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Received: 11 July 2018 | Revised: 9 November 2018 | Accepted: 16 December 2018

DOI: 10.1111/ajt.15241

ORIGINAL ARTICLE

AJT

In situ normothermic perfusion of livers in controlled circulatory death donation may prevent ischemic cholangiopathy and improve graft survival

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Funding information
National Institute for Health Research; Evelyn Trust; National Institute for Health Research Blood and Transplant Research Unit (NIHR BTRU)

Livers from controlled donation after circulatory death (DCD) donors suffer a higher incidence of nonfunction, poor function, and ischemic cholangiopathy. In situ normothermic regional perfusion (NRP) restores a blood supply to the abdominal organs after death using an extracorporeal circulation for a limited period before organ recovery. We undertook a retrospective analysis to evaluate whether NRP was associated with improved outcomes of livers from DCD donors. NRP was performed on 70 DCD donors from whom 43 livers were transplanted. These were compared with 187 non-NRP DCD donor livers transplanted at the same two UK centers in the same period. The use of NRP was associated with a reduction in early allograft dysfunction

Abbreviations: ALT, alanine transaminase; A-NRP, abdominal normothermic regional perfusion; CIT, cold ischemic time; CKD-Epi, Chronic Kidney Disease Epidemiology Collaboration; DCD, donation after circulatory death; eGFR, estimated Glomerular Filtration Rate; MELD, model for end-stage liver disease; NHSBT, National Health Service Blood and Transplant; NMP, normothermic machine perfusion; NRP, normothermic regional perfusion; TA-NRP, thoracoabdominal normothermic regional perfusion; UKELD, United Kingdom End Stage Liver Disease score; UK, United Kingdom.

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(12% for NRP vs. 32% for non-NRP livers, $P = .0076$), 30-day graft loss (2% NRP livers vs. 12% non-NRP livers, $P = .0559$), freedom from ischemic cholangiopathy (0% vs. 27% for non-NRP livers, $P < .0001$), and fewer anastomotic strictures (7% vs. 27% non-NRP, $P = .0041$). After adjusting for other factors in a multivariable analysis, NRP remained significantly associated with freedom from ischemic cholangiopathy ($P < .0001$). These data suggest that NRP during organ recovery from DCD donors leads to superior liver outcomes compared to conventional organ recovery.

KEYWORDS
clinical research/practice, donors and donation: donation after circulatory death (DCD), extracorporeal membrane oxygenation (ECMO), liver transplantation/hepatology, surgical technique

1 | INTRODUCTION

The success of liver transplantation as a treatment for patients with liver disease is limited by a shortage of suitable donor organs, such that of those patients listed for a liver transplant in 2014 and 2015 in the UK, 13% died and 8% were removed from the list within 2 years without undergoing liver transplantation.[1] The situation is similar in the United States where 12% of patients listed in 2013 died and 17% were removed from the list in the subsequent 2 years.[2] This high waiting list attrition has led surgeons to use organs that were previously considered to be at higher risk of failure,[3] balancing the risk of death without a transplant against the risk of complications from a suboptimal graft.[4] Livers donated after circulatory death (DCD) are one such higher risk category.

Following withdrawal of life-supporting treatment in a potential DCD donor, the autonomic response to falling cerebral perfusion is characterized by release of catecholamines which cause an alpha-adrenergic mediated peripheral and mesenteric vasoconstriction, resulting in reduced blood flow to the liver.[5,6] Hence during the withdrawal period before circulatory arrest the liver suffers significant ischemia. This is then compounded by a further period of warm ischemia after circulatory arrest (the asystolic period) during which the donor is verified dead and is transferred to the operating room where, following a rapid laparotomy, the organs are flushed and cooled in situ with preservation fluid. Cold perfusion reduces, but does not stop the metabolic processes that were active in the warm, and leads to further depletion of high energy phosphates that occurs rapidly during warm ischemia. The successive insults of warm ischemia followed by cold storage account for the suboptimal nature of livers donated after circulatory death (DCD), with a higher incidence of primary nonfunction and initial poor function compared to those from brain dead donors. In addition, DCD donor livers are associated with a higher incidence of biliary complications and in particular nonanastomotic biliary strictures ("ischemic cholangiopathy") which can be seen in 20% to 30% of DCD livers.[7,8] DCD donors comprise 41% of deceased donors in the UK, and 17% in the United States.[1,2] The high proportion of DCD donors has forced increasing utilization of DCD donor livers to address the waiting list mortality, such that 24% of transplanted livers in the UK in 2016 and 2017 were from DCD donors; this compares to just 6% in the United States in 2016.[1,2,9]

In situ normothermic regional perfusion is a technique that restores a circulation of oxygenated blood to the abdominal organs via cannulas in the aorta and vena cava using an extracorporeal circuit containing a membrane oxygenator, heater, and pump. Restoration of a blood supply arrests the ischemic damage to the liver and allows it to recover before being cooled down for transport to the recipient center. In this way, it converts DCD donation into a situation more akin to that seen in DBD donation. In an effort to improve the outcomes of DCD liver transplantation, we started a program of normothermic regional perfusion (NRP) in two UK centers in 2011 and 2012.[10,11]

Several groups have described encouraging outcomes of DCD organ transplantation following NRP,[12-14] initially with kidneys but, latterly, cases of successful liver transplantation.[15-19] Originally pioneered in uncontrolled DCD donation in Spain and Taiwan,[20,21] NRP is increasingly used for controlled DCD donation in Spain, France and the UK.[11,19,22] In contrast to Spain and France, where premortem cannulation and heparinization are permitted, current guidance does not permit either in the UK.

This paper describes the experience of the two pioneering UK centers with the use of NRP for DCD liver transplantation and focuses on the recognized complications of DCD liver transplantation, namely early allograft dysfunction and ischemic cholangiopathy. NRP was used in two settings, one solely by the abdominal transplant team and the other in collaboration with cardiac surgeons to facilitate heart transplantation from DCD donors.[23]

2 | MATERIALS AND METHODS

This retrospective study used prospectively collected data collated from the UK Transplant Registry and hospital records on patients undergoing transplantation using livers recovered from DCD donors after a period of NRP. NRP was considered in donors when trained staff were available, and was initially conducted as part of an approved clinical research study in which donor families consented to NRP treatment

of the donor and the recipients consented to receive an organ treated by NRP. Latterly, NRP has been performed as part of a service evaluation by the National Health Service Blood and Transplant (NHSBT) organization in the UK. In this latter period, when the safety of the technique had been confirmed, recipients gave consent for an organ from a DCD donor, regardless of whether the donor had undergone NRP or not. Interest in utilization of hearts from DCD donors led to a bias in favor of younger donors at one center where the cardiothoracic teams were able to call on different staff to perform the perfusions.

