1 year		Patient death within 3 years	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Etiology of liver disease (ref; ALD)				
HCV	1.432 (0.919–2.231)	0.113	1.362 (0.963–1.927)	0.081
MASH	1.696 (1.199–2.398)	0.003	1.604 (1.226–2.098)	<0.001
Biliary disease	2.035 (1.192–3.472)	0.009	2.259 (1.498–3.406)	0.001

ALD, alcohol-related liver disease; HCV, hepatitis C virus infection; MASH, metabolic dysfunction-associated steatohepatitis; BMI, body mass index; MELD, Model for End-Stage Liver Disease.

the post-transplantation period, recipients with MASH had worse 1-year and 3-year patient survival compared to those with ALD.

Prior studies have reported higher LT waitlist mortality in patients with MASH [6, 7, 18], which are consistent with our findings. MASH, previously known as non-alcoholic fatty liver disease, affects an estimated 30% of the global population, although prevalence varies considerably based on individual risk profiles [19]. MASH carries a risk of progression to advanced fibrosis and cirrhosis, and is associated with increased likelihood of HCC, LT, and liver-related mortality [20]. The impact of MASH extends beyond hepatic involvement, such as CVD, CKD, and type 2 diabetes mellitus [21]. Indeed, MASH has been related to an increased risk of CKD, with a recent meta-analysis indicating nearly a twofold higher risk of CKD among patients with MASH [22]. In addition, the majority of deaths among patients with MASH are attributable to CVD [21]. Thus, MASH is a clinically significant condition with systemic involvement, contributing to multi-organ dysfunction and increased mortality. In our study, post-SLKT mortality was higher in recipients with MASH compared with those with ALD. In cause-specific analyses, patients with MASH were associated with a higher risk of CVD-related death than those with ALD, suggesting that CVD risk may have contributed to the increased mortality in this group.

In contrast, several previous reports have suggested that MASH was not independently associated with increased mortality among LT waitlist candidates. Thuluvath et al reported that adjusted cumulative incidence of death on LT waitlist was comparable between MASH and autoimmune hepatitis cirrhosis [23]. However, dialysis and serum creatinine were identified as negative predictors of receiving LT. Thus, the renal dysfunction may play a vital role, particularly among candidates for SLKT. Wong et al analyzed waitlist outcomes among LT alone by disease etiology among patients registered in the United States between 2004 and 2013 [18]. In their report, patients with HCV were associated with higher 1-year waitlist mortality, compared to those with MASH. It should be noted that their study period ended in 2013, prior to the widespread availability of direct-acting antivirals (DAA) for HCV [24]. With the introduction of DAAs as a curative therapy, LT in patients with HCV now shows improved GS compared to the pre-DAA era [25]. This reflects a key difference in the historical context between that study and our current analysis, as our analysis was conducted during the DAA era.

In our study, ALD patients demonstrated the highest 90-day and 1-year LT alone (without receiving KT) probability. When adjusted for clinical factors at listing, this difference was no longer significant among liver etiologies. The reasons for not receiving KT were not clearly identifiable, whether due to renal recovery or the decision of dialysis. Thus, it was difficult to determine which etiology was more likely to be associated with kidney recovery and avoidance of SLKT. However, patients with ALD may have a higher potential for renal recovery than those with other etiologies. Meanwhile, according to the report by Singal et al, OPTN database analysis confirms the rising use of SLKT, particularly among patients with MASH cirrhosis [26]. Notably, the study highlights that SLKT has increased most rapidly in this group and that patients with MASH have worse renal outcomes compared to those with cholestatic or ALD. Future research is warranted to clarify whether the course of renal function differs by etiology among SLKT candidates.

Then, how should our findings be applied to optimize clinical management of SLKT candidates? When evaluating outcomes according to MELD score categories at listing, among SLKT candidates in the middle MELD subgroup, patients with MASH had higher 1-year waitlist mortality than those with ALD, while SLKT probability was comparable. Earlier referral for SLKT and more intensive management during the waiting period may be beneficial for patients with MASH in the middle MELD group. Although incorporation of liver etiology into the MELD score is challenging because overlap exists among etiologies, risk stratification cannot be fully achieved using the MELD score alone. MELD score-based assessment for urgency may be complemented by individual additional risk evaluation, careful follow-up while on the waitlist, including comorbidities, disease trajectory, and the likelihood of renal recovery. A strategy incorporating delayed KT and the safety-net may also be considered for patients with potential for renal recovery.

Since the adoption of the MELD score for organ allocation in 2002, the system of liver allocation and distribution has been changing dynamically with the goal of decreasing waitlist mortality and minimizing geographic variability in median MELD score at the time of transplantation, without compromising graft or patient survival [27]. We previously evaluated the impact of the MELD score, introduced in January 2016 for LT allocation, on waitlist and post-transplantation outcomes, using OPTN data [28]. The introduction of the MELD score improved LT waitlist outcomes by reducing mortality and increasing transplantation rates. In July 2023, MELD 3.0 was implemented to address sex-based disparities by incorporating additional variables such as serum albumin and female sex.[1] According to retrospective analysis of MELD 3.0 reclassification using OPTN data, female SLKT candidates were more likely to have higher MELD 3.0 scores than their listing MELD/MELD-Na score, suggesting potential benefits for female candidates [29]. Other than the introduction of MELD score, changes to the system of liver allocation and distribution, including Regional Share 15, Regional Share for Status 1, Regional Share 35/National Share 15, and, most recently, the Acuity Circles Distribution Model, have been made [30–32]. On February 4, 2020, the United States implemented a new liver allocation policy, replacing Donation Service Areas (DSAs) with acuity circles as the primary geographic unit for organ distribution, shifting from a primarily local to a broader sharing system [33]. After this allocation policy change, transplantation volume increased nationwide and increased for both high MELD and low MELD centers [8]. Regarding the effect of the acuity circle policy on SLKT, Okumura et al demonstrated that, following the policy change, SLKT candidates had higher MELD scores and were more likely to receive SLKT, with no increase in 90-day waitlist mortality [34]. Additionally, post-transplantation outcomes including one-year patient, liver, and kidney graft survival were comparable between the pre- and post-AC periods, suggesting that the AC policy improved access to SLKT without compromising outcomes [34]. In our analysis, the differences in both 90-day and 1-year waitlist mortality by etiology were no longer observed after the 2020 allocation policy change. This finding suggests a temporal association between the 2020 allocation policy revision and mortality differences by etiology. This observation may also be influenced by the increasing use of marginal livers, including older donors or DCD (donation after circulatory death) [35], as well as growing use of DCD donors and machine perfusion in SLKT [36].

There are several limitations in our study using OPTN database. First, the etiology in this study was based solely on classifications within OPTN database, and cases with overlapping liver diseases could not be fully identified. In OPTN dataset, only 101 cases were identified as having both ALD and HCV during the study period. Given the limited sample size, this group was excluded in this analysis. Second, due to the retrospective cohort, a direct causal relationship with the acuity circle policy could not be determined. Of note, this study focused on patients without exception scores. The number of donors has been increasing consistently since 2010, reaching 14,905 in 2022, which represents a 7.5% increase compared with the previous year [37]. The increase in donor numbers may also have contributed to reducing disparities in mortality across different liver etiologies. Third, given the lack of comprehensive longitudinal data on serum creatinine and GFR prior to listing and throughout the waitlist period, precise classification of renal phenotypes (CKD or sustained AKI) was not available and thus could not be included in the analysis. Dialysis duration was also not included in the analysis due to a substantial amount of missing data. Fourth, there may be potential confounding by era effects such as the COVID-19 period, donor supply shifts and the MELD 3.0 implementation. However, it is difficult to precisely define discrete time periods for factors such as the COVID-19 era and shifts in donor supply. MELD 3.0 was implemented on July 13, 2023 and this study period was from January 2018 to March 2024. As the post-implementation period was relatively short (approximately 8 months), it was also difficult to evaluate its impact adequately. Finally, OPTN database lacks certain detailed clinical information, and unmeasured confounders may have influenced waitlist outcomes.

In conclusion, waitlist outcomes varied significantly depending on the etiology in SLKT. One-year waitlist mortality was higher in patients with MASH compared to those with ALD, despite similar SLKT probabilities. When stratified by MELD score at listing, patients with MASH in the middle score group had significantly higher mortality compared to those with ALD. The revised 2020 allocation policy may be temporally associated with

[1]https://www.hrsa.gov/optn/data/allocation-calculators/meld-calculator

changes in mortality disparities across different liver etiologies; however, further approaches to risk assessment tailored to the etiology of liver disease in SLKT are needed to ensure greater equity.

DATA AVAILABILITY STATEMENT

The data reported here have been supplied by the UNOS as the contractor for the OPTN. The interpretation and reporting of these data are the responsibility of the author(s) and in no way should be seen as an official policy of or interpretation by the OPTN or the U.S. Government. This data can be found here: https://unos.org/data/.

ETHICS STATEMENT

The requirement of ethical approval was waived by Henry Ford Institutional Review Board for the studies involving humans because this study used the OPTN dataset provided by the United Network for Organ Sharing (UNOS), in which all individually identifiable information is encrypted. 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 because this study used the OPTN dataset provided by the United Network for Organ Sharing (UNOS), in which all individually identifiable information is encrypted.

AUTHOR CONTRIBUTIONS

RO and SN conceived the idea of the study. RO developed the statistical analysis plan and conducted statistical analyses. RO, LR, DK, AY, MA and SN contributed to the interpretation of the results. RO drafted the original manuscript. SN and MA supervised the conduct of this study. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

The author(s) declared that this work 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) declared that generative AI was not 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.2026.16153/full#supplementary-material

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Impact of Early Post-Transplantation Diabetes Mellitus and Changes in Diabetic Status on Graft Failure and Mortality in Kidney Transplant Recipients

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Post-transplantation diabetes mellitus (PTDM) is a common complication following kidney transplantation (KT), but the prognostic significance of early PTDM and changes in diabetic status remains uncertain. Using the Korean National Health Insurance Service (NHIS) database, we analyzed 8,486 KT recipients (KTRs) who underwent national health screening from 2009 to 2017. Early PTDM was defined as new-onset diabetes between 3 months and 1 year after KT. Cox regression was used to estimate the risk of graft failure and all-cause mortality. Early PTDM and preexisting DM were present in 12.2% and 28.5% of KTRs, respectively. Early PTDM was significantly associated with mortality (aHR 1.309, 95% CI 1.015–1.689) but not with graft failure. Changes in diabetic status were not significantly associated with graft failure. However, transitioning from non-DM to PTDM (aHR 1.646, 95% CI 1.080–2.510) and having persistent early PTDM (aHR 1.755, 95% CI 1.325–2.377) were associated with increased mortality, whereas regression from early PTDM to non-DM was not. Preexisting DM was associated with increased mortality, regardless of subsequent changes; the risk was relatively lower in those who regressed to non-DM. Changes in diabetic status were found to have a greater impact on outcomes than early PTDM, highlighting the importance of continuous glycemic monitoring and individualized care in KTRs.

Keywords: all-cause mortality, diabetic status, early post-transplantation diabetes mellitus, graft failure, kidney transplantation

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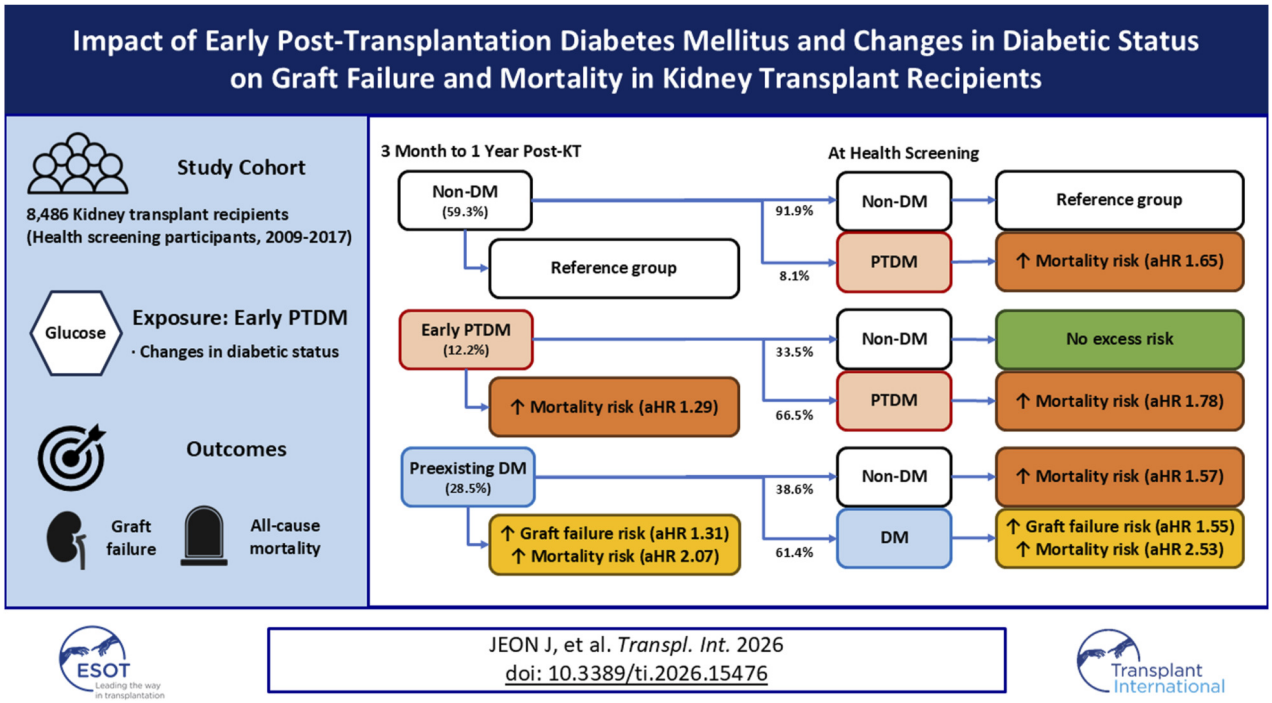
Abbreviations: aHR, adjusted hazard ratio; BMI, Body mass index; CI, confidence intervals; CNI, calcineurin inhibitor; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HR, hazard ratio; HTN, hypertension; ICD-10, International Statistical Classification of Diseases and Related Health Problems, 10th Revision; KTRs, kidney transplant recipients; KT, kidney transplantation; NHIS, National Health Insurance Service; PTDM, post-transplant diabetes mellitus.



GRAPHICAL ABSTRACT |

INTRODUCTION

Given its ability to provide a better quality of life and long-term survival compared with dialysis, kidney transplantation (KT) remains the gold standard of renal replacement therapy for eligible patients with end-stage kidney disease [1]. The improvement in the prognosis of KT recipients (KTRs) over the decades highlights the importance of managing long-term complications. Post-transplantation diabetes mellitus (PTDM), defined as newly diagnosed diabetes mellitus (DM) following organ transplantation, is a common complication of KT that occurs in 8%–39% of KTRs with no history of diabetes [2].

Previous studies have revealed an association between PTDM and poor graft or patient outcomes [3–9]. However, some recent studies have found no association between the prognosis of PTDM and KTRs [10–12]. PTDM may serve as an indirect indicator of common factors affecting the overall prognosis, such as the use of high doses of glucocorticoids or calcineurin inhibitors and sarcopenia [13, 14]. Notably, in some studies, PTDM was defined as newly diagnosed DM following KT, regardless of the timing of diagnosis [5, 7, 8]. However, PTDM that develops early after KT may be attributed to the use of immunosuppressants or general health conditions. Furthermore, its impact on patient outcomes differs from that of PTDM that develops later. Thus, the clinical implications of PTDM, particularly early-onset PTDM, remain to be established.

Although attempts have been made to clarify the prognostic impact of PTDM in large cohorts using national health insurance databases, these data contain limited information regarding patient characteristics, such as the glomerular filtration rate and body mass index (BMI) [8, 9]. Therefore, this study aimed to determine the prognostic impact of PTDM among KTRs who participated in a national health screening program, with a focus on the development of early PTDM within 1 year post-KT and the change in diabetic status, to overcome this limitation.

MATERIALS AND METHODS

Data Sources and Study Setting

Data from the Korean National Health Insurance Service (NHIS), which provides mandatory universal coverage to approximately 97% of the Korean population (the Medical Aid Program covers the remaining 3% of the population), were used in this nationwide retrospective cohort study. The NHIS database comprises two databases: an eligibility database (containing information such as age, sex, type and severity of disability, socioeconomic variables, income level, and type of eligibility) and a medical treatment database (containing medical bills submitted by medical service providers). Several epidemiological studies have used data from the Korean NHIS database [15–17].

The NHIS conducts an annual or biennial national health screening program that comprises a standardized self-report questionnaire regarding health behaviors, anthropometric

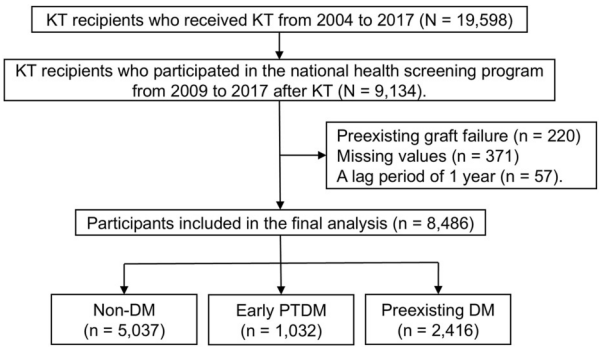


FIGURE 1 | Participant selection process. KT, kidney transplantation.

measurements, and laboratory tests. Furthermore, BMI, waist circumference, blood pressure, fasting blood glucose, serum creatinine, triglyceride, and urine protein levels are measured after overnight fasting in accordance with the South Korean Association of Laboratory Quality Control guidelines. To receive reimbursement, medical institutions must adhere to NHIS protocols and undergo certification for quality control procedures [18].

Ethics Approval

This study was approved by the Institutional Review Board of the Samsung Medical Center (IRB No. 2023-01-006) and adhered to the Declaration of Helsinki. The requirement for obtaining informed consent from the patients was waived owing to the use of anonymized and de-identified data.

Study Population

A total of 19,598 patients who had undergone KT between 2004 and 2017 were identified. A combination of the *International Statistical Classification of Diseases and Related Health Problems, 10th Revision* (ICD-10) code Z94.0 (Kidney transplant status) and a special code for KT (V005) was used to define KT status [19]. The NHIS has registered patients through the Rare and Incurable Disease Registration Program since 2006 to identify patients requiring additional assistance for the management of rare or intractable diseases, including KT status. Patients under this registration are beneficiaries of special medical aid. KTRs who participated in the national health screening program between 2009 and 2017 (N = 9,134) were eligible for inclusion in the present study. Participants with preexisting graft failure (n = 220) and missing values (n = 371) were excluded. Furthermore, a lag period of 1 year was applied to exclude newly diagnosed cases of graft failure or death within 1 year of the national health screening date (n = 57). Thus, the final analysis included 8,486 participants. The participant selection process is shown in **Figure 1**.

Exposure: Early PTDM

KTRs were divided into three groups: non-DM, early PTDM, and preexisting DM. Early PTDM was defined as DM newly diagnosed between 3 months and 1 year after KT. Patients who were diagnosed with DM within the first 3 months post-transplant but did not have DM thereafter were classified as non-DM [20]. Preexisting DM was defined as a diagnosis of DM prior to KT. The diagnosis of DM was defined based on the recorded use of at least one oral antidiabetic agent or insulin, with ICD-10 codes E11–E14, or a fasting glucose level of ≥126 mg/dL.

Covariates

The glomerular filtration rate (eGFR) was determined by assessing the serum creatinine-based eGFR calculated using the Modification of Diet in Renal Disease equation [21]. The dipstick test was used to assess proteinuria, with the detection of traces classified as negative. The presence of at least one claim per year for antihypertensive agents with ICD-10 codes I10–I13 or I15 or a systolic/diastolic blood pressure of ≥140/90 mmHg was defined as hypertension (HTN). The presence of at least one claim per year for antihyperlipidemic agents with ICD-10 code E78 or total cholesterol levels of ≥240 mg/dL was defined as dyslipidemia. A BMI of ≥25 kg/m^2 was defined as obesity. The ICD-10 codes F32 or F33 indicated a diagnosis of depression. Smoking status was categorized as never, former, or current smoker. Alcohol consumption was categorized as none, moderate (<30 g/day), and heavy (≥30 g/day). Regular exercise was defined as engaging in moderate physical activity for >30 min ≥5 times per week or engaging in strenuous physical activity for >20 min ≥3 times per week. The low-income group comprised those in the bottom 25% of earners. The requirement for rituximab, thymoglobulin, and intravenous glucocorticoids for >3 days; intravenous immune globulin; or plasmapheresis following readmission after KT was defined as acute rejection therapy.

Outcomes and Follow-Up

The primary outcome measures were graft failure and all-cause mortality. Graft failure, defined as receiving dialysis at least 3 months post-KT (hemodialysis > at least 25 times or peritoneal dialysis at least 3 months), was assessed using death-censored graft failure. The follow-up period commenced on the date of the national health screening examination and ended at the occurrence of the outcome on 31 December 2021.

Subgroup Analysis

Subgroup analyses were conducted based on age (<40, 40–49, 50–59, 60–69, and ≥70 years old), sex, low income, smoking status (never or former vs. current), alcohol consumption, regular exercise, hypertension, dyslipidemia, depression, proteinuria, hospitalization within 1 year post-KT, and acute rejection therapy within 1 year post-KT.

Sensitivity Analysis

The majority of previous studies defined PTDM to include DM diagnosed within the first 3 months after KT. For comparison, we performed an additional sensitivity analysis using this conventional definition, classifying all patients diagnosed with DM within 1 year post-transplant as having PTDM.

TABLE 1 | Baseline characteristics of KT recipients.

Variables	All (N = 8,486)	Non-DM (n = 5,037)	Early PTDM (n = 1,032)	Preexisting DM (n = 2,416)	P- value
Age, y	50.1 ± 10.4	47.68 ± 10.46	53.01 ± 9.27	53.74 ± 9.32	<0.0001
<40	1,263 (14.88)	1,022 (20.29)	75 (7.27)	166 (6.87)	<0.0001
40–49	2,506 (29.53)	1,693 (33.60)	251 (24.32)	562 (23.26)	
50–59	3,080 (36.3)	1,646 (32.67)	449 (43.51)	985 (40.77)	
60–69	1,484 (17.49)	631 (12.52)	230 (22.29)	623 (25.79)	
≥70	153 (1.80)	46 (0.91)	27 (2.62)	80 (3.31)	
Male sex	5,062 (59.65)	2,841 (56.39)	634 (61.43)	1,587 (65.69)	<0.0001
BMI, kg/m^2	22.90 ± 3.28	22.66 ± 3.22	23.11 ± 3.17	23.32 ± 3.42	<0.0001
Waist circumference, cm	79.97 ± 9.59	78.66 ± 9.24	81.06 ± 9.2	82.22 ± 9.99	<0.0001
Systolic BP, mmHg	125.48 ± 14.91	124.89 ± 14.23	126.19 ± 14.98	126.4 ± 16.15	<0.0001
Diastolic BP, mmHg	78.12 ± 10.22	78.84 ± 10.02	79.11 ± 10.41	76.2 ± 10.32	<0.0001
Hypertension	5,651 (66.59)	3,203 (63.58)	743 (72.00)	1,705 (70.57)	<0.0001
Dyslipidemia	4,880 (57.51)	2,767 (54.92)	667 (64.63)	1,446 (59.85)	<0.0001
Depression	504 (5.94)	240 (4.76)	71 (6.88)	193 (7.99)	<0.0001
eGFR, mL/min/1.73 m^2	65.49 ± 42.96	65.73 ± 42.94	63.41 ± 41.01	65.89 ± 43.78	0.248
Proteinuria, positive	858 (10.11)	471 (9.35)	126 (12.21)	261 (10.8)	0.0087
Fasting glucose, mg/dL	107.89 ± 35.29	97.68 ± 18.05	117.07 ± 35.40	125.26 ± 51.04	<0.0001
Total cholesterol, mg/dL	183.38 ± 36.08	186.21 ± 35.25	181.72 ± 34.97	178.17 ± 37.63	<0.0001
HDL-C, mg/dL	58.76 ± 20.87	60.71 ± 23.38	55.69 ± 15.33	56.00 ± 16.47	<0.0001
LDL-C, mg/dL	99.07 ± 31.02	100.51 ± 30.92	97.31 ± 30.29	96.83 ± 31.38	<0.0001
Triglyceride[a], mg/dL	115 (114–117)	114 (113–116)	129 (126–133)	112 (110–114)	<0.0001
Smoking status					<0.0001
Never smoker	5,208 (61.37)	3,227 (64.05)	627 (60.76)	1,354 (56.04)	
Former smoker	2,731 (32.18)	1,512 (30.01)	337 (32.66)	882 (36.51)	
Current smoker	547 (6.45)	299 (5.93)	68 (6.59)	180 (7.45)	
Alcohol consumption					0.001
None	7,291 (85.92)	4,277 (84.89)	888 (86.05)	2,126 (88.00)	
Moderate	1,127 (13.28)	725 (14.39)	137 (13.28)	265 (10.97)	
Heavy	68 (0.8)	36 (0.71)	7 (0.68)	25 (1.03)	
Regular exercise	2,128 (25.08)	1,201 (23.84)	270 (26.16)	657 (27.19)	0.0052
Low income (<20%)	2,434 (28.68)	1,383 (27.45)	318 (30.81)	733 (30.34)	0.0097
Time from KT to screening, y	3.04 ± 2.41	3.25 ± 2.52	3.10 ± 2.36	2.59 ± 2.13	<0.0001

Categorical variables and continuous variables are presented as numbers (percentages) and as mean ± standard deviation.

[a]*Triglyceride values are shown as a geometric mean (95% confidence interval).*

BMI, body mass index; BP, blood pressure; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein-cholesterol; KT, kidney transplantation; KTRs, recipients of KT; LDL-C, low-density lipoprotein-cholesterol; PTDM, post-transplantation diabetes mellitus.

Statistical Analysis

Categorical and continuous variables are presented as numbers (percentages) and mean ± standard deviation or median (interquartile range), respectively. Intergroup comparisons were conducted using a t-test (for continuous variables) and a Chi-square test (for categorical variables). The incidence rate (IR) was presented per 1,000 person-years. Cumulative graft failure and all-cause mortality were expressed using Kaplan–Meier curves and compared using the log-rank test. Hazard ratios (HR) with 95% confidence intervals (CI) were estimated using Cox proportional hazards regression analysis to estimate the risk of graft failure and all-cause mortality. Multivariable analysis was conducted for the following variables: sex, age, low income, smoking status, alcohol consumption, regular exercise, HTN, dyslipidemia, depression, BMI, eGFR, the time from KT to screening, proteinuria, induction therapy, and acute rejection within 1 year post-KT. All statistical analyses were conducted using SAS 9.4 (SAS Institute, Cary, North Carolina, USA). A two-sided *P*-value of <0.05 was considered statistically significant.

RESULTS

Characteristics of the Study Population

Table 1 presents the characteristics of KTRs at the time of participating in the national health screening program. Of the 8,486 KTRs, 1,032 (12.2%) and 2,416 (28.5%) KTRs had early PTDM and preexisting DM, respectively. The mean interval between KT and health screening was 3.04 ± 2.41 years. The mean age was 50.1 ± 10.4 years, and 5,062 (59.63%) of the KTRs were men. Patients in the preexisting DM group were the oldest and most likely to be men, followed by those in the early PTDM group. The BMI and waist circumference were highest in the preexisting DM group, followed by the early PTDM group. Systolic blood pressure was lowest in the non-DM group and similar between the early PTDM and preexisting DM groups; however, diastolic blood pressure and the proportion of HTN were highest in the early PTDM group. The eGFR was similar across the three groups, whereas the early PTDM group had the highest proportion of proteinuria. Total HDL-, and LDL-cholesterol levels were highest in the

TABLE 2 | Treatment history during the first year post-KT.

Variables	All	Non-DM	Early PTDM	Preexisting DM	P value
Induction therapy					<0.0001
None	983 (11.58)	724 (14.37)	106 (10.27)	153 (6.33)	
Thymoglobulin	675 (7.95)	340 (6.75)	69 (6.69)	266 (11.01)	
Basiliximab	6,617 (77.98)	3,858 (76.58)	825 (79.94)	1,934 (80.05)	
Both	211 (2.49)	116 (2.30)	32 (3.1)	63 (2.61)	
Acute rejection within 1 year	623 (7.34)	311 (6.17)	112 (10.85)	200 (8.28)	<0.0001
OPD visits within 1 year, n	25.26 ± 14.04	23.01 ± 12.41	25.58 ± 12.55	29.82 ± 16.51	<0.0001
<10	60 (0.71)	54 (1.07)	1 (0.10)	5 (0.21)	<0.0001
10–19	3,296 (38.84)	2,343 (46.51)	362 (35.08)	591 (24.46)	
20–29	2,963 (34.92)	1,671 (33.17)	391 (37.89)	901 (37.29)	
30–39	1,222 (14.4)	583 (11.57)	175 (16.96)	464 (19.21)	
≥40	945 (11.14)	387 (7.68)	103 (9.98)	455 (18.83)	
Hospitalizations within 1 year, n					<0.0001
0	1,923 (22.66)	1,344 (26.68)	181 (17.54)	398 (16.47)	
1	2,846 (33.54)	1,768 (35.09)	316 (30.62)	762 (31.54)	
≥2	3,717 (43.80)	1,926 (38.23)	535 (51.84)	1,256 (51.99)	

Categorical variables are presented as numbers (percentages).
Abbreviations: DM, diabetes mellitus; KT, kidney transplantation; PTDM, post-transplantation diabetes mellitus; OPD, outpatient department.

non-DM group; however, triglyceride levels and the proportion of dyslipidemia were the highest in the early PTDM group. The proportion of KTRs with low income was the lowest in the non-DM group. The median (interquartile range) follow-up durations for graft failure and all-cause mortality were 6.12 (4.22–8.40) years and 6.00 (4.06–8.14) years, respectively.

Treatment History During the First Year Post-KT

Table 2 presents the treatment history during the first year post-KT. The proportion of KTRs who did not receive induction therapy was the highest in the non-DM group (14.4%), followed by the early PTDM group (10.3%) and the preexisting DM group (6.3%). The proportion of KTRs who underwent thymoglobulin induction in the preexisting DM group (11.0%) was significantly higher than that in the non-DM (6.8%) and early PTDM (6.6%) groups. Acute rejection therapy commenced within 1 year post-KT in 7.34% of KTRs. The incidence of acute rejection was the highest in the early PTDM group (10.9%), followed by the preexisting DM (8.3%) and non-DM (6.2%) groups.

The mean number of outpatient department visits within 1 year post-KT was the highest in the preexisting DM group (29.8 ± 16.5), followed by the early PTDM (25.6 ± 12.6) and non-DM (23.0 ± 12.4) groups. Multiple hospitalizations (≥2 times) within 1 year post-KT were similar in the early PTDM (51.8%) and preexisting DM (52.0%) groups, followed by the non-DM (38.2%) group.

Risk of Graft Failure and All-Cause Mortality According to Early PTDM within 1 Year Post-KT

Compared with the non-DM group, the early PTDM group exhibited no association with the risk of graft failure but a significant association with the risk of all-cause mortality (adjusted hazard ratio [aHR] 1.044, 95% CI 0.833–1.309 for graft failure and 1.309, 95% CI 1.015–1.689 for all-cause mortality) (**Table 3**). **Figures 2A,B** present the cumulative incidences of all-cause mortality and graft failure according to the diabetic status 1 year post-KT. Compared with that in the non-DM group, an increased risk of graft failure and all-cause mortality was observed in the preexisting DM group (**Table 3**).

Risk of Graft Failure and All-Cause Mortality According to Changes in Diabetic Status

The risk of graft failure and all-cause mortality was evaluated according to changes in diabetic status from 1 year post-KT to the time of health screening (**Table 4**). An analysis was conducted after excluding KTRs who underwent health screening within 1 year post-KT to distinguish between early diabetic status and the changes in diabetic status. While 8.1% of non-DM patients progressed to PTDM, 33.5% of those with early PTDM and 38.6% of those with preexisting DM regressed to a non-DM status. KTRs who remained non-diabetic from KT until health screening served as the reference group.

KTRs who transitioned from non-DM to PTDM or from early PTDM to non-DM showed a significantly higher risk of graft failure in the univariable analysis. However, in the multivariable model, these transition groups did not exhibit a statistically significant increase in risk of graft failure, although transition from non-DM to PTDM tended to be associated with an increased risk (non-DM → PTDM: aHR 1.314, 95% CI 0.934–1.848; early PTDM → non-DM: aHR 1.221, 95% CI 0.818–1.824). Patients who remained in the PTDM status (early PTDM → PTDM) did not show a significantly elevated risk of graft failure. Among KTRs with preexisting DM, those who remained diabetic had a significantly higher risk of graft failure compared with those who remained non-diabetic

TABLE 3 | Risk of graft failure and mortality according to diabetic status within 1 year post-KT.

Outcome	Diabetic status	IR[a]	Hazard ratio (95% confidence interval)			
			Model 1	Model 2	Model 3	Model 4
Graft failure	Non-DM	12.49	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)
	Early PTDM	15.49	1.240 (0.995, 1.545)	1.359 (1.086, 1.699)	1.284 (1.026, 1.606)	1.044 (0.833, 1.309)
	Preexisting DM	15.57	1.266 (1.071, 1.496)	1.400 (1.176, 1.666)	1.344 (1.129, 1.600)	1.287 (1.079, 1.535)
All-cause mortality	Non-DM	6.33	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)
	Early PTDM	12.62	2.003 (1.560, 2.573)	1.424 (1.107, 1.833)	1.422 (1.105, 1.830)	1.309 (1.015, 1.689)
	Preexisting DM	19.29	3.169 (2.648, 3.792)	2.064 (1.715, 2.483)	2.005 (1.665, 2.414)	2.065 (1.712, 2.491)

Model 1 was unadjusted; Model 2 was adjusted for sex and age; Model 3 was further adjusted for low income, smoking status, alcohol consumption, regular exercise, and hypertension, dyslipidemia, and depression; and Model 4 was additionally adjusted for body mass index, estimated glomerular filtration rate, proteinuria, time from kidney transplantation to health screening, induction therapy, and acute rejection within 1 year.

[a]*Incidence rates are expressed per 1,000 person-years.*

DM, diabetes mellitus; IR, incidence rate; KT, kidney transplantation; PTDM, post-transplantation diabetes mellitus.

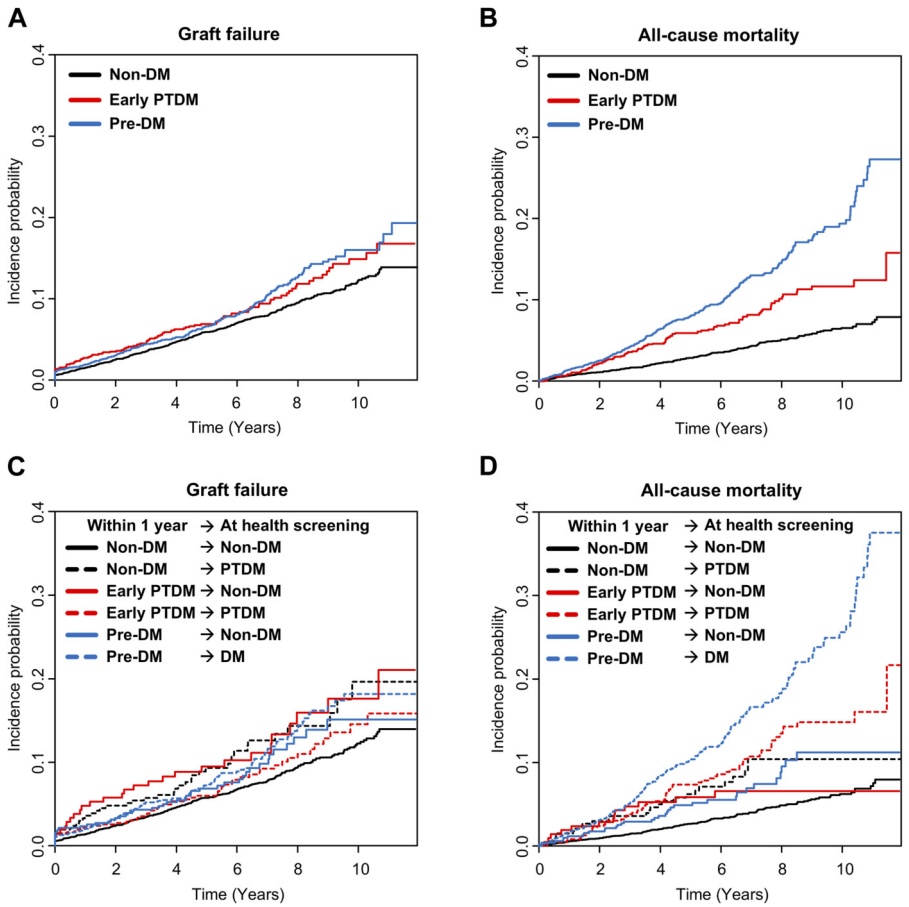


FIGURE 2 | Cumulative incidence of graft failure and all-cause mortality according to diabetic status within 1 year post-KT and changes in diabetic status in KT recipients who underwent health screening following KT. **(A)** Graft failure according to the diabetic status within 1 year post-KT, **(B)** all-cause mortality according to diabetic status within 1 year post-KT, **(C)** graft failure according to the changes in diabetic status, and **(D)** all-cause mortality according to the changes in diabetic status. DM, diabetes mellitus; KT, kidney transplantation; PTDM, post-transplantation diabetes mellitus.

throughout (aHR 1.545, 95% CI 1.231–1.940), whereas those who transitioned to non-DM did not show a significantly increased risk (aHR 1.298, 95% CI 0.948–1.779). **Figure 2C** presents the cumulative incidence of graft failure according to the changes in diabetic status.

