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Infusion of human mesenchymal stromal cells during normothermic machine perfusion of discarded human kidneys: a randomized, blinded, paired, preclinical study

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Abbreviations page

CIT	Cold ischemia time
CrCl	Creatinine clearance
DAPI	4',6-diamidino-2-phenylindole
DBD	Donation after brain death
DCD	Donation after circulatory death
DMSO	Dimethyl sulfoxide
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
EV	Extracellular vesicle
FDR	False discovery rate
FENa	Fractional excretion of sodium
GMP	Good Manufacturing Practice
HGF	Hepatocyte growth factor
HLA	Human Leukocyte Antigen
hMSC	Human mesenchymal stromal cell
IFN-γ	Interferon-γ
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-18	Interleukin-18
IRR	Intrarenal resistance
KIM-1	Kidney injury molecule-1
LTCG	Laboratory of Cell and Gene Therapy
MSC	Mesenchymal stromal cell
NGAL	Neutrophil gelatinase-associated lipocalin
NMP	Normothermic machine perfusion
RBF	Renal blood flow
SCS	Static cold storage
SEM	Standard error of the mean
TGF-α	Transforming growth factor-α
TNF-α	Tumor necrosis factor-α
VEGF-A	Vascular endothelial growth factor-A
WIT	Warm ischemia time
%FIAT	Percentage of fibrosis and interstitial atrophy/tubular atrophy

Abstract

Normothermic machine perfusion (NMP) enables pre-transplant organ assessment and therapeutic delivery. Mesenchymal stromal cells (MSCs) may limit ischemia-reperfusion injury, but their delivery to human kidneys during NMP has not been tested in a controlled setting.

Seven pairs of discarded human kidneys underwent 6-hour NMP. In each pair, one kidney received intra-arterial injection of 10 million clinical-grade bone marrow-derived human MSCs 45 minutes after perfusion onset; the paired kidney received placebo. Primary outcomes were feasibility and safety; secondary outcomes included functional, injury, cytokine, and histological parameters, assessed blindly.

Renal blood flow and intrarenal resistance improved significantly over time ($p<0.0001$; $p=0.04$), with no significant between-group difference, though MSC-treated kidneys trended toward higher flow and lower resistance early in perfusion. Urine output and creatinine clearance increased over time in both groups ($p=0.009$; $p=0.01$) and were numerically higher with MSCs, without reaching significance ($p=0.61$; $p=0.40$). NGAL was numerically lower in MSC-treated kidneys later in perfusion; cytokine and histological profiles were comparable.

This first blinded, randomized, paired, controlled study shows clinical-grade hMSC delivery during human kidney NMP is safe and translatable. Consistent functional trends suggest a biological signal, supporting an adequately powered confirmatory trial using this paired discarded kidney model.

1. Introduction

Kidney transplantation is the treatment of choice for patients with end-stage kidney disease, offering superior survival and quality of life compared to dialysis.[1–3] However, the persistent shortage of donor organs remains a major limitation, leading to prolonged waiting times and increased mortality on the transplant waiting list.[4] To mitigate this shortage, organs from extended criteria donors, particularly elderly donors, are increasingly considered for transplantation.[5,6] Although this strategy might expand the deceased donor pool, extended criteria kidneys are more vulnerable to ischemia–reperfusion injury and are associated with higher rates of delayed graft function and poorer long-term outcomes.[7–9] Strategies aimed at improving the evaluation and preservation of marginal donor kidneys are therefore of considerable interest.[10–12]

Normothermic machine perfusion (NMP) has recently emerged as a promising approach for organ preservation and viability assessment.[13–17] By restoring near-physiological conditions through oxygenated perfusion at body temperature, NMP allows metabolic activity to resume and enables real-time evaluation of graft function.[18–20] Beyond its role in preservation, NMP also provides an attractive platform for delivering therapeutic interventions directly to the organ before transplantation.[21–23] Such strategies may help limit ischemia–reperfusion injury and potentially improve graft quality prior to transplantation.[24–26]

Mesenchymal stromal cells (MSCs) have attracted significant attention as a potential therapeutic tool because of their immunomodulatory and tissue-protective properties. In preclinical models of renal ischemia–reperfusion injury, MSC administration has been associated with reduced tubular damage, decreased pro-inflammatory cytokine release, and improved renal function recovery.[27–30] These effects are thought to be largely mediated

through paracrine mechanisms, including the release of anti-inflammatory and trophic factors, rather than direct cell engraftment. [31,32] While systemic intravenous delivery has been the most used route of administration, it is limited by pulmonary sequestration and poor renal targeting, highlighting the need for more direct delivery strategies. [33–35] The pivotal kidney transplantation study reported by our group in 2019 concern a phase I–II, open-label, single-centre clinical study in which ten deceased-donor kidney transplant recipients received a single intravenous infusion of third-party allogeneic BM-MSC on day 3 post-transplantation. The intervention was safe and well-tolerated. Compared to historical controls, MSC-treated patients showed improved eGFR trajectories in the first year post-transplant alongside an increased proportion of circulating regulatory T cells.[31]

The use of NMP as a platform for MSC delivery offers several potential advantages. Compared to intravenous injection, direct intra-arterial administration during NMP allows targeted delivery to the kidney vasculature, bypassing pulmonary sequestration and maximizing local cell concentration, with a reduced risk of immunization. Furthermore, the controlled *ex vivo* environment of NMP enables real-time monitoring of immediate functional and biological responses, providing a unique opportunity to assess the early effects of MSC therapy before transplantation. [36–39]

In this study, we aimed to evaluate the feasibility and biological effects of administering allogeneic human MSCs (hMSCs) during NMP of discarded human kidneys as a preclinical model. To our knowledge, this represents the first report of intra-arterial delivery of clinical-grade, bone marrow-derived hMSCs during NMP in a paired kidney model in humans, enabling direct intra-donor comparisons under controlled conditions. Leveraging this design, we

performed an integrated assessment of renal function and inflammatory responses over a 6-hour perfusion period.

