SCIENTIFIC Publishing Limited

Trends in Immunotherapy

https://ojs.ukscip.com/index.php/ti

Article

Morphofunctional and Immuno-Inflammatory Assessment of Renal Allografts from Asystolic Donors: A Multicriteria Model Comparing Early and Extended Warm Ischemia

Zhanybek Ashimov [1,2], Kanat Mamakeev [1,*], Isabek Ashimov [2], Batyrkhan Niyazov [2] and Taalai Akmatov [1]

[1] Department of Scientific, National Surgical Center named after M.M. Mamakeev of the Ministry of Health of the Kyrgyz Republic, Bishkek 720000, Kyrgyzstan

[2] Department of General Surgery, Kyrgyz State Medical Institute of Retraining/Advanced Training named after S.B. Daniyarov, Bishkek 720017, Kyrgyzstan

[*] Correspondence: mamakeevkanatnsc@gmail.com

Received: 27 April 2026; **Revised:** 18 May 2026; **Accepted:** 11 June 2026; **Published:** 18 June 2026

Abstract: Global organ shortages have intensified interest in donation after circulatory death (DCD). However, DCD kidneys are affected by warm ischemia, which amplifies ischemia-reperfusion injury via a reactive oxygen species-damage-associated molecular pattern-cytokine-endothelial cascade, increasing the risk of microvascular dysfunction and delayed graft function. This study developed an objective postmortem *ex vivo* model to compare asystolic early donors/rapid death (AED; warm ischemia ≤30 min) and asystolic extended/delayed donors (ASED; 60–120 min) using integrated morphofunctional, biochemical, and immuno-inflammatory indicators during hypothermic perfusion. In an *ex vivo* design, 145 DCD renal allografts (71 AED, 74 ASED) underwent standardized hypothermic rehabilitation, with evaluations at 30, 60, and 120 min. Oxidative stress was quantified by lipid peroxidation and antioxidant capacity, in addition to histopathology (tubular, endothelial, podocyte, nuclear injury) and composite indices for cytokine activation, endothelial injury, and immune activation. A multicriteria scoring algorithm was used to categorize rehabilitation effectiveness, and group differences were assessed using comparative tests. These findings support a dynamic, multicriteria viability framework, indicating that ASED kidneys are at a higher risk yet potentially salvageable under comprehensive perfusion rehabilitation, warranting prospective transplantation-linked validation. Lipid peroxidation increased over time, rising more in the ASED group, whereas glutathione showed limited compensatory change. The ASED kidneys demonstrated greater tubular degeneration, endothelial swelling, podocyte injury, and nuclear alterations, with higher oxidative, inflammatory, and immune activation scores. Despite the higher biological burden, positive integrated outcomes remained frequent (AED 89.2% vs. ASED 84.5%; RR: 0.982; OR: 1.07–1.16), and morphology-function correlations were strong (AED $r = 1.0$; ASED $r = 0.9$). These findings support a dynamic, multicriteria viability framework, indicating that ASED kidneys are at a higher risk yet potentially salvageable under comprehensive perfusion rehabilitation, warranting prospective transplantation-linked validation.

Keywords: Donation after Circulatory Death; Ischemia-Reperfusion Injury; Delayed Graft Function; Hypothermic Machine Perfusion; Normothermic Machine Perfusion; Reactive Oxygen Species; Toll-like Receptors

1. Introduction

Worldwide, the need for organs suitable for transplantation exceeds the supply, leading to extended waiting periods and deaths among patients awaiting transplantation. To address this disparity, donation after circulatory death (DCD), or asystolic donation, has become key to increasing the number of deceased donors and reducing waiting lists [1, 2]. This challenge is particularly significant in India, where the demand for transplants greatly exceeds organ availability [1].

The main drawback of DCD organs is warm ischemia between circulation cessation and organ preservation. This ischemic period worsens ischemia-reperfusion injury, making DCD grafts more susceptible than donation after brain death, especially in kidney transplants [3,4]. This susceptibility manifests as increased delayed graft function, which has been linked to poorer long-term outcomes [5, 6]. Ischemic injury increases the risk of rejection and decreases graft survival [5]. While DCD donation provides a vital solution to organ shortages, success depends on minimizing ischemic injury and improving preservation techniques to enhance transplant outcomes [4,7].

Ischemia-reperfusion injury (IRI) occurs when oxygen-rich blood returns to oxygen-deprived tissue, transforming an ischemic event into an oxidative and immune-related injury. Reactive oxygen species (ROS) during reperfusion trigger lipid peroxidation (LPO), destabilize mitochondrial membranes, and worsen mitochondrial dysfunction, leading to cell death and the release of damage-associated molecular patterns (DAMPs), such as mitochondrial products. These products activate innate immune sensors, including Toll-like Receptors (TLRs)-dependent pathways and inflammasome signaling [8–10]. In kidney transplantation, early reperfusion causes local IL-6 release, whereas extended warm ischemia increases interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), complement component 5a (C5a) receptor expression, and neutrophil infiltration [11, 12]. The complement system amplifies this response: C3, C3a, and C5a recruit leukocytes, cause microvascular injury, and spread inflammation, whereas complement inhibition can improve graft perfusion [13, 14]. Neutrophils worsen damage through adhesion, transmigration, and extracellular trap formation, leading to increased renal injury [13,15]. Endothelial dysfunction and capillary blockage impair microcirculatory flow and create conditions for delayed graft function in DCD kidneys [16]. Thus, IRI represents a coordinated ROS–DAMP– TLRs–complement–neutrophil axis that affects graft outcomes [14,17].

Donation after circulatory death (DCD), also known as asystolic donation, involves the retrieval of organs following an irreversible circulatory halt. Donors were categorized based on asystolic warm ischemia into asystolic early donors/rapid death (AEDs) [≤30 min] and asystolic extended/delayed donors (ASEDs) [60–120 min]. The 30-min limit is applied because extended warm ischemia is linked to increased ischemia-reperfusion injury, delayed graft function, and damage to the endothelium/microvasculature in kidney transplantation [4, 17, 18]. However, this threshold is not definitive. Evidence shows that extended warm ischemia can impair early graft function while allowing satisfactory long-term outcomes when donor quality and preservation conditions are optimized [4, 19]. The ASED remains contentious due to the heightened biological risk versus the potential clinical benefit. Centers remain cautious, as extended ischemia requires greater expertise and advanced preservation techniques, such as normothermic regional perfusion or hypothermic machine perfusion, to mitigate damage [20–23]. Regulatory differences affect practices in different countries. Italian DCD programs require a 20-min no-touch period, while other systems have different criteria, showing how policies influence donor utilization [20,24]. ASED kidneys may be viable when selection and preservation are optimized; thus, prolonged asystolic exposure indicates a higher risk but does not preclude successful transplantation [4,22,23].

Emerging preservation technologies are biologically active interventions that alter the inflammatory and immunological states of grafts before implantation. Hypothermic machine perfusion (HMP) benefits kidney transplantation by decreasing the incidence of delayed graft function compared to static cold storage. Oxygenated end-hypothermic perfusion aids in ATP restoration and minimizes ischemic injury [25, 26]. In liver transplantation, hypothermic oxygenated perfusion before implantation reduces reperfusion injury and perioperative metabolic disturbances, indicating that oxygen delivery during cold preservation mitigates early graft injury [27].

Normothermic machine perfusion (NMP) enhances organ preservation by maintaining body-like conditions and allowing functional evaluation during preservation. In donor livers, NMP modifies graft immune characteristics, with changes in leukocyte populations and cytokines, suggesting altered inflammatory signaling [28]. Mechanistically, NMP reduces hepatic IRI by preventing Cold-Inducible RNA-binding Protein (CIRP)-mediated oxidative

stress, mitochondrial division, and cell death, linking perfusion to suppressed danger signal release and immune activation [29]. In kidney preservation, normothermic *ex vivo* perfusion serves to preserve and evaluate injury, with initial human trials showing safety and potential for enhanced graft function [30,31].

These technologies can diminish cytokine storm-like amplification, adjust oxidative stress, and prevent immune priming before reperfusion. This is significant because IRI triggers complement activation, immune signaling, and endothelial inflammation, contributing to dysfunction and an increased risk of alloimmune response. In discarded kidneys under NMP, complement activation products correlated with pro-inflammatory cytokines such as IL-6, IL-8, and TNF-α, showing that the perfusion environment affects inflammatory signaling [32].

