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Application of Ex Vivo Normothermic Machine Perfusion in Deceased Donors With Acute Kidney Injury With Successful Renal Transplantation: A Preliminary Experience

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Background. Ex vivo normothermic machine perfusion (NMP) has improved organ preservation and viability assessment among heart, liver, and lung transplantation. However, literature regarding the application of NMP in human clinical kidney transplantation remains limited. Numerous kidneys, especially from donors with stage 3 acute kidney injury (AKI), are not utilized concerning the high rate of delayed graft function (DGF) and primary nonfunction. The present study investigated the impact of NMP (135–150 min) on short-term outcomes after kidney transplantation from deceased donors with AKI. **Methods.** Graft outcomes of NMP kidneys were compared with contralateral kidneys stored in static cold storage (SCS) from the same donor with AKI during December 2019–June 2021. The study's primary aim is to assess the safety and feasibility of NMP in deceased donors with AKI. The primary outcome was DGF. Secondary outcomes were duration of DGF, biopsy-proven rejection, postoperative intrarenal resistive index, postoperative infections, hospital stay duration, primary nonfunction, and kidney function estimated glomerular filtrate rate at discharge, 3 mo, and 1 y. **Results.** Five pairs of AKI kidneys (NMP versus SCS) were included in the final analysis. The results show no statistically significant differences in clinical outcomes between NMP versus SCS kidneys; however, NMP kidneys demonstrated slightly improved estimated glomerular filtrate rate at 3 mo (59.8 ± 5.93 [59] versus 75.20 ± 14.94 [74]) mL/min/1.73 m^2 ($P < 0.065$) and at the last follow-up (12–29 mo) (72.80 ± 10.71 [75]) versus (94 ± 22.67 [82]) mL/min/1.73 m^2 ($P < 0.059$) as compared with SCS kidneys. A higher proportion of NMP kidneys had normal intrarenal resistive index (0.5–0.7) and mild acute tubular injury on protocol biopsy, suggesting NMP is safe and feasible in deceased donors with acute kidney injury. **Conclusions.** NMPs of AKI donor kidneys are safe and feasible. A larger cohort is required to explore the reconditioning effect of NMP on AKI kidneys.

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Kidney transplantation is the gold-standard therapy for individuals suffering from end-stage renal disease.[1] The prevalence of chronic kidney disease has increased worldwide, and by 2040, it will be the fifth leading cause of death.[2] There is a severe shortage of kidneys available for transplantation. Among the end-stage renal disease population in the United States, <20% receive a kidney transplant, whereas the rest die prematurely on dialysis.[3] This prevailing shortage of organ donors across the globe, with a growing waitlist for kidney transplantation has led to an increase in organ procurement and acceptance of kidneys from marginal and high-risk donors, which are more susceptible to ischemia-related reperfusion injury with subsequent high rates of delayed graft function (DGF) and primary nonfunction (PNF).[4,5]

Kidneys obtained from deceased organ donors with early stages of acute kidney injury (AKI) have long-term outcomes similar to those donors without AKI.[6] However, there is a high discard rate of >40% and a very high DGF rate of over 75% among donors with stage 3 AKI kidneys.[5,7] Limited studies have shown satisfactory graft outcomes with proper utilization of these high-risk kidneys by experienced centers.[8] Ex vivo normothermic machine perfusion (NMP) is an emerging technology that has shown to be successful in utilizing organs like the heart, lung, and liver from marginal donors by safe preservation, reduction of ischemia-reperfusion injury (IRI), and DGF compared with conventional static cold storage (SCS) leading to successful organ transplantation.[9-11] However, the use of NMP in clinical kidney transplantation remains extremely limited.

The Cambridge study group has shown the potential of NMP in decreasing DGF in marginal human kidneys compared with SCS and had also successfully transplanted declined donation after circulatory determination of death donors' kidneys using NMP.[12,13] NMP is a technically challenging procedure and is now gaining popularity, with an increasing number of experienced transplant centers around the globe using it for clinical kidney transplantation.[14-17] However, the literature on the outcomes following transplantation of AKI kidneys using NMP remains nonexistent. Therefore, this preliminary clinical study aimed to evaluate the safety and feasibility of NMP in kidney transplantation while using deceased donor kidneys with AKI and its impact on short-term outcomes, that is, DGF and graft function, till 1 y after kidney transplantation.

MATERIALS AND METHODS

This single-center clinical study prospectively investigated the effect of 135–150 min of NMP on AKI kidneys retrieved from donation after brain death (DBD) organ donors with AKI and compared it with the contralateral AKI kidneys from the same donor stored in SCS with subsequent kidney transplantation. The study period was December 2019 to June 2021, with follow-ups ranging from 12 to 29 mo. The study was approved by the Institutional Ethics Committee, ethical approval no (INT/IEC/001788/NK/6725/394), with a project grant (71/2-16/Edu4895) also received from the study institute. All possible patients during the stipulated time were planned for the recruitment and written informed consent was taken from the patients recruited in the study. Inclusion criteria were:DBD donor with AKI defined as an increase in serum creatinine (sCr) by ≥0.3 mg/dL for ≤48 h or requirement of renal replacement therapy prior to organ retrieval.[18] Exclusion criteria were patients receiving second or subsequent kidney transplantation or those with multiple donor arteries.

