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Transplantation of Kidneys After Normothermic Perfusion: A Single Centre Experience

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Running head: NRP of kidneys: a UK centre experience
Abbreviations:

AKI – Acute kidney injury
ANOVA - Analysis of variance
ATP - Adenosine triphosphate
CI – Confidence interval
CIT – Cold ischemic time
DBD – Donation after brainstem death
DCD – Donor after circulatory death
DGF – Delayed graft function
ECD – Extended criteria donor
ECMO - Extra-corporeal membrane oxygenation
eGFR – Estimated glomerular filtration rate
FWIT – Functional warm ischemic time
IQR – Interquartile range
IVC – Inferior vena cava
NORS – National organ retrieval service
NRP – Normothermic regional perfusion
PNF – Primary non-function
RRT – Renal replacement therapy
SCS – Static cold storage
SD – Standard deviation
WIT – Warm ischemic time
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Abstract

In order to expand the pool of usable donors from circulatory death (DCD) there is increasing interest in normothermic regional perfusion (NRP) to assess and improve liver viability (1,2). NRP may also improve outcomes in kidney transplantation. We present our single centre experience of outcomes in imported kidneys following NRP. Data was obtained from a prospectively maintained database between December 2012 and September 2018. Primary endpoints were incidence of delayed graft function (DGF) and estimated glomerular filtration rate (eGFR). 632 decease donor kidneys were transplanted, 229 from DCD donors, 29 of which had NRP. The DGF rate was lower for NRP vs DCD (6 of 29, 20.7% vs 70 of 200, 35.0%) with reduced duration of DGF. Multivariate analysis demonstrated transplant type to be a statistically significant independent predictor of eGFR at 7 and 14 days. Early transplant function in NRP kidneys was comparable to DBD. There were no graft losses within 30 days in the NRP group. One-year graft loss rate was 3.4% for NRP and 6.0% for standard DCD. This data suggests NRP is safe, and reduces rates of DGF and improves early renal function.

Keywords: Normothermic Regional Perfusion (NRP); Organ preservation; Delayed Graft Function; Donors from circulatory death (DCD)

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1. Introduction

Despite sustained efforts to increase numbers of organ donors, the greatest challenge for kidney transplantation remains how best to bridge the discrepancy between the increasing demand of those in need of a kidney transplant and the utilizable supply of grafts. Many European countries, including the UK, have substantially increased their deceased donor pool with routine use of donors from circulatory death (DCD), however, there remains a substantial and clinically significant shortfall. There are currently over 6000 patients on the waiting list for a kidney in the UK alone(3). The use of extended criteria donors (ECD), i.e., those from donors over 60 years of age, has increased the number of donors but at the cost of inherent challenges of reduced graft survival and increased rates of delayed graft function (DGF). The proportion of ECD donations here in the UK has progressively increased over time, with 35% deceased donations being provided by donors over 60 years of age(4).

The nature of DCD kidney transplantation, with inevitable warm ischaemia time and unpredictable perfusion and oxygenation during the agonal phase, creates the environment for ischemia-reperfusion injury(1,5,6). Organs recovered in this setting are typically obtained through rapid laparotomy following circulatory arrest, and following organ explantation, in situ cold perfusion fluid is circulated through the grafts, before the graft is then transported to the recipient transplantation unit in static cold storage (SCS). The period of warm ischaemia, followed by cold storage, is thought to be a key contributory factor underpinning ischaemia-reperfusion injury and the resulting increased rates of primary non-function (PNF), delayed graft function (DGF) and long-term graft survival rates are well described(7).

