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Normothermic Ex Vivo Kidney Perfusion for Human Kidney Transplantation: First North American Results

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Background. Normothermic ex vivo kidney perfusion (NEVKP) has shown promising results for preservation, assessment, and reconditioning of kidney allografts in preclinical studies. Here, we report the first North American safety and feasibility study of deceased donor kidneys grafts transplanted following preservation with NEVKP. **Methods.** Outcomes of 13 human kidney grafts that received 1 to 3 h of NEVKP after being transported in an anoxic hypothermic machine perfusion device were compared with a matched control group of 26 grafts that were preserved with anoxic hypothermic machine perfusion alone. **Results.** Grafts were perfused for a median of 171 min (range, 44–275 min). The delayed graft function rate in NEVKP versus control patients was 30.8% versus 46.2% ($P=0.51$). During the 1-y follow-up, no differences in postoperative graft function, measured by serum creatinine, necessity for dialysis, and urine production, were found between the study group and the control group. There were no differences in 1 y posttransplantation graft or patient survival between the 2 groups. **Conclusions.** Our study demonstrates the safety and feasibility of NEVKP for human deceased donor kidney transplantation. Further studies are warranted to explore how this technology can minimize cold ischemia, improve posttransplant graft function, and assess and repair expanded criteria kidney grafts.

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INTRODUCTION

Kidney transplantation offers better outcomes than dialysis as a kidney replacement treatment for patients with kidney failure, resulting in improved survival and quality of life.[1,2] To address the increasing gap between demand and supply of kidneys for transplantation,[3] grafts from donation after circulatory death (DCD) donors and expanded criteria donors (ECDs) have been increasingly used for transplantation.[4]

Although the benefits of kidney transplantation using ECD and DCD grafts still outweigh the risks of

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long-term dialysis, several trials have shown that these kidneys tolerate static cold storage (SCS) poorly, with a higher rate of delayed graft function (DGF), primary nonfunction (PNF), and premature graft loss.[4-6] Recent preclinical studies in porcine models have demonstrated that reducing cold storage by normothermic ex vivo kidney perfusion (NEVKP) reduces graft injury and improves kidney function after kidney transplantation.[7-11] In human lung and liver transplantation, the potential benefits of normothermic ex vivo perfusion for organ preservation and assessment have been clearly demonstrated.[12,13]

Although elimination or minimization of cold storage in clinical kidney transplantation is currently limited by a lack of portable NEVKP devices that can be transported to the site of deceased donor organ procurement, a period of normothermic perfusion at the end of cold storage may be useful for graft reconditioning, assessment, and repair before transplantation. Hosgood et al have demonstrated the beneficial effects of this approach in an animal model as well as in a pilot clinical trial.[14-16]

Here, we report the first North American results of a safety and feasibility study of NEVKP for human kidney transplantation.

MATERIALS AND METHODS

Study Design

The current study was designed as a pilot study to assess the safety and feasibility of NEVKP for human deceased donor kidney transplantation. Before the beginning of the trial, the study was registered at clinicaltrials.gov (NCT03136848). All transplants were performed at the Ajmera Transplant Program, Toronto General Hospital. Between November 2017 and January 2020, all deceased donor kidney grafts (including DCDs, ECDs, and standard criteria donor kidneys) that were considered transplantable with conventional cold perfused storage were eligible for NEVKP. Kidneys from donors with evidence of hepatitis B, hepatitis C, or grafts with multiple renal arteries were excluded from the study. All waitlisted kidney transplant candidates aged 18 y or older who had provided written informed consent before the organ offer were eligible for participation in the study. Recipient candidates waiting for multiple organ transplants or retransplantation and highly sensitized recipients with a calculated panel reactive antibodies >99% were excluded from the study. The study was approved by the research ethics board of the Toronto General Hospital (REB number 15-9907). A historic control group that received only anoxic hypothermic machine perfusion (HMP) was retrospectively matched 2 to 1. Matching was done on the basis of donor type (DCD or donation after neurological death), donor age (difference <6 y), recipient age (difference <5 y), cold ischemia time (CIT), and donor province. To achieve a well-matched control group of adequate sample size, we included transplants performed between 2012 and 2020. Similar to the NEVKP group, recipients with retransplantation, multiorgan transplantation, multiple arteries, and calculated panel reactive antibodies >99% were excluded. Written informed consent was obtained from all patients who received a graft subjected to NEVKP. For the control group, the research ethics board waived the need for consent.

