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Normothermic Machine Perfusion and Inflammatory Mediators Adsorption: First Kidney Transplant Experience

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Kidney transplantation faces challenges due to the shortage of donor organs, leading to the increased use of extended criteria donor (ECD) organs. Recent advancements in *ex-situ* organ perfusion technologies have facilitated the use of ECD kidneys by preserving organs in near-physiological conditions to tackle ischemia-reperfusion injury (IRI), a process that leads to long-term graft injury. This study focuses on the application of an inflammatory mediators' removal (IMR) integrated in a normothermic machine perfusion (NMP) for the recovery of an ECD kidney before transplantation. This IMR device, designed to adsorb inflammatory molecules, demonstrated effective removal of cytokines during the perfusion process. An ECD kidney underwent 320 minutes of NMP, allowing detailed organ viability assessments and cytokine modulation. A significant volume of urine output and successful post-transplantation outcomes, with no delayed graft function (DGF), highlight the efficacy of this approach. Additionally, the adsorption of inflammatory cytokines was characterized by concentration-dependent removal, suggesting a balanced modulation of both pro- and anti-inflammatory responses. The integration of IMR device into the perfusion process might offer a promising option for evaluating organ viability and mitigating IRI. Further studies are needed to explore the long-term clinical impact of this approach on graft survival and function. *ASAIO Journal* 2025; XX:XX–XX

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The use of extended criteria donor (ECD) kidneys is supported by the introduction of *ex-situ* perfusion techniques.[1] Ischemia-reperfusion injury (IRI) involves various triggers (cytokines, chemokines, complement factors, *etc*.) and inflammatory pathways that can also be targeted during *ex-situ* perfusion leading to an overwhelming inflammation and, consequently, worsening IRI.[2–4] The complex processes involved in this setting can also contribute to the accumulation of the inflammatory mediators in the organ, their endogenous release and an auto-alimented inflammatory loop, resulting in poor transplant outcomes.[5]

The adsorption of inflammatory mediators (AIM) in the perfusate using sorbents has been applied in experimental and clinical *ex-situ* settings for different organs showing and promising results for improved clinical post-transplant outcomes.[3–6]

Overall, the AIM is successfully performed for the treatment of many life-threatening conditions, some also present in the complications in transplant recipients.[7] Hence, we present the first experience using for inflammatory mediators' removal (IMR) exclusively integrated in a normothermic machine perfusion (NMP). The aim of this study was to explore the safety and feasibility of the IMR during NMP for better ECD kidney recovery for transplantation.

Materials and Methods

Donor and Recipient Characterizations

A 75 year old female donor (height, 153 cm; weight, 70 kg), hemodynamically unstable with extreme hypotension, was

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D.P. and R.B. had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. D.P., R.B., P.G.C., S.D.S., I.V., and S.G. contributed to concept and design. D.P., R.B., M.L.P., S.P., P.S., B.B., S.C., F.d.F., S.L.P. contributed to acquisition, analysis, or interpretation of data. D.P., R.B., M.L.P., and A.J. contributed to drafting of the manuscript. D.P., V.A., and M.P. contributed to critical revision of the manuscript for important intellectual content. R.B. and M.L.P. contributed to statistical analysis. D.P., V.A., and S.G. contributed to administrative, technical, or material support. D.P., R.B., and S.G. contributed to supervision.

The data that support the findings of this study are available from ISMETT, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the corresponding (S.G.) and first authors (D.P.). The patient involved in the study provided written informed consent.

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Table 1. Perfusion Solution Composition

Component	Volume
RBC (Ht 60%)	750 ml
Fresh frozen plasma	900 ml
Human albumin 200 g/l (Albuman, Prothya Biosolutions, formerly Sanquin Plasma Products, Amsterdam, the Netherlands)	100 ml
Modified parenteral nutrition (Clinimix N17G35E, Baxter, Deerfield, IL)	7.35 ml
Multivitamins for infusion (Cernevit, Baxter, Heidelberg, Germany)	7 µl (1 cc)
Concentrated trace elements for infusion (Nutritrace, BBraun, Milan, Italy)	7.35 ml
Metronidazole for i.v. administration (5 mg/ml) (Flagyl, Sanofi-Aventis, Toronto, Canada)	40 ml
Cefazolin 1,000 mg flask 5 ml powde for i.v. administration (Servazolin, Biochemie, Florence, Italy)	2 ml
Fast-acting insulin (100 IU/ml) (Actrapid, Novo Nordisk Pharmaceutique S.A., Chartres, France)	20 ml
Calcium gluconate, intravenous solution 10%, 137.5 mg/ml (Sandoz, Mumbai, India)	40 ml
Sterile H_2O	51.3 ml
NaCl 0.9% solution	160 ml
Sodium bicarbonate 8.4% solution	31 ml
Heparin 5,000 IU/ml for i.v.	5 ml
Total volume	2,115 ml