2.1 | Normothermic regional perfusion (NRP)

NRP was undertaken in two contexts, one to assess and retrieve the abdominal organs alone and the other to also assess and recover the heart for transplantation; the abdominal organs received the same treatment in each case. All donors were controlled DCD donors (Maastricht III).[24] In all cases the mode of withdrawal of treatment was left to the intensivist caring for the patient, but usually comprised extubation and discontinuation of inotropes. Death was confirmed no less than 5 minutes after cessation of the circulation following which the patient was transferred to the operating room. Abdominal NRP (A-NRP) was performed by cannulating the aorta or right common iliac artery, and the inferior vena cava or right common iliac vein, with an endovascular or external clamp occluding the descending thoracic aorta. Thoracoabdominal NRP (TA-NRP) was performed either by cannulating the ascending aorta and IVC via the right atrial appendage, or cannulating the abdominal aorta and IVC, with a clamp placed across the origins of the brachiocephalic trunk, left common carotid and left subclavian arteries; there was no simultaneous abdominal cannulation in these cases. Current preference for DCD heart recovery involves abdominal aortic cannulation, since that affords better access for clamping the arch vessels to prevent cerebral perfusion.

The prime solution comprised compound sodium lactate (Hartmann's solution, Baxter Healthcare Ltd, Thetford, UK) and succinylated gelatin (Gelofusine, BBraun), and contained antimicrobials, 1 mmol/kg sodium bicarbonate, and 25 000 units heparin as previously described,[10] with additional mannitol and heparin for TA-NRP. Antemortem cannulation and heparinization are not permitted in the UK.

The circuit comprised an oxygenator, heat exchanger, pump, and latterly, a leucocyte filter (LeukoGuard, Pall Corporation, Portsmouth, UK), either using bespoke cardiopulmonary bypass equipment from Medtronic (Watford, UK), the Cardiohelp (Maquet, Sunderland, UK), or the Extra-Corporeal Organ Procurement System (ECOPS) or the Donor Assist (both from Organ Assist, Groningen, Netherlands). Irrespective of the equipment, the target for abdominal NRP flow was 2.5-3 L/min, with higher flow rates (4-6 L/min) being employed for TA-NRP. The leucocyte filter was excluded from the circuit for the first 2 minutes to allow adequate mixing of blood with heparin to minimize the risk of clot formation.

The intention was to restore a circulation to the abdominal organs for 2 hours before in situ perfusion with cold University of Wisconsin preservation solution. Where TA-NRP was performed and cardiac output was restored, the extracorporeal perfusion was stopped at 30 to 60 minutes and the heart allowed to support the limited thoracoabdominal circulation while its function was evaluated. If the cardiac function was considered inadequate and failing to sustain an adequate mean arterial pressure, extracorporeal perfusion was recommenced.

The decision to use the liver was based on subjective and biochemical factors. The appearance of the liver during NRP was assessed, with cirrhotic and severely steatotic livers being declined. Alanine transaminase (ALT) was measured in the perfusate every 30 to 60 minutes during NRP. In our early experience an ALT over 200 iu/L would result in the liver being declined, but latterly perfusion ALTs up to 500 iu/L have been accepted, providing there was no continued rise in ALT between the first and second hour. Perfusate lactate concentrations were measured every 30 minutes, as part of the blood gas profile used to manage gas delivery to the circuit. A fall in lactate was also considered encouraging, but deoxygenated lactate-rich blood draining back into the circuit from nonperfused areas together with the lactate content in supplementary Hartmann's solution made this less reliable as an indicator; in later perfusions Hartmann's was not administered once NRP had commenced. Typically, the lactate fell between 3 and 14 mmol/L over the 2 hours of perfusion.

2.2 | NRP and comparator cohorts

A contemporaneous comparator cohort comprised all DCD liver transplants performed at both centers since the start of the NRP program. Livers subject to ex situ machine perfusion were excluded.

2.3 | Definitions

Ischemic cholangiopathy was defined as the presence of any nonanastomotic biliary stricture on endoscopic or magnetic resonance cholangiopancreatography (ERCP or MRCP) in the absence of arterial thrombosis or stenosis. Cholangiography was undertaken when clinically indicated by pruritus, cholangitis, raised bilirubin or ALP posttransplant. Protocol cholangiograms were also performed as part of the initial evaluation of NRP. Patients with evidence of strictures but without symptoms sufficiently severe to warrant retransplantation were treated symptomatically, often with attempts at endoscopic or percutaneous duct clearance of any debris.

An anastomotic stricture was defined as any stricture at the biliary anastomosis requiring treatment, and anastomotic leaks were defined as a bile leak confirmed at laparotomy; no suspected leak was managed without surgery in either group. Glomerular filtration rate was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) equation.[25]

Organ damage was graded separately by the donor surgeon and recipient center as minor (trivial) or moderate (needs repair); the worst degree of damage reported is recorded here.

2.4 | Statistical analysis

Descriptive data were presented as median, interquartile range or range (continuous variables) or number, percentage (categorical

variables) and were compared using the Kruskal-Wallis test (continuous variables) and Fisher's exact test (categorical variables). Analysis of variance (ANOVA) was used to compare CKD-Epi GFR over time between NRP and comparator cohorts. A random effect was included in the ANOVA model to allow for correlation between GFR results at different time points for the same patient. Kaplan Meier estimates of graft survival were compared using the log-rank test. Graft survival was defined as the time from transplant to graft failure with patient deaths with a functioning graft censored. Logistic regression was used to determine the factors associated with developing ischemic cholangiopathy. Analyses were undertaken using SAS/STAT, version 9.4 (SAS Institute Inc., Cary, NC) and Prism version 7.0c (GraphPad, La Jolla, CA).