Setup and Priming of the Device

For the clinical study, we adapted our preclinical NEVKP system, which has been previously described.[7-9] As a perfusion device, we used an S3 heart-lung machine and a neonatal cardiopulmonary bypass machine (Biomedicus pump console from Medtronic). The device includes a Revolution centrifugal pump, a Dideco Kids D100 neonatal oxygenator, a venous reservoir, a Dideco Kids D10 40-μm neonatal arterial filter, and polyvinyl chloride tubing (LivaNova Canada Corp, Markham, ON, Canada). In addition, a heat exchanger (ParaTherm heater cooler from Chalice Medical) was added to the system. A closed system was used for the perfusion, so both the vein and the artery were cannulated. The perfusate solution contained 215 mL of dextran/albumin solution (Steen Solution, XVIVO Perfusion AB, Goteborg, Sweden), 400 mL of packed red blood cells, 2 mL of calcium gluconate 10%, 1300 U/L heparin, and 400 mg of cefazolin. Oxygen/carbon dioxide gas (95%/5%; 2 L/min) was provided continuously during perfusion, resulting in pO_2 levels of 650 mm Hg during the entire preservation time. Also, verapamil and an infusion of amino acids, glucose, and insulin were administered continuously during perfusion. To account for the loss of circulating volume because of urine production, Ringer's lactate was continuously infused in the perfusate with a rate of 10 mL/h.

"Back-to-Base" NEVKP

Donor kidneys were flushed in situ with cold University of Wisconsin solution, procured using standard techniques, and placed on LifePort HMP for transport. Upon arrival of the kidney to the Toronto General Hospital, HMP was terminated, and a standard back-table preparation of the allograft was performed. The renal vessels and ureter were cannulated and then connected to the previously primed circuit, and NEVKP was initiated. The recipient was brought to the operating room, and surgery was initiated according to standard practices. Once the iliac vessels were prepared for implantation of the graft, NEVKP was terminated, and the kidney was flushed with cold University of Wisconsin solution until the effluent became clear. Following this, the graft was immediately implanted and reperfused in the recipient according to standard procedures.

Perfusion Characteristics

Perfusion characteristics were monitored continuously. Our system includes a centrifugal pump, so the grafts were perfused with continuous pressure. By adjusting the rate of the centrifugal pump, arterial pressure was initially set at 75 mm Hg and maintained at 65 mm Hg. Venous pressure was maintained at approximately 0 mm Hg by adjusting the height of the reservoir relative to the kidney. The perfusion temperature was maintained at 37 °C during the entire procedure. Blood gas analyses were performed every 30 min. Urine production was assessed hourly; however, the urine produced during NEVKP was not recirculated in the perfusate. A perfusate sample was taken for culture at the end of the perfusion.

Donor and Recipient Perioperative Characteristics

Donor type, age, sex, body mass index (BMI), cause of death, history of diabetes, and creatinine before retrieval were documented at the time of donation. For the recipients, the following preoperative data were collected: age, sex, BMI, preoperative biochemical profile, underlying cause of kidney disease, dialysis type and duration, and history of medical comorbidities, including hypertension, cardiovascular disease, diabetes, dyslipidemia, and systemic illness. Intraoperative CIT and warm ischemia time were also documented.

Recipient Outcome Data

Recipient variables of the NEVKP and control groups were collected from the research database of our center and analyzed for short-term and long-term outcomes.[17] PNF, DGF, and 1-y death-censored graft and patient survival were assessed. DGF was defined as the need for at least 1 session of dialysis within the first week after transplantation. PNF was defined as lack of function of the transplanted graft with the need for dialysis. Kidney function was determined by serum creatinine and estimated glomerular filtration rate (eGFR) in the absence of dialysis. For the calculation of eGFR, the Chronic Kidney Disease Epidemiology Collaboration equation was used.[18] Postoperative hospital length of stay was recorded. Complications were graded according to the Clavien-Dindo score.[19] CIT did not include the time spent on NEVKP.

Immunosuppression Protocol

Immunosuppression was administered according to our institutional standard protocols for kidney transplant recipients and was not modified for patients who received grafts that had been exposed to NEVKP. The regimen consisted of induction with either basiliximab or Thymoglobulin and maintenance immunosuppression with tacrolimus or cyclosporine, steroids, and mycophenolate mofetil. Antimetabolite drugs were used in a calcineurin inhibitor–sparing regimen when there was concern for calcineurin inhibitor–induced neurotoxicity or nephrotoxicity, as well as in candidates who were considered to be at high risk for rejection.

