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THE FIRST EXPERIENCE IN NORMOTHERMIC *EX VIVO* KIDNEY PERFUSION (CASE REPORT)

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Objective: to assess if normothermic *ex vivo* machine perfusion (NMP) of a kidney from an expanded criteria donor (ECD) is feasible and safe. **Materials and methods.** NMP of the right kidney from an ECD was performed on a device developed at Botkin Hospital. A solution based on donor's blood with the addition of Ringer's lactate solution and human albumin was used for perfusion. The temperature in the circuit was maintained at 37 °C. Perfusion lasted for 203 minutes, after which the renal resistive index was almost halved from 0.33 to 0.16. 120 ml of urine was obtained. Results. The right kidney was successfully transplanted after perfusion. There was immediate function of the right renal graft in the postoperative period. The recipient's serum creatinine level was 530 μmol/L on day 1 following transplantation and 170 μmol/L on day 14 of discharge. The left kidney was preserved by static cold storage and further transplanted to the recipient. **Conclusion.** The use of NMP to preserve grafts obtained from ECDs is safe and feasible in clinical practice. Further studies are required to determine the clear indications for its use and to formulate an optimal procedure for its implementation.

Keywords: expanded criteria donor, kidney transplantation, perfusion devices.

INTRODUCTION

Organ transplantation is a remarkable achievement of the 20th century that has prolonged the lives of many thousands of patients. However, a major challenge in this field remains the persistent imbalance between the demand for donor organs and their limited availability [1]. One potential strategy to address this shortage is the use of expanded criteria donors (ECDs) [2]. In the context of kidney transplantation, ECDs are typically defined as donors aged 60 years and above, or those aged 50–59 years who present with at least two of the following comorbidities: cerebrovascular cause of death, a history of hypertension, or a serum creatinine level exceeding 132 μmol/L [3]. Despite this approach, organs from ECDs are often considered suboptimal due to concerns over their pathological condition and uncertainties regarding their functional adequacy post-transplant.

Traditional kidney preservation techniques rely primarily on hypothermia. By lowering tissue temperature, enzymatic activity is significantly slowed, with metabolic rates decreasing by approximately two- to threefold for every 10 °C decrease in temperature [4]. Hypothermia slows down adenosine triphosphate (ATP) depletion in the cell, preventing the breakdown of cellular structures. However, as the duration of cold preservation increases, ATP levels continue to decline, eventually leading to cellular necrosis [4, 5].

Cold ischemia time (CIT) is a well-established independent risk factor for post-transplant organ dysfunction and is closely associated with delayed graft function [6]. This is particularly relevant for organs retrieved from ECDs, which are inherently more susceptible to ischemic damage due to pre-existing risk factors that contribute to graft vulnerability [7–9]. While organ donation programs implement various strategies to minimize CIT – especially for ECD organs – logistical constraints often limit the effectiveness of these efforts. As a result, there is a growing interest in modifying mechanical preservation methods, such as dynamic perfusion, to reduce static cold storage duration and improve graft outcomes.

In the past decade, organ perfusion at subnormothermic and normothermic temperatures has garnered significant research interest. Unlike traditional approaches that suppress cellular activity, normothermic conditions aim to preserve aerobic metabolism and promote the restoration of cellular function. This strategy offers several potential advantages over static cold storage (SCS) and hypothermic machine perfusion, including minimizing or avoiding cold ischemia-induced injury. By maintaining physiologic conditions, normothermic machine perfusi-

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on (NMP) can activate cellular recovery mechanisms and enable functional assessment of donor kidneys.

To explore the clinical applicability of NMP within our hospital setting, we developed a preliminary perfusion protocol. Using this approach, we perfused one kidney from a donor older than 60 years prior to transplantation. Both kidneys were subsequently transplanted into recipients at Botkin Hospital. The clinical details and outcomes of this case are presented below.

CLINICAL CASE

Donor characteristics

The donor was a 65-year-old male who succumbed to a traumatic brain injury. He remained in the intensive care unit for 55 hours prior to organ procurement. During this period, there were no episodes of circulatory arrest or hypotension. Peak vasopressor support did not exceed 350 ng/kg/min. Initial laboratory values showed a urea level of 4.3 mmol/L and a serum creatinine level of 92.0 μmol/L.