Impact of COVID-19 Pandemic

Organ donations at the study center reduced drastically because of the ongoing COVID-19 pandemic. Deceased organ donation was affected more than live donation during the COVID-19 pandemic, resulting in a small sample size.

Patients Group

DBD donors with AKI were included. The kidneys were allocated after they were deemed macroscopically transplantable on retrieval. In congruence with the institutional criteria, the right and left kidneys were randomly distributed among the NMP or SCS in a 1:1 ratio. The contralateral kidneys undergoing SCS acted as a comparator group. The recipients were blinded for the kidney preservation method until the study follow-up duration was completed.

NMP

The kidneys were stored in SCS before the NMP. The CIT mentioned in the NMP group corresponds to the time spent on SCS after retrieval. Kidneys underwent pulsatile pressure-controlled perfusion (60 bpm at a temperature of 35–37 °C) on the KIDNEY ASSIST (Organ Assist, CE-certified open pump system) before transplantation using a red cell-based oxygenated perfusion solution (based on Cambridge clinical trial protocol; Table 1).[19] The pressure was manually adjusted on KIDNEY ASSIST from 45 to 90 mm Hg over 0–150 min, keeping the intrarenal resistance (IRR) of machine-perfused kidneys over the higher side of normal physiological range (0.7). An overall quality assessment score (QAS) score (from 1 = highest quality to 5 = lowest quality) (Table 2) based on the macroscopic appearance of kidneys, renal blood flow (RBF), and urine output on NMP was recorded as defined in the Cambridge clinical study.[20] The NMP was carried out in a separate operation theater with gradual oxygenated rewarming at the beginning of NMP for 15–30 min. Considering the pre-existing ischemic insult in AKI kidneys, a period of 2 h of NMP was chosen on KIDNEY ASSIST as it has been shown to replenish the depleted ATP levels fully.[21] The NMP was further prolonged to 150 min and was terminated just before the transplantation to minimize the secondary CIT (<10 min) on SCS while the recipient's surgery was going on. A urine

TABLE 1.

Perfusion solution

Two units of ABO compatible leuco-depleted PRBC mixed with + 750 mL of RL	Red blood cell-based perfusates for adequate tissue oxygenation
Mannitol 10% 50 mL	Diuresis
Dexamethasone 16 mg	Anti-inflammatory
Heparin 1000 IU/mL, 2 mL	Anticoagulation
Sodium bicarbonate 8.4%+ additional as required	Buffer
Nitroglycerine 10 μg/min	Vasodilator
Glucose 5% at 5 mL/h + insulin 100 IU	Nutrition
95% oxygen+ 5% CO_2 at 1.5 L/min	Oxygenation
Cefuroxime 750 mg + calcium gluconate 10% 10 mL	Antibiotics
Supplements, 100 mL of 5% albumin	Prevent cellular edema

TABLE 2.

Quality assessment score on NMP

Macroscopic grade	Score
Grade I: excellent perfusion (global pink appearance)	1
Grade II: moderate perfusion (patchy appearance)	2
Grade III: poor perfusion (global mottled and purple/black appearance)	3
Renal blood flow (mL per min per 100 g)	
Threshold ≥50	0
Threshold <50	1
Total urine output (mL/h)	
Threshold ≥43	0
Threshold <43	1

NMP, ex vivo normothermic machine perfusion.

TABLE 3.

Urine output and perfusion time of each kidney on NMP

Recipients	Urine output in (mL/h)	Total perfusion time on NMP in (min)
KB-S3	22–38	135
KC-S1	68–120	135
KD-S3	24–42	150
KG-S3	10–30	135
KH-S3	32–42	150

NMP, ex vivo normothermic machine perfusion.

recirculation approach was followed (Table 3). Perfusion parameters like RBF, urine output, IRR, perfusate sample to measure electrolytes, acid-base balance, glucose, lactate, and amount of urine recirculated were recorded until the end of the perfusion. Logistic and technical difficulties during NMP were also recorded. The decision to transplant or discard the kidney based on the high QAS score >4 of the NMP kidneys was left to the transplanting surgeon.

Post-NMP

The kidneys were flushed using histidine-tryptophan-ketoglutarate solution before and after NMP. Kidney transplantation was done as per the standard technique. ATG induction (at 1 mg/kg body weight for 3 d) was followed by standard triple-drug regimen tacrolimus (0.2 mg/kg/d divided into 2 doses) + mycophenolate mofetil (1 g BD) + prednisolone (0.4 mg/kg OD). Resistive index/IRR was measured at the level of arcuate or interlobar arteries in the upper, mid, and lower poles of kidneys during the immediate postoperative period (days 1–5). Antimicrobial and antithrombotic prophylaxis was given as per the usual practice. Protocol biopsies were performed in patients with DGF on day 5 and were assessed by a senior consultant pathologist blinded to the kidney preservation method. An in-person follow-up was recorded.

