Viability Assessment and Utilization of Declined Donor Kidneys with Rhabdomyolysis using Ex Vivo Normothermic Perfusion Without Preimplantation Biopsy

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Financial disclosures: Nothing to declare

Acknowledgments / Funding: Nothing to declare

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List of abbreviations:

AKI – Acute Kidney Injury

AKIN - Acute Kidney Injury Network

DCD - Donor from circulatory death

DGF – Delayed graft function

ECD – Extended criteria donor

eGFR – Estimated glomerular filtration rate

EPTS score - Estimated Post Transplant Survival score

EVNP – Ex vivo normothermic perfusion

KDPI – Kidney donor profile index

KDRI – Kidney donor risk index

PNF – Primary non-function

RIFLE - Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease

Abstract

The role of ex vivo normothermic perfusion (EVNP) in both organ viability assessment and reconditioning is increasingly being demonstrated. We report the use of this emerging technology to facilitate the transplantation of a pair of donor kidneys with severe acute kidney injury (AKI) secondary to rhabdomyolysis. Donor creatinine was 10.18mg/dL with protein (30mg/dL) present in urinalysis. Both kidneys were declined by all other transplantation units and subsequently accepted by our unit. The first kidney was perfused with red cell-based perfusate at 37°C for 75 minutes, mean renal blood flow was 110 ml/min/100g and produced 85mls of urine. Having demonstrated favourable macroscopic appearance and urine output, the kidney was transplanted into a 61yo peritoneal dialysis-dependent without complication. Given the reassuring information from the first kidney provided by EVNP, the second kidney was not perfused with EVNP and was directly implanted to a 64yo patient. The first kidney achieved primary function and the second functioned well after delayed graft function. Recipient eGFR have stabilized at 88.5 and 55.3, respectively (mL/min/1.73m^2), at two months post-transplant.

Introduction

Ex vivo normothermic perfusion (EVNP) is becoming an established method of both assessing and potentially reconditioning marginal kidneys.(1) Despite the emerging reconditioning techniques, which hope to expand the pool of potential donor organs, there remains a significant shortfall between the number of utilizable grafts and recipient demand.(2) The increasing use of 'extended-criteria' donors (ECD) has been one strategy to increase the availability of grafts. Although it has been widely shown that kidneys from ECDs have inferior outcomes(3), the proportional impact of donor terminal creatinine is less certain. Terminal creatinine is a predictive component of the US-based kidney donor profile index (KDPI, formerly KDRI)(4), however, it is not part of the more recently implemented UK allocation donor risk indices.(5)

Despite the aforementioned uncertainty, terminal donor creatinine has long been thought of as a negative indicator of post-transplant function. Studies have shown higher rates of delayed graft function and prolonged index hospital admission. (6–9) These studies have, however, used varying absolute serum creatinine levels to stratify between presence and severity of AKI. Most studies adopt the commonly used stratification tools RIFLE criteria or the more recent Acute Kidney Injury Network (AKIN) criteria(10), both of which are designed for assessment and treatment of native kidney injury and, although relevant, are not designed for use in real-time transplant decision making. A retrospective analysis of the UK transplant registry assessed transplant outcomes in relation to the AKIN criteria and similarly, that donors with AKI were associated with higher rates of DGF, but also reported increased graft loss and primary non function (PNF) with AKIN stage 3 kidneys.

The recommendations were that careful selection of AKIN stage 1 and 2 is safe, but caution is advised with AKIN stage 3 donors.(11)

As a result, donor acute kidney injury (AKI) is commonly cited as a reason to decline a donor organ. A retrospective study by Kayler (2009) found that where terminal creatinine exceeded 2.0mg/dL, neither kidney was retrieved in 44% of occasions, in contrast to 2.3% when levels were less 1.99mg/dL or lower, and those >2.0mg/dL were associated with a seven fold increased risk of organ decline post-retrieval compared to grafts with terminal creatinine <1.5mg/dL.(8) Corroborating this, a European group previously reported donor creatinine to be the second most common reason for organ decline.(12)

Rhabdomyolysis is a well-known cause of AKI which is characterized by a syndrome of muscle injury, myoglobinuria and electrolyte imbalance. Myoglobin is thought to be the primary toxin responsible for the resultant renal tubular injury by way of tubular obstruction, inflammation, and vasoconstriction. The aetiology of rhabdomyolysis is commonly trauma with or without crush injuries, however, it is also well described in illicit drug use and with medication side effects.(13) Kidneys from donors with severe AKI secondary to rhabdomyolysis have been transplanted with varying results. There are limited data on the outcomes of kidneys transplanted from deceased donors with AKI due to rhabdomyolysis. DGF is common, however long-term graft outcomes have been reported to be acceptable.(14,15,16) Although pre-implantation histological analysis may aid the decision-making process, in many transplant centers, the resources are not in place to routinely perform pre-implantation analysis.