KTRs who transitioned from non-DM to PTDM, along with those with persistent early PTDM, exhibited a significantly higher risk of all-cause mortality (non-DM → PTDM: aHR 1.646, 95% CI 1.080–2.510; early PTDM → PTDM: aHR 1.755, 95% CI 1.325–2.377) (**Table 4**). In contrast, KTRs who transitioned from

TABLE 4 | Risk of graft failure and mortality according to changes in diabetic status.

Outcome	Changes in diabetic status		N	IR[a]	Hazard ratio (95% confidence interval)			
	With 1 year after KT	At health screening			Model 1	Model 2	Model 3	Model 4
Graft failure	Non-DM	Non-DM	3,807	12.24	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)
	Non-DM	PTDM	336	19.77	1.625 (1.164, 2.268)	1.707 (1.221, 2.385)	1.506 (1.074, 2.111)	1.314 (0.934, 1.848)
	Early PTDM	Non-DM	211	20.37	1.655 (1.116, 2.454)	1.803 (1.215, 2.678)	1.695 (1.141, 2.518)	1.221 (0.818, 1.824)
	Early PTDM	PTDM	629	14.38	1.175 (0.883, 1.563)	1.349 (1.009, 1.804)	1.236 (0.923, 1.654)	1.034 (0.770, 1.389)
	Pre-DM	Non-DM	514	15.47	1.283 (0.940, 1.750)	1.350 (0.989, 1.842)	1.302 (0.953, 1.779)	1.298 (0.948, 1.779)
	Pre-DM	DM	1,332	17.10	1.422 (1.154, 1.753)	1.701 (1.359, 2.128)	1.608 (1.284, 2.013)	1.545 (1.231, 1.940)
All-cause mortality	Non-DM	Non-DM	3,807	6.02	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)
	Non-DM	PTDM	336	12.31	2.076 (1.370, 3.144)	1.891 (1.248, 2.866)	1.809 (1.191, 2.749)	1.646 (1.080, 2.510)
	Early PTDM	Non-DM	211	9.00	1.478 (0.839, 2.602)	1.127 (0.639, 1.988)	1.099 (0.623, 1.938)	0.864 (0.488, 1.530)
	Early PTDM	PTDM	629	16.24	2.719 (2.041, 3.621)	1.899 (1.422, 2.535)	1.886 (1.412, 2.521)	1.775 (1.325, 2.377)
	Pre-DM	Non-DM	514	10.86	1.864 (1.286, 2.703)	1.629 (1.123, 2.363)	1.556 (1.073, 2.257)	1.566 (1.077, 2.275)
	Pre-DM	DM	1,332	25.67	4.455 (3.607, 5.504)	2.577 (2.066, 3.214)	2.465 (1.973, 3.080)	2.532 (2.022, 3.169)

Model 1 was unadjusted; Model 2 was adjusted for sex and age; Model 3 was further adjusted for low income, smoking status, alcohol consumption, regular exercise, and hypertension, dyslipidemia, and depression; and Model 4 was additionally adjusted for body mass index, estimated glomerular filtration rate, proteinuria, time from kidney transplantation to health screening, induction therapy, and acute rejection within 1 year.

[a]*Incidence rates are expressed per 1,000 person-years.*

DM, diabetes mellitus; IR, incidence rate; KT, kidney transplantation; PTDM, post-transplantation diabetes mellitus.

early PTDM to non-DM showed no significant association with all-cause mortality. Among those with preexisting DM, the risk of all-cause mortality was increased regardless of subsequent changes in diabetic status, although those who transitioned from preexisting DM to non-DM exhibited a relatively lower HR for all-cause mortality compared with those who remained diabetic. **Figure 2D** presents the cumulative incidence of all-cause mortality according to the changes in diabetic status.

A sensitivity analysis conducted after excluding KTRs who underwent health screening within 2 years post-KT yielded comparable results, except that transitioning from non-DM to PTDM was significantly associated with graft failure (aHR 1.473, 95% CI 1.025–2.118) (**Supplementary Table S1**).

Subgroup Analysis

Figure 3 and **Supplementary Tables S2, S3** present the results of the subgroup analyses. The relationship between early PTDM and graft failure or all-cause mortality exhibited no significant interaction with respect to age, sex, income, smoking status, alcohol consumption, regular exercise, HTN, dyslipidemia, proteinuria, hospitalization, or acute rejection therapy.

Outcomes With an Early PTDM Definition, Including the First 3 Months Post-KT

When the first 3 months post-KT were included in the definition of early PTDM, the number of early PTDM cases increased by 921, from 1,032 to 1,953. Under this definition, the HRs of early PTDM were attenuated and were not significantly associated with the risk of either graft failure or all-cause mortality (**Supplementary Table S4**). In the analysis of changes in diabetic status, HRs were amplified in both directions; that is, higher HRs became higher, and lower HRs became lower. Consequently, KTRs who transitioned from non-DM to PTDM showed a significantly increased risk of graft failure and all-cause mortality (non-DM → PTDM: aHR 1.578, 95% Cl 1.074–2.320 for graft failure and aHR 1.784, 95% Cl 1.100–2.893 for all-cause mortality). Notably, patients who transitioned from early PTDM to non-DM demonstrated an even lower risk of all-cause mortality compared with those who consistently remained non-DM (early PTDM → non-DM: aHR 0.678, 95% Cl 0.464–0.992) (**Supplementary Table S5**).

Analysis of all KTRs Irrespective of Health Screening Participation

We further analyzed all 24,524 KTRs who underwent KT between 2004 and 2020, regardless of their participation in the national health screening program, after excluding those with missing values (n = 536) and those who died <3 months post-KT (n = 309). **Supplementary Table S6** summarizes the characteristics of all KTRs who underwent KT between 2004 and 2020; the characteristics were similar to those of KTRs who participated in the health screening. Treatment history patterns of all KTRs

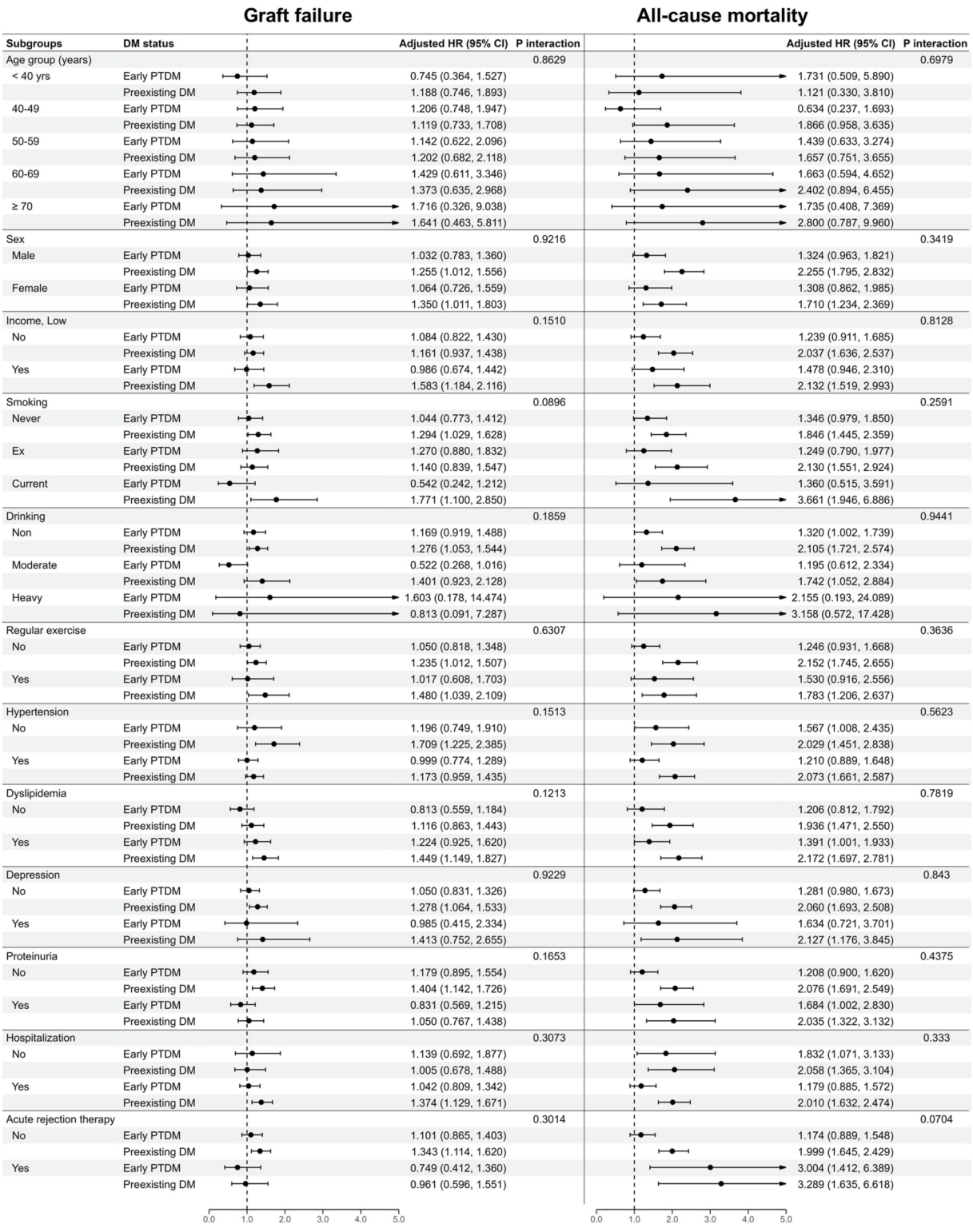


FIGURE 3 | Subgroup analysis for graft failure and all-cause mortality according to diabetic status within 1 year post-KT. CI, confidence interval; DM, diabetes mellitus; HR, hazard ratio; KT, kidney transplantation; PTDM, post-transplantation diabetes mellitus.

TABLE 5 | Risk of graft failure and mortality by diabetic status within 1 year of KT in all KTRs who received a KT from 2004 to 2020, regardless of health screening participation.

Outcome	Diabetic status	N	IR[a]	Hazard ratio (95% confidence interval)		
				Model 1	Model 2	Model 3
Graft failure	Non-DM	13,546	11.39	1 (Ref.)	1 (Ref.)	1 (Ref.)
	Early PTDM	2,742	17.32	1.525 (1.349, 1.725)	1.708 (1.506, 1.936)	1.524 (1.344, 1.729)
	Preexisting DM	8,954	15.24	1.385 (1.264, 1.519)	1.579 (1.433, 1.741)	1.462 (1.324, 1.614)
All-cause mortality	Non-DM	13,546	6.22	1 (Ref.)	1 (Ref.)	1 (Ref.)
	Early PTDM	2,742	13.24	2.142 (1.861, 2.465)	1.474 (1.279, 1.700)	1.339 (1.153, 1.535)
	Preexisting DM	8,954	20.03	3.432 (3.113, 3.783)	2.161 (1.952, 2.392)	2.108 (1.900, 2.338)

Model 1 was unadjusted; Model 2 was adjusted for sex and age; Model 3 was further adjusted for low income, hypertension, dyslipidemia, induction therapy, and acute rejection within 1 year.

[a]*Incidence rates are expressed per 1,000 person-years.*

DM, diabetes mellitus; IR, incidence rate; KT, kidney transplantation; PTDM, post-transplantation diabetes mellitus.

according to the diabetic status were also similar to those of KTRs who participated in the national health screening program. However, the proportions of KTRs who underwent thymoglobulin induction (13.51% in all KTRs vs. 7.95% in KTRs who participated in the national health screening program) and acute rejection therapy within 1 year (10.69% in all KTRs vs. 7.34% in KTRs who participated in the health screening) tended to be higher. Furthermore, the mean number of hospitalizations within 1 year was higher among all KTRs compared with that among those who participated in the national health screening program (2.05 in all KTRs vs. 1.68 in KTRs who participated in the national health screening program).

Compared with non-DM, early PTDM was associated with an increased risk of graft failure and all-cause mortality in all KTRs who underwent KT between 2004 and 2020 (aHR 1.524, 95% CI 1.344–1.729 for graft failure and 1.339, 95% CI 1.153–1.535 for all-cause mortality) (**Table 5**).

DISCUSSION

This nationwide study in the Republic of Korea demonstrated that early PTDM, defined as the onset of DM between 3 months and 1 year post-KT, was not associated with graft failure but was significantly associated with a higher risk of all-cause mortality in KTRs who participated in a national health screening program post-KT. However, early PTDM was associated with a higher risk of graft failure in the overall KTR population. Changes in diabetic status from within to beyond 1 year after KT may serve as a more significant prognostic factor than early PTDM alone. Notably, KTRs with early PTDM who no longer had diabetes later exhibited no increased risk of mortality, whereas those who transitioned from non-DM to PTDM showed a higher risk of mortality. Notably, over one-third of patients with preexisting DM regressed to non-DM status. Nevertheless, preexisting DM was associated with a higher risk of mortality even among those who transitioned to non-DM, whereas graft failure risk was not elevated in this group.

Few studies have explored the association between early PTDM within 1 year post-KT and outcomes, and their results have been conflicting [3, 11]. One study reported that PTDM developing within 1 year post-KT was not associated with adverse outcomes, whereas PTDM developing after 1 year was associated with adverse outcomes [11]. This suggests that the prognostic impact of PTDM may vary depending on the timing of the onset, consistent with our findings. The timing of PTDM diagnosis is closely related to how PTDM is defined. The majority of studies included early post-transplant hyperglycemia as PTDM, and only a few excluded the first 1–3 months post-KT [9, 22, 23]. A recent consensus recommends performing the first diagnostic glucose tolerance test at 10–13 weeks post-KT, classifying earlier hyperglycemia as persistent hyperglycemia rather than PTDM [20]. Our study addressed this issue by defining early PTDM as new-onset diabetes occurring between 3 months and 1 year post-KT. When patients with hyperglycemia within 3 months were classified as non-DM, the HRs of PTDM for outcomes increased, suggesting that these patients may have a lower risk of complications. These findings support the current consensus recommending exclusion of hyperglycemia within 3 months of a diagnosis of PTDM.

The present study investigated the prognostic impact of the changes in diabetic status in the first year post-KT and beyond. Regression from early PTDM to non-DM was not associated with an increased risk of graft failure or mortality, whereas new-onset PTDM and persistent PTDM beyond 1 year post-KT were linked to worse outcomes. Preexisting DM conferred a persistent risk of mortality even after regression to non-DM, although the HR was lower than that of those with persistent DM. Some degree of hyperglycemia may be an appropriate compensatory response to acute stress conditions such as the early post-KT state. Hyperglycemia may develop as an appropriate response to glucose utilization by tissues in the presence of insulin resistance under acute stressful conditions such as sepsis [24]. In addition, early PTDM may also lead to more frequent medical visits and multidisciplinary care during the early post-transplantation period, thereby facilitating more optimal management. Few studies have evaluated the impact of changes in diabetic status after KT, and their findings have been conflicting [4, 25]. Transient post-KT hyperglycemia (corresponding to early PTDM to non-DM in the present study) was not associated with adverse outcomes in a previous

study [25]. In contrast, transient PTDM was associated with poorer patient and graft outcomes than sustained PTDM in another study, irrespective of its onset [4]. Our findings suggest that persistent DM or late-onset PTDM has a greater impact on outcomes than early post-transplant diabetic status. Our study revealed a more comprehensive picture of the dynamic nature and important clinical impact of changes in diabetic status on long-term outcomes post-KT. These findings underscore the importance of monitoring the changes in diabetic status over time after KT and the role of multidisciplinary care in improving the overall outcomes of KTRs with early PTDM or preexisting DM.

Predisposing factors for PTDM in KTRs include the use of glucocorticoids and calcineurin inhibitors, along with viral infections, such as cytomegalovirus [13, 26–28]. Thus, KTRs with high immunological risk or acute rejection who require more intense immunosuppressants are at a higher risk for PTDM and poor outcomes. Glucocorticoids and immunosuppressants can induce sarcopenia [29, 30]. Sarcopenia, which is associated with an increased risk of DM, is also associated with an increased risk of graft failure and mortality among KTRs [31–33]. In contrast, hyperglycemia after KT itself can promote oxidative stress and subsequent endothelial dysfunction, thereby accelerating allograft dysfunction and overall mortality [34]. Determining whether early PTDM is an independent prognostic factor or a marker of poor general status is therefore challenging. KTRs in poor condition after KT were unlikely to participate in the national health screening program. Indeed, the rates of thymoglobulin induction, acute rejection therapy, and hospitalization within 1 year post-KT observed among all KTRs tended to be higher than among national health screening program participants. This selection bias may explain why early PTDM was associated with graft failure among all KTRs but not among national health screening program participants among KTRs. Therefore, our findings might support the importance of actively managing PTDM in KTRs with a higher immunologic risk or poorer general conditions.

The present study has some limitations. First, the retrospective design and the limitations of the NHIS database hindered the capture of more detailed information, such as HLA matching status, donor information, and cause of death. Consequently, the possibility of unmeasured confounders that affected DM status and the associated outcomes cannot be excluded. Second, rather than using HbA1c levels, blood glucose levels, or glucose tolerance test results, the operational definition of DM status was based on diagnostic codes and antidiabetic medication use. However, this operational definition of DM using health insurance claims data has been soundly validated as a reliable tool in a recent study of the Korean population [35]. Third, the KTRs who participated in the national health screening program may be subject to selection bias, as they likely represent only relatively healthy or health-conscious KTRs. However, the relationship between early PTDM and clinical outcomes was assessed more accurately by excluding KTRs with extremely poor post-KT health status or poor compliance. This is a major strength of the present study.

In conclusion, early PTDM was associated with an elevated risk of mortality, while the development of PTDM beyond 1 year was associated with poor outcomes, and the resolution of early PTDM was associated with favorable outcomes. These findings underscore the importance of active surveillance, early intervention, and personalized management strategies for PTDM after KT to improve long-term patient and graft outcomes.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because they are owned by the Korea NHIS. Requests to access the datasets should be directed to https://nhiss.nhis.or.kr/bd/ab/bdaba000eng.do.

ETHICS STATEMENT

The studies involving humans were approved by Institutional Review Board of the Samsung Medical Center. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because the use of anonymized and de-identified data.

AUTHOR CONTRIBUTIONS

JJ, HJ, KH, and WH had full access to all aspects of the study and take responsibility for the integrity of the data and the accuracy of the data analysis. WH and KH conceptualized the study. JJ, HJ, KH, and WH designed the study. KH and YP acquired the data and performed the formal analysis. JJ, HJ, KL, JL, and WH interpreted the data. JJ drafted the initial manuscript, and JJ, HJ, YP, KL, and WH revised it. JL and KH supervised the study. All authors read and approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2026.15476/full#supplementary-material

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ORIGINAL RESEARCH
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Urinary VP1 Flow Cytometry as a Complementary Approach for BK Polyomavirus Monitoring: A Proof-Of-Concept Study

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Polyomavirus nephropathy (BKPyVAN) is a major cause of allograft dysfunction after kidney transplantation (KTX). While plasma BKPyV-PCR is the diagnostic gold standard, it may not fully reflect tissue injury. We conducted a prospective observational proof-of-concept study in 30 KTX recipients with BKPyV reactivation (November 2022–February 2024); 21 underwent kidney biopsy, 11 were diagnosed with biopsy-proven (BP)-BKPyVAN. Urine samples were analyzed by flow cytometry to quantify the potential of VP1-positive reno-urinary epithelial cells as a novel non-invasive marker of active tubular damage. The control cohort included 21 virology-negative patients. Median urinary VP1-positivity was higher in BP-BKPyVAN (33%, IQR 27–46) vs. non-BKPyVAN patients (5%, IQR 1–13; $p < 0.001$). The assay achieved an AUC of 0.98 (95% CI 0.93–1.00, p = 0.0003; cut-off: 11.7%; sensitivity = 91%, specificity = 89%) for BP-BKPyVAN. Longitudinally, median VP1-burden declined from 13% (IQR 4–29) at baseline to 0% (IQR 0–0.4). BKPyV-DNAemia declined rapidly, but plateaued at ~4×10^2–7×10^2 copies/mL, whereas urinary VP1-positive cells became undetectable. Our preliminary results suggest that combining urinary VP1-positivity with plasma BKPyV-PCR may help distinguish BP-BKPyVAN from non-BKPyVAN within a BKPyV-reactivation cohort. Longitudinal VP1 tracking may indicate resolution of viral infection earlier than DNAemia. These findings are hypothesis-generating and require validation in larger independent cohorts.

Keywords: BK polyomavirus–associated nephropathy, kidney transplantation, liquid biopsy, urinary VP1 flow cytometry, viral biomarkers

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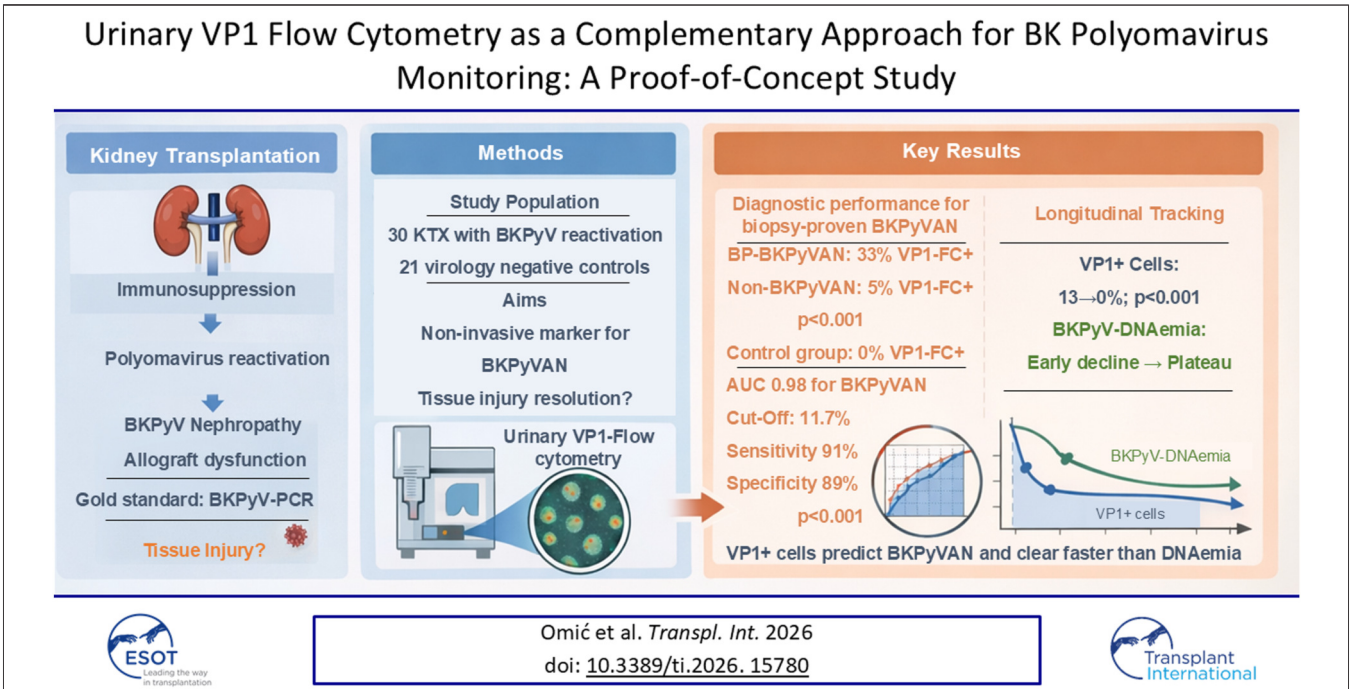
Abbreviations: ATG, anti-thymocyte globulin; BKPyV, BK polyomavirus; BKPyVAN, BK polyomavirus-associated nephropathy; CI, confidence interval; CMV-IgG, cytomegalovirus immunoglobulin G; DAPI, 4′,6-diamidino-2-phenylindole; DSA, donor-specific antibodies; ESRD, end-stage renal disease; FC, flow cytometry; FCS, fetal calf serum; HLA, human leukocyte antigen; HE, hematoxylin–eosin; IFL, immunofluorescence; IgG, immunoglobulin G; IQR, interquartile range; JCPyV, JC polyomavirus; JJ, double-J stent; KTX, kidney transplant; lTag, large tumor antigen; LOOCV, leave-one-out cross-validation; MMF, Mycophenolate/Mofetil; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; qPCR, quantitative polymerase chain reaction; ROC, receiver operating characteristic; SD, standard deviation; SV40, simian virus 40; TP, timepoint; VP1, viral capsid protein 1.



GRAPHICAL ABSTRACT |

INTRODUCTION

BK polyomavirus (BKPyV) is a highly prevalent human-specific pathogen residing in urogenital cells without pathogenicity in immunocompetent individuals [1–4]. However, its tropism and pathogenicity towards reno-urinary cells may change due to immunosuppression after kidney or stem cell transplantation [5, 6]. BKPyV may undergo a replicative surge, leading to lysis of tubular epithelial cells [6–8]. Infected cells are shed from the basement-membrane and can appear in urine as decoy cells [9, 10]. Despite the presence of BKPyV-DNAemia and high-level BKPyV shedding in the urine, affected patients typically do not exhibit systemic symptoms aside from kidney injury [11–13]. Physicians usually monitor BKPyV-replication after KTX to detect uncontrolled replication potentially leading to BK polyomavirus-associated nephropathy (BKPyVAN) [14, 15]. The gold standard for monitoring BKPyV uses quantitative polymerase chain reaction (qPCR) in plasma to assess BKPyV-DNAemia. In some centers urine sediment may also be assessed for the presence of decoy cells [16, 17]. In cases of worsening graft function, diagnostic proof of BKPyVAN requires KTX-biopsy, with evaluation of typical signs of lytic infection as well as immunohistochemical detection of simian virus 40 large T-antigen (SV40) in kidney cells [11, 18–21]. However, it has been observed that approximately 20% of BKPyVAN cases are still undetected using this method [13]. Consequently, alternative approaches such as qPCR for detecting BKPyV in urine and its sediment and the BK Haufen test were introduced to enhance sensitivity in detection strategies but are not part of routine care [22–24].

In this study, morning urine samples from KTX recipients with incipient or ongoing BKPyV-DNAemia and concomitant adaptation of immunosuppressive medication were collected longitudinally. Urine sediment cells were fixed, permeabilized, and stained via immunofluorescence by a novel BKPyV-specific capsid protein VP1 staining method to detect viral cellular presence, thereby allowing for a quantitative readout by flow cytometry (urinary-VP1-FC). This proof-of-concept study aims to evaluate a novel non-invasive FC-based immunofluorescence approach from urine sediment to enhance sensitivity for the success of ongoing viral clearance after the reduction of immunosuppression (IS). We investigated whether our biologically-sound approach may complement plasma PCR with its known lag time of viral clearance.

MATERIALS AND METHODS

Patient Selection

All KTX recipients (>18 years) at the outpatient clinic of the Department of Nephrology and Dialysis at the Medical University of Vienna between 11/2022 and 02/2024 were eligible for inclusion if they met one of the following criteria: Detection of BKPyV and/or JC polyomavirus (JCPyV) viremia on routine PCR; identification of decoy cells in

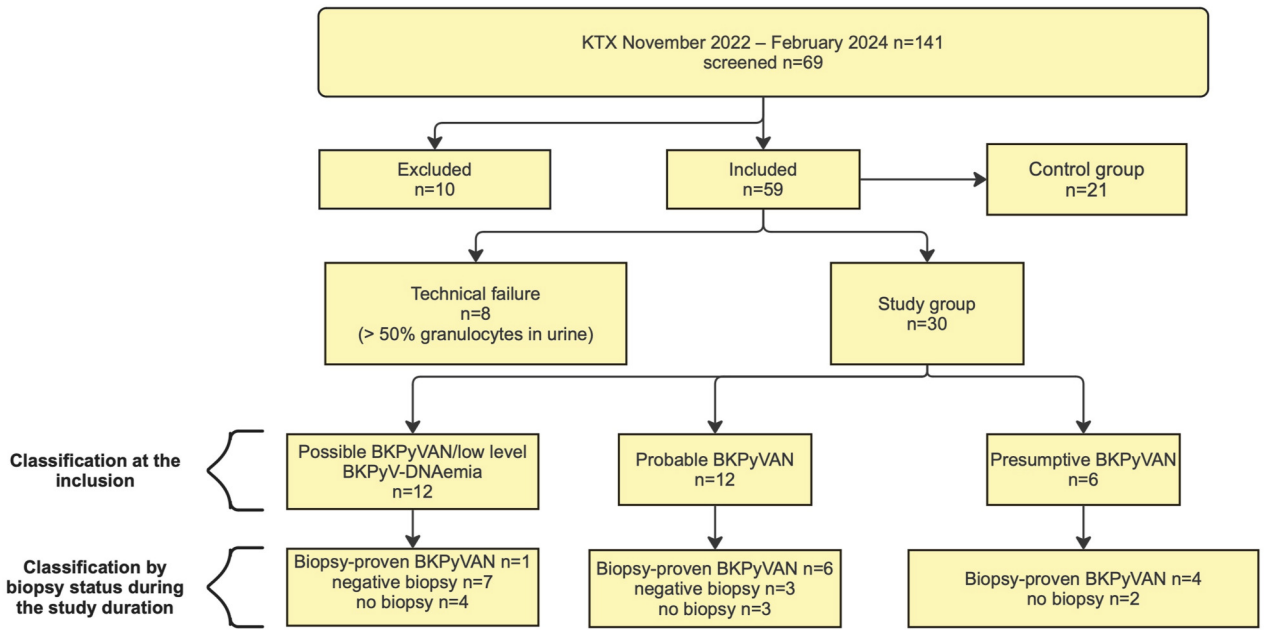


FIGURE 1 | Study Flowchart. Ten patients did not meet the inclusion criteria and were therefore excluded from flow cytometry analysis due to prolonged urine storage time beyond the predefined limit (n = 3) or missing essential clinical data (n = 7). Classification according to BKPyV DNAemia was based on plasma BKPyV DNAemia levels at study inclusion, whereas classification by biopsy status was based on biopsy results obtained within the study period.

routine urine sediment; a urinary viral load of BKPyV and/or JCPyV $>10^8$ U/mL; biopsy-proven BKPyVAN (BP-BKPyVAN), which was performed in line with current consensus recommendations in the context of allograft dysfunction [11]. Patients were included at the time of clinically relevant BKPyV reactivation prompting diagnostic evaluation, rather than at a predefined virological stage, resulting in variable timing relative to transplantation and viral kinetics. In addition, a control group of clinically stable KTX patients without evidence of relevant BKPyV/JCPyV replication was included. Controls had no detectable DNAemia and no clinically relevant DNAuria according to current consensus definitions (urinary DNA $<10^7$ copies/mL). The study flowchart is presented in **Figure 1**.

Test Methods for Urine and Plasma BKPyV

Urinary and plasma BKPyV and JCPyV-DNA were quantified as part of routine post-transplant surveillance at our center in all kidney transplant recipients included in the study, following international screening recommendations, acknowledging that PyVAN may rarely be caused by JCPyV (for details, see **Supplementary Materials**) [25, 26].

Definition of BKPyV-Associated Endpoints

BKPyVAN was categorized according to the most recent international consensus framework into four grades: possible, probable, presumptive, and definite BKPyVAN [13] (see details for each diagnostic tier in the **Supplementary Materials**).

Flow-Cytometric Analysis of VP1-Positive Urinary Epithelial Cells

Urine cell pellets were ethanol-fixed and immunolabeled for BKPyV capsid protein VP1, with DAPI nuclear counterstain, and acquired on a FACSCanto flow cytometer [16]. Flow-cytometry data were processed using custom Python scripts in JupyterLab. Samples with marked granulocyturia were pre-specified as technical exclusions. The detailed protocol is provided in the **Supplementary Materials**.

Cytopreparations

Urine sediment cells were maintained in culture medium as described above (RPMI 1640 supplemented with 2% FCS and ampicillin-streptomycin). A 60 µL aliquot was applied to the funnel of a cytocentrifuge and centrifuged at 1,200 rpm for 3 min. The resulting cytopreparation was air-dried for 1 h, followed by fixation in acetone for immunostaining or in the fixation solution of the Hemacolor® (Merck, Merck KGaA, Darmstadt, Germany; Millipore, SIG 1116740001) three-component kit for 4 min (see **Supplementary Materials**).

Immunofluorescence of Urinary Cells on Cytopreparations

Cytopreparations were acetone-fixed and immunostained for BKPyV VP1 using either a mouse monoclonal primary (Invitrogen 4942, 1:60) or a rabbit anti-VP1 primary (1:1,000) on separate slides. After incubation (overnight at 4 °C or 2 h at

room temperature) and PBS washes, Alexa Fluor 594 goat anti-mouse or Alexa Fluor 488 goat anti-rabbit secondary antibodies (1:700) were applied with DAPI nuclear counterstain. Slides were mounted in Vectashield and imaged on a Zeiss inverted confocal microscope; images were post-processed in Adobe Photoshop. Full step-by-step details are provided in the **Supplementary Materials**.

Decoy Cell Staining

One cytopreparation was sequentially stained with eosin, followed by hematoxylin and a washing buffer, using the Hemacolor® staining kit (Merck, Merck KGaA, Darmstadt, Germany; Millipore, SIG 1116740001). Cell enumeration was performed via light microscopy (see **Supplementary Materials**).

Statistical Analysis

Continuous variables were assessed for normality with the Shapiro-Wilk test and are presented as means ± standard deviations (SD) when normally distributed or as medians with interquartile ranges (IQR) for skewed data. Group comparisons were performed using the student's t-test or the Mann-Whitney U test. Kruskal-Wallis's test was used to compare the differences in BKPyV-DNAemia and urinary-VP1-FC counts in more than two groups. Complete statistical analysis is described in **Supplementary Materials**.

RESULTS

Study Cohort

Between November 1st, 2022 and February 29th, 2024, 30 KTX recipients meeting our inclusion criteria for the study group and 21 KTX for the control group were analyzed. A flowchart of patient inclusion and exclusion is depicted in **Figure 1**. **Table 1** provides all relevant patient dispositions and transplant-specific baseline characteristics. Median age was 59 years (IQR 49.8–64.0) and 19 (63%) recipients were male. Donor-specific antibodies were detected in one (3%) patient. The median HLA A/B/DR mismatch was three (IQR 2–4). As induction therapy the majority (97%) received IL-2 antibody basiliximab (20 mg on days 0 + 4). Maintenance immunosuppression consisted of tacrolimus/MMF/steroids in all patients (**Table 1**). The first flow-cytometry assay was performed at a median of 11.5 months after KTX (IQR 2.7–18.1). At baseline, BKPyV-DNA was detectable at high levels in urine (median 3.3×10^6 c/mL, IQR 2.5×10^1–4.0×10^9) and well detectable in plasma (median 4.6×10^3 c/mL, IQR 2.5×10^2–8.8×10^3). Tacrolimus trough levels at the time of the first urinary VP1-FC sample (baseline) were at a median of 7.8 ng/mL (IQR 6.9–9.3), which was followed by a constant reduction due to significant BKPyV infection. In 29 (96%) patients the MMF dose was either reduced or MMF was paused. Serum creatinine mirrored this, with a median of 1.86 mg/dL (IQR 1.09–4.82) at baseline and a subsequent increase from months one to three, which was afterwards followed by a stabilization phase (**Table 1**; **Supplementary Table S1**). Renal allograft biopsy was performed in 21 of 30 patients (70%) with 10 being classified as biopsy-negative and 11 as biopsy-proven BKPyVAN. With respect to the timing of the biopsy, eight (38%) were performed at a median of 1 month (IQR 1.0–2.0) before the first urinary VP1-FC assessment - of which none showed signs of BKPyVAN - and 13 biopsies (62%) were performed at a median of 1 month after urinary VP1-FC (IQR 0.0–4.0, **Supplementary Figure S1**). Protocol biopsies accounted for 40% (4/10) of the biopsy-negative group and 18% (2/11) of the biopsy-proven group (p = 0.36). Three of the eight biopsies performed prior to VP1 sampling were protocol biopsies.

Our control cohort comprises 21 KTX recipients screened during the study, of whom 62% were male, with a median age of 63 years (IQR 50.0–67.5). All had negative BKPyV-DNAemia and no clinically relevant BKPyV-DNAuria (DNA-uria centered at zero; median 0 copies; IQR 0–145) and low JCPyV-DNAuria (median 0 copies; IQR 0–2,850). Correspondingly, FC measurements were low and clustered around baseline, with a median value of 0.0 (IQR 0.0–3.2). In the control cohort 9 (43%) biopsies were performed, 7 (33%) after the VP1-FC sampling. All were negative for BKPyVAN.