Adjunctive methods enhance the translational potential of machine perfusion. Combining hypothermic oxygenated perfusion with normothermic perfusion shows promise, integrating metabolic stabilization with functional recovery and reducing oxidative damage markers compared with normothermic perfusion alone [33]. Machine perfusion serves as a platform for pharmacological, genetic, and cell-based interventions. Reviews have highlighted the administration of therapeutic agents during perfusion, such as mesenchymal stem cells and anti-inflammatory interventions, to enhance reconditioning while minimizing oxidative damage [34,35]. These strategies suggest that preservation technologies could decrease cytokine amplification, limit oxidative stress, inhibit complement activation, and prevent immune priming, thereby enhancing graft immunologic quality before implantation.

Recent multimodal toxicology studies show that combining structural, biochemical, and imaging endpoints improves injury assessment and supports more objective viability frameworks [36,37]. This gap is significant because IRI involves endothelial damage, tubular injury, inflammatory signaling, and delayed graft dysfunction. Research shows that IRI triggers immune pathways, including cytokine release and complement activation, linking early damage to outcomes. Current graft quality assessments rely on donor values, risk scores, inspection, biopsy, and perfusion parameters, which cannot fully capture the complexity of the injury. The relationship between morphological changes, metabolic disturbances, and immune activation remains unclear. While extended warm ischemia correlates with early injury, it does not always preclude acceptable outcomes.

This study aimed to create a model to evaluate AED and ASED based on the morphofunctional and immunoinflammatory potential of renal allografts postmortem renal allograft assessment. By integrating morphological, biochemical, and risk-based parameters, this study aimed to provide an objective assessment framework. This approach reflects that renal viability can be evaluated using combined markers during machine perfusion. Studies have shown that perfusion resistance, oxygenation status, and mitochondrial preservation are related to graft quality. A combined morphofunctional-immunologic model may improve viability prediction and promote the rational use of asystolic donor kidneys.

2. Methods

This study involved an *ex vivo* postmortem examination of renal allografts sourced from donors after circulatory death (DCD). This study aimed to assess the morphofunctional, biochemical, and immunoinflammatory properties of kidneys exposed to varying periods of warm ischemia, followed by hypothermic perfusion-based rehabilitation. Animal testing, computational modeling, and transplantation into recipients were excluded. This study followed the Declaration of Helsinki principles (2013) with the Bioethics Committee of the National Surgical Center of the Ministry of Health of the Kyrgyz Republic approval (Protocol No. 3, dated June 13, 2025). All participants respondents or guardians provided written informed consent.

The analysis included 145 renal allografts, with 71 grafts identified as asystolic early donors (AED; warm ischemia ≤30 min) and 74 grafts identified as asystolic extended/delayed donors (ASED; warm ischemia 60–120 min). Each graft underwent standardized *ex vivo* rehabilitation and evaluation. Morphological, ultrastructural, biochemical, oxidative stress, endothelial damage, and integrated morphofunctional analyses were conducted according to a predefined multicriteria evaluation framework.

To clarify, the term "postmortem renal allograft" refers to kidneys retrieved after circulatory death and assessed during *ex vivo* preservation and rehabilitation. No grafts were transplanted in this study, and no clinical outcome data related to the recipients were gathered.

Postmortem DCD renal allografts were included if they had recorded warm ischemia duration, anatomical integrity, and suitability for perfusion-based evaluations. Grafts with significant mechanical damage, irreversible structural damage preventing evaluation, or incomplete morphologic or biochemical assessment data were ex-

cluded.

Renal allografts underwent *ex vivo* cold protective rehabilitation using hypothermic perfusion-based preservation to reduce IRI. Functional and biochemical evaluations were conducted at 30, 60, and 120 min. During rehabilitation, oxidative stress markers, antioxidant capacity indicators, morphological features, and surrogate immunoinflammatory indices were assessed. The protocol evaluates graft viability, injury progression, and the links between structural changes, metabolic disruptions, and combined morphofunctional outcomes. Morphological integrity and metabolic condition were integrated into the multicriteria scoring system to assess overall rehabilitation effectiveness.

Oxidative stress and LPO were quantified using biochemical markers, such as malondialdehyde (MDA) and diene conjugates (DC). Antioxidant capacity was assessed by measuring the levels of reduced glutathione (GSH). These markers were analyzed at 30, 60, and 120 min and expressed in relative units or $nmol \cdot mL^{-1}$. The balance between pro-oxidant (MDA, DC, and ROS proxies) and antioxidant (GSH) systems characterizes oxidative injury and compensatory responses. Surrogate markers of immune activation, including cytokine activation indices (IL-6 proxy) and endothelial injury scores, reflect immunoinflammatory involvement.

Histopathological analysis was used to evaluate structural damage in nephron components, including tubular degeneration, endothelial swelling, podocyte injury, and nuclear alterations. Tissue samples were examined using light microscopy and ultrastructural examination to identify changes. Morphological integrity was graded using a standardized scoring system: optimal (preserved structure), suboptimal (mild alterations), and poor (severe damage), with scores of 5, 3, and 1, respectively.

The multicriteria assessment framework included distinct analytical areas, such as morphological integrity, metabolic dysfunction, rehabilitation effectiveness, and morphofunctional compliance. The results will show unique outcomes based on various scoring criteria, not repeated datasets. Morphology, metabolism, and rehabilitation parameters were examined independently to delineate the aspects of graft injury and rehabilitation potential.

This study used both quantitative (IN, RR, FR, FF, OR, points, and %) and qualitative criteria related to morphology and metabolism to evaluate the rehabilitation potential of various technologies. We believe that the proposed evaluation algorithm, along with the relevant criteria and indicators (both optimal and suboptimal) for assessing the effectiveness of various postmortem renal allograft assessment rehabilitation technologies, with their standardized gradation in both qualitative and quantitative terms, enables us to comprehensively assess the resource potential of the ASED (**Table 1**). The outcome of the postmortem renal allograft assessment rehabilitation effectiveness evaluation was categorized as follows: 1) Satisfactory (4–5 points); 2) Weak (2–3 points); and 3) Negative (0–1 point). Therefore, this evaluation method assumes a standardized gradation for each criterion in quantitative terms. The assessment results, expressed in points, were totaled (Σ). In the final phase, the degree of alignment of the ASED and AED potentials was determined as follows: 1) potentials fully aligned—5 points; 2) potentials partially aligned—3 points; 3) potentials did not align—0–1 point.

Table 1. Criteria for assessing the effectiveness of cold protective rehabilitation of postmortem renal allograft assessment.

Domain	Criteria	Description	Score
Morphological integrity	Optimal	Preserved morpho- and ultrastructure	5
	Suboptimal	Mild structural alterations	3
	Poor	Severe structural damage	1
Metabolic status	Optimal	Minimal metabolic disturbances	5
	Moderate	Moderate metabolic alterations	3
	Severe	Pronounced metabolic dysfunction	1

This study introduces the ASED category to create a new group of potential ex-mortuary cadaveric donors. It is important to note that this multicriteria approach was developed under the assumption of limited information regarding the nature of morphofunctional abnormalities in the ex-mortuary cadaveric parenchyma and their changes when rehabilitation technologies are applied. We pioneered the use of a multi-criteria approach to identify asystolic donor types, such as AED and ASED, enhancing our understanding of the constraints and opportunities in balancing theoretical precision with the practical application of simulated reality. To assess the efficacy of rehabilitation, the results were arranged in a four-field contingency table (**Table 2**). Grafts were categorized by donor type into

the AED group (asystolic early donors; warm ischemia ≤ 30 min) and the ASED group (asystolic extended/delayed donors; warm ischemia 60–120 min). Rehabilitation outcomes were compared between the groups. After calculating the totals, the incidence (IN), relative risk (RR), attributable risk (AR), attributable fraction (AF), and odds ratio (OR) were computed to assess the differences in effectiveness and morphofunctional outcomes between AED and ASED allografts.

Table 2. Correlation between outcomes of rehabilitation efficacy and morphofunctional compliance scores in AED and ASED kidney transplants based on multicriteria assessment.

Group	Positive Outcome (+)	Negative Outcome (−)	Total
AED group	a	b	a + b
ASED group	c	d	c + d
Total	a + c	b + d	A + b + c + d = n

Note: AEDs = Asystolic early donors/rapid death; ASEDs = Asystolic extended/delayed donors; Incidence (IN) = a/(a + b), c/(c + d); RR = Relative Risk; AR = Attributable Risk; AF = Attributable Fraction; OR = Odds Ratio.

To define the immuno-inflammatory characteristics of kidney transplants, surrogate and integrated markers of innate immune activation were analyzed post-mortem. Recognizing IRI as a key factor in immune responses, this study focused on the ROS–DAMP–cytokine–endothelial axis as a graft immunogenicity indicator.