Following the declaration of brain death, the heart, liver, and both kidneys were retrieved. Examination of the right and left kidneys revealed medium-sized organs with a homogeneous color and no tumor-like formations. Each kidney had a single renal artery originating from the aorta, which exhibited atherosclerotic changes, and a single renal vein. Both kidneys were deemed suitable for transplantation and were subsequently transported to Botkin Hospital.

Kidney preservation under normothermic machine perfusion

The NMP circuit was developed at Botkin Hospital and is based on a Maquet heart-lung machine equipped with a roller pump and a Skipper AF Plus oxygenator (Eurosets). A custom-designed perfusion container, created using 3D modeling, was used to accommodate the kidney during NMP. A view of the right kidney within this container during perfusion is shown in Fig. 1.

The perfusion circuit was primed with 1200 ml of perfusate composed of donor blood collected prior to in situ cold perfusion (800 ml), Ringer's lactate solution, and human albumin, adjusted to achieve a target hematocrit of 15–25%. The right kidney graft was positioned in the perfusion container and connected to both arterial and venous circuits. A thin venous catheter was inserted into the ureter to facilitate urine drainage. Synthetic glucocorticoids, heparin, glucose, insulin, prostaglandin E1, amino acid solution, antibiotics, and sodium bicarbonate were injected into the circuit during perfusion. The perfusion temperature was maintained at 37 °C using a stationary Maquet temperature control device.

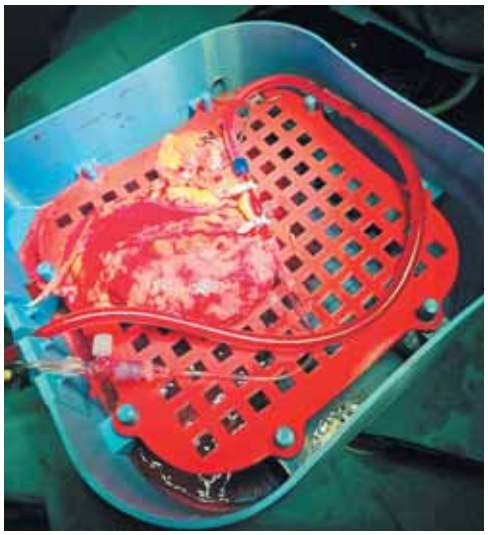

Fig. 1. View of a donor kidney in a normothermic perfusion container

Arterial blood samples were taken periodically to monitor acid–base balance, electrolyte levels, and other biochemical parameters. Renal artery pressure was continuously measured and displayed on a separate monitor. Perfusion pressure increased from 90 mmHg at the start to 130 mmHg by the end of the procedure. Urine output was quantitatively measured throughout the perfusion.

The interval between initiation of in situ cold perfusion during organ retrieval and commencement of ex vivo NMP was 300 minutes (5 hours). NMP lasted for 203 minutes. Details are presented in Table 1.

At 15 minutes after initiation of perfusion, urine output was 10 ml. Over the full duration of perfusion, a total of 120.0 ml of urine was produced. Clinical and biochemical analysis of the urine was performed, as detailed in Table 2.

Upon completion of NMP, the right kidney was deemed suitable for transplantation and was successfully transplanted into the recipient. The left kidney was transplanted without undergoing additional perfusion.

Result of zero-time kidney biopsies

The right kidney biopsy revealed 15 glomeruli, which appeared anemic and ischemic. Tubular structures demonstrated granular protein dystrophy and epithelial cell necrosis. Arterioles and interstitium showed no pathological features. The left kidney biopsy contained 7 glomeruli, one of which was globally sclerotic. Several glomeruli showed signs of anemia and ischemia. Tubules exhibited granular protein dystrophy and epithelial cell necrosis. Two muscular arteries and the arterioles were without pathological findings. Microfocal interstitial sclerosis was observed.

Conclusion: Findings are consistent with mild to moderate acute tubular necrosis in both kidneys.

Right kidney transplant recipient: *The recipient was a 52-year-old woman with end-stage CKD secondary to polycystic kidney disease. She had been undergoing renal replacement therapy (RRT) via hemodialysis since 2019 and was placed on the transplant waiting list on January 9, 2020. Kidney transplantation was performed on October 10, 2023.*

Cold kidney storage lasted 300 minutes prior to NMP and 132 minutes following NMP. Upon restoration of blood flow, there were no visible signs of reperfusion syndrome, and urine outflow via the ureter was observed.

