Usage guidelines

Ex vivo perfusion characteristics of DCD kidneys predict long-term graft survival

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Running title: Machine perfusion in DCD kidney transplantations

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Abstract

Background: Ex vivo perfusion is used in our unit for kidneys donated after cardiac death (DCD). Perfusion flow index (PFI), resistance (RT) and perfusate Glutathione S Transferase (GST) can be measured to assess graft viability. We assessed whether measurements taken during perfusion could predict long-term outcome after transplantation.

Methods: All DCD kidney transplants performed between 2002 and 2014 were included in this study. The exclusion criteria were unavailable data, kidneys not machine perfused, kidneys perfused on continuous mode and dual transplantation. There were 155 kidney transplants included in the final analysis. Demographic data, ischemia times, donor hypertension, graft function, survival and machine perfusion parameters after 3 hours were analysed. Each perfusion parameter was divided into three groups as high, medium, and low. Estimated glomerular filtration rate (eGFR) was calculated at 12 months and then yearly after transplantation.

Results: There was a significant association between graft survival and PFI, GST (p=0.020 and 0.022, respectively). PFI was the only independent parameter to predict graft survival

Conclusions: A low PFI during *ex vivo* hypothermic perfusion is associated with inferior graft survival after DCD kidney transplantation. We propose that PFI is a measure of the health of the graft vasculature and that a low PFI indicates vascular disease and therefore predicts a worse long-term outcome.

Keywords: Ex vivo machine perfusion, graft survival, donation after cardiac death (DCD), perfusion flow index (PFI), pulsatile machine perfusion

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Introduction

The number of patients with end stage renal disease (is increasing [1] leading to a greater demand for renal replacement therapies. Transplantation is the most effective treatment for end stage renal disease providing a significant survival advantage over dialysis [2], however, the demand for kidney transplantation outstrips the supply of organs. Decreased numbers of donation after brain death (DBD) kidneys has led to increased interest in donation after cardiac death (DCD) kidney transplantation [3]. In 2007, only 9 countries performed DCD transplantation, performing 778 transplants, but by 2014 this number had increased to 11 countries transplanting 3401 DCD kidneys [4, 5]. In 2007, 907 DBD and 300 DCD kidney transplants were performed in the UK. In 2013, whilst there was only a moderate increase in UK DBD transplants to 1160, the number of DCD transplants had more than doubled to 794 [1, 6, 7].

The main concern about DCD kidney transplantation is the magnitude of the ischaemic injury. It is greater than with DBD transplants because of the primary warm ischaemia time (WIT) between cardiac arrest and perfusion of the organs. The Maastricht Category was introduced to describe the clinical characteristics of DCD donors [8, 9]. Uncontrolled cardiac death (Maastricht Category I) describes donors who are declared dead outside of hospital. Maastricht Category II refers to donors unresponsive to resuscitation who are declared dead within hospital. Controlled cardiac death includes two subgroups: Maastricht Category III for donors awaiting cardiac arrest and Maastricht Category IV for donors suffering cardiac death after diagnosis of brain death. WIT is generally longer in uncontrolled categories. Gok et al found the longest WIT in Maastricht Category II donors with decreasing WIT in Maastricht Category III and IV. Delayed graft function was seen for 83.8 % of Maastricht Category II, 67.4 % of Maastricht Category III and 0% of Maastricht Category IV. However, one year graft survival was similar among these groups [10].

In an attempt to reduce the damage caused by ischaemia, machine perfusion has been used in Newcastle since 1998 [11].

One advantage of machine perfusion is its potential to assess the viability of a kidney, identifying organs at risk of primary non-function. Three parameters are measured by the Lifeport® perfusion machine: flow, pressure and resistance. Ischaemic injury causes loss of vasodilatation and potentially vessel occlusion due to thrombosis. Flow will be reduced as ischaemic injury increases so lower flow

rates could predict poorer graft function. Higher flow rates could be obtained by increasing perfusion pressure, however, may damage the kidney. To correct for pressure dependent variation in flow rate, we have used perfusion flow index (PFI) in our unit. Resistance (RT) is the second parameter that can be used to assess viability [8, 17]. It is an intrinsic property of the kidney that could be a biomarker of future function [18]. Resistance alsoreflects loss of vasodilatation caused by ischaemic injury. Glutathione S Transferases (GST) are a family of enzymes involved in toxin metabolism and found in different tissues [19]. GST levels may predict magnitude of cell membrane damage and cell death caused by ischaemic injury and has been shown to be a marker of kidney injury during machine perfusion [20].