RBC, red blood cells.

admitted 6 days earlier for a cerebrovascular accident. The serum creatinine on presentation was 1.3 mg/dl. Organ procurement was performed according to standard procedures. Both kidneys showed visible and palpable narrowing and irregularity of the cortical surface, without anatomical abnormalities such as fetal lobulations or splenic humps. The right kidney (RK) was considered suitable for transplantation despite the presence of severe aortic atherosclerosis without a renal cortex color uniformity after recovery with suspicious areas of remnant donor blood, such as lividities, and the presence of a double ureter.[2] The discrepancy (Left Kidney Karpinski score 7, RK Karpinski score 3) in the histological evaluation (Supplementary Figure 1, Supplemental Digital Content, https://links.lww.com/ASAIO/B581) was a key factor in the decision to perform NMP and have a more comprehensive assessment of the organ's viability.

The kidney was successfully transplanted (Supplementary Figure 2, Supplemental Digital Content, https://links.lww.com/ASAIO/B582) in a 74 year old woman with end-stage renal disease caused by insulin-dependent diabetes and hypertension and treated with hemodialysis. The postoperative course was uncomplicated. At 6 months follow-up, the patient is alive and well.

Perfusion Protocol

After the back table and 715 minutes of static cold storage, the graft was perfused using PerLife (Aferetica srl, Bologna, Italy) for NMP and integrating the IMR PerSorb device (Cytosorbents Inc., Princeton, NJ). Cannulation was performed with a 10 Fr cannula (Carnamedica, Warsaw, Poland) through the renal artery. Normothermic machine perfusion system was primed adapting the perfusate composition from established NMP liver protocols (Table 1) with target hemoglobin level of 4–7 mg/dl. PerSorb is a polymer-based technology unselectively modulating inflammatory mediators' levels. With a total active surface of major then 45,000 square meters, it adsorbs molecules by means of size exclusion and hydrophobic interactions: small and mid-size hydrophobic molecules up to a size of approximately 55–60 kDa. The unselective adsorption is concentration-dependent. These mechanisms of actions and their efficacy were shown both in experimental and clinical settings in several clinical fields.[6,7] To characterize the IMR effectiveness and efficiency, perfusate samples were collected, pre- and post-PerSorb at the following time points: 60 minutes after the perfusion start (T1), after the stabilization of the perfusion dynamics (pressures, flows), 120 minutes after the perfusion start (T2), 320 minutes after the perfusion start (Tend).

Luminex Assays and proteomic analysis were performed to quantify target molecules (cytokines, chemokines, growth factors, proteins) in the perfusate (Supplementary Figure 3, Supplemental Digital Content, https://links.lww.com/ASAIO/B583).

The total amount (pg) of the molecules removed through the mass balance (MB) calculation was performed according to the following formula:

$\text{MASS BALANCE}_{IL} = (\overline{\text{Cpre}} - \overline{\text{Cpost}}) \times \text{flow} \times \text{time}$, where $\overline{\text{Cpre}} = [(C_x + C_{x+1})/2]_{pre}$ and $\overline{\text{Cpost}} = [(C_x + C_{x+1})/2]_{post}$, respectively, pre- and post-IMR, at t_x and t_{x+1}, flow is the perfusate (in ml/min) in the cartridge and time is duration (in minutes) of the period between t_x and t_{x+1}.

This approach was already reported by several authors for evaluations on the actual effectiveness of the removal, on the possible saturation of the sorbent, and, consequently, on the number of sorbents to be used, as well as on the convenient timing for replacement.[7]

Mass balance offers a more comprehensive understanding of the dynamics involving substance release/generation and removal in the organ, allowing the evaluation interventions like adsorption. It offers the distinction of the components of equilibrium between the input and output of solutes in the organ during the adsorption process. The only evaluation of trends in those settings cannot allow this characterization resulting in misleading for therapy application decisions. The production of inflammatory mediators during the application of purification therapies can apparently diminish the effectiveness of the removal if the only evaluation parameter is the trend over time, because the trend does not show the amount of the removed molecule. This was observed in various studies, who noted the persistence of cytokine activation, which challenges traditional models of inflammation resolution.[8] The cytokines are not necessarily removed uniformly, and their continuous generation during the therapy can wrongly result in diminished effectiveness of cytokine removal.