ROS production was indirectly evaluated using LPO markers, such as malondialdehyde (MDA) and diene conjugates (DC), indicating oxidative membrane damage and triggering of DAMPs. Antioxidant capacity, measured by GSH, determines the redox balance that affects immune activation. These biochemical markers, analyzed with inflammatory indices, illustrate the connection between oxidative stress and immune response.

To assess inflammatory activity, an inflammatory index linked to cytokines was incorporated into the framework. This index does not directly measure cytokines; instead, it estimates the inflammatory load from oxidative stress and endothelial damage indicators. Therefore, these indices should be viewed as indirect indicators, not measures of cytokine expression or signaling activity. High scores indicate increased inflammatory signaling and potential graft immune priming.

Endothelial damage was assessed using a composite index of endothelial dysfunction, representing microvascular damage, leukocyte adhesion potential and complement-mediated injury. This index correlates with complement activation (C3a/C5a axis) and neutrophil recruitment, which are key in IRI-induced inflammation. Higher scores indicate increased microcirculatory impairment and immune-mediated graft dysfunction. The integrated immune activation score combined oxidative stress parameters, cytokine activation indices, and endothelial injury markers. This composite measure quantified the overall immunological burden of the graft and compared immune activation between the AED and ASED groups.

Statistical evaluations were used to assess differences in morphologic, biochemical, and integrated rehabilitation outcomes between the AED and ASED groups. Continuous data are presented as mean ± standard deviation (SD), and categorical data are presented as frequencies and percentages. Group comparisons were performed using Student's t-test or Mann–Whitney U test for continuous data and chi-square or Fisher's exact test for categorical data. Relative risk (RR), odds ratio (OR), attributable risk (AR), and attributable fraction (AF) were computed with 95% confidence intervals (CI). Pearson's correlation coefficient was used to explore the relationships between morphological and functional abnormalities, with coefficients (r) ranging from −1 to +1. A strong positive correlation indicates a direct link between structural damage and functional impairment. A two-sided p-value of less than 0.05 was considered significant.

All procedures involving postmortem renal allografts adhered to institutional and national guidelines for donation after circulatory death and the scientific use of donor organs. This study met the ethical standards for postmortem tissue research. As it focused solely on ex vivo organ evaluation, it did not involve any living human participants, recipient interventions, or clinical follow-up activities.

3. Results

Figure 1 shows the progression of oxidative stress markers and morphological and functional outcomes in the AED and ASED groups. LPO markers, such as MDA and DC, increased in both groups from 30 to 120 min, with a more

significant rise in the ASED group. Meanwhile, GSH levels slightly increased, indicating a compensatory, antioxidant response. Morphological and functional scores also increased, with the ASED group consistently scoring higher. A temporary dip in ASED functional parameters at 60 min suggests acute oxidative fluctuations before recovery and an increase at 120 min.

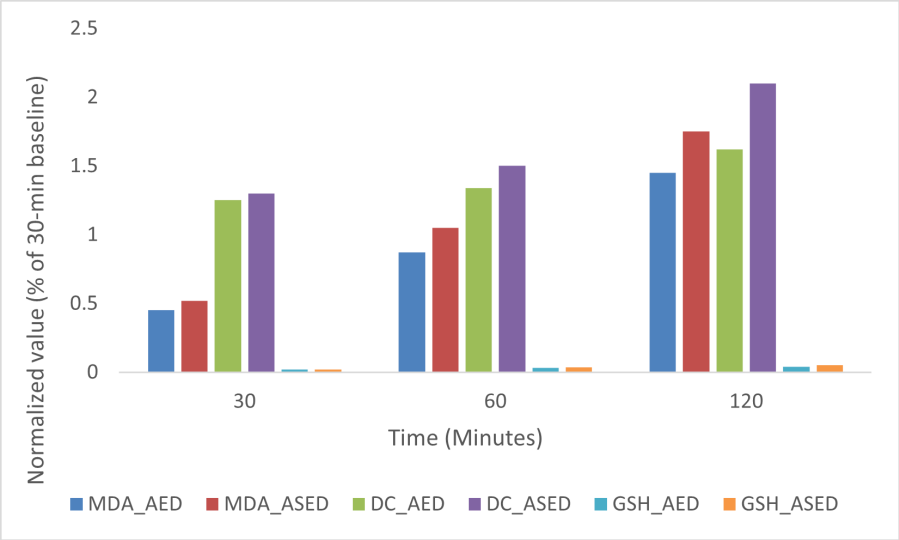

Figure 1. Temporal changes in oxidative stress biomarkers and functional outcomes in AED and ASED groups.

Figure 2 shows the histopathological damage in key renal parameters. The ASED group exhibited more tubular degeneration, endothelial swelling, podocyte damage, and nuclear changes than the AED group. Nuclear changes were the most severe, followed by podocyte damage and endothelial swelling. The increase from AED to ASED indicates worsening cellular and subcellular damage under oxidative stress. These results highlight the structural manifestations of biochemical and immunological injuries.

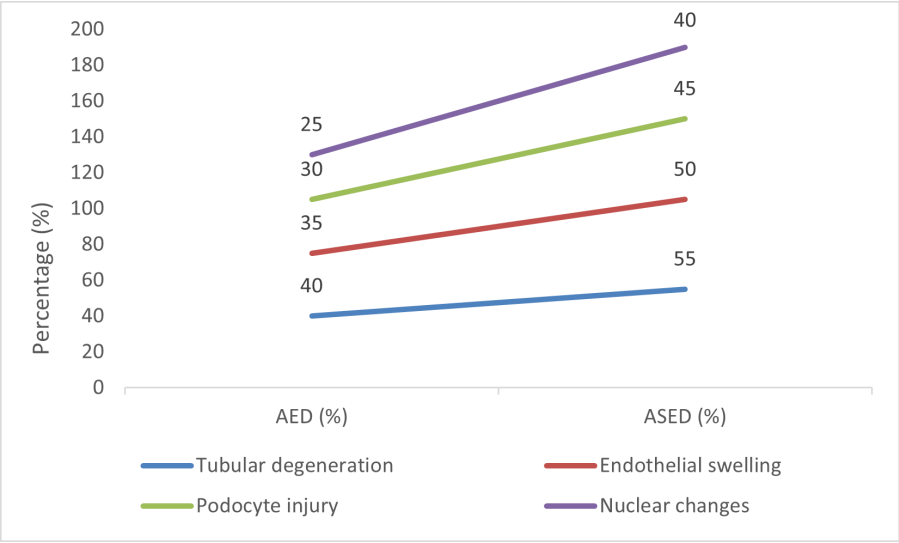

Figure 2. Histopathological alterations in renal structures.

Figure 3 shows the percentages of positive and negative results in the two study groups. The AED group had a higher rate of positive results (89.2%) than the ASED group (84.5%). Negative results were more common in the ASED group (15.5%) than in the AED group (10.8%). These findings suggest that AED has a better clinical or

experimental outcome profile. The increased negative outcomes in the ASED group align with its higher oxidative stress and inflammatory burden, supporting the link between biochemical imbalances and negative outcomes.

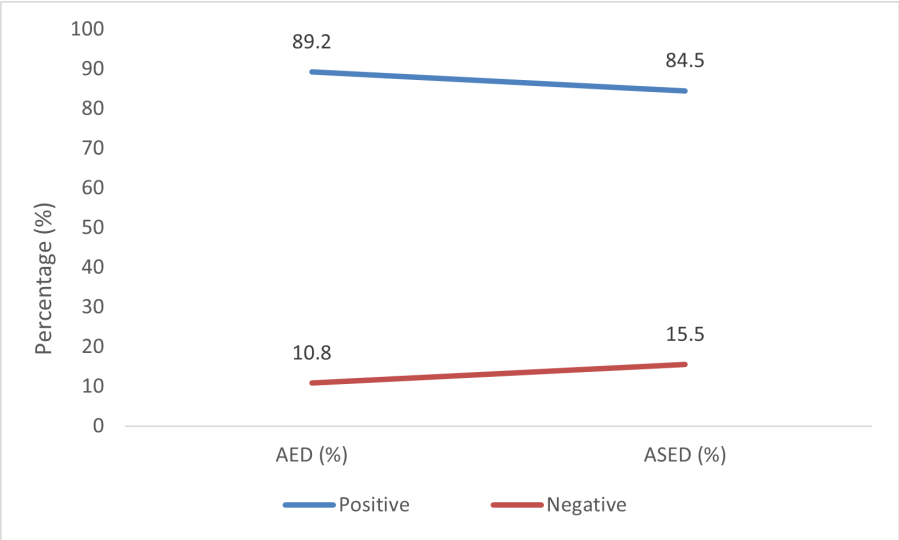

Figure 3. Distribution of positive and negative outcome percentages.

