

## CASE REPORT

# Forty-eight hours of normothermic kidney preservation applying urine recirculation

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## 1 | INTRODUCTION

End-stage renal disease (ESRD) is a major global health burden. It is projected that 5.4 million people will receive renal replacement therapy (RRT) by 2030 and up to approximately seven million adult people will have died due to lack of access to RRT.<sup>1</sup> The preferable type of RRT for most ESRD patients is kidney transplantation. Due to the evident organ scarcity and the great disparity between transplantable kidneys and patients on RRT and the waiting list, access to kidney transplantation is limited. The transplant community has been working on enlarging the donor pool by recovering and transplanting more marginal organs from extended criteria and donors after circulatory death. Implementing kidney preservation technologies like hypothermic machine perfusion (HMP)<sup>2</sup> and moving kidney viability assessment to a clinically applicable level by normothermic machine perfusion (NMP) of deceased donor kidneys<sup>3</sup> have been relevant measures contributing to increase the supply of transplantable kidneys. In the clinical setting, kidney NMP has been applied for one to two hours before such organs have been either declined or transplanted. Short-term kidney NMP cannot improve transplant logistics and currently, no transportable stand-alone perfusion device enabling long-term preservation is commercially available.

The aim of our perfusion experiment was to preserve a discarded human kidney normothermically for up to 48 h proving the principle of urine recirculation in terms of stable perfusion hemodynamics and perfusate homeostasis according to the protocol published by Weissenbacher et al.<sup>4,5</sup> Achieving long-term kidney preservation might be a key element required for the realization of an international or even global exchange of solid organs to allocate reconditioned and repaired organs, which are discarded otherwise, to the most suitable recipients. Besides possible treatment of the organ, preserving kidneys beyond 24 h offers potential treatment of immunologically complex recipients prior to transplantation.

## 2 | CASE DESCRIPTION

The human kidney graft included in this case report was retrieved for transplant but discarded at the recipient center. The perfusion was performed in the laboratory of the Organ Regeneration Center of Excellence, organLife™, Medical University of Innsbruck. Hemodynamic and biochemical perfusion parameters were analyzed. Perfusate, urine, and biopsy samples were obtained during perfusion. The study was evaluated and approved by the institutional ethics committee (EK Nr. 1216/2019).

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## 2.1 | Preparation of kidney graft, the composition of perfusate, and perfusion set-up

The kidney arrived at the transplant center in an icebox, preserved in Custodiol® HTK (histidine-tryptophan-ketoglutarate) solution, and was placed on the LifePort kidney transporter (Organ Recovery Systems) immediately after arrival. After deemed untransplantable, the organ was taken off the HMP device and prepared for connection to the NMP circuit. For NMP, the Organ Assist Kidney Assist (XVIVO Perfusion) device was used. The renal artery was cannulated with a 10-Fr cannula. The ureter was cannulated with a T-tube to recirculate the urine. The disposable set was adapted by implementing an in-line blood gas analyzer (CDI500, Terumo Medical Corporation). Perfusate temperature was set at 37°C. Oxygenation of the circuit was facilitated by manual regulation of air (21% oxygen) and CO<sub>2</sub>. The perfusion circuit was primed with three units of packed RBCs of the same blood group as the kidney, resuspended in 1000 ml 5% human albumin solution (Octapharma), resulting in a total perfusate volume of approximately 1800 ml; adapted from the protocol published by Weissenbacher et al.<sup>4</sup> Before connecting the kidney, the perfusate was supplemented with 750 mg cefuroxime, 10 ml calcium gluconate, and 8000 IE enoxaparin, low molecular weight heparin (LMWH). For pH adjustment prior to initiation of NMP, 10 ml of sodium bicarbonate 8.4% were added to the perfusate to achieve a physiological pH level of 7.3. Immediately after perfusion start, 5 ml verapamil was administered directly into the arterial line. For glucose and electrolyte monitoring, regular blood gas analyzer measurements were performed and 5ml of total parenteral nutrition (Nutriflex Special, containing 0.24 g/ml glucose) was administered once perfusate glucose levels dropped below 70 mg/dl.

