Comparison of the Outcome of Kidney Transplant After Pulsatile or Continuous Ex Vivo Hypothermic Machine Perfusion of Kidneys Donated After Cardiac Death: Analysis of Kidney Pairs

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ABSTRACT

Background. Hypothermic machine perfusion is used to improve renal perfusion and reduce the rate of early and late graft dysfunction. It has been used in our unit since 2001. It has 2 modes of flow: continuous or pulsatile. The aim of this study is to compare the modes of perfusion in terms of perfusion-related parameters, graft survival, and estimated glomerular filtration rate.

Methods. All donation after cardiac death kidneys between 2002 and 2014 were reviewed. A total of 64 pairs of kidneys were identified of which one kidney underwent pulsatile and the other continuous perfusion. Machine parameters including resistance and perfusion flow index levels at 0, 1, 2, 3, and 4 hours were recorded and glutathione S-transferase was measured in perfusate. Estimated glomerular filtration rate from the first week of transplant until the fifth year and graft survival rates were determined.

Results. Machine parameters were similar at all time points. Estimated glomerular filtration rates and graft survival were the same irrespective of perfusion mode.

Conclusion. Pulsatile perfusion may be regarded as more physiological. However, we could not identify difference in outcome following transplant of kidneys from the same donor that had been perfused under pulsatile or continuous conditions.

THE BURDEN of chronic kidney disease is increasing steadily worldwide. Chronic kidney disease was the 27th most common cause of death in 1990, but by 2010 it was the 18th most common [1]. More than 2 million people are now treated for kidney failure, either by dialysis or transplant [2]. Transplant offers superior survival and quality of life compared with dialysis [3]. Unfortunately, demand for transplant outweighs supply of donor organs, with long waiting times for transplant. To overcome this issue, there has been increasing use of organs donated after cardiac death (DCD), which now represents an important source of kidneys in many countries including the United Kingdom. In the United Kingdom, the number of DCD kidney transplants was 37 in 2000, whereas this increased to 619 in 2017 [4,5].

The challenge with DCD kidneys is minimizing ischemia reperfusion injury induced by longer warm ischemia times (WIT). Ex vivo machine perfusion has been extensively investigated and used as an intervention to reduce the impact of ischemia and therefore reduce the severity of reperfusion injury, which may be particularly important for DCD kidneys. There are 2 main types of machine perfusion: normothermic and hypothermic perfusion. Normothermic machine perfusion is a more recent development, and its benefit is still being evaluated [6]. In contrast, hypothermic

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perfusion has been evaluated in several clinical trials. Moers et al showed that machine perfusion is superior to static cold storage in improving 1-year graft survival and reducing the duration of delayed graft function [7,8]. There are other studies reporting an advantage of machine perfusion over static cold storage [9–13]. Hypothermic perfusion has even been used for living donor kidneys with estimated glomerular filtration rate (eGFR) higher at 1 year in recipients of kidneys that had undergone hypothermic machine perfusion compared with static cold storage [14]. Two types of hypothermic perfusion machines are available: the RM3 Renal Preservation System (Waters Medical Systems, Rochester, Minn, United States) and the Lifeport Kidney Transporter (Organ Recovery Systems, Itasca, Ill, United States). Lifeport machine perfusion has been used in Newcastle upon Tyne since 2001. The latter has 2 modes of perfusion: continuous or pulsatile. The pulsatile mode perfuses kidney at a set systolic pressure 30 times per minute. The continuous mode perfuses kidneys at a constant pressure [15].

There is limited data comparing the modes of perfusion. To the best of our knowledge, the effects of the different modes of perfusion available on the Lifeport machine on transplant outcome have not been compared. The aim of this study is to assess the effect of pulsatile and continuous machine perfusion modes on eGFR and graft survival, uniquely using pairs of kidneys from the same donor.