EVNP is an alternative method, which has been shown to be an effective point-of-care tool for assessing marginal grafts prior to transplantation. Depending on perfusion

parameters, macroscopic appearance and urine output, the kidney can evaluated (EVNP quality assessment score 1-5), with a score of 3 or less considered safe for transplantation.(1) The assessment tool has been demonstrated with declined donor after circulatory death (DCD) kidneys due to ‘poor in situ perfusion’ whereby a favourable quality assessment score facilitated subsequent successful transplantation.(17)

Case Detail

A pair of kidneys from a 37-year-old male donor from circulatory death (DCD) were offered to our transplant unit. Past medical history included recreational drug use only. Serum creatinine on admission to intensive care was 2.1mg/dL and increased to 3.2, 5.0, 8.4 over the subsequent days prior to donation offer. Historic serum creatinine prior to admission was 0.9mg/dL. A clinical diagnosis of rhabdomyolysis was made based on serum creatinine kinase (peak value 36,284 U/L) and urine colour (etiology unknown). At time of offer, terminal serum creatinine was 10.2mg/dL.

Both intended recipients were counselled and consented for the use of these kidneys on the proviso that the kidneys have an acceptable viability assessment with EVNP. The first kidney was accepted for a 61-year-old with end stage renal failure treated with peritoneal dialysis (see Table 1. for full donor and recipient demographics). EVNP was conducted as per the previously described Nicholson protocol(17); pressure was maintained at 75mmHg, with a single unit of 0 negative packed red cells suspended in crystalloid solution with multivitamin, sodium bicarbonate, dextrose and dexamethasone

administered, warmed to a temperature of 37°C with no additional filtration of the perfusion circuit (Figure 1.).

The kidney maintained a good appearance throughout the 75 minutes of perfusion. Mean renal blood flow was 110ml/min/100g and produced 85mls of urine (EVNP quality assessment score of 2: Moderate appearance (2 points); Mean renal blood flow >50ml/min/100g (0 points), Total urine output (ml) >43mL/hour (0 points)). Urine was clear and demonstrated normal appearance, however was not tested for myoglobin. Blood gas analysis from the arterial limb of the perfusion circuit demonstrated potassium and lactate within normal levels. To minimise overall ischemic time, at 20 minutes of perfusion, the decision was made to proceed with the operation for the planned recipient. The kidney was removed from the perfusion device at 75 minutes, flushed with 2L of cold hyperosmolar citrate perfusion solution and maintained on ice until required. The operation was performed without complication.

There were 9 minutes of warm ischemic time at organ procurement, and a total of 10 hours 20 minutes cold ischemic time, 45 minutes of which followed EVNP when placed back on ice prior to implantation. Whilst acknowledging that pairs of kidneys do not always perform in an identical manner, the implanting surgeon for the second kidney was given confidence by the period of assessment provided by EVNP of the first kidney. With this additional information, and wishing to minimise further cold ischemic time, the decision was made to implant the second graft without EVNP. The recipient was a 64-year-old haemodialysis-dependent patient. Unfortunately, on reperfusion of the kidney there was evidence of hilar bleeding which resulted in an additional 14 minutes of warm ischaemic time whilst control was established and kidney perfusion recommenced. The patient

required dialysis in the immediate post-operative period due to hyperkalaemia and delayed graft function persisted for 7 days. Both grafts are now functioning well, now at two months post-transplant, with serum creatinine of 0.71mg/dL and 1.38mg/dL for Patient 1 and Patient 2, respectively (corresponding to an eGFR of 88.5 and 55.3mL/min/1.73m^2, see Figure 2.).

Discussion

Kidneys are commonly declined due to donor AKI, particularly when classified as severe (AKIN 3 criteria), as the literature strongly advises caution with such grafts. Pre-implantation biopsies may offer additional information in cases with otherwise favourable donor characteristics, and a reversible cause of the apparent AKI. The availability of real-time biopsy information in many transplant centers, however, is limited. Provision of a 24-hour service is complex and costly and evidence for the ability of pre-implantation histology to improve kidney utilization remains lacking. Similarly, the overall cost-effectiveness of this approach remains in question. A UK national study, PITHIA(18), has been instituted to address this question in DCD kidneys but kidneys from younger donors with AKI, such as those described in this report, fall outside its scope. Neither kidney in this report were biopsied prior to implantation.