## 2.2 | Kidney characteristics

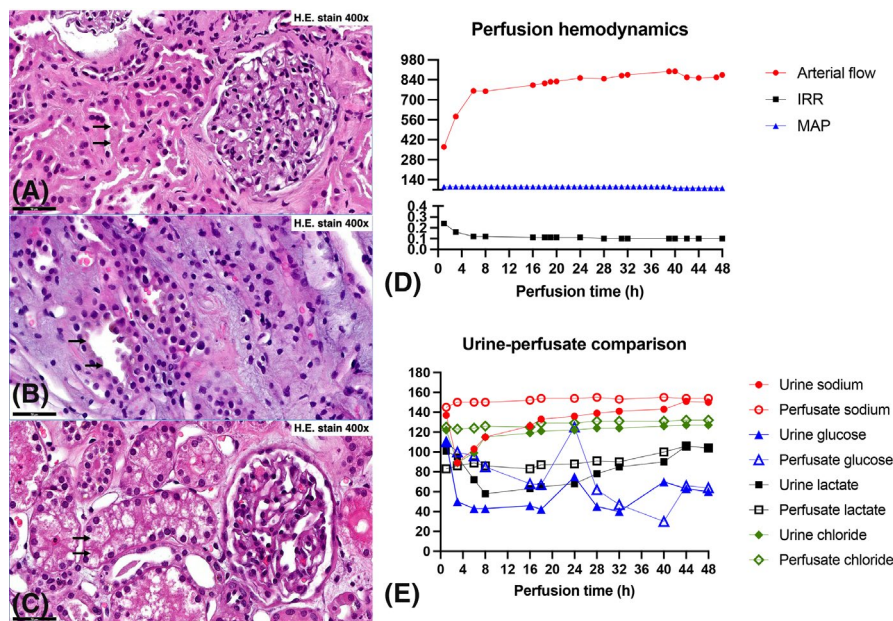
A 62-year-old kidney from a male donation after brain death donor was allocated for experimental long-term NMP after being declined by all transplant centers within Eurotransplant due to a malignant neoplasm of the partner kidney. The cause of death was a cerebrovascular accident caused by a spontaneous intracerebral hematoma, last donor serum creatinine and urea were 0.9 mg/dl and 4.4 mmol/L, the last urine output was 600 ml over 6 h, history of hypertension was unknown and there was no evidence of diabetes mellitus. The kidney donor risk and profile indexes were 1.33 and 78%. Remuzzi score of the zero-biopsy was 0 (Figure 1A). Overall cold ischemia time, until NMP-start, was 27 h and 3 min including 4.5 h on the HMP device.

## 2.3 | Perfusion characteristics

The mean arterial pressure was fixed at 90 mm Hg right from the start of NMP and the perfusate temperature was between 36 and 37°C. The kidney was perfused for 48 h and the urine recirculated. Figure 1B–C is displaying biopsy results after 6 (medulla only) and 48 h. No glomerular and/or tubular necrosis occurred over the 48 h NMP period according to the histological assessment of the whole kidney. Figure 2A–D shows photographs of the perfused kidney 30 min, 6, 24, and 48 h after NMP-start.

Median (IQR) pO<sub>2</sub> and pCO<sub>2</sub> levels were in the physiological range of 113.5 (6.3) and 38 (3.7) mm Hg throughout; median (IQR) pH was 7.44 (0.11). Table 1 outlines hemodynamic and metabolic function parameters. Arterial flow 30 min after NMP-start was 370 ml/min with a corresponding intrarenal resistance (IRR) of 0.25 mm Hg/ml/min. The median (IQR) arterial flow was 850.5 (79) ml/min and the median IRR was 0.11 (0.02) mm Hg/ml/min. At hour 40, the arterial flow reached 900 ml/min and the perfusion device alarmed, without interrupting NMP, as this flow level was the maximum that could be reached by the device. As a consequence, the MAP was decreased and set to 80 mm Hg and perfusion continued without any complications. Figure 1D displays arterial flow, MAP, and IRR per hour, and Figure 2E–I shows photographs of the graphical user interface at 90 min, 6, 24, 40, and 48 h after NMP-start.

Biochemistry parameters of the perfusate before NMP-start were: Hemoglobin 8.8 g/l, potassium 8.9 mmol/L, sodium 146 mmol/L, chloride 125 mmol/L, glucose 126 mg/dl, and lactate 81 mg/dl (normal range 4–14 mg/dl). Median (IQR) perfusate lactate was 88.5 (11.75) mg/dl and similar to median urine lactate with 81.5 (33.8) mg/dl over time;  $p = 0.2$ . Median (IQR) perfusate sodium and chloride were significantly higher with 153.5 (4) mmol/L and 129 (6) mmol/L compared to median urine sodium with 136.5 (24.7) mmol/L and 122 (9.5) mmol/L over time,  $p < 0.001$  and 0.009. Median (IQR) perfusate glucose was insignificantly higher with 67.5 (36.5) mg/dl compared to median urine glucose with 48 (25.3) mg/dl during 48 h of NMP,  $p = 0.047$ . A total of 55 ml TPN was administered during 48 h of NMP, equivalent to an overall amount of 13.2 g glucose. Figure 1E illustrates the differences between sodium, chloride, glucose, and lactate in perfusate and urine per perfusion hour. Anti-Xa levels over time were 10.4 IU/ml after 1, 6.2 IU/ml after 6, 3.9 IU/ml after 24, and 2.1 IU/ml after 48 h of NMP. Hemoglobin level at the end of perfusion was 7.7 g/l and potassium 7.9 mmol/L. Free hemoglobin was rising over time from 0.06 g/dl at hour 1 after NMP-start to 0.14 g/dl at hour 48 and was the reason to terminate the perfusion. Excreted and recirculated urine volume per hour was approximately 22.5 ml until hour 48 of NMP.