Baseline Cross-Assay Quantification of VP1-Positive Urinary Cells

Urinary-VP1-FC using the rabbit polyclonal anti-VP1 antibody detected a median of 13% (14%–29%, n = 30) VP1-positive urinary epithelial cells. When the same cytospin was examined by indirect immunofluorescence, the rabbit-derived immunoreagent produced a median signal of 10% positive cells (0%–84%, n = 30). In contrast, the subset stained with the mouse monoclonal antibody averaged 6% (2%–40%, n = 19). Classical hematoxylin-eosin morphology identified decoy-like nuclei in 3.5% (0%–40%, n = 30) of cells, and the pathologist's semi-quantitative decoy cell scoring showed a median of 2% (0%–25%, n = 21) decoy cells. In the BK-negative control group, all baseline cross-assay measurements were centered at background levels, with median values of 0.0 for VP1-FC, indirect immunofluorescence, and hematoxylin–eosin assessment. **Supplementary Figure S2** shows the representative confocal immunofluorescence of urine sediment from a 58-year-old male KTX recipient with BKPyVAN, with numerous VP1-positive cells at low magnification. The detailed fluorescence intensity measurements of five representative transplant recipients are provided in **Supplementary Figure S3**, presenting the technical performance of the urinary VP1 assay.

Urinary-VP1-Positive Cell Burden and Diagnostic Certainty of BKPyVAN

Among 30 recipients stratified by baseline plasma BKPyV-DNAemia (**Figure 1**), urinary VP1-positive cell percentages differed significantly across groups ($p = 0.04$). In Dunn's post-hoc comparisons, recipients with presumptive BKPyVAN showed a greater VP1 burden than those with possible BKPyVAN (adjusted $p = 0.03$). When BK-negative controls were included in the same model, controls showed

TABLE 1 | Patient disposition and baseline characteristics.

Variable		
Age, years (median, IQR)	59	50–64
Recipient male sex (n, %)	19	63
Donor male sex (n, %)	12	55
Cause of ESRD		
Congenital* (n, %)	10	33
Glomerular** (n, %)	4	13
Metabolic/vascular*** (n, %)	11	37
Unknown etiology (n, %)	4	13
Pre-transplant immunology		
DSA-positive (n, %)	1	3.3
Recipient CMV-IgG positive (n, %)	17	56
Donor CMV-IgG positive (n, %)	18	60
Transplant-related variables		
First transplant (n, %)	27	90
Second transplant (n, %)	3	10
HLA A/B/DR mismatch sum (median, IQR)	3	2–4
Lymphocyte-depleting agent (n, %)	1	3.3
Desensitization: IA (n, %)	1	3.3
Tacrolimus (n, %)	30	100
MMF/MPA (n, %)	30	100
Steroids	30	100
Time to Double-J removal (days, median, IQR)	56	43–75
BKPyV-associated data		
Time to first VP1-urinary-FC post Tx (months, median, IQR)	10	2–16
Time to peak urinary-VP1-FC post Tx (months, median, IQR)	11.5	2.7–18.1
BKVPyV-DNAuria at first urinary-VP1-FC (c/mL, median, IQR)	3.3×10^6	2.5×10^1–4.0×10^9
BKPyV-DNAemia at first urinary-VP1-FC (c/mL, median, IQR)	4.0×10^3	4.0×10^2–9.0×10^3
BKPyV-DNAemia peak after first urinary-VP1-FC (c/mL, median, IQR)	6.0×10^3	1.0×10^3–2.0×10^4
Patients with JCPyV-DNAemia-only (n/%)	2	6
Patients with combined JCPyV + BKPyV-DNAemia (n/%)	2	6
JCPyV-DNAuria positive (n/%)	8	28
JCPyV-DNAuria (c/mL) (median, IQR)	7.1×10^6	1.1×10^6–2.2×10^8
JCPyV-DNAemia positive (n/%)	4	13
JCPyV-DNAemia (c/mL) (median, IQR)	3.2×10^3	1.0×10^2–8.4×10^4
Tacrolimus trough levels after sampling (ng/mL, median, IQR)		
Month 0 (=baseline urinary-VP1-FC sampling)	7.8	6.9–9.3
Month 1	6.9	5.6–8.9
Month 2	5.9	4.5–9.0
Month 3	6.5	5.4–8.5
Month 4	6.6	5.9–7.1
Month 5	6.7	5.8–8.5
Month 6	6.8	5.7–8.1
Serum creatinine after sampling (mg/dL, median, IQR)		
Month 0 (=baseline urinary-VP1-FC sampling)	1.9	1.1–4.8
Month 1	1.9	1.3–4.3
Month 2	2.0	1.2–3.9
Month 3	2.1	1.1–4.1
Month 4	1.9	1.3–3.4
Month 5	1.9	1.3–3.6
Month 6	2.0	1.3–3.7
MMF/MPA reduction by 50% during episode (n, %)	16	53
MMF/MPA reduction by >50% during episode (n, %)	13	43

Baseline variables of study population:

[a]*Congenital causes of end-stage renal disease (ESRD): agenesis, Alport syndrome, autosomal dominant polycystic kidney disease, autosomal recessive polycystic kidney disease, urethral valve, anatomic malformations.*

[b]*Glomerular: FSGS, glomerulonephritis, IgA nephropathy.*

[c]*Metabolic/vascular causes: diabetes mellitus, hypertension, amyloidosis, EPH, gestosis; ESRD, end-stage renal disease; DSA, donor-specific antibody; CMV, cytomegalovirus; HLA, human leukocyte antigen; ATG, antithymocyte globulin; IA, immunoadsorption; MMF, mycophenolate mofetil; MPA, mycophenolic acid; BKPyV, BK, polyomavirus; VP1-FC, VP1-flow cytometry; Tx, transplantation; JCPyV, JC, polyomavirus; DNAuria, viral DNA, detected in urine; DNAemia, viral DNA, detected in plasma.*

consistently lower VP1 levels than all BKPyVAN categories (**Figure 2A**). Among recipients stratified by diagnostic certainty (possible BKPyVAN with low-level BKPyV-DNAemia, probable BKPyVAN, and biopsy-proven BKPyVAN), urinary VP1-positive cell percentages increased progressively across tiers, with a significant overall group

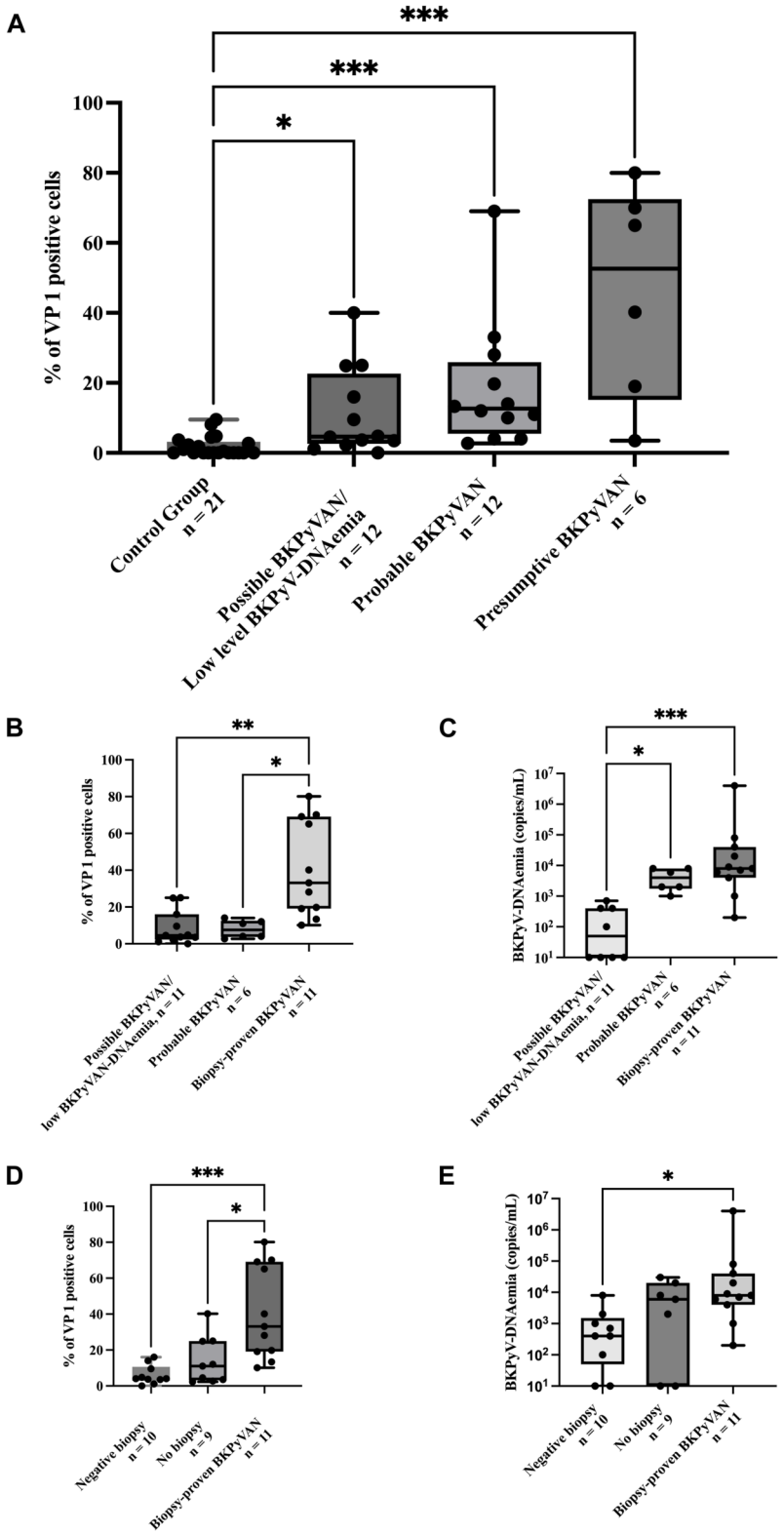


FIGURE 2 | Urinary VP1-positive cell burden and BKPyV-DNAemia by BKPyVAN status; VP1 positivity was calculated as the percentage of VP1-positive cells among 200 DAPI-positive, non-polymorphic nuclei per sample, excluding debris and polymorphonuclear cells; **(A)** Urinary VP1-positive cell percentages in viremic recipients at study inclusion across BKPyV diagnostic certainty categories and the control group ($p < 0.001$). **(B)** Box-and-whisker plots of urinary VP1-positive cell

(*Continued*)

FIGURE 2 | percentages in patients with possible BKPyVAN with low-level BKPyV DNAemia (n = 11), probable BKPyVAN (n = 6), and biopsy-proven BKPyVAN (n = 11), demonstrating a significant overall group difference (Kruskal–Wallis p < 0.001). Median VP1 positivity increased stepwise across categories [possible: 4.5% (IQR 3.5–14.0), probable: 12.0% (IQR 6.0–22.0), biopsy-proven: 33.0% (IQR19.0–69.0)]. **(C)** Plasma BKPyV DNAemia across the same diagnostic tiers, showing significant differences between groups (Kruskal–Wallis p < 0.001). **(D)** Urinary VP1-positive cell percentages stratified by biopsy status: biopsy-negative (n = 10), no biopsy (n = 9), and biopsy-proven BKPyVAN (n = 11), with significantly higher VP1 burden in biopsy-proven cases (Kruskal–Wallis p = 0.0009). **(E)** Plasma BKPyV DNAemia according to biopsy status, demonstrating significant differences across groups (Kruskal–Wallis p < 0.001).All panels depict baseline measurements obtained at study inclusion.

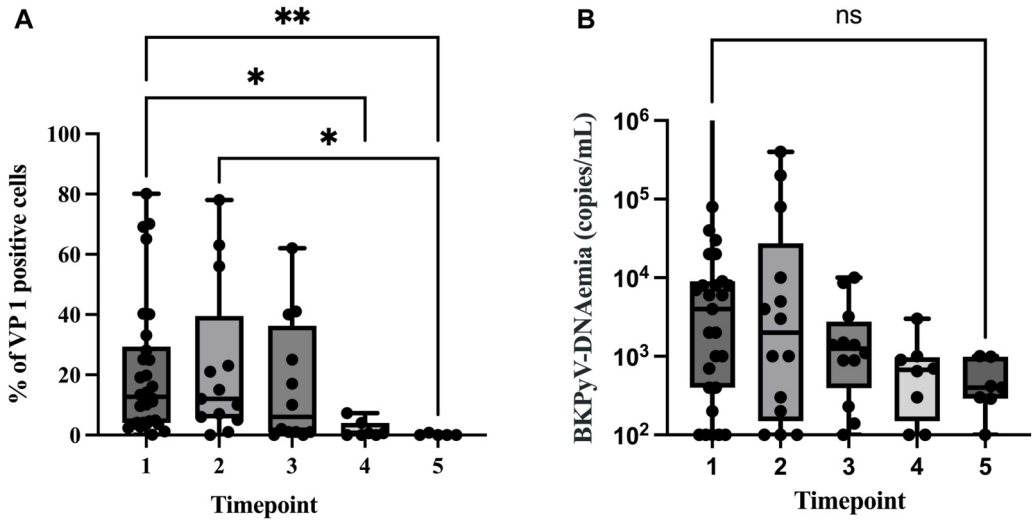


FIGURE 3 | Longitudinal decline in urinary VP1-positive cells and BKPyV-DNAemia. **(A)** Box-and-whisker plots depict the percentage of VP1-positive epithelial cells at each of the five scheduled samplings (Timepoints, TP1-5). Boxes indicate the inter-quartile range (IQR), horizontal bars represent medians. Medians (IQR) fell from 13% (4%–29%) at Timepoint 1 (TP1) to 0% (0%–0.4%) at TP5, illustrating a progressive loss of detectable VP1 antigen. Median timepoints after the study inclusion for the five collections were: baseline (as Timepoint1; TP 1), 21d for TP2, 53d for TP3, 109d for TP4, and 203d for TP5. The corresponding inter-quartile ranges (IQR) were 16–65 days (d), 34-111d, 59-220d and 153-218d. Pairwise Dunn tests (family-wise $\alpha = 0.05$) identified significant reductions between early and late measurements: TP1 vs. TP4 (p = 0.02*), TP1 vs. TP5 (p = 0.005**), TP2 vs. TP4 (p = 0.05), and TP2 vs. TP5 (p = 0.01). Asterisks above the boxes mark these differences (*<0.05; **<0.01); non-significant comparisons are omitted for clarity. **(B)** Box-and-whisker plots of parallel BKPyV DNAemia measurements (copies/mL) at five sequential urine collections (TP1-TP5).

difference (**Figure 2B**; p < 0.001). Plasma BKPyV-DNAemia likewise varied significantly by diagnostic tier (**Figure 2C**; p < 0.001). Stratification by biopsy status demonstrated significant differences in urinary VP1 burden (**Figure 2D**; p = 0.0009) and plasma BKPyV-DNAemia (**Figure 2E**; p < 0.001). Finally, patients with isolated BKPyV-DNAuria had significantly lower urinary VP1 positivity than those with concurrent BKPyV-DNAemia (p = 0.02).

Temporal Decline in Urinary VP1-Positive Cells and Parallel BKPyV-DNAemia Measurements

Across the five flow-cytometric assessments, the distribution of VP1-positive cells became progressively narrower and shifted toward lower values (**Figure 3**). Collection timepoints (TPs) for the FC samples are described in **Figure 3**. At baseline (TP1), the median VP1 burden was 13% (IQR 4%–29%). At TP2 the median fell to 11% (IQR 4%–31%), then to 6% (IQR 1%–36%) at TP3, and to 0.6% (IQR 0%–4%) at TP4. At TP5 the median had declined to 0% (IQR 0%–0.4%); $p < 0.001$. Collectively, these data indicate that the bulk of VP1 clearance occurred between months two and four post-urinary-VP1-FC sampling (i.e., between TP3 and TP4), after which the viral signal plateaued to nearly background levels in almost all patients (**Figure 3A**). Parallel to the flow-cytometric quantification of VP1-positive cells, at TP1, the median viral load was 4.0×10^3 copies/mL (IQR 4.0×10^2–9.0×10^3), which declined to 2.0×10^3 copies/mL (IQR 1.5×10^2–2.8×10^4) at TP2 and to 1.3×10^3 copies/mL (IQR 4.0×10^2–2.9×10^3) at TP3. By TP4 the median had fallen further to 5.0×10^2 copies/mL (IQR 1.3×10^2–1.0×10^3) and at TP5 to 4.0×10^2 copies/mL (IQR 2.0×10^2–1.0×10^3), despite complete loss of VP1 signal by FC at TP5. **Figure 3B**, shows a rapid initial drop in DNAemia between TP1 and TP2, followed by a gradual plateau towards low-level DNAemia thereafter. Across visits, urinary VP1% showed a positive correlation with BKPyV-DNAemia. At TP1, the correlation was moderate (rho = 0.59, $p = 0.001$), but it was strong at TP2 (rho = 0.75, $p = 0.01$). At later visits, correlations were weaker and not statistically significant.

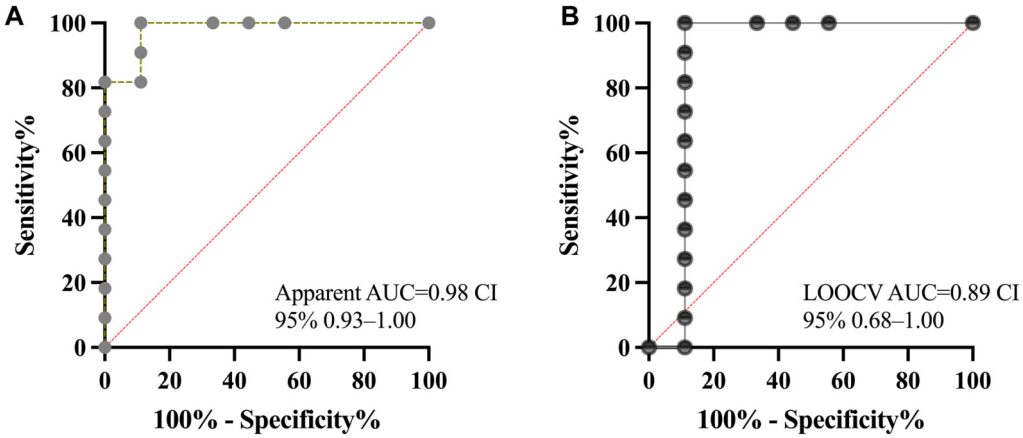


FIGURE 4 | Receiver-operating-characteristic curves for urinary-VP1-FC in detecting biopsy-proven BKPyVAN. **(A)** depicts the apparent ROC obtained when the model was both trained and tested on all 20 recipients with available biopsy after the sampling (13 biopsies from study group and 7 biopsies from the control group; AUC = 0.98). **(B)** shows the leave-one-out cross-validated ROC, in which each patient was predicted by a model that excluded his or her data (AUC = 0.89). Diagonal line = reference for random classification (AUC = 0.50).

ROC Analysis for the Diagnostic Performance of VP1 Liquid Biopsy

Receiver-operating-characteristic analysis for urinary VP1-FC, using biopsy-proven BKPyVAN as the endpoint of interest and restricted to 20 recipients biopsied after study inclusion (including 7 available biopsies from the control group), demonstrated excellent diagnostic performance when using baseline (TP1) values. The AUC was 0.98 (95% CI 0.93–1.00, p = 0.0003; **Figure 4A**). A VP1 threshold of 7.4% achieved 100% sensitivity and 88.9% specificity. Increasing the cutoff to 11.7% resulted in a sensitivity of 90.9% and specificity of 88.9%, whereas a stricter rule-in threshold of 17.5% yielded sensitivity of 81.8% with 100% specificity, thereby favoring confirmation over detection. In LOOCV, urinary VP1-FC retained good discriminatory performance (AUC 0.89, 95% CI 0.68–1.00; p = 0.0034) despite the small cohort (**Figure 4B**). However, when separating groups based on possible, probable, and presumptive BKPyVAN using viremia levels at TP1, AUC for the prediction of BKPyVAN by urinary VP1-FC applying a cutoff of 17.5% was 0.74 for possible, 0.52 for probable, and 0.80 for presumptive BKPyVAN. Corresponding values for sensitivity were 47%, 33%, and 83%, and for specificity were 73%, 56%, and 71%. The optimal VP1-burden thresholds maximizing Youden's index were >10% for possible BKPyVAN (sensitivity 79%, specificity 73%), >4% for probable BKPyVAN (sensitivity 92%, specificity 33%), and >40% for presumptive BKPyVAN (sensitivity 67%, specificity 96%). The secondary analysis using the combined logistic model of urinary VP1 and BKPyV-DNAemia demonstrated the strongest predictive performance for biopsy-proven BKPyVAN (n = 13, AUC = 0.97). Predicted probability surfaces revealed that probabilities exceeded 0.95 already at approximately 18%–20% VP1 positive cells, even at low viral loads, and approached 0.99 beyond 25% VP1 across any BKPyV-DNAemia (**Supplementary Figure S4**).

Longitudinal FC Kinetics

A total of 68 urine samples from 30 KTX recipients were available for mixed-effects modelling. At TP1, VP1 load was 21% (geometric mean, 95% CI 13%–35%). Time after diagnosis (start of the reduction of immunosuppression) was an independent predictor for VP1 decline: $\beta = -0.044 \pm 0.022$, $p = 0.047$. Exponentiation of the coefficient indicated a 4% weekly decline in VP1-positive cells ($\exp\beta = 0.96$, 95% CI 0.92–1.00). Raw observations are displayed in **Figure 5A**, while a population-level fitted curve with its 95% CI is shown in **Figure 5B**. To define a clinically meaningful threshold for VP1 positivity, we reclassified VP1 negativity as ≤1% to avoid trace analytical noise. We then examined VP1-positivity across predefined BKPyV-DNAemia bins. For the BKPyV-DNAemia in a range of 200–700 copies/mL, VP1 was almost always absent, with a median of 0% (IQR 0%–0.95% in 2×10^2–4×10^2; 0%–0.87% in 4×10^2–5×10^2) and positivity observed in only 3 of 12 cases (25%). By contrast, VP1 positivity increased substantially with higher viremia. At 7×10^2–1×10^3 copies/mL, 67% of samples were positive (median 2.65%, IQR 0.6–12.2), and at 1×10^3–3×10^3 copies/mL, 58% were positive (median 3.35%, IQR 0.9–12.9). Above 3×10^3 copies/mL, VP1 positivity was frequent (3×10^3–10×10^4: 86%; 1×10^4–1×10^5: 88%), with median VP1 levels rising steeply (11.3% and 60.6%, respectively). These results indicate that VP1 positivity becomes unlikely once plasma BKPyV DNAemia falls into the ≤500–700 copies/mL range.

DISCUSSION

In this prospective cohort of KTX recipients, we demonstrate that VP1-positive cell quantification via urinary-VP1-FC might

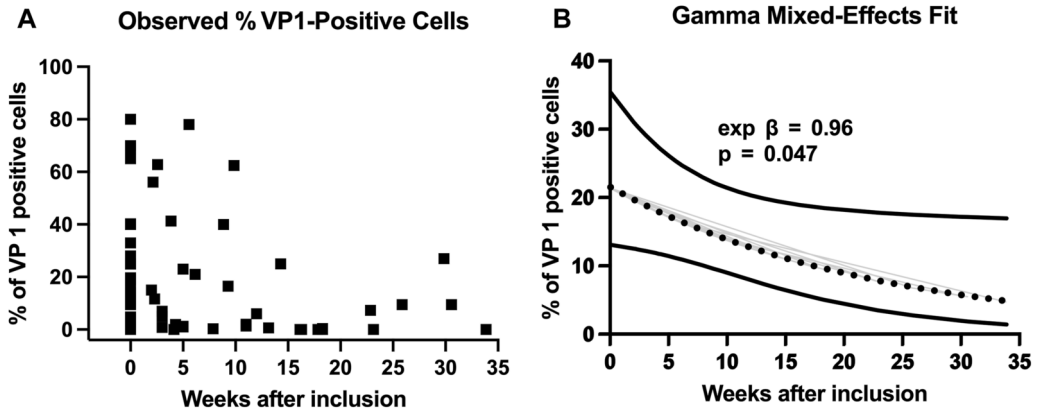


FIGURE 5 | Time course of urinary VP1-positive cells and mixed-effects model fit. **(A)** Observed percentages of VP1-positive epithelial cells measured in 68 urine samples from 30 kidney-transplant recipients. **(B)** The dotted black line shows the population-level trajectory predicted by the gamma-log mixed-effects model, and the area above and below it denotes its 95% confidence interval. Grey lines indicate the individual VP1 trajectories. According to the model, VP1 positivity declines by ≈ 4% per week (expβ = 0.96, p = 0.047), mirroring gradual viral clearance after diagnosis.

provide a sensitive non-invasive marker to monitor the course of BKPyV infection across different BKPyV-associated endpoints. At baseline, recipients across diagnostic certainty tiers exhibited a stepwise increase in median VP1 burden. Biopsy-proven BKPyVAN revealed the highest VP1 levels, underscoring the relationship between cellular antigen load and disease severity. Our control cohort demonstrated that urinary VP1-detection was largely confined to BKPyV-affected recipients with controls being at assay background-levels only. Moreover, longitudinal sampling revealed a rapid decline in VP1-positive cells between months 2 and 4 post-sampling, paralleling, but ultimately outlasting reductions in plasma BKPyV-DNAemia. Finally, ROC analysis confirmed excellent discriminative performance (AUC 0.98; 95% CI 0.93–1.00) of baseline VP1-FC for BKPyVAN, with an optimal cut-off at 11.6% VP1-positive cells yielding both high sensitivity and specificity. Our preliminary findings support further validation of urinary VP1-FC trajectories for monitoring the course of BKPyV infection. Our results align with earlier reports that direct enumeration of infected cells in conjunction with qPCR may more accurately reflect viral injury [27–29]. Traditional decoy-cell scoring is limited by subjective interpretation and low throughput as well as the requirement for trained pathologists [30]. Immunofluorescence assays targeting VP1 may improve specificity when compared to urinary diagnostic methods [27, 31]. Our urinary VP1-FC assay builds on the availability of standard flow cytometers in routine laboratories. Once established, it may deliver rapid (<24 h), quantitative high-throughput detection of infected reno-urinary epithelial cells, overcoming logistical and technical constraints of other biomarkers for BKPyVAN [23]. The *Haufen test*, which detects viral aggregates in urine by electron microscopy, has shown good diagnostic accuracy in independent cohorts. However, its reliance on electron microscopy and labor-intensive protocols has limited its clinical adoption [23, 32]. Similarly, early transcriptomic assays measuring urinary VP1, granzyme B, and PI-9 mRNA reported high sensitivity and specificity for biopsy-proven BKPyVAN, but these methods were technically demanding, rarely reproduced, and did not address viral clearance kinetics [33]. In contrast, urinary VP1-FC provides direct quantification of infected epithelial cells and may offer the advantage to observe viral clearance in advance to the resolution of DNAemia. This may aid in the evaluation of success or failure during/after reduction of immunosuppression and its potential re-installation.

Temporal kinetics of VP1-positivity mirror the clinical course of BKPyVAN. At baseline we observed a median burden of 13% VP1-positive cells, which declined to 0% by the fifth sampling (≈7 months after study inclusion). Notably, the steepest drop occurred after the second month, coinciding with immunosuppression reduction. Additionally, our threshold analysis demonstrated that urinary VP1-FC is negative in low range viremia, whereas positivity is frequently observed above 10^3 c/mL. Persisting viremia with or without further graft damage due to BKPyV has already been reported [34–37]. Those findings show a possible dissociation between the clearance of cell-associated antigen and the persistence of circulating viral genomes. Such compartmentalized kinetics suggest that urinary VP1-FC may report on the resolution of intrarenal infection, capturing the disappearance of actively replicating epithelial reservoirs, whereas PCR-based DNAemia measurements can remain elevated owing to free virions, non-cell-associated viral debris, concentration, and genomic variation or mutations in primers [38–40]. Therefore, in scenarios where DNAemia plateaus, reliance on PCR alone may underestimate viral clearance on the tissue-level, underscoring the added value of our approach for guiding immunosuppression (re)-adjustments. ROC-derived thresholds for urinary VP1-FC compared favorably with those reported for urine PCR and decoy-cell counts. An AUC of 0.98 for biopsy-proven BKPyVAN is even higher than previously reported in small-series estimates for urinary cell assays (AUC≈0.85–0.92) and

exceeds typical performance of urinary viral load cut-offs alone (AUC≈0.75–0.85) [27, 31, 41, 42]. The 11% VP1 burden threshold optimizes Youden's index, yet a lower cut-off of 7% captures all cases at the expense of specificity. This trade-off mirrors clinical dilemmas in BKPyV management, where early detection must be balanced against overtreatment risks [43]. Additionally, the absence of BKPyVAN in early biopsies indicates that preceding histological findings did not bias VP1 measurements via changes in immunosuppressive therapy. However, while ROC analyses suggested excellent discrimination, the low sample size increases the risk of optimistic performance estimates despite LOOCV, and confidence intervals remain wide. Accordingly, these results should be interpreted with caution and only as hypothesis-generating and require confirmation in larger, independent cohorts. Future work will focus on assay standardization (pre-analytical handling, gating strategy, and inter-run calibration) and prospective multicenter validation with pre-specified thresholds and quality-control procedures.

Mixed-effects modeling quantified a weekly 4% decline in VP1 positivity post-diagnosis, reinforcing the assay's potential for dynamic monitoring. When combined with cross-validated discrimination, the joint probability modeling with BKPyV-DNAemia confirmed that urinary VP1-positivity >20% already corresponds to >95% predicted probability of biopsy-proven BKPyVAN. Together, these kinetics support further exploration with respect to biopsy-sparing approaches and non-invasive monitoring of therapeutic re-escalation of immunosuppression.

Despite these promising findings, several limitations merit discussion. First, 30 recipients from a single center limit generalizability and may introduce selection bias. Second, 30% of patients were not biopsied, potentially misclassifying some as BKPyVAN-negative; although non-invasive assays may obviate biopsy in many cases, such missing histology reduces certainty in diagnostic accuracy estimates. Additionally, the observed heterogeneity in biopsy timing relative to VP1-FC sampling reflects routine clinical workflows and supports interpretation of VP1-FC as a dynamic marker of polyomavirus activity rather than a biopsy-synchronized diagnostic test. While we included a virologically negative, stable transplant control cohort, this group does not capture all inflammatory or ischemic graft pathologies that may confound urinary cellular readouts. Consequently, the reported specificity and AUC primarily reflect performance within a BKPyV-reactivation framework and should not be interpreted as real-world specificity across all causes of graft dysfunction. Larger validation studies incorporating relevant differential diagnoses are required prior to clinical implementation. Further, the flow-cytometric assay did not include epithelial lineage markers (e.g., pan-cytokeratin or EpCAM); although urinary samples in BKPyV infection are predominantly epithelial, inflammatory cell admixture in injured grafts cannot be excluded, and future studies should confirm the cellular origin of VP1-positive events. Another limitation is that the polyclonal anti-VP1 antibody recognizes conserved VP1 epitopes across polyomaviruses, including BKPyV and JCPyV. While this precludes viral species discrimination at the cellular level, it aligns with routine diagnostic practice, where SV40 immunohistochemistry similarly detects polyomavirus infection without species specificity. Consequently, the assay should be interpreted as reflecting active polyomavirus replication rather than BKPyV-exclusive disease. In addition, as demonstrated by other research groups, BKPyV frequently acquires mutations - particularly in the VP1 protein - potentially leading to false negative results in our assay [44, 45]. Lastly, urine samples with more than 50% granulocytes were excluded because high granulocyte content interferes with reliable flow-cytometric acquisition and VP1 signal interpretation. This exclusion may introduce selection bias, as granulocyturia can occur in the context of concurrent infection or acute rejection, conditions that may overlap clinically with BKPyVAN. However, this limitation reflects a technical constraint of the current assay rather than *post hoc* selection, and the study population remains representative of patients in whom non-invasive urinary monitoring is clinically applicable.

Strengths of the study include the current application of the hierarchical BKPyVAN diagnostic algorithm, parallel comparison of urinary VP1-FC with multiple detection modalities (PCR, IFL, decoy cells), and robust statistical methods to avoid overfitting. Moreover, the direct measurement of VP1 on exfoliated cells target the pathogen at its site of action, arguably offering greater pathophysiological relevance than surrogate markers.

If future studies confirm our findings, integration of urinary VP1-FC into post-transplant surveillance may complement current BKPyV monitoring strategies. This proof-of-concept cohort lacks a fully representative control population and the observed diagnostic performance requires validation in larger cohorts before conclusions regarding real-world specificity or clinical utility can be drawn. Standardization of assay protocols and interlaboratory calibration will be essential for broader implementation. While PCR remains the gold standard for detecting BKPyV replication, VP1-FC directly quantifies cell-associated viral antigen in exfoliated tubular epithelial cells and may provide complementary insight into intrarenal infection dynamics, particularly in the setting of persistent low-level or plateauing DNAemia. The potential role of urinary VP1-FC in informing immunosuppression management, including re-escalation strategies after apparent cellular viral clearance, should therefore be evaluated in future prospective studies.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by Ethics Committee of the Medical University of Vienna (No. 1065/2021) and conducted following the Declaration of Helsinki

and Declaration of Istanbul. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HO: Conceptualization, study design, data collection, data analysis, interpretation of results and drafting of the manuscript. DV: Laboratory analysis, data interpretation, visualization and manuscript editing. ME: Interpretation of results and manuscript editing. KAE-G: Data collection, laboratory analysis, data interpretation and drafting of the manuscript. WW: study design, data interpretation and drafting of the manuscript. AS: data interpretation, drafting of the manuscript and critical revision. SK: study design, data interpretation and drafting of the manuscript. DG: Data collection, statistical analysis and critical revision. RS: Data collection and critical revision. LW: Laboratory analysis, data interpretation and manuscript editing. FE: Study supervision, study design, data analysis, interpretation of results, drafting of the manuscript and final approval of the manuscript.

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CONFLICT OF INTEREST

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2026.15780/full#supplementary-material

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ORIGINAL RESEARCH
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Long-Term Outcomes Across Age and Risk Profiles in a Caucasian Living Kidney Donor Cohort

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Living kidney donation achieves excellent recipient outcomes, but increasingly involves older and medically complex donors, while long-term data across age groups remain limited. The Heidelberg Kidney Donor Study followed 632 donors (1991–2020), stratified by age <40 (n = 93), 40–60 (n = 424), and >60 years (n = 115). Primary outcomes were a ≥50% eGFR decline and an eGFR <45 mL/min/1.73 m^2 at long-term follow-up. Early post-donation adaptation, long-term eGFR trajectories, cardiovascular events, and risk patterns were evaluated. Mean donor age was 50.6 ± 10.6 years (62.5% female). eGFR declined by 26.0% after donation and remained stable thereafter. At a median follow-up of 12 years, ≥50% eGFR decline occurred in 4.8%, 5.3%, and 14.4% of donors aged <40, 40–60, and >60 years, respectively, an eGFR <45 mL/min/1.73 m^2 in 1.2%, 5.3%, and 20.4%. An eGFR <30 mL/min/1.73 m^2 occurred in 1.2%, major adverse cardiovascular events in 4.3%. Age, hypertension, and baseline-eGFR independently predicted renal impairment. Younger donors with hypertension or obesity had up to a 14.3% risk of ≥50% eGFR decline, exceeding the risk in healthy older donors (12.5%). Living kidney donation was associated with stable long-term kidney function after early adaptation, with substantial heterogeneity driven more by baseline renal reserve and comorbidity than chronological age alone.

Keywords: age, cardiovascular risk, living kidney donation, renal function, risk score

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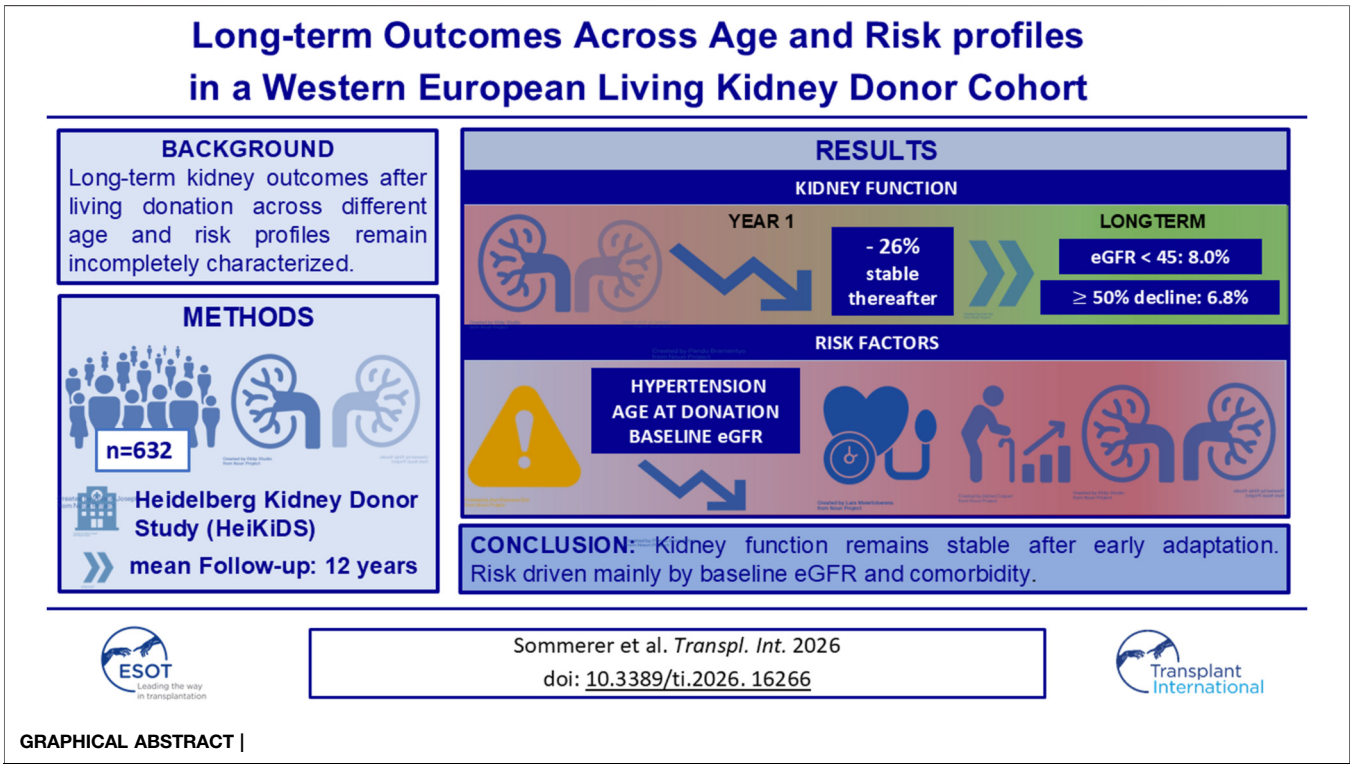
INTRODUCTION

Living kidney donation (LKD) is the optimal treatment for renal failure, increasing organ availability, shortening waiting times and enabling preemptive transplantation with superior graft and patient survival [1–3]. Most international and national cohort studies have demonstrated no significant long-term harm to donors [4–9]. However, two more recent studies identified a modestly higher risk of endstage renal disease (ESRD) in living kidney donors compared with the general population [10, 11], providing limited data on outcomes for older donors and those with cardiometabolic comorbidities.