Machine perfusion characteristics and perfusate total GST were routinely used in our unit to assess whether kidneys are suitable for transplant [21]. This was found to be more important for Maastricht Category II kidneys as for controlled donors very few are considered too poor to transplant [10]. The aim of this study is to determine whether machine perfusion characteristics measured prior to transplant are predictive of long-term outcome.

Materials and Methods

Patient cohort

All DCD kidney transplants between 2002 and 2014 were assessed for inclusion in the study. Dual transplants, kidneys not machine perfused, patients with incomplete data sets (<2/3 measurements available) and kidneys perfused in continuous mode were excluded from the study. Figure 1 summarises patient numbers and the reason for the exclusion from the final analysis. Details regarding donors' sex, age, and blood pressure history were collected from donor request forms. Maastricht Category, first and second WIT, cold ischaemic time and total ischaemic time were obtained from the clinical notes.

Details about recipients were collected from the hospital's medical record system. The recipients' age, sex, graft and patient survival times, creatinine values at 12 months and yearly until 5 years after transplantation and estimated glomerular filtration rates (eGFR) were calculated using the 4 point MDRD equation.

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Perfusion data

Machine perfusion data including machine type, PFI, RT and GST concentrations were collected. Once the kidney was connected to the Lifeport, systolic pressure was increased to a maximum of 30 mmHg and fixed at that pressure. PFI is calculated by dividing flow by systolic pressure and expressed as ml/min/mmHg per 100 g of kidney [16]. RT is calculated as mean pressure divided by flow at specified time and presented as mmHg/ml/min. Total GST level were measured from the perfusate fluid [21]. Perfusion measurements and 10 mls of perfusate for GST analysis were taken at the third hour of perfusion.

All parameters were categorized into 3 groups empirically; low, medium and high. PFI values were: low ≤ 0.8, medium 0.81 to 1.2 and high ≥ 1.21 ml/min/mmHg per 100 g of kidney. RT groups were: low ≤ 0.2, medium 0.21 to 0.3 and high ≥0.31 mmHg/ml/min. GST groups were: low ≤ 50, medium 51 to 75 and high ≥ 76 IU/L per 100g of kidney.

Statistical analysis

Scientific Package for Social Science (version 15.0; SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Chi-square test, independent-samples t test and Kruskall-Wallis tests were used for comparison of variables. Bonferonni correction was applied for pairwise comparisons. Kaplan–Meier curves and log rank method were used for graft survival. Cox regression models were used to evaluate independent parameters of graft survival. A linear regression was performed to find independent predictors of eGFR.

Results

All patients undergoing DCD kidney transplantation between 2002 and 2014 at the Freeman Hospital, Newcastle upon Tyne were reviewed (n=403). After exclusion criteria were applied 155 kidney transplant recipients were included in the final analysis (Figure 1).

Demographic characteristics of all patients included in the analysis are presented in Table 1. The median age of recipients was 55 years and of donors was 50 years. Males were predominant, both as donors and recipients. Kidneys were Maastricht Category III in 92.9 % of transplants. Hypertension was present in 18.1% of donors.

The effect of donor hypertension on perfusion parameters and transplant outcome

Mean age of hypertensive donors was significantly higher than non-hypertensive donors (p<0.001). The distribution of PFI status was significantly different in hypertensive donor group compared to the non-hypertensive donor group (p=0.03), with a higher proportion of kidneys with low PFI from hypertensive donors, presumably due to changes in renal vasculature. There was no significant relationship between RT or GST grouping and donor hypertension (p=0.051 and 0.342, respectively) (Table 2). eGFR was significantly higher in recipients of a kidney from a non-hypertensive donor 12 months after transplant (p=0.049). No differences were seen in eGFR beyond 12 months. There was no relationship between donor hypertension and graft survival (p=0.781) (Figure 2).

The relationship between perfusion parameters, donor and graft characteristics

PFI, RT, and GST measurements of DCD kidneys after 3 hours of perfusion (n=131, 130 and 87 respectively) were grouped into three categories as detailed in the methods section. Demographic and other parameters for the PFI groups are presented in Table 3. A Kruskal-Wallis test showed that there was a significant difference in PFI related to age (p=0.001) Donor age was 55.5 years in low PFI group, () 46.5 years in high PFI group and 47 years in medium PFI group () indicating lower flow rate for a given perfusion pressure in kidneys from older donors (Supplementary Figure 1). Pairwise comparisons showed significant differences in age between low versus medium and low versus high PFI groups. 1st WIT was similar among PFI groups.