A Doppler ultrasound scan performed immediately post-transplantation revealed a resistive index (RI) of 0.75 (see Fig. 2).

Table 1

Parameters of normothermic *ex vivo* kidney perfusion

Measurement time points / Perfusion parameters	Donor blood from the bag (before perfusion)	0 min, start of perfusion	15 min	45 min	90 min	180 min
Renal artery pressure, mmHg		60	90	96	134	130
Perfusion rate, mL/min		180	400.0	430.0	800.0	800.0
Perfusion pressure/rate ratio, resistive index		0.33	0.22	0.22	0.16	0.16
Perfusion temperature, °C		–	37.0	37.1	37.1	37.0
Diuresis, mL		–	10.0	–	30.0	80.0
pH	6.83	6.72	7.22	7.48	7.56	7.99
pO_2, mmHg	153.0	190.0	180.0	197.0	278.0	293.0
pCO_2, mmHg	120.8	34.4	16.8	9.3	9.4	7.0
K^+, mmol/L	2.6	3.0	2.9	2.8	3.0	3.3
Na, mmol/L	153.0	152.0	149.0	151.0	154.0	162.0
BE, mmol/L	–13.0	–30.0	–21.0	–17.0	–14.0	2.0
Hct, %	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0
Glucose, mmol/L	35.2	10.7	8.3	8.3	9.4	15.0
Urea, mmol/L			2.0			1.7
Creatinine, μmol/L			44.0			44.0

Note: pO_2, partial pressure of oxygen; pCO_2, partial pressure of carbon dioxide; K^+, potassium; Na, sodium; BE, base excess; Hct, hematocrit.

Table 2

Analysis of a urine sample produced by kidney during *ex-vivo* normothermic perfusion

Parameters	120th minute of perfusion
Urinalysis	
Color	Light yellow
Transparency	Full
Specific weight	1.018
pH	5.5
Glucose, mmol/L	not detected
Protein, g/L	0.1
Ketone bodies, mmol/L	not detected
Urobilinogen, mmol/L	3.4
Leukocytes, count/μL	15.0
Epithelium, per field of view	0–5
Erythrocytes, per field of view	40.0
Cylinders, per field of view	0
Urine chemistry	
K^+, mmol/L	49.0
Na^+, mmol/L	62.0
Albumin, mg/L	573.72
Creatinine, μmol/L	181.0

Note: K^+, potassium; Na, sodium.

Urine output on postoperative day 1 was 600 mL. Postoperative hemodialysis was not required. By the time of discharge on postoperative day 14, daily urine output had increased to 1900 mL. Laboratory values at discharge showed a blood urea level of 25.1 mmol/L and a serum creatinine level of 170 μmol/L (see Table 3).

Left kidney transplant recipient (non-perfused): *A 54-year-old woman with end-stage CKD secondary to chronic glomerulonephritis and nephrosclerosis. She had been receiving RRT via hemodialysis since September 2021 and was placed on the transplant waiting list on May 13, 2022. Her first kidney transplantation was performed on November 18, 2022; however, the graft was removed on postoperative day 7. A kidney re-transplantation was successfully performed on October 10, 2023, with evidence of primary graft function. On postoperative day 1, serum creatinine was 752 μmol/L and blood urea was 20.6 mmol/L. Urine output reached 600 mL within the first 24 hours. No postoperative hemodialysis was required. At the time of discharge on day 14, the patient had a daily urine output of 1900 mL, a blood urea level of 25.1 mmol/L, and a serum creatinine level of 170 μmol/L (see Table 3).*