Primary Outcome

DGF defined as a requirement of dialysis during the first posttransplant week.

Secondary Outcomes

DGF duration (from transplant to the last day of dialysis), biopsy-proven acute rejection, postoperative resistive index/IRR, postoperative surgical complications and infection (Clavien Dindo > 3a),[22] duration of hospital stay, PNF (defined as failure to ever function or failure to achieve an estimated glomerular filtration rate [eGFR] of over 20 mL/min/1.73 m^2 over 3 mo), eGFR at the end of the first postoperative week, at hospital discharge, at 3 mo, and at the last follow-up.

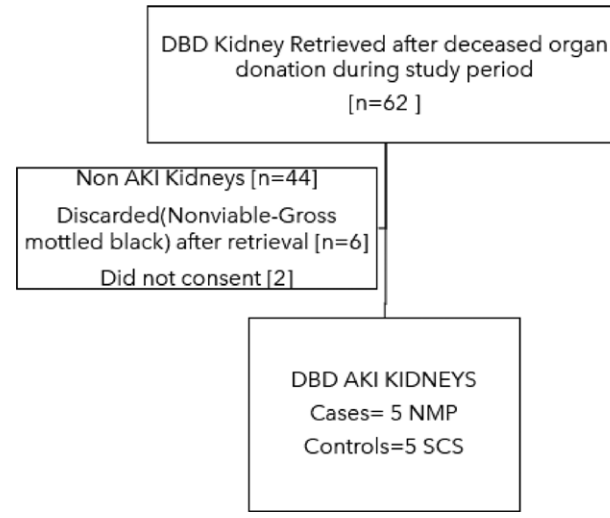

FIGURE 1. Five DBD-AKI kidneys underwent NMP. AKI, acute kidney injury; DBD, donation after brain death; NMP, ex vivo normothermic machine perfusion.

Statistical Analysis

The Shapiro–Wilk lambda test tested the normality of the data. Chi-square and Fisher's exact association tests were used to analyze the relationship between 2 categorical variables. The nonparametric Mann–Whitney U test compared differences between 2 continuous variables. Parametric continuous data and paired nonparametric continuous data were compared by Student's t-test and Wilcoxon sign rank test, respectively. Two-sided statistical tests were employed, with $P < 0.050$ considered statistically significant. The study analysis also reported the uncertainty in the estimates to complement the *P* values. Analyses were performed by IBM SPSS Statistics for Windows, Version 22 software.

RESULTS

NMP was performed on 5 DBD AKI kidneys (Figure 1).

AKI DBD Donor Baseline Characteristics

All donors were brought to the study center with an unrecordable BP except KC-S1 donor and were managed in the intensive care unit with organ protective therapy (Table 4). Since admission, all potential donors had a high inotropic requirement with persistent hemodynamic instability. All donors' blood, urine, and tracheal aspirate cultures were negative. Most of the donors (80%) were in stage 3 AKI at the time of organ donation.[18,23,24] No medical comorbidities were recorded among the donors.

Recipient's Baseline and Posttransplant Characteristics

All the study participants were on hemodialysis (HD) for a period ranging from 25 to 62 mo (Table 5). SCS kidneys underwent transplantation first, followed by the NMP kidneys after NMP. All perfusion fluid cultures sent during NMP were sterile.

TABLE 4.

DBD donors characteristics

Baseline characteristics	KB-S3	KC-S1	KD-S3	KG-S3	KH-S3
Age, y	50	26	27	12	65
Sex	Male	Female	Male	Male	Male
Blood group	B+	AB+	B+	AB+	O+
BMI	26.7	20.2	25.3	18	27.6
Cause of death	CNS trauma	CNS trauma	CNS trauma	CNS trauma	CNS trauma
Days on mechanical ventilation in ICU	3	6	4	7	2
Donor and perfusion cultures	Sterile	Sterile	Sterile	Sterile	Sterile
Se. creatinine on the d of donation (mg/dL)	3.02	1.4	4.3	3.2	2.2
Acute kidney injury stage	Stage 3	Stage 1-2	Stage 3	Stage 3	Stage 3
Hourly urine output (mL/min)	25	50	10	50	75
Inotropic support[a]	3+	2+	3+	3+	2+
KDPI[b]	0.59	0.3	0.33	0.31	0.82
KDRI[b]	1.08	0.82	0.84	0.84	1.4
QAS on NMP (macroscopic appearance + RBF + urine output)	(1 + 1 + 1) = 3	(1 + 0 + 0) = 1	(1 + 1 + 1) = 3	(1 + 1 + 1) = 3	(1 + 1 + 1) = 3

[a]1+, 2+, 3+ (noradrenaline[0.5 μg/kg/min], vasopressin [8U/h], adrenaline [0.5 μg/kg/min]).
[b]KDPI/KDRI kidney donor profile index and kidney donor risk index were calculated using the organ procurement and transplant network calculator.
K-kidney B, C, D, G, H- Donors S – Quality assessment score (QAS) obtained on NMP at 1 h.
BMI, body mass index; DBD, donation after brain death; KDPI, kidney donor profile index; KDRI, kidney donor risk index; NMP, ex vivo normothermic machine perfusion; RBF, renal blood flow.