Ex-vivo normothermic perfusion offers an alternative method of assessing such kidneys providing real-time information on the appearance whilst on perfusion, intra-renal vascular resistance via pump parameters, and urine output.(17) This approach has the potential for expanding the pool of utilizable grafts through the implantation of currently discarded

kidneys as in this case. Furthermore, in this case where the pair of kidneys was offered, the viability assessment of the first kidney whilst on perfusion was deemed sufficient for the paired kidney to be considered transplantable without EVNP. It is important to note that the cost of perfusing a kidney *ex vivo* as per established protocols is approximately £1000 within the UK (approximately $1250) and requires a considerable amount of hardware and expertise. In circumstances where there is uncertainty regarding graft suitability, delivering EVNP to one of the paired grafts, could provide clinicians with helpful information on both grafts, even if destined for a different transplantation unit.

An important further consideration is the reconditioning potential of EVNP, a benefit now demonstrated in pre-clinical studies(19), and under evaluation in an ongoing randomized control trial comparing EVNP to static cold storage.(20) Importantly, this report documents the use of EVNP with only a single pair of DCD grafts and therefore generalizability of these findings are limited. Furthermore, in this case, due to the surgical complication evident during the implantation to Patient 2, it is not possible to draw any conclusions from the apparent differences between the two grafts, much less the therapeutic/reconditioning effect of EVNP. Moreover, there were many recipient differences between these two cases. Pre- and post-EVNP biopsies were not taken in this instance, which represents a further limitation of this report. Histological evidence of ‘washout’ of the obstructing casts seen in rhabdomyolysis-induced kidney injury, for example, may have contributed to the understanding of this perfusion technique’s therapeutic utility.

We believe, however, that this report illustrates the ability for EVNP to assess grafts at the extremes of common graft acceptance, where favourable evaluation would provide

clinicians with sufficient information to proceed with transplantation. The new National Kidney Allocation Scheme (commenced September 2019) in the UK offers paired kidneys to transplantation units where donors are categorized as the marginal (so called ‘D4’) and donor age exceeds 70 years. As a result, more units will be offered pairs of kidneys in this manner, and therefore, assessment of one kidney may be sufficient to provide assurance to transplant both grafts.

Table 1. **Donor and recipient demographics including details of procurement and cold ischaemic time**

	Age	**Primary Renal Disease**	**Renal Replacement Therapy**	**Past medical history**	**WIT (mins)**	**CIT (Hours: mins)**	**KDPI / KDRI**	**UK Donor /Rec Risk Indices**	**EPTS score**
Donor	37	-	-	Nil**	-	-	KDRI 56% - 1.06*	D2	-
Recipient 1	61	Lithium toxicity	Peritoneal Dialysis	Ischaemic heart disease	9	10:20	-	R3	30%
Recipient 2	64	IgA Nephropathy	Haemodialysis	Ischaemic heart disease	23 (9 + 14)	13:40	-	R3	48%

*KDPI creatinine level capped at 8mg/dL so unable to enter accurate value of 10.2mg/dL

**Cause of death – Intracranial haemorrhage

WIT – Warm Ischaemic time

CIT – Cold Ischaemic time

EPTS score - Estimated Post Transplant Survival

Figure 1. ***First paired kidney pre- and post-perfusion with normothermic red cell-based perfusate***