Statistical Analysis

Statistical analyses were performed with R software (version 1.1.463). Descriptive statistics were calculated (mean, median), and relevant variables were compared between study groups using the *t* test, Kruskal-Wallis test, and Fisher exact test, when appropriate. Continuous variables that were normally distributed were summarized as mean ± SD, and the *t* test was used for comparing groups. In the case of nonnormally distributed data, the median (interquartile range), the Kruskal-Wallis test was used for analysis. In the case of categorical variables, the Fisher exact test was used for examining differences between the 2 groups. The Kaplan-Meier estimator was used to calculate cumulative survival probabilities, and the log-rank test was used to compare the survival functions. Patient and graft survival were calculated from the time of kidney transplantation. A 2-sided *P* value <0.05 was considered statistically significant.

RESULTS

Thirteen organs were included in the perfusion group. This group was matched to a control group of 26 grafts, which were preserved with anoxic HMP alone. All patients had at least 1 y of follow-up posttransplant.

Donor Characteristics

Donor type, age, BMI, cause of death, and creatinine before retrieval were similar between the NEVKP and the control group (Table 1). There were more male donors in the NEVKP group. Seven grafts in the NEVKP group and 14 grafts in the control group were retrieved from DCD donors (53.8%).

Perfusion Characteristics

Median perfusion time was 171 min (44–275 min). Graft perfusion was performed while the recipient was prepared for surgery, and perfusion was stopped when the recipient was ready for transplantation. No technical problems occurred during machine perfusion. Median renal artery flow was 279 mL/min (range, 60–547 mL/min) at the beginning of perfusion (0 h) and increased during the duration of perfusion (median flow at 1 h was 346 mL/min; range, 206–680 mL/min; Figure 1). Median arterial pO_2 levels were 562 mm Hg (median 0 h, 585 mm Hg; range, 500–616 mm Hg; median 1 h, 588 mm Hg; range, 474–638 mm Hg; median 2 h, 573 mm Hg; range, 474–628 mm Hg; median 3 h, 503 mm Hg; range, 461–626 mm Hg). Because the perfusate was acidotic at the beginning of perfusion (median pH 0 h, 7.10; range, 6.68–7.33), 8.4% sodium bicarbonate was added to the perfusate to correct the pH (mean, 6.4 mL ± 4.7 mL). After the initial phase, pH values remained stable (median pH 1 h, 7.32; range, 7.25–7.38; median pH 2 h, 7.37; range, 7.33–7.46; median pH 3 h, 7.4; range, 7.31–7.49); therefore, no further bicarbonate administration was necessary (Figure 1). Urine production was not present in 2 cases, whereas urine production

TABLE 1.
Donor characteristics

	HMP (n = 26)	NEVKP (n = 13)	*P*
Donor type			1.000
DCD	14 (53.8)	7 (53.8)	
NDD	12 (46.2)	6 (46.2)	
Donor age	60.0 (51.5–64.0)	61.0 (52.0–66.0)	0.765
Donor BMI	27.8 (24.2–31.8)	26.9 (23.9–31.4)	0.721
Donor sex			0.276
Female	9 (34.6)	2 (15.4)	
Male	17 (65.4)	11 (84.6)	
Donor cause of death			0.309
Anoxia	4 (15.4)	3 (23.1)	
Cerebrovascular accident	10 (38.5)	2 (15.4)	
Trauma	1 (3.85)	2 (15.4)	
Other	11 (42.3)	6 (46.2)	
Donor terminal creatinine	61.5 (48.8–76.8)	56.0 (35.0–68.0)	0.216

Data are provided as median (interquartile range) or n (%).
BMI, body mass index; DCD, donation after circulatory death; HMP, hypothermic machine perfusion; NDD, donation after neurological death; NEVKP, normothermic ex vivo kidney perfusion.