TABLE 5.

DBD recipients baseline and posttransplant characteristics

Characteristic of primary renal pathology unknown in 80% of study group	NMP (n = 5) (mean ± SD) [median]	SCS (n = 5) (mean ± SD) [median]	*P*	95% confidence interval of the difference	Estimate
Age, y	44.80 ± 12.0 [48]	43.80 ± 11.25 [49]	0.895	−15.974 to 17.974	0.136
Sex (n female vs n male)	(1 vs 4)	(3 vs 2)	0.524	0.010 to 2.821	0.167
BMI (kg/m^2)	23.24 ± 1.81	22.4 ± 3.71	0.622	−3.4231 to 5.1031	0.454
H/o diabetes (n)	0	2	0.444	0.815 to 3.409	1.667
H/o hepatitis C (n)	1	1	1	0.045 to 22.175	1.000
H/o sensitization(n)	1	1	1	0.045 to 22.175	1.000
Cold ischemia time (min) {IQR}	546.20 ± 197.91 [450]{271}	441.60 ± 192.89 [380]{273}	0.076	−264.000 to 488.000	109.000
Anastomosis time (min)	35.60 ± 2.60 [34]	33.2 ± 4.14 [32]	0.305	−2.652 to 7.452	1.095
Right kidney vs left kidney	(2vs3)	(3vs 2)	0.527	0.179 to 28.254	2.250
Postoperative RI/IRR <0.7 (d 1–5) [normal (0.5–0.7) vs high (>0.7)]	(4 vs 1)	(0 vs 5)	0.052	0.035 to 1.154	0.200
Delayed graft function (n)	3	2	0.527	0.080 to 12.557	1.000
DGF duration, d	2.80 ± 2.95 [3]	3.80 ± 3.89 [4]	0.660	−6.042 to 4.042	-0.457
eGFR at d 7 (mL/min/1.73 m^2)	32.60 ± 22.42 [32]	19.20 ± 15.61 [13]	0.462	−0.300 to 0.000	0.000
Adverse events complications SSI (≥Clavien Dindo 3a) (n)	0	2	0.429	0.815 to 3.409	1.667
Best creatinine at discharge (mg/dL)	1.82 ± 1.07 [1.1]	1.80 ± 0.45 [1.5]	0.913	−1.000 to 1.000	0.000
eGFR at discharge (mL/min)	51.40 ± 20.2 [52]	43.40 ± 6.69 [46]	0.425	−13.952 to 29.952	0.840
Hospital stay, d	14 ± 8.2 [12]	18.80 ± 7.56 [20]	0.366	−16.362 to 6.762	-0.957
Creatinine at 1 mo (mg/dL)	1.20 ± 0.47 [1]	1.4 ± 0.58 [1.3]	0.513	−1.000 to 0.000	0.000
eGFR at 3 mo (mL/min)	75.20 ± 14.94 [74]	59.8 ± 5.93 [59]	0.065	−1.178 to 31.978	2.142
Creatinine at follow-up (mg/dL)	0.94 ± 0.13 [1]	1.00 ± 0.00 [0.9]	0.317	−0.300 to 0.000	0.000
eGFR at follow-up (mL/min)	94 ± 22.67 [82]	72.80 ± 10.71[75]	0.059	0.000 to 54.000	15.000

BMI, body mass index; DBD, donation after brain death; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; IQR, interquartile range; IRR, intrarenal resistance; NMP, ex vivo normothermic machine perfusion; RI, resistive index; SCS, static cold storage; SSI, surgical site infection.

Primary and Secondary Outcomes

The incident of DGF was (3 of 5) in NMP versus (2 of 5) in the SCS group. The 2 DGF incidents in SCS kidneys coincided with their NMP kidneys. An additional DGF incident in the NMP group was attributed to the technical event. There were no biopsy-proven acute rejections or PNF in either group. Immediate postop IRR in NMP group ranged ([RI (0.5–0.7)] versus SCS [RI (0.8–1)]) (P = 0.052) (Table 6). All protocol biopsies during DGF period (**Figures S-HPE 1–5, SDC,** http://links.lww.com/TXD/A456) showed mild-moderate acute tubular injury (ATI) in NMP kidneys and severe ATI in SCS, except for 1 KG-S3 donor NMP kidney, which had a technical event during NMP and showed thrombotic microangiopathy (TMA) on protocol biopsy. Two SCS recipients had surgical site infections requiring antimicrobial therapy for a week. Although not statistically significant, eGFR at 3 mo

($P < 0.065$) and the last follow-up (12–29 mo) ($P < 0.059$) were better in the NMP group versus SCS (Figures 2 [Boxplot] and 3 [machine-perfused kidney]). Patient and graft survival was 100% in both groups (**Figures S-NMP [1–5], SDC,** http://links.lww.com/TXD/A456).