In order to mitigate this pathophysiology there is increasing interest in perfusion techniques, both at the time of organ procurement, and following explantation i.e. ex vivo perfusion. The optimal timing, duration, temperature and contents of the perfusate are all independent areas of ever-emerging research. Normothermic regional perfusion (NRP) is a method of in situ perfusion of the infra-celiac viscera with warmed (37 °C) oxygenated blood. This in situ method works in three main ways. Firstly, it allows restoration of blood flow following the confirmation of death and prior to organ recovery(8), thus minimizing warm ischemia time.
Furthermore, once the perfusion circuit is established, NRP changes a rapid organ recovery technique into a controlled unhurried procedure, with the potential benefit of reducing organ damage at procurement. Secondly, at a cellular level the perfusion enables a period of rehabilitation with restoration of mitochondrial and adenosine triphosphate (ATP) stores (9–11). Thirdly, this period of time on the circuit also allows for graft viability assessment, theoretically enhancing the transplantation decision-making process, although with current technology the viability assessment is primarily only possible for the liver (6).

In the context of liver transplantation, Hessheimer et. al (2018) demonstrated NRP to reduce postoperative biliary complications and graft loss (8). Adding to this, Minanbres et al. (2017) reported NRP to reverse the poor results seen in controlled DCD (cDCD) liver transplantation whilst also presenting comparable rate of DGF and kidney graft survival with cDCD kidneys to that of a matched group of DBD transplants (12). With regards to kidney transplantation, the UK report on the experience of NRP (reporting on 32 kidney transplants) added to this evidence with favourable rates of DGF but, of concern, demonstrated a higher-than-expected graft loss (12%) within 30 days (1).

Although more work is necessary, there is an increasing body of evidence that NRP may have a multi-organ benefit in ameliorating the effects of ischaemia-reperfusion injury. We present our single centre experience, as a non-retrieval unit, of imported kidney grafts perfused with NRP at time of organ procurement, to assess this techniques impact on outcomes in kidney transplantation.

2. Methods

This was a retrospective analysis of all kidney transplants between December 2012 and September 2018 implanted in our transplantation unit. Our prospectively-maintained regional renal electronic patient record (SERPR) was used to obtain endpoint data. DCD kidneys were separated into two distinct groups, those that had NRP at time of organ procurement (NRP) and those that did not receive this treatment modality (DCD). Organs from donation after brainstem death (DBD) were also analysed. Primary endpoints were incidence and duration of delayed graft function (DGF) and estimated glomerular filtration rate (eGFR) at one year. Secondary endpoints included graft loss and eGFR at two and three years.
The NRP process was performed by a group National Organ Retrieval Service (NORS) centres across the UK utilizing a standardized protocol as published by Oniscu et al. (2014)(1). As NRP was part of a clinical service evaluation and not a funded core activity for NORS, it was not available for every DCD retrieval, and indeed we had no control over whether NRP would be performed at the time of accepting DCD kidney offers. Once NRP completed, the organs were flushed with cold University of Wisconsin preservation solution and removed as per standard DBD practice. Organs were then allocated in accordance with the current United Kingdom kidney deceased donor matching criteria (as per NHS Blood and Transplant) and transferred to our transplantation unit on static cold storage.

Outcome data of all kidneys was recorded at 7, 14 and 28 days, and 1, 2- and 3-years post-transplant. Delayed graft function was defined as the need for dialysis within the first seven days, excluding within the first 24 hours for hyperkalemia. Cold ischaemia time (CIT) was defined at the time from end of NRP to organ perfusion in the recipient patient. Warm ischaemia time (WIT) was defined as time from asystole to NRP circuit perfusion, in addition to anastomosis time.

Statistical analysis was performed using R version 3.5.2 for Linux, using Rstudio and the dplyr, ggplot2, survival, survminer, matchit and car packages. Univariate analysis of continuous variables was performed using ANOVA where parametric and equality of variance assumptions could be met; Tukey HSD test was used for post-hoc inter-group comparisons as this test adjusts the p value for multiple comparisons. Analysis of covariance (ANCOVA) was used to control for confounding variables. The Kruskal-Wallis test was used to compare continuous variables where non-parametric conditions applied. Categorical variables were compared using Fisher’s exact test, and survival analysis using the Kaplan-Meier method with differences compared using the log-rank test. Propensity score matching was used to account for covariates at a 4:1 ratio due to the sample size within the NRP group; the NRP group was compared directly to DCD group, given that it was not possible to control for the inherent differences in DCD and DBD donation. Donors with severe AKI requiring RRT at time of offering were excluded from this propensity score matching analysis as they were significant outliers.
3. Results