Table 2. Perfusion Time Table

Perfusion Time (Minutes)	pH	pO_2 (mm Hg)	pCO_2 (mm Hg)	HCO_3 (mmol/L)	Be (mmol/L)	Ht (%)	K+ (mmol/L)	Glucose (mg/dl)	Lactate (mmol/L)	Urine Output (ml)
5	7.48	184.6	11.5	13.0	−14.1	15	3.26	101	4.91	
30	7.56	235.5	9.6	13.7	−13.1	15	2.88	97	6.37	15
60	7.55	138.8	8.7	13.0	−14.3	15	4.06	88	8.03	
90	7.51	151.3	9.7	12.7	−14.5	15	3.72	84	8.53	
120	7.48	166.7	10.0	12.0	−15.4	14	3.45	86	8.97	80
150	7.44	156.2	10.3	11.4	−16.3	14	3.10	82	9.71	100
180	7.42	164.6	10.3	11.0	−16.3	14	2.74	82	10.55	
210	7.40	165.4	10.5	10.8	−17.1	14	2.39	81	12.24	130
240	7.38	195.1	10.2	10.3	−17.7	15	2.38	78	11.50	150
270	7.20	197.4	13.7	8.5	−20.8	21	2.61	110	12.42	
300	7.37	201.5	23.2	15.6	−10.9	21	2.35	105	12.33	160
320	7.45	202.8	19.8	16.6	−9.6	21	2.16	106	14.38	

At the 30 minute mark, K^+ administration (40 mEq) was given to counteract a decrease in potassium levels (3.26 mmol/L at the 5 minute mark, dropping to 2.88 mmol/L at 30 minutes). This intervention helped to stabilize the potassium concentration, though it gradually decreased to 2.16 mmol/L by the 320 minute mark, indicating continued electrolyte loss likely due to urine output. The perfusate pH remained in the alkaline range, particularly during the first 270 minutes (7.48–7.56). After 270 minutes, the pH dropped to 7.20, likely due to rising CO_2 and decreased bicarbonate (8.5 mmol/L). Hematocrit levels remained low throughout the perfusion, around 14–15%, but dropped to 21% by 270 minutes, requiring packed red blood cells transfusion at the 240 minute mark. This was crucial for ensuring oxygen-carrying capacity and preventing hypoxic damage to the graft. Additionally, the pO_2 levels remained sufficiently high (184.6 mm Hg at 5 minutes, up to 202.8 mm Hg by the end), indicating adequate oxygenation of the graft.

pCO2, partial pressure of carbon dioxide; pO2, partial pressure of oxygen.