3 | RESULTS

Between January 1, 2011 and June 30, 2017, 43 DCD liver transplants were performed from 70 DCD donors undergoing NRP. These were compared with 187 non-NRP DCD liver transplants performed over the same period. Details of the donors are given in Table 1, as are details of the 27 NRP donors whose livers were not used. Reasons for not using livers included donor encephalitis of unknown cause (n = 1), abnormal liver appearance (n = 7: steatosis [n = 3], fibrosis, cirrhosis, trauma, lesion), thromboemboli (n = 3), bleeding (n = 1), abnormal liver function tests pre-NRP (n = 2), rising ALT (n = 5, including 3 donors over 70 years old), recipient unfit perioperatively (n = 1) and prolonged withdrawal period (n = 7: withdrawal period durations 76, 90, 90, 113, 127, 133, and 176 minutes). The liver utilization rates over time are shown in the supplementary data, Figures S1A,B. As experience accrued, livers from donors with longer withdrawal periods and with higher biochemistry thresholds were used. There was no difference in UK donor liver index between NRP and non-NRP livers, nor in the donors where the livers were not used.[3] The US donor risk index for livers was higher in the non-NRP livers reflecting the longer cold ischemic time and fewer local donors (Table 1).[4,26] Of the 43 cases where NRP was performed and the liver transplanted, TA-NRP was performed in 10 cases from which 9 hearts were also transplanted.

3.1 | Donor demographics and timings

The median donor age in the NRP liver transplants was 41, compared to 50 in the comparator group (Table 1). There was a greater proportion of head injury liver donors undergoing NRP (23% NRP vs. 12% non-NRP) and more donors dying from hypoxic brain injury in the non-NRP comparator cohort (37% non-NRP, 28% NRP), but these differences were not significant ($P = .5358$). A greater proportion of the liver donors were in the transplant center in the NRP group compared to the non-NRP comparators (77% vs. 16%, $P < .0001$), and similarly most NRP livers were retrieved by the transplanting center, unlike comparator livers (93% NRP vs. 55% non-NRP, $P < .0001$). While the median withdrawal period was slightly shorter in the NRP livers (NRP 13 minutes, non-NRP 14 minutes, $P = .0707$) the median asystolic period was slightly longer (NRP 16 minutes, non-NRP 13 minutes, $P < .001$) reflecting the extra time taken to establish the donor on the extracorporeal circuit. NRP was performed for a median of 123 minutes (Figure 1). As a consequence of distant procurement being more common in the comparator livers, the median cold ischemic times experienced by the comparator livers were 62 minutes longer than the NRP livers (444 vs. 382 minutes, $P = .004$).

3.2 | Recipient demographics and outcomes

The liver recipients in each study group were of similar age, severity of liver disease and had similar indications for transplantation (Table 2). The incidence of early allograft dysfunction, defined by the Olthoff criteria,[27] was significantly lower in the NRP group (12% vs. 32%, $P = .0076$), largely as a consequence of the significantly lower peak ALT in the first week posttransplant (633 iu/L compared to 1154 iu/L, $P < .0001$). Similarly, the Model for Early Allograft Function (MEAF) score was lower in NRP treated livers (3.7 vs. 5.0, $P < .0001$; Figure 2).[28] Twelve percent of the comparator livers failed within the first 30 days (7% primary nonfunction, 3% hepatic artery thrombosis) compared to 2% of NRP livers (hepatic artery thrombosis, n = 1) ($P = .0559$).

Biliary complications were much more common in livers recovered without NRP. Of the 171 non-NRP liver recipients whose livers lasted more than 8 days, 26 (15%) developed both anastomotic strictures and ischemic cholangiopathy, 21 (12%) developed ischemic cholangiopathy alone, and 21 (12%) anastomotic strictures alone. Where NRP was used, none of the recovered livers developed cholangiopathy compared to a 27% total incidence of cholangiopathy in non-NRP livers ($P < .0001$). 7% of the NRP DCD livers developed an anastomotic stricture compared to a 27% anastomotic stricture rate in the comparator group ($P = .0069$). The actual 90-day death-censored graft survival and patient survival were comparable between groups, although the 5-year actuarial death-censored graft survival was significantly better for NRP livers (Figure 3A, log rank $P = .0386$). There was no difference in actuarial patient survival or graft survival not censored for death with a functioning graft (Figure 3B,C).

Although there was less deterioration in renal function in recipients of livers recovered using NRP, with a median fall in estimated glomerular filtration rate of 13 mls/min compared to 26 mls/min in the non-NRP liver recipients, the difference in CKD-GFR did not reach significance ($P = .6229$; Table 2).

3.3 | Factors determining ischemic cholangiopathy

Given the differences in donor and recipient populations in each group, a multivariate analysis was undertaken to determine whether NRP was a significant factor in preventing ischemic cholangiopathy (IC).

Tables 3-5 detail the recipient, donor and transplant factors considered. Recipients who developed cholangiopathy had, on average, a lower serum sodium at the time of transplant. Donors whose

TABLE 1 Donor demographics and donation data

	Comparator cohort (n = 187)	NRP liver donors (n = 43)	NRP non-liver donors (n = 27)	*P* value
Age (y) (median; IQR; range)	50 (37-58; 11-76)	41 (33-57; 16-69)	54 (38-63; 22-78)	.1317
Cause of death, n (%)				
Head injury	23 (12%)	10 (23%)	5 (18%)	.5358
Hypoxia	69 (37%)	12 (28%)	8 (30%)	
Cerebrovascular accident	90 (48%)	20 (47%)	13 (48%)	
Other	5 (3%)	1 (2%)	1 (4%)	
Agonal period (minutes)[b] (median, IQR)	14 (10-18)	13 (11-17)	18 (10-89)	.0707
Asystolic period (minutes)[b]	13 (11-16)	16 (13-20)	17 (13-22)	<.0001
Withdrawal to in situ perfusion (minutes)[b]	27 (22-32)	30 (26-36)	34 (24-108)	.0046
Normothermic regional perfusion duration (minutes)[c] (median; IQR)		123 (103-130)	122 (86-127)	.3657
Cold ischemic period (minutes)[d]	444 (395-493)	382 (303-502)		.0035
Preservation period (minutes)[c]	444 (395-493)	510 (423-631)		.0008
US donor risk index[c]	2.5 (2.0-2.9)	1.8 (1.7-2.4)		<.0001
UK donor liver index[e]	1.9 (1.6-2.2)	1.9 (1.7-2.2)	2.0 (1.8-2.5)	.4275
Location of donor				<.0001
Local	29 (16%)	33 (77%)	18 (67%)	
Regional	68 (36%)	10 (23%)	9 (33%)	
National	90 (48%)	0 (0%)	0 (0%)	
Proportion of occasions the transplanting center performed the donor organ recovery	102 (55%)	40 (93%)		<.0001

IQR, interquartile range; NRP, normothermic regional perfusion.