Due to organ shortages and long waiting times, transplant centers increasingly accept medically complex living kidney donors – older individuals or those with hypertension and obesity [12], consistent with recent registry observations [13]. This shift may increase donors' risk for renal and cardiovascular events and affect transplant ouctomes [12, 14]. Such donors now account for 25%–50% of LKD program [15]. Acceptance criteria vary widely between transplant centers [16].



GRAPHICAL ABSTRACT |

Despite the importance of this issue, high-quality studies with long-term outcomes are scarce [9, 14, 17, 18]. Most available data derive from retrospective registry-based analyses focusing on hard endpoints, with limited insight into longitudinal kidney function trajectories or clinically relevant heterogeneity among donors.

The aim of the present study was to characterize long-term renal and cardiovascular outcomes after living kidney donation across donor age groups using detailed longitudinal follow-up data. A central objective was to disentangle whether advanced donor age predominantly affects the level of kidney function achieved after donation or whether it is associated with an accelerated rate of subsequent kidney function decline over time.

In addition, the study aimed to assess the modifying role of baseline renal reserve and common comorbidities and to describe heterogeneity in long-term renal risk based on routinely collected donor characteristics.

MATERIALS AND METHODS

Study Design

The Heidelberg Kidney Donor Study (HeiKiD) is a prospective cohort study established to evaluate long-term outcomes after LKD and to enhance shared decision-making and informed consent.

The study was approved by the ethics committee of the University Hospital Heidelberg (S104-2011), and all participants provided written informed consent. Data collected prior to 2011 were obtained retrospectively from medical records. Since 2011, donors have been followed prospectively within a structured cohort study with predefined follow-up intervals.

Data handling complied with the European General Data Protection Regulation.

Eligible donors were aged ≥18 years, had completed the pre-donation evaluation, and had at least 5 years of follow-up after donation. The analysis included 632 caucasian donors who donated between 01/1991 and 06/2020 for recipients across 48 dialysis centers in southern and central Germany. Demographic data were obtained via structured questionnaires. Clinical and laboratory data were collected before donation, 1 year post-donation, and annually thereafter. In accordance with German living donor evaluation standards, all potential donors underwent comprehensive cardiovascular assessment before donation, including echocardiography and exercise ECG; if coronary heart disease was known or non-invasive testing was abnormal, coronary angiography was performed. Donors with clinically significant or uncontrolled cardiovascular disease were generally excluded, and only mild, stable, or previously treated cardiovascular conditions were accepted after interdisciplinary evaluation.

Study Outcomes

Primary outcomes were:

- ≥50% decline in estimated glomerular filtration rate (eGFR) from baseline to last follow-up
- prevalence of eGFR <45 mL/min/1.73 m^2 at long-term follow-up.

The threshold corresponds to CKD stage 3b and was selected because this level of kidney function has been consistently associated with a substantially increased risk of cardiovascular events, CKD progression, and mortality in large population-based studies [19, 20].

Secondary endpoints included the incidence of eGFR <30 mL/min/1.73 m^2, the need for renal replacement therapy, composite major adverse cardiovascular events (MACE), individual components of MACE (myocardial infarction, stroke, cardiovascular death, peripheral artery disease events (revascularization, amputation)), and death.

Beyond binary renal endpoints, analyses focused on early post-donation change, achieved post-adaptation kidney function, and long-term eGFR trajectories.

Analyses were stratified by donor age (<40 years, 40–60 years, and >60 years) to explore age effects. In addition, the impact of baseline comorbidity on longterm outcome was analyzed. A dedicated subanalysis was performed in donors aged ≥70 years.

Statistical Methods

Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR) and compared using the Student's t-test or Mann–Whitney U test. Categorical variables were presented as counts and percentages and compared using the chi-square or Fisher's exact test. Bias was minimized through consecutive enrollment and consistent data collection. Analyses were performed using complete case data without imputation.

Linear mixed-effects models were used to evaluate the association of age at donation with eGFR levels and longitudinal eGFR trajectories.

To separate the expected early post-nephrectomy adaptation from subsequent long-term changes, early eGFR change was quantified from baseline to 12 months after donation. Early change was defined as ΔeGFR0–12 (eGFR1y − eGFRbaseline) and, in sensitivity analyses, as percent change (%ΔeGFR). Determinants of early eGFR change were assessed using multivariable linear regression with prespecified predictors: baseline eGFR (CKD-EPI), age at donation (continuous), preexisting hypertension, BMI, sex, and donation era (grouped into 1991–2000, 2001–2010, 2011–2020). To facilitate comparison of effect sizes across predictors, standardized regression coefficients were calculated for continuous variables.

To assess determinants of achieved kidney function at long-term follow-up, multivariable linear regression models were fitted with eGFR at last follow-up as the dependent variable and adjusted for baseline eGFR, early eGFR change (ΔeGFR0–12), follow-up duration, age at donation, hypertension, BMI, and sex.

Risk Grouping and Regression Analysis

Donors were classified by age and presence of established risk factors for kidney function decline. Associations between baseline variables and outcomes were assessed using univariate logistic regression. Multivariate models included prespecified clinically relevant variables.

Exploratory Risk Stratification Using Baseline Clinical Variables

An exploratory composite score was constructed to illustrate the combined association of key baseline factors with long-term outcomes. Independent predictors from the multivariate analysis were used in an exploratory manner to summarize their joint association with long-term renal outcomes. Regression coefficients (β) were used to assign relative weights to each predictor, reflecting their contribution within this cohort. An individual composite score was calculated as the sum of weighted predictors. For descriptive purposes, risk scores were categorized into tertiles representing low, intermediate, and high-risk groups, and the observed incidence of both outcomes was calculated for each category. Model calibration was assessed exploratorily by comparing predicted and observed outcome frequencies across tertiles and deciles, including calibration plots, calibration slope and intercept, and the Hosmer–Lemeshow test. Discriminative performance was quantified using the area under the receiver operating characteristic curve (AUC).

All analyses were performed in R (Version 2024.12.0).

RESULTS

Cohort Characteristics

A total of 632 living kidney donors with a minimum follow-up of 5 years were included. Of these, 93 were aged <40 years (14.7%), 424 were aged 40–60 years (67.1%), and 115 were aged >60 years (16.6%), **Table 1**. The mean age at donation was 50.6 ± 10.6 years (range 19–77). Mean donor age increased from 43.9 years in 1990–1994 to 53.4 years in 2015–2020.

Overall, 62.5% were female, ranging from 53.9% among donors >60 years of age to 71.0% in the youngest group. Nearly two-thirds were related to their recipients (392/632; 62.0%), most frequently parent-to-child, especially mother-to-son (16.9%), **Table 1**.

The median follow-up time after donation was 12 years (IQR 9–16).

Cardiometabolic History and Baseline Renal Findings

Cardiometabolic profiles showed clear age-related trends. Mean BMI was comparable across all groups, but overweight and obesity were more common in older donors. Smoking was more prevalent in younger individuals, while hypertension and the use of antihypertensives increased with age. Accordingly, office and 24-h blood pressure measurements were higher in older donors. Dyslipidemia and thyroid disorders also increased with age (**Table 1**).

Hematuria was observed in 9.6% of donors and occurred more frequently in female than male donors (12.8% vs. 4.2%). In cases of unexplained hematuria, a pre-donation kidney biopsy was performed in 14 donors. Histopathological findings were generally mild and major glomerulopathies were excluded. Thin basement membrane alterations were reported in 3 donors.

TABLE 1 | Baseline Characteristics of the Living Kidney Donor Cohort and Median Follow-Up, stratified by Age.

Parameter	n	Total cohort	<40 years	40–60 years	>60 years	p-value
n		632	93	424	115	
Demographics						
Age (mean ± SD)	632	50.6 ± 10.6	33.3 ± 5.3	50.3 ± 5.6	65.5 ± 3.7	**<0.001**
Male gender (%)	632	37.5 (237)	29.0 (27)	37.0 (157)	46.1 (53)	**0.0387**
BMI (kg/m^2)	615	26.2 ± 4.1	25.4 ± 4.8	26.4 ± 4.0	26.3 ± 3.6	**0.0405**
18-25 kg/m^2		41.1 (253)	49.4 (44)	40.6 (168)	36.6 (41)	
<18 kg/m^2		1.0 (6)	3.4 (3)	3.1 (13)		
>25 kg/m^2		40.5 (249)	27.0 (24)	43.2 (175)	44.6 (50)	
Obesity class I (BMI 30-35 kg/m^2)		14.1 (87)	15.7 (14)	13.3 (55)	16.1 (18)	
Obesity class II (BMI 35-40 kg/m^2)		2.8 (17)	3.4 (3)	3.1 (13)	0.9 (1)	
Obesity class III (BMI >40 kg/m^2)		0.5 (3)	1.1 (1)	0.5 (2)	1.8 (2)	
Nicotine use (active smoker)	603	24.2 (146)	31.4 (27)	26.6 (107)	10.5 (12)	**0.0008**
Donated to	630					
Related		63.7 (392)	80.0 (72)	61.7 (259)	58.4 (66)	**0.0012**
First-degree relatives		61.1 (385)	73.1 (68)	60.0 (253)	55.7 (64)	**0.0150**
Unrelated		36.2 (226)	20.0 (18)	38.3 (161)	51.6 (47)	**0.0012**
Spouse		31.6 (200)	17.2 (16)	34.0 (144)	34.8 (40)	**0.0051**
Sibling		13. 9 (88)	29.0 (27)	12.3 (52)	7.8 (9)	**<0.001**
Mother -- child		29.3 (185)	35.5 (33)	28.5 (121)	27.0 (31)	0.3427
Father -- child		18.2 (115)	9.6 (8)	19.3 (82)	21.7 (25)	**0.0288**
Friend		1.9 (12)	1.1 (1)	2.6 (11)	5.2 (6)	0.4978
Blood pressure parameters						
Mean arterial pressure (mmHg)	618	96.8 ± 9.8	93,2 ± 9.3	96.6 ± 9.4	99.9 ± 10.4	**<0.001**
Systolic BP (mmHg)	618	128.5 ± 15.1	123.2 ± 13.2	127.9 ± 14.6	134.9 ± 16.2	**<0.001**
Diastolic BP (mmHg)	618	80.8 ± 8.5	78.3 ± 8.6	81.0 ± 8.2	82.5 ± 9.2	**0.0097**
24h systolic BP mean (mmHg)	467	125.6 ± 13.0	121.9 ± 13.5	125.2 ± 13.1	130.4 ± 10.6	**<0.001**
24h diastolic BP mean (mmHg)	466	77.4 ± 8.9	74.5 ± 7.2	77.7 ± 9.2	78.7 ± 8.5	**0.0054**
Medical history						
Hypertension	632	44.5 (281)	24.7 (23)	43.2 (183)	65.2 (75)	**<0.001**
Diabetes mellitus	632	0.0 (0)	0.0 (0)	0.0 (0)	0.0(0)	-
Prediabetes	595	5.2 (31)	3.4 (3)	4.8 (19)	8.1 (9)	0.2691
Hyperlipidemia	632	8.6 (54)	2.2 (2)	7.8 (33)	16.5 (19)	**<0.001**
Metabolic syndrome	611	7.7 (49)	10.2 (9)	7.5 (31)	6.3 (7)	0.5624
Thyroid disease	632	17.3 (109)	5.4 (5)	18.0 (76)	24.0 (26)	**0.001**
Cardiovascular disease I	632	0.9 (6)	0.0 (0)	0.7 (3)	2.6 (3)	0.15
Malignant diseases	632	2.2 (14)	0.0 (0)	2.4 (10)	2.6 (3)	0.400
COPD/Asthma/Chronic bronchitis	632	3.8 (24)	2.2 (2)	4.3 (18)	3.6 (4)	0.6256
Urological diseases	632	4.6 (29)	1.1 (1)	4.5 (19)	7.8 (9)	0.062
Gynecological diseases	632	6.3 (40)	5.4 (4)	7.8 (33)	1.7 (2)	0.057
Psychiatric disorders	632	2.5 (16)	0.0 (0)	2.8 (12)	3.5 (4)	0.4181
Family medical history						
Kidney disease	388	24.5 (95)	32.5 (13)	21.1 (58)	32.9 (24)	0.0528
Medication use						
Antihypertensive medication	632	24.4 (154)	3.2 (3)	23.1 (98)	46.1 (53)	**<0.001**
Number of antihypertensives	632	0.36 ± 0.74	0.04 ± 0.25	0.33 ± 0.67	0.76 ± 1.03	**<0.001**
Lipid-lowering drugs	632	5.1 (32)	0.0 (0)	4.0 (17)	13.0 (15)	**<0.001**
Thyroid medication	632	12.5 (79)	1.1 (1)	13.4 (57)	18.3 (21)	**0.0006**
Pain medication (non-NSAIDs)	632	0.2 (1)	0.0 (0)	0.2 (1)	0.0 (0)	0.7822
Antidepressants	632	2.1 (13)	0.0 (0)	2.4 (10)	2.6 (3)	0.3138
Kidney data						
Kidney length right (mm) (mean ± SD)	575	112.0 ± 9.2	112.5 ± 13.6	112.4 ± 8.2	110.5 ± 8.5	**0.041**
Kidney length left (mm) (Mean ± SD)	574	112.6 ± 9.6	112.2 ± 14.3	113.1 ± 8.7	111.3 ± 8.2	**0.0049**
MAG3-clearance (mL/min/1.73m^2)	513	232.2 ± 44.4	254.9 ± 43.0	232.9 ± 44.2	212.6 ± 37.1	**<0.001**
Renal function right side (%, mean ± DS)	559	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04	0.5434
Renal function left side (%, mean ± SD)	559	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04	0.50 ± 0.04	0.5282
Renal function donated kidney (%, mean ± SD)	559	0.49 ± 0.04	0.48 ± 0.04	0.49 ± 0.04	0.49 ± 0.04	0.8883
Hematuria	628	9.6 (60)	4.3 (4)	11.2 (47)	7.9 (9)	0.1005

(*Continued*)

TABLE 1 | Continued

Parameter	n	Total cohort	<40 years	40–60 years	>60 years	p-value
Proteinuria[a] (g/L) (Median IQR)	613	0.037 (0.018–0.065)	0.034 (0.013–0.067)	0.037 (0.0195–0.0645)	0.043 (0.020–0.062)	0.626
Proteinuria[a] >0.15 (g/L)	613	2.9 (18)	5.6 (5)	2.9 (12)	0.91 (1)	0.1408
Pre-donation biopsy	631	2.2 (14)	0.0 (0)	2.1 (9)	4.3 (5)	0.1039
Donated kidney left side	598	53.0 (318)	46.0 (55)	53.0 (216)	50.0 (56)	0.8000
Median follow-up years (Md, IQR)	632	12.3 (8.8–16.2)	13.9 (10.0–18.6)	12.1 (8.6–16.2)	11.8 (8.6–14.9)	**0.0046**

Continuous variables are presented as mean ± standard deviation (SD), and group comparisons were performed using the non-parametric Kruskal-Wallis test. Categorical variables are shown as percentages with the absolute number in parentheses, and were compared using the Chi-square test or Fisher's exact test, as appropriate. A p-value less than 0.05 was considered statistically significant.

Abbreviations: BMI -Body Mass Index; Obesity class I: BMI, 30–34.9 kg/m^2; Obesity class II: BMI, 35–39.9 kg/m^2; Obesity class III: BMI ≥40 kg/m^2. BP: blood pressure; SPB: COPD: chronic obstructive pulmonary disease; MAG3: Mercaptoacetyltriglycine (renal scintigraphy agent); NSAIDs: Non-Steroidal Anti-Inflammatory Drugs; n–number; SD: standard deviation. Bold values indicate statistical significance.

[a]*Measured in spot urine samples.*

History of Malignancy and Psychiatric Disorders

A history of malignancy was documented in 14 donors (2.2%), including breast cancer (n = 2), cervical cancer (n = 3), colon cancer (n = 1), papillary thyroid carcinoma (n = 1), renal cell carcinoma (n = 1), seminoma (n = 1), appendiceal carcinoid tumor (n = 1), pleural tumor (n = 1), and basal cell carcinoma (n = 3). Psychiatric disorders were reported in 16 donors (2.5%), predominantly depressive disorders (n = 12), followed by bipolar disorder (n = 2), panic attacks (n = 1), and adjustment disorder/burnout (n = 1). The prevalence of these comorbidities at baseline and at long-term follow-up is shown in **Figure 1**, both overall and stratified by age group (<40, 40–60, >60 years).

Descriptive Course of Kidney Function

Baseline-eGFR decreased progressively with age and fell by 0.58 mL/min/1.73 m^2 per year of age (β = −0.58; 95% CI −0.67 to −0.48; p = 3.26 × 10^{-2}), which corresponds to −5.76 mL/min/1.73 m^2 per decade.

During the study, CKDepi eGFR decreased from 95.8 ± 14.3 to 66.5 ± 16.6 mL/min/1.73 m^2 (Δ-30.6%, p < 0.001, **Table 2**). This decline was observed in all age groups but was most pronounced in donors >60 years (−35.1%, p < 0.001). Transitions between eGFR categories are shown in **Figure 2**. Within the first year after donation, eGFR decreased by an average of 26.0% (Δ−22.4% < 40 years; 24.9% 40–60 years; −31.8% > 60 years) and remained stable with minimal long-term change (+0.4%, −1.2%, +3.2%, respectively), **Figure 3**.

Across longitudinal follow-up, older age at donation was associated with lower eGFR values (−0.65 mL/min/1.73 m^2 per year of age, p < 0.001), whereas the rate of eGFR decline did not differ by age group (interaction p = 0.54). Donors with hypertension demonstrated the largest early decline and slightly lower long-term eGFR values (**Figure 4**).

≥ 50% Decline in eGFR at Long-Term Follow-Up and Prevalence of eGFR <45 mL/min/1.73 m^2

A ≥50% eGFR decline at long-term was detected in 6.8% of donors, with the prevalence increasing sharply with age (4.8% < 40 years, 5.3% 40–60 years, 14.4% > 60 years, p = 0.0053), **Table 3**. Even among younger donors, the incidence ranged from 3.0% in donors with a favorable profile to 14.3% in donors with both hypertension and obesity. In older donors, the risk was 12.5% in donors without risk factors, 22.4% in donors with one risk factor, and 45.5% in hypertensive obese donors (**Figure 5**).

eGFR <45 mL/min/1.73 m^2 was identified in 8.0% of donors, with the prevalence increasing sharply with age (1.2% < 40 years, 5.3% 40–60 years, 20.4% > 60 years). The incidence of an eGFR <45 mL/min/1.73 m^2 rose further with the presence of risk factors such as hypertension and obesity. In older donors, the risk was 12.5% in donors without risk factors, 22.4% in donors with one risk factor, and 45.5% in hypertensive obese donors (**Figure 5**).

Factors Associated With a ≥50% Decline in eGFR at Long-Term Follow-Up

In the univariate analysis (**Table 4**), age at donation strongly predicted a ≥50% decline in eGFR: each additional year increased the probability (≈odds) of a substantial GFR loss by about 4.7% (OR 1.047, p = 0.006). Donors >60 years had a more than three times higher risk compared to donors <40 years (OR 3.34, p = 0.038).

Among preexisting comorbidities, hypertension and thyroid disease proved to be significant predictors, while hyperlipidemia, cardiovascular disease, and prediabetes were not predictive. The use of antihypertensive, lipid-lowering, and thyroid medications were also linked to a higher risk.

Each additional month since donation, increased the risk. Older age at follow-up also remained a strong predictor. At long-term follow-up, hypertension (both preexisting and new-onset) was associated with GFR loss, and the presence of malignancy also increased the risk.

Factors Associated With an eGFR <45 mL/min/m^2 at Long-Term Follow-Up

Older age at donation increased the long-term risk of an eGFR <45 mL/min/1.73 m^2 by approximately 10.6% per years (OR 1.11, p < 0.001), **Table 5**. Donors older than 60 years had a

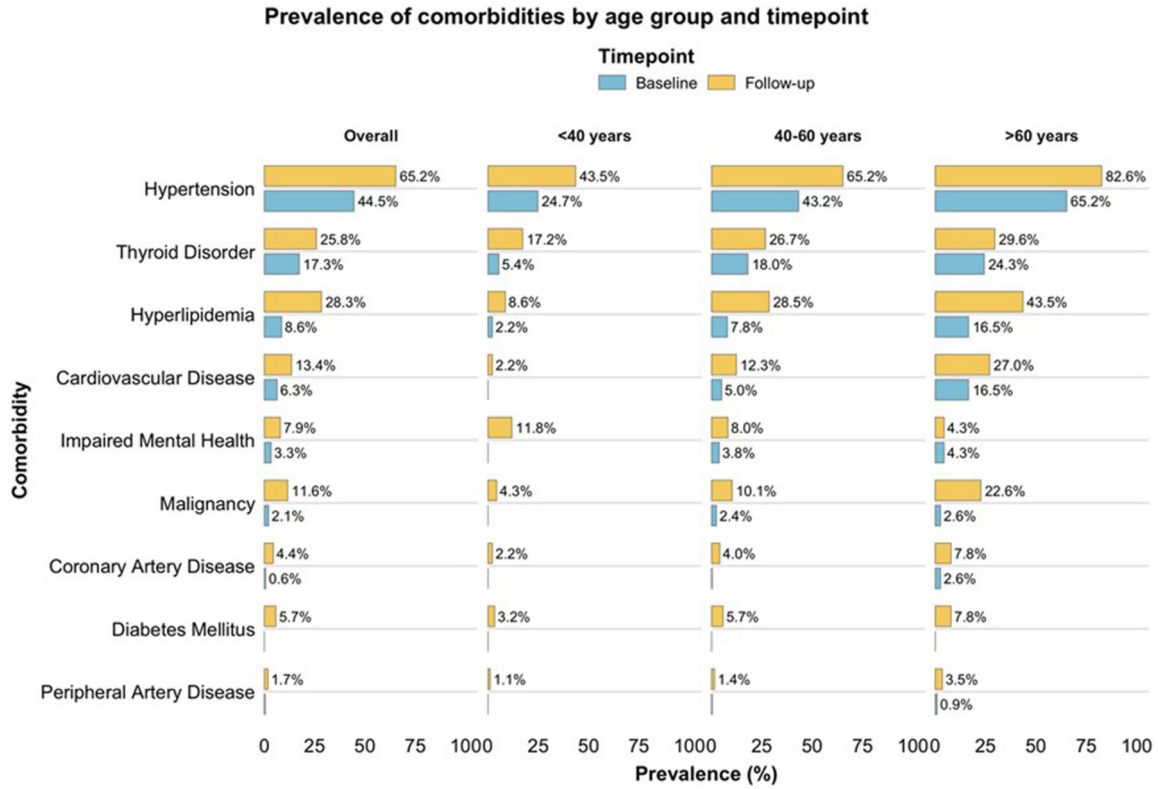


FIGURE 1 | Prevalence of comorbidities at baseline and at long-term follow-up after living kidney donation. Stacked horizontal bar plots show the percentage of donors with selected comorbidities before donation (blue) and at last follow-up (yellow), presented overall and by age group (<40, 40–60, >60 years).

substantially higher risk compared with those younger than 40 years (OR 6.62, $p < 0.001$). Pre-existing hypertension was associated with an approximately twofold increased risk of an eGFR <45 mL/min/1.73 m^2 (OR 2.07, $p = 0.002$. A similar increase was observed for hyperlipidemia (OR 2.40, $p = 0.036$). Thyroid disease was also associated with a significantly higher risk of reduced eGFR (OR 2.30, $p = 0.020$).

A lower baseline GFR as measured by MDRD, CKDepi, and Cockcroft-Gault formulas predicted a higher risk (all $p < 0.001$).

At long-term follow-up, older age continued to increase the risk by approximately 10.5% per year. Hypertension, hyperlipidemia, and malignant diseases were also significantly associated with increased risk.

In the multivariate analysis, age at donation, hypertension and baseline eGFR (OR 0.93), remained independent predictors (**Figure 6**).

Determinants of Early Post-Donation eGFR-Decline

In multivariable linear regression analyses with absolute early eGFR change (ΔeGFR 0–12 months) as the dependent variable, baseline eGFR showed the strongest association with early decline (standardized $\beta = -0.29$, $p < 0.001$). Age at donation was independently associated with early decline but with a smaller effect size (standardized $\beta = -0.16$, $p = 0.002$). Hypertension showed a modest additional association (standardized $\beta = -0.10$, $p = 0.023$), whereas BMI and donation era were not independently associated with early eGFR change. When early eGFR change was expressed as a percentage of baseline function, age at donation and baseline eGFR showed comparable but smaller effect sizes (standardized $\beta = -0.16$ and -0.12, respectively), while BMI and donation era remained non-significant.

Clinical Consequences and Preservation of Kidney Function

Severe renal impairment was rare, an eGFR <30 mL/min/1.73 m^2 was observed in 1.2% and <15 mL/min/1.73 m^2 in 0.3% of donors. Two patients required renal replacement therapy. Preservation of renal function (≤20% decline) was noted in 20.0% overall (32.4% in donors <40 years, 22.0% in donors 40–60 years, and 15.4% in donors >60 years).

MACE occurred in 4.3% (n = 27) of donors (**Table 2**), increasing with age (mean age at donation 55 years) and longer follow-up.

Twenty-one deaths occurred predominantly among older donors (mean age 73 ± 8.7 years at death), with malignancies accounting for 52.4%. Median time from donation to death was 14 years (IQR 10–19). Overall long-term survival was high across all age groups (**Table 3**).

TABLE 2 | Longitudinal Changes in Laboratory Parameters, stratified by Age.

Laboratory Values	Group	Baseline (mean ± SD)	Last follow-up (mean ± SD)	p-value (longitudinal)	p-value (age groups)
CKDepi GFR (mL/min/1.73m^2)	Total	95.8 ± 14.3	66.5 ± 16.6	<0.001	<**0.001**
	<40	104.9 ± 19.3	77.9 ± 19.5	<0.001	
	40–60	96.3 ± 12.6	66.7 ± 15.0	<0.001	
	>60	86.7 ± 11.0	56.3 ± 13.3	<0.001	
MDRD GFR (mL/min/1.73m^2)	Total	100.6 ± 20.7	62.3 ± 15.2	<0.001	<**0.001**
	<40	107.0 ± 27.0	69.8 ± 15.5	<0.001	
	40–60	100.5 ± 19.2	61.9 ± 14.4	<0.001	
	>60	95.8 ± 18.6	57.4 ± 15.5	<0.001	
Creatinine (mg/dL)	Total	0.78 ± 0.16	1.07 ± 0.32	<0.001	**0.001**
	<40	0.79 ± 0.19	0.99 ± 0.22	<0.001	
	40–60	0.78 ± 0.15	1.06 ± 0.33	<0.001	
	>60	0.79 ± 0.16	1.14 ± 0.30	<0.001	
Cholesterol (mg/dL)	Total	203 ± 41	208 ± 38	0.287	
	<40	202 ± 42	191 ± 39	0.0584	
	40–60	206 ± 40	210 ± 36	0.746	
	>60	196 ± 44	210 ± 40	0.00168	
LDL cholesterol (mg/dL)	Total	126 ± 32	120 ± 35	0.0084	0.616
	<40	118 ± 37	123 ± 34	0.0614	
	40–60	127 ± 31	120 ± 35	0.0668	
	>60	126 ± 32	115 ± 36	0.0005	
HDL cholesterol (mg/dL)	Total	59 ± 16	59 ± 17	0.1502	0.093
	<40	55 ± 17	57 ± 17	0.7374	
	40–60	60 ± 17	59 ± 18	0.0459	
	>60	59 ± 15	61 ± 16	0.5115	
Triglycerides (mg/dL)	Total	114 ± 73	139 ± 123	<0.001	0.370
	<40	101 ± 73	129 ± 81	0.0066	
	40–60	117 ± 76	144 ± 134	<0.001	
	>60	110 ± 58	130 ± 104	0.0826	
TSH (mU/L)	Total	1.48 ± 1.44	1.82 ± 1.20	<0.001	0.891
	<40	1.84 ± 1.18	2.06 ± 1.59	0.0982	
	40–60	1.45 ± 1.58	1.73 ± 0.97	<0.001	
	>60	1.33 ± 0.97	2.00 ± 1.57	0.0016	
Hemoglobin (g/dL)	Total	14.07 ± 1.29	14.02 ± 1.51	0.7636	0.139
	<40	13.86 ± 1.41	13.97 ± 1.74	0.2376	
	40–60	14.08 ± 1.30	14.11 ± 1.46	0.1775	
	>60	14.15 ± 1.10	13.70 ± 1.49	0.0092	
HbA1c (%)	Total	5.45 ± 0.40	5.56 ± 0.58	<0.001	0.440
	<40	5.34 ± 0.37	5.36 ± 0.55	0.301	
	40–60	5.42 ± 0.38	5.59 ± 0.64	<0.001	
	>60	5.62 ± 0.43	5.57 ± 0.58	0.905	
Urea (mg/dL)	Total	27.8 ± 7.6	35.2 ± 13.6	<0.001	<**0.001**
	<40	26.2 ± 7.5	30.3 ± 9.7	<0.001	
	40–60	27.8 ± 7.8	34.9 ± 12.9	<0.001	
	>60	29.3 ± 6.5	39.9 ± 16.9	<0.001	
Phosphate (mg/dL)	Total	1.03 ± 0.23	1.06 ± 0.32	0.102	0.230
	<40	1.05 ± 0.18	1.01 ± 0.32	0.265	
	40–60	1.03 ± 0.24	1.06 ± 0.30	0.0304	
	>60	1.02 ± 0.21	1.09 ± 0.41	0.563	

Mean ± SD, values represent the average laboratory measurements and their Standard deviation (SD) for each parameter, stratified by age group and timepoint (baseline before donation and long-term follow-up. Longitudinal comparisons between T0 and LFUP, within each age group were performed using paired Wilcoxon signed-rank tests to assess whether changes over time are statistically significant. Between-group comparisons at the LFUP, timepoint were conducted using.

Kruskal-Wallis tests to evaluate differences across age groups. P-values are reported for both the longitudinal and between-group (age group) tests. A p-value less than 0.05 was considered statistically significant.

Abbreviations: Hb–hemoglobin; Hct–hematocrit; MDRD GFR, glomerular filtration rate estimated by Modification of Diet in Renal Disease equation; CKDepi GFR, glomerular filtration rate estimated by Chronic Kidney Disease Epidemiology Collaboration equation; HbA1c–glycated hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; n - number; iPTH, intact parathyroid hormone; TSH, thyroid-stimulating hormone.

Bold values indicate statistical significance.

Longitudinal Changes in Laboratory Parameters

Glycemic control remained stable, although HbA1c levels increased slightly within the normal range from 5.45% ± 0.40% to 5.56% ± 0.58% ($p < 0.001$), mainly in donors aged 40–60 years.

Total cholesterol levels remained largely stable across the entire cohort, except for a small but significant increase in older donors ($p = 0.0017$), **Table 2**. Hemoglobin and hematocrit showed minimal changes, apart from a mild decline in hemoglobin among donors >60 years ($p = 0.0092$), **Table 2**.

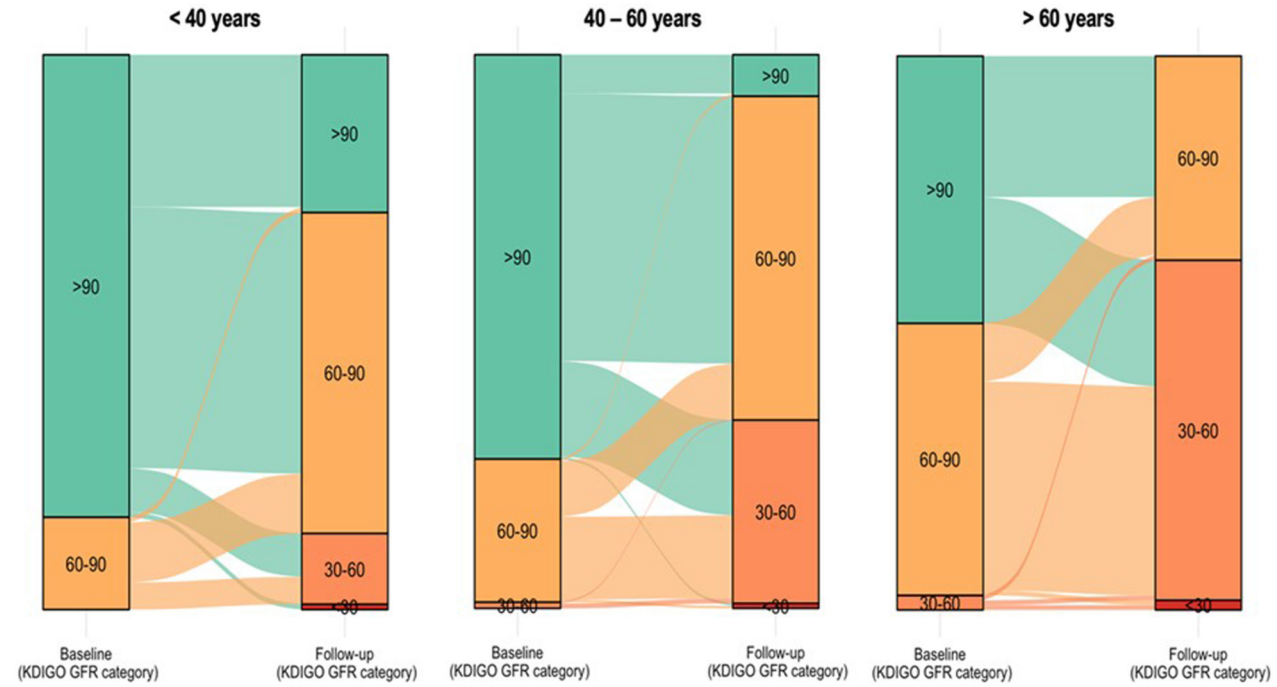


FIGURE 2 | Transition of CKD stages before donation and at long-term follow-up in donors <40 years, 40-60 years and >60 years. Alluvial plots show transitions of donors between KDIGO eGFR categories (>90, 60–90, 45–60, 30–45, <30 mL/min/1.73 m^2) from baseline (left) to last follow-up (right) in three age groups: <40 years, 40–60 years, and >60 years. The width of each flow represents the number of donors moving between categories.

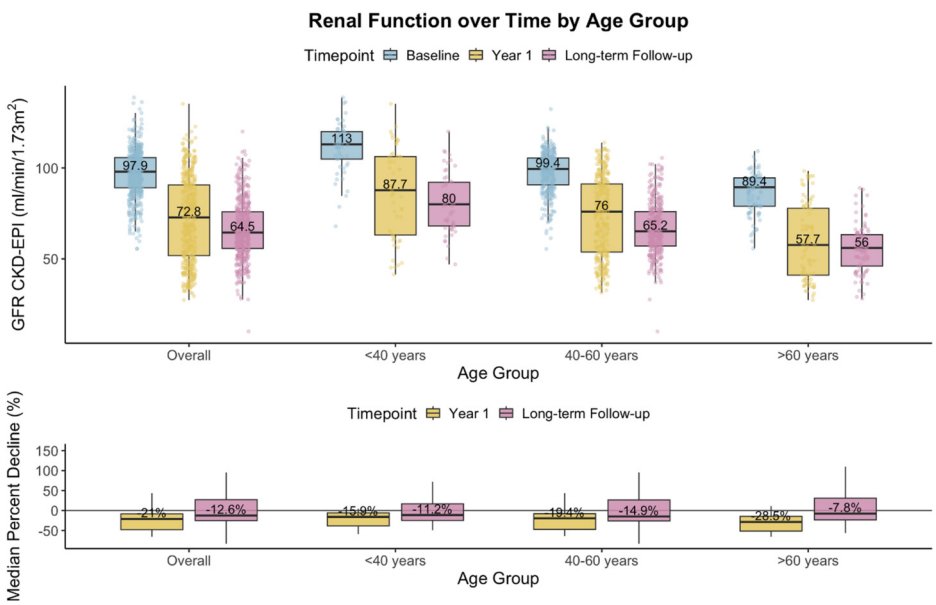


FIGURE 3 | Changes in estimated glomerular filtration rate (eGFR, CKDepi) after living kidney donation. Top panel: Boxplots show eGFR (mL/min/1.73 m^2) at baseline (blue), 1 year after donation (yellow), and long-term follow-up (pink) overall and by donor age group (<40, 40–60, >60 years). Numbers within boxes indicate group medians. Bottom panel: Median percent decline in eGFR relative to baseline (year 1) and relative to year 1 at long-term follow-up. Values represent median (interquartile range). The greatest early decline occurred in donors >60 years (–28% at year 1), with minimal further change thereafter.

