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Case Report

Successful Transplantation of Human Kidneys Deemed Untransplantable but Resuscitated by *Ex Vivo* Normothermic Machine Perfusion

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We report the successful transplantation of a pair of human kidneys that were declined for transplantation due to inadequate *in situ* perfusion but subsequently transplanted after perfusion and assessment using *ex vivo* normothermic perfusion (EVNP). The kidneys were from a 35-year-old man, a donation after circulatory death donor. Both kidneys were declined by all UK transplant centers. On arrival, the kidneys had significant areas of incomplete clearance of blood from the microcirculation that did not clear after a further attempt to flush them. Kidneys underwent 60 min of EVNP with an oxygenated packed red blood cell–based solution warmed to 35.2°C. During EVNP, the patchy areas cleared in both kidneys. The mean renal blood flow and total urine output were 68.0 mL/min/100 g and 560 mL in the left kidney and 59.9 mL/min/100 g, 430 mL in the right, respectively. Based on the EVNP perfusion parameters, both kidneys were deemed suitable for transplantation. They were transplanted without any complications, and both recipients had initial graft function. The serum creatinine levels at 3 months were 1.2 mg/dl in the recipient of the left kidney and 1.62 mg/dl in the recipient of the right kidney. EVNP technology can be used to assess and rescue kidneys previously deemed unsuitable for transplantation.

Abbreviations: DCD, donation after circulatory death; EVNP, *ex vivo* normothermic perfusion; RBF, renal blood flow; U/O, urine output; UW, University of Wisconsin

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Introduction

Although there is a severe shortage of human kidneys for transplantation, around 17% of kidneys obtained from donation after circulatory death (DCD) donors in the United Kingdom are deemed unsuitable for transplantation and discarded (1). The most common reason is inadequate perfusion during *in situ* flushing with preservation fluid following procurement (2).

Inadequate *in situ* perfusion can be due to the accumulation of intravascular thrombosis after the withdrawal of treatment and during circulatory arrest, technical issues during retrieval, the anatomy, vasospasm, or donor factors such as atherosclerosis. The microcirculation is compromised, increasing the exposure to warm ischemia and damage to the endothelium, which in combination with a period of hypothermic preservation can result in irreversible injury (3,4). The majority of these inadequately flushed DCD kidneys are regarded as unsuitable for transplantation.

Preservation techniques such as *ex vivo* normothermic machine perfusion (EVNP) can be used to resuscitate and assess the quality of the kidney before transplantation (5–7). In an earlier laboratory study, we assessed the quality of 22 human kidneys declined for transplantation primarily due to inadequate *in situ* perfusion using EVNP (3,5). After assessment and grading, we considered that 19 of these 22 kidneys may have been suitable for transplantation.

Here, we report the successful transplantation of human kidneys that were declined by all UK transplant centers due to inadequate *in situ* perfusion following resuscitation and assessment using EVNP. Ethical approval was granted by the National Research Ethics Committee in the United Kingdom for this research study.

Case Report

Both kidneys procured from a 35-year-old DCD donor who died after an intracranial hemorrhage were offered to all UK transplant centers but deemed untransplantable due to poor *in situ* flushing at the time of procurement.

The liver and pancreas were also declined for transplantation for the same reason. The right kidney was initially accepted by one UK transplant center. After inspection, it was declined due to the appearance. The donor terminal serum creatinine was 0.6 mg/dl and warm ischemic time 13 min.

Permission for research was granted by the donor family, and the kidneys were allocated to our center for research. Initially, each of kidneys was offered and accepted into different studies for destructive research. On consultation with our group, the decision was made to allocate them into our study for assessment and potential transplantation. On arrival, surface inspection showed that around 50% of the renal parenchyma of each kidney had failed to flush following both *in situ* perfusion with University of Wisconsin (UW) cold preservation solution in the donor and perfusion with cold UW solution on the back table after procurement (Figures 1A and B).

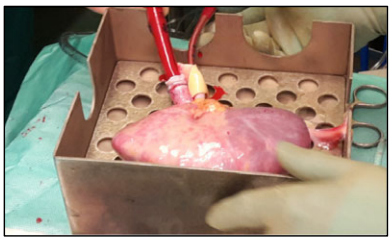
Ex vivo *normothermic perfusion*

Each kidney underwent EVNP for a period of 60 min using a technique that has previously been described in detail (6–8). In brief, the organs were perfused with a warmed (35.2°C), oxygenated plasma-free red cell–based solution at a pressure of 75 mmHg. Nutrients, glucose, and prostacyclin were infused at a set rate to maintain homeostasis. During EVNP, the quality of each kidney was assessed. The assessment was based on the macroscopic appearance, the mean renal blood flow (RBF), and the total amount of urine produced.

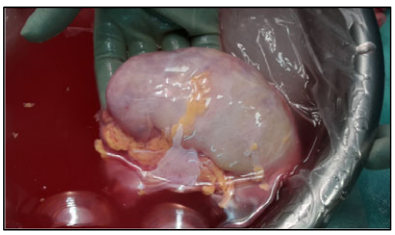
For macroscopic assessment, each kidney was categorized into one of three groups according to its macroscopic appearance as follows: grade I, excellent perfusion (global pink appearance); grade II, moderate perfusion (patchy pink/purple appearance that either remained or improved during EVNP); or grade III: poor perfusion (global mottling and purple/black appearance that remained throughout EVNP).