P values are for Kruskal-Wallis test (continuous variables) and Fisher's exact test (categorical variables). Data are median (IQR) or number (percentage).

US Donor Risk Index from Schaubel et al[4]; Cold ischemic time did not include the period on NRP.

Agonal period: from withdrawal of life-supporting treatment to death. Asystolic period: from circulatory arrest to in situ perfusion. Normothermic regional perfusion: from restoration of circulation to the abdominal organs to cold in situ perfusion. Cold ischemic period: from in situ cold perfusion to reperfusion in recipient. Preservation period: from in situ perfusion (cold or normothermic) to reperfusion in the recipient.

[a]Not reported for 1 NRP non-liver donor and 2 non-NRP liver donors.

[b]Not reported for 1 NRP non-liver donor and 3 non-NRP liver donors.

[c]Not reported for 1 NRP liver donor.

[d]Not reported for 3 non-NRP liver donors.

[e]UK Donor Liver index from Collett et al[3] A liver with a DLI >1.31 is in the upper quartile of UK donor livers and has a 3-fold higher risk of graft failure than one with a DLI≤1.31.

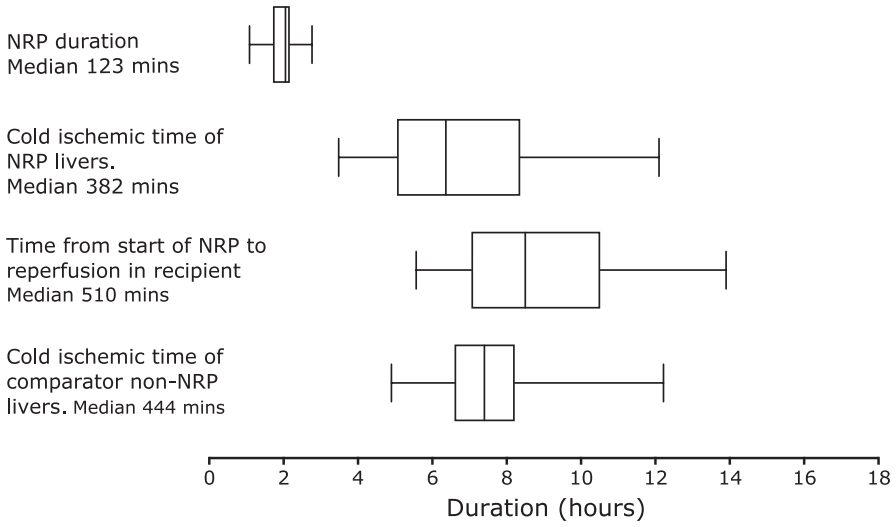

FIGURE 1 Duration of NRP, cold ischemia, and total preservation. NRP, normothermic regional perfusion

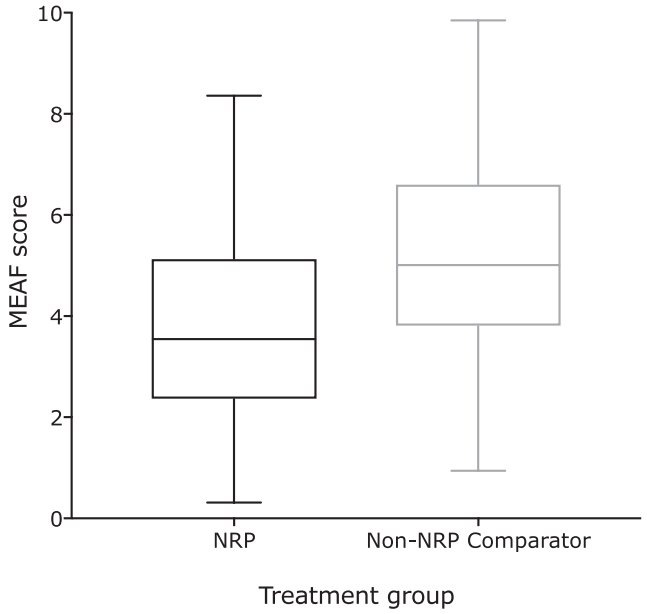

FIGURE 2 Box and whisker plot showing difference in model for early allograft function (MEAF) score between groups

recipients developed cholangiopathy were on average older, had a lower peak ALT before donation, spent less time on intensive care prior to donation, were not local to the recipient center, and their livers were recovered without using NRP compared to donors whose recipients did not develop cholangiopathy. Of note, neither the withdrawal period, the asystolic period, nor the cold ischemic time was associated with cholangiopathy in this study, probably because the variables were relatively short and similar in each group.

A logistic regression model was then built to identify the odds of developing IC. Donor age, the recipient sodium and locality of the liver predicted the development of IC posttransplant (Table 6). Although NRP was highly significant ($P < .0001$) in not developing IC, no meaningful odds ratio could be determined since there were no cases of IC in livers from donors where organ recovery used NRP.

3.4 | Retrieval damage

Five (11.6%) of the NRP livers were reported to be damaged, 3 (7%) minor, 2 (4.7%) moderate; 48 (25.7%) of the non-NRP livers were reported to be damaged, 36 (19.3%) minor and 12 (6.4%) moderate ($P = .0689$ for any damage; $P = .999$ for moderate damage).

4 | DISCUSSION

This paper describes our experience with liver transplantation from controlled DCD donors who have undergone in situ normothermic regional perfusion (NRP) before organ recovery, and compares those cases to a contemporaneous cohort of liver transplants from DCD donors who did not undergo NRP but were also transplanted in the two study centers. The study has shown that NRP is an independent factor in reducing the incidence of ischemic cholangiopathy in DCD livers following transplantation, so much so that no liver from a donor undergoing NRP prior to recovery developed cholangiopathy. Our study has also shown that lower recipient sodium at the time of surgery, older donor age, and a donor outside the local hospital was independently associated with the development of ischemic cholangiopathy.