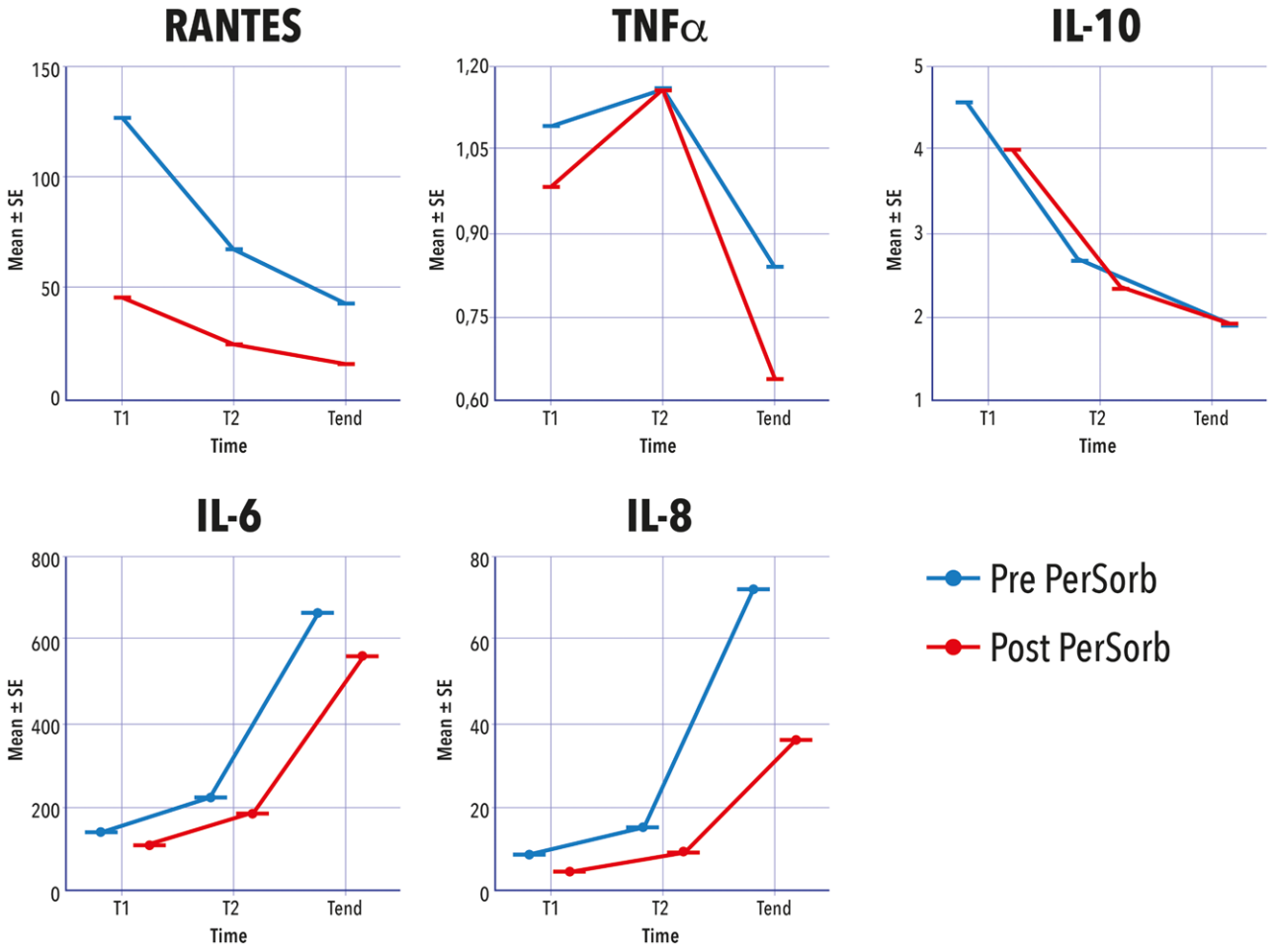

Figure 1. Cytokine profiles. The plots represent the levels of RANTES, TNFα, IL-10, IL-6, and IL-8 in the perfusion liquid, expressed as means of pg/ml (± SE), at three different time points: 60 minutes after the perfusion start (T1) 120 minutes after the perfusion start (T2), 320 minutes after the perfusion start (tend). The major amount of specific cytokines, and particularly, the major concentration represents the pre-IMR, while inferior curves represents the post-IMR values. In the cytokine profile, description is reported the total and specific amount of adsorbed cytokines during the treatment. IL, interleukin; IMR, inflammatory mediators' removal; RANTES, regulated on activation, normal T cell expressed and secreted; TNF-α, tumor necrosis factor alpha.