A combination of the macroscopic and functional parameters was used to create an index of organ quality. Based on previous data (8), receiver operating characteristic curves were used to determine thresholds of RBF and urine output (U/O). These thresholds were combined with the macroscopic grade to give an overall EVNP assessment score of 1 to 5. Macroscopic grades I, II, and III were assigned scores of 1, 2, and 3, respectively. Kidneys with a mean RBF below the threshold (<50 mL/min/100 g) were given an additional score of 1. Kidneys producing less than the threshold volume of urine (<43 mL/h) were also given an additional score of 1. Therefore, overall EVNP assessment scores ranged from 1, indicating the least injury, to 5, the most severe.

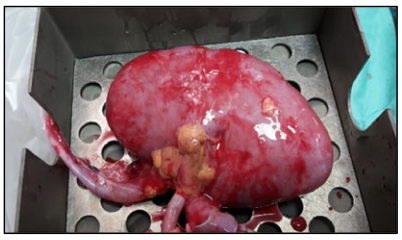
The left kidney had an EVNP score of 1, and the right kidney had a score of 2. Both kidneys had RBF (Figure 2) and U/O over the threshold. The left kidney appeared evenly perfused throughout EVNP with a macroscopic score of 1 (Figure 1C). The right kidney appeared patchy to start but had a global pink appearance thereafter (Figure 1D). The macroscopic score was 2.



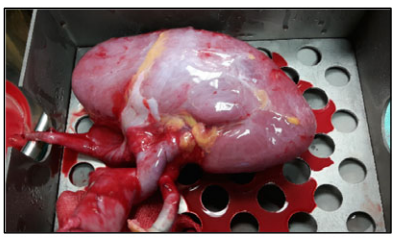
A Left kidney pre Ex-vivo normothermic perfusion (EVNP)



B Right kidney pre EVNP



C Left kidney post EVNP



D Right kidney post EVNP

Figure 1: Kidneys before and after *ex vivo* normothermic perfusion (EVNP). (A) Left kidney before EVNP. (B) Right kidney before EVNP. (C) Left kidney after EVNP. (D) Right kidney after EVNP.

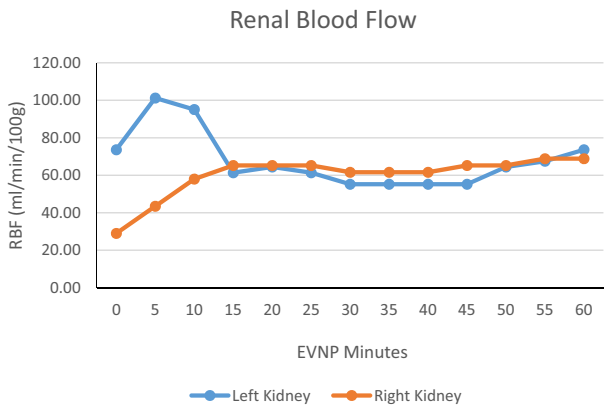


Figure 2: Renal blood flow during normothermic perfusion in the left and right kidneys.

Table 1: Recipient demographics, ischemic times, and serum creatinine levels up to 3 months posttransplantation

	1 (left kidney)	2 (right kidney)
Recipient		
Age (years)	30	68
Sex	Male	Female
Cold ischemic time, 1st	17 h 24 min	19 h 44 min
Cold ischemic time, 2nd	132 min	247 min
Anastomosis	33 min	33 min
Total ischemic time	21 h 09 min	25 h 24 min
Length of hospital stay (days)	5	9
Outcome		
Serum creatinine (mg/dL)		
Before	9.43	6.31
Creatinine day 7	1.3	6.22
Creatinine day 14	1.13	2.62
Creatinine 1 month	1.14	1.62
Creatinine 3 months	1.2	1.62

A wedge biopsy specimen was taken from each kidney after EVNP and before implantation. Both the left and right kidney had mild acute tubular necrosis but no evidence of vascular thrombi.

Outcome

The recipient demographics, ischemic times, and outcome are presented in the Table 1. The cold ischemic time was 17 h 24 min for the left kidney and 19 h 44 min for the right. After EVNP, the left kidney had an added cold ischemic time of 132 min and the right, 247 min.

The kidneys were transplanted without any complications. The left kidney had immediate graft function. The right kidney had slow graft function defined as a less than 10% fall in serum creatinine for 3 consecutive days during the first week after transplantation but the patient remained dialysis independent. The recipients had 3-month serum creatinine levels of 1.2 and 1.62 mg/dl, respectively.