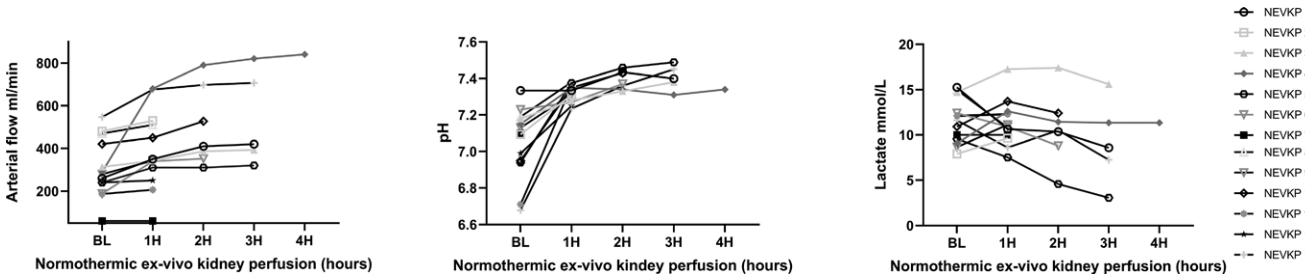

FIGURE 1. Perfusion characteristics—arterial flow, pH, and lactate. BL, baseline; NEVKP 1–NEVKP 13, individual curves for each of the 13 cases.

varied widely in the other 11 cases (median 16 mL; range, 1–104.5 mL during the course of perfusion). Lactate levels in the perfusate were constant during the perfusion (median lactate at 0 h was 11.6 mmol/L; range, 7.9–15.25 mmol/L and at the end of perfusion it was 10.13 mmol/L; range, 3.06–15.6 mmol/L; Table 2; Figure 1).

No significant differences in perfusion characteristics were noted between grafts that developed DGF after transplantation and those that did not. Renal flow and intrarenal resistance at baseline (313 versus 260 mL/h, $P = 0.23$; 0.25 versus 0.31, P=0.41) and at the end of perfusion (550 versus 372 mL/h, P=0.12; 0.14 versus 0.19, P=0.12) were similar in the group that developed DGF versus the group that did not develop DGF. Perfusate pH, lactate, pO_2, pCO_2, and urine production during perfusion were similar between the 2 groups. Urine production during perfusion did not correlate with graft function and urine production after transplantation. All cultures taken from perfusate samples at the end of the perfusion were negative. No episodes of graft infections related to NEVKP were present.

Recipient Demographics and Preoperative Status

Recipient characteristics, such as cause of kidney disease, age, sex, BMI, and history of hypertension and dyslipidemia, were similar between both groups. Also, the dialysis type and duration before transplantation were similar between groups (Table 3).

Perioperative Characteristics and Outcomes After Kidney Transplantation

CIT and intraoperative warm ischemia time were comparable in NEVKP and control patients (Table 4). None of the transplanted grafts developed PNF. No difference in graft function was observed between both groups after transplantation. DGF occurred in 4 (30.8%) patients in the NEVKP group compared with 10 (38.5%) patients in the control group ($P = 0.73$). More than 1 dialysis session was needed in 4 (30.8%) patients in the NEVKP group and 7 (26.9%) in the control patients (Table 4).

In the DCD subgroup, DGF occurred in 2 (28.6%) patients in the NEVKP group compared with 8 (57.1%) patients in the control group ($P = 0.36$). More than 1 dialysis session was needed in 2 (28.6%) patients in the NEVKP group versus 8 (57.1%) in the control patients ($P = 0.66$).

Serum creatinine values after transplantation were also similar in both groups. Creatinine continued to decrease in both groups during the first week posttransplantation without reaching significance. During the first year after transplantation, serum creatinine in the NEVKP versus the control group was comparable at 1, 6, and 12 mo (115 versus 128 μmol/L, $P = 0.43$; 118 versus 112 μmol/L, $P = 0.65$; 130 versus 127 μmol/L, $P = 0.76$, respectively) and had a similar trend in both groups (Figure 1). eGFR increased in both groups during the first year after transplantation, and at 1-y, eGFR was 75.3 mL/min/1.73 m^2 in the NEVKP group and 76.5 mL/min/1.73 m^2 in the control group (Figure 2). Length of hospital stay posttransplantation (median stay, NEVKP versus control group; 9.5 versus 9 d; $P = 0.85$) was similar in both groups (Table 4). Urine production in the first 4 d after transplantation was similar between the 2 groups (Table 4).

Severe perioperative complications classified as Clavien-Dindo ≥3a were present in 3 patients (23.1%) in the NEVKP group versus 3 patients (11.5%) in the control group ($P = 0.38$). Patient and death-censored graft survival at 1 y after transplantation were similar between the 2 groups (Figure 3). One patient in the NEVKP group developed moderate hydronephrosis because of an obstruction of the ureteral stent and required a percutaneous nephrostomy. After this, kidney function recovered well, and the patient was dialysis-free. At 6-wk posttransplantation, 1 patient developed severe pneumonia and expired because of subsequent heart failure. In the control group, there was 1 cardiac-related death in the first 3 mo after transplantation. The patient had a functioning graft at the time of death. In 1 control patient, the graft had to be removed on POD3 because of venous thrombosis. Also, 2 control patients required drainage placement for perinephric collections.