The study is limited by being a retrospective analysis of prospectively collected data, rather than being a randomized controlled trial. We have compensated for this by including a contemporaneous comparator group of all the DCD livers transplanted in the study centers over the same 7-year study period. The regression analysis that we undertook allowed for other potentially confounding factors where the two groups differed, such as the variations in cold ischemic time and locality of the donor, to be taken into account when determining that NRP was an independent factor in the nonoccurrence of cholangiopathy. Differences in donor age, cold ischemic time, and locality contributed to the difference in US DRI between groups, which favored the NRP livers; neither locality nor CIT contribute to the UK Donor Liver Index which showed the two groups to be similar. The supplementary data illustrate that even at the extremes of donor age, CIT and withdrawal time, NRP appears superior. The other limitation is in the identification of the endpoint, given that not every patient underwent cholangiography. Although none of the NRP group had cholangiopathy detected, 31% of the NRP group had cholangiograms performed which did not show a cholangiopathy compared to 25% in the non-NRP group that showed no cholangiopathy, suggesting that there was no bias in investigating for possible cholangiopathy. This is in addition to the 27% of non-NRP patients who had positive cholangiograms. The apparently high incidence of cholangiopathy in the non-NRP arm is similar to the 26.3% incidence seen in the DCD livers in the control group of a recently published normothermic machine perfusion (NMP) study. Moreover, the absence of cholangiopathy with NRP contrasts with the 11.1% incidence in DCD livers undergoing NMP from the point of retrieval at the donor hospital.[8] This suggests that NRP in DCD liver donors may be superior to NMP in the prevention of biliary complications, an observation that will need further exploration; other observations also suggest that NMP does not protect DCD livers from cholangiopathy.[29]

Our cholangiopathy data mirror the Spanish experience presented at the 2018 International Liver Transplantation Society Congress, where they described a 2% incidence of cholangiopathy in 95 cases of NRP compared to 12% in 124 conventionally recovered DCD livers; 1 year graft survival was also not significantly better in that study also (87% NRP, 78% non-NRP).[30] Assuming the apparent difference in cholangiopathy rates is real, and the presence of similar results from the Spanish series suggests, it is interesting to speculate what may have contributed to it. One theory of cholangiopathy in DCD livers suggests the etiology to be related to ischemia of the bile ducts and the possible presence of thrombi in the biliary plexus.[31,32] Restoration of a heparinized circulation may hasten their dispersal, and there is some evidence that DCD donors exhibit fibrinolysis during NRP, which may contribute.[33] However, a high rate of

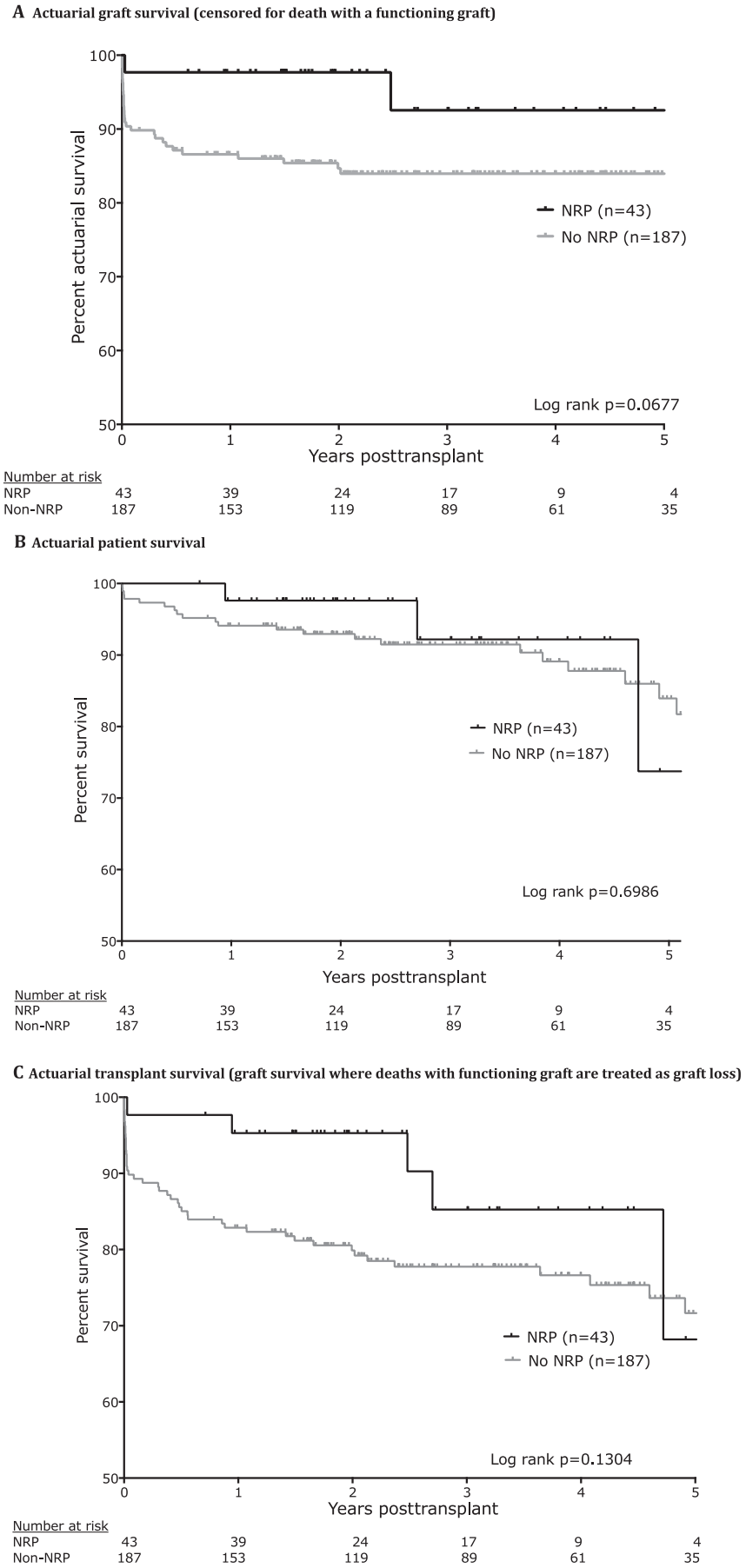

FIGURE 3 Kaplan Meier plots showing (A) death-censored graft survival, (B) patient survival, and (C) graft survival where deaths with functioning grafts were treated as graft loss