Demographic and graft characteristics in the GST groups are given in Table 4. Data were not available for all patients (n=87). All kidneys in the low GST group were Maastricht Category III type. In contrast, in the high GST group 17.2% of donors were Maastricht Category II (p=0.02, Chi-square test). The first WIT was longer in the high GST group (18.3±5.9, 18.7±5.8, 22±5.3, low, medium, high GST groups respectively, p=0.016). Maastricht type and the duration of first WIT are therefore the major parameters influencing perfusate GST concentration.Most of the donors from the high RT group were female (64.7%) compared to 29.7% and 32.1% in the low and medium groups respectively (p=0.002). There was no significant difference in frequency of donor hypertension among the GST or RT groups (Tables 4 and 5).

Relationship between perfusion parameters and long- term graft survival and function

PFI was significantly associated with outcome (p=0.02 by log rank analysis). Patients receiving a kidney with a high PFI had superior transplant survival (Figure 3A). Pairwise comparison demonstrated a statistically significant survival difference in survival between low and high PFI groups (p=0.005) and medium and high PFI groups (p=0.048). Cox regression analysis demonstrated that PFI was an independent predictor of graft survival (p=0.021) in a model including donor age, recipient age,RT group, cold ischaemia time (Table 6). The hazard ratio for graft loss was 4.752 (CI: 1.283-17.594 p=0.02) in the medium PFI and 10.733(CI 1.919-60.021 p=0.007) in the low PFI group compared to high PFI group. Graft survival was significantly different among GST groups (Figure 3B). Patients in the medium GST group had the worst graft survival (p=0.022). Graft survival did not differ between RT groups (p=0.472) (Figure 3C).

There was a significant association between eGFR measured at 12, 24, 36, 48 months post transplant and PFI grouping (p<0.05 for all) (Figure 4A). Comparison of mean eGFR between low versus medium PFI groups and low versus high PFI groups were also significant at all time points (p<0.05 for all analyses). Pairwise comparisons of eGFR between medium and high PFI groups were similar (p>0.05 for all analyses). A low PFI is therefore a predictor of a lower eGFR up to 4 years post-transplant. There was no significant association between GST or RT and eGFR at 12, 24, 36, 48 months post-transplant (p>0.05 for all analyses) (Figure 4B and C). However, using multiple linear regression analysis with the variables donor age and sex, recipient age and sex, donor hypertension and PFI, GST, RT grouping, PFI was not independently associated with eGFR. The main independent effect on eGFR was donor age (12 months: p=0.003, B=-0.486; 24 months: p=0.005, B=-0.553; 36 months: p=0.021, B=-0.492) (see the Supplementary Table 1).

Discussion

Measurements taken during machine perfusion have been used in our centre to define the viability of kidneys from DCD donors. This has been most useful in Maastricht Category II donor kidneys as the viability of kidneys from Maastricht Category III and IV donors is usually good [3, 11]. In this study, we used machine perfusion data primarily from Maastricht Category III DCD kidneys that have been transplanted to assess the relationship between these measurements and longer term graft survival

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and function. We found that PFI can predict graft survival whereas donor age is a more important for predictor of eGFR. To the best of our knowledge, this is the first evaluation of the relationship between PFI and graft survival.

Evidence of the benefits of machine perfusion is conflicting. Moers et al have shown better 1 year graft survival and lower delayed graft function rates after machine perfusion MP in a cohort composing both DCD (n=42) and DBD kidneys (n=294) [12] and these findings were replicated in other studies [13, 14]. However, Moers have stated later on that three years after transplantation machine perfusion MP had no advantage over cold storage in terms of graft survival in DCD kidney transplantation. In addition, a multicentre study conducted in the UK showed no advantage of machine perfusion MP over cold storage in terms of incidence of delayed graft function, renal function and 1 year graft and patient survival [15]. A meta-analysis also showed that machine perfusion decreased the incidence of delayed graft function compared to static cold storage but graft and patient survival at 1 year were similar [23]. The main focus of our study, however, was exploring the relationship between machine perfusion parameters and graft survival rather than comparison of machine perfusion with cold storage.