Figure 4 compares essential biochemical and immunological markers between AED and ASED groups. The ASED group exhibited elevated levels of ROS, MDA, and cytokine activation (IL-6), indicating increased oxidative stress and inflammation. The markers of endothelial damage were significantly elevated, suggesting more severe vascular dysfunction. Immune activation scores were also higher, indicating a stronger systemic inflammatory response. In summary, ASED is associated with a more pronounced oxidative and immunopathological profile than AED.

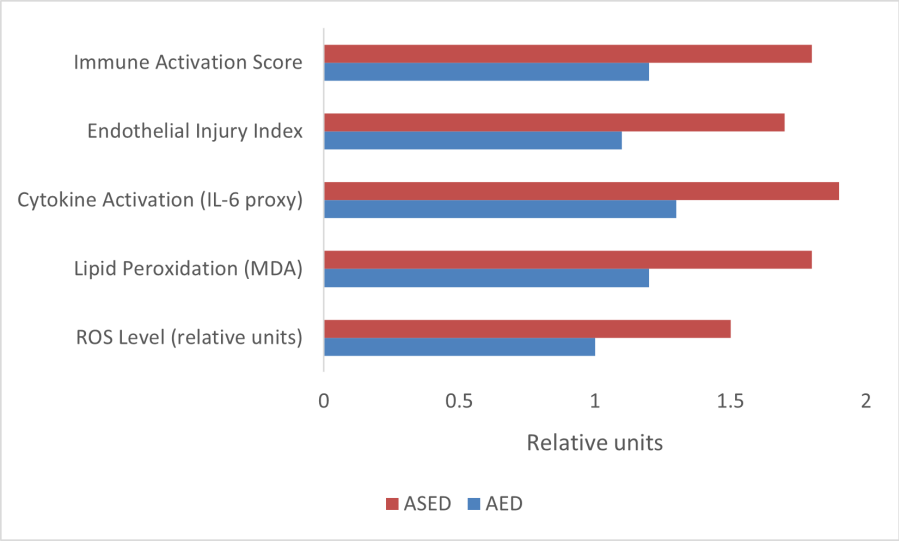

Figure 4. Comparative analysis of oxidative stress and immune-inflammatory markers.

Figure 5 shows the normalized MDA, diene conjugates (DC), and GSH measurements compared to the baseline at 30, 60, and 120 min. Both the AED and ASED groups exhibited a time-dependent increase in MDA and DC, with ASED showing greater changes, indicating increased LPO. GSH levels increased less, indicating limited antioxidant compensation. The pro-oxidant and antioxidant trend gap was more pronounced in ASED, suggesting an oxidative imbalance. These patterns underscore the progressive oxidative damage and inadequate antioxidant defense under stress.

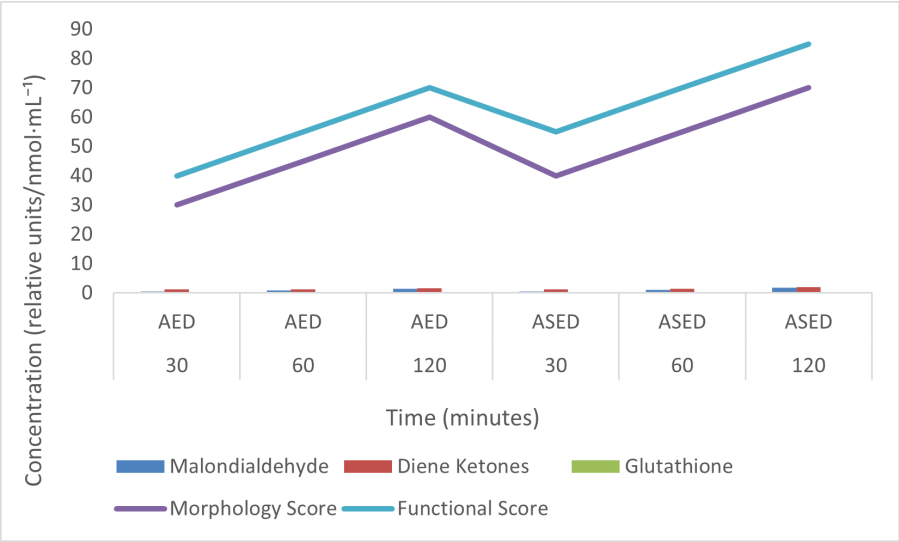

Figure 5. Normalized changes in LPO and antioxidant markers over time.

MDA changes: 2.2 times decrease at 30 min, 3.7 times at 60 min, and 1.4 times increase from 60 min. The 30-min MDA drop was due to the protective effect of hypothermia. Continued intermediate product decrease is related to hypothermia and waste removal from perfusion. The limit of hypothermia was 60 min, after which LPO in membranes increased by 120 min. Morphological and biochemical data showed destructive dystrophic changes dependent on LPO, peaking with 120-min hypothermic perfusion. Nephron analysis post-120-min rehabilitation showed reversible convoluted tubule changes and less glomerular filtration impact, manifested as epithelial degeneration and glomerular swelling. Electron microscopy confirmed glomerular endothelium edema and organelle changes. Podocytes exhibited similar changes, including pedicle thickening and organelle transformation, particularly in the mitochondria.

DC LPO levels showed that after 120 min of rehabilitation, the average was 0.54 relative units, 3.5 times lower than that at 30 min. At 60 min, the glutathione peroxidase level was 1.5 times lower than that at 30 min. MDA levels were 1.6 times higher (1.45–0.87 nmol/ml), while GSH levels were 2.2 times lower (0.014–0.031 relative units). The increase in MDA levels is likely due to LPO catalyzed by free oxygen.

By 60 min, the antioxidant capacity was exhausted and had returned to baseline. By 120 min, LPO stabilized as free radical formation ceased. The data showed destructive dystrophic changes linked to LPO, peaking during 120 min of hypothermic perfusion. Nephron changes after 120 min of rehabilitation showed reversible convoluted tubule changes and less glomerular impact, as evidenced by epithelial degeneration and glomerular swelling. Electron microscopy confirmed endothelial edema and organelle alterations. Podocytes exhibited similar changes, including pedicle thickening and organelle transformation, particularly in mitochondria.

Returning to the conceptual aspect of the issue, it is crucial to recognize that the primary problem with postmortem renal allograft donation from asystolic donors is warm ischemia, which develops and damages the postmortem renal allograft after asystole. This is evidenced by the pathogenesis of structural and functional disorders in postmortem renal allograft assessment.

The use of donor postmortem renal allograft assessment is permitted in certain cases. In AED, absolute thermal ischemia should not exceed 30 min, and relative thermal ischemia should stay under 120 min (ASED). Russian and Kyrgyz laws differ: Russia's approach is optimal (postmortem renal allograft sampling 30 min post-asystole), whereas Kyrgyzstan's is less ideal (120 min post-asystole).

Table 3 shows that the ASED group had a postmortem renal allograft rehabilitation score of 232 points versus 154 in the AED group. The ASED group had slightly more positive outcomes than the AED group (95.9% vs. 92.9%). The risk of negative outcomes was lower in the AED group, with an RR of 0.977. AR (−0.02) and AF (0.002%) indicate favourable rehabilitation results in both groups, which are linked to morphological abnormalities in the postmortem renal allograft parenchyma with hypoxic factors (RF +). The OR of 2.51 suggests a higher likelihood

of negative outcomes in the AED group.

Table 3. Morphological integrity assessment outcomes based exclusively on structural and ultrastructural injury scoring criteria.

Parameters	ASED	AED	Total
Positive (+)	66 (92.9%); 232 points	71 (95.9%); 154 points	137
Negative (−)	5 (7.1%); 5 points	3 (4.1%); 3 points	8
Comparative Indicators			
Metric		**Value**	
IN (ASED)		0.978	
IN (AED)		0.980	
RR		0.997	
AR		−0.020	
AF		0.002%	
OR		2.51	

Note: Data presented as n (%); points. AEDs = Asystolic early donors/rapid death; ASEDs = Asystolic extended/delayed donors; IN = Incidence; RR = Relative Risk; AR = Attributable Risk; AF = Attributable Fraction; OR = Odds Ratio.

According to **Table 4**, the ASED group achieved a positive postmortem renal allograft rehabilitation score of 419 points, whereas the AED group achieved 410 points. The ASED group had an 8.7% higher proportion of negative outcomes than the AED group. The likelihood of a negative outcome was lower in the AED group than in the ASED group (RR = 0.987). The AR (−0.012) and AF (0.0013%) values suggest that favorable rehabilitation results in both groups are strongly linked to the degree of metabolic disturbances in the postmortem renal allograft parenchyma caused by hypoxic factors (RF+). Additionally, the OR (1.3) indicates that the odds of a negative outcome were greater in the AED group than in the ASED group.