**FIGURE 1** (A) Hematoxylin & eosin (H&E) stained zero-biopsy taken (4-mm skin punch biopsy) during back table preparation with a Remuzzi score of 0. (B) H&E-stained biopsy, displaying distal tubules, 6 h after NMP-start; biopsy was taken with a needle biopsy and only medulla was obtained. (C) H&E-stained biopsy (4-mm skin punch biopsy) 48 h after NMP-start; the glomerulus and the proximal tubules are comparable to their status before perfusion starts with unspecific anisometric cytoplasmatic changes. Acute tubular damage is indicated by arrows in (A–C). (D) Perfusion hemodynamics (arterial flow in ml/min, IRR = intrarenal resistance in mm Hg/ml/min, MAP = mean arterial pressure in mm Hg) per hour until the end of NMP at hour 48; (E) Comparison of sodium (in mmol/L), chloride (in mmol/L), glucose, and lactate (in mg/dl) in perfusate and urine over time until the end of NMP at hour 48

### 3 | DISCUSSION

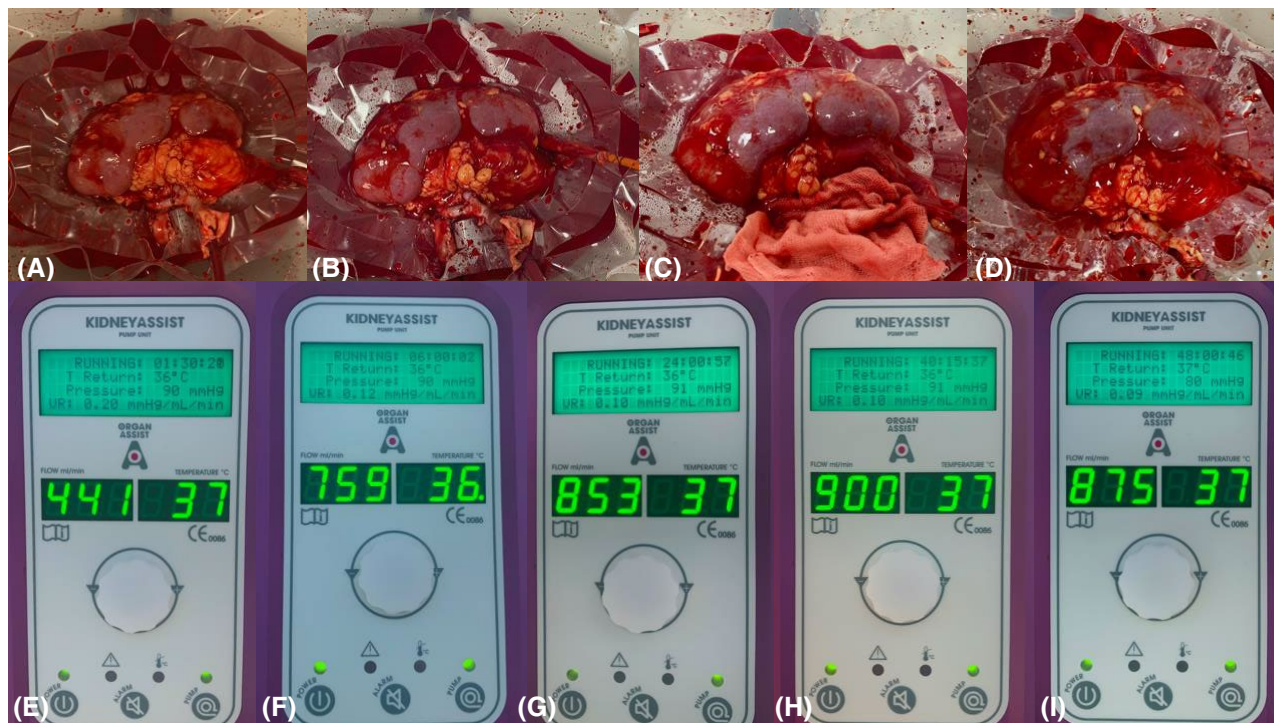
This is the first report of 48 h of normothermic ex-situ kidney perfusion applying urine recirculation to enable stable perfusion hemodynamics and homeostasis. Urine recirculation was chosen in this setting as the feasibility of this technique to control perfusate volume has been successfully demonstrated in 24-h kidney NMP before.<sup>4,5</sup> We considered that the lack of excretory kidney function, as a result of urine recirculation, would be unlikely to have any severe physiological consequences within the 48-h time frame. Comparing the ex-situ environment with an adult and anephric person requiring hemodialysis sessions three times a week, the expected urea accumulation would be insignificant and acceptable in a single-kidney-only milieu.<sup>4</sup> The histology results at 48 h illustrate intact morphological tubular and glomerular structures. There were no signs of hemosiderin deposits, caused by severe hemolysis, and no thrombosis. Some areas of the kidney (Figure 1C) showed unspecific anisometric cytoplasmatic changes of the proximal tubules, which could be an effect of osmotic changes of the perfusate, with increasing sodium and chloride levels (Figure 1E), over time. More distinct tubular vacuolization was described by the Oxford group<sup>4</sup> as a mannitol effect that is causing osmotic imbalance. All of their