A minority of donors were hypertensive. There was no relationship between donor hypertension and graft survival. This contrasts with earlier work that found donor hypertension may have an effect on graft survival [24-26]. The number of patients in our study with hypertension may have been insufficient to demonstrate this effect and diagnosis or reporting of donor hypertension may have been incomplete; this result should therefore be explored in further research with a larger group of patients. As would be expected hypertensive donors were older than non hypertensive donors. Donor hypertension was more frequent in the low PFI and high RT group. The reason for this might be increased stiffness of vessels relating to high blood pressure and possible comorbid conditions including atherosclerosis. As stiffness increases, loss of vascular compliance will result in increased resistance to flow and lower PFI.

PFI groups were different in terms of graft survival time with recipients of a kidney from the high PFI group having better graft survival compared to the other groups. Pairwise comparisons showed that survival of kidneys in the high PFI group was superior to those from the low and medium PFI groups. PFI level was concordant with eGFR values at 12, 24, 36, 48 months post transplantation with lower

eGFRs in recipients of kidneys from the low PFI group compared to medium and high PFI groups. There was a negative correlation between PFI and donor age, with PFI increasing with decreasing donor age, perhaps reflecting age related changes in the renal vascular bed (Supplementary Figure 1). This suggests that the changes in renal vasculature associated with low perfusate flow are also associated with and predictive of worse long-term graft survival.

Donor age has been found as a factor affecting graft survival in DCD kidney transplantation in other studies. Although there was a relationship between donor ages in univariate analysis, PFI was the only independent predictor of graft survival in multivariate analysis.

There was no association between graft survival, eGFR and RT. Similar to our study, Vries et al also found that renovascular resistance during machine perfusion did not relate to graft and patient survival [27]. In contrast, Jochmans et al has found that RT was an independent risk factor for delayed graft function and 1-year graft failure [28]. Patients in the medium GST group had worse graft survival and similar eGFR values compared to low and high groups.). The reason for this is unclear and cannot be explained by injury to the graft prior to recovery of organs. This relationship was no longer significant when multivariate analysis was performed and the absence of a relationship with eGFR suggests that this is observation is not biologically significant. Moers et al has looked for a relationship between perfusate parameters (lactate dehydrogenase, aspartate aminotransferase, total glutathione-S-transferase, alanine-aminopeptidase, N-acetyl-D-glucosaminidase, and heart type fatty acid binding protein) and graft survival. None were predictive of graft survival [20]. Gok et al found similar results for GST levels and graft survival [29]. PFI therefore appears unique in its ability to predict survival, however this needs to be validated in a second cohort.

There were some limitations of our study. Although we data from 155 patients into the analysis, this was after having excluded almost half of our initial population due to missing data. It is possible, though we believe unlikely, that there were systematic differences between those patients who were excluded from the analysis due to missing data, and those who were included. As data were not available for all patients some of the confidence intervals were wide, increasing the risk of a type 2 error. We also did not have data regarding delayed graft function which was related with machine perfusion parameters in some studies [15].

In summary, PFI is a unique and independent parameter to predict graft survival in DCD kidney transplantation. Donor age is still important for prediction of eGFR.

Legends to Figures

Figure 1. Details of patient cohort.

All DCD kidney transplantations from 2002-2014 were included into the study.

Figure 2. Donor hypertension and graft survival.

Solid line represents donors with hypertension. Dashed line represents donors without hypertension. Graft survival was not statistically different (p=0.781).

Figure 3. Relationship between machine perfusion parameters and graft survival.

Solid line represent high, dotted line represent medium, dashed line represent low group of machine parameter in each figure. Kaplan Meier curves of **A.** PFI groups (P=0.020), **B**. GST groups (P=0.022), **C.** RT groups (P=0.472).

Figure 4. Relationship between machine perfusion parameters and eGFR.

Comparison of eGFR among PFI (A), RT (B) and GST (C) groups at 12, 24, 36, 48 months post transplantation. Solid columns represent low, dotted columns represent medium, striped columns represent high group for each parameter. The number of patients in each group is given at the bottom of each figure. Estimated glomerular filtration rate (eGFR) is given as mean±standard deviation (SD). [*]P<0.05, **P<0.01.

Abbreviations:

DBD: Donation after brain death

DCD: Donation after cardiac death

eGFR: Estimated glomerular filtration rate

GST: Glutathione S Transferase

PFI: Perfusion flow index

RT: Resistance

WIT: Warm ischaemia time

Author Contributions:

Mustafa Sevinc: Collected data, analyzed data, performed study, wrote the paper

Susan Stamp: Collected data

Jonathan Ling: Analyzed data

Noel Carter: Designed study

David Talbot: Designed study, interpretation of the data, critical revision

Neil Sheerin: Designed study, interpretation of the data, critical revision

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