TABLE 2.
Perfusion characteristics

	NEVKP (n = 13)
Perfusion time, min	171 (44–275)
Renal artery flow, baseline, mL/min	279 (60–547)
Renal artery flow, 1 h, mL/min	346 (206–680)
Urine production, mL during the perfusion period	16 (0–105)
Baseline lactate, mmol/L	11.6 (7.9–15.25)
Final lactate, mmol/L	10.13 (3.06–15.6)
pH, baseline	7.1 (6.68–7.33)
pH, last value	7.39 (7.05–7.49)
Bicarbonate added before starting perfusion, mL	10 (0–15)
Bicarbonate added just after starting perfusion, mL	5 (0–15)

Data are provided as median and range.
NEVKP, normothermic ex vivo kidney perfusion.

DISCUSSION

This is the first North American report of clinical NEVKP and the first North American human study demonstrating that back-to-base NEVKP is safe and feasible for kidney

TABLE 3.

Recipient characteristics and perioperative data

	HMP (n = 26)	NEVKP (n = 13)	P
Recipient age	66.5 (58.5–70.8)	66.0 (54.0–71.0)	0.788
Recipient BMI	25.6 (23.8–28.7)	25.6 (24.3–28.4)	0.899
Recipient sex			0.704
Female	6 (23.1)	4 (30.8)	
Male	20 (76.9)	9 (69.2)	
Cause of kidney failure			0.827
Diabetes	10 (38.5)	6 (46.2)	
Glomerulonephritis	7 (26.9)	2 (15.4)	
Hypertension	2 (7.69)	2 (15.4)	
Other	7 (26.9)	3 (23.1)	
Hypertension			0.98
No	3 (11.5)	1 (7.7)	
Yes	23 (88.5)	12 (92.3)	
Dyslipidemia			0.910
No	14 (53.8)	6 (46.2)	
Yes	12 (46.2)	7 (53.8)	
Dialysis type			1.000
Hemodialysis	18 (69.2)	9 (69.2)	
Peritoneal dialysis	8 (30.8)	4 (30.8)	
Pretransplant dialysis duration	1509 (1012–1971)	1686 (1457–2161)	0.371
WIT, min	32.0 (30.0–36.0)	29.0 (26.0–35.0)	0.184
CIT, min	616 (488–893)	537 (504–615)	0.450

Data are provided as median (interquartile range) or n (%).
BMI, body mass index; CIT, cold ischemia time; HMP, hypothermic machine perfusion; NEVKP, normothermic ex vivo kidney perfusion; WIT, warm ischemia time.

transplantation. No technical issues were encountered during perfusion, and all grafts that were perfused could be transplanted.

Most previous clinical trials of NEVKP have been conducted in the United Kingdom, and 1 study was conducted in The Netherlands (Table 5). Nicholson and Hosgood[14] reported a clinical trial including 18 ECD kidneys that received NEVKP for an average of 63 min for reconditioning before transplantation. The results were compared with a matched group of 47 ECD kidneys preserved by SCS. The NEVKP group showed a significantly lower incidence of DGF than the SCS-preserved grafts (NEVKP 5.6% versus SCS 36.2%, $P = 0.014$). Graft and patient survival at 1 y were similar in both groups. The same group also proposed a scoring system for evaluating kidneys during a short period of end-ischemic NEVKP[21] based on the macroscopic appearance of the kidney, mean renal blood flow, and the total urine output. Based on this score, 5 kidneys that had been considered unsuitable for transplantation were successfully transplanted. In another recent report, Callaghan et al described their experience with NEVKP for the assessment of marginal kidney grafts before transplantation using the above-mentioned scoring system.[22] Of the 14 grafts that underwent NEVKP for approximately 60 min, 12 were transplanted into 10 recipients. The incidence of DGF was 30%, and there were no cases of PNF. At 1 y, patient and graft survival were 100%. Finally, a multicenter trial is currently investigating the benefits of 1 h of NEVKP compared with cold storage in DCD kidneys. The trial is expected to be completed in 2022 (ISRCTN15821205).[24]

More recently, a study from The Netherlands described their experience with NEVKP.[23] Eleven grafts received 2 h of end-ischemic NEVKP after being preserved with anoxic HMP. All grafts were eligible for transplantation based on standard criteria. No cases of PNF were reported, and the incidence of DGF was 36%. Graft survival at 1 y was 81%, and patient survival at 1 y was 91%. Graft outcomes of NEVKP perfused grafts were compared with a control group, and no differences were found between groups.