24-h kidney biopsies, without mannitol as a supplement, showed normal preserved tubules. The main difference of the work published by the Oxford group,<sup>4,5</sup> using a fully closed circuit with a cannulated renal vein and a soft-shell reservoir, is the open perfusion setting with the renal vein draining directly into the kidney bowl which serves as a reservoir in our case. This open setting might contribute substantially to the increasing amount of free hemoglobin when a flow of approximately 800 ml/min is considered over a full 2-day period. With the current knowledge, it is more difficult to interpret perfusate lactate in the kidney setting compared to the liver as the physiology is different and lactate itself depending on the metabolic state of the organ.

Enoxaparin was the preferred anticoagulant as it can be administered once as a bolus and due to the characteristic that low molecular weight heparin is cleared by the renal route, after primary metabolism in the liver (missing in an isolated ex-situ kidney NMP circuit), known to accumulate in patients suffering from chronic kidney disease. Enoxaparin offers, therefore, the possibility to measure anti-factor Xa activity over time, serving as a potential biomarker.

In summary, it is feasible to perfuse and preserve a human kidney ex-situ normothermally for 48 h. Currently, it is unknown whether a more extended





**FIGURE 2** (A) Kidney immediately after the start of NMP. (B) Kidney at hour 6 after NMP-start. (C) Kidney at hour 24 after NMP-start. (D) Kidney at end of NMP, after 48 hours of perfusion. (E–I) Photographs of the kidney assist graphical user interface displaying perfusion hemodynamics at 90 min, 6, 24, 40, and 48 h after NMP-start

**TABLE 1** Hemodynamic and metabolic parameters

	Hour 1	Hour 6	Hour 12	Hour 18	Hour 24	Hour 30	Hour 36	Hour 42	Hour 48
Arterial pressure in mm Hg	90	90	90	90	90	90	90	80	80
Arterial flow in ml/min	370	762	786	815	853	870	897	858	874
IRR in ml/min/mm Hg	0.24	0.12	0.11	0.11	0.11	0.1	0.1	0.1	0.1
pH	7.47	7.39	7.35	7.45	7.32	7.44	7.42	7.42	7.45
Perfusate lactate level in mg/dl	83	89	83	87	88	90	100	106	104
Urine lactate level in mg/dl	101	72	63	65	68	85	90	106	104
Perfusate glucose level in mg/dl	110	96	68	67	125	47	30	66	64
Urine glucose level in mg/dl	110	43	46	42	75	40	70	63	60
Perfusate sodium in mmol/L	145	150	152	154	154	153	155	154	154
Urine sodium in mmol/L	137	103	126	133	136	139	141	147	150
Perfusate chloride in mmol/L	125	124	125	129	129	131	131	132	132
Urine chloride in mmol/L	122	99	119	121	122	124	125	127	127

criteria donor organ could be preserved effectively for 48 h and this is an important question to answer, as such marginal organs are likely to be the ones requiring extended preservation periods to enable therapeutic interventions in future. Furthermore, more experimental work needs to be dedicated to define the most optimal and physiological perfusate composition to implement long-term ex-situ kidney preservation in the clinical routine.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with the contents of this article.


## AUTHOR CONTRIBUTIONS

Annemarie Weissenbacher: concept and design of perfusion set-up and performing the organ perfusion, data collection and analysis, drafting the article; Franka Messner, Silvia Gasteiger: supporting the perfusion and



data collection; Afschin Soleiman: histological analyses; Dietmar Öfner, Stefan Schneeberger: revision of the article.

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