Table 4. Metabolic dysfunction assessment outcomes based on oxidative stress and biochemical disturbance parameters.

Parameters	ASED	AED	Total
Positive (+)	52 (73.2%); 419 points	60 (81.1%); 430 points	112
Negative (−)	19 (26.8%); 19 points	14 (18.9%); 14 points	33
Comparative Indicators			
Metric		**Value**	
IN (ASED)		0.956	
IN (AED)		0.968	
RR		0.987	
AR		−0.012	
AF		0.0013%	
OR		1.30	

Note: Data presented as n (%); points. AEDs = Asystolic early donors/rapid death; ASEDs = Asystolic extended/delayed donors; IN = Incidence; RR = Relative Risk; AR = Attributable Risk; AF = Attributable Fraction; OR = Odds Ratio.

As shown in **Table 5**, the AED group had a 4.7% higher proportion of positive outcomes than the ASED group. This is supported by the positive postmortem renal allograft rehabilitation score of 232 points in the AED group compared to 298 points in the ASED group. The RIs were 0.954 and 0.973, respectively. The risk of a negative outcome was lower in the AED group than in the ASED group (RR = 0.982). The negative AR (−0.01) and minimal AF (0.0013%) suggest that successful rehabilitation outcomes in both groups are reliant on the efficacy of the rehabilitation technologies employed. The OR (1.07) revealed that the likelihood of a negative outcome was nearly the same for both groups.

Table 5. Integrated rehabilitation effectiveness outcomes derived from combined morphologic and metabolic rehabilitation scoring criteria.

Parameters	ASED	AED	Total
Positive (+)	60 (84.5%); 232 points	66 (89.2%); 298 points	243
Negative (−)	11 (15.5%); 11 points	8 (10.8%); 8 points	306

Table 5. *Cont.*

Parameters	ASED	AED	Total
Comparative Indicators			
Metric		**Value**	
IN (ASED)		0.954	
IN (AED)		0.973	
RR		0.982	
AR		−0.01	
AF		0.005%	
OR		1.07	

Note: Data presented as n (%); points. AEDs = Asystolic early donors/rapid death; ASEDs = Asystolic extended/delayed donors; IN = Incidence; RR = Relative Risk; AR = Attributable Risk; AF = Attributable Fraction; OR = Odds Ratio.

Table 6 shows that the intervention group had a higher percentage of positive postmortem renal allograft rehabilitation outcomes than the ASED group (89.2% vs. 84.5%). The ASED group achieved 232 points, whereas the AED group reached 298 points. The IN values were 0.954 and 0.973, respectively. The AED group had a lower likelihood of negative results (RR = 0.984). The negative AR (−0.015) and minimal AF (0.003%) indicate that rehabilitation success depends on technology efficacy. The OR (1.16) suggests similar odds of negative outcomes in both groups. The selection of ASED criteria is crucial for the national pool of potential cadaveric donors. We believe that using criteria based on numerical characteristics from morpho-, ultrastructural, and functional-physiological studies of postmortem renal allograft assessment is feasible and essential. This should occur dynamically within acceptable (up to 30 min) and conditionally acceptable (60–120 min) timeframes. Within acceptable timeframes, postmortem morphofunctional changes in the postmortem renal allograft offer hope for adequate restoration after reperfusion injury. However, within conditionally acceptable timeframes, this is only possible using comprehensive rehabilitation technologies.

Table 6. Final morphofunctional compliance analysis integrating structural, metabolic, and rehabilitation effectiveness domains.

Parameters	ASED	AED	Total
Positive (+)	60 (84.5%); 252 points	66 (89.2%); 298 points	263
Negative (−)	11 (15.5%); 11 points	8 (10.8%); 8 points	306
Comparative Indicators			
Metric		**Value**	
IN (ASED)		0.958	
IN (AED)		0.973	
RR		0.984	
AR		−0.015	
AF		0.003%	
OR		1.16	

Note: Data presented as n (%); points. AEDs = Asystolic early donors/rapid death; ASEDs = Asystolic extended/delayed donors; IN = Incidence; RR = Relative Risk; AR = Attributable Risk; AF = Attributable Fraction; OR = Odds Ratio.

It is essential to elucidate the relationship between morphological and functional abnormalities in the postmortem renal allograft when modeling the AED and ASED. Functional abnormalities in the postmortem renal allograft correspond to morphological abnormalities. Earlier, we detailed the nature and extent of morphological abnormalities in the postmortem renal allograft under AED and ASED conditions. How closely do these abnormalities correlate with AED and ASED? We performed a correlation analysis of the correspondence.

Let X represent the mathematical expectation at the level (A, i = 57.7% + 70.4%/n = 59.7%) of morphological disorders, and at the level (B, i = 67.5% + 83.7%/n = 68.6%): $X = 1/nx\ (\Sigma\ A \times B)$. Note that r in the overall series of measurements (n) can take any value from −1 to +1, as $-1 \leq r \leq +1$. When $r > 0$, there is a positive correlation in the assessment results, and when $r < 0$, there is a negative relationship (**Table 7**). Our calculations indicated that in A, r = 1.0, and in B, r = 0.9. Therefore, both AED and ASED exhibit a strong positive correlation between morphological and functional disorders and the timing of postmortem renal allograft removal.

Thus, maximizing the quantitative characteristics of morphological and metabolic studies enables the identification of the pathophysiological mechanisms of both disorders and their sanogenesis. The qualitative and quantitative criteria presented in this study, both effective and subeffective, allow for a more precise and reliable evaluation

of the effectiveness of perfusion and cryoprotective rehabilitation of postmortem renal allograft. Overall, the developed criteria can be used to determine ASED's status.

Table 7. Correlation between morphological and functional disorders.

Model	Morphological	Functional	r
AED (n–71)	41 (57.7%)	50 (70.4%)	1.0
ASED (n–74)	50 (67.5%)	62 (83.7%)	0.9

Note: Correlation coefficients are presented together with sample size (n), 95% confidence intervals, and corresponding *p*-values. Correlation strength interpreted according to standard statistical criteria. AEDs = Asystolic early donors/rapid death; ASEDs = Asystolic extended/delayed donors; r = Correlation.

4. Discussion

This study highlights the link between oxidative stress, structural damage, and immune activation in kidney transplantation from asystolic donors. The ASED group experienced extended warm ischemia, leading to increased LPO markers (MDA and diene conjugates) and insufficient antioxidant compensation (GSH), indicating an oxidative imbalance.

Extended warm ischemia before transplantation sensitizes the immune response: longer bloodlessness depletes ATP, impairs mitochondrial function, and disrupts redox balance, making reperfusion immunologically charged. This supports research showing that IRI is driven by DAMP release, PRR/TLR detection, and inflammatory signaling, especially via the TLR–NF-κB pathway [38,39]. Elevated MDA, an IL-6 indicator, and endothelial damage support this hypothesis. MDA, a measure of LPO, indicates oxidative membrane damage before graft use. Reviews on hepatic IRI highlight hepatocyte damage, triggering redox loops, DAMP release, TLR signaling, and cytokine production [40].

In liver ischemia-reperfusion, extended ischemia induces ER stress in Kupffer cells, increasing TLR stimulation response, raising pro-inflammatory cytokines, and decreasing anti-inflammatory signaling [41]. In renal IRI, prolonged ischemia raises labile heme levels, which are linked to increased IL-6, TNF-α, complement receptor expression and neutrophil infiltration. Heme enhances IL-6 levels in LPS-stimulated macrophages, linking oxidative damage to cytokine amplification. In human kidney transplantation, early reperfusion showed significant local IL-6 release from the graft, indicating immediate and localized cytokine activation [12].

This study supports that oxidative stress transforms ischemic tissue into an immunogenic substrate. Increased ROS levels cause cell membrane damage, mitochondrial injury, endogenous danger signal release, immune receptor activation, and changes in vascular endothelial activation and permeability. This aligns with the TLR-focused IRI literature. Endothelial damage is significant as it marks and drives injury; the activated endothelium expresses adhesion molecules, facilitates leukocyte adhesion, and increases vascular leakage, worsening reperfusion damage [42]. The complement fits into this cascade. Oxidative damage and DAMP release promote complement activation by exposing altered self-structures and increasing inflammatory signaling. Studies on renal and cardiac IRI have associated complement activation with severe injury, neutrophil accumulation, and reduced graft function [12]. In intestinal IRI, complement initiation varies by sex, reinforcing complement as a major effector of sterile inflammatory injury [43].