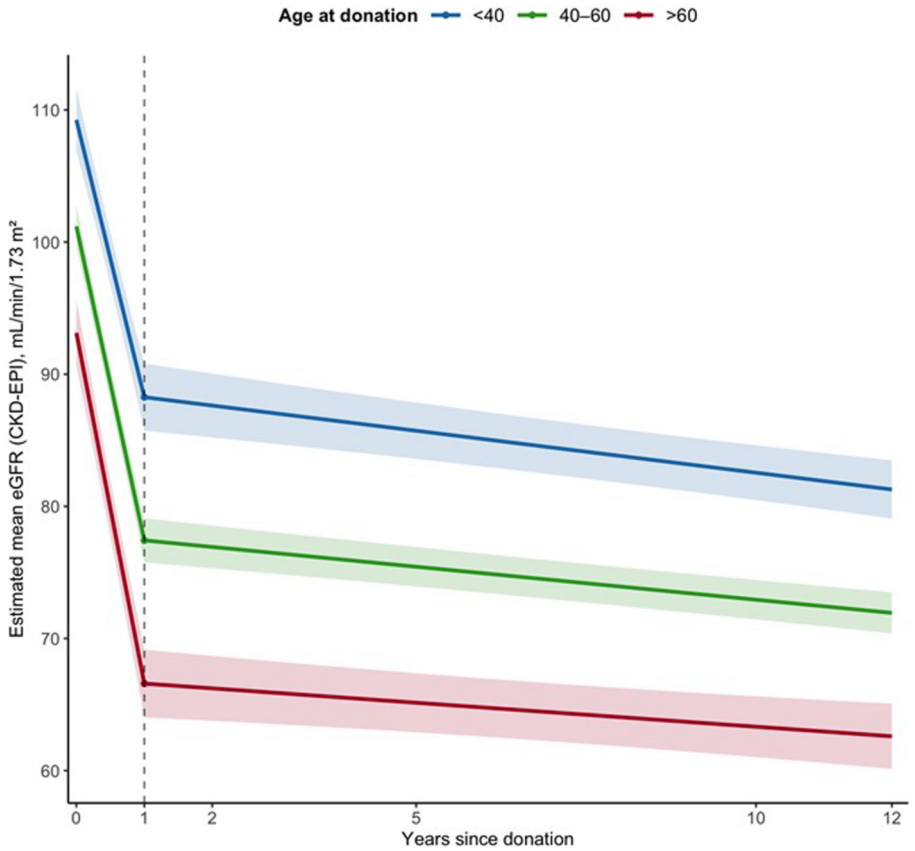


FIGURE 4 | Change in eGFR from baseline after living kidney donation according to cardiometabolic risk profile and age. Mean ΔeGFR from baseline at 1 year and long-term follow-up is shown for donors with no risk factors, hypertension, obesity, or both obesity and hypertension, stratified by age group (<40, 40–60, >60 years). All groups demonstrate the expected early decline in eGFR after donation followed by relative stabilization. Error bars indicate 95% confidence intervals.

TABLE 3 | Long-Term Outcome after Living Kidney Donation, stratified by Age.

Outcome	Total cohort	<40 years	40–60 years	>60 years	p-value
≥50% eGFR decline	6.8 (40)	4.8 (4)	5.3 (21)	14.4 (15)	**0.0053**[a]
eGFR <45 mL/min/1.73 m^2	8.0 (47)	1.0 (1)	5.5 (21)	20.4 (25)	**<0.001**
eGFR <30 mL/min/1.73 m^2	1.2 (7)	1.2 (1)	1.0 (4)	1.9 (2)	0.6090[a]
Renal replacement therapy	0.2 (2)	0.1 (1)	0.2 (1)	0.0 (0)	—
MACE (composite)	4.3 (27)	2.2 (2)	4.0 (17)	7.0 (8)	0.2300[b]
Myocardial infarction	0.6 (4)	0.1 (1)	0.7 (3)	0.0 (0)	0.3968
Stroke	1.7 (11)	0.0 (0)	1.2 (6)	4.3 (5)	**0.0432**
Cardiovascular death	0.3 (2)	0.0 (0)	0.2 (1)	0.9 (1)	0.4603
Peripheral artery disease events	1.4 (9)	1.2 (1)	0.7 (5)	1.7 (3)	0.7045
Death	3.3 (21)	0.0 (0)	2.6 (11)	8.7 (10)	**0.0012**[b]

[a]*Trend p-value (Cochran–Armitage).*
[b]*Group p-value (chi-square/Fisher, as appropriate).*
eGFR, estimated glomerular filtration rate; MACE, major adverse cardiac event.
Bold values indicate statistical significance.

Exploratory Risk Stratification Based on Baseline Donor Characteristics

An exploratory risk stratification was performed to describe patterns of long-term renal outcomes according to baseline donor characteristics. Using baseline variables that were independently associated with an eGFR <45 mL/min/1.73 m^2, including age (β = 0.062 per year), hypertension (β = 1.113), and baseline eGFR (β = −0.069 per mL/min/1.73 m^2), a composite risk score was constructed to summarize their combined effects.

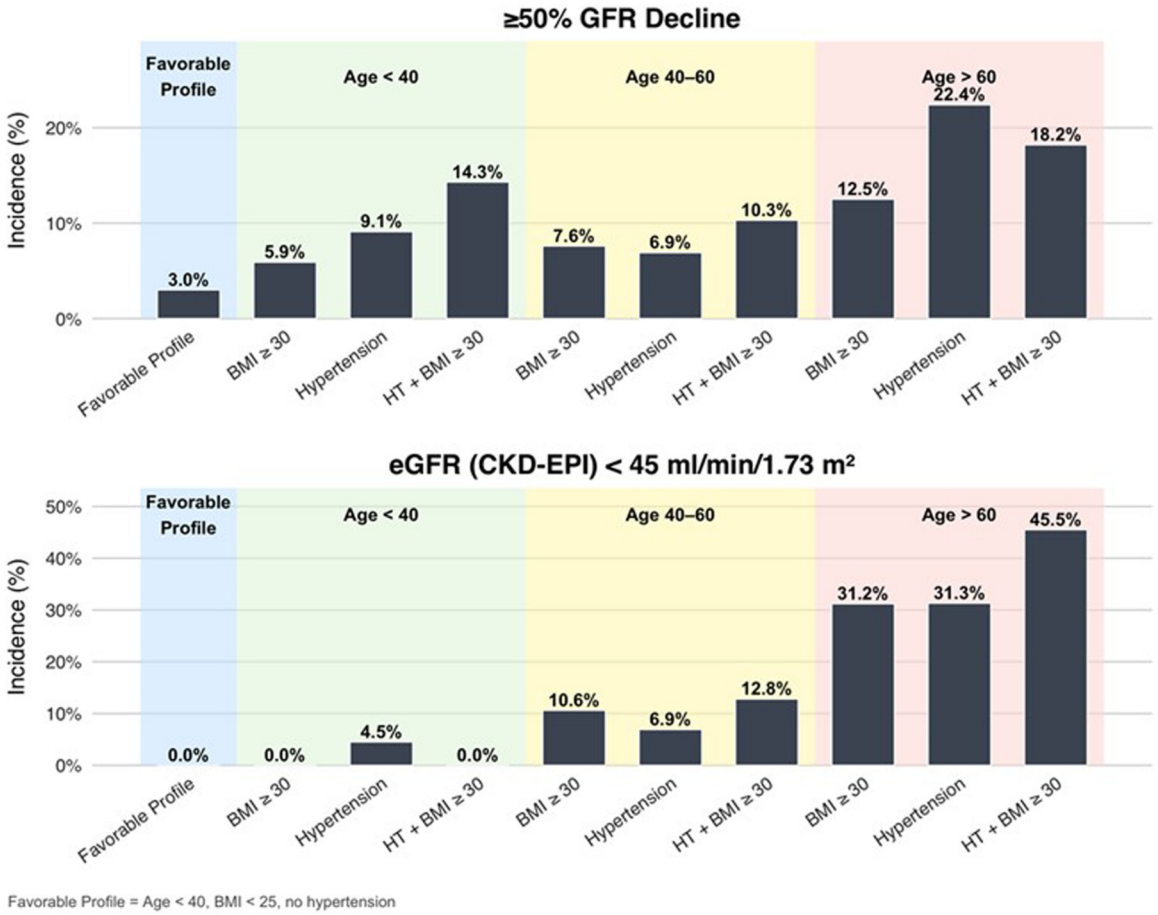


FIGURE 5 | Incidence of adverse renal outcomes by age group and baseline risk profile. Bar plots show the percentage of donors who developed (top) ≥ 50% eGFR decline from baseline and (bottom) an eGFR <45 mL/min/1.73 m^2 at long-term follow-up. Results are stratified by age group (<40, 40–60, >60 years) and baseline risk profile. "Favorable profile" = age <40 years, BMI <25 kg/m^2, and no hypertension. The combination of older age (>60 years), baseline hypertension, and BMI ≥30 kg/m^2 was associated with the highest incidence of both outcomes, reaching 22.4% for ≥50% eGFR decline and 45.5% for eGFR <45 mL/min/1.73 m^2.

A two-dimensional risk map illustrated the joint association of age, hypertension, and baseline eGFR with the prevalence of eGFR <45 mL/min/1.73 m^2, showing a progressive increase with advancing age and lower baseline kidney function, particularly among hypertensive donors (**Figure 7**). When grouped into tertiles, the observed prevalence of eGFR <45 mL/min/1.73 m^2 increased from 1.0% in the lowest to 20.0% in the highest risk category (**Figure 8A**). Across age strata, predicted and observed prevalences were closely aligned (<40 years: 1.4% vs. 1.2%; 40–60 years: 5.8% vs. 5.3%; >60 years: 21.9% vs. 24.0%; **Figure 7B**).

Model discrimination was quantified for descriptive purposes and showed an AUC of 0.84 (95% CI 0.78–0.89; **Figure 9**).

Cardiovascular Disease in Older Donors

Cardiovascular disease at baseline was uncommon overall but more frequent in older donors. Among donors >60 years, 19 of 115 (16.5%) had cardiovascular disease at baseline. Baseline kidney function was comparable between donors with and without cardiovascular disease. Long-term renal outcomes were also similar: eGFR <45 mL/min/1.73 m^2 occurred in 21.1% versus 21.9%, and a ≥50% decline in eGFR in 23.5% versus 13.8%, respectively. Major adverse cardiovascular events occurred in 10.5% versus 6.3%.

Outcome of Donors Aged ≥70 years

Among donors aged ≥70 years (mean 72, range 70–78 years), 55% were male. Most donated to their children (45%) or partners (35%). The mean follow-up period was 10.1 ± 2.5 years (range 7.1–14.7). Baseline eGFR (CKDepi) was 84.7 ± 9.0 mL/min/1.73 m^2, and 75% had at least one comorbidity, most commonly hypertension (65%), hyperlipidemia (30%), and cardiovascular disease (30%), **Table 6**.

After donation, the mean eGFR decreased by 37.7% and subsequently remained stable (year 1: 54.5 ± 20.0 mL/min/1.73 m^2 at year one, last follow-up: 53.6 ± 14.7 mL/min/

TABLE 4 | Univariate Logistic Regression Analysis of Predictors for eGFR Decline ≥50% at Long-Term Follow-Up.

Characteristics	OR	95%-CI	p
Demographics			
Age (years) at donation	1.047	1.013; 1.082	**0.006**
<40 years	Reference		
40-60 years	1.159	0.388:3.460	0.791
>60 years	3.337	1.068; 10.427	**0.038**
Male gender	1.043	0.583; 2.163	0.736
BMI (kg/m^2)	1.016	0.940; 1.099	0.684
BMI >30 kg/m^2	1.243	0.555; 2.786	0.597
Active smoker	1.046	0.480; 2.279	0.909
Blood pressure parameters			
Mean arterial pressure (mmHg)	1.040	1.009; 1.072	**0.012**
Systolic BP (mmHg)	1.030	1.010; 1.050	**0.003**
Diastolic BP (mmHg)	1.029	0.992; 1.067	0.124
24h systolic BP (mmHg)	1.035	1.009; 1.062	**0.009**
24h diastolic BP (mmHg)	1.035	0.992; 1.081	**0.112**
History of comorbidities			
Hypertension	3.557	1.744; 7.256	**<0.001**
Prediabetes	1.075	0.246; 4.696	0.923
Hyperlipidemia	1.580	0.592; 4.217	0.361
Metabolic syndrome	0.935	0.219; 2.730	0.931
Thyroid disease	2.910	1.419; 5.965	**0.004**
Cardiovascular disease	3.010	0.34; 26.36	0.321
Malignant diseases	1.14	0.15; 8.94	0.901
COPD/Asthma/Chronic bronchitis	1.344	0.305–5,932	0.696
Medication use			
Antihypertensive medication	2.19	1.133; 4.246	**0.020**
Number of antihypertensives	1.390	0.981; 1.969	**0.064**
Lipid-lowering drugs	2.99	1.085; 8.236	**0.034**
Thyroid medication	2.527	1.183; 5.394	**0.017**
Baseline laboratory			
Creatinine (mg/dL)	0.730	0.095; 5.583	0.761
MDRD eGFR (mL/min/1.73 m^2)	0.997	0.981; 1.012	0.666
CKDepi eGFR (mL/min/1.73 m^2)	0.991	0.970; 1.013	0.433
Cockroft-gault-eGFR (mL/min)	0.991	0.979; 1.003	0.168
MAG3-clearance (mL/min/1.73m^2)	0.996	0.988; 1.004	**0.302**
Creatinine-clearance (mL/min/1.73m^2)	0.989	0.976; 1.002	**0.094**
Hemoglobin (g/dL)	1.089	0.846; 1.402	0.509
Cholesterol (mg/dL)	0.995	0.986; 1.004	0.256
Triglycerids (mg/dL)	0.999	0.993; 1.004	0.680
LDL cholesterol (mg/dL)	0.995	0.982; 1.007	0.413
HDL cholesterol (mg/dL)	0.992	0.967; 1.018	0.539
Blood glucose (mg/dL)	1.000	0.979; 1.023	0.968
HbA1c (%)	1.312	0.517; 3.376	0.561
TSH (µIU/mL)	1.170	0.997; 1.374	0.055
Phosphate	1.399	0.370; 5.291	0.806
iPTH	1.011	0.652; 1.567	0.962
Urea (mg/dL)	0.994	0.951; 1.040	0.806
Urine albumine (mg/L)	0.983	0.863; 1.121	0.803
Urine protein (g/L)			
<0.03 g/L	Reference		
0,03-0.15 g/L	1.008	0.518; 1.986	0.981
>0.15 g/L	3.348	0.872; 12.858	0.078
Long-term follow-up data			
Time since donation (months)	1.006	1.002–1.011	**0.002**
Age at follow-up (years)	1.075	1.040; 1.112	**<0.001**
Comorbidities			
Hypertension	2.136	1.001; 4.560	**0.049**
Diabetes mellitus	9.200	0.213; 3.964	0.910
Hyperlipidemia	1.693	0.872; 3.286	**0.120**
Thyroid disease	6.280	0.273; 1.445	0.273
Cardiovascular disease	2.320	0.927; 5.806	0.072
Malignant diseases	2.391	1.095; 5.223	**0.029**
Major cardiovascular events	-	0.00-Inf	0.984

Bold values indicate statistical significance.

TABLE 5 | Univariate Logistic Regression Analysis: eGFR CKDepi <45 mL/min/1.73 m2 at Long-Term Follow-Up.

Characteristics	OR	95%-CI	p
Demographics			
Age (years) at donation	1.11	[1.07; 1.15]	**<0.001**
<40 years	Reference		
40-60 years	4.794	[0.637; 36.098]	**0.128**
>60 years	6.62	[3.56; 12.31]	**<0.001**
Male gender	1.256	[0.688; 2.294]	0.458
BMI (kg/m^2)	1.028	[0.958; 1.104]	0.441
BMI >30 kg/m^2	1.707	[0.855; 3.409]	0.130
Active smoker	0.540	[0.236; 1.234]	0.144
Blood pressure parameters			
Mean arterial pressure (mmHg)	1.039	1.010; 1.069	**0.008**
Systolic BP (mmHg)	1.032	1.014; 1.051	**<0.001**
Diastolic BP (mmHg)	1.024	0.990; 1.059	0.175
24h SBP (mmHg)	1.033	1.009; 1.057	**0.008**
24h DBP (mmHg)	1.022	0.984; 1.062	**0.255**
Comorbidities			
Hypertension	2.073	1.130; 3.802	**0.002**
Prediabetes	1.890	0.631; 5.661	0.256
Hyperlipidemia	2.399	1.059; 5.437	**0.036**
Metabolic syndrome	1.150	0.333; 3.030	**0.799**
Thyroid disease	2.296	1.142; 4.617	**0.020**
Cardiovascular disease	1.47	0.50; 4.36	0.484
Malignant diseases	2.119	0.460; 9.759	0.335
COPD/Asthma/Chronic bronchitis	-	0.000; Inf	
Medication use			
Antihypertensive medication	2.493	1.355; 4.586	**0.003**
Number of antihypertensives	1.474	1.073; 2.024	**0.017**
Lipid-lowering drugs	3.920	1.598; 9.617	**0.003**
Thyreoid medication	2.021	0.962; 4.246	0.063
Baseline laboratory			
Creatinine (mg/dL)	53.750	8.808; 327.982	**<0.001**
MDRD eGFR (mL/min/1.73 m^2)	0.953	0.936; 0.970	**<0.001**
CKDepi eGFR (mL/min/1.73 m^2)	0.924	0.903; 0.946	**<0.001**
Cockroft-gault eGFR (mL/min)	0.962	0.947; 0.977	**<0.001**
MAG3-clearance (mL/min/1.73m^2)	0.992	0.984; 0.999	0.2500
Creatinine-clearance (mL/min/1.73m^2)	0.985	0.972; 0.997	0.1600
Hemoglobin (g/dL)			
Cholesterol (mg/dL)	0.997	0.989; 1.005	0.432
Triglycerids (mg/dL)	0.998	0.993:1.003	0.484
LDL cholesterol	0.995	0.984; 1.021	0.964
HDL cholesterol (mg/dL)	1.000	0.978; 1.337	0.074
Blood glucose	1.012	0.993; 1.031	0.210
HBA1c (%)	2.017	0.863; 4.710	0.105
TSH (mU/mL)	1.149	0.987; 1.337	0.074
Urea (mg/dL)	1.022	0.983; 1.062	0.280
Urine albumine (mg/L)	1.020	0.927; 1.124	0.681
Urine protein (g/L)			
<0.03 g/L	Reference		
0,03–0.15 g/L	0.956	0.523; 1.750	0.885
>0.15 g/L	0.729	0.092; 5.772	0.765
Long-term follow-up data			
Time since donation (months)	1.002	0.998; 1.006	0.401
Age at follow-up (years)	1.105	1.069; 1.006	**<0.001**
Comorbidities			
Hypertension	2.319	1.133; 4.746	**0.021**
Diabetes mellitus	0.768	0.179; 3.292	0.722
Hyperlipidemia	1.948	1.060; 3.578	**0.032**
Thyroid disease	1.087	0.527; 2.243	0.821
Cardiovascular disease	2.794	1.232; 6.336	**0.014**
Malignant diseases	1.381	0.597:3.195	0.451
Major cardiovascular events	0.996	0.228; 4.338	0.995

BMI, Body Mass Index; BP, blood pressure; CI, confidence interval; CKDepi eGFR, glomerular filtration rate estimated by Chronic Kidney Disease Epidemiology Collaboration equation; COPD, chronic obstructive pulmonary disease; GFR, glomerular filtration rate; HbA1c–glycated hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MDRD eGFR, glomerular filtration rate estimated by Modification of Diet in Renal Disease equation; TSH, thyroid-stimulating hormone; OR, odds ratio: p–significance p.

Bold values indicate statistical significance.

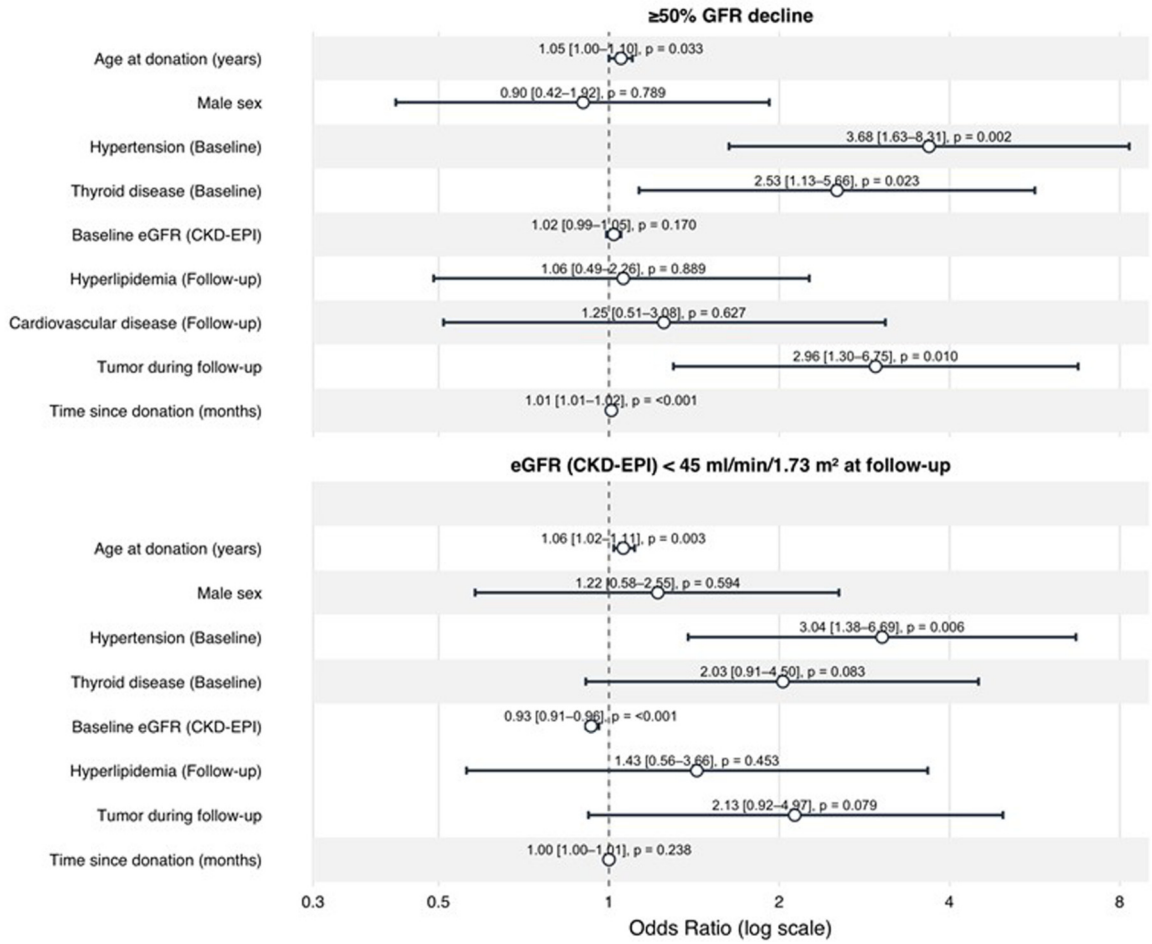


FIGURE 6 | Multivariable logistic regression for adverse renal outcomes after living kidney donation. Forest plots display adjusted odds ratios (ORs) with 95% confidence intervals for predictors of (top) eGFR <45 mL/min/1.73 m^2 at long-term follow up and (bottom) ≥50% eGFR decline from baseline at long-term follow-up.

1.73 m^2; mean age 82 years). A ≥50% decline in eGFR occurred in 18.8%, and 25% had eGFR <45 mL/min/1.73 m^2, but none dropped below 30 mL/min/1.73 m^2 or required renal replacement therapy.

MACE occurred in 15%, while diabetes, malignancy, and psychiatric disorders were rare. One donor died from prostate cancer during long-term follow-up.

DISCUSSION

In this prospective caucasian cohort with long-term follow-up, living kidney donation demonstrated favorable clinical outcomes across a broad age spectrum. The mean donor age of 51 ± 11 years was 5–10 years higher than in most international cohorts (e.g., US: 41 ± 12 years; New Zealand: median 44 years) [21–23]. The donor population in this study closely resembled contemporary European cohorts, including the SoLKiD German National Registry (GNR) [24] and other European cohorts [7, 24, 25].

Importantly. living kidney donors do not represent a uniformly healthy cohort, but rather reflect an aging population and the gradual expansion of donor eligibility criteria driven by long waiting time for a brain-death donor due to donor shortage in Germany. Overweight and obesity were widespread, affecting 40.5% of donors and cardiometabolic risk factors were already prevalent at baseline. Hypertension was present in nearly half of donors, hyperlipidemia and prediabetes were less frequent, and approximately one-quarter of donors were active smokers, particularly among younger individuals. In contrast, established cardiovascular disease, malignancy, and mental health impairment were rare at the time of donation.

Women constituted 62.5% of donors, particularly in the youngest age group (71%), likely reflecting sociocultural factors and clinical considerations. This requires particular attention as donation by women of child-bearing age bears special risks due to potential pregnancy-related complications following nephrectomy [26, 27]. Sex was not associated with long-term

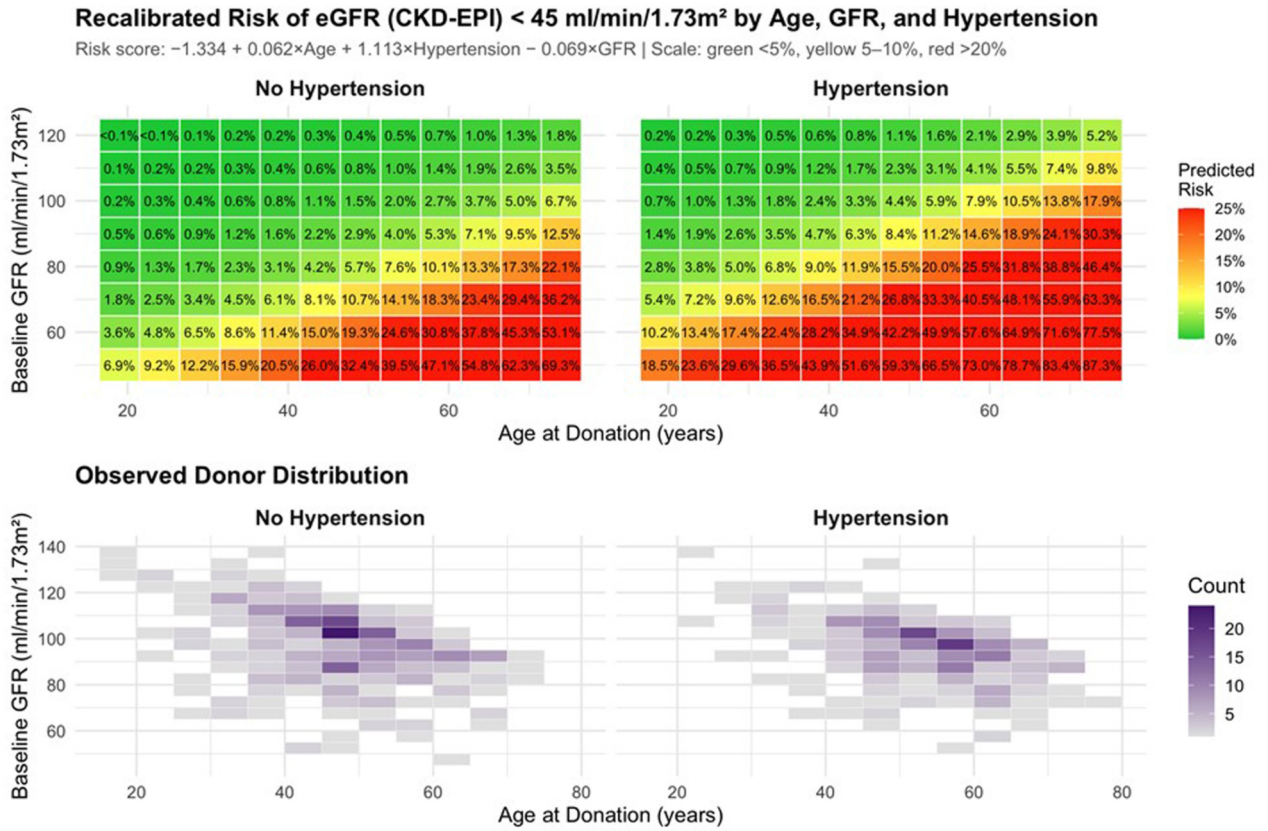


FIGURE 7 | Predicted long-term risk of an eGFR <45 mL/min/1.73 m^2 after living kidney donation, according to age at donation, baseline eGFR, and presence of hypertension. Top panels: Heatmaps display the predicted probability (%) of developing an eGFR <45 mL/min/1.73 m^2 at long-term follow-up based on the recalibrated risk model (risk score = −1.334 + 0.062 × Age +1.113 × Hypertension − 0.069 × baseline eGFR). Colors indicate risk level (green <5%, yellow 5%–10%, orange 10%–20%, red >20%). Left: donors without baseline hypertension; right: donors with hypertension. Bottom panels: Observed distribution of donors by age and baseline eGFR, stratified by baseline hypertension status; shading reflects donor density.

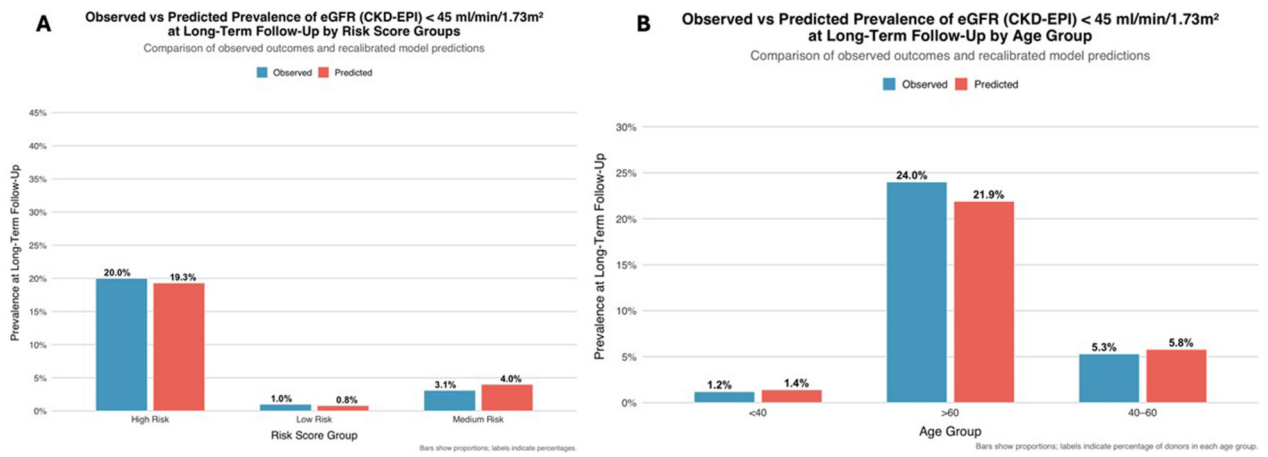


FIGURE 8 | (A) Observed versus predicted prevalence of CKDepi eGFR <45 mL/min/1.73 m^2 by risk-score groups. **(B)** Observed versus predicted prevalence of CKDepi eGFR <45 mL/min/1.73 m^2 by age group. **(A)** Observed (blue) and predicted (orange) prevalence across low-, medium-, and high-risk tertiles. Predicted and observed rates (0.8% vs. 1.0%; 4.0% vs. 3.1%; 19.3% vs. 20.0%) confirmed accurate model calibration. **(B)** Model calibration across donor age categories (<40, 40–60, >60 years). Observed and predicted prevalence were closely aligned (1.2% vs. 1.4%; 5.3% vs. 5.8%; 24.0% vs. 21.9%, respectively).

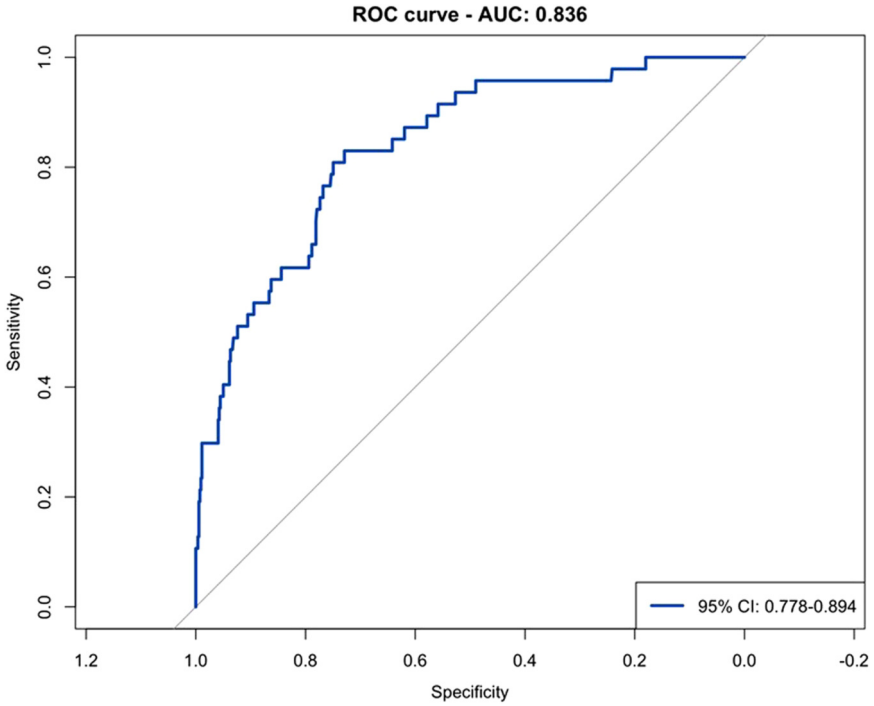


FIGURE 9 | Receiver operating characteristic (ROC) curve for prediction of CKDepi eGFR <45 mL/min/1.73 m^2. Observed (blue) and predicted (orange) prevalence across low-, medium-, and high-risk tertiles. Predicted and observed rates (0.8% vs. 1.0%; 4.0% vs. 3.1%; 19.3% vs. 20.0%) confirmed accurate model calibration.

TABLE 6 | Baseline characteristics and long-term outcome of living kidney donors aged ≥70 years (n = 21).

Parameter	Baseline	Last follow-up	Significance baseline – last follow up
Hypertension	65.0	85.0	0.125
Diabetes mellitus	0.0	5.0	0.004
Prediabetes	20.0	35.0	0.480
Hyperlipidemia	30.0	50.0	0.125
Cardiovascular disease	30.0	40.0	0.500
Malignancy	0.0	10.0	0.487
Thyroid disease	25.0	25.0	-
COPD/Asthma/Chronic bronchitis	0.0	0.0	
Impaired mental health	5.0	5.0	0.997
Family history of kidney disease	40.0	40.0	0.998
CKDepi GFR (mean +/- SD)	84.7 ± 9.0	53.6 ± 14.7	**<0.01**
Drop of 50% eGFR	-	18.75	
eGFR <45 mL/min/1.73 m^2	0.0	25.0	-
eGFR <30 mL/min/1.73 m^2	0.0	0.0	-
Renal replacement therapy	0.0	0.0	-
Death	-	4.8	-
Composite MACE	0.0	15.0	0.25

Continuous variables are presented as mean ± standard deviation (SD), and group comparisons were performed using the non-parametric Kruskal-Wallis test. Categorical variables are shown as percentages, and were compared using the Chi-square test or Fisher's exact test, as appropriate. A p-value less than 0.05 was considered statistically significant. Abbreviations: CKDepi GFR, glomerular filtration rate estimated by Chronic Kidney Disease Epidemiology Collaboration equation; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; MACE, major adverse cardiac event; SD, standard deviation.
Bold values indicate statistical significance.

kidney function decline in this cohort, consistent with several prior studies, although sex-specific effects remain inconsistently reported in the literature [10, 11, 28–30]. Nearly two-thirds of donors were biologically related to their recipients, which may modestly increase long-term renal risk through shared genetic or environmental factors [10, 11, 31, 32].

A key finding is the clear separation between early post-donation kidney function changes and long-term trajectories. As expected, eGFR declined by approximately 26% within the

first year after donation, consistent with physiological adaptation to nephrectomy. The magnitude of early eGFR decline during the first year after donation was largely driven by baseline eGFR and, to a lesser extent, by age and preexisting hypertension. Donors with higher baseline eGFR experienced greater absolute early declines, consistent with a physiological ceiling effect of renal adaptation [33, 34]. In stratified analyses, donors with obesity alone showed slightly higher baseline eGFR values and comparable long-term trajectories. This likely reflects selection effects and higher baseline renal reserve, as obese individuals are accepted for donation only after careful screening, and higher baseline eGFR may partly reflect adaptive hyperfiltration.