A total of 632 deceased donation kidneys were transplanted during this time period. 29 of which received NRP at organ procurement, 388 DBD, and 215 DCD (see Table 1). Mean donor age in the NRP group was 46.9 years (range 19 - 70), compared to 53.2 in the DCD group, and 49.7 in the DBD group (see Figure 1). Male to female ratio in the NRP group was 1.6:1. There was a statistically significant difference (Tukey’s HSD) in donor age between DBD and DCD group only (49.7 v 53.2 years, respectively; p=0.021); Donor age for NRP vs DCD was non-significant (p=0.09). 9 of 29 (31%) donors within the NRP group were 60 years or older and therefore extended-criteria donors. There were cases of pediatric donation in the DBD and DCD group. Endpoint analysis was made on a comparison of all groups filtered for age between 19 and 70, in line with the NRP group. This removed paediatric and extreme age donors and groups for analysis were as follows, DCD n=200, DBD n=337 and NRP n=29.

Mean donor eGFR in the NRP group was 99.2, compared to 95.0 and 88.2 in the DCD and DBD groups, respectively. There was statistical difference between donor eGFR in the DBD and DCD groups (p = 0.015), however the difference observed between NRP and DCD was not significant (p = 0.80).

Recipient age in the NRP group was 49.8 years (mean, range 11-72 years), compared to 53.6 years in the DCD group (p=0.34), and 47.8 years in the DBD group (p=0.72) (see Table 2). One of the NRP kidneys was transplanted into a pediatric recipient.

Median cold ischemic time was 9h18 in the NRP group, 11h07 in the DCD group, and 11h50 DBD group (see Table 2 and Figure 2). There was found to be a statistically significant difference between the cold ischemic time of NRP compared to DCD (p = 0.025, Kruskal Wallis test with multiple pairwise comparison). The difference between NRP and DBD, and DCD and DBD was also statistically significant (p<0.001 and p=0.001, respectively, Kruskal Wallis test with multiple pairwise comparison). Median anastomosis time was 33 minutes, 31 minutes and 32 minutes for NRP, DCD and DBD respectively.
Three donors were receiving renal replacement therapy (RRT) at time of donation; two in the NRP group and one in the DBD group. To avoid a disproportionate effect, we present data on all donors, whilst also, where stated, present data with donors receiving RRT excluded from the analysis.

### 3.1 Delayed graft function

Incidence of delayed graft function (DGF) was 20.7% (6 of 29) in the NRP group, compared to 35% (70 of 200) in standard DCD, and 19.5% (66 of 337) DBD (see table 3). Duration of DGF was defined as the amount of days total for which dialysis was required post-transplantation. The reduced rate of DGF in NRP group vs DCD did not reach statistical significance (p=0.1). The mean duration of DGF was 2.5 days in the NRP group (range 1 - 27), compared to 3.4 days in the DCD group (range 1 - 29). This did not reach statistical significance (p= 0.087, Kruskal-Wallis test with Dunn test for multiple comparison).

When excluding for all donors receiving RRT at time of donation, the rate of DGF in the NRP group was 14.8% (two NRP grafts were from donors on CVVH at time of donation). Comparing NRP to DCD in isolation, the reduction in rate of DGF was statistically significant (4 of 27 in NRP group, vs 70 of 200 for DCD, p = 0.047). Furthermore, when RRT donors excluded, the mean duration of DGF in the NRP group was 1.5 days. NRP compared with DCD in this context (1.5 days vs 3.4 days) did reach statistical significance (p = 0.032, Kruskal-Wallis test with Dunn test for multiple comparison). DBD vs DCD was statistically significant; when donors with AKI excluded, the difference between DBD and NRP was non-significant (p=0.588).