TECHNICAL EVENTS IN THE COHORT

One technical event requiring interruption of machine perfusion and recannulation of NMP kidney (KG-S3) due to a leak occurred on a smaller pediatric donor kidney carrel patch with an additional warm ischemia time (WIT) of of 5 min. Protocol biopsy of this kidney showed TMA, but subsequent graft biopsies showed features suggestive of ATI with no evidence of antibody-mediated rejection or any other prothrombotic disorder, atypical hemolytic uremic syndrome, or drug toxicity (**Figure S-HPE 5, SDC,** http://links.lww.com/TXD/A456). The patient was discharged after 4 wk. Baseline sCr (1–1.1 mg/dL) was recorded at a 1-y follow-up. Several technical difficulties faced during NMP are detailed in Table 7, and perfusion parameters recorded on NMP are given in Table 8. (**Figures S-NMP 1–5, SDC,** http://links.lww.com/TXD/A456).

TABLE 6.

IRRs represent mean values recorded in NMP vs SCS kidneys in the immediate postoperative period (d 1–5)

Recipients	NMP (IRR)	SCS (IRR)
KB-S3	0.65–0.70	0.90–1.0
KC-S1	0.50–0.70	0.70–0.80
KD-S3	0.56–0.65	0.8–0.85
KG-S3	0.90–1.0	0.80–0.90
KH-S3	0.60–0.70	1.0

IRR, intrarenal resistance; NMP, ex vivo normothermic machine perfusion; SCS, static cold storage.

DISCUSSION

There has been an increase in organ utilization from high-risk donors like donors with AKI, which are more susceptible to IRI with high rates of DGF and PNF following prolonged CIT on SCS.[6,7] Recent recommendations favor the utilization of stage 1–2 AKI kidneys at experienced centers considering high mortality in prospective recipients remaining on the waiting list while safeguarding against the usage of stage 3 donor AKI kidneys for transplantation due to their high DGF, PNF, and discard rate.[6,25] NMP has the potential to emerge as a therapeutic reconditioning tool for these high-risk AKI kidneys, as NMP provides more objective evidence for organ viability assessment and organ preservation in a near-physiological environment. Ex vivo NMP preservation technology has shown level 1 evidence in reducing graft injuries in liver transplantation and has shown mitigation of IRI in marginal grafts in heart transplantation.[9,11]

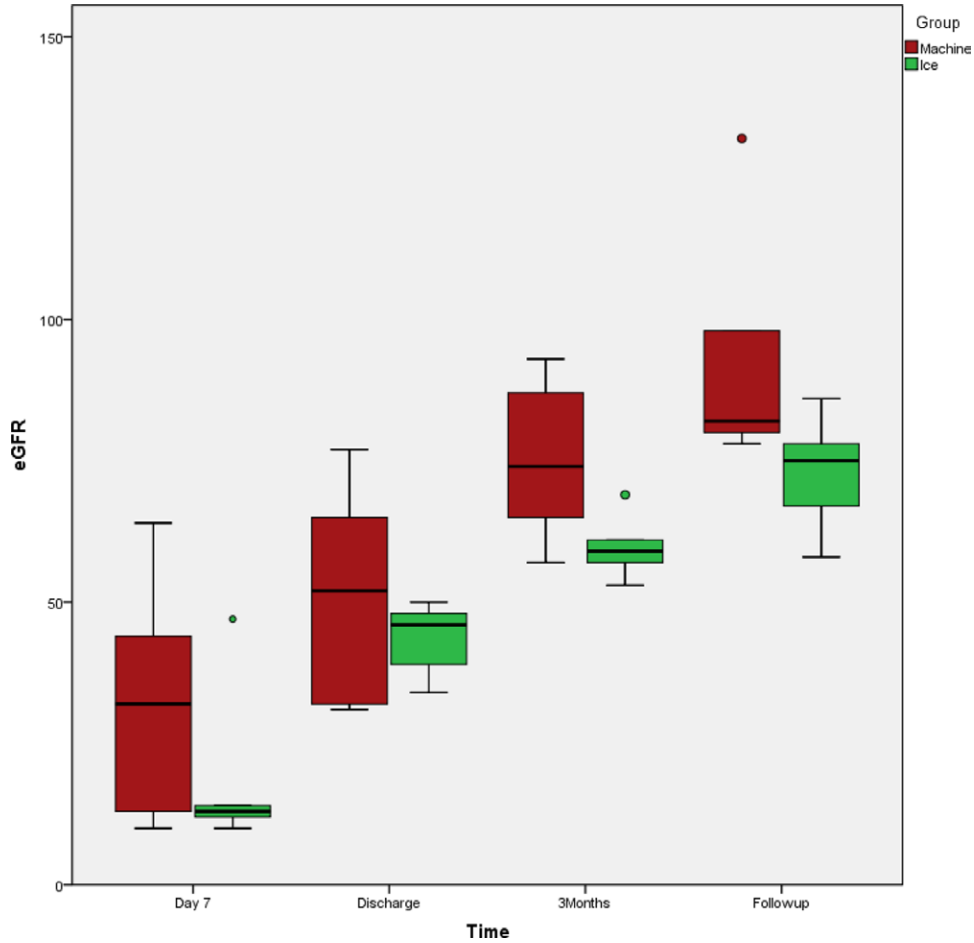

FIGURE 2. Box plot showing renal function (estimated glomerular filtration rate [eGFR]) in kidneys that underwent normothermic machine perfusion (NMP) in red and the control static cold storage (SCS) kidneys in green from POD-7 till the last follow-up.