MATERIAL AND METHODS

When 2 Lifeport machines were purchased from Organ Recovery Systems, the perfusion mode was set as pulsatile for one machine and continuous for the second. Therefore, for DCD donors providing 2 kidneys, each kidney had a different mode of perfusion. All DCD kidney transplants between 2002 and 2014 at the Freeman Hospital, Newcastle upon Tyne NHS Foundation Trust were retrospectively reviewed. Exclusion criteria were patients who received dual transplants, kidneys without machine perfusion, kidneys in which 1 of the pair was transplanted at another center, and kidney pairs where both perfused on same mode. Figure 1 summarizes details of the 2 groups. Details about donor's age, sex, Maastricht category, presence of donor hypertension, first and second WIT, cold ischemia time were collected from donor request forms and clinical notes. Details of recipient's sex, age, transplant date, graft survival details, creatinine values, presence of delayed graft function (DGF) and eGFRs at the 1st week; 1st, 3rd, 6th, 9th, and 12th months; and 2nd, 3rd, 4th, and 5th years were collected from hospital record

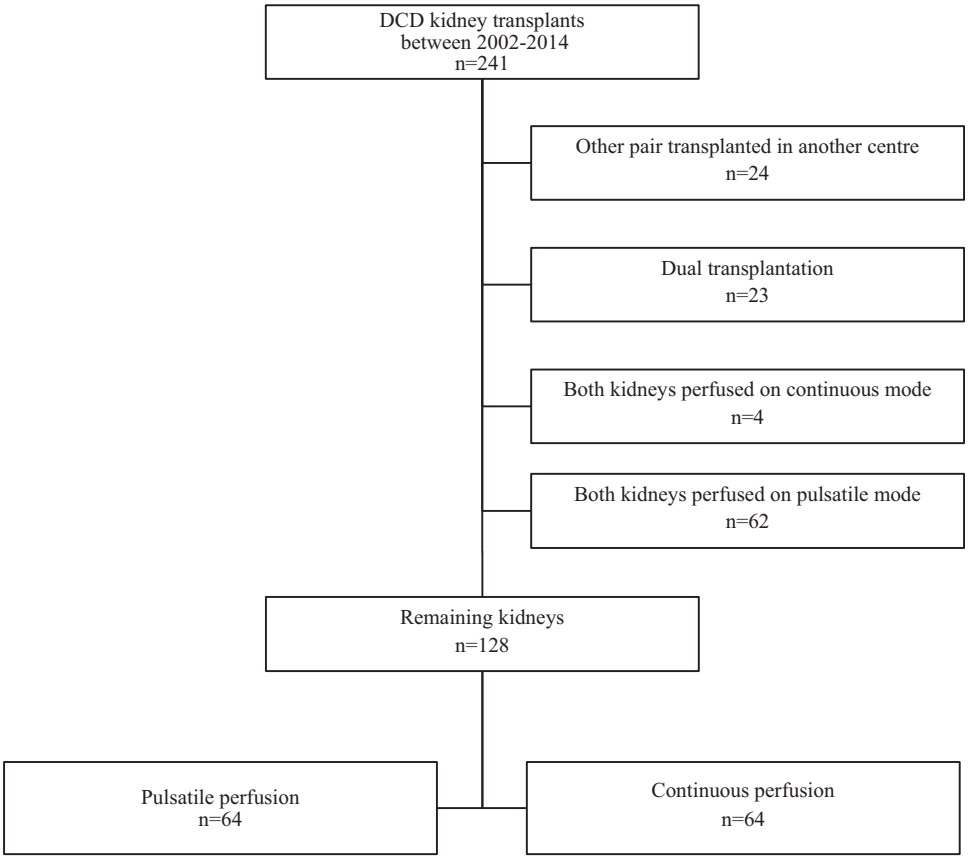

Fig 1. Details of patient cohort. All donation after cardiac death (DCD) kidney transplants from 2002–2014 were included.