Regarding all grafts, 61 of 632 (9.6%) had DGF with a duration of 10 days or greater. The rate of this prolonged DGF was highest in the DCD group (33 of 215, 15.3%), compared to 2 of 29 (8.3%) for NRP and 26 of 388 (6.7%) for DBD. The difference between DBD and DCD was statistically significant (p=<0.001), however NRP vs DCD did not reach statistical significance.
Multivariate analysis demonstrated donor age (p=0.019), male donor sex (p=0.079), DCD donor type (p=<0.01) and cold ischaemic time (p=<0.01) to be contributing factors in the development of delayed graft function. Propensity matching NRP vs DCD controlling for the covariates donor age, donor sex and cold ischemic time (4:1 ratio) demonstrated that NRP remained a significant covariate and grafts treated with NRP had a lower rate of DGF (p=0.03).

3.2 Transplant function

Regarding early transplant function (see Table 4), NRP median eGFR at 7 days was 28.55, compared to 38.0 for DBD and 11.5 for DCD (all units mL/min per 1.73 m²). The differences observed between groups were statistically significant (Tukey multiple comparisons of means; NRP v DCD p= 0.0016; DCD v DBD p = <0.001), except NRP vs DBD (p= 0.78). At 14 days, median eGFR was 52.2 in the NRP group, 45.6 for DBD and 26.6 in DCD (all units mL/min per 1.73 m²; NRP v DCD p = <0.001)(see Figure 3).

Regarding medium term transplant function, the median eGFR at 1 year was 61.0 in the NRP group, compared to 56.1 for DBD and 48.1 for DCD (all units mL/min per 1.73 m²)(see Figure 4). The observed difference between NRP and DCD at this time point was non-significant (p=0.060), however DCD vs DBD did reach statistical significance (p = <0.001). At 2 years post-transplant, median eGFR in the NRP group was 55.7, compared to 53.90 for DBD and 45.95 for DCD (all units mL/min per 1.73 m²). Statistical significance was reached for NRP vs DCD at 2 years (p=0.018). Transplant function at 3 years was 52.0 for NRP, 53.9 for DBD and 46.6 for DCD, the difference between NRP and DCD was non-significant (p=0.19; all units mL/min per 1.73 m²). Mean eGFR in the NRP group outperformed DCD at all time points (see Figure 5), however only reached statistical significance during the early post-operative period.

3.3 Graft and patient survival
None of the NRP grafts were lost within 30 days (see Figure 6). One of the NRP grafts was lost within the first year (1-year graft loss rate of 3.4%) at 82 days post-transplant, a second graft was lost at approximately 18 months (526 days). Graft survival at one year, therefore, was 96.6% in the NRP group. In contrast, 92.4% of DBD and 92.7% of DCD kidneys were functioning at one year. These results are all censored for death with functioning transplant. Patient survival was 96.6% at 5 years in the NRP group. Cox regression models show transplant type alone is a poor predictor of both graft and patient survival. Donor age was, however, found to be an independent predictor of graft survival (p=0.018). Cold ischemic time and NRP were non-significant.

3.4 Multivariate Analysis

With correction for the covariates, age, donor eGFR, and transplant type (i.e., NRP, DCD etc.) were independent predictors of eGFR at 7 days (p=0.001). Notably, cold ischemic time nearly reached statistical significance (p=0.088). Although a difference was observed, post-hoc analysis demonstrated NRP vs DCD to be non-significant (p=0.052).

At 14 days, again transplant type was found to be an independent predictor of eGFR (p=<0.001). In this model, cold ischemic time (p=0.002) and donor age (p=0.007) were also found to be highly significant predictors of early transplant function. At 14 days post-transplant, the eGFR in NRP kidneys was significantly better than standard DCD (p=0.005, CI 13.1 to 85.3), controlled for other variables.