TABLE 2 Recipient demographics and outcomes

	NRP, n = 43	Comparator cohort, n = 187	*P* value
Recipient age (median, IQR, range)	60 (51-64; 34-73)	57 (51-63; 18-72)	.3192
Liver disease necessitating transplant			
Alcohol related liver disease	8 (19%)	41 (22%)	.6155
Hepatocellular carcinoma	17 (39%)	67 (36%)	
Hepatitis C cirrhosis	2 (5%)	13 (7%)	
Primary sclerosing cholangitis	3 (7%)	15 (8%)	
Primary biliary cholangitis	6 (14%)	22 (12%)	
Nonalcoholic fatty liver disease (NAFLD)	4 (9%)	12 (6%)	
Retransplant	2 (5%)	2 (1%)	
Other	1 (2%)	15 (8%)	
UKELD at transplant (median [IQR])[a]	56 (52-59)	54 (50-58)	.2389
MELD at transplant (median [IQR])[c]	15 (12-23)	15 (11-20)	.4169
Peak ALT in first 7 d (median [IQR])	633 (319-1070)	1154 (667-2099)	<.0001
Early allograft dysfunction[c]	5/43 (12%)	55/173 (32%)	.0076
Model for early allograft function (median [IQR])[f]	3.5 (2.4-5.1)	5.0 (3.8-6.6)	<.0001
Bile duct complications[d]			
Biliary leak	3/43 (7%)	18/174 (10%)	.7731
Anastomotic stricture	3/42 (7%)	46/171 (27%)	.0041
Ischemic cholangiopathy	0/42 (0%)	47/171 (27%)	<.0001
Proportion undergoing cholangiography (M/ERCP)	13/42 (31%)	91/171 (53%)	.0102
Graft loss			
Primary nonfunction	0	13 (7%)[e]	.1347
Hepatic artery thrombosis in first 28 d	1 (2%)	5 (3%)	>.99
Ischemic cholangiopathy	0	11 (6%)	.2253
Other	0	2 (1%)	>.99
Graft survival at 90 days % (95% CI) (deaths with a functioning graft censored)	97.7 (84.6, 99.7)	89.8 (84.5, 93.4)	.1019
Graft survival at 90 days % (95% CI) (deaths with a functioning graft classed as events)	97.7 (84.6, 99.7)	88.8 (83.3, 92.1)	.0760
Patient survival at 90 days % (95% CI)	100 (-)	97.3% (93.7, 98.9)	.2810
CKD-Epi GFR mls/min/1.73 m^2 (median, IQR)			.6229
Baseline (n = 43/43; 187/187)	84 (64-100)	95 (73-105)	
1 mo (n = 43/43; 177/182)	70 (52-87)	77 (53-97)	
2 mo (n = 42/42; 173/182)	65 (46-81)	73 (55-91)	
3 mo (n = 41/42; 161/181)	63 (50-75)	69 (52-85)	
4 mo (n = 36/42; 165/180)	62 (52-77)	68 (53-85)	
6 mo (n = 40/40; 160/176)	67 (54-87)	72 (55-89)	
12 mo (n = 38/39; 163/166)	72 (53-84)	70 (52-87)	

P-values are for Kruskal-Wallis test (continuous variables) and Fisher's exact test (categorical variables). Tests not performed for graft failures or deaths. Analysis of variance (ANOVA) was used to compare CKD-Epi GFR over time and between the two groups. A random effect was included in the model to allow for correlation between GFR at different time points for the same patient. Data are median (IQR) or number (percentage).

[a]UKELD = United Kingdom end-stage liver disease score.[39] A UKELD of ≥49 corresponds to a survival benefit in favor of transplantation. UKELD not available for one NRP liver and two non-NRP livers.

[b]MELD = Model for End-stage Liver Disease.[40]

[c]Early allograft dysfunction defined as ALT > 2000 u/L in first week, or INR ≥ 1.6 or Bilirubin ≥171 μmol/L on day 7.[27] Excludes 14 grafts lost in first week in comparator cohort due to primary nonfunction, hepatic artery thrombosis or death, and one in NRP cohort with hepatic artery thrombosis.

[d]Denominators exclude livers with early failure from consideration for anastomotic stricture or ischemic cholangiopathy.

[e]Includes 2 patients dying from PNF without a retransplant.

[f]Model for early allograft function.[28] Data not available for two patients in NRP group, and for eight patients in non-NRP group including four with PNF.

cholangiopathy has been reported in controlled DCD livers receiving ante-mortem heparinization and undergoing "super-rapid recovery," where thrombi should not be forming.[30] It is also possible that bile ducts are more sensitive to ischemia than hepatocytes, with less regenerative capacity, and benefit most from early reperfusion in the donor and avoidance of consecutive periods of warm followed by cold ischemia.

Another possible explanation for the absence in cholangiopathy relates to the composition of the bile. Treatment withdrawal in the donor is followed by a period of reduced organ perfusion secondary to catecholamine release in response to falling systemic pressure and cerebral perfusion.[5] With decreasing perfusion, and decreasing oxygenation, the donor becomes more acidotic, and the ability of the cholangiocytes to produce bicarbonate may be impaired. By restoring a circulation promptly following arrest it is possible that the biliary tree has time to recover and produce the bicarbonate "umbrella" that has been proposed to stop direct bile salt injury of the biliary epithelium.[34,35]

Several additional observations are noteworthy. The non-NRP livers suffered a numerically higher (7%) incidence of primary nonfunction, compared to no primary nonfunction in the NRP livers (P = .1347). This is in spite of the liver utilization rate in NRP-treated livers being 61%, in contrast to the UK national rate without NRP which varied between 27% and 36% per annum in the period of the study,[1,36] suggesting that selection bias was probably not the reason for the better initial outcomes. Instead the difference, albeit not significant in this small study, was probably accounted for by the ability to test liver viability and function in the period after the warm ischemic insults that characterize the withdrawal and asystolic periods.[5,6]

The ability to test viability of the liver in situ is the main benefit of NRP. Our small series does not provide sufficient information to define viability criteria, but we followed similar parameters to those published to help assess uncontrolled DCD donors in Spain,[20] namely lactate fall as a marker of function and ALT release as a marker of damage, although we adopted a more liberal interpretation of ALT levels to inform liver utilization. It is likely that with more experience clear parameters will be defined.