2. Material and Methods

2.1. Ethical Statement

This study was approved by the University Hospital–Faculty Ethics Committee of Liege (reference ID: 2023/20) and conducted in accordance with the Belgian law of 19 December 2008 governing the procurement and use of residual human body material.

2.2. Study design

Seven pairs of discarded human kidneys procured from the same deceased organ donors underwent 6 hours of simultaneous NMP, allowing paired comparisons between treated and untreated kidneys. Kidneys declined for clinical transplantation were considered for inclusion in the study if they exhibited sufficient residual function (calculated GFR of the deceased organ donor > 40 mL/min), a comparable size at donor computed tomography and a vascular anatomy suitable for arterial cannulation. Following deceased donor aortic cold flush with IGL-1 solution and classical organ procurement, the kidneys were transported to the surgical laboratory on static cold storage (SCS) and connected to the NMP circuits using an autologous red blood cell–based perfusion solution. In donation after circulatory death (DCD) donors, procurement warm ischemia time was defined as the interval between circulatory arrest and initiation of cold perfusion via aortic flush with preservation solution. Cold ischemia time (CIT) was defined as the interval between aortic cold flush and the onset of NMP. After 45 minutes of NMP, kidneys within each donor pair were randomized in a blinded manner to receive

either placebo (non-treated kidneys) or an intra-arterial injection of 10×10^6 clinical-grade bone marrow–derived human MSCs (hMSC) (MSC treated kidneys) (**Fig 1**).

2.3. Human kidney NMP setup

The complete NMP setup and protocol have been previously described.[40] Upon arrival at the laboratory, renal arteries of both kidneys were cannulated with 14 Fr cannulas (Medtronic, Brussels, Belgium) and ureters were cannulated to allow urine collection for volume measurement and sampling. The kidney pairs were perfused simultaneously for 6 hours using an oxygenated, autologous red blood cell–based perfusion solution and two—clinical cardiopulmonary extracorporeal circulation systems (Stockert GmbH, Freiburg, Germany). Red blood cells were recovered from the deceased organ donor during surgical procurement using a cell-saver device (Xtra autotransfusion system; LivaNova, Zaventem, Belgium. Pressure-controlled NMP was performed at 37 °C with a target arterial pressure of 70-75 mmHg. The volume of perfusate in the circuit was maintained constant by replacing urine output with an equivalent volume of crystalloid solution administered hourly. Perfusate and urine samples were collected hourly throughout the perfusion period. Renal function was assessed by measuring the clearance of an exogenous bolus of creatinine (75 mg/L, Sigma-Aldrich, St. Louis, MO, USA) and iohexol (518 mg/L, GE Healthcare, Diegem, Belgium) added to the perfusate during circuit priming. Creatinine clearance (CrCl) was calculated using the standard formula: CrCl = (urinary creatinine concentration × urine flow rate) / perfusate creatinine concentration. Additionally, the fractional excretion of sodium (FENa) was calculated as: FENa (%) = (urinary sodium concentration × perfusate creatinine concentration) / (perfusate sodium concentration × urinary creatinine concentration) × 100, to assess tubular sodium reabsorption capacity. Physiological parameters were continuously recorded throughout the 6-hour

perfusion period, including perfusate blood gas parameters, urine output, and renal arterial pressure and renal blood flow (RBF). Intrarenal Resistance (IRR) was calculated by dividing the pressure by the flow and was expressed in mmHg/mL/min/100g of tissue. Chronic histological lesions were assessed on post-perfusion biopsies by a single nephropathologist (Pauline Erpicum) in a blinded fashion, using a modified Banff scoring system adapted to the pre-transplant setting, as previously described.[41,42] Parameters included arteriolar hyalinosis (mm), arterial intimal thickening (ah), vascular changes (cv), interstitial fibrosis (ci), tubular atrophy (ct), and the percentage of cortical fibrosis and tubular atrophy (%FIAT). Acute rejection-related Banff parameters (e.g., tubulitis, intimal arteritis, glomerulitis) were not assessed, as they are not applicable in the pre-transplant setting and cannot be distinguished from ischemia-reperfusion injury under NMP conditions.

2.4. Human Mesenchymal Stromal Cell treatment

The allogeneic, off the shelf, clinical-grade, hMSCs used in this study were isolated, cultured, banked and delivered by the Cell and Gene Therapy Laboratory (LTCG) of the Department of Haematology at the University Hospital of Liege, a Good Manufacturing Practice (GMP)-accredited facility. The use of these cells in clinic was approved by the institutional ethics committee (reference 2025/470). The hMSCs were isolated from the bone marrow of healthy unrelated donors. The methodology for hMSC isolation, expansion, characterization and banking has been previously described.[31,43,44] The hMSCs were thawed immediately prior to use and diluted in a saline–human albumin solution. The final hMSCs product was infused within one hour after thawing to preserve cell stability and viability. The placebo consisted of a 20 mL syringe containing saline, human albumin, and dimethyl sulfoxide (DMSO), identical in appearance and volume to the syringe containing 10×10^6 thawed hMSCs. Syringes

containing either hMSCs or placebo were administered intra-arterially, as close as possible to the graft, by a 5-minutes injection. Treatment allocation was randomized and performed in a blinded manner. All investigators involved in the study remained blinded to group allocation until completion of the study.

2.5. MSC tracking in kidney tissue

In a separate experiment, one donor kidney pair was exclusively used for MSC tracking, to avoid any confounding effect on the primary experimental outcomes. Prior to infusion, hMSCs were prelabelled with CellTracker CMPTX Red fluorescent dye (C34552, Thermo Fisher Scientific, Massachusetts, United States) to enable subsequent cell tracking within the renal tissue. Renal biopsies were collected at 30 minutes, 1, 2, 3, 4, 5, and 6 hours post-infusion and immediately embedded in Tissue-Tek OCT compound for cryopreservation. Cryosections of 5 µm thickness were prepared and counterstained with DAPI (4',6-diamidino-2-phenylindole) to visualize cell nuclei. Fluorescence imaging was performed using a Zeiss Axioscan slide scanner, and image analysis was carried out with the Zeiss ZEN image analysis software.