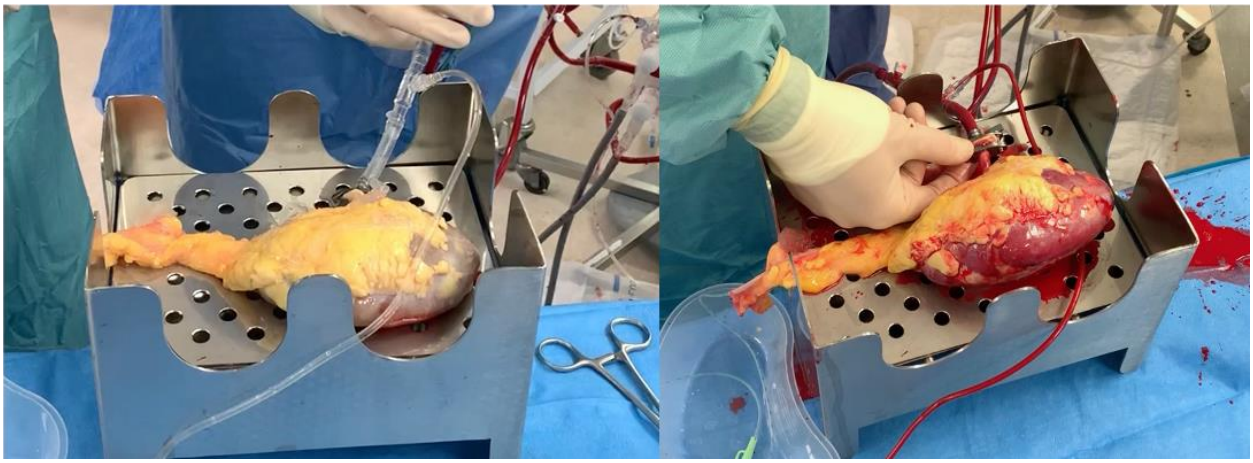
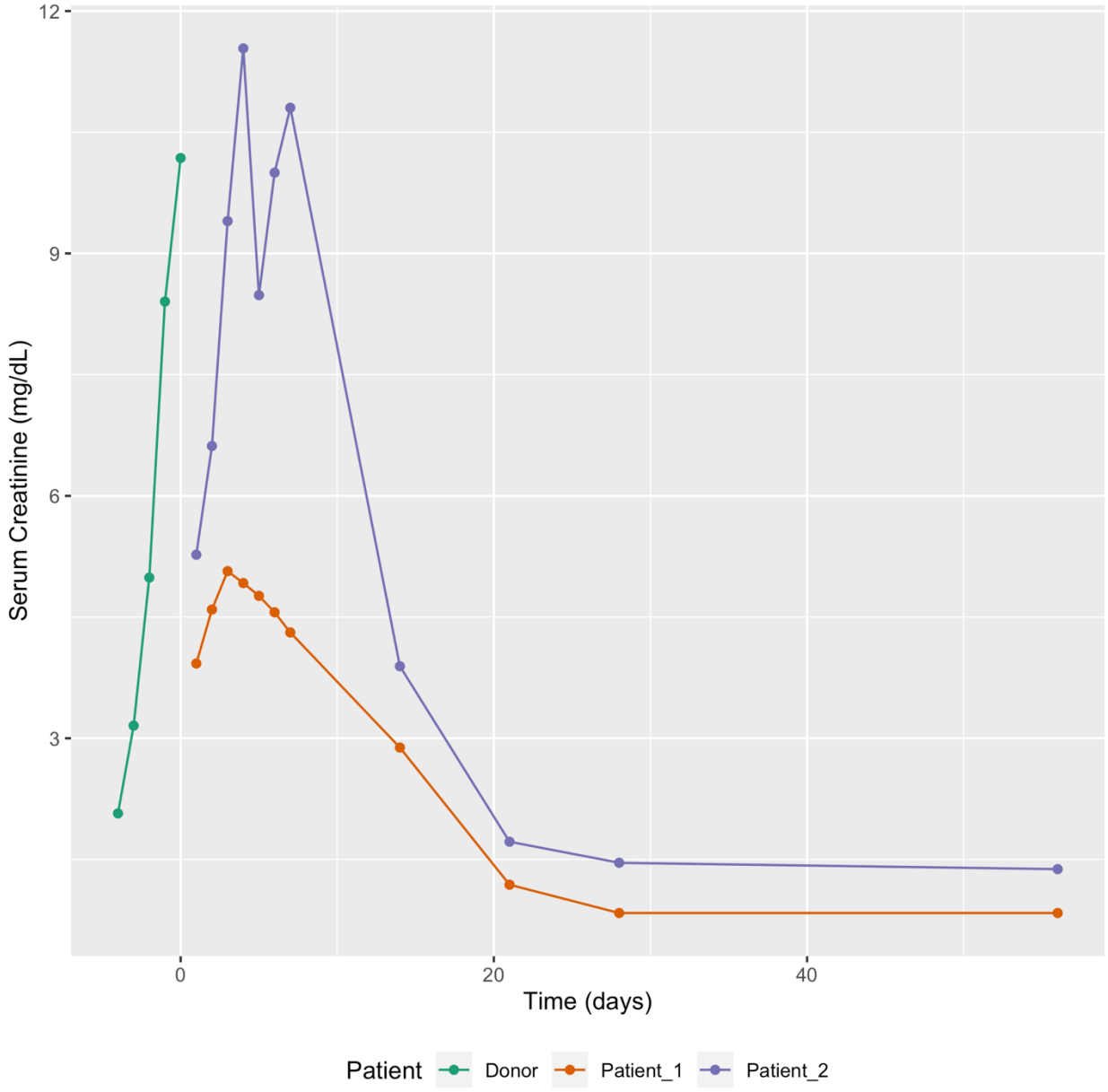


Figure 2. ***Serum Creatinine (mg/dL) over time for Patient 1, Patient 2 and the donor kidney***



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