By 30 days the transplant type was still a significant predictor (p=0.044). At this time point, the cold ischemic time was no longer significant as a predictor (p=0.42), however, donor age has become highly significant (p=0.0002). The reduction in the significance of both transplant type and cold ischemic time may be explained by recovery from ischaemia-reperfusion injury by this time.
4. Discussion

Normothermic perfusion was first established over 20 years ago where an extracorporeal membrane oxygen device (ECMO) was used for 29 days to successfully oxygenate a liver graft in the context of a Donation after Brainstem Death(13). The technology, while fundamentally unchanged, has been adapted, and widely investigated, for its role for both in situ regional perfusion (i.e., NRP) and more recently ex-vivo isolated perfusion (i.e. post-procurement, commonly referred to as ex vivo normothermic perfusion). The mainstay of current DCD practice remains rapid laparotomy followed by transport on static cold storage, and the developing perfusion techniques are required to demonstrate any or ideally all of the following: reduction in delayed graft function, improvement in medium term transplant function (i.e. eGFR) and/or facilitation of an increase in graft utilization.

The systematic review by Shapey et. al (2013) assessed regional perfusion (including both normothermic and hypothermic studies in liver and kidney) and concluded that 1-year patient and graft survival was superior with DCD grafts that had a period of in situ regional perfusion, and for this endpoint were comparable to DBDs, irrespective of perfusion temperature(6). DGF rates were found to be hugely variable between groups, with no clear relationship to temperature or duration of perfusion. More specifically, Valera et. al (2000) found normothermic recirculation to reduce both DGF and primary non-function, with a quoted DGF rate of 12.5% (1 of 8)(14). Utilizing hypothermic perfusate, Farney et. al (2008) demonstrated extracorporeal support of DCD grafts reduced DGF rates to 20%(15). A recent Cochrane review confirmed superiority of hypothermic machine perfusion to static cold storage but reported that further work is required to elucidate the benefits of normothermic techniques(16).

In the UK, due to longstanding ethical considerations, cannulation of femoral/iliac vessels in the context of controlled category III(17) DCD is performed following the withdrawal of treatment and crucially the confirmation of death. Spanish law (Real Decreto 2070/1999), however, permits the vessels to be cannulated prior to the withdrawal of treatment and, if required, intravenous heparin can be administered to facilitate organ procurement. This premortem cannulation has the potential to reduce functional warm ischaemic time (FWIT) by 10-15 minutes(12).
The work by Minambres et al. (2017) in Spain was integral in the demonstration of the beneficial effects of NRP in controlled DCD. It compared NRP DCD transplants to a matched group of DBDs, with comparable rates of DGF (27% vs 33.3%, respectively) and good medium-term transplant function with no difference in graft survival during the 18 months follow-up. However, although premortem heparinization was permitted in this protocol, 5% (2 of 37) of grafts were lost with primary non-function, due to arterial thrombosis(12).

Rojas-Pena et al. (2014) presented work from the United States, again in controlled DCD with normothermic extracorporeal support (akin to NRP) with premortem cannulation and heparinization; rates of DGF were 31% and PNF 3.5%, no cause was specified(18).

Molina et al. (2019) reported on the Spanish experience in which 241 kidneys were transplanted from uDCD donors whereby normothermic in situ perfusion was used as a preservation strategy. Despite reporting a PNF rate of 6.8% (with 43% attributed to venous thrombosis) and DGF rate of 73.4 (including any need for dialysis in first seven days), the group reported satisfactory outcomes with regards to short- or long-term graft function and survival. It is important to note, however, that the uDCD group received augmented immunosuppression with ATG and cold ischaemic time was lower in this group (12h vs 20hrs compared to DBD). In recognition of the rate of venous thrombosis, prophylactic anticoagulation became routine and following this change the rate of PNF reduced to 5.8% (less than 25% attributed to venous thrombosis).(19)

Data from the French Transplant Registry detailing 499 transplants (49% treated with NRP), published by Antoine et al. (2020), demonstrated the contribution of NRP in the context of uncontrolled DCD (uDCD) donation. The group demonstrated in situ normothermic method to be protective against graft failure and poor function at one year compared to in situ cooling. Of interest, during the study period the use of NRP increased from 9% of cases in 2008 to 84% of cases in 2014, and is now the mainstay of treatment in uDCD.(20)
The UK study group (Oniscu et al. 2014), restricted to postmortem vessel cannulation and heparinization, demonstrated a DGF rate of 40% in NRP-DCD (13 of 32). Of which four were from donors who were on hemofiltration at time of procurement. Overall, four kidneys (12.5%) were lost, two due to venous thrombosis, one to thrombotic microangiopathy and one due to infarction(1). Our work presents our non-retrieval centre experience in which NRP was administered at time of procurement in line with the same UK protocol and demonstrate zero graft loss in 30 days, and one graft loss within the first year (3.4%).