2.6. Cytokines measurement (ELISA)

Levels of neutrophil gelatinase-associated lipocalin (NGAL), interleukin-10 (IL-10), and tumor necrosis factor-α (TNF-α) were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA). Cytokine concentrations in perfusate samples were further assessed using a custom multiplex assay (Human Luminex Discovery Assay, LXSAHM-09, R&D Systems, Minneapolis, MN, USA). The 9-plex panel included hepatocyte growth factor (HGF), interferon-γ (IFN-γ), interleukin-6 (IL-6), interleukin-18 (IL-18), vascular endothelial growth factor-A (VEGF-A), kidney injury molecule-

1 (KIM-1), transforming growth factor-α (TGF-α), interleukin-1β (IL-1β), and interleukin-8 (IL-8). All assays were performed according to the manufacturer's instructions and the assessor of these endpoints remained blinded to the treatment allocation throughout the experiments.

2.7. Statistical Analysis

Statistical analyses were performed using GraphPad Prism (version 10, GraphPad Software, San Diego, CA, USA). Data are presented as mean ± standard error of the mean (SEM), unless otherwise specified. Longitudinal perfusion parameters were analysed using a mixed-effects model (restricted maximum likelihood, REML) to account for repeated measurements over time and pairing between MSC-treated and non-treated kidneys. The Geisser–Greenhouse correction was applied where appropriate, and multiple comparisons between groups at individual time points were performed using Šídák's correction. Cytokine levels were normalized to baseline (T0) and expressed as log2 fold change. Given the small sample size and the non-normal distribution of cytokine data, paired comparisons between MSC-treated and non-treated kidneys were performed at each time point using the Wilcoxon matched-pairs signed-rank test. To control for multiple testing across time points and cytokines, false discovery rate (FDR) correction was applied. A two-sided p-value <0.05 was considered statistically significant.

3. Results

3.1. Organ donors and kidney grafts characteristic

Seven pairs of human kidneys (n = 14) were included in the study (Table 1). Donors included both donation after circulatory death (DCD, n=5) and donation after brain death (DBD, n=2) cases, comprising both male (n=3) and female individuals aged between 59 and 84 years.

Causes of death were predominantly cerebrovascular accidents or cardiac arrest. The primary reason for discard was advanced donor age, frequently in combination with DCD status or additional comorbidities such as arterial hypertension or type 2 diabetes mellitus. In one case, calcification of the renal artery was also reported. Last serum creatinine levels ranged from 0.83 to 1.18 mg/dL. Cold ischemia times ranged from 2h10min to 3h46min, with one exception in which perfusion was performed the following day, resulting in a prolonged cold ischemia time of 18h04min. In DCD donors, the mean procurement warm ischemia time was 9.2 minutes.

3.2. Physiological and functional parameters during NMP

Comparison within kidney pairs showed no major differences in physiological parameters between MSC-treated and non-treated kidneys (**Fig 2**). All kidneys produced urine and demonstrated homogeneous perfusion throughout NMP. Although renal blood flow (RBF) varied significantly over time ($p < 0.0001$), no statistically significant difference was detected between groups (treatment effect: $p=0.66$; time × treatment interaction: $p=0.83$; Fig. 2A). However, a trend toward higher RBF was apparent in the treated group throughout the perfusion period. Similarly, while intrarenal resistance (IRR) showed a significant time effect ($p=0.04$), no significant between-group differences were found (treatment: $p=0.33$; interaction: $p=0.71$; Fig. 2B), although a tendency toward lower resistance was observed in the treated group during the two first hours of perfusion. Perfusate sodium concentrations increased significantly over time ($p< 0.0001$), while potassium concentrations declined ($p< 0.001$), with no differences detected between MSC-treated and non-treated kidneys for sodium (treatment: $p=0.97$; interaction: $p=0.30$; Fig 2C). Although no significant treatment effect was observed for potassium ($p=0.50$), a borderline significant interaction between time

and treatment was detected (p=0.048), suggesting a subtle divergence in the temporal evolution of potassium concentrations between groups (Fig 2D).

Urine output, expressed as mL/h/100 g of kidney tissue, increased over time in both groups (p=0.009), with no significant differences between MSC-treated and non-treated kidneys (p=0.61; interaction p=0.93; Fig. 3A). Higher urine output values were observed in MSC-treated kidneys at 2, 3, 5, and 6 hours of perfusion, although these differences did not reach statistical significance. To further evaluate renal function during perfusion, filtration capacity was assessed by creatinine clearance (Fig. 3B). Creatinine clearance increased significantly over time (p=0.01), reflecting dynamic changes in filtration throughout NMP. No significant differences were observed between groups (p=0.40; interaction p=0.33), although creatinine clearance values were consistently higher in MSC-treated kidneys throughout perfusion. Tubular function was assessed by the fractional excretion of sodium (Fig. 3C), which showed lower values in MSC-treated kidneys, without reaching statistical significance (p=0.47; interaction p=0.96). NGAL concentrations increased markedly over time in both groups, consistent with progressive tubular stress during perfusion. Although NGAL levels were lower in MSC-treated kidneys at later time points, these differences did not reach statistical significance (treatment: p=0.81; interaction: p=0.96; Fig 3D). Perfusate creatinine concentrations declined progressively over time in both groups (p<0.001), with no significant difference between MSC-treated and non-treated kidneys (p=0.55; interaction p=0.99; Fig. 3E), reflecting effective creatinine elimination during perfusion. Similarly, iohexol concentrations decreased markedly over time in both groups (p<0.001), with no significant difference between groups (p=0.69; interaction p=0.95; Fig. 3F). Across all parameters analysed, post hoc

comparisons at individual time points confirmed the absence of significant differences between MSC-treated and non-treated kidneys.