After this initial adaptive phase, kidney function remained stable over long-term follow-up across all age groups. This observation aligns with prior work describing rapid compensatory hyperfiltration within the first months to years after donation, followed by a plateau phase without evidence of progressive decline in most donors [35]. Given the comparatively high donor age in many European countries [7, 15], our analyses extend prior work by disentangling age-related differences in achieved post-donation kidney function from age-related differences in the rate of kidney function change over time. Linear mixed-effects models demonstrated that older age at donation was associated with lower eGFR values throughout follow-up, whereas the slope of eGFR change did not differ significantly by age. Thus, age primarily influenced the level of kidney function prior to donation and immediately after donation rather than accelerating post-donation decline. The average decline of 35.1% was comparable to previous studies reporting a post-donation GFR decrease of 25%–40% with a similar or only marginally higher long-term risk of ESRD compared to the general population [4, 28, 32]. Even among donors aged ≥70 years, severe renal impairment was rare, and no donor progressed to ESRD.

Long-term renal impairment, as assessed by two primary endpoints, followed a clear age-related gradient but remained clinically reassuring in terms of severe outcomes. The primary endpoint of an eGFR <45 mL/min/1.73 m^2 - was observed in 8.0% overall, 20.4% in donors age >60 years. Severe renal impairment was rare, with eGFR <30 mL/min/1.73 m^2 in 1.2% and eGFR <15 mL/min/1.73 m^2 in 0.3% of donors. The second primary endpoint - aGFR decline ≥50% at long-term - occurred in 6.8% overall and increased with age (4.8% < 40 years, 5.3% 40–60 years, 14.4% > 60 years). Beyond age, renal risk was highly heterogeneous and strongly modified by baseline risk profile. In younger donors with favorable risk profiles (no hypertension, BMI <25 kg/m^2), the prevalence of reduced eGFR was negligible, but increased to 4.5%–10.6% in young donors with hypertension or obesity. In older donors, risk likewise varied considerably, ranging from low levels in donors without risk factors up to 45.5% in donors with hypertension and a BMI ≥30 kg/m^2. These findings underscore that chronological age alone is insufficient for individualized risk assessment and that long-term renal outcomes after donation are shaped by the interaction between baseline renal reserve and comorbidity burden—such that younger donors with adverse metabolic profiles may experience less favorable outcomes than some older but otherwise healthy donors.

Cardiovascular risk factors were also common in younger donors. Smoking was more prevalent among younger donors, whereas hypertension affected more than 65% of donors older than 60 years at follow-up. Although cardiovascular disease was rare at donation, its prevalence increased over time, especially among older donors. At time of donation, only 0.9% had relevant cardiovascular disease, increasing to 6.6% overall and 16.5% among donors over 60 years. Similarly, Colucci et al. reported only 3.2% new-onset cardiovascular disease after 10 years of follow-up [25]. A Norwegian registry study observed a modestly increased risk of ischemic heart disease in donors (3.5%) compared with controls (1.7%) [36], while other large studies found no significant difference in cardiovascular morbidity and mortality between donors and healthy non-donors [37, 38]. Overall, the available evidence suggests that living kidney donation, when donors are carefully selected and monitored, is not associated with a substantial excess cardiovascular risk. The observed rise in modifiable risk factors such as hypertension and hyperlipidemia over time underscores the importance of structured long-term care, including lifestyle counseling and optimized blood-pressure management.

Longitudinal follow-up highlighted additional clinically relevant aspects. Younger age does not protect against the development of new comorbidities, including mental health impairment, which remains an often underrecognized aspect of donor follow-up. Psychological screening and support should be integral components of long-term donor care, even for individuals initially classified as low risk. Similarly, the rise in malignancy incidence, particularly among older donors, appears age-related rather than donation-related but supports the need for age-appropriate cancer surveillance.

Finally, the present study explores whether data-informed approaches may help to characterize heterogenity of long-term renal risk after living kidney donation. While earlier models, such as that by Grams et al., estimated lifetime ESRD risk in potential donors [31], the present analyses addressed post-donation renal outcomes within a well-characterized donor cohort. Exploratory analyses suggested that a limited set of routinely available baseline clinical parameters - particularly baseline eGFR and hypertension status, togethter with age - captured a substantial proportion of the variation in long-term renal outcomes. Although not intended as a predictive tool, this approach illustrates how readily available clinical information may support more individualized counseling and risk-adapted follow-up strategies. Given that the HeiKiD approach was derived from a predominantly Caucasian European donor cohort, its applicability is likely most relevant to similar donor populations. Such approaches may complement clinical judgment but require external validation before broader application.

This study has limitations. The cohort represents caucasian donors mainly from Germany; however, donor characteristics

were comparable to national and other European cohorts [7, 24, 25], suggesting reasonable generalizability within similar healthcare settings. In addition, the absence of a non-donor control group precludes direct estimation of donation-attributable risk. Accordingly, the observed outcomes describe post-donation trajectories within the donor population rather than comparative risk relative to non-donors.

In conclusion, this long-term prospective cohort demonstrates stable kidney function after the expected early adaptive decline following living kidney donation, even in an aging donor population. Clinically relevant renal impairment increased with age, yet severe renal impairment and renal replacement therapy were rare. Long-term outcomes were not driven by chronological age alone but reflected the combined influence of baseline renal reserve, hypertension, and comorbidity burden. Using a small number of routinely available clinical parameters, long-term renal risk could be meaningfully characterized, supporting individualized donor counseling and risk-adapted lifelong follow-up.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because partial restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Requests to access the datasets should be directed to claudia.sommerer@med.uni-heidelberg.de.

ETHICS STATEMENT

The studies involving humans were approved by Ethics committee of the University Hospital Heidelberg. 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

CS designed and conducted the study, recruited patients, collected and analyzed data, and wrote the manuscript; IS collected and analyzed data, and wrote the manuscript; NK and ZB collected data; MZ recruited patients and supervised the study. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

The authors(s) declared that this work 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) declared that generative AI was not used in the creation of this manuscript.

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ORIGINAL RESEARCH
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Anesthetic Management of Brain-Dead Donors During Organ Retrieval: Hemodynamic Effects and Potential Organ-Protective Implications – A Retrospective Analysis of 85 Cases

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Currently, no evidence-based recommendations for anesthetic management of brain-dead organ donors exist. Hemodynamic responses to surgical stimulation and potential organ-protective effects of anesthetic agents have been reported inconsistently. We retrospectively analyzed anesthetic management of all donors at University Hospital Münster between 2010 and 2025. Heart rate (HR) and mean arterial pressure (MAP) were assessed before, during, and up to 15 min after first incision. Eighty-five donors were included; volatile anesthetics were administered in 41%, opioids in 80%, and neuromuscular blocking agents in 92%. HR (bpm) remained unchanged from before (94 [85–105]) to during (93 [84–104]) and post-incision (95 [85–103]). MAP (mmHg) decreased from 5 minutes (86 [76–95]) to 15 min post-incision (80 [72–89]; $p = 0.034$). Sufentanil did not affect HR or MAP at any point. Sevoflurane was associated with lower HR at all time points ($p < 0.001$) and lower MAP during incision ($p = 0.020$), but independent of surgical stimulation. Anesthetic management varied substantially. Hemodynamics did not increase following incision, and our findings do not support opioid administration, whereas hemodynamic effects of sevoflurane must be carefully managed to ensure sufficient organ perfusion during retrieval. Evidence-based recommendations for anesthetic management are needed to support organ-protective strategies in organ donation.

Keywords: anesthesia, brain death, donor management, hemodynamics, organ retrieval

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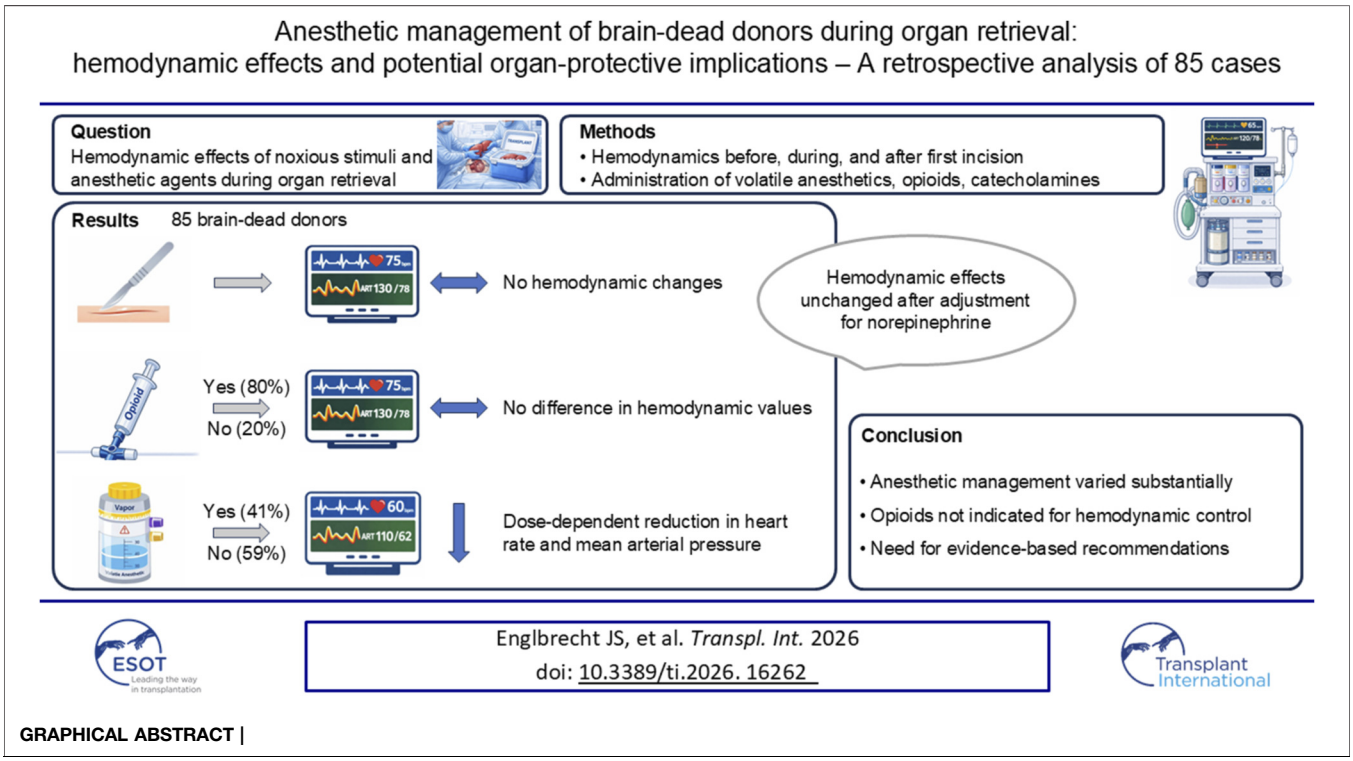
INTRODUCTION

The supply of transplantable organs remains insufficient worldwide [1]. Thus, adequate donor management is essential to increase the number and quality of available organs for donation [2]. However, evidence-based recommendations for organ-protective measures during intensive care unit (ICU) stay and organ retrieval for donation after brain death (DBD) donors are very limited.



GRAPHICAL ABSTRACT |

Due to the paucity of available studies, recommendations for ICU therapy are mainly based on pathophysiological reasoning, epidemiological observations, or extrapolations from general ICU management strategies [2, 3]. Surprisingly few studies have examined the role and influence of anesthesia during organ retrieval [4, 5]. Accordingly, no evidence-based recommendations are currently available regarding the anesthetic management of DBD donors [4–6]. This results in non-standardized management, especially concerning the indications for the administration of anesthetic agents, as well as targeted hemodynamic values and indication for and selection of catecholamines [4, 7, 8].

Previous studies have demonstrated that DBD donors exhibit both a hemodynamic reaction with increase in heart rate (HR) and mean arterial pressure (MAP), and an endocrine stress reaction to surgical stimuli [9–12]. It has been suggested that volatile anesthetics might attenuate these responses [13]. However, a recent study reported no consistent changes in HR or MAP in donors receiving anesthetic agents at the time of skin incision [4]. In addition to their possible hemodynamic effects, volatile anesthetics have been suggested to mitigate ischemia–reperfusion injury (IRI), a critical pathological process and an unavoidable challenge in organ transplantation. Thus, the use of volatile anesthetics during organ retrieval may offer potential organ-protective benefits through anesthetic preconditioning beyond possible hemodynamic effects [6, 14–17].

The indication for opioids in this context is even less well studied. The limited available evidence suggests that opioids do not significantly alter hemodynamic responses or the release of stress hormones due to surgical stimuli under these conditions [18, 19]. Some authors thus recommend avoiding their use during organ retrieval [6], while others support it [20]. Whether opioids influence IRI in the context of transplantation remains unknown [21]. The available evidence is derived mainly from animal studies demonstrating, for example, a reduction in acute renal injury mediated by pharmacological preconditioning with opioids [22].

Additionally, administering anesthetic agents to brain-dead patients is a matter of an ongoing ethical debate. While the German Ethics Council states that these drugs are unnecessary in DBD donors [23], some authors argue that using anesthetic agents during organ retrieval represents an ethical obligation to ensure the patient's dignity and to alleviate the moral distress of clinicians faced with autonomic reflexes to nociceptive stimuli [24, 25].

The present study therefore aimed to address two key questions. First, whether the administration of volatile anesthetics and opioids in DBD donors might influence a possible hemodynamic response to surgical stimuli. Second, how anesthesiologists manage organ retrieval, particularly regarding the use of anesthetic agents and hemodynamics.

PATIENTS AND METHODS

This study was performed in accordance with the Declaration of Helsinki. The Ethics Committee of the University of Muenster approved the study protocol (file number 2021-801-f-S) on 28 May 2025. The need for informed consent was waived due to the retrospective analysis of routinely collected patient data.

We retrospectively identified all utilized DBD donors at University Hospital Münster from January 2010 to October 2025 and included all donors aged ≥14 years into further analysis. For each case, basic demographic parameters (sex, age, height, weight) and the etiology of the devastating brain injury were obtained through review of the medical records.

To analyze hemodynamics and anesthetic management, five time points during organ retrieval were defined:

- pre (baseline, 5 min before first incision)
- 0 min (during first incision)
- 5 min, 10 min, 15 min (five, 10, and 15 min after first incision)

The following parameters were extracted from the anesthesia records at each time point:

- HR (bpm)
- MAP (mmHg)
- Type and infusion rate of administered catecholamines
- Average end-tidal concentration of volatile anesthetics from pre to 15 min

Additionally, the type and cumulative dose of administered opioids and neuromuscular blocking agents (NMBA) from 30 min before until first incision (0 min) were recorded.

Statistical Methods

All statistical analyses were performed using SPSS (IBM, Version 31). Additional verification of results and figure generation were conducted in Python (version 3.11). Normality of all continuous variables was assessed using the Shapiro–Wilk test. Data following a normal distribution were expressed as mean ± SD, and non-normally distributed data as median [IQR, 25th-75th percentile]. Within-subject comparisons of continuous variables across time points were conducted using repeated-measures ANOVA for normally distributed variables or Friedman test for non-normally distributed data. Post-hoc pairwise comparisons were performed using paired *T*-tests or Wilcoxon signed-rank tests, respectively. Between-group comparisons were analyzed using Mann–Whitney U tests for both absolute values and relative changes from baseline (baseline = pre, Δ values). To increase group sizes and reduce the number of necessary comparisons, the end-tidal concentrations of desflurane and isoflurane, which were used in only a few cases, were converted into equipotent sevoflurane concentrations [26]. Associations between continuous variables were assessed using Spearman rank correlation for both absolute and Δ values. To evaluate potential additive or interaction effects of volatile anesthetics and opioids on HR and MAP, a two-way analysis of variance was performed with both agents as factors and a nonparametric aligned-rank transform ANOVA was applied. Both absolute values and Δ values were analyzed at each time point. To account for potential confounding by catecholamine administration, time-matched partial Spearman correlations were computed, evaluating the relationship of catecholamine infusion rate and hemodynamic parameters while controlling for opioid dose and volatile anesthetics concentration, and *vice versa*. All variables were rank-transformed before partial correlation, and residuals after regression on covariates were correlated to yield partial Spearman's ρ. All tests were two-tailed. To control for multiple testing, Bonferroni correction was applied, and a corrected $p \leq 0.05$ was considered statistically significant. The detailed description and results of all subsequent statistical analyses are provided in the **Supplementary Material**.

TABLE 1 | Demographics of the study cohort.

Parameter	value
Male/female, *n* (%)	56/29 (66/34)
Age (years)	45.0 [31.5–63.0]
Height (cm)	177 ± 8.7
Weight (kg)	80.0 [70.0–90.0]
Etiology of devastating brain injury	
Intracranial hemorrhage, *n* (%)	31 (36)
Hypoxic brain injury, *n* (%)	28 (33)
Traumatic brain injury, *n* (%)	20 (24)
Stroke, *n* (%)	4 (5)
Others, *n* (%)	2 (2)

Data are presented as mean ± SD, in normally distributed data, otherwise as median [IQR].

RESULTS

In total, 96 DBD donors were utilized between January 2010 and October 2025 at our institution. Six cases were excluded due to age at admission (<14 years), and an additional five cases due to missing data. Accordingly, 85 cases were included in the further analysis (**Table 1**). The minimum monitoring standard for all donors during organ retrieval comprised ECG, pulse oximetry, invasive arterial blood pressure monitoring, and central venous catheterization.

Anesthetic Management During Organ Retrieval

Since no evidence-based recommendations are available, management was mainly determined by the attending senior anesthesiologist. Volatile anesthetics were administered in 41% DBD donors during organ retrieval, with sevoflurane used in all but three cases. No intravenous hypnotic agents were used in any of the cases. Sufentanil was the only opioid used and was administered in 80% of the cases prior to incision, while NMBA were used prior to incision in 92% (**Table 2**). At least one catecholamine was used in 88% of the cases (**Table 3**).

Infusion rates of catecholamines were analyzed across all time points. Group sizes for epinephrine, dobutamine, and dopamine were small, and therefore the Friedman test was used as a more robust approach although distributions appeared to be normal. Only vasopressin infusion rates showed a significant difference over time ($p = 0.016$, partial $\eta^2 = 0.171$). However, *post hoc* analysis showed no significant differences between time points ($p \geq 0.206$ for all, Cohen´s $d_z = 0.243–0.512$) (**Table 4**; **Supplementary Table S1**).

TABLE 2 | Administered anesthetic agents during organ retrieval.

Anesthetic agent	"No", *n* (%)	"Yes", *n* (%)	Dosage
Volatile anesthetics	50 (59)	35 (41)	
- Sevoflurane		32 (91)	1.2 ± 0.6*
- Desflurane		2 (6)	3.9*
- Isoflurane		1 (3)	0.7*
Opioids	17 (20)	68 (80)	
- Sufentanil		68 (100)	0.56 μg kg^{-1} [0.31–0.67]
Neuromuscular blocking agents	7 (8)	78 (92)	
- Cisatracurium		46 (59)	0.17 mg kg^{-1} [0.13–0.24]
- Rocuronium		32 (41)	0.86 mg kg^{-1} [0.65–1.25]

*Data are presented as mean ± SD, in normally distributed data, otherwise as median [IQR]. *mean end-tidal concentration (%) from pre to 15 min after incision.*

TABLE 3 | Distribution of administered catecholamines during organ retrieval.

Agent	Administered, n (%)	Norepinephrine	Epinephrine	Dobutamine	Dopamine	Vasopressin
None, *n* (%)	10 (12)	-	-	-	-	-
One, *n* (%)	56 (66)	49	0	1	1	5
Two, *n* (%)	14 (16)	14	3	2	2	7
Three, *n* (%)	5 (6)	5	2	2	1	5
Total, *n* (%)	85 (100)	68	5	5	4	17

Type and number of administered catecholamines.

TABLE 4 | Dosage of administered catecholamines during organ retrieval.

Substance	*n (%)*	pre	0 min	5 min	10 min	15 min
Norepinephrine (μg kg^{-1} min^{-1})	68 (80)	0.071 [0.021–0.154]	0.072 [0.024–0.154]	0.074 [0.021–0.143]	0.073 [0.026–0.143]	0.071 [0.029–0.143]
Epinephrine (μg kg^{-1} min^{-1})	5 (6)	0.083 ±0.042	0.081 ±0.045	0.081 ±0.045	0.085 ±0.045	0.084 ±0.042
Dobutamine (μg kg^{-1} min^{-1})	5 (6)	2.667 ±1.979	2.667 ±1.979	2.667 ±1.979	3.167 ±1.354	3.167 ±1.354
Dopamine (μg kg^{-1} min^{-1})	4 (5)	2.412 ±0.626	2.412 ±0.626	2.412 ±0.626	2.412 ±0.626	2.412 ±0.626
Vasopressin (I.E., h^{-1})	17 (20)	1.629 ±0.696	1.600 ±0.712	1.559 ±0.743	1.541 ±0.774	1.541 ±0.774

Data are presented as mean ± SD, in normally distributed data, otherwise as median [IQR]. Infusion rates over time from baseline (pre), during incision (0 min) until 15 min after incision.

Hemodynamics During Organ Retrieval

HR remained stable throughout the period from before incision (pre) until 15 min after incision (**Figure 1A**; **Supplementary Table S2**). There were no significant differences between time points (p = 0.308, Kendall´s W = 0.014). A significant difference in MAP was found across all time points (p = 0.007, Kendall´s W = 0.042), with *post hoc* analysis revealing only a modest decrease at 15 min compared with 5 min after incision (p = 0.034, r = −0.317) (**Figure 1B**; **Supplementary Table S2**). Six (7%) DBD donors exhibited a MAP <65 mmHg at one time point, seven (8%) at two consecutive time points, and two (2%) at four time points.

Effects of Sufentanil on Heart Rate and Mean Arterial Pressure

Sufentanil administration showed no significant influence on either HR or MAP at any time point. Across all comparisons, median absolute HR values were comparable between donors with and without sufentanil (p = 1.000 for all, r = −0.092 to 0.024) (**Figure 2A**; **Supplementary Table S3**). For absolute MAP values, no time point reached significance, though a weak tendency toward higher values at 15 min in the group with sufentanil was observed (p = 0.345, r = −0.287) (**Figure 2B**; **Supplementary Table S3**). Changes from baseline HR (Δ HR, p = 1.000 for all, r = −0.116 to 0.064) and MAP (Δ MAP, p ≥ 0.600 for all, r = −0.228 to −0.156) were likewise similar between groups (**Figures 2C,D**; **Supplementary Table S3**).

Correlation analyses revealed no clear dose-dependent effects of sufentanil on HR or MAP when considering absolute values (p ≥ 0.754 for all, ρ = −0.003–0.138). When changes relative to baseline were analyzed, no time point reached statistical significance for Δ HR (p ≥ 0.919 for all, ρ = −0.073–0.132). For Δ MAP, a weak positive association between sufentanil dose and Δ MAP was observed at 0 min (p = 0.079, ρ = 0.252) and 10 min (p = 0.094, ρ = 0.245) (**Supplementary Table S3**).

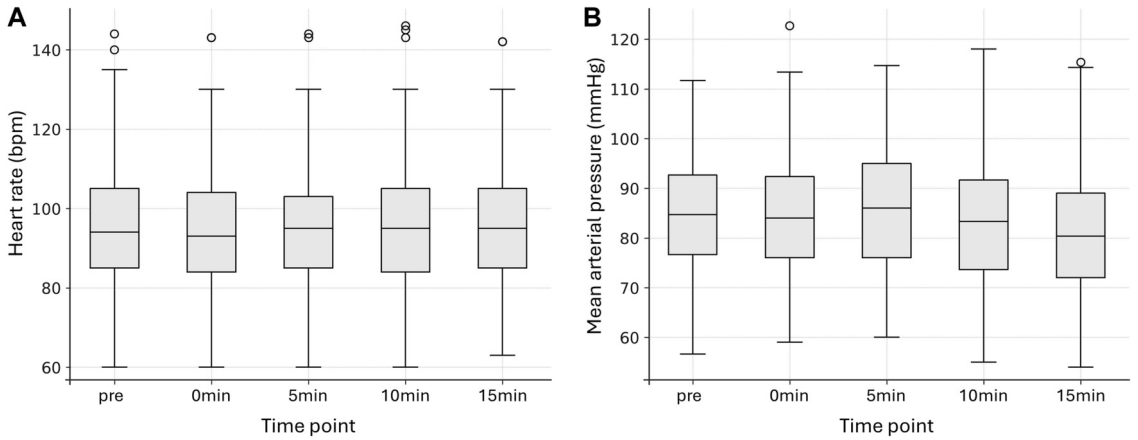


FIGURE 1 | Hemodynamics during organ retrieval. Boxplots showing heart rate (bpm) **(A)** and mean arterial pressure (mmHg) **(B)** at baseline (pre), during incision (0min), and 5, 10, and 15 min after incision. Boxes represent the IQR, horizontal lines denote the median, and whiskers indicate values within 1.5 × IQR. Open circles represent data points classified as outliers (beyond 1.5 × IQR).

Effects of Sevoflurane on Heart Rate and Mean Arterial Pressure

In contrast, sevoflurane administration was associated with consistently lower HR and MAP.

Absolute HR values were significantly lower in the sevoflurane group at all time points ($p \leq 0.001$ for all, $r = 0.470–0.571$). Absolute MAP values were likewise reduced, but only significantly at 0 min ($p = 0.020$, $r = 0.386$), and with trends at pre ($p = 0.093$, $r = 0.302$), 5 min ($p = 0.114$, $r = 0.292$), 10 min ($p = 0.075$, $r = 0.312$), and 15 min ($p = 0.057$, $r = 0.325$), respectively (**Figures 3A,B**; **Supplementary Table S4**). No significant changes were observed for Δ HR ($p = 1.000$ for all, $r = -0.114$ to 0.048) or Δ MAP ($p \geq 0.193$ for all, $r = 0.030–0.253$) at any time point (**Figures 3C,D**; **Supplementary Table S4**).

Dose–response analyses confirmed a negative correlation between sevoflurane concentration and both HR and MAP. For absolute HR values, correlations were strongly negative across all time points ($p < 0.001$ for all, $\rho = -0.499$ to -0.415). For absolute MAP values, significant negative correlations occurred at pre, 0 min, 10 min, and 15 min ($p \leq 0.032$ for all, $\rho = -0.322$ to -0.294), and a trend was seen at 5 min ($p = 0.059$, $\rho = -0.272$). There were no significant correlations for Δ HR ($p = 1.000$ for all, $\rho = -0.026–0.123$) or Δ MAP ($p \geq 0.270$ for all, $\rho = -0.199$ to -0.040) (**Supplementary Table S4**).

Interaction Analysis Between Sufentanil and Sevoflurane

Across all time points, no significant interaction effects between sufentanil and sevoflurane were observed for absolute values of HR ($p \geq 0.466$ for all, partial $\eta^2 = 0.007–0.035$) and MAP ($p = 1.000$ for all, partial $\eta^2 = 0.001–0.005$) (**Figures 4A,B**; **Supplementary Table S5**). Analyses of relative changes yielded similar results, with no interaction between sufentanil and sevoflurane on the magnitude or direction of changes in Δ HR ($p \geq 0.887$ for all, partial $\eta^2 = 0.000–0.018$) or Δ MAP ($p \geq 0.453$ for all, partial $\eta^2 = 0.003–0.031$) (**Supplementary Table S5**).

Effect of Norepinephrine on Hemodynamics

Since norepinephrine was the predominant catecholamine, and dosing of all catecholamines did not change significantly over time, subsequent analyses focused solely on the effects of norepinephrine. Partial correlation analyses revealed time-specific relationships between norepinephrine infusion rate and hemodynamics. After controlling for sufentanil and sevoflurane, norepinephrine positively correlated with HR in the early phase from pre to 5 min ($p \leq 0.038$ for all, $\rho = 0.287–0.318$) and negatively with MAP during the later phase from 10 min to 15 min ($p \leq 0.032$ for all, $\rho = -0.303$ to -0.294) (**Supplementary Figures S1A, S1B**; **Supplementary Table S6**). Thus, higher norepinephrine rates were associated with increased HR and decreased MAP at corresponding times. No significant partial correlations were found between sufentanil dose and HR or MAP after norepinephrine adjustment ($p \geq 0.547$ for all, $\rho = -0.048–0.175$) (**Supplementary Figures S1C, S1D**; **Supplementary Table S6**), whereas the inverse relationship between sevoflurane concentration and HR remained significant throughout all time points ($p < 0.001$ for all, $\rho = -0.502$ to -0.414), and significant for MAP for 0 min, 10 min, and 15 min ($p \leq 0.033$ for all, $\rho = -0.316$ to -0.292) (**Supplementary Figures S1E, S1F**; **Supplementary Table S6**).

DISCUSSION

This study is among the few to comprehensively analyze both the type and dosage of anesthetic agents and catecholamines during organ retrieval, along with their corresponding hemodynamic effects. Administration of anesthetic agents varied considerably among donors. Neither HR nor MAP showed a marked stress-related change in response to surgical stimulation, although MAP decreased slightly at 15 min compared with 5 minutes after

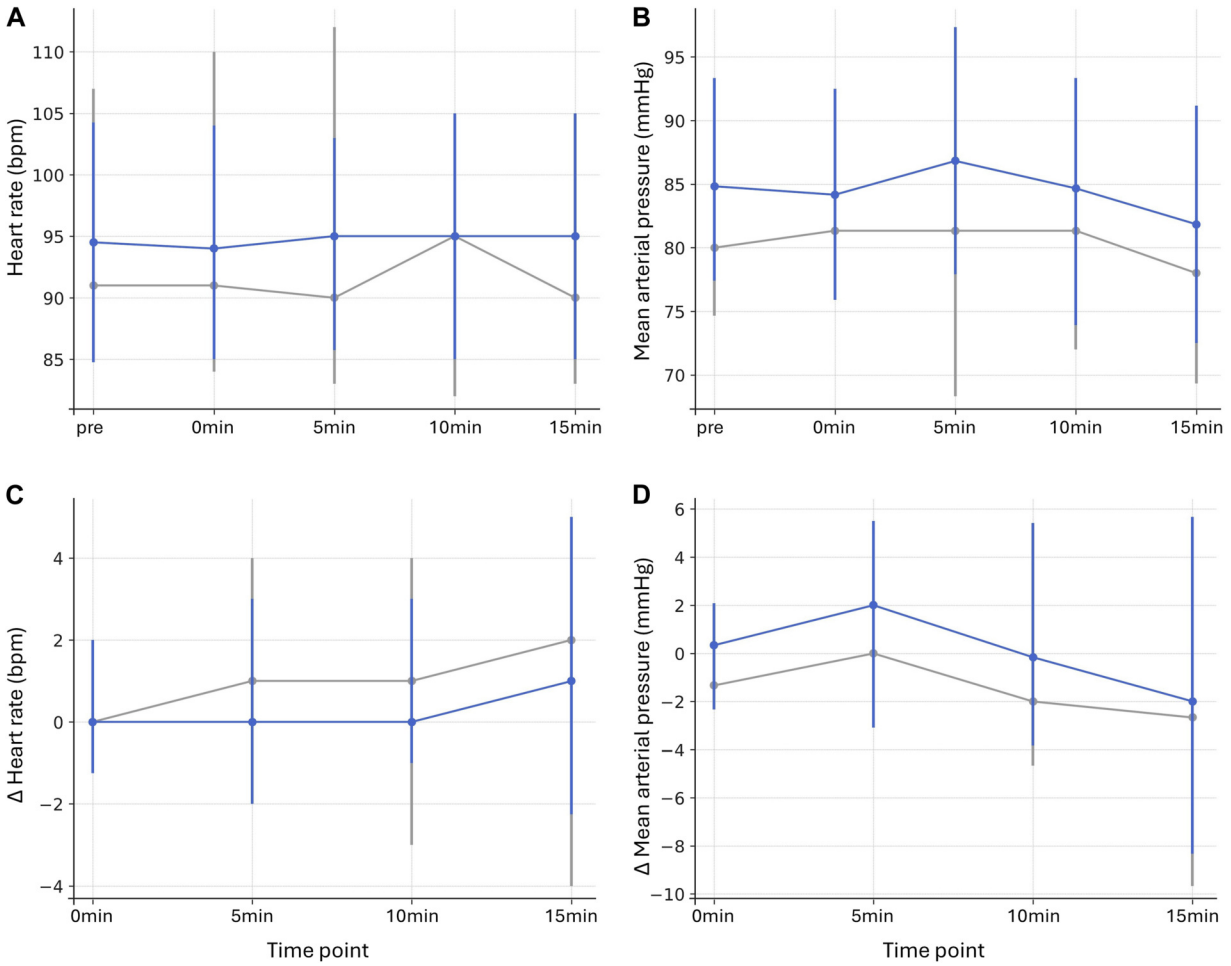


FIGURE 2 | Heart rate and mean arterial pressure in donors with and without sufentanil Heart rate (bpm). **(A)** and mean arterial pressure (mmHg) **(B)** at baseline (pre), during incision (0min), and 5, 10, and 15 min after incision. Changes from baseline (pre, Δ values) are displayed for heart rate **(C)** and mean arterial pressure **(D)** for corresponding time points. Data are presented as median values with interquartile ranges (vertical lines). Gray lines represent cases without, and blue lines with sufentanil administration.

incision. Sufentanil had no significant impact on hemodynamics. Sevoflurane was associated with consistently lower absolute HR and MAP values. However, compared with baseline, no significant differences were observed during or after incision. The hemodynamic effects of anesthetic agents remained unchanged after adjustment for concomitant use of norepinephrine.

Hemodynamic Response During Organ Retrieval

Only few studies have systematically examined hemodynamic responses to surgical stimuli in DBD donors. The earliest available investigation reported an immediate increase in HR and MAP after incision in 10 donors but provided detailed information on anesthetic agent or catecholamine administration for only one case. In this donor, HR and MAP decreased 11 minutes after incision following administration of enflurane, while no opioids or catecholamines were given. In all other cases, only an unspecified reduction in catecholamine dosing was described, with HR and MAP normalizing within 25 min in all donors [13]. A subsequent case report documented similar increases in HR and MAP after incision in two donors but did not specify anesthetic or catecholamine use. Notably, only these two analyzed cases of a total of 30 reviewed DBD donors exhibited a hemodynamic response [11]. Another study involving 14 DBD donors reported elevations in HR and MAP 6 minutes after incision, without administration of anesthetic agents to any donor, but with dopamine in nine cases at constant doses [12]. Fitzgerald and colleagues reported on 11 DBD donors without anesthetic agents, seven of whom received dopamine. MAP increased immediately after incision and then decreased, while HR remained stable [9]. In a more recent study, donors with anesthetic agents exhibited higher HR and more frequent episodes of hypotension, although maximal MAP did not differ between groups. However, the study only distinguished

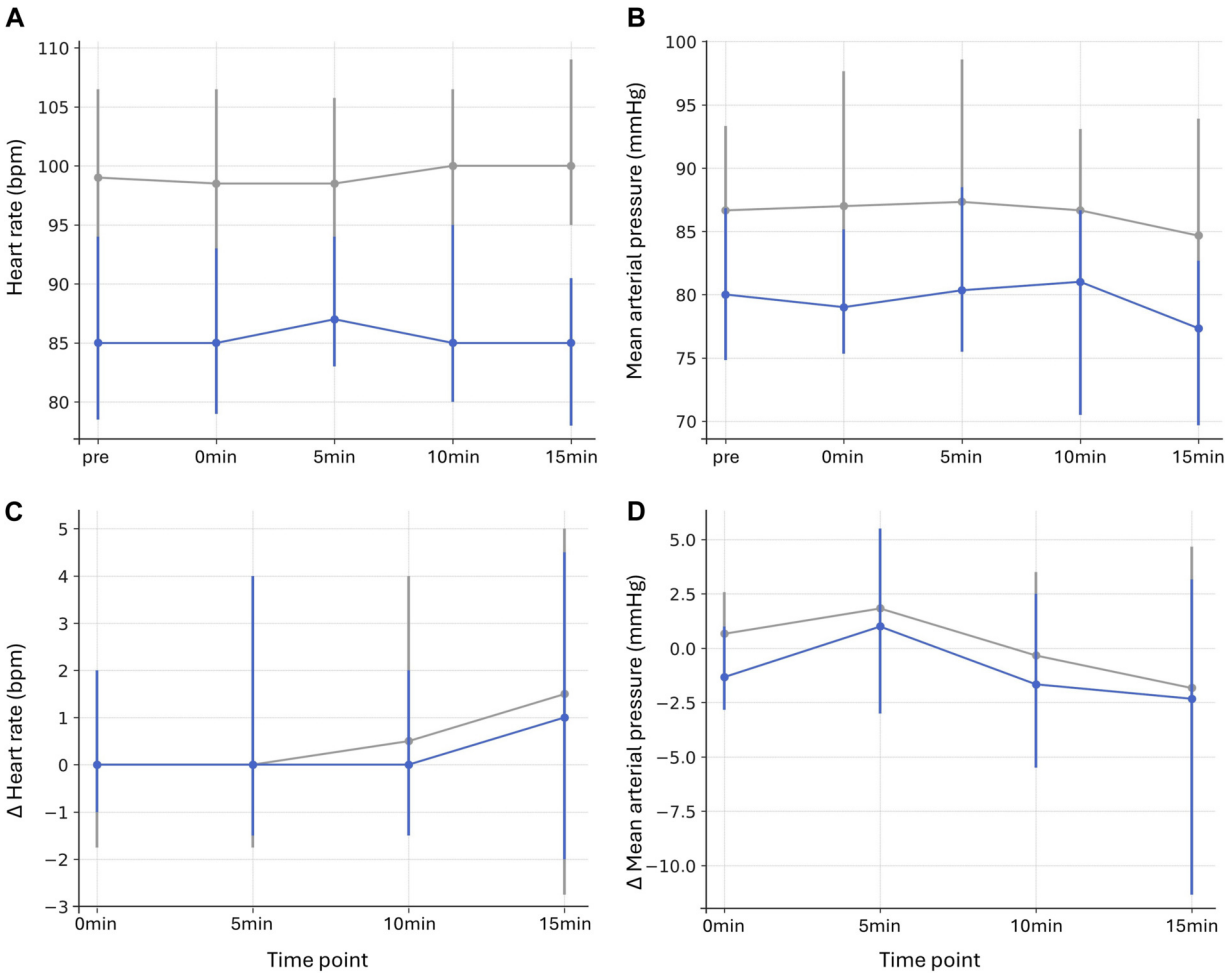


FIGURE 3 | Heart rate and mean arterial pressure in donors with and without sevoflurane Heart rate (bpm). **(A)** and mean arterial pressure (mmHg) **(B)** at baseline (pre), during incision (0min), and 5, 10, and 15 min after incision. Changes from baseline (pre, Δ values) are displayed for heart rate **(C)** and mean arterial pressure **(D)** for corresponding time points. Data are presented as median values with interquartile ranges (vertical lines). Gray lines represent subjects without, and blue lines with sevoflurane.

between donors with and without anesthetic agent administration. Among those receiving agents (62% in total), the majority were given opioids alone (72%), while volatile anesthetics were administered in only 16% of cases. Additionally, hemodynamic parameters were not compared with baseline values prior to incision. Consequently, the observed hemodynamic effects cannot be attributed to any specific drug class or a possible reaction to surgical stimuli [4]. Taken together, these studies highlight the lack of standardized methodology for assessing hemodynamic parameters during organ retrieval, which are likely to contribute to inconsistent findings.