Our approach to NEVKP differed from that used in the UK trials. First, all of the studies presented above used a red blood cell–based perfusate that consisted of 1 pack of red blood cells, Ringer's lactate, nutrients, dexamethasone, mannitol, insulin, prostacyclin, and bicarbonate.[14,22] In contrast, our perfusate consisted of dextran/albumin (Steen solution) with packed red blood cells, which had been widely adopted for clinical ex vivo lung and liver perfusion.[12,25] This results in a high physiological oncotic pressure, and as a consequence, urine production during perfusion was minimal. In our pilot study, urine production during NEVKP was not correlated with posttransplant kidney function. These findings were consistent with our observations from preclinical porcine studies that we used to develop our NEVKP system.[7,8] Second, acceptance of the kidney for transplantation in the UK trials is mainly based on the macroscopic appearance, mean renal blood flow, and the total urine output during NEVKP. In contrast, in our study, kidneys were all accepted for transplantation independent of the preservation method, and all kidneys had an excellent macroscopic appearance during perfusion with low intra-arterial resistance.

Our results are in line with the results published by The Netherlands group. Interestingly, although their perfusate was different from the one we used (red blood cell–based perfusate that consisted of 250 mL of red blood cells, Sterofundin solution, nutrients, dexamethasone, mannitol, insulin, prostacyclin, and bicarbonate), perfusion characteristics were quite similar. In contrast to the Cambridge experience, this study used longer NEVKP perfusion times of 2 h. This is more alike to the perfusion times we used in the present study and shows that perfusion times >1 h are safe and feasible in a clinical context.

In the current study, we used NEVKP for end-ischemic reconditioning following a period of prolonged anoxic cold storage. Although several animal studies from our group have demonstrated that shortening the period of SCS by replacing it with NEVKP resulted in significantly better outcomes after transplantation,[7,10] this approach is not yet feasible in human kidney transplantation because of the absence of a portable, automated NEVKP device. The perfusion system used in this study required a highly skilled user and the constant presence of both a surgeon and perfusionist familiar with the NEVKP protocol. Similar to our porcine studies, no beneficial effects of short-duration end-ischemic NEVKP were observed when compared with a control group with an identical cold storage time.

Short-duration end-ischemic NEVKP could have benefits that were not explored in this study. Even if significance was not reached, we observed less DGF in the DCD subgroup of the patients who received NEVKP, suggesting that NEVKP could result in an improved graft function after transplantation. Graft assessment during a short period of NEVKP could provide important information

TABLE 4.
Recipient outcomes

	HMP (n = 26)	NEVKP (n = 13)	*P*
Creatinine, µmol/L			
POD 1	496 (295–554)	371 (268–541)	0.701
POD 2	407 (219–489)	247 (179–469)	0.455
POD 3	236 (176–495)	270 (156–392)	0.953
1 wk	130 (92.2–212)	149 (116–196)	0.509
1 mo	128 (93.5–229)	115 (104–134)	0.430
3 mo	105 (84.0–147)	107 (93.8–122)	0.987
6 mo	112 (89.0–158)	118 (108–134)	0.650
1 y	127 (85.0–183)	130 (104–138)	0.918
eGFR, mL/min/1.73 m^2			
POD 1	9.35 (7.70–17.5)	13.7 (6.80–15.5)	0.886
1 wk	75.4 (43.1–100)	48.3 (42.3–89.4)	0.483
1 mo	73.6 (40.9–106)	75.2 (71.5–89.8)	0.677
6 mo	88.0 (61.6–121)	78.5 (70.2–86.5)	0.475
1 y	76.5 (50.3–112)	75.3 (71.9–77.6)	0.595
Urine 24 h, mL			
POD 1	1671 (674–2259)	2870 (774–3542)	0.500
POD 2	1900 (1017–2296)	2080 (1086–2544)	0.670
POD 3	2505 (1181–3139)	1544 (47.0–2475)	0.271
POD 4	1862 (243–2388)	1300 (67.5–2242)	0.414
Dialysis post-Tx			0.733
Yes	10 (38.5)	4 (30.8)	
No	16 (61.5)	9 (69.2)	
Dialysis post-Tx ≥2			0.98
Yes	7 (26.9)	4 (30.8)	
No	19 (73.1)	9 (69.2)	
Length of hospital stay post-Tx	9.50 (6.00–17.5)	9.00 (7.00–10.0)	0.845
	HMP, DCD (n = 14)	**NEVKP, DCD (n = 7)**	
Dialysis post-Tx			
Yes	8 (57.1)	2 (28.6)	0.361
No	6 (42.9)	5 (71.4)	
Dialysis post-Tx ≥2			
Yes	6 (42.9)	2 (28.6)	0.656
No	8 (57.1)	5 (71.4)	