3.3. Perfusate cytokine assessment

To assess whether MSC administration during NMP modulates the inflammatory milieu of human kidney grafts, we quantified, in the perfusate, a panel of pro- and anti-inflammatory cytokines as well as injury-associated markers (**Fig 4**). Across all conditions, cytokine levels exhibited a time-dependent increase during perfusion. Despite observable variability and differences in magnitude between MSC-treated and non-treated kidneys for selected analytes, no statistically significant differences were identified at any time point after correction for multiple comparisons. Although non-significant trends were noted for certain mediators, including lower levels of IL-6 and TNF-α in MSC-treated kidneys at later time points, these findings were not consistent across the cytokine panel and did not reach statistical significance. Similarly, anti-inflammatory cytokines and injury-related markers, including IL-10, KIM-1, and VEGF-A, followed comparable temporal trajectories in both groups. Collectively, these data indicate that, under the present experimental conditions, MSC delivery during NMP does not result in a measurable modulation of the cytokine profile during *ex vivo* perfusion.

3.4. Histological assessment of kidney injury

Biopsies from all 7 donor pairs were sampled after 6hours of NMP and scored according to the modified Banff classification **(Fig S1, Table S1**). Both non-treated and MSC-treated kidneys displayed mild pre-existing chronic changes, including low-grade mesangial matrix expansion, arteriolar hyalinosis, and interstitial fibrosis with tubular atrophy. The extent of fibrosis and

interstitial atrophy (%FIAT) remained below 10% in most samples, except for Pair 6, which showed 15–20% in the non-treated kidney. Within each donor pair, the histological profiles of the two kidneys were comparable, confirming that pre-existing lesions did not confound the assessment of MSC treatment effect.

3.5. Renal distribution of hMSCs

Fluorescence microscopy of cryosections revealed the presence of CellTracker CMPTX Red-labelled hMSCs (red puncta, indicated by white arrows) dispersed among renal tubular cells counterstained with DAPI (blue) **(Fig 5).** Red-labelled cells were detectable as early as 30 minutes post-infusion. At 1-hour, fluorescent cells appeared more numerous, although their distribution was heterogeneous across the tissue sections, with some areas showing higher cell density than others. From 2 hours onwards, the number of visible labelled cells appeared to gradually decrease, though isolated, red-labelled cells remained detectable up to 6 hours post-infusion. These qualitative observations suggest that infused hMSCs reach the renal parenchyma rapidly and can be detected over a 6-hour period, although the heterogeneity of the biopsies precludes any definitive quantitative conclusion regarding their spatiotemporal distribution.

4. Discussion

While MSC-based therapies have shown promising results in preclinical transplantation models, their translation to human grafts under clinically relevant perfusion conditions remains largely unexplored. To our knowledge, the present study is the first to evaluate the effect of clinical-grade, bone marrow–derived, allogeneic hMSC treatment during NMP in a preclinical model in humans using discarded kidneys and a paired design, thereby providing

an internal control for inter-donor variability. Our study demonstrates that intra-arterial hMSC delivery during NMP is technically feasible and safe: functional parameters — including renal blood flow, intrarenal resistance, creatinine clearance, and NGAL levels — evolved comparably between MSC-treated and non-treated kidneys throughout the perfusion period, with no detectable adverse effects on NMP dynamics. Notably, consistently higher creatinine clearance values and greater urine output were observed in MSC-treated kidneys across multiple time points throughout perfusion, alongside a trend toward higher renal blood flow and lower intrarenal resistance in the early perfusion phase. While these differences did not reach statistical significance, their consistency across independent functional parameters is biologically coherent and may reflect a real, through modest, paracrine reparative signal. The sample size of the present study — inherent to the use of discarded organ pairs — was not powered to detect differences of such magnitude, and the possibility that a larger cohort would reveal statistically significant improvements in creatinine clearance or urine output cannot be excluded.

The clinical grade hMSCs used in our study were produced under GMP conditions and administered immediately after thawing, without prior *in vitro* culture or expansion. This approach differs from some earlier studies in which MSCs were cultured prior to administration, which, while potentially beneficial for viability and function, introduces significant practical barriers to clinical implementation and represents a departure from standard GMP release practice.[36,45–47] This ready-to-use, off-the-shelf approach is closely aligned with the logistical constraints of solid organ transplantation *and reflects standard GMP release practice.* Importantly, the laboratory's GMP manufacturing protocol relies on a highly standardized culture process with rigorous batch qualification, which substantially reduces

inter-batch variability compared to non-GMP cultured cells used in other studies. These hMSCs have already demonstrated clinical safety in two phase I-II, controlled trials conducted by the same group, in which a single infusion of third-party hMSCs were infused after transplantation in 10 liver and 10 kidney recipients, respectively.[31,48] In the kidney recipient cohort, hMSC infusion was associated with improved early allograft function and increased regulatory T cell, but also with a risk of immunization against the unrelated hMSC donor Human Leucocyte Antigens (HLA).[31] This prior clinical experience provides a strong rationale for the hMSC use during NMP to decrease the risk of immunization and supports the biological plausibility of an effect in the present study.