The observed hemodynamic responses in DBD donors have traditionally been interpreted as sympathetic reflexes to noxious stimuli mediated *via* spinal cord pathways in the absence of supraspinal control [20, 27]. Moreover, increases in systemic vascular resistance and endogen catecholamine levels after incision have been demonstrated [9, 12]. These findings have led to the hypothesis that anesthetic agents may attenuate such hemodynamic responses and thereby exert organ-protective effects [6].

Our findings add new evidence to this conflicting body of literature. The total cohort in our study exhibited no increase in HR or MAP over time or after incision compared with baseline. Sufentanil did not alter hemodynamic values. There was even a non-significant trend to higher MAP values in donors receiving sufentanil, which aligns with results from previous studies [18, 19]. Thus, our results do not support the use of opioids for hemodynamic control in DBD donors, contrary to prior recommendations [20].

Opioid receptors are widely distributed within the central nervous system (CNS), including the substantia gelatinosa of the dorsal horn and to a lesser extent in peripheral tissues. Within the CNS, activation of opioid receptors in the midbrain is thought to be a major mechanism of opioid-induced analgesia. They stimulate descending inhibitory pathways, which results in a

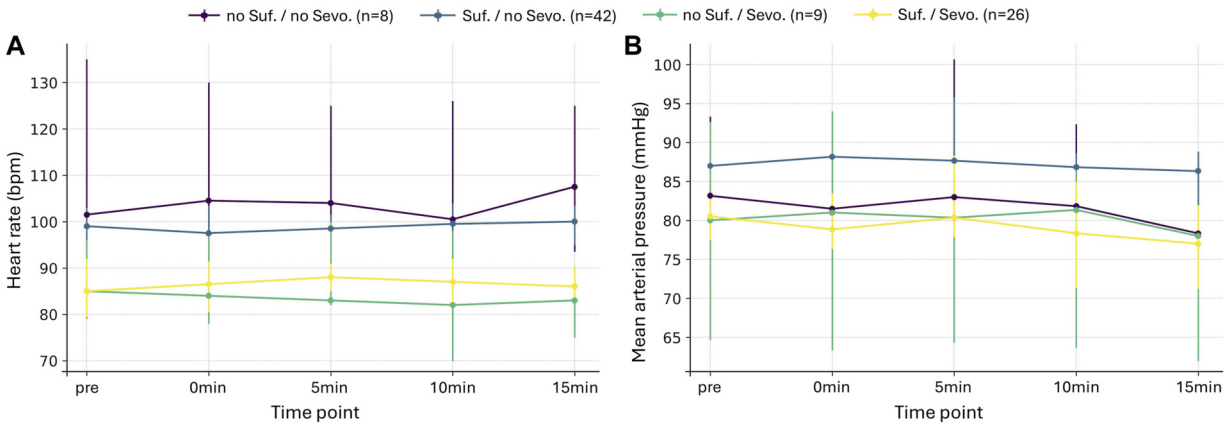


FIGURE 4 | Interaction of sufentanil and sevoflurane Heart rate (bpm). **(A)** and mean arterial pressure (mmHg) **(B)** over time in four groups defined by the presence or absence of sufentanil (Suf.) and sevoflurane (Sevo.) administration. Values represent the group median at baseline (pre), during incision (0min), and 5, 10, and 15 min after incision. Vertical lines indicate 95% CI, displayed instead of IQR to allow precise interval estimates even in subgroups with small sample sizes.

reduction of nociceptive transmission from the periphery to the thalamus [28]. Peripheral opioid effects are variable and are primarily observed under conditions of tissue injury, such as inflammation or neuropathy [29]. Although opioids can exert direct inhibitory effects when administered epidural, spinal or peripheral, clinically relevant analgesia following systemic administration seems to be predominantly mediated *via* central mechanisms [28]. In DBD donors, the absence of supraspinal integration and functioning descending inhibitory pathways likely limits the ability of systemically administered opioids to attenuate nociceptive transmission and autonomic responses. Moreover, the absence of effective cerebral perfusion in the brain-dead state prevents systemically administered opioids from reaching the brain, rendering potential central antinociceptive and reflex-modulating effects unlikely [6]. Hence, the isolated use of opioids during organ retrieval appears ineffective for controlling possible neurovegetative reflexes or conferring tissue protection [4]. In contrast, sevoflurane significantly decreased HR and MAP, but these characteristics were already seen at baseline and were independent of surgical stimuli. This pattern is consistent with the general pharmacodynamic properties of volatile anesthetics, which induce vasodilation predominantly through actions at the spinal cord level, independent of any effects on the CNS [30], and may also involve direct myocardial effects or reductions in systemic vascular resistance [31]. Importantly, this effect does not reflect modulation of responses to surgical stimuli.

Organ Protective Effects of Anesthetic Agents

Administering anesthetic agents during organ retrieval could nonetheless be considered an anesthetic preconditioning strategy to attenuate IRI. Several studies have suggested that volatile anesthetics exert cardioprotective effects in cardiac surgery [32]. Under brain death conditions, these mechanisms have only been investigated scarcely. Experimental data suggest that pharmacological modulation could possibly mitigate IRI [33], and volatile anesthetics have been proposed to exert similar protective effects through distinct pathways [14, 17]. One single clinical study showed that preconditioning with sevoflurane during organ retrieval improved liver graft function [34], whereas a later study was unable to confirm a protective effect in early or long-term graft survival for kidney, liver, lung, or heart transplantation [16]. Another study likewise found no association between the use of volatile anesthetics and/or opioids and kidney graft function [4]. Overall, both approaches - administering or omitting anesthetic agents - lack robust clinical evaluation and high-quality evidence regarding their impact on graft outcomes [4].

Hemodynamic Management During Organ Retrieval

Norepinephrine was the predominant catecholamine used in our cohort, while other catecholamines were administered in only a few donors, consistent with previous findings [4, 8]. Whether their use was intended as part of an organ-protective strategy, for hemodynamic stabilization, or simply continued from preexisting ICU therapy cannot be determined in the retrospective setting of our study. Overall, catecholamine dosing did not change significantly over time, and a MAP ≥65 mmHg was maintained in most donors throughout the observation period.

Very few other studies have investigated hemodynamic management during organ retrieval. One study reported that the target MAP of 65 mmHg was not maintained for a considerable period in 62% of DBD donors [4]. In another study, 24% of donors experienced hypotension lasting from a minimum of 10 min to a maximum of 96 min [8]. Interestingly, our data show that shortly after incision, a decline in MAP was particularly evident in the cohort receiving VA. Notably, higher norepinephrine doses correlated with lower MAP values, likely reflecting the reactive use of vasopressors in response to

hypotension rather than a direct hypotensive effect of norepinephrine.

This phenomenon is consistent with earlier studies demonstrating a rapid decline in endogenous catecholamine levels shortly after incision [9, 11]. The combination of reduced catecholamine concentrations and the vasodilatory properties of volatile anesthetics may increase the risk of hypotension and should therefore be carefully considered when volatile anesthetics are administered. Opioids, in contrast, did not contribute to hemodynamic modulation in our cohort.

Continuous waveform monitoring enables real-time detection of hemodynamic changes in critically ill patients and is essential for timely intervention [35]. Its use in DBD donors has not been described in the literature but may represent a promising approach to ensure hemodynamic stability during organ retrieval and to improve understanding of the effects of anesthetic agents and catecholamines in this setting.

Ethical Considerations

Beyond physiological considerations, the choice to administer anesthetic agents may reflect clinicians' unease with the concept of brain death—a difficulty frequently noted in the literature [24, 25]. Administering anesthesia to a DBD donor for reasons other than a potential protective effect risks undermining the conceptual and ethical clarity of brain death and could have detrimental implications for both public perception and healthcare professionals [36]. Consequently, Turner suggested that the term 'anesthesia′ itself should perhaps be avoided in this context to minimize misunderstanding of the transplantation process [37].

Limitations

Potential confounders could not be controlled for, such as details of preceding ICU management strategies or preexisting cardiovascular or other organ specific conditions. Hemodynamic measurements were referenced to the time of the first incision, which may not correspond to the most relevant noxious stimulus. The administration of other drugs (e.g., corticosteroids) or fluids was not considered, although these may have influenced hemodynamic responses to an unknown extent. As a single-center study, generalizability is limited, and management strategies may also have evolved over the long observation period, introducing potential heterogeneity. The decision to administer anesthetic agents was not randomized and may have been influenced by clinical judgement or donor characteristics. Finally, graft outcomes could not be analyzed. Therefore, no conclusions can be drawn regarding the potential effect of anesthetic management on transplantation results.

CONCLUSION

Anesthetic management of organ retrieval varied considerably. Neither the surgical stimulus nor sufentanil had a measurable impact on hemodynamics in DBD donors. This suggests that opioids are not indicated during organ retrieval until further evidence about other pharmacological organ-protective effects is available. Sevoflurane was associated with consistently lower absolute HR and MAP values, but this was independent of the surgical stimulus. These effects of volatile anesthetics in DBD donors must be carefully considered to maintain hemodynamic stability. Thus, our findings highlight two key points. First, there is an urgent need for evidence-based recommendations on anesthetic management during organ retrieval, encompassing both pharmacological and ethical considerations. Second, such recommendations should be based on future research elucidating the relationship between anesthetic management and graft outcomes. This may represent a promising strategy to enhance organ-protective measures and should be evaluated in prospective studies to determine whether it ultimately improves transplant outcomes.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Anonymized raw data supporting the conclusions of this article will be made available on reasonable request. Requests to access these datasets should be directed to jan.englbrecht@ukmuenster.de.

ETHICS STATEMENT

The studies involving humans were approved by Ethical Committee of the University of Münster. 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

All authors participated in the design, interpretation and analysis of the data and review of the manuscript; JE, TP, and SZ were involved in planning and supervised the work, JE, MK, CM, and SZ processed the data, and performed the analysis. JE drafted the manuscript and designed figures. TP, MK, and CM aided in interpreting the results and worked on the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

Author TP was employed by CSL Behring and Edwards Lifesciences and has received speaker's honoraria unrelated to the current study.

The remaining author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

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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.2026.16262/full#supplementary-material

SUPPLEMENTARY FIGURE S1 | Partial correlation analyses between norepinephrine, sufentanil, sevoflurane and hemodynamic parameters. Scatterplots display the relationship between norepinephrine (μg kg^{-1} min^{-1}) and heart rate (HR, bpm) **(A)** and mean arterial pressure (MAP, mmHg) **(B)**; sufentanil (μg kg^{-1}) and HR **(C)** and MAP **(D)**; sevoflurane (et %) and HR **(E)** and MAP **(F)**. Data are shown for baseline (pre), during incision (0 min), and 5, 10, and 15 minutes after incision. For each time point, individual data points represent partial residuals of HR and MAP after statistical adjustment for both sufentanil and sevoflurane **(A,B)**, and norepinephrine **(C–F)**, respectively. Each panel contains a linear regression line, and a shaded band depicts the 95% CI. Raw predictor values were plotted against partial residuals of HR or MAP obtained after adjustment for covariates. Accordingly, x-axes display variables in their original physical units, while y-axes represent dimensionless residuals reflecting adjusted deviations from model-predicted values.

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BRIEF RESEARCH REPORT
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Beyond a Biomarker: Investigations of a Proinflammatory Role for Cell-Free DNA in Liver Transplant Ischemia and Reperfusion

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Donor-derived cell-free DNA (dd-cfDNA) is a biomarker for rejection after organ transplantation. We hypothesized that high release of cfDNA immediately after liver transplant also has a biologic role in inflammation in ischemia and reperfusion injury (IRI). To investigate this concept, C57BL/6 mice were subjected to 90 min *in situ* liver ischemia. After 6 h reperfusion, cfDNA was purified from serum and used to stimulate macrophages *in vitro*, which resulted in production of high levels of inflammatory cytokines TNFα and IL-6, and chemokine CXCL10. Enzymatic degradation of cfDNA by DNase I inhibited these inflammatory responses (e.g., TNFα: DNase I 48.1 ± 37.4 vs. untreated 1,030 ± 206 pg/mL, p = 0.0001). cfDNA from netosis-deficient PAD4KO mice was found to be equally pro-inflammatory compared to wild type cfDNA (TNFα: PAD4KO 1048 ± 199 vs. wild-type 1,162 ± 150 pg/mL, p = 0.64), indicating its mechanism is not dependent on neutrophils undergoing netosis. Next, a single dose of DNase I was added to the perfusate during rat liver normothermic machine perfusion (NMP) to significantly reduce perfusate cfDNA levels (384 ± 132 to 129 ± 18 ng/mL, p = 0.026). In conclusion, our data suggest that cfDNA can have pro-inflammatory effects during liver IRI beyond being a biomarker. DNase I may be a promising therapeutic intervention during NMP to reduce the graft's inflammatory propensity prior to implantation.

Keywords: cell free DNA, inflammatory cytokines, ischemia and reperfusion, liver transplantation, macrophages

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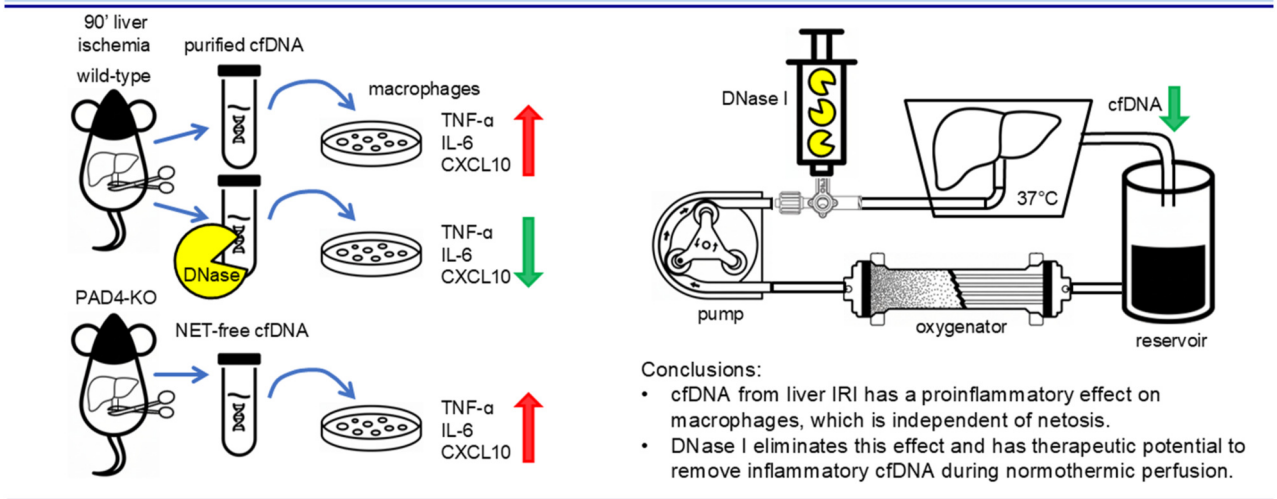
INTRODUCTION

In organ transplantation, circulating donor-derived cell-free DNA (dd-cfDNA) has been established as a reliable biomarker for rejection, and "liquid biopsy" blood tests have been developed for screening and early detection [1]. In liver transplantation, dd-cfDNA is also associated with non-rejection liver graft cellular injury [2]. The greatest insult causing cellular injury occurs during ischemia and reperfusion at the time of liver implantation: at this time, cfDNA levels are more than 10 times higher than in controls [3] and 80%–90% of cfDNA is donor-derived (compared to ~30% during a biopsy proven rejection episode) [4]. It is still largely unknown if cfDNA has biologic activity in the pathogenesis of organ graft injury. In other inflammatory conditions however, extracellular DNA has been investigated as a damage-associated molecular pattern (DAMP) that can activate innate immune cells to initiate and/or propagate the sterile inflammatory response [5, 6].



GRAPHICAL ABSTRACT |

Ischemia and reperfusion injury (IRI) is inherent to liver donation and transplantation [7]. While a young, healthy liver graft can readily recover from IRI, marginal liver grafts have a lower tolerance for IRI. After transplantation of a marginal liver, IRI can result in reperfusion syndrome, early allograft dysfunction, acute kidney injury, and prolong a recipient's hospital stay and recovery [8]. Because of organ donor shortage, we depend on the use of marginal donors for liver transplantation (older, having fatty liver, donation after circulatory death). Therefore, understanding the mechanisms of IRI remains important and elucidating a bioactive role for cfDNA can be of significance in developing treatment strategies to inhibit IRI.

Here we investigated the hypothesis that liver-derived cfDNA has a pro-inflammatory role beyond a biomarker in ischemia-reperfusion injury. We also explored if DNase I can be used to neutralize pro-inflammatory cfDNA, and whether this could be applied during NMP to clean the liver from cfDNA even prior to implantation.

METHODS

Animals and Surgical Procedures

Animal protocols were in adherence with the NIH Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee. Details on mouse strains and surgical procedures are available in the Supplementary Methods. In mice, 90 min of *in situ* partial warm-ischemia to the liver was applied [9]. Reperfusion was initiated, and mice were euthanized at various time points for serum collection. The rat donor hepatectomy procedure included laparotomy, hepatic artery ligation, bile duct and portal vein cannulation, and *in situ* liver flush with cold heparinized PBS until the effluent from the vena cava appeared clear [10].

Cell-Free DNA Purification

cfDNA was purified from mouse serum using the QIAamp Circulating Nucleic Acid Kit, Qiagen, Hilden, Germany, to exclude other pro-inflammatory stimuli such as cytokines and chemokines. cfDNA was quantified using Quant-iT PicoGreen dsDNA Assay Kit, ThermoFisher Scientific, Waltham, MA. DNase I, Millipore Sigma, St. Louis, MO, was added to purified cfDNA at escalating concentrations of 0.1, 0.5, 1, and 5 mg/mL and incubated at 37 °C for 30 min, followed by measurement of remaining cfDNA concentrations.

Macrophage Culture and Stimulation

RAW 264.7 cells (ATCC, Manassas, VA) were seeded onto 96-well plates at 75,000 cells/well and incubated overnight at 37 °C. Peritoneal macrophages were collected from C57BL/6J mice, and cultured as previously described [11], and as detailed in the Supplementary Methods. Macrophages cultures were washed once and incubated with medium containing 5000 ng/mL purified cfDNA. cfDNA was complexed with lipofectamine for intracellular delivery as was previously suggested by Kaczorowski et al. [12] cfDNA from wild type mice was compared to DNase I-treated cfDNA, and cfDNA from PAD4KO mice. After 24 h

stimulation, supernatants were analyzed for TNF-α, IL6, and CXCL10 using ELISA (ThermoFisher Scientific).

Normothermic Machine Perfusion

A custom-designed NMP system for rat livers was developed together with Harvard Apparatus, Holliston, MA. Livers were perfused via the cannulated portal vein under controlled conditions for oxygenation, flow, and pressure at temperature of 37 °C. Details on the perfusate composition are provided in the Supplementary Methods. Perfusate samples were collected every 15 min. After 120 min, DNase I was added to the perfusate at a concentration of 0.5 mg/mL.

Statistical Analysis

Continuous variables were expressed as mean ± SD or mean ± SEM. A two-tailed independent t-test was used to compare experimental groups. Repeated measures ANOVA was used to compare cfDNA values during NMP. All analyses were performed with GraphPad Prism and a P value < 0.05 was considered statistically significant.

RESULTS

Liver Ischemia Causes High Levels of Circulating cfDNA

In our experience, 90 min of portal clamping results in significant IRI without being lethal [9]. All mice survived the operation and returned to normal activity upon emergence from anesthesia. Quantification of cfDNA in serum revealed that cfDNA levels steeply rise upon reperfusion, peak at 6 h post-reperfusion (15,594 ± 3,728 vs. 1,048 ± 289 ng/mL after sham laparotomy, n = 6, p < 0.0001), and return to baseline levels by 24 h (**Supplementary Figure S1**). A similar pattern has been observed in liver transplant patients in whom cfDNA levels are highest at completion of the operation and have cleared 24 h postoperatively [3].

Purified cfDNA has a Direct Pro-Inflammatory Effect on Macrophages

Purification of cfDNA from serum containing peak levels of cfDNA (6 h post-reperfusion) resulted in eluates containing 12,000–35,000 ng/mL cfDNA. Purified cfDNA was used to stimulate RAW cells and primary peritoneal macrophage cultures at 5000 ng/mL, a clinically relevant concentration observed in serum of patients undergoing liver transplant [3]. **Supplementary Figure S2** represents our initial experiments where a release of TNF-α into the supernatant was observed upon stimulation of both RAW cells and peritoneal macrophages. This response was increased by complexing cfDNA with lipofectamine, which may indicate a role for an intracellular receptor mechanism [13]. In subsequent experiments, we therefore used cfDNA with lipofectamine to stimulate fresh peritoneal macrophage cultures. Macrophages were significantly activated to produce high levels of inflammatory cytokines TNF-α (1,155 ± 166 vs. unstimulated 36.8 ± 7.3 pg/mL, p < 0.0001) and IL-6 (2,932 ± 374 vs. unstimulated 190 ± 45 pg/mL, p < 0.0001) (**Figures 1A,B**). We investigated whether macrophages produced type I IFN-β [14]. IFN-β in supernatants was detected, however, the levels were variable (data not shown). As macrophages themselves express type I IFN receptors (IFNAR-1) [15], we hypothesized that IFN-β was partially being removed from supernatant by autocrine binding to INFAR-1. We therefore tested supernatants for CXCL10, a chemokine produced upon interferon pathway stimulation. Indeed, levels of CXCL10 were highly elevated (851 ± 74 vs. unstimulated 6.7 ± 1.7 pg/mL, p < 0.0001) (**Figure 1C**).

DNase I Degrades cfDNA and Eliminates Its Inflammatory Effect

Thus far, our results showed that circulating cfDNA resulting from liver IRI can have a pro-inflammatory effect on innate immune cells such as macrophages. As we previously used DNase I to limit IRI and steatohepatitis in mouse models [16, 17], we investigated the effect of DNase I in the *in vitro* macrophage stimulation assay. First, the effect of various concentrations of DNase I was tested and a dose-dependent degradation of cfDNA during 30min incubation at 37 °C was found (**Supplementary Figure S3**). We then used DNase I at 5 mg/mL concentration to pretreat cfDNA prior to macrophage stimulation. **Figure 2** shows that cfDNA that had been digested by DNase I nearly completely lost its capacity to stimulate macrophages (TNFα: 48.1 ± 37.4 vs. untreated 1,030 ± 206 pg/mL, p = 0.0001; IL-6: 44.2 ± 15.5 vs. untreated 2090 ± 457 pg/mL, p = 0.0002; CXCL10: 14.4 ± 6.5 vs. untreated 888 ± 54 pg/mL, p < 0.0001), demonstrating again that cfDNA is inflammatory, and providing proof of concept that DNase I can be a potential treatment option to limit cfDNA-induced inflammation in liver transplant IRI.

cfDNA Is Inflammatory Independent of Netosis

In previous work we focused on the inflammatory properties of extracellular DNA from neutrophils that form neutrophil extracellular traps (NETs) [17, 18]. Although it is known that netosis can release proinflammatory DNA into circulation [18], the very high levels of circulating cfDNA from liver IRI are unlikely the result of netosis alone. Thus, we tested whether cfDNA can induce inflammation independent of netosis. Liver IRI was applied to PAD4KO mice that are deficient in forming NETs. PAD4KO confers a degree of protection against IRI [18] but 90 min of liver ischemia still resulted in high levels of circulating cfDNA. Purified cfDNA from NET-free PADKO mice, used at the same concentration as wild type cfDNA, induced the production of TNF-α, IL-6, and CXCL10 to comparable levels (TNFα: PAD4KO 1048 ± 199 vs. wild-type 1,162 ± 150 pg/mL, p = 0.64; IL-6: PAD4KO 1952 ± 359 vs. wild-type 2,472 ± 354 pg/mL, p = 0.31; CXCL10: PAD4KO 820 ± 66 vs. wild-type 749 ± 69 pg/mL, p = 0.48) (**Figure 3**), indicating that the pro-inflammatory activity of cfDNA is independent of neutrophils undergoing netosis. To further investigate the composition of cfDNA, we applied PCR using specific primers for nuclear and mitochondrial DNA and found that liver IRI

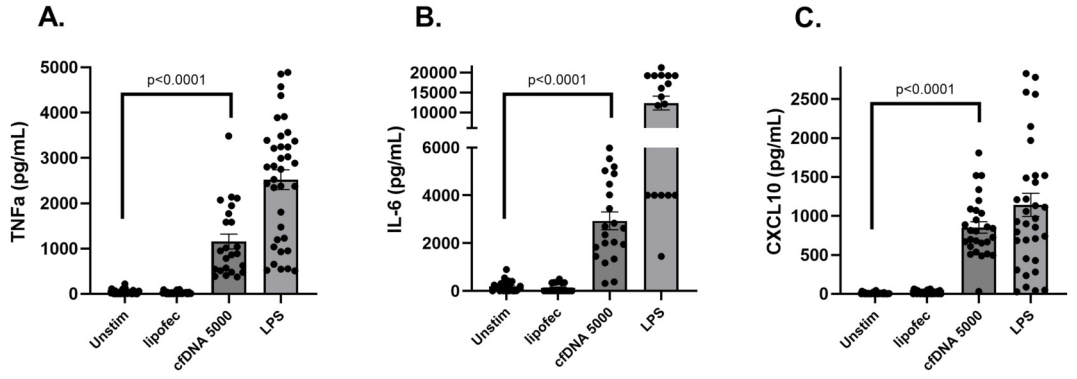


FIGURE 1 | Inflammatory cytokine levels in mouse peritoneal macrophage cultures stimulated with purified cfDNA after liver ischemia and reperfusion. cfDNA-stimulated macrophages produced high levels of inflammatory cytokines TNF-α **(A)** and IL-6 **(B)**, and chemokine CXCL10 **(C)** compared to unstimulated and lipofectamine controls. Data represent the mean ± SEM of 5 or more independent experiments, each experiment performed in triplicate.

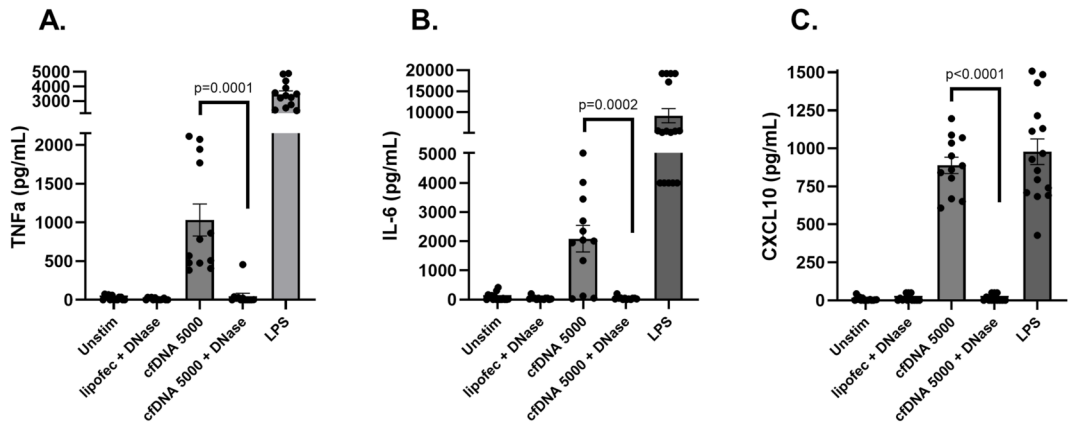


FIGURE 2 | Cytokine production in mouse peritoneal macrophage cultures exposed to cfDNA that was pretreated with 5 mg/mL DNase I. DNase I-degraded cfDNA nearly completely lost its ability to induce the production of TNF-α **(A)**, IL-6 **(B)**, and CXCL10 **(C)** by macrophages. Data represent the mean ± SEM of 3 independent experiments, each experiment performed at least in triplicate.

causes the release of both (details in Supplementary Methods and Supplementary Results).

NMP as a Platform for *Ex Vivo* Treatment With DNase I

NMP offers a promising platform to deliver *ex vivo* treatment to the organ, and NMP at 37 °C would provide the optimal conditions for the enzymatic activity of DNase I. We established an NMP system for rat livers. Flows of 2 mL/g liver tissue were obtained while portal pressures remained low at 5–7 mmHg. Perfusate PaO_2 was kept >80 mmHg and livers produced bile while being perfused (**Figure 4A**). During NMP, ALT levels gradually increased over time, comparable to the pattern of ALT release in our clinical liver NMP program. Adding a single dose of DNase I to the perfusate for a concentration of 0.5 mg/mL (10x lower than used in our *in vitro* experiment) resulted in a reduction of perfusate cfDNA levels from 384 ± 132 to 129 ± 18 ng/mL, indicating that DNase I has activity in the currently used NMP setup. Repeated measures ANOVA demonstrated that post-DNase I cfDNA levels were significantly lower than pre-DNase I levels, p = 0.031 (**Figure 4B**). The performance of the liver during NMP appeared unaffected by DNase I addition. While flow was kept consistent, no difference in portal vein pressure was noted (5.77 ± 0.51 mmHg pre-DNase I vs. 5.69 ± 0.41 mmHg post-DNase I, p = 0.92). After DNase I administration at 120min, lactate clearance continued for all livers, in a pattern that did not appear different from liver perfusions during which DNase I was not applied (**Figure 4C**). Although not quantified, bile production was not altered by DNase I.

DISCUSSION

Donor-derived cell-free DNA (dd-cfDNA) has diagnostic value as a biomarker for early detection of organ rejection after transplantation. Here we investigated a biologic activity of

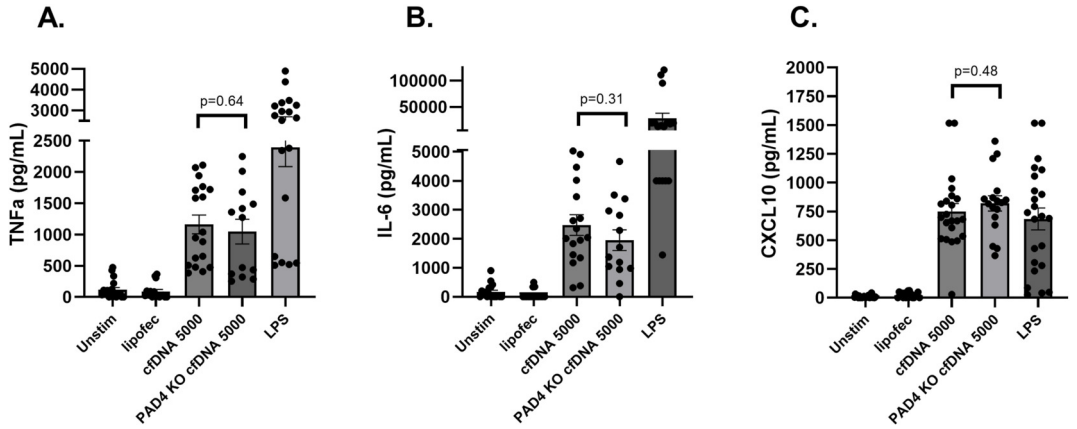


FIGURE 3 | Comparison of cytokine production in mouse peritoneal macrophage cultures exposed to cfDNA purified from wild type vs. PAD4 KO mice. The production of TNF-α **(A)**, IL-6 **(B)**, and CXCL10 **(C)** induced by cfDNA from PAD4 KO mice was not significantly different than by cfDNA from wild type mice, indicating that inflammatory cfDNA is likely to originate from cell types other than the known inflammatory DNA released by neutrophils during netosis. Data represent the mean ± SEM of 4 independent experiments, each experiment performed at least in triplicate.

cfDNA beyond being a biomarker. We found that liver-derived cfDNA purified from serum after liver ischemia and reperfusion is a direct pro-inflammatory stimulus for macrophages. DNase I effectively degraded cfDNA and eliminated its pro-inflammatory effect *in vitro*. Applying DNase I during liver NMP reduced cfDNA levels in the perfusate, suggesting that addition of DNase I during NMP can be further explored as a strategy to clean the liver graft from inflammatory cfDNA even prior to transplantation.

After reperfusion of a liver, serum dd-cfDNA levels are multiple times higher than levels that would indicate rejection [4]. With our mouse model of *in situ* liver IRI, we confirmed that ischemia-reperfusion is indeed the responsible phenomenon for this massive release of cfDNA. Excessive cfDNA release can overwhelm natural serum DNases and result in inflammatory immune stimulation. In trauma and severe sepsis for example, the levels of serum cfDNA correlate with higher levels of inflammation and worse clinical outcomes [19, 20]. Under those conditions, cfDNA may act as a DAMP that activates pattern recognition receptors on innate immune cells. Conversely, when a genetic mutation results in DNase deficiency, low levels of cfDNA from physiologic cell turnover lead to autoimmune disease (systemic lupus erythematosus) in humans and mice, and immune activation leads to the presence of anti-DNA antibodies [21, 22]. Although the significance of DAMPs in transplant immune responses has been recognized [23, 24], a biologic activity for cfDNA in liver IRI has not been established, and to our knowledge, there have also been no investigations of targeting cfDNA to reduce IRI. While we have yet to confirm this in *in vivo* models of liver transplant, our data provide the initial mechanistic proof of this concept.

DNase I has previously been used for its anti-inflammatory effects in rodent models of IRI [16, 25], and steatohepatitis [17]. We have previously shown that treatment with exogenous DNase I can alleviate NET-induced inflammation [16, 17]. In liver transplantation, the high levels of circulating cfDNA after reperfusion are unlikely the result of netosis alone, and we hypothesized that cfDNA can be inflammatory independent of netosis. NET-free cfDNA from PAD4KO serum retained its capability of activating macrophages, indicating that cfDNA from other cell types and other mechanisms than netosis can be pro-inflammatory. Using PCR we found that cfDNA contains both nuclear and mitochondrial DNA; defining the specific role of each in liver IRI will be important, as mitochondrial DNA has been shown to be a DAMP in transplant inflammation [26].

Ideally, one would limit the liver graft DAMP-burden even prior to organ implantation. NMP offers a promising yet greatly underutilized platform to deliver *ex vivo* treatment, and normothermia provides the optimum conditions for DNase, an enzyme active at body temperature. We found that the addition of DNase I to the perfusate greatly reduced cfDNA levels in a rat liver NMP model, without affecting pump parameters and lactate levels. If this were to be developed toward a clinical application, DNase I seems to have a favorable safety profile. Patients with lupus nephritis have been safely treated with recombinant human DNase [27]. Moreover, *ex vivo* use during NMP would avoid direct exposure of the recipient patient.