Data are provided as median (interquartile range) or n (%).
Dialysis post-Tx ≥2 is ≥2 sessions of dialysis after transplantation.
DCD, donation after circulatory death; eGFR, estimated glomerular filtration rate; HMP, hypothermic machine perfusion; NEVKP, normothermic ex vivo kidney perfusion; POD, postoperative day; Tx transplantation.

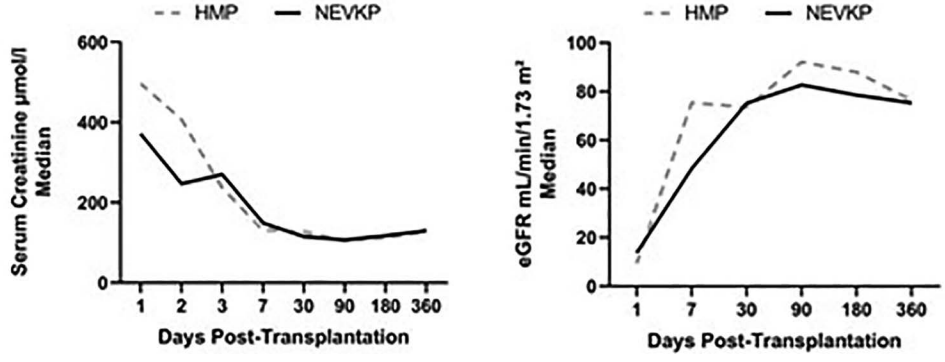

FIGURE 2. Serum creatinine and eGFR following transplantation with NEVKP or HMP as the preservation technique. All values are presented as median. Both creatinine and eGFR were similar in both groups. eGFR, estimated glomerular filtration rate; HMP, hypothermic machine perfusion; NEVKP, normothermic ex vivo kidney perfusion.

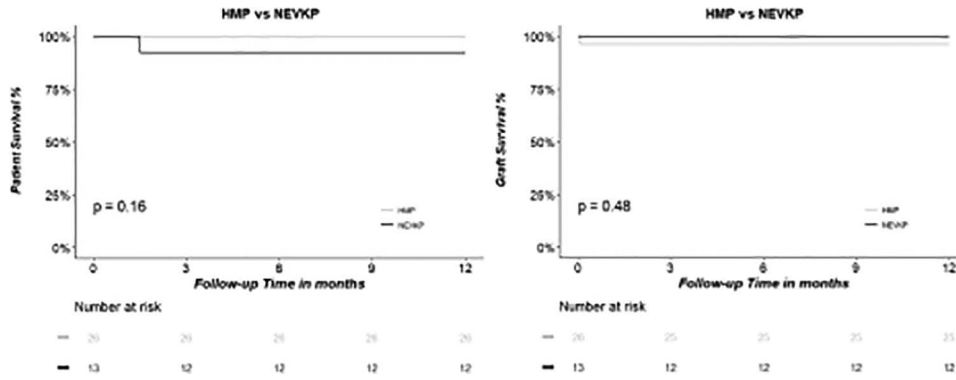

FIGURE 3. Patient and graft survival at 1 y after transplantation. Graft survival rates as analyzed by Kaplan-Meier estimation and compared between recipients who have received a graft preserved with end-ischemic NEVKP (black line) vs a graft preserved with HMP (gray line). Patients at risk are shown in the table below the graph. HMP, hypothermic machine perfusion; NEVKP, normothermic ex vivo kidney perfusion.