The time-dependent increase in cytokine levels observed in both groups reflected the expected pattern of ischemia–reperfusion injury during *ex vivo* perfusion, without significant modulation by MSC treatment after correction for multiple comparisons, although non-significant trends were noted in selected cytokines. Histological assessment revealed comparable levels of tubular injury, inflammation, and vascular changes across groups. These findings are in line with those reported by Lohmann et al. in a porcine auto transplantation model, in which MSC infusion during NMP was equally well tolerated and similarly did not yield measurable improvements in renal function or histological injury parameters within a 14-day post-transplant follow-up, despite confirmed MSC retention in the renal cortex.[49]

The interpretation of these findings warrants consideration of several contributing factors. First, intra-arterial MSC delivery during machine perfusion is inherently constrained by cell loss within the perfusion circuit: Sierra-Parraga et al. showed that circulating MSC numbers decline to approximately 10% of the infused dose within hours of NMP onset, [37] and Pool et al.

demonstrated that the few intact cells remaining were predominantly trapped in glomerular capillaries rather than distributing to the tubular compartments most affected by ischemic injury.[36] Consistent with these observations, fluorescence microscopy tracking of CMPTX Red-labelled hMSCs in our study revealed a sparse and heterogeneous distribution of MSCs within the renal parenchyma, with an apparent reduction in detectable MSCs over the 6-hour observation period, suggesting limited and transient engraftment following intra-arterial infusion. The dose of 10×10^6 MSCs was selected based on the work of Pool et al., who identified this as the minimum effective dose for MSC detection and retention in the kidney during NMP, and subsequently used it to demonstrate reduced injury markers and immunomodulatory effects in an ex vivo porcine model.[36,50] Second, the NMP duration may have been insufficient to allow MSC-derived paracrine mediators to exert detectable reparative effects. The growing feasibility of prolonged NMP protocols extending to 24–48 hours opens a broader therapeutic window that future studies combining extended perfusion with biological interventions should explore. [51–54] Third, inter-donor variability — even within a paired design — may have obscured signals of modest magnitude, a limitation inherent to all discarded organ studies. Finally, the optimal MSC dose, infusion timing, and perfusion composition for human kidney NMP remain poorly defined, and as Lohmann et al. acknowledged, the absence of detectable benefit should not be equated with biological inactivity.[49] The present findings, combined with converging mechanistic evidence, suggest that intact MSC delivery may not be the optimal strategy for harnessing MSC biological potential during machine perfusion. As argued by Gilbo *et al.,* the rapid loss of viable cells in the perfusion circuit and their predominantly glomerular trapping — rather than distribution to injured tubular compartments — represent fundamental barriers unlikely to be overcome

by dose optimization alone, making extracellular vesicles (EV)-based or secretome-based strategies inherently better suited to this context.[55]

In conclusion, this study demonstrates that clinical-grade, allogeneic, hMSC delivery during human kidney NMP is technically feasible, safe, and directly translatable to a clinical setting — establishing a rigorous methodological framework and an ethically grounded experimental platform that future interventional studies can build upon. These findings contribute valuable groundwork for the field, clarifying what remains to be optimized and in which direction future efforts should be directed. The maturation of NMP as an active therapeutic platform, combined with the expanding availability of prolonged perfusion protocols and the growing mechanistic evidence supporting EV-based approaches, identifies realistic and well-supported directions for pre-transplant organ conditioning. The paired discarded kidney model validated here — spanning both DBD and DCD donors under clinically representative conditions — offers an ideal infrastructure to pursue these strategies, with the objective of improving graft quality and outcomes for transplant recipients.

Declarations

Ethics approval: This study was reviewed and approved by the Hospital-Faculty Ethics Committee of the University of Liège (approval number 2023/200) under the approved project entitled “Impact of mesenchymal stromal cell–enriched normothermic perfusion on renal ischemic injury”, approved on July 25, 2023. Human mesenchymal stromal cells were obtained from donors who provided written informed consent for the use of their biological samples for research purposes, in accordance with institutional and national regulations. The use of Human MSCs was reviewed and approved by the Hospital–Faculty Ethics Committee of the University of Liège under the approved project entitled “TB0202F11-Consent-collecte moelle allo MSC”, with approval reference number 2025-470, approved on November 7, 2024.

Consent for publication: Not applicable

Availability of data and materials: All data analysed during this study are included in this published article. The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Disclosure: The authors declare no conflicts of interest. The authors declare that they have not use AI-generated work in this manuscript.

5. Figure legends

Fig 1. Study design overview. Fourteen discarded human kidneys (7 donor pairs; WIT 0–11 min, CIT ±3 h) underwent 6 hours of NMP at 37°C. Within each pair, one kidney received an intra-arterial MSC injection at 45 minutes after NMP initiation, while the contralateral kidney received placebo. Perfusate and urine samples were collected at serial time points (T0–T6), and a renal biopsy was obtained at the end of perfusion. CIT, cold ischemia time; MSC, mesenchymal stromal cell; NMP, normothermic machine perfusion; WIT, warm ischemia time.

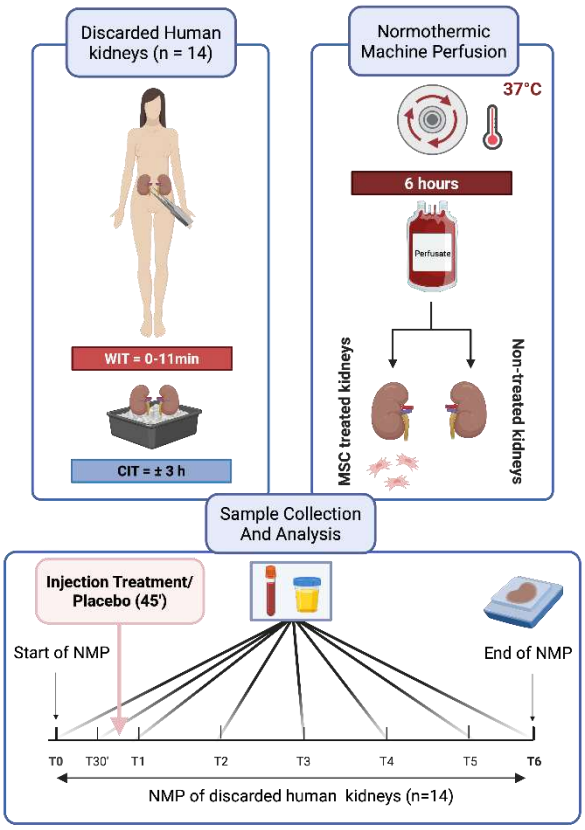


Fig 2. Hemodynamic and electrolyte parameters during normothermic machine perfusion. Longitudinal assessment of perfusion dynamics in paired human kidneys treated or not with MSCs. (A) Renal blood flow (RBF), showing a trend toward higher flow in the MSC-treated group throughout perfusion. (B) Intrarenal resistance (IRR), with a tendency toward lower resistance in the treated group during the early phase of perfusion. (C) Sodium concentration in the perfusate over time. (D) Potassium concentration in the perfusate over time. Data are presented as mean ± SEM (n = 7 pairs). Statistical

analysis was performed using a linear mixed-effects model accounting for repeated measures within kidney pairs. No significant treatment effect or time × treatment interaction was observed for any parameter.