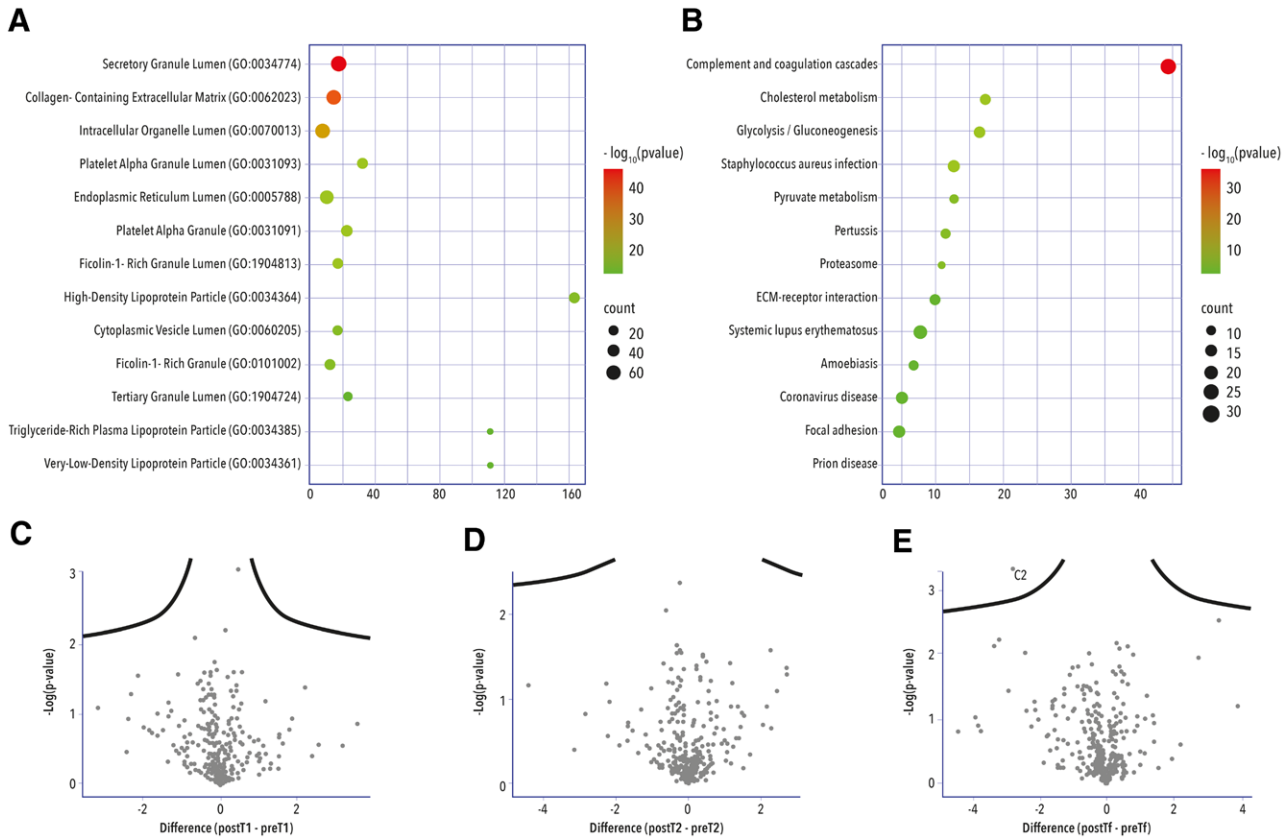

Figure 2. Bubble plot of GOCC analysis. Each sample was analyzed in triplicate, and 50 µg of protein per sample processed with the iST-kit from PreOmics (PreOmics GmbH, Martinsried, Germany) following the manufacturer's protocol. Samples were dissolved in 50 µl of LYSE buffer (PreOmics), with proteins reduced, alkylated, and then digested for 2 hours at 37°C using Lys-C and trypsin. The resulting peptides were washed, eluted from the cartridge, and vacuum-dried. Peptides were re-suspended in LC-LOAD buffer (PreOmics) and sonicated in a water bath. Peptide concentration was measured with a Nanodrop 2000 (Thermo Scientific, Wilmington, DE), and 1 µg of peptides per sample was separated on the Vanquish Neo UHPLC nanoLC system (Thermo Scientific), coupled online to an Exploris 480 mass spectrometer, using a 25 cm × 75 µm Acclaim PEPMap C18 column (Thermo Fisher Scientific). Protein quantification was performed using LFQ. Peptides were separated over a 132 minute binary gradient of water and acetonitrile, each containing 0.1% formic acid. DIA was performed with an MS1 full scan from 400 to 1,200 m/z, followed by 60 sequential DIA windows with 1 m/z overlap and optimized window placement. Full scans were acquired at a resolution of 120,000, with an automatic gain control (AGC) target of 3 × 106 and a maximum injection time of 50 ms. The 60 isolation windows were acquired at a resolution of 30,000, with an AGC target of 8 × 105 and the maximum injection time set to "auto" for optimal cycle time. Fragmentation was achieved with 30% normalized HCD collision energy. Data analysis was conducted using DIA-NN (version 1.8.1) with a predicted library generated from an *in silico*-digested human UniProt reference database (proteome ID UP000005640_9606), allowing for K* and R* cleavages, up to two missed cleavages, and a minimum peptide length of six amino acids. The FDR for peptide and protein identification was set at 0.01%. The plot highlights the 13 most significant GOCC terms derived from the 331 proteins identified through mass spectrometry analysis. The varying color intensities of the bubbles represent the different adjusted *p* values, while the bubble sizes indicate the number of genes associated with each term. **B**: Bubble plot of KEGG analysis. The plot highlights the 13 most significant KEGG pathways derived from the 331 proteins identified through mass spectrometry analysis. **C**–**E**: Volcano plot analysis showing the $\log_2$ of protein ratio between perfusate after and before IMR device at time T1 (**C**), T2 (**D**), and Tf (**E**). The black hyperbolas indicate the FDR and proteins displayed above the FDR curves were considered significantly different between the two conditions (FDR < 0.05). DIA, data-independent acquisition; FDR, false discovery rate; GOCC, gene ontology-cellular component; HCD, higher-energy collisional dissociation; IMR, inflammatory mediators' removal; LFQ, label-free quantification.