Three livers were lost in settings where they may have been utilized if NRP had not been performed. In one case, there was severe aortic atheroma and a nonfunctioning shrunken left kidney secondary to ostial atheroma. During NRP, atheromatous emboli occluded the origin of the celiac trunk and right renal ostium, something that may not have happened if retrograde cold perfusion had been used from the start. In two other cases clots were noted in the circuit, in one case associated with the leucocyte filter and in the other in

TABLE 3 Summary of recipient and donor continuous variables and their association with ischemic cholangiopathy for the 231 DCD donor livers transplanted

	Ischemic cholangiopathy						
	Yes (n = 47)		No (n = 183)			Total (n = 230)	
Continuous variable	No missing	Median (IQR)	No missing	Median (IQR)	*P*-value	No missing	Median (IQR)
Recipient factors							
Age (y)	0	58 (54-62)	0	57 (51-63)	0.9	0	57 (51-63)
Creatinine (μmol/L)	0	69 (56-89)	0	74 (63-95)	0.2	0	73 (62-95)
Bilirubin (μmol/L)	0	46 (30-83)	0	43 (20-88)	0.5	0	43.5 (22-86)
International normalised ratio (INR)	0	1.4 (1.2-1.6)	0	1.4 (1.14-1.6)	0.7	0	1.4 (1.14-1.6)
Sodium (mmol/L)	0	135 (132-138)	0	137 (134-139)	0.01	0	136 (134-139)
Potassium (mmol/L)	1	4.2 (4-4.5)	2	4.1 (3.9-4.5)	0.6	3	4.1 (3.9-4.5)
Albumin (gm/L)	1	29 (25-34)	1	29 (25-33)	0.6	2	29 (25-34)
Waiting time to transplant (d)	0	94 (23-248)	4	74 (28-183)	0.4	4	77.5 (26-193)
Donor factors							
Donor age (y)	0	52 (45-63)	0	48 (35-58)	0.02	0	49 (36-58)
Donor BMI (kg/m^2)	0	25.8 (22.3-28.3)	0	25.7 (22.9-28.3)	0.96	0	25.7 (22.8-28.3)
Donor height (cm)	0	172 (167-177)	0	172 (165-179)	0.97	0	172 (165-178)
Maximum ALT (iu/L)	0	29 (20-55)	4	40 (22-84)	0.04	4	36 (21-73)
Maximum bilirubin (μmol/L)	0	9 (6-12)	0	9 (6-14)	0.4	0	9 (6-14)
ITU stay (d)	0	2 (1-4)	2	3 (2-5)	0.04	2	3 (2-5)
Hospital stay prior to donation (d)	0	3 (1-5)	0	3 (2-5)	0.12	0	3 (2-5)

TABLE 4 Summary of categorical recipient factors for 231 DCD donor livers transplanted by ischemic cholangiopathy

Variable	Level	Ischemic cholangiopathy Yes (N = 47)	Ischemic cholangiopathy No (N = 183)	Fishers exact *P*-value
Sex	Male	31 (66.0%)	119 (65%)	>.99
	Female	16 (34%)	64 (35.0%)	
Disease group	Alcohol related liver disease	14 (29.8%)	35 (19.1%)	.9
	HCC	15 (31.9%)	69 (37.7%)	
	HCV	2 (4.3%)	13 (7.1%)	
	Primary sclerosing cholangitis	4 (8.5%)	14 (7.7%)	
	Primary biliary cholangitis	6 (12.8%)	22 (12.0%)	
	NAFLD	2 (4.3%)	14 (7.7%)	
	Retransplant	0 (0%)	4 (2.2%)	
	Other	4 (8.5%)	12 (6.6%)	
HCV status	No HCV	38 (80.9%)	136 (74.3%)	.4
	HCV	9 (19.1%)	47 (25.7%)	
Renal support	Hemodialysis	1 (2.1%)	13 (7.1%)	.6
	Hemofiltration	0 (0%)	2 (1.1%)	
	Not required	46 (97.9%)	166 (91.8%)	
Inpatient status	Out-patient	39 (83.0%)	155 (84.7%)	.8
	In-patient	8 (17.0%)	28 (15.3%)	
Previous abdominal surgery	No	43 (91.5%)	158 (86.3%)	.5
	Yes	4 (8.5%)	25 (13.7%)	
Encephalopathy	Not present	30 (63.8%)	137 (74.9%)	.18
	Compromised; altered mood/ behavior; psychometric defects	16 (34%)	39 (21.3%)	
	Drowsy; inappropriate behavior	0 (0%)	5 (2.7%)	
	Coma; cannot be aroused	0 (0%)	1 (0.5%)	
	Unknown	1 (2.2%)	1 (0.5%)	
Ascites	No ascites	24 (51.1%)	102 (55.7%)	.6
	Ascites	23 (48.9%)	81 (44.3%)	
Diabetes	No	33 (70.2%)	126 (68.9%)	.9
	Yes	14 (29.8%)	53 (29.0%)	
	Not reported	0 (0%)	4 (2.2%)	

the reservoir. Both cases were in conjunction with thoracic cannulation for recovery of the heart. The etiology of the clots is unclear, but their occurrence suggests inadequate mixing of returning blood with heparin before reaching thrombogenic surfaces such as the leucocyte filter, oxygenator or particulate filter in the reservoir. In the original Cambridge series, a shunt was used to divert blood away from the oxygenator for a few minutes until adequate mixing of the blood with the heparinized prime solution had occurred,[10] while Edinburgh also used heparin-coated components in its circuit. In the current circuit only the leucocyte filter is bypassed for the first 2 minutes of perfusion. Ensuring adequate heparinization at the start of NRP is paramount if clots and emboli are to be avoided, and this may be best achieved by premortem heparinization in countries where this is permitted.

NRP has other benefits, and in this series it was used to facilitate DCD heart transplantation.[37] We have also been able to use livers with longer withdrawal periods than currently accepted by many centers,[38] reassured by the knowledge that the liver's function can

TABLE 5 Summary of categorical donor and transplant related variables for 231 DCD donor livers