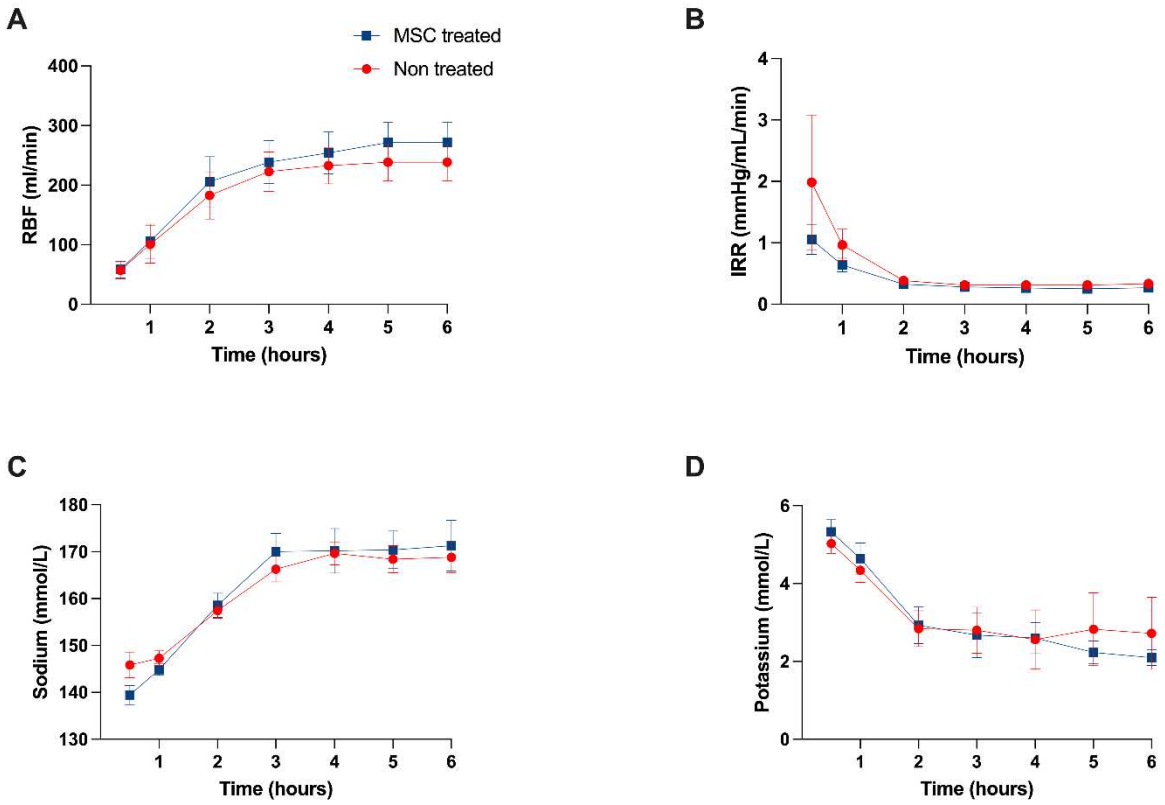


Fig 3. Functional parameters during normothermic machine perfusion. Longitudinal assessment of renal function in paired human kidneys treated or not with MSCs. (A) Urinary volume normalized to kidney weight, with a trend toward higher diuresis in the MSC-treated group. (B) Creatinine clearance (CrCl) per 100g of kidney, as an estimate of glomerular filtration rate, showing a tendency toward higher values in the treated group at later time points. (C) Fractional excretion of sodium (FeNa), reflecting tubular function over time. (D) Neutrophil gelatinase-associated lipocalin (NGAL) levels in the perfusate, as a marker of renal tubular injury. (E) Perfusate creatinine concentrations over time, reflecting progressive creatinine elimination during perfusion. (F) Perfusate iohexol concentrations over time, used as an exogenous marker of glomerular filtration rate. Data are presented as mean ± SEM (n = 7 pairs). Statistical analysis was performed using a linear mixed-effects model accounting for

repeated measures within kidney pairs. No significant treatment effect or time × treatment interaction was observed for any parameter.