Discussion

Inadequate *in situ* perfusion is a common cause of organ discard, particularly in kidneys from DCD donors (2,3). A procoagulant state and hypotension after withdrawal of life-supporting treatment together with the cession of circulation after cardiac arrest can result in accumulation of intravascular thrombosis. *In situ* perfusion with cold preservation solution rapidly flushes the blood from the kidneys. However, under cold conditions, the cell membranes stiffen, which makes complete clearance difficult. Additional flushing on the backtable can help to clear the microcirculation, but often residual blood cells remain. Platelets adhering to the vascular endothelium can induce endothelial apoptosis and aggravate reperfusion injury after transplantation (4). Gok et al reported an exceptionally high rate of primary nonfunction from inadequately perfused kidneys: 66.7% compared with 4.8% in those with adequate perfusion (9). Therefore, there is much reluctance to use these organs.

EVNP has an advantage over cold techniques in that circulation is reestablished at a near body temperature. Cell membrane fluidity is restored, and this facilitates clearance of the renal capillary beds. Preliminary evidence from our work in porcine kidneys and clinical studies suggest that EVNP upregulates protective mechanisms that may improve early graft function (10,11). Normothermic perfusion with a red cell–based solution also provides the ideal environment to assess the quality of the kidney using a measure of the macroscopic appearance to identify areas of poor perfusion, combined with thresholds of RBF and U/O. A higher quality assessment score indicates more injury and higher risk of delayed graft function (8). In this report, both kidneys were declined for transplantation due to inadequate *in situ* perfusion by all UK transplant centers. Fifty percent of the surface area of each kidney had a purple blotchy appearance. The logistics of recruiting declined kidneys into this research study are problematic, and at the time of arrival an added difficulty was the prolonged cold ischemic time. In the United Kingdom, kidneys for research are offered through at national system based on the geographical area. Although there is discussion to change the system, at present there is no priority for any individual study. Despite the appearance of the kidneys and prolonged cold ischemic time, within minutes of EVNP the left kidney was pink and evenly perfused with no evidence of microvascular disruption. The right kidney was slower to start. Nonetheless, by 15 min the kidney was pink and evenly perfused. Both kidneys had a

good level of RBF and produced a large volume of urine with EVNP quality assessment scores of 1 and 2, respectively. After EVNP, the kidneys were flushed with cold preservation solution and packed in ice until transplantation. Both kidneys had initial graft function and good levels of renal function 3 months posttransplantation. In normal circumstances, EVNP is carried out immediately before transplantation with an added cold ischemic time of on average 30 min. In this case, the left kidney was stored for approximately a further 2 h, and the right, just over 4 h. We have previously reported a successful case of intermediate EVNP in which the kidney was stored for a further 5 h before transplantation with no adverse effects. This present case emphasizes how EVNP can used to assess and judge the suitability of a kidney for transplantation before committing the recipient to surgery. More prolonged periods of EVNP are possible to avoid the additional cold ischemic insult. However, there is currently no data to support this. To our knowledge, this is the first report of the successful transplantation of human kidneys deemed unsuitable for transplantation but resuscitated by EVNP.

Conclusion

EVNP has the potential to rescue kidneys previously deemed untransplantable because of inadequate flushing with cold preservation solution, thereby reducing their discard rate.

Acknowledgments

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

References

1. NHS Blood and Transplant, Organ donation and activity report 2014–2015. [cited 2016 Jan 21]. Available from: http://www.odt.nhs.uk/uk-transplant-registry/annual-activity-report/.
2. Callaghan CJ, Harper SJ, Saeb-Parsy K, et al. The discard of deceased donor kidneys in the UK. Clin Transplant 2014; 28: 345–353.
3. Hosgood SA, Barlow AD, Dormer J, Nicholson ML. The use of *ex-vivo* normothermic perfusion for the resuscitation and assessment of human kidneys discarded because of inadequate in situ perfusion. J Transl Med 2015; 13: 329.
4. Sindram D, Porte RJ, Hoffman MR, Bentley RC, Clavien PA. Platelets induce sinusoidal endothelial cell apoptosis upon reperfusion of the cold ischemic rat liver. Gastroenterology 2000; 118: 183–191.
5. Hosgood SA, Nicholson ML. *Ex vivo* normothermic perfusion of declined human kidneys after inadequate in situ perfusion. Am J Transplant 2014; 14: 490–491.
6. Hosgood SA, Nicholson ML. First in man renal transplantation after *ex vivo* normothermic perfusion. Transplantation 2011; 92: 735–738.
7. Nicholson ML, Hosgood SA. Renal transplantation after *ex vivo* normothermic perfusion: The first clinical study. Am J Transplant 2013; 13: 1246–1252.
8. Hosgood SA, Barlow AD, Hunter JP, Nicholson ML. *Ex-vivo* normothermic perfusion for quality assessment of marginal donor kidney transplants. Br J Surg 2015; 102: 1433–1440.
9. Gok MA, Bhatti AA, Asher J, et al. The effect of inadequate in situ perfusion in the non heart-beating donor. Transpl Int 2005; 18: 1142–1146.
10. Hosgood SA, van Heurn E, Nicholson ML. Normothermic machine perfusion of the kidney: Better conditioning and repair? Transpl Int 2015; 28: 657–664.
11. Hosgood SA, Patel M, Nicholson ML. The conditioning effect of *ex vivo* normothermic perfusion in an experimental kidney model. J Surg Res 2013; 182: 153–160.