Two large European studies quoted DGF rates of 50.2% and 63.4% for controlled DCDs (21,22). This contrasts to the aforementioned reported rates with NRP (12.5% to 40%)(12,14,18,23,24). In this work we compare NRP to DCDs, with DGF rates of 20.7% vs 35% with a significant reduction in duration of DGF (14.8% DGF rate in NRP group when donors receiving RRT excluded). The NRP kidneys performed comparably to DBD kidneys with analogous DGF rates, short- and medium-term function and graft survival. This reduction in DGF appears to be comparable to the results reported by Demiselle et. al (2015) in which NRP of kidneys demonstrated a DGF rate of 10% (n=19) with reduced ‘delay to creatinine <250µmol/day’ compared to in-situ cold perfusion(7).

In comparison to DBD donors, DCD procurement is associated with fewer grafts being recovered and subsequently transplanted. By utilizing a DCD procurement protocol with normothermic ECMO, Magliocca et al. (2005) demonstrated an increase in the potential pool by 33% (61 vs 81 patients)(23). Furthermore, the aforementioned American group reported an organ recovery rate of 2.59 per donor, with no reported procurement-related injuries (18), which in context of the relatively nascent DCD programme is significant progress. Utilising NRP, the number of organs procured in the last 12-month period by the Spanish group is 3.2 per donor, which is in contrast to the published rate of standard DCD in the UK of 2.8(4).

There is increasing interest in post procurement organ normothermic perfusion - so called Ex vivo normothermic perfusion, (EVNP) - driven principally but not exclusively in the context of liver
transplantation. The OrganOx Metra device was recently used in a pioneering randomised control trial by Nasralla et al (2018) and demonstrated 50% reduced graft discard rate compared to static cold storage, resulting in 20% more transplanted livers, with no detrimental effect of transplant function (2). The benefits of this treatment, performed at the recipient centre, are compelling. In the UK there are 24 renal transplantation units, less than half (10) of which are also national organ retrieval service (NORS) centres. The ethical considerations, expertise and cost to local health boards will be significant barriers to setting up perfusion techniques in recipient units. NRP, i.e., regional perfusion to all transplantable abdominal viscera, may represent an intervention that is deliverable by experienced units and has multiple organ benefit irrespective of the location of the allocated recipient.

Multiple pathways are implicated in the ischaemia-reperfusion injury seen in deceased donor transplantation: e.g. inflammatory response, oxygen free radicals, T cell activation. The multivariate analysis performed in this study found that up to 14 days post-transplant, transplant type, cold ischaemia time, and donor age were all independent predictors of transplant function. NRP also demonstrated superior transplant function compared to DCD at this time point. At 30 days, however, cold ischaemic time was no longer a significant predictor of transplant function, and transplant type was less significant. This is likely to represent the recovery from ischaemia-reperfusion injury by this time. In this study the benefit to transplant function in the NRP group was present up to 3 years post-transplant, although was not found to be statistically significant.

In conclusion, NRP has been shown to decrease DGF rates and protect against the effects of ischaemia-reperfusion injury compared to standard DCD practice, and behaves analogous to DBD donation with respect to DGF, short- and medium-term function and patient/graft survival. There remain considerable ethical considerations surrounding DCD donation, yet in the context of UK practice, this study found no increased rate of thrombosis and had no graft loss within 30 days. Further work is required to delineate the how best to reproduce the delivery of this intervention and to compare this regional technique to post-procurement techniques such as ex vivo normothermic perfusion.
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6. **Disclosures**

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