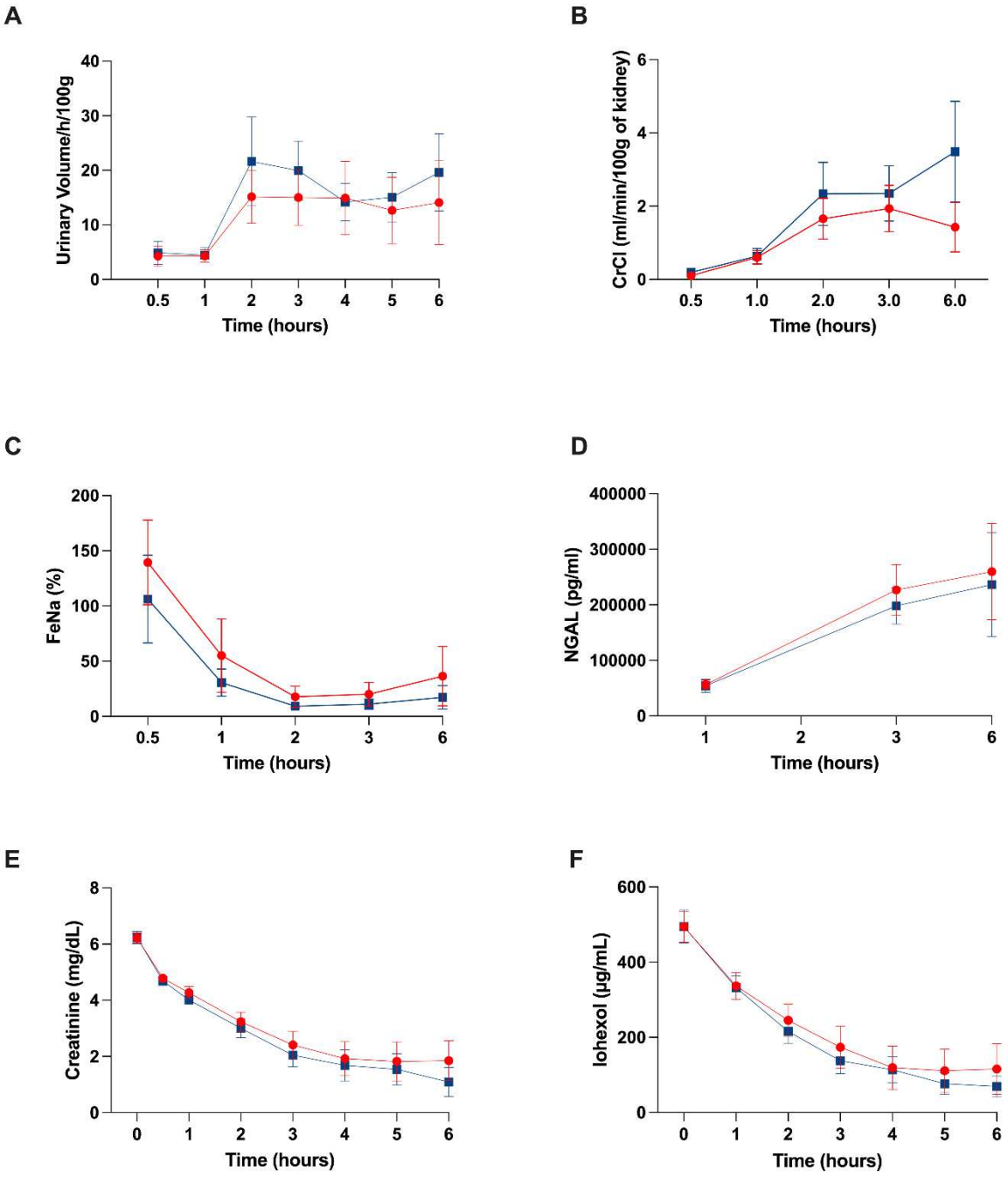


Fig 4. Temporal profile of inflammatory and injury-related biomarkers during NMP expressed as fold change from baseline. Time-dependent changes in perfusate cytokines and injury markers during normothermic machine perfusion (NMP) in MSC-treated and non-treated kidneys, expressed as fold

change relative to baseline (T0). (A), IL-1β ; (B), IFN-γ ; (C), IL-6 ; (D), IL-10 ; (E), IL-18 ; (F), TGF-α ; (G), KIM-1 ; (H), VEGF-A ; (I), TNF-α. Data are presented as mean ± SD. Fold change was calculated relative to baseline values for each kidney. Due to the wide dynamic range across analytes, y-axis scales differ between panels. Comparisons between MSC-treated and control kidneys at each time point were performed using paired Wilcoxon signed-rank tests on log_2-transformed fold change values, with false discovery rate (FDR) correction for multiple testing. No statistically significant differences were observed between groups.