ROS during ischemia, especially upon reperfusion, harm the endothelial glycocalyx, increase permeability, and trigger adhesion molecules like ICAM-1, VCAM-1, P-selectin, and E-selectin, transforming the vascular lining from a barrier to an inflammatory platform [44]. Endothelial activation facilitates leukocyte tethering, rolling, adhesion, and transmigration, worsening microvascular inflammation and tissue damage.

Following endothelial injury, leukocyte adhesion increases as endothelial cells display selectins and adhesion molecules, supporting neutrophil recruitment [44, 45]. Platelet adhesion and leukocyte-platelet interactions intensify local inflammation and thrombosis-like obstruction. Capillary plugging and stasis occur when leukocytes, platelets, and damaged cells impede microvascular flow, leading to uneven perfusion. Glycocalyx loss enhances adhesion receptor access, making adhesion more likely under reperfusion [46].

In transplantation, this explains the delayed graft function: the graft is present, but its microcirculation is impaired, so reperfusion does not restore organ performance [47].

The experimental C1 inhibitor preserved endothelial integrity, showing that complement is an active participant in vascular injury. In kidney transplantation, elevated soluble terminal complement complex and tissue com-

plement deposition correlate with the severity of delayed graft function [48].

Your correlation of r ≈ 0.8–1.0 between morphological damage and functional impairment aligns with this understanding. In ischemia-reperfusion, endothelial disruption, leukocyte infiltration, and microvascular collapse diminish perfusion, oxygen delivery, and filtration capacity. Thus, worsening histology is a determinant of declining organ performance [49].

This finding suggests a shared causal cascade rather than separate endpoints. More extensive endothelial and parenchymal injuries lead to severe microcirculatory failure and poorer graft function. Interventions that preserve endothelial integrity can enhance morphology and function [49].

Immune activation mediates the transition from structural injury to functional loss. Oxidative stress damages the endothelium; the injured endothelium promotes leukocyte adhesion, cytokine amplification, and complement activation; these processes worsen microvascular flow and propagate tissue injury, manifesting as delayed graft function [44,50].

The pattern from 30 to 120 min shifts from temporary redox control to antioxidant failure and increased inflammation. At 30 min, reduced MDA levels suggested the initial protective effect of hypothermia. Early cooling delays antioxidant depletion and inhibits LPO, reducing MDA levels and maintaining GSH defenses. By 60 min, the system enters a compensatory phase, but the antioxidant response is insufficient against ROS. Early enzyme alterations are observed, but tissue damage continues as the oxidative demand exceeds the response. Increased LPO indicates membrane damage that surpasses the buffering capacity. At 120 min, the injury pattern was maladaptive. Prolonged ischemia leads to GSH collapse and oxidative damage, overwhelming the antioxidants. Studies on warm ischemia have shown that longer periods cause severe necrosis, suppressed signaling, and stronger injury, reflecting lost resilience. Antioxidant failure results from systemic breakdown: ATP depletion limits regeneration (GSH recycling and synthesis require energy, and energy failure reduces the restoration of reduced antioxidants). Despite cooling, ROS generation persists (mitochondrial dysfunction and reperfusion-associated ROS drive LPO). Membrane peroxidation generates danger signals (lipid damage promotes DAMP release, triggering immune activation; oxidative injury and inflammatory signaling are linked through NF-κB pathways and redox-sensitive immune loops).

Endothelial injury worsens the response (damaged endothelium encourages leukocyte adhesion, capillary dysfunction, and microcirculatory collapse, turning stress into organ dysfunction). The ASED category is considered high-risk; however, it is not irreversibly poor. Prolonged warm ischemia increases the oxidative damage. Antioxidant reserves are depleted. This implies that the graft is damaged but not beyond recovery. Evidence shows that hypothermia, GSH supplementation, and antioxidant/anti-inflammatory strategies can reduce LPO, maintain flow, and decrease leukocyte adhesion, supporting the therapeutic modification of some injuries.

Hypothermic and normothermic machine perfusion mitigates IRI by disrupting the ROS–cytokine–endothelium cascade. This supports the findings that hypothermic oxygenated perfusion decreases oxidative damage and maintains graft function in marginal livers; combined HOPE–NMP protocols lower oxidative injury and inflammation compared to normothermic perfusion [35]. Normothermic perfusion facilitates aerobic metabolism and functional reassessment, reducing CIRP-mediated oxidative stress and inflammation in DCD liver models, with CIRP levels correlating with postoperative IL-6 and liver function [29]. Perfusion limits cytokine amplification, as IRI involves TNF-α and IL-6 reinforcement. Machine perfusion studies have shown reduced inflammatory mediators and enhanced graft recovery, including intestinal HMP, which decreases IL-1, IL-6, IFN-γ, and TNF-α levels while increasing IL-10 levels and preserving epithelial barrier proteins [51]. Both perfusion types steer the graft from a proinflammatory state to a regulated phenotype. Preserving endothelial integrity is crucial because endothelial injury links oxidative stress and immune dysfunction. In kidney perfusion, complement activation and inflammatory signaling during normothermic perfusion support the endothelium as the primary immunologic interface [32].

Perfusion biomarkers differentiate salvageable grafts from non-salvageable grafts. For example, real-time hypothermic perfusion lactate at 120 min predicts early allograft dysfunction and correlates with post-transplant injury and extended hospitalization [52]. This supports the notion that ongoing metabolic failure during perfusion indicates immunometabolic vulnerability.

The literature supports the comparison that IRI is linked with elevated IL-6 and TNF-α levels, complement activation is common in DCD and marginal grafts, and machine perfusion reduces inflammation. NMP mitigated hepatic IRI by inhibiting CIRP-associated oxidative stress and mitochondrial fission, with CIRP connected to IL-

6 and its function [29]. Complement activation during normothermic machine perfusion has been observed in discarded kidneys and is linked to inflammatory cytokines and tissue damage [32]. Hypothermic and combined machine perfusion strategies decrease inflammatory activation and enhance functional recovery in liver, kidney, and intestinal models.

The current results indicate that renal allografts undergoing extended warm ischemia sustain greater structural damage, oxidative stress, and immunoinflammatory challenges than those with shorter ischemic periods. Despite this, many ASED grafts achieved positive scores in the ex vivo rehabilitation setting. These findings suggest the potential for preservation and rehabilitation under experimental perfusion conditions; however, they do not prove post-transplant graft function, reduced delayed graft function, or long-term graft survival. Therefore, transplantation-focused studies are needed.

Immunocompromised donor or recipient conditions may affect inflammatory responses to ischemia-reperfusion and graft outcomes. Although the model did not assess immunocompromised status, altered immune function could affect oxidative stress, cytokine signaling, endothelial activation, and tissue repair during graft preservation and reperfusion. Reduced inflammatory activation might lessen IRI, but decreased repair abilities, altered antioxidant defenses, and increased risk of endothelial dysfunction or infection could harm graft recovery and viability. Immunosuppressive treatments may influence ROS-DAMP-cytokine interactions and the balance between damage and remodeling.

This study impacts kidney transplantation by increasing the use of DCD. Despite increased oxidative stress and immune activation, ASED grafts show potential for recovery, suggesting they are high-risk but salvageable with better preservation. Integrating oxidative, morphological, and immunological parameters into one model provides a comprehensive framework for assessing viability compared with traditional criteria. Clinically, this approach could improve donor selection, reduce organ discards, and enhance availability, especially in regions with shortages. Machine perfusion technologies, particularly hypothermic and normothermic ones, are crucial for preservation, *ex vivo* immunomodulation, real-time assessment, and targeted interventions.

These findings should be interpreted with awareness of the limitations. This study focused on graft rehabilitation in an *ex vivo* postmortem context. Thus, morphofunctional outcomes indicate preservation potential, not direct evaluations of post-transplant function, delayed graft function, rejection, or long-term survival. However, transplantation studies are required to confirm the clinical significance of these findings. This study used a historical ex vivo dataset, and certain donor variables, perfusate parameters, and procedural details were inconsistently available. Therefore, these elements were excluded and should be considered when evaluating the results.

Experimental, model-based designs require caution when applying the results to clinical transplantation outcomes. The absence of long-term graft survival data and post-transplant clinical follow-up limits the association of pre-implantation findings with outcomes such as delayed graft function or rejection. Although comprehensive, the multicriteria scoring system involves semi-quantitative grading, risking observer variability and requiring validation in independent cohorts. Sample size limitations and lack of stratification for donor-specific factors (age and comorbidities) might affect generalizability. While addressing key aspects of IRI, this study did not include advanced molecular analyses (e.g., transcriptomics and proteomics), which could provide detailed insights into immunological mechanisms. Because of the retrospective multicriteria assessment framework, some historical datasets lacked the information needed to reconstruct variance measures and inferential statistical parameters. Thus, the findings should be viewed as comparative evaluations of graft rehabilitation potential, not as conclusive hypothesis-testing results.