There are limited published prospective studies on ex vivo NMP of kidneys to date.[12,14,15,17,26] The present study is the first human clinical kidney NMP study reporting the study center's preliminary experience with NMP of deceased donor kidneys with AKI (predominantly 80% stage 3 AKI kidneys), detailing successful kidney transplants performed after 135–150 min of NMP using an oxygenated red cell-based perfusate and also describes their short-term outcomes. The primary purpose of applying NMP was to establish its safety and reduce the DGF rate in the DBD-AKI kidneys.

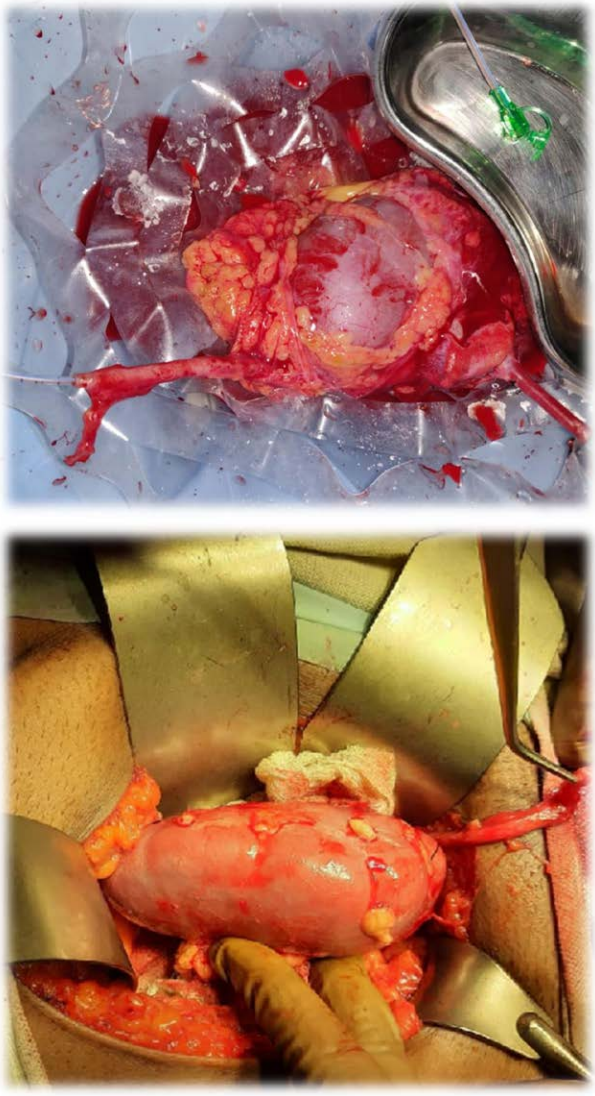

FIGURE 3. Machine perfused kidney and after transplantation.

However, the rate of DGF was higher at 60% (3 of 5) in the present study compared with 0%–36% in pilot kidney NMP studies.[12,14,15,17,26] The higher incidence of DGF in the present study is mainly attributed to stage 3 AKI in 80% of the donors, whereas the reported NMP studies did not include donors with stage 3 AKI. It is well known that DGF increases with progressive stages of donor AKI with a range between 25% and 35% in stage 1, 32% and 43% in stage 2, and 51% and 55% in stage 3 AKI.[6,27] NMP of AKI kidneys may not be able to reduce the incidence of DGF because of the severe pre-existing ischemic insult, especially in the stage 3 AKI kidneys. Stage 3 AKI kidneys are also 20 times more likely to be discarded as compared with donors with no AKI because of an associated higher DGF ($P < 0.005$) and PNF ($P = 0.04$).[27] The discard rates of stage 3 kidneys are as high as 44% among US centers.[7] However, perfusion parameters with good RBF on NMP helped us utilize all stage 3 AKI kidneys with a QAS of 3 in 80% of NMP kidneys.