Table 1. Demographic Characteristics of All Patients

	All Patients (n = 128)	Pulsatile Perfusion (n = 64)	Continuous Perfusion (n = 64)	*P* Value
Recipient age, median (IQR), y	55.5 (47–63)	55.5 (47–63)	55.5 (45–63)	.89
Female recipient, %	30.5	21.9	39.1	.04
Donor age, median (IQR), y	51 (39–63.75)			
Female donor, %	35.9			
Maastricht category, %				
II	4.7			
III	92.2			
IV	3.1			
Donor hypertension, %				
Yes	25			
No	68.8			
Unknown	6.2			
1st WIT, mean (SD), min	19.55 (5.85)	19.7 (5.92)	19.4 (5.83)	.77
2nd WIT, mean (SD), min	40.64 (12.75)	40.19 (8.87)	41.06 (15.64)	.76
Total WIT, mean (SD), min	60.5 (13.93)	60.43 (11.09)	60.56 (16.23)	.63
Minimum CIT, mean (SD), min	1206.11 (292.25)	1219.48 (296.10)	1193.58 (290.38)	.62

Abbreviations: CIT, cold ischemia time; IQR, interquartile range; WIT, warm ischemia time.

systems. Delayed graft function was defined as the requirement for dialysis in the first 7 days after transplant.

Machine perfusion data including machine type, perfusion flow index (PFI), resistance (RT) [16], and glutathione S-transferase (GST) concentrations were collected. During perfusion, systolic pressure was increased to 30 mm Hg and fixed at that pressure. The PFI is calculated by dividing flow by systolic pressure and expressed as mL/min/mm Hg per 100 g of kidney. The RT is calculated as mean pressure divided by flow at specified time and presented as mm Hg/mL/min. Total GST level was measured from the perfusate fluid. Perfusion measurements and 10 mL perfusate for GST analysis were taken at 0, first, second, third, and fourth hour of perfusion. Belzer UW machine solution (Preservation Solutions, Inc, Elkhorn, WI, USA) was used for both modes of perfusion.

All patients had received induction with basiliximab and standard triple maintenance immunosuppression consisting of tacrolimus, mycophenolate mofetil, and prednisolone as regulated by Freeman Hospital guideline. The target trough tacrolimus levels were 6 to 10 μg/L for the first 6 months, 5 to 8 μg/L for the second 6 months, and 3 to 7 μg/L thereafter.

Statistical Analysis

Statistical analysis was performed by SPSS version 15.0 (IBM, Armonk, NY, United States). Continuous variables were compared by independent samples *t* test and Kruskal-Wallis test. Categorical variables were compared by χ^2 test. Graft survival was analyzed by Kaplan-Meier curves and log-rank method.

RESULTS

Demographic Data

In total, data from 241 kidneys transplanted between 2002 and 2014 were analyzed. After exclusion of 113 kidneys for reasons detailed Fig 1, a total of 64 pairs of kidneys were included of which one of the pair was perfused in pulsatile mode and the other was perfused in continuous mode.

Demographic characteristics of donors and recipients and details about ischemia times are given in Table 1. Pulsatile and continuous groups were similar in comparison of recipient age, first and second WIT, total WIT, and cold ischemia time ($P > .05$ for all). Female recipients were 21.9% of the pulsatile group and 39.1% of the continuous group ($P = .04$). Donor age, hypertension, and Maastricht category were identical as pairs of kidneys were analyzed.

Effect of machine mode on perfusion parameters and outcome. A summary of all perfusion parameters is given in Table 2. The RT values decreased gradually in both groups, and comparison showed they were similar ($P > .05$). The GST values from 0 hours until 4 hours were also similar between pulsatile and continuous mode ($P > .05$ for all analyses). Although PFI values were all higher in continuous mode for all times, these did not differ significantly from pulsatile mode ($P > .05$ for all). Delayed graft function occurred in 22.8% of transplants following pulsatile perfusion and in 25.4% following continuous perfusion ($P = .74$). Estimated glomerular filtration rates between pulsatile and continuous groups were similar from just after transplant (1 week) until the fifth year ($P > .05$ for all time points) (Fig 2). Graft survival was also similar between 2 modes of perfusion (log-rank $P = .80$) (Fig 3).