Future research should validate the morphofunctional-immunological model in clinical transplantation and link it to graft outcomes. Including immunological biomarkers, such as IL-6, TNF-α, High Mobility Group Box 1, C3a, and C5a, through enzyme-linked immunosorbent assay and flow cytometry would enhance the model's insights and predictive accuracy. Protocols for graft assessment using biomarkers, integrating real-time perfusion data with immunological profiling, are needed for precision donor selection. Studies should explore *ex vivo* therapeutic strategies during machine perfusion, such as antioxidants, complement inhibitors, anti-inflammatory drugs, and cell-based therapies, such as mesenchymal stem cells, to adjust graft immunogenicity. This need for validated multimodal readouts parallels recent preclinical work integrating imaging, histology, and biomarker endpoints to quantify glucocorticoid induced tissue remodeling disruption, as well as a recent review of emerging toxicity technologies, both of which support combining structural and functional endpoints when evaluating organ injury and

recovery [36,37]. Future studies should explore how donor and recipient immune statuses, including immunosuppressive therapy and baseline dysfunction, affect rehabilitation, signaling, and post-transplant outcomes in kidney transplantation after circulatory death. Large-scale multicenter studies are required to standardize scoring systems and define graft viability thresholds. Advanced technologies, including Artificial Intelligence and predictive modeling, can refine decision-making in transplant medicine. The goal is to move from static evaluation to a dynamic, precision immunology-based approach to organ transplantation.

5. Conclusions

This study shows that IRI in kidney transplants is an immuno-oxidative process driven by the ROS–DAMP–cytokine–endothelial pathway. ASED grafts exhibit a stronger oxidative and inflammatory response than AED grafts, yet retain some functional recovery, indicating that the damage is not entirely irreversible and can be altered. Patterns of oxidative stress, endothelial damage, and morphofunctional decline align with the mechanisms outlined in IRI, such as inflammatory signaling linked to ROS–DAMP. Nonetheless, directly assessing these pathways was outside the scope of this study and requires confirmation in future mechanistic research.

Combining biochemical, morphological, and immunological metrics into a single evaluation model provides a clinically significant method for assessing graft viability. These results suggest a shift in transplantation views, recognizing graft quality as a dynamic and adaptable condition that can be improved through interventions. By redefining marginal donor evaluation and emphasizing immunomodulation, this study advances precision transplantation medicine, potentially increasing the donor pool, improving graft success, and alleviating the organ shortage crisis.

Author Contributions

Conceptualization, Z.A.; methodology, K.M.; software, I.A.; validation, B.N.; data curation, T.A.; writing—original draft preparation, Z.A., K.M., and I.A.; writing—review and editing, B.N. and T.A. All authors have read and agreed to the published version of the manuscript.

Funding

This work received no external funding.

Institutional Review Board Statement

This study followed the Declaration of Helsinki principles (2013) with the Bioethics Committee of the National Surgical Center of the Ministry of Health of the Kyrgyz Republic approval (Protocol No. 3, dated June 13, 2025).

Informed Consent Statement

All participants respondents or guardians provided written informed consent.

Data Availability Statement

Not applicable.

Conflicts of Interest

The authors declare that there is no conflict of interest.

AI Use Statement

While drafting this manuscript, the authors used Paperpal solely to refine the language, grammar, spelling, and scientific readability. No AI tools were used for data generation, statistical analyses, result interpretation, content fabrication or conclusion formulation. All scientific interpretations, clinical analyses, and final revisions were independently reviewed and approved by the authors, who assumed full responsibility for the manuscript's integrity, originality, and accuracy.

References

1. Talbot, D.; D'Alessandro, A.M.; Muiesan, P. Organ Donation and Transplantation after Cardiac Death. *Ann. R. Coll. Surg. Engl.* **2010**, *92*, 719.
2. Caldwell, J.S.; Cheng, X.S.; Chertow, G.M.; et al. Kidney Transplant Wait Times Under Waiting List Expansion Scenarios. *JAMA Netw. Open* **2025**, *8*, e251665.
3. Haque, O.; Yuan, Q.; Uygun, K.; et al. Evolving Utilization of Donation after Circulatory Death Livers in Liver Transplantation: The Day of DCD Has Come. *Clin. Transplant.* **2021**, *35*, e14211.
4. Alghannam, K.; Fine, J.; Howard, B.; et al. Impact of Warm Ischemia Time on Donation after Circulatory Death Kidney Transplant Outcomes. *Clin. Transplant.* **2024**, *38*, e15436.
5. Walls, D.O.; Lee-Riddle, G.S.; Bover Manderski, M.; et al. Kidney Transplant Outcomes from Donation after Circulatory Death Donors of Advanced Age. *Clin. Transplant.* **2020**, *34*, e13881.
6. Kim, D.W.; Tsapepas, D.; King, K.L.; et al. Financial Impact of Delayed Graft Function in Kidney Transplantation. *Clin. Transplant.* **2020**, *34*, e14022.
7. Negret, M.A.; Rodriguez, M.P.; Díaz-Brochero, C.; et al. Impact of Delayed Graft Function on Overall and Graft Survival in Recipients of Deceased Donor Kidney Transplants. *BMC Nephrol.* **2025**, *26*, 633.
8. Tang, S.P.; Mao, X.L.; Chen, Y.H.; et al. Reactive Oxygen Species Induce Fatty Liver and Ischemia-Reperfusion Injury by Promoting Inflammation and Cell Death. *Front. Immunol.* **2022**, *13*, 870239.
9. Liao, S.; Luo, J.; Kadier, T.; et al. Mitochondrial DNA Release Contributes to Intestinal Ischemia/Reperfusion Injury. *Front. Pharmacol.* **2022**, *13*, 854994.
10. Hirao, H.; Dery, K.J.; Kageyama, S.; et al. Heme Oxygenase-1 in Liver Transplant Ischemia-Reperfusion Injury: From Bench-to-Bedside. *Free Radic. Biol. Med.* **2020**, *157*, 75–82.
11. Nieuwenhuijs-Moeke, G.J.; Pischke, S.E.; Berger, S.P.; et al. Ischemia and Reperfusion Injury in Kidney Transplantation: Relevant Mechanisms in Injury and Repair. *J. Clin. Med.* **2020**, *9*, 253.
12. Wang, L.; Vijayan, V.; Jang, M.S.; et al. Labile Heme Aggravates Renal Inflammation and Complement Activation After Ischemia Reperfusion Injury. *Front. Immunol.* **2019**, *10*, 2975.
13. Wu, X.; You, D.; Cui, J.; et al. Reduced Neutrophil Extracellular Trap Formation During Ischemia Reperfusion Injury in C3 KO Mice: C3 Requirement for NETs Release. *Front. Immunol.* **2022**, *13*, 781273.
14. Delaura, I.F.; Gao, Q.; Anwar, I.J.; et al. Complement-Targeting Therapeutics for Ischemia-Reperfusion Injury in Transplantation and the Potential for Ex Vivo Delivery. *Front. Immunol.* **2022**, *13*, 1000172.
15. Kaltenmeier, C.; Yazdani, H.O.; Handu, S.; et al. The Role of Neutrophils as a Driver in Hepatic Ischemia-Reperfusion Injury and Cancer Growth. *Front. Immunol.* **2022**, *13*, 887565.
16. Zaphiros, N.H.; Nie, J.; Alchaer, M.W.; et al. Outcomes of DCD Kidneys with CIT-Induced Delayed Graft Function. *Clin. Transplant.* **2023**, *37*, e14918.
17. Zhang, M.; Liu, Q.; Meng, H.; et al. Ischemia-Reperfusion Injury: Molecular Mechanisms and Therapeutic Targets. *Signal Transduct. Target. Ther.* **2024**, *9*, 12.
18. Asaka, M.; Imumura, H.; Sato, K.; et al. Ischemia–Reperfusion Injury as the Leading Cause of Primary Non-Function in Renal Transplantation Using Donors with Prolonged Warm Ischemic Time. *Clin. Transplant.* **2008**, *22*, 19–24.
19. Heylen, L.; Pirenne, J.; Samuel, U.; et al. The Impact of Anastomosis Time During Kidney Transplantation on Graft Loss: A Eurotransplant Cohort Study. *Am. J. Transplant.* **2017**, *17*, 724–732.
20. De Carlis, R.; Di Sandro, S.; Lauterio, A.; et al. Successful Donation After Cardiac Death Liver Transplants with Prolonged Warm Ischemia Time Using Normothermic Regional Perfusion. *Liver Transpl.* **2017**, *23*, 166–173.
21. Savoye, E.; Legeai, C.; Branchereau, J.; et al. Optimal Donation of Kidney Transplants after Controlled Circulatory Death. *Am. J. Transplant.* **2021**, *21*, 2424–2436.
22. Dondossola, D.; Ravaioli, M.; Lonati, C.; et al. The Role of Ex Situ Hypothermic Oxygenated Machine Perfusion and Cold Preservation Time in Extended Criteria Donation after Circulatory Death and Donation After Brain Death. *Liver Transpl.* **2021**, *27*, 1130–1143.
23. Nagaraju, S.; Savilla, R.; Moore, M.; et al. Successful Transplantation of LUCAS Device Assisted Uncontrolled DCD Kidneys with Prolonged Relative Warm Ischemia Time: An Underutilized Option in North America. *Clin. Transplant.* **2023**, *37*, e15058.
24. De Carlis, R.; Di Sandro, S.; Lauterio, A.; et al. Liver Grafts from Donors After Circulatory Death on Regional Perfusion with Extended Warm Ischemia Compared with Donors After Brain Death. *Liver Transpl.* **2018**, *24*, 1523–1535.
25. Hameed, A.M.; Pleass, H.C.; Wong, G.; et al. Maximizing Kidneys for Transplantation Using Machine Perfusion: From the Past to the Future: A Comprehensive Systematic Review and Meta-Analysis. *Medicine* **2016**, *95*,