The Cambridge group devised a QAS score to grade the quality of NMP kidneys with a reported DGF rate of 6% among QAS-1, 0% in QAS-2 as compared with 38% in QAS-3 NMP kidneys with a significantly higher sCr ($P = 0.030$) and lower eGFR ($P = 0.015$) at 1 y in QAS-3 kidneys compared with QAS-1,2 kidneys.[20] In the present study, 80% of the kidneys (all stage 3 AKI kidneys) scored a QAS-3 on NMP. Although no statistically significant differences were found between the 2 groups, NMP kidneys demonstrated slightly improved eGFR at 3 mo (59.8 ± 5.93 [59] versus 75.20 ± 14.94 [74], $P = 0.065$) and at the last follow-up (72.80 ± 10.71 [75] versus 94 ± 22.67 [82] mL/min/1.73 m^2) ($P = 0.059$), respectively, in comparison to SCS kidneys. In contrast, the reported studies on NMP in the literature showed similar eGFR in NMP versus SCS kidneys at 6 mo and 1-y follow-up; nevertheless, none selectively included AKI donor kidneys.[14,15,17]

IRR on doppler aids in measuring blood flow changes in kidneys, with a high IRR/RI suggestive of tubulointerstitial damage, acute tubular injury (ATI), necrosis, and DGF with impaired renal graft recovery.[28] High IRR during the first posttransplant week is a marker for increased vascular impedance, can also be used to predict the duration of DGF, and correlates well with reduced eGFR, raised sCr, graft loss, and recipient mortality within 1 y of kidney transplantation.[29-31] An important finding in the index study was a normal immediate postoperative IRR/RI among the NMP kidneys (RI <0.7 in 80%) versus SCS kidneys ($P = 0.052$). This improved IRR correlated with histology of mild-moderate ATI in NMP kidneys (n = 2) versus severe ATI in SCS (n = 2) on protocol biopsies despite having a higher mean CIT in the NMP group as compared with the SCS group (Table 5).[32,33] The published literature shows that severe ATI in reperfusion biopsies is associated with a low eGFR at 5 y ($P = 0.04$).[34] Although not statistically significant, improved IRR in the NMP kidney group may have halted or dampened

TABLE 7.

Technical difficulties faced during NMP.

I. Air bubbles in the system during NMP were managed by de-airing.
II. Dryness of the exposed adventitia of kidney vasculature and kidney surface was managed by pouring NMP perfusion solution over the dry surface every h.
III. Fogging of the cover of the cradle reduced the visibility were cleaned with a sterile gauge every h.
IV. It took 15–30 min for the NMP kidney with raised vascular resistance to reach normothermia after it was placed on NMP.
V. One incident of perfusion fluid leak and incomplete de-airing reversed rapidly within min.
VI. There was no autoregulatory pH or PCO_2 settings, and it required manual bicarbonate infusion and assistance every 15 min.

NMP, ex vivo normothermic machine perfusion.

TABLE 8.

Perfusion parameters at the start and the end of NMP

Perfusion parameters	Start of NMP mean ± SD [median] (IQR)	End of NMP mean ± SD [median] (IQR)	*P*	95% confidence interval of the difference	Estimate
Renal blood flow (mL/min)	26 ± 5.65 [28] (11.00)	132 ± 49.29 [114] (71.00)	0.043	74.000 to 186.000	92.000
Perfusate pH	7.16 ± 0.35 [7.2] (0.61)	7.36 ± 0.21 [7.4] (0.41)	0.225	−0.170 to 0.700	0.210
Perfusate lactate (mmol/L)	15.5 ± 7.10 [15.27] (13.46)	21.08 ± 3.94 [21.30] (6.45)	0.043	1.390 to 15.200	4.200
Perfusate glucose (mmol/L)	158.60 ± 94.82 [200] (183.50)	227.34 ± 60.19 [159] (110)	0.680	−70.000 to 106.000	−18.000
Perfusate sodium (mmol/L)	134.48 ± 6.58 [131.2] (12.4)	139.36 ± 7.79 [138.5] (13.25)	0.225	−2.500 to 20.700	3.800
Perfusate potassium (mmol/L)	4.6 ± 1.2 [4.9] (2.29)	5.56 ± 1.18 [5.6] (1.98)	0.138	−0.570 to 2.110	1.045
Perfusate PO_2, mm Hg	435.24 ± 43.21 [412.6] (80.8)	400.54 ± 14.46 [394] (238.65)	0.890	−229.300 to 119.400	−54.950
Intrarenal resistance on NMP	1.8 ± 2.31 [1.6] (0.8)	0.7 ± 0.19 [0.70] (0.33)	0.043	−1.540 to −0.640	−1.075
Perfusate PCO_2, mm Hg	33.86 ± 25.40 [45.8] (48.35)	33.2 ± 19.27 [36.9] (38.25)	0.500	−23.700 to 7.300	3.700

IQR, interquartile range; NMP, ex vivo normothermic machine perfusion.

the ensuing damage to the ischemic AKI kidneys resulting in earlier recovery and improved graft function (eGFR) at 3 mo and 1 y after transplantation in the NMP group.