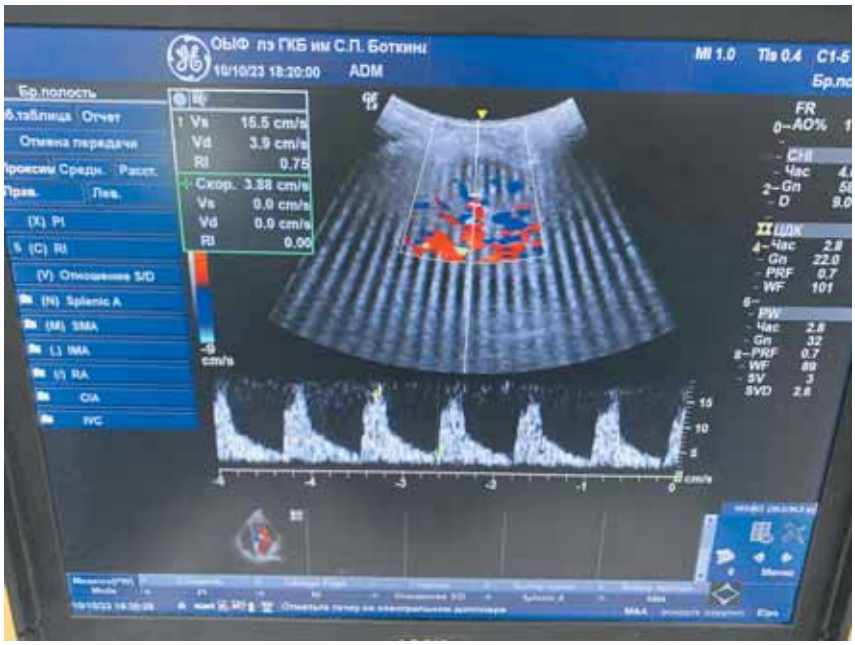

Fig. 2. Doppler ultrasound of the right kidney graft upon completion of transplantation to the recipient

Table 3

Characteristics of right kidney recipient

Characteristics	Right kidney recipient
Gender, male/female	Female
Age, years	52
Diagnosis	Stage 5 CKD, Polycystic kidney disease
Start of hemodialysis	2019
Number of HLA-A, B, Dr mismatches	4
Total cold ischemia time, min	432.0
Resistive index RI, at the end of surgery	0.75
RI, day 1	0.8
RI, day 7	0.76
RI , at discharge	0.67
Graft function	Primary
Number of hemodialysis sessions after transplantation	0
Urea/creatinine, day 1, mmol/L, μmol/L	14.1/530
Urea/creatinine, day 6, mmol/L, μmol/L	26.7/300
Urea/creatinine at discharge, mmol/L, μmol/L	25.1/170
Diuresis, day 1, mL	600
Diuresis at discharge, mL	1900
Inpatient stay, bed days	14

Note: RI, resistive index.

DISCUSSION

NMP represents a paradigm shift in donor organ preservation, offering the dual advantage of organ recovery and real-time functional assessment prior to transplantation. This report presents the first documented case of normothermic kidney perfusion (NKP) in clinical practice in Russia. The choice of a donor aligns with the current international strategy of using NMP for ECDs and donors after circulatory death. In this case, the donor was a 65-year-old individual who died from traumatic brain injury, with biochemical markers of renal function within normal reference ranges.

The perfusion circuit, composition of the perfusate, and perfusion protocol are similar to those described by Nicholson and Hosgood [10], leading experts from the United Kingdom with extensive experience in NKP. In 2013, Nicholson and Hosgood [10] published the results of the first clinical application of NMP. Between December 2010 and August 2012, they subjected 18 kidneys

from ECDs to NMP. The transplant outcomes were compared with a control group of 47 recipients who received kidneys from ECDs preserved using static cold storage between March 2008 and August 2012 at the same transplant center. Both groups were comparable in terms of donor and recipient age, cold ischemia time, and were limited to first-time kidney transplant recipients.

During perfusion, renal blood flow was continuously monitored. The intrarenal resistive index (RI) was calculated as the ratio of mean arterial pressure to perfusion rate, measured every 5 minutes during the first 15 minutes and then every 15 minutes until the end of perfusion. Total urine output was recorded, and blood gas analysis was performed before and after perfusion to assess acid-base balance [10].

In our case, kidney perfusion was initiated at an arterial pressure of 60 mmHg, which gradually increased throughout the procedure, reaching 130 mmHg by the 180th minute. The perfusion rate also rose progressively, from 180.0 ml/min to 800.0 ml/min. RI decreased almost twofold during the perfusion, from 0.33 to 0.16. The temperature within the perfusion circuit was consistently maintained at 37 °C.

Nicholson et al. [10] reported more moderate perfusion parameters, maintaining mean arterial pressures between 52 and 70 mmHg during NMP. While all kidneys in their study demonstrated some fluctuations in RI during the initial 15 minutes of perfusion, a general downward trend in RI was observed. The authors found a statistically significant correlation between RI and donor age ($p = 0.027$), as well as between RI and reduced urine output during perfusion ($p = 0.035$).