about the suitability of the donor kidney for transplantation. In contrast to other studies, our data suggest that urine production during NEVKP is an unreliable indicator of future graft function following transplantation. NEVKP lacks important components required for physiological urine production, such as hormonal support. In addition, urine production during NEVKP is highly dependent on the oncotic pressure of the perfusate, and even suboptimal kidneys will excrete fluid if exposed to a perfusate that has a subphysiological oncotic pressure.[8] A perfusate with higher oncotic pressure is less likely to promote urine production but reduces organ edema and swelling. In future studies, we hope to gain a better understanding of posttransplant kidney function by analyzing urine composition rather than volume. Based on our experience with NEVKP in an animal model, the urine produced during perfusion was not recirculated; however, we acknowledge that recirculating urine could represent a valuable option, and we are considering to implement this change in our future studies. Recirculating urine, instead of replacing it with Ringer's lactate, might ensure better homeostasis of the perfusate. On the downside, if kidney perfusion is performed for a prolonged period, urine recirculation could lead to the accumulation of waste products that might potentially harm the graft.

Weissenbacher et al[26] presented data from 11 kidneys that were rejected for transplantation and that were perfused for 24 h. Eight kidneys were perfused using urine recirculation and 3 were perfused using Ringer's lactate to replace the excreted urine volume. Kidneys that were perfused with urine recirculation demonstrated stable perfusion parameters and could be perfused longer than kidneys without urine recirculation. Moreover, kidneys perfused with urine recirculation demonstrated physiological sodium levels and acid–base stability. This study demonstrates the feasibility of using urine recirculation during ex vivo normothermic machine perfusion. None of the kidneys were transplanted, and therefore, the safety of this method has to be evaluated in a clinical trial.

The current study achieved its primary objective of demonstrating the safety and feasibility of NEVKP. There are several reasons why no clinically significant differences were observed between the NEVKP and cold storage groups. Most importantly, the current study was not designed to evaluate the efficacy of NEVKP to reduce preservation injury in marginal grafts or to assess grafts rejected for transplantation. All grafts used in this study fulfilled our criteria for transplantation and were expected to provide satisfactory graft function with standard preservation. Application of NEVKP to a uniformly higher risk group of kidney grafts might be necessary to detect any protective effects of NEVKP. In addition, our study was not powered to assess clinical differences, and a larger study cohort would be required for this purpose. Also, it is possible that prolonged NEVKP replacing and therefore shortening cold storage will be required to provide important benefits over standard continuous cold storage. Another strength of this study is the long duration of the NEVKP perfusion, with a median of 171 min and a maximum of 275 min. Most studies have perfused grafts for an average of 60 min, with only 1 study showing data from grafts that were perfused for 120 min. This is extremely important, as it may allow for further extension of perfusion time, necessary to adequately assess function, and apply therapies during perfusion.

TABLE 5.

Clinical outcomes from single-center series of kidney grafts that had been subjected to normothermic ex vivo perfusion before transplantation

Author	N	DGF (%)	PNF (%)	1-y graft survival (%)	Perfusion time (min)	Donor type	Data collection time, site
Hosgood and Nicholson[20]	1	0	0	NR	35	ECD	2011, United Kingdom
Nicholson and Hosgood[14]	18	5.6	0	NR	63 ± 16	ECD	2010–2012, United Kingdom
Hosgood et al[21]	5	20	0	NR	60	DCD	2012–2014, United Kingdom
Chandak et al[22]	12	30	0	100	60	DCD, ECD	2016–2017, United Kingdom
Rijkse et al[23]	11	36	0	81	120	DCD, DBD	2018, The Netherlands

DCD, donation after circulatory determination of death; DBD, donation after brain death; DGF, delayed graft function; ECD, expanded criteria donation; NR, not reported; PNF, primary nonfunction.

The present study has several limitations. The study group has a small sample size, and the control group was selected retrospectively. Moreover, perfusion was performed for only a short period, and the SCS period was not shortened. In addition, the study group included kidneys that would be deemed suitable for transplantation with cold storage alone. Biopsies could reveal important information and should ideally be performed 12 to 24 h after reperfusion. Routine biopsies after reperfusion are difficult to justify in a clinical trial and could lead to significant morbidity and were therefore not performed.

In conclusion, this pilot study suggests that NEVKP with dextran/albumin solution (Steen solution) as perfusate is safe and feasible as a preservation technique for human kidney transplantation. Possible benefits might become apparent if, in future studies, the period of SCS is reduced by the prolonged usage of NEVKP. Moreover, future studies need to determine the NEVKP potential for assessing graft function and to evaluate novel repair strategies during end-ischemic NEVKP.

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