Results

Tables 1 and 2 show NMP basic characteristics and dynamics.

Cytokine Profiles

The analysis of cytokine levels revealed a marked increase between the start of the perfusion (T1) and the end (Tend) for interleukin (IL)-6, IL-8, and monocyte chemoattractant protein 1 (MCP-1). Conversely, levels of IL-10, G-colony stimulating factor (CSF), hepatocyte growth factor (HGF), IL-1 receptor antagonist (RA), and Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES) showed a decreasing trend (Figure 1). Other cytokines remained relatively stable with near-physiological values. The heatmap highlights all cytokine levels (pg/ml) at different timepoints (Supplementary Figure 3, Supplemental Digital Content, https://links.lww.com/ASAIO/B583). Cytokines that were present at elevated levels were effectively adsorbed. The MB calculation showed an abnormal cytokine release and consequent adsorption by the IMR device (Figure 1).

Proteomic Analysis

A total of 331 proteins were identified pre- and post-IMR device. Gene ontology (GO) analysis revealed that most of these proteins were located in the "secretory granule lumen" or within the collagen-containing extracellular matrix (Figure 2A). Additionally, kyoto encyclopedia of genes and genomes (KEGG) pathway analysis indicated that a majority of the identified proteins were involved in the complement and coagulation cascade, including complement components (C1R, C1S, C2, C3, and others), several serpins, kallikrein, and kininogen (Figure 2B). Although the Luminex assay revealed significant changes in levels of several cytokines, proteomics showed no significant differences in the dosed proteins at T1 and T2 (Figure 2, C and D). At T3, only C2 was reduced in the perfusate after filtration (Figure 2E). Altogether, these data demonstrate IMR device's specificity for cytokines over other proteins present in the perfusate.

Discussion

The present study reports the use of an IMR device during NMP of an ECD kidney for transplantation. This first preliminary experience was focused on the safety and feasibility of the use of an AIM technology to try to limit the inflammatory burden of the organ before the intervention. Normothermic machine perfusion could provide a real-time evaluation and supports cellular metabolism by perfusing the organ with oxygenated blood at physiological temperature, which is particularly valuable in ECD kidneys undergone warm ischemia. Adding the IMR could furtherly improve the organ viability reducing the inflammation induced by all the organ *ex-situ* management NEXT-Kidney Study demonstrated that NMP is safe, feasible, and potentially beneficial in reducing delayed graft function (DGF) in suboptimal kidneys from circulatory death donors.[4] The high urine output observed during perfusion and the excellent early graft function post-transplant are particularly encouraging signs.[8] Inflammatory mediators' removal resulted in safe and feasible treatment with an unselective, concentration-dependent modulation of inflammatory mediators' levels. In consistence with previously reported experiences, the inflammatory mediators showed increasing trends during NMP. The data about the mass of cytokines adsorbed during the treatment show both the performances of IMR and the amount of inflammatory response expressed by the organ during NMP. The MB allowed to compare pre- and post-sorbent levels, showing the effective removal of target molecules during NMP, with no evidence of the sorbent saturation, even though the cytokine levels raised over time.[6–8]

Our experience showed the huge inflammatory response in the organ during NMP, but it is still to be explored whether this response is a result of the accumulation of the inflammatory mediators during organ *ex-situ* management or it is the endogenous production during NMP. Probably, considering also the previously published experiences and the complex underlying IRI mechanisms, it is a combination of accumulation, release and endogenous production generating an inflammation amplification strengthening IRI. This makes IMR potentially useful also for other organs.

This is a preliminary experience reporting a safe, feasible, and effective application of IMR during NMP to recover an ECD graft and transform NMP in reconditioning, allowing to manipulate organs, and to achieve proper reconditioning. As the inflammatory response has been shown to have a central role on graft function after transplantation, an active adsorption IMR strategy during perfusion is very attractive. The clinical impact of IMR must be furtherly validated in rigorous clinical trials, to extend its application and fully exploit its potential for organ recoveries.

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