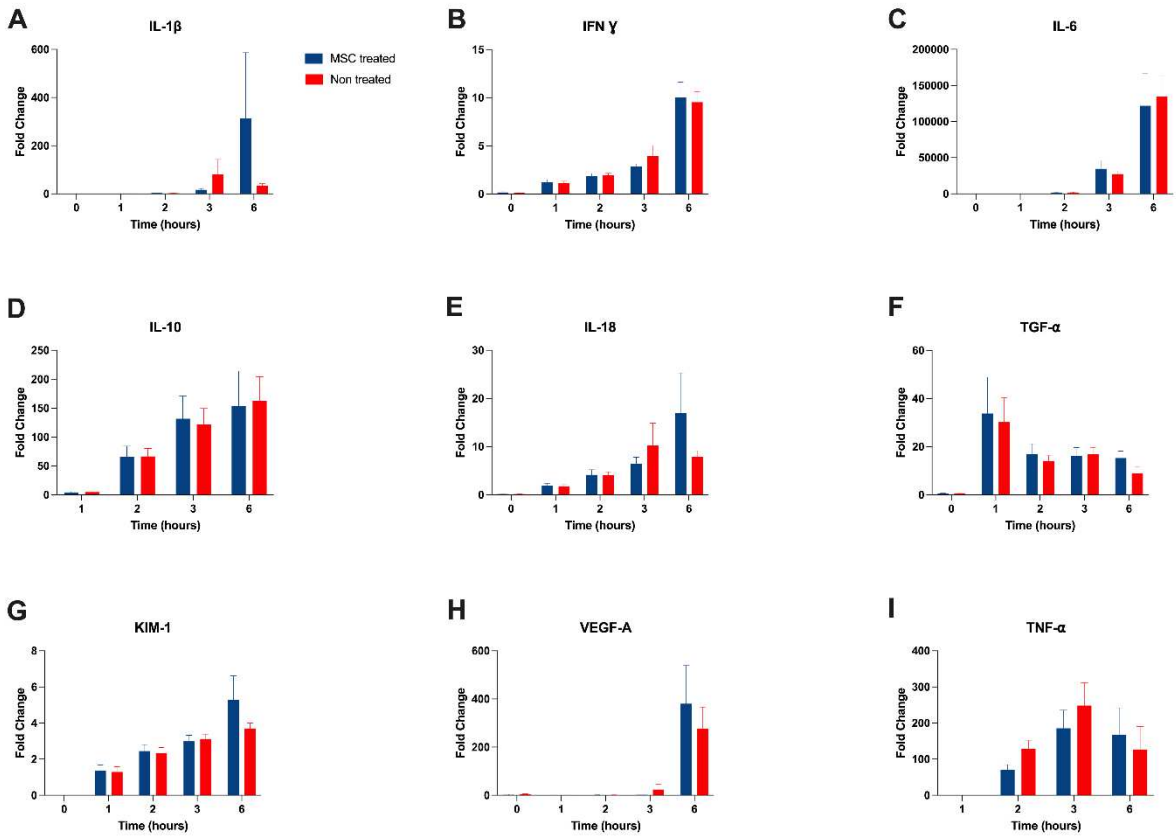
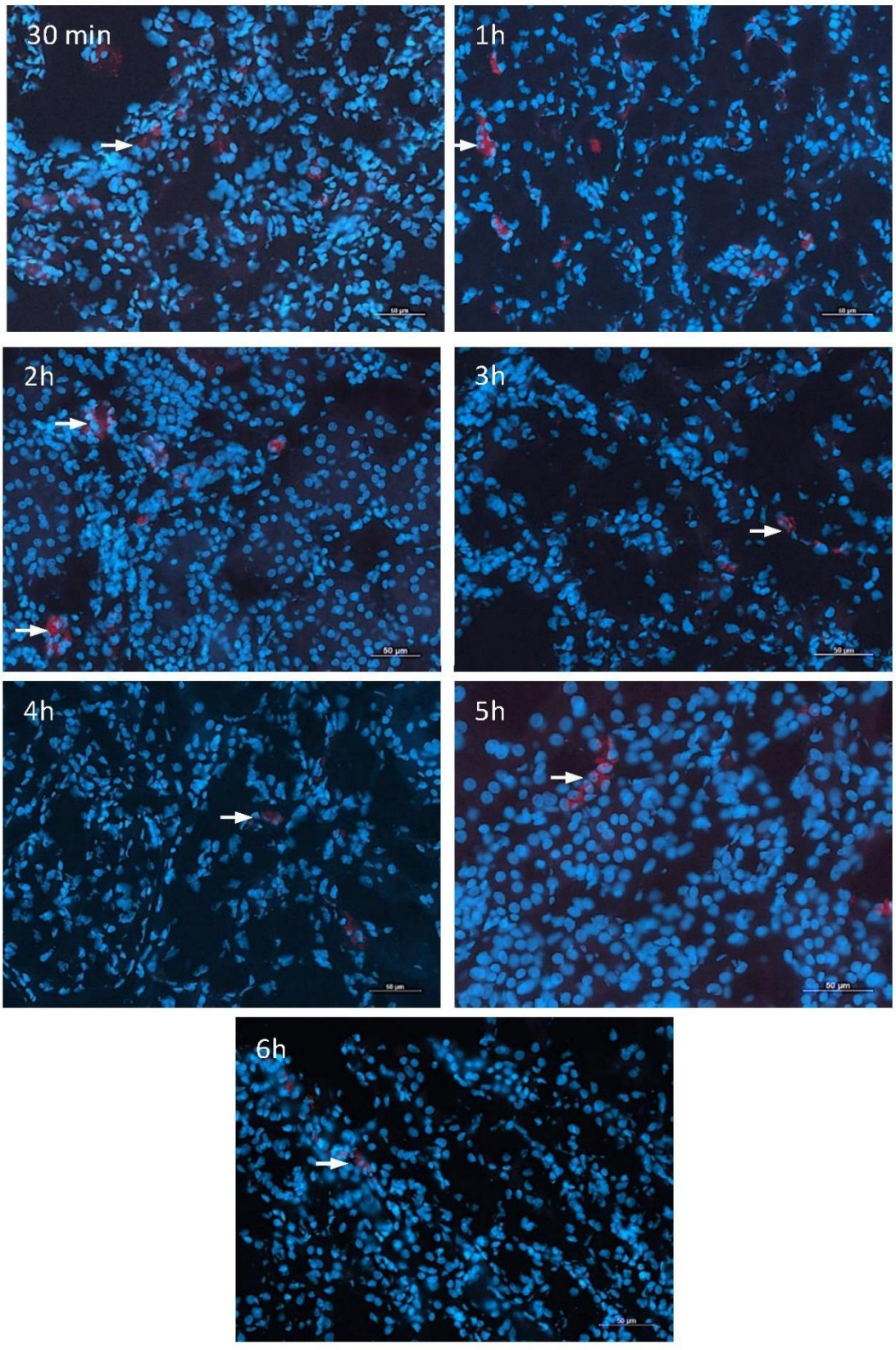


Figure 5. Fluorescence microscopy tracking of hMSCs. Cryosections (5 µm) of renal biopsies collected at 30 minutes, 1, 2, 3, 4, 5 and 6 hours post-infusion were examined by fluorescence microscopy using a Zeiss Axioscan slide scanner. hMSCs prelabelled with CellTracker CMPTX Red (red, white arrows) are visible within the renal tissue counterstained with DAPI (blue). The heterogeneous distribution of labelled cells across tissue sections reflects the inherent variability of the biopsy sampling. Scale bar = 50 µm.



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Figure S1. Representative histological images of chronic lesions in discarded human kidneys (Periodic Acid Schiff staining). (A) Low-magnification view showing multiple arteries with concentric fibrous intimal thickening (cv ≥ 1) leading to vascular luminal narrowing. (B) Intermediate-magnification view showing areas of low-inflammatory fibrosis and tubular atrophy, along with glomeruli exhibiting mild focal and segmental mesangial expansion. Lesions were graded according to the modified Banff classification.

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