The study-center perfusion solution is based on the Cambridge group study protocol with a perfusion time until 150 min and follows a urine recirculation approach as it is shown to be superior in maintaining perfusate homeostasis over a longer duration of perfusion by upregulating the protective pathways.[35-37] A longer duration of NMP was built on the superiority of more extended periods of NMP in decreasing the cold storage-induced injuries compared with brief duration NMP and the inability of brief NMP to predict viability and optimize graft repair within a short 1-h perfusion period, especially in high-risk kidneys.[38-40] Further findings from magnetic resonance imaging of kidneys undergoing NMP reveal that renal cortical perfusion proceeds only after the first hour of NMP as the kidney flow during this period on NMP is mainly to the renal medulla.[41] Besides, the kidneys placed on NMP in the index study initially took 15–30 min to reach normothermia upon gradual rewarming. At the same time, mean arterial pressure was gradually raised to prevent injury in the cold kidneys by pumping against a raised IRR, much similar to the controlled oxygenation rewarming of the kidneys NMP studies but over a shorter period of <30 min.[26] Most of the current literature on clinical NMP uses 1 h for NMP of kidneys, with only the Toronto and Netherlands study groups performing 120 min of NMP.[15] The Toronto study group reached up to a maximum of 275 min in NMP in a recently published first North American results of NMP in kidney transplantation.[17] The benefits and harms of extending the duration of NMP of kidneys beyond the established criteria envisage further human clinical studies.

The lactate metabolism reveals the metabolic viability of the kidney on NMP as the renal cortex is the major lactate-consuming organ in the body, second only to the liver. In the index study, albeit nonsignificant increasing lactate on the NMP of kidneys suggests an impaired cortical function among the AKI kidneys as the renal cortex suffers major blunt in ischemic AKI as total RBF reduces strikingly by >60% in the renal cortex compared to 11% in the renal medulla.[23] However, a significant increase in post-NMP perfusate lactate levels ($P < 0.001$) was observed among the Netherlands (NMP-2hr) study group, where NMP were performed on older donor kidneys >65 y using kidney assist with perfusate composition primarily based on the Cambridge group.[15] The progressive lactate accumulation during NMP may result from PRBC-derived lactate and is also a marker of renal lactate production by renal medulla during the initial hours of impaired oxidative phosphorylation on NMP.[42] An extended period of NMP possibly would have been better for ischemic reconditioning and functional recovery amongst these high-risk grafts.

Technical challenges also exist with the NMP technique. An arterial leak during NMP of a pediatric kidney requiring interruption of perfusion highlights the need for continuous observation during NMP. This event led to an additional DGF incident in the NMP group with an unknown cause TMA, which was also reported previously in the NMP literature with a resultant poor graft function at 1 y, although it was not attributed to the NMP technique.[14] NMP interruption in the present case with a resultant IRI in the pre-existing detrimental endothelium of AKI kidney might have contributed to the posttransplant TMA. As within 15 min of NMP, a rapid release of fibrinogen is known to occur from the renal tubular epithelium with a resultant high concentration of fibrinogen in peritubular capillaries and has been considered a major contributory factor for an increased RBC aggregation and has shown to decrease the renal cortical perfusion of the kidneys undergoing NMP. This deleterious process is known to be reduced significantly by including plasminogen and tissue plasminogen activators in the NMP perfusate.[43] TMA in a setting of NMP interruption could represent a potential concern for the safety of NMP in kidney transplantation.

The index study results will further expand the duration of clinical NMP, providing sufficient time for active repair, reconditioning, and access to graft viability in high-risk kidneys with an optimum window for on-pump therapies before kidney transplantation.[44,45]

Limitation of the Study

This is an early experience with a clinical pilot study design with a small sample size with the recruitment of only DBD-AKI donors limiting the statistical analysis and the conclusions drawn. Longer CIT in the NMP group is an important limiting factor attributed to the NMP logistics despite following a strict NMP protocol. Requirements for an additional workforce with a separate operation theater and technical challenges exist with the NMP technique.

Strength

This study includes a high-risk group of donors to implement a longer duration of NMP into clinical practice and has

shown that NMP of AKI kidneys is safe and feasible. This prospective pilot study adds to a small body of evidence for clinical kidney NMP literature. Paired statistical analysis with the in-built contralateral donor kidney in SCS as control with resultant more power in small numbers and a longer duration of follow-up. The same NMP surgeons team performed the technicalities of NMP. Uncertainties in the estimates have also been reported, which will help design more extensive and robust randomized controlled trials in the future.

Future Focus

Reconditioning of AKI kidneys using NMP can significantly improve long-term kidney function among recipients of these high-risk kidneys, resulting in considerable long-term benefits. The findings from this study should be reciprocated and confirmed in the future with more extensive studies. Cost-benefit analyses will also be required at some stage, and a much longer follow-up is desirable to study the effect on long-term graft outcomes. The ongoing randomized controlled trial of NMP in the United Kingdom will provide further evidence, guidelines, and additional safety measures on various aspects of kidney NMP.[19]

Conclusion

Although NMP of the kidney is technically challenging, it is safe and feasible in stage 3 AKI kidneys. Although not statistically significant, NMP kidneys demonstrated slightly improved eGFR at 3 and 12 mo following kidney transplantation. Larger multicentric randomized controlled trials need to be designed to address the reconditioning effect of NMP in AKI kidneys.

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