Overall, we found that cfDNA from liver IR can be more than a biomarker for cellular injury. We acknowledge that our results thus far are based on *in vitro* and *ex vivo* observations and that there are several remaining questions to be answered. First, we next need to perform experiments of liver reperfusion and transplantation after DNase I treatment to understand the clinical benefit of targeting cfDNA for therapeutic purposes. Second, we acknowledge that our studies have used peritoneal macrophages as target cells, as they can be consistently obtained and cultured. We have not yet been able to establish Kupffer cell cultures. We believe that our model is relevant since both peritoneal macrophages and Kupffer cells are tissue resident macrophages derived from common yolk sac progenitors [28]. However, it will be important to define the specific responses to cfDNA of Kupffer cells versus infiltrating macrophages, as the roles of macrophage subtypes in response to inflammation and

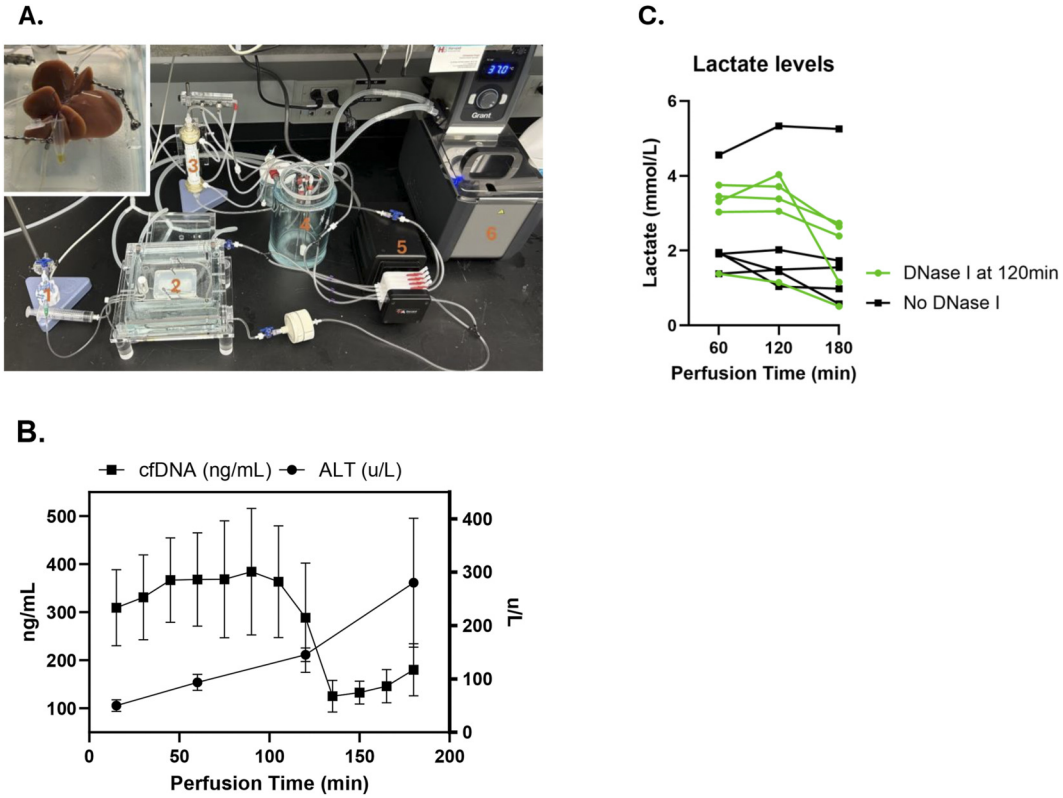


FIGURE 4 | (A) Rat liver normothermic perfusion pump system. 1. Pressure transducer, 2. Organ chamber, 3. Oxygen membrane, 4. Perfusate reservoir, 5. Peristaltic pump, 6. Temperature-controlled water bath. Insert: rat liver during NMP with production of bile. **(B)** Perfusate ALT and cfDNA levels during rat liver NMP. cfDNA and ALT levels gradually increased over time. A single dose of DNase I for a concentration of 0.5 mg/mL administered at 120 min significantly reduced cfDNA levels (repeated measures ANOVA comparing cfDNA pre- and post-DNase I, $p = 0.031$). **(C)** Administration of DNase I did not affect ongoing clearance of lactate between 120 and 180 min (lactate data available for 5 independent experiments).

resolution are increasingly becoming understood [29, 30]. Third, we need to investigate the signaling mechanisms in further detail. Although lipofectamine was not required to elicit a cytokine response, creation of a lipid bilayer (liposome) for intracellular delivery of cfDNA increased the pro-inflammatory response. Several known intracellular nucleotide receptors exist and include Toll-like receptor 9 (TLR9), stimulator of interferon gene (STING), and absent in melanoma 2 (AIM2). These receptors all have been implied in liver IRI [31–33], but their exact response to cfDNA has yet to be determined. *In vivo* equivalents of liposomes to fuse with the macrophage membrane could include DNA packaged in extracellular vesicles for which a role has been suggested [34], but not yet confirmed.

In conclusion, here we show that cfDNA has a pathogenic role beyond being a marker of graft injury. cfDNA resulting from liver IRI was directly inflammatory in macrophage cultures and DNase-digestion greatly eliminated this effect. It was applied during NMP to actively reduce the level of cfDNA. Understanding the pathophysiologic role of cfDNA has significance in the development of treatment strategies to inhibit IRI, and to explore the tremendous potential for *ex vivo* treatment during liver NMP.

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 the Institutional Animal Care and Use Committee. The study was conducted in accordance with the local legislation and institutional requirements, and the NIH Guide for the Care and Use of Laboratory Animals.

AUTHOR CONTRIBUTIONS

MS, YW, CZ, YK, and HE-S contributed to data collection and methods. TH and JS contributed to data interpretation. YZ and TM contributed data interpretation and methods. Funding was acquired by DW who wrote the original draft of the report. MS and DW are responsible for final submission of the manuscript for publication and all authors approved the final version before submission. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

The authors(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2026.15340/full#supplementary-material

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LETTER TO THE EDITOR
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Exploring Tissue Expander Utility in Kidney Transplant Allograft Nephrectomy

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Keywords: allograft nephrectomy, end stage kidney disease, kidney transplantation, repeat transplant, tissue expander

Dear Editors,

Kidney transplantation remains the treatment of choice for patients with end-stage renal disease (ESRD), with 5-year graft survival rate of 77%–88% [1, 2]. As patient survival improves, retransplantation now represents a growing proportion of transplant activity. Allograft nephrectomy may be required prior to retransplantation to create space for the subsequent kidney allograft [3, 4]. The procedure is challenging due to dense scarring, distortion of surgical planes, and risk of injury to adjacent vascular, neural, and visceral structures [4]. When retransplantation is performed in the same iliac fossa, surgeons must re-enter a hostile operative field, increasing procedural complexity and risk.

We report a novel application of a breast tissue expander to preserve the iliac fossa following allograft nephrectomy in anticipation of staged kidney retransplantation.

A 37-year-old man with type-1 diabetes and ESRD previously underwent simultaneous kidney-pancreas transplantation, with the pancreas in the right iliac fossa and the renal allograft in the left. Following repeated infections, acute cellular rejection and progressive renal dysfunction, he returned to hemodialysis. After identifying a suitable living donor, we planned to extirpate the failed renal allograft and reimplant the living donor kidney back into the left iliac fossa. Recognizing the technical complexity of allograft nephrectomy and the challenges of reopening a re-scarred fossa for repeat transplantation, the surgical team proposed a novel approach: placement of a tissue expander in the resected fossa after graft removal to preserve space and hopefully limit further scar formation (**Figures 1A–C**). Following completion of the allograft nephrectomy, a breast tissue expander was soaked in iodine solution, filled with 200 mL of diluted tobramycin and placed into the left iliac fossa. The wound was closed in standard fashion. The patient recovered uneventfully and remained stable during the two-month interval prior to retransplantation.

At the time of living-donor transplantation, the expander was encountered within a well-formed pseudocapsule. Extraction was straightforward, and the capsule provided a smooth-walled cavity corresponding to the prior graft bed (**Figures 1D-F**). Notably, the external iliac vessels and bladder were situated outside the borders of the expander and pseudocapsule, necessitating careful posterior dissection through residual scar tissue to obtain access to the vasculature and bladder. The donor kidney was anastomosed to the external iliac vessels in standard fashion. Immediate graft perfusion and urine output were observed following clamp release. The ureteroneocystostomy was completed over a stent without complication. The patient demonstrated immediate graft function and was discharged on postoperative day seven with stable renal function.

Allograft nephrectomy may be required in cases of failed kidney allograft to create space for subsequent retransplantation, either as a staged procedure or performed simultaneously at the time of retransplantation [5]. The operation is often challenging due to scarring and inflammation around the failed graft, which can increase the risk of increased blood loss, injury to muscle, nerve,

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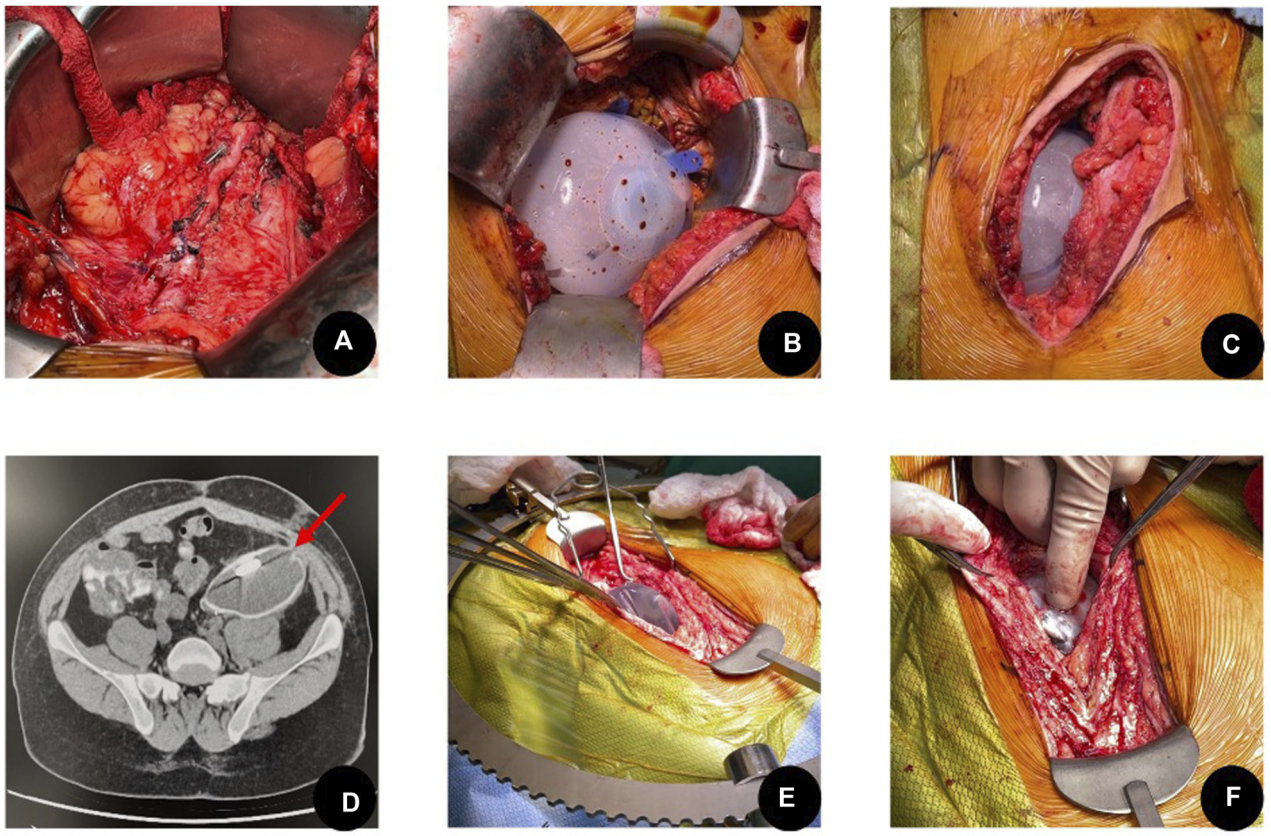

FIGURE 1 | Intraoperative images of the insertion and removal of a breast tissue expander in the left iliac fossa. **(A)** Completed allograft nephrectomy in the left iliac fossa, highlighting the pelvic vessels ready for repeat transplant procedure, **(B)** Insertion of the tissue expander and appropriate sizing with instillation fluid, **(C)** Closure of allograft nephrectomy wound around tissue expander, **(D)** Pre-transplant CT scan demonstrating the implantation of the tissue expander in the left iliac fossa. **(E,F)** Removal of the tissue expander during the retransplantation procedure, highlighting the pseudocapsule created and ease of extraction.

vasculature and intraabdominal structures [5]. To our knowledge, this is the first case report describing the use of a tissue expander to limit excessive scarring in the iliac fossa and facilitate subsequent dissection for repeat kidney transplantation on the same operative side. While the tissue expander improved access to the implant bed, the pseudocapsule complicated dissection of the pelvic vessels and bladder, which lay outside it.

The choice between simultaneous or staged nephrectomy during kidney retransplantation remains debated. Simultaneous explant and retransplantation has the advantage of minimizing the number of surgeries and avoiding the anephric state [6]. However, this procedure can result in prolonged anesthesia time, increased risk of intraoperative complications and potentially poor hemodynamic states to support kidney retransplantation [6]. The practice at our center is to stage an allograft nephrectomy, when required, two to three months before repeat transplantation. We found that implantation of the tissue expander in the excised space can potentially redirect scar formation to the periphery of the operative field preserving iliac fossa space. Prolonged implantation of tissue expanders is not a new concept in breast reconstructive surgery, as they are often left *in situ* for between 2–12 months [7]. However, longer periods of implantation are associated with increased scar tissue formation and risk of tissue expander rupture [7]. Immunocompromised patients are at a higher risk of post-operative infection after tissue expander implantation, and in our center all patients considered for retransplantation remain on their maintenance immunosuppression following allograft nephrectomy. In this case, we thoroughly bathed the tissue expander with iodine solution, filled it with diluted tobramycin and gave IV prophylactic meropenem and micafungin at the time of surgery to minimize the risk of bacterial and fungal infection throughout this patient's course given their past history of infection.

The formation of the pseudocapsule is consistent with previous studies on breast tissue expanders, where capsular contracture is a well-documented phenomenon [8]. The presence of scar tissue around the vessels and bladder underscores the complexity of the fibrotic response. Strategies aimed at reducing pseudocapsule formation, such as antifibrotic agents, may help mitigate scarring and facilitate future dissection, potentially increasing the appeal of tissue expander use. However,

the challenges posed by the pseudocapsule were significant enough that our group will likely avoid using tissue expanders in this patient population unless effective strategies to minimize pseudocapsular formation are available.

This case demonstrates a novel application of a tissue expander to preserve iliac fossa space in kidney retransplantation. Although limited to a single case, the technique was feasible and safe, resulting in successful retransplantation with immediate graft function. The pseudocapsule, while helpful in maintaining iliac fossa space, complicated dissection of the iliac vessels and bladder, limiting the broader applicability of this approach at present.

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 University British Columbia Research Ethic Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. 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

CN and DH contributed to the study design, data collection, manuscript writing and review. AH and AG contributed to manuscript writing and literature review. All authors contributed to the article and approved the submitted version.

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Mesoamerican nephropathy as a potential contributor to chronic allograft dysfunction in tropical settings

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Dear Editors,

Chronic kidney disease (CKD) represents a major and growing global health burden, affecting well over 850 million individuals worldwide and contributing substantially to morbidity and mortality [1, 2]. Despite advances in diagnostic approaches, a significant proportion of end-stage renal disease (ESRD) cases remains classified as of unknown etiology, particularly in low-resource and tropical settings. This diagnostic uncertainty is especially problematic in the context of kidney transplantation, where unidentified causal factors may persist after transplantation and compromise long-term graft survival.

The under-recognition of environmental and occupational nephropathies may exacerbate this issue in tropical regions like French Guiana. Among these, Mesoamerican nephropathy (MeN) emerges as a plausible but largely overlooked contributor. Initially described in Central American agricultural workers, MeN predominantly affects young individuals without diabetes or hypertension and is characterized by chronic tubulointerstitial injury, low-grade proteinuria, and progressive decline in renal function [3]. Its pathogenesis is complex and multifactorial, involving repeated exposure to heat stress, chronic dehydration, environmental nephrotoxins such as pesticides and heavy metals, and recurrent subclinical acute kidney injury [4, 5] (Table 1).

Although environmental determinants are central to current models, Mesoamerican nephropathy is increasingly recognized as a multifactorial condition in which individual susceptibility may also play a role. Emerging evidence suggests that genetic and epigenetic factors may modulate vulnerability to renal injury and influence disease progression [6]. Candidate genes involved in endothelial function and vascular regulation, such as *APOE* and *NOS3*, have been proposed as potential contributors to interindividual variability in susceptibility. In addition, epigenetic mechanisms, including DNA methylation changes—particularly in regions of chromosome 7—may influence gene expression in response to chronic environmental stressors such as heat exposure and dehydration [6].

The environmental and occupational conditions observed in French Guiana—including high ambient temperatures, persistent humidity, and physically demanding labor in agricultural, construction, or forest-related activities—closely resemble those described in endemic regions of MeN. These similarities strongly support the hypothesis that a comparable entity, sometimes referred to as "Meso-Amazonian nephropathy," may be present but underdiagnosed in this setting. Epidemiological data indicate that a non-

TABLE 1 Pathophysiological mechanisms of Mesoamerican nephropathy and potential impact on kidney allograft function.

Mechanism	Renal effects	Potential impact on kidney allograft
Recurrent dehydration and renal hypoperfusion	Tubular ischemia, reduced GFR	Repeated subclinical injury leading to chronic graft dysfunction
Recurrent subclinical acute kidney injury	Progressive tubulointerstitial fibrosis	Accelerated decline in graft function
Heat stress	Tubular inflammation, cellular stress	Increased susceptibility of graft to environmental injury
Environmental nephrotoxins (pesticides, heavy metals)	Direct tubular toxicity	Synergistic damage with immunosuppressive nephrotoxicity
Oxidative stress	Cellular damage, fibrogenesis	Promotion of chronic allograft fibrosis
Chronic inflammation	Interstitial remodeling and fibrosis	Long-term structural deterioration of graft
Hyperosmolarity and electrolyte disturbances	Tubular stress and injury	Functional instability of the graft

negligible proportion of ESRD cases in French Guiana remain of indeterminate origin, raising the possibility that unrecognized MeN contributes to this burden [7].

This hypothesis has important implications for kidney transplantation. When the etiology of native kidney disease is unknown, transplant recipients may remain exposed to the same pathogenic factors that led to initial renal failure. In the case of MeN, continued exposure to heat stress, inadequate hydration, and physically demanding occupational conditions may directly affect the transplanted kidney. Such exposure may result in repeated episodes of subclinical renal hypoperfusion and injury, ultimately leading to progressive functional decline despite appropriate immunosuppressive therapy and standard clinical follow-up.

In this context, genetic susceptibility may also influence transplant outcomes. Variability in donor and recipient genetic profiles has been shown to affect graft survival through mechanisms related to immune response, vascular integrity, and susceptibility to injury [8]. While polygenic risk scores are currently primarily used for highly heritable renal diseases, their potential extension to multifactorial conditions such as MeN could provide a more refined framework for risk stratification. In particular, incorporating genetic susceptibility into donor evaluation may, in the future, contribute to improved donor selection and better anticipation of graft vulnerability. Although the implementation of such approaches remains challenging in resource-limited tropical settings due to economic and infrastructural constraints, they may represent a valuable long-term strategy for optimizing transplant outcomes in high-risk populations, particularly by enhancing the understanding of genetic factors that influence transplant success.

The present letter highlights MeN as a potential non-immunological and underappreciated determinant of chronic allograft dysfunction in tropical settings. Unlike traditional causes of graft failure, such as acute or chronic rejection, immunological incompatibility, or drug-related nephrotoxicity, this mechanism is primarily environmental and therefore theoretically preventable. However, it remains largely absent from current transplant evaluation frameworks and follow-up strategies, which rarely incorporate detailed environmental or occupational risk assessments, potentially leading to missed opportunities for improving graft outcomes in tropical settings, such as the failure to identify and mitigate specific environmental factors that could contribute to chronic allograft dysfunction (Figure 1).

In French Guiana and similar tropical territories, this issue is further compounded by a range of contextual challenges. Transplant recipients are exposed to a high burden of infectious diseases, including tuberculosis, HIV, and endemic parasitic infections, which may reactivate or worsen under immunosuppressive therapy [9]. In addition, healthcare delivery is often constrained by geographical remoteness, limited access to specialized care, and reduced availability of diagnostic tools, which may delay the detection and management of graft dysfunction [9]. Socioeconomic factors also play a critical role, as many patients face financial pressures that necessitate early return to physically demanding work after transplantation, thereby increasing exposure to heat stress and dehydration [10]. These factors may interact synergistically with MeN-related mechanisms to accelerate graft injury and functional decline, leading to poorer long-term outcomes for transplant patients, particularly in those who are already vulnerable due to their socioeconomic status and limited access to healthcare resources.

Importantly, the relevance of Mesoamerican nephropathy may extend beyond tropical regions to the European context. Increasing migration from Central and South America, as well as from other tropical regions exposed to extreme occupational heat stress, may result in more kidney transplant candidates or recipients with unrecognized environmentally mediated kidney injury in European healthcare systems. In parallel, climate change is contributing to more frequent and intense heat waves across Europe, particularly in Southern regions, potentially recreating environmental conditions similar to those implicated in Mesoamerican nephropathy. These converging phenomena underscore the need for transplant programs in Europe to incorporate environmental and occupational exposure histories into pre- and post-transplant risk assessment strategies.

Recognizing MeN as a potential contributor to graft outcomes calls for a more comprehensive and context-adapted approach to transplant care. Pre-transplant evaluation should include a detailed assessment of occupational history, environmental exposures, and lifestyle factors, particularly in patients with ESRD of unknown etiology. Identifying individuals at risk could allow for targeted counseling and implementation of preventive strategies. Post-transplant management should emphasize strict hydration, avoidance of prolonged heat exposure, and adaptation of occupational activities whenever feasible. Patient education is essential, as awareness of environmental risk factors remains limited in many affected populations, which can lead to

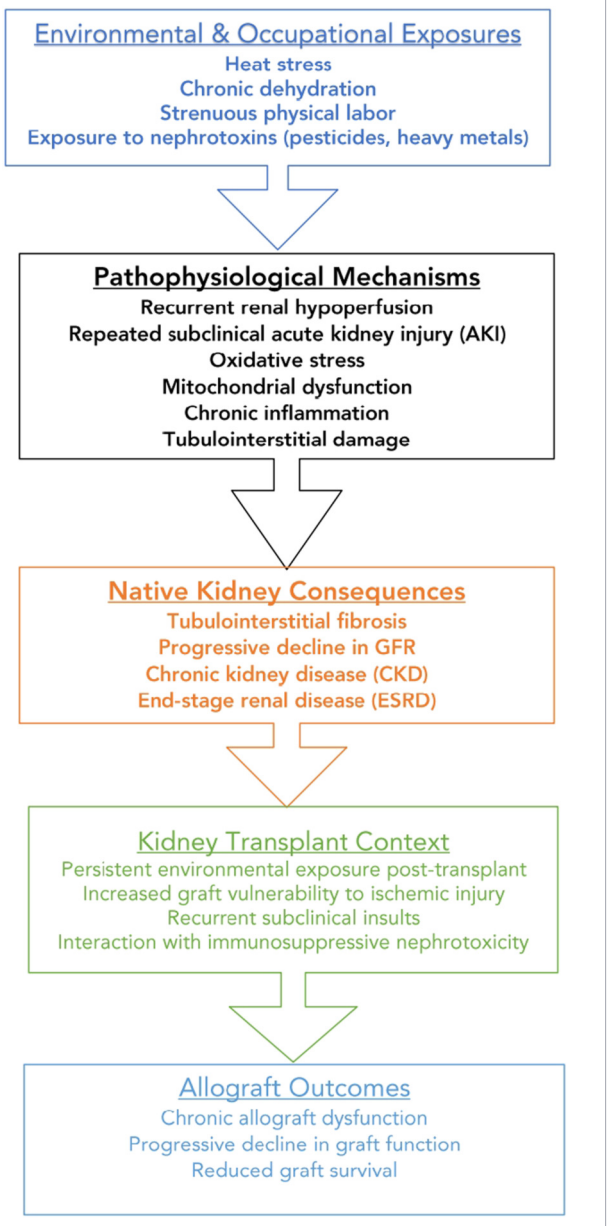

FIGURE 1
Conceptual framework of environmental and pathophysiological mechanisms linking Mesoamerican nephropathy to native and kidney allograft dysfunction.

increased complications and poorer health outcomes if not addressed through effective communication and resources.

From a pathophysiological standpoint, the mechanisms underlying MeN are relevant to transplanted kidneys, which may be particularly vulnerable to repeated subclinical insults. These mechanisms include recurrent renal hypoperfusion due to dehydration, cumulative tubular injury, oxidative stress, mitochondrial dysfunction, and progressive interstitial fibrosis [4]. A concise summary of these mechanisms and their potential impact on graft function is provided below.

In addition to conventional clinical approaches, emerging technologies such as digital health tools and artificial intelligence may offer new opportunities to improve risk stratification and follow-up in resource-limited settings. By integrating environmental, occupational, and biological data, predictive models could help identify patients at increased risk of graft dysfunction and enable earlier and more personalized interventions [11]. Remote monitoring strategies may also help overcome geographical barriers and improve continuity of care.

Considering MeN in the evaluation of ESRD etiology and in post-transplant follow-up represents an important step toward more personalized and context-specific care in tropical environments. Beyond its clinical implications, this perspective emphasizes the necessity to broaden current paradigms of graft dysfunction by incorporating environmental determinants, such as local disease prevalence and socio-economic factors, into routine practice. Additional epidemiological, clinical, and translational studies are required to validate the existence and assess the impact of MeN in French Guiana and analogous areas, as well as to formulate targeted preventive and therapeutic strategies [12].

Raising awareness of this potential entity among transplant clinicians and integrating environmental risk assessment into clinical practice may ultimately contribute to improved graft survival and better long-term outcomes for patients living in tropical settings.

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

AM conceived the study, conducted the literature review, drafted the manuscript, and approved the final version for submission.

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LETTER TO THE EDITOR
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Totally Video-Assisted Thoracoscopic Surgery for Lung Transplantation: A Case Series

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Dear Editors,

Lung transplantation remains the only effective treatment for end-stage lung disease. Traditionally, the Clam-Shell incision is the standard approach for bilateral sequential lung transplantation [1]; however, it requires a long incision and sternotomy, resulting in significant trauma [2] and extended recovery [3]. Accordingly, less invasive strategies have been explored, including bilateral anterolateral incisions without sternotomy [3], thoracoscopic-assisted techniques [4, 5], and robotic-assisted lung transplantation [6, 7]. Notably, most published experiences to date describe hybrid minimally invasive approaches, rather than totally video-assisted thoracoscopic surgery (VATS) as a standalone technique for lung transplantation.

In this context, we report our initial experience with totally VATS lung transplantation without rib retraction. Five consecutive patients with end-stage lung disease underwent this procedure at West China Hospital, Sichuan University, since December 2023. All operations were performed under VA-ECMO support. Baseline and clinical characteristics are summarized in **Figure 1F**.

In our practice, we generally prioritize right-sided lung transplantation first. If the first-side procedure is excessively time-consuming, we convert to open thoracotomy for the contralateral side to avoid prolonged cold ischemia time. The surgical technique of totally VATS right lung transplantation is illustrated using Patient 1 as a representative case.

Patient was positioned supine with shoulders and back elevated to the nipple plane, arms abducted. A three-port incision design was used: an 8 cm main incision in the 4th intercostal space, a camera port in the 6th along the anterior axillary line, and an accessory port in the 6th along the midclavicular line (**Figure 1A**). The 8 cm incision was unnecessary for totally VATS resection and transplantation but required for donor lung implantation. The accessory and camera ports were interchangeable, with both used for chest tube placement.

The right pneumonectomy was performed using a single-direction thoracoscopic technique [8], proceeding anteriorly to posteriorly. The anterior apical branch of the pulmonary artery was addressed first, followed by the superior pulmonary vein, main pulmonary artery, inferior pulmonary vein, and right main bronchus. The vessels were stapled with an ETHICON ECR45W stapler. The main bronchus was dissected with an ultrasonic scalpel and scissors, then trimmed before anastomosis using scissors. A longitudinal pericardial incision anterior to the right phrenic nerve exposed the hilum and facilitated anastomoses. The incision was sutured and was brought out near the sternal edge of the incision, retracting the pericardium toward the anterior chest wall and suspending it. Then, an oval forceps was placed around the pericardial fold near the pulmonary veins, and the pericardium around the incision and veins was gradually separated to expose the left atrium.

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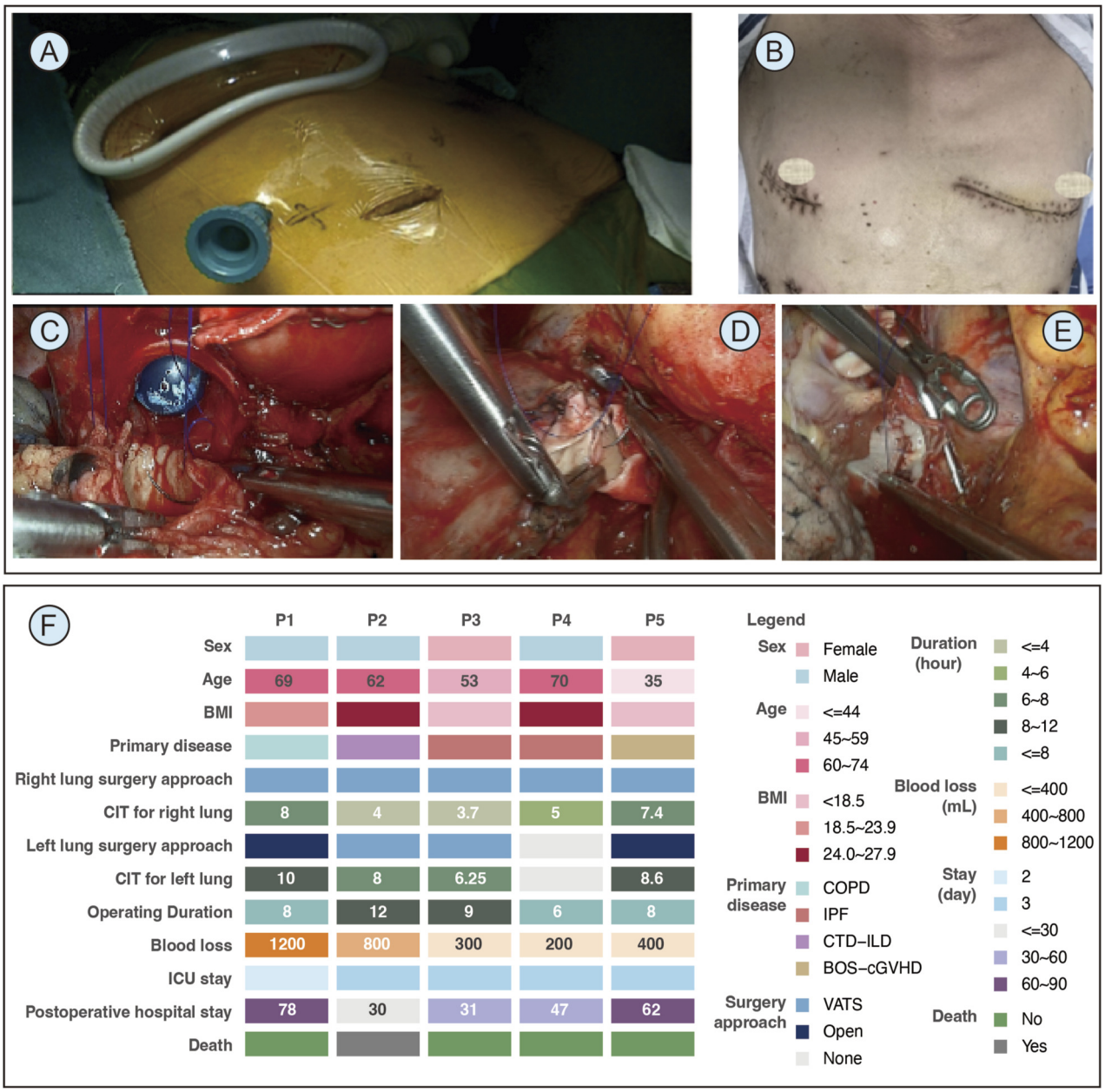

FIGURE 1 | Surgical steps and patient outcomes of the totally video-assisted thoracoscopic surgery for lung transplantation. **(A)** The three-port incision design for totally VATS right lung transplantation. **(B)** Postoperative wound healing at the Patient 1 incision site; the right-side shows thoracoscopic incisions, while the left side shows open surgical incisions. **(C)** Right bronchial anastomosis. **(D)** Right pulmonary artery anastomosis. **(E)** Left atrial cuff anastomosis performed on the right side. **(F)** Waffle plot depicting patient characteristics as well as perioperative and long-term outcomes. Abbreviation: ICU, intensive care unit; CIT, cold ischemia time; BMI, body mass index; COPD, chronic obstructive pulmonary disease; IPF, idiopathic pulmonary fibrosis; CTD-ILD, connective tissue disease–related interstitial lung disease; BOS-cGVHD, bronchiolitis obliterans syndrome related to chronic graft-versus-host disease; VATS, video-assisted thoracoscopic surgery.

The donor lung was inserted through the main incision, with an oval forceps through the accessory port to retract the apex and position the lung optimally. Anastomosis was performed in the same order as open surgery (**Figures 1C–E**). Pulmonary artery occlusion was achieved with a releasable Bulldog clamp (Aesculap, Inc., Center Valley, PA, USA) inserted through the accessory port. Left atrial occlusion was performed using an auricular appendage clamp. All anastomoses were performed using Prolene sutures with a continuous technique. The procedure began at the dorsocranial side, progressing downward to totally three-quarters of the circumference, then returning to finish the final quarter. Knotting was performed at

the ventral midpoint. Suture traction was assisted using atraumatic forceps through the main or accessory ports. The anastomosis was primarily done under thoracoscopic guidance, with direct visualization via the main incision when necessary.

Perioperative and long-term clinical outcomes are detailed in **Figure 1F**. All cases maintained acceptable cold ischemia times and operative durations. No intraoperative emergency was observed. For postoperative complications, Patient 1 developed infections with multidrug-resistant *Pseudomonas aeruginosa*, *Candida* albicans, and *Corynebacterium striatum* 2 weeks postoperatively, requiring prolonged antimicrobial treatment, and was discharged on POD78, fully recovered and off supplemental oxygen. For long-term outcomes, Patient 2 died of gastrointestinal hemorrhage 6 months postoperatively, while the remaining recipients achieved successful recovery.

We preliminarily evaluated the potential benefits of totally VATS lung transplantation by comparing perioperative outcomes between the conventional open approach (n = 101) and totally VATS surgery (n = 5). Postoperative length of hospital stay was comparable between the open and VATS groups (41.70 ± 30.33 vs. 48.60 ± 20.22 days). No anastomotic fistula or stenosis was observed in the VATS group, whereas the rates in the open group were 3.96% and 2.97%, respectively. These observations should be interpreted with caution given the limited sample size.

Additionally, intraoperative parameters were compared within Patient 1, who underwent totally VATS transplantation on the right side and an anterolateral open approach on the left side (**Figure 1B**). Operative time was shorter for the VATS side (231 vs. 256 min), with reduced blood loss (400 vs. 800 mL). Postoperative incision-related pain, assessed by VAS, was also lower on the VATS side upon awakening (4 vs. 6).

Unlike previous "minimally invasive lung transplant" approaches involving mini thoracotomy [4, 5], the technique described herein employs a totally thoracoscopic method without rib spreading, minimizing chest wall disruption and promoting faster recovery.

Successful completion of thoracoscopic procedures relies on several key factors. First, a supine position is required for thoracoscopic pneumonectomy, with precise anatomical identification essential, particularly when freeing the left atrium to avoid injury. For anastomosis, the limited instrument angle due to the unexpanded incision may necessitate combining thoracoscopic guidance with direct visualization through the incision.

Patients with a large thoracic cavity, wide intercostal spaces, and a relatively small heart are generally more suitable candidates for VATS lung transplantation. In recipients with chronic obstructive pulmonary disease (COPD), anatomical characteristics associated with hyperinflated lungs may facilitate thoracoscopic manipulation. However, the presence of dense pleural adhesions can significantly limit the feasibility of a totally thoracoscopic approach.

Our findings suggest that VATS lung transplantation does not substantially prolong cold ischemia time or operative duration compared with conventional open surgery. Recent advances in donor lung preservation, such as 10 °C cold storage, or 4 °C–8 °C portable device for static hypothermic preservation may further enhance the feasibility of this minimally invasive approach [9], potentially enabling its application in more complex cases. Finally, Prospective comparative studies and long-term follow-up are needed to confirm its advantages over conventional thoracotomy.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The requirement for ethical approval was waived by the Ethics Committee of Biomedical Research, West China Hospital, Sichuan University, for this retrospective case series, as it did not meet the definition of "human subject research" according to institutional policies. All patient data were anonymized to protect privacy, and the study posed minimal risk to participants. Written informed consent was obtained from all participants for study participation and for the publication of any potentially identifiable data or images.

AUTHOR CONTRIBUTIONS

(I) Conception and design: LL and QP; (II) Administrative support: QP; (III) Provision of study materials or patients: QP, XZ, DT, CG, QY, JM, and LM; (IV) Collection and assembly of data: JZ, XT, ND, and JC; (V) Interpretation of results and derivation of insights: QP, XZ, JH, JZ, and YH; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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CONFLICT OF INTEREST

The authors(s) declared that this work 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 author(s) declared that generative AI was used in the creation of this manuscript. During the preparation of this work, the authors used ChatGPT-4, to assist in improving the readability and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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