e5083.
26. Husen, P.; Boffa, C.; Jochmans, I.; et al. Oxygenated End-Hypothermic Machine Perfusion in Expanded Criteria Donor Kidney Transplant: A Randomized Clinical Trial. *JAMA Surg.* **2021**, *156*, 517–525.
27. Horné, F.; Drefs, M.; Schirren, M.J.; et al. Hypothermic Oxygenated Machine Perfusion (HOPE) Prior to Liver Transplantation Mitigates Post-Reperfusion Syndrome and Perioperative Electrolyte Shifts. *J. Clin. Med.* **2022**, *11*, 7381.
28. Lee, A.C.H.; Edobor, A.; Lysandrou, M.; et al. The Effect of Normothermic Machine Perfusion on the Immune Profile of Donor Liver. *Front. Immunol.* **2022**, *13*, 788935.
29. Liu, W.; Fan, Y.; Ding, H.; et al. Normothermic Machine Perfusion Attenuates Hepatic Ischaemia-Reperfusion Injury by Inhibiting CIRP-Mediated Oxidative Stress and Mitochondrial Fission. *J. Cell. Mol. Med.* **2021**, *25*, 11310–11321.
30. Mazilescu, L.I.; Urbanellis, P.; Kim, S.J.; et al. Normothermic Ex Vivo Kidney Perfusion for Human Kidney Transplantation: First North American Results. *Transplantation* **2022**, *106*, 1852–1859.
31. Urbanellis, P.; Hamar, M.; Kaths, J.M.; et al. Normothermic Ex Vivo Kidney Perfusion Improves Early DCD Graft Function Compared with Hypothermic Machine Perfusion and Static Cold Storage. *Transplantation* **2020**, *104*, 947–955.
32. Jager, N.M.; Venema, L.H.; Arykbaeva, A.S.; et al. Complement Is Activated During Normothermic Machine Perfusion of Porcine and Human Discarded Kidneys. *Front. Immunol.* **2022**, *13*, 831371.
33. Boteon, Y.L.; Laing, R.W.; Schlegel, A.; et al. Combined Hypothermic and Normothermic Machine Perfusion Improves Functional Recovery of Extended Criteria Donor Livers. *Liver Transpl.* **2018**, *24*, 1699–1715.
34. Li, J.; Peng, Q.; Yang, R.; et al. Application of Mesenchymal Stem Cells During Machine Perfusion: An Emerging Novel Strategy for Organ Preservation. *Front. Immunol.* **2021**, *12*, 713920.
35. Dengu, F.; Abbas, S.H.; Ebeling, G.; et al. Normothermic Machine Perfusion (NMP) of the Liver as a Platform for Therapeutic Interventions during Ex-Vivo Liver Preservation: A Review. *J. Clin. Med.* **2020**, *9*, 1046.
36. Tuladhar, A.; Carter, C.; Vaughan, E.; et al. Multimodal Preclinical Assessment of Glucocorticoid-Induced Disruption of Bone Remodeling in Sprague-Dawley Rats. *Toxicol. Sci.* **2026**, *209*, kfag026.
37. Tuladhar, A.; Guffroy, M.; Finnema, S.J.; et al. Investigation of Bone Toxicity in Drug Development: Review of Current and Emerging Technologies. *Toxicol. Sci.* **2025**, *208*, 207–224.
38. Li, Q.; Lan, P. Activation of Immune Signals During Organ Transplantation. *Signal Transduct. Target. Ther.* **2023**, *8*, 110.
39. Wang, S.; Zhang, K.; Huang, Q.; et al. TLR4 Signalling in Ischemia/Reperfusion Injury: A Promising Target for Linking Inflammation, Oxidative Stress and Programmed Cell Death to Improve Organ Transplantation Outcomes. *Front. Immunol.* **2024**, *15*, 1447060.
40. Dery, K.J.; Chiu, R.; Kasargod, A.; et al. Feedback Loops Shape Oxidative and Immune Interactions in Hepatic Ischemia-Reperfusion Injury. *Antioxidants (Basel)* **2025**, *14*, 944.
41. Rao, J.; Yue, S.; Fu, Y.; et al. ATF6 Mediates a Pro-Inflammatory Synergy Between ER Stress and TLR Activation in the Pathogenesis of Liver Ischemia-Reperfusion Injury. *Am. J. Transplant.* **2014**, *14*, 1552–1661.
42. Guo, Y.; Dong, L.; Gong, A.; et al. Damage to the Blood Brain Barrier and Activation of Neuroinflammation by Focal Cerebral Ischemia Under Hyperglycemic Condition. *Int. J. Mol. Med.* **2021**, *48*, 142.
43. Wu, M.; Rowe, J.M.; Fleming, S.D. Complement Initiation Varies by Sex in Intestinal Ischemia Reperfusion Injury. *Front. Immunol.* **2021**, *12*, 649882.
44. Ampofo, E.; Berg, J.J.; Menger, M.D.; et al. Maslinic Acid Alleviates Ischemia/Reperfusion-Induced Inflammation by Downregulation of NFκB-Mediated Adhesion Molecule Expression. *Sci. Rep.* **2019**, *9*, 6119.
45. Sun, Y.; Lu, Y.; Ge, F.; et al. Deciphering the Anti-Atherosclerotic Mechanisms of Danggui-Chuanxiong Herb Pair: A Synergistic Approach Combining Computational Prediction and In Vivo Verification. *Front. Cardiovasc. Med.* **2026**, *13*, 1717999.
46. Bongoni, A.K.; Lu, B.; McRae, J.L.; et al. Complement-Mediated Damage to the Glycocalyx Plays a Role in Renal Ischemia-Reperfusion Injury in Mice. *Transplant Direct* **2019**, *5*, e341.
47. Mannon, R.B. Delayed Graft Function: The AKI of Kidney Transplantation. *Nephron* **2018**, *140*, 94–98.
48. Arias-Cabrales, C.E.; Riera, M.; Pérez-Sáez, M.J.; et al. Activation of Final Complement Components After Kidney Transplantation as a Marker of Delayed Graft Function Severity. *Clin. Kidney J.* **2020**, *14*, 1190–1196.
49. Collett, J.A.; Mehrotra, P.; Crone, A.; et al. Endothelial Colony-Forming Cells Ameliorate Endothelial Dysfunction via Secreted Factors Following Ischemia-Reperfusion Injury. *Am. J. Physiol. Renal Physiol.* **2017**, *312*, F897–F907.
50. Zhao, H.; Perez, J.S.; Lu, K.; et al. Role of Toll-Like Receptor-4 in Renal Graft Ischemia-Reperfusion Injury. *Am.*

J. Physiol. Renal Physiol. **2014**, *306*, F801–F811.
51. Hou, W.; Yang, S.; Lu, J.; et al. Hypothermic Machine Perfusion Alleviates Ischemia-Reperfusion Injury of Intestinal Transplantation in Pigs. *Front. Immunol.* **2023**, *14*, 1117292.
52. Zhylko, A.; Morawski, M.; Rykowski, P.; et al. Real-Time Biomarkers of Liver Graft Quality in Hypothermic Oxygenated Machine Perfusion. *J. Clin. Med.* **2025**, *14*, 471.