DISCUSSION

This study evaluated the possible relationship between machine mode during hypothermic perfusion and long-term outcomes of DCD kidney transplants. Selection of the study population to contain only pairs of kidneys excluded many possible biases in this study. Perfusing one kidney on pulsatile and the other kidney of the pair in continuous mode made donor characteristics including

Table 2. Machine Perfusion Parameters at 0, 1, 2, 3, and 4 Hours

	Pulsatile Perfusion (n = 64)	Continuous Perfusion (n = 64)	*P* Value
GST0, mean (SD), IU/L/100 g of kidney	24.7 (15.5)	25.8 (17.1)	.74
GST1, mean (SD), IU/L/100 g of kidney	52.1 (23.6)	59.7 (26.5)	.29
GST2, mean (SD), IU/L/100 g of kidney	61.9 (31.5)	72.2 (31.2)	.10
GST3, mean (SD), IU/L/100 g of kidney	69 (34.9)	76.2 (37.2)	.30
GST4, mean (SD) IU/L/100 g of kidney	80.6 (46.4)	83.4 (38.1)	.44
PFI0, mean (SD), mL/min/mm Hg/100 g kidney	0.71 (0.48)	0.84 (0.58)	.18
PFI1, mean (SD), mL/min/mm Hg/100 g kidney	1.32 (0.72)	1.43 (0.76)	.41
PFI2, mean (SD), mL/min/mm Hg/100 g kidney	1.36 (0.72)	1.55 (0.69)	.13
PFI3, mean (SD), mL/min/mm Hg/100 g kidney	1.45 (0.90)	1.52 (0.7)	.25
PFI4, mean (SD), mL/min/mm Hg/100 g kidney	1.63 (0.93)	1.9 (1.1)	.34
RT0, mean (SD), mm Hg/mL/min	0.67 (0.34)	0.67 (0.52)	.85
RT1, mean (SD), mm Hg/mL/min	0.30 (0.15)	0.32 (0.15)	.40
RT2, mean (SD), mm Hg/mL/min	0.28 (0.14)	0.30 (0.15)	.46
RT3, mean (SD), mm Hg/mL/min	0.26 (0.13)	0.31 (0.13)	.06
RT4, mean (SD), mm Hg/mL/min	0.22 (0.12)	0.27 (0.12)	.26
DGF, %	22.8	25.4	.74

Mann-Whitney test was used for all comparisons.
Abbreviations: DGF, delayed graft function; GST, glutathione S-transferase; PFI, perfusion flow index; RT, resistance.

donor age, donor sex, donor hypertension, and Maastricht Category identical in the 2 groups. Ischemia times were also similar between groups. Recipients received the same induction and standard triple maintenance immunosuppression according to local guidelines. Therefore, any difference in outcome is likely because of the mode used for hypothermic perfusion.

There are some studies comparing static cold storage with hypothermic perfusion using the Lifeport machine without citing mode of perfusion [9,10, 13], with continuous mode [11] or with pulsatile mode [12]. These studies showed benefit of machine perfusion over static cold storage in terms of recovery of renal function and length of hospital stay [9], graft survival [10,12], and DGF [11,13], although a benefit has not been seen in all studies [17]. In addition, Kozaki et al reported their experience of kidneys perfused on continuous mode to define viability of donated kidneys [18]. Two meta-analyses have been performed comparing hypothermic machine perfusion with static cold storage [19,20]. Machine perfusion had short-term benefits with decreased rate of DGF. Long-term results were similar. However, these studies did not address the question of whether continuous or pulsatile perfusion is superior. Perfusion modes have been compared in 2 animal studies. Implantation of left ventricular assistance devices was used to measure the effects of pulsatile and continuous systemic perfusion on renal sympathetic nerve activity in mongrel dogs [21]. Sympathetic nerve activity and peripheral vascular resistance were decreased by pulsatile perfusion. The authors concluded that pulsatile perfusion may