Categorical variable	Level	Ischemic cholangiopathy		Fishers exact *P*-value
		Yes (n = 47)	No (n = 183)	
Donor grouped cause of death	Head injury	5 (10.6%)	28 (15.3%)	.4
	Hypoxia	13 (27.7%)	68 (37.2%)	
	CVA	28 (59.6%)	82 (44.8%)	
	Other	1 (2.1%)	5 (2.7%)	
Donor sex	Male	28 (59.6%)	110 (60.1%)	>.99
	Female	19 (40.4%)	73 (39.9%)	
Donor ethnicity	White	46 (97.9%)	177 (96.7%)	>.99
	Asian	1 (2.1%)	6 (3.3%)	
History of diabetes	No	45 (95.7%)	174 (95.1%)	>.99
	Yes	2 (4.3%)	8 (4.4%)	
	Unknown	0 (0%)	1 (0.5%)	
History of smoking	No	23 (48.9%)	99 (54.1%)	.6
	Yes	24 (51.1%)	84 (45.9%)	
Organ appearance	Healthy	35 (74.5%)	147 (80.3%)	.4
	Suboptimal	12 (25.5%)	33 (18%)	
	Unknown	0 (0%)	3 (1.6%)	
Steatosis	No	27 (57.4%)	113 (61.7%)	.8
	Yes	20 (42.6%)	67 (36.6%)	
	Unknown	0 (0%)	3 (1.6%)	
Steatosis degree	No	27 (57.4%)	113 (61.7%)	.4
	Yes, mild	12 (25.5%)	46 (25.1%)	
	Yes, moderate	7 (14.9%)	21 (11.5%)	
	Yes, severe	1 (2.1%)	0 (0%)	
	Unknown	0 (0%)	3 (1.6%)	
Normal anatomy	No	16 (34.0%)	50 (27.3%)	.5
	Yes	30 (63.8%)	130 (71%)	
	Unknown	1 (2.1%)	3 (1.6%)	
Grade of retrieval damage	None	40 (85.1%)	150 (82.0%)	.9
	Mild	3 (6.4%)	18 (9.8%)	
	Moderate	2 (4.3%)	6 (3.3%)	
	Unknown	2 (4.3%)	9 (4.9%)	
Retrieval team	A	2 (4.3%)	10 (5.5%)	.11
	B	7 (14.9%)	15 (8.2%)	
	C	7 (14.9%)	8 (4.4%)	
	D	0 (0%)	6 (3.3%)	
	E	13 (27.7%)	72 (39.3%)	
	F	5 (10.6%)	21 (11.5%)	
	G	13 (27.7%)	51 (27.9%)	
Donation year	2011	8 (17.0%)	20 (10.9%)	.5
	2012	4 (8.5%)	21 (11.5%)	
	2013	6 (12.8%)	22 (12.0%)	
	2014	7 (14.9%)	32 (17.5%)	
	2015	13 (27.7%)	32 (17.5%)	
	2016	7 (14.9%)	40 (21.9%)	
	2017	2 (4.3%)	16 (8.7%)	

(Continues)

TABLE 5 (Continued)

Categorical variable	Level	Ischemic cholangiopathy		Fishers exact *P*-value
		Yes (n = 47)	No (n = 183)	
Locality of donor	Local	6 (12.8%)	56 (30.6%)	.03
	Regional	17 (36.2%)	61 (33.3%)	
	National	24 (51.1%)	66 (36.1%)	
Blood group compatibility	Identical	46 (97.9%)	181 (98.9%)	.5
	Compatible	1 (2.1%)	2 (1.1%)	
Perfusion used	Comparator	47 (100%)	140 (76.5%)	<.0001
	NRP	0 (0%)	43 (23.5%)	

TABLE 6 Summary of continuous transplant related factors for DCD donor liver transplants, by ischemic cholangiopathy

Variable	Ischemic cholangiopathy					Total (n = 230)	
	Yes (n = 47)		No (n = 183)				
	No missing	Median (IQR)	No missing	Median (IQR)	*P*-value	No missing	Median (IQR)
Asystolic period[a]	1	14 (12-16)	0	13 (11-16)	0.4	0	13 (11-16)
Withdrawal period (min)[b]	0	28 (22-32)	2	28 (23-33)	0.7	0	28 (22-33)
Cold ischaemia time (min)	0	428 (385-477)	2	438 (382-499)	0.94	0	433.5 (383-496.5)

[a]Asystolic period: circulatory arrest to in situ cold perfusion with preservation solution or commencement of NRP.
[b]Withdrawal period, from withdrawal of treatment to circulatory arrest.

TABLE 7 Logistic regression model for odds of developing ischemic cholangiopathy

Factor	Level	N	Odds ratio (95% CI)	*P*-value
Donor age (y)	Linear	207	1.03 (1.00, 1.06)	.015
Recipient sodium at transplant (mmol/L)	Linear	207	0.90 (0.83, 0.98)	.02
Locality of liver	Local	58	0.31 (0.11, 0.93)	.03
	Regional	68	1.00	
	National	81	1.09 (0.51, 2.32)	

CI, confidence interval; NRP, Normothermic regional perfusion.
NRP use was statistically significant after adjusting for other factors (*P* = .0001). However, we were not able to produce meaningful odds ratios as there were no cases of ischemic cholangiopathy in the NRP group.

be checked in situ before removal. This further increases the available pool of DCD donor livers that may be used to address the high mortality of those on the waiting list. The functional assessment also allows for the discard of livers that may develop primary nonfunction but which previously would have been used based solely on predonation data.

In summary, we have described 43 cases of liver transplantation from DCD donors subject to NRP, with improved early allograft function, absence of ischemic cholangiopathy, and improved graft survival. The multivariate analysis emphasizes the potential of NRP as an independent factor preventing ischemic cholangiopathy, and the study supports continued implementation and evaluation of the technique.

ACKNOWLEDGMENTS

The authors wish to acknowledge the invaluable support of Andy Nichols, Scott Melvin, Dave Gifford, and Simon Colah in developing NRP in Cambridge and John Stirling and Andy Stone in developing NRP in Edinburgh. This work would have not been possible without the cooperation and enthusiasm of the specialist nurses in organ donation in the Eastern Region and Scotland. We are also grateful to Sophie Hughes, Brian Davidson, Moira Perrin, and Bridget Gunson for the follow-up on patients transplanted outside of Cambridge and Edinburgh. We are indebted to the support of Lynn Calvert and Sagar Haval of Maquet and Arjan van der Plaats and Martin Kuizenga of Organ Assist for their help with the equipment used for NRP. The work in Cambridge was supported by grants from the Evelyn Trust and the National Institute for Health Research Blood and Transplant Research Unit (NIHR BTRU) in Organ Donation and Transplantation at the University of Cambridge in collaboration with Newcastle University and in partnership with NHS Blood and Transplant (NHSBT). The Joan Kendrick legacy supported the purchase of a near patient blood chemistry analyzer. The University of Cambridge has received salary support in respect of Professor Watson from the NHS in the East of England through the Clinical Academic Reserve. The work in Edinburgh was supported by grants from the Scottish

Government Health and Social Care Directorate and The Edinburgh and Lothian Health Foundation, which enabled the purchase of the NRP equipment. Mr. Oniscu and Mr. Currie are supported by NHS Research Scotland (NRS) Fellowships from the Chief Scientist Office. Both Cambridge and Edinburgh were supported by NHS Blood and Transplant to further evaluate NRP. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, CSO, Scottish Government, the Department of Health or NHSBT.

AUTHOR CONTRIBUTIONS

CJEW, AJB, GCO conceived of the study; CJEW, FH, SM, IC, SL, AS, KC, SJW, CF, SC, LVR, JDT, SU, AJB, GCO all