Similar perfusion characteristics were reported by Canadian authors in their publication detailing the first clinical experience with normothermic perfusion in North America [11]. In their protocol, arterial pressure was initially set at 75 mmHg and maintained at 65 mmHg by adjusting the centrifugal pump speed. At the start of perfusion (0 hour), median renal artery blood flow was 279 mL/min (range: 60–547 mL/min), which increased over time. After one hour of perfusion, median flow had risen to 346 mL/min (range: 206–680 mL/min).

In our case, the partial pressure of oxygen (pO_2) at the beginning of NMP was below 200.0 mmHg (measured at 190.0 mmHg) and progressively increased throughout the procedure, reaching 293.0 mmHg by the end. The perfusate was prepared using the donor's blood, which was transported in a blood collection bag. Initial analysis of the acid-base status of the perfusate revealed severe acidosis, with a pH of 6.83, markedly elevated pCO_2 (120.8 mmHg), significant base deficit, and extremely high glucose levels. These findings are consistent with anaerobic conditions during donor blood preservation.

Most of these abnormalities were rapidly corrected following the initiation of perfusion, owing to the combined effects of the roller pump and oxygenator, as well as the introduction of sodium bicarbonate solution, potassium chloride to correct hypokalemia, and short-acting insulin to manage hyperglycemia. However, a persistent base (alkali) deficit was observed throughout most of the perfusion, despite repeated sodium bicarbonate administration. We hypothesize this may be due to impaired hydrogen ion excretion and bicarbonate reabsorption, indirectly indicating suboptimal renal function during perfusion – though this hypothesis requires further investigation.

Urine output was first noted at the 15th minute of perfusion. Due to the absence of further active diuresis, furosemide was introduced into the circuit, resulting in a positive diuretic response.

Overall, analysis of both hemodynamic and metabolic parameters during perfusion underscores the importance of strict adherence to protocol. Introduction of drugs into the circuit should be performed through the designated perfuser to prevent abrupt fluctuations in perfusate composition, especially during extended perfusion sessions. In this case, NMP lasted for 203 minutes.

In the study by Mazilescu et al. [11], 13 human kidney grafts were perfused for a median duration of 171 minutes (range: 44–275 minutes). One of the notable findings was the consistently high oxygen level in the perfusate, with a reported median pO_2 of 562 mmHg. A single dose of bicarbonate solution was administered, following which the pH remained stable throughout the perfusion period. Urine output was not observed in 2 of the 13 cases. The authors highlighted a high degree of variability in urine production across the cohort, with a median of 16 mL and a range of 1–104.5 mL during perfusion.

In our study, lactate levels in the perfusate were not measured. However, Mazilescu et al. [11] reported that lactate concentrations remained relatively stable during perfusion, with a median value of 11.6 mmol/L at baseline (range: 7.9–15.25 mmol/L) and 10.13 mmol/L at the end of perfusion (range: 3.06–15.6 mmol/L). These findings suggest that stable lactate levels, despite being relatively high, may be indicative of satisfactory renal perfusion. The authors found no significant differences in perfusion characteristics between grafts that developed delayed graft function and those with immediate (primary) function. Specifically, renal blood flow and intrarenal RI at baseline (313 *vs.* 260 mL/min, $P = 0.23$; RI 0.25 *vs.* 0.31, $P = 0.41$) and at the end of perfusion (550 *vs.* 372 mL/min, $P = 0.12$; RI 0.14 *vs.* 0.19, $P = 0.12$) were comparable between the two groups. Similarly, perfusate parameters, including pH, lactate, pO_2, pCO_2, and urine production during perfusion did not differ significantly

and showed no correlation with post-transplant graft function or urine output.

It is also worth noting that Mazilescu et al. used a perfusate based on dextran/albumin (Steen solution) supplemented with red blood cells – a composition commonly used in *ex vivo* lung and liver perfusion studies [12, 13]. This solution provides high oncotic pressure, which may account for the generally low urine output observed during perfusion. The authors highlight that future research should shift focus toward analyzing the composition of urine produced during perfusion, rather than volume alone, as a more meaningful indicator of post-transplant kidney function.

CONCLUSION

Our initial experience with normothermic perfusion of a donor kidney demonstrated the safety and technical feasibility of this technique in clinical practice. Moving forward, there is a need to develop a structured study protocol to assess the applicability and effectiveness of NMP in kidneys retrieved from donors following an out-of-hospital cardiac arrest.

The authors declare no conflict of interest.

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