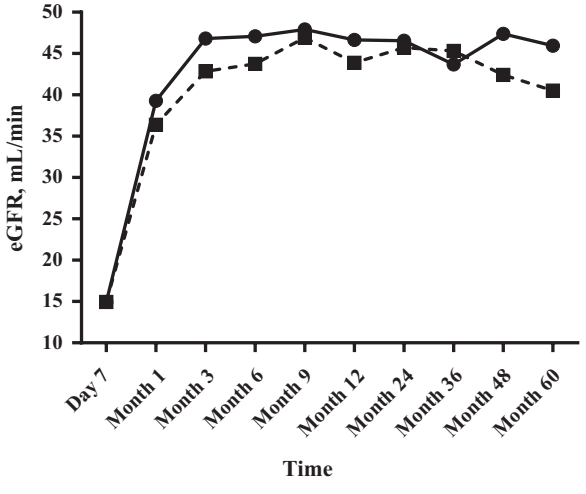

Fig 2. eGFR values according to machine mode. The solid line represents pulsatile mode, dotted line represents continuous mode. eGFR was similar at all time points ($P > .05$ for all). eGFR, estimated glomerular filtration rate.

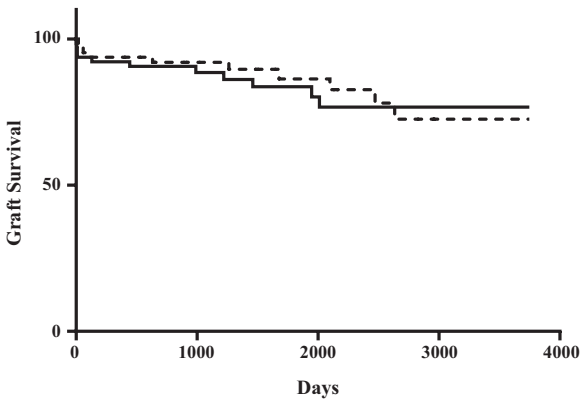

Fig 3. Comparison of graft survival according to perfusion mode. Kaplan-Meier analysis of graft survival following pulsatile (solid line) or continuous (dotted line) hypothermic machine perfusion ($P = .80$). Data are censored for death with a functioning graft.

therefore improve both microcirculation and organ function. Lindell et al compared modes of Lifeport machine perfusion beagle dogs [22]. After a warm ischemia period of 45 minutes, the left kidneys of 12 dogs were removed. Eight kidneys were maintained by pulsatile machine perfusion and 4 by continuous perfusion for 24 hours. Kidneys were autotransplanted after machine perfusion at which stage the right kidneys were removed. Serum creatinine levels were significantly lower in the pulsatile group. The survival was 50% in the continuous group and 100% in the pulsatile perfusion group.

This current study is the first to our knowledge to compare the 2 perfusion modes in clinical transplantation. No difference in either short (DGF) or long-term transplant outcomes (eGFR and transplant survival) were identified. Although it is a retrospective analysis of data, this study is strengthened by the ability to compare outcomes in pairs of kidneys from the same donor, one of which underwent continuous and the other pulsatile perfusion. Therefore, any theoretical advantage of more physiological pulsatile perfusion, supported by preclinical data, does not translate into clinical benefit.

In summary, whatever the mode of perfusion, machine perfusion has been superior to static cold storage in many studies. Animal studies comparing perfusion modes favor pulsatile perfusion. Pulsatile perfusion is perhaps more physiological, but despite this, we did not identify any difference in resistance or PFI between pulsatile of continuous flow. The GST concentrations in the perfusate, as a biomarker of renal cell injury, were similar in kidneys undergoing pulsatile or continuous perfusion. This suggests that nonphysiological continuous perfusion is not causing pressure-related injury to the perfused kidney. Most importantly, and in contrast to animal studies, we have found similar graft survival rates and eGFR values between different modes of perfusion.

CONCLUSION

This is the first study comparing the mode of hypothermic machine perfusion in human kidneys. Results of this study reveal that machine perfusion modes are not superior to each other, with similar DGF rates, graft function, and survival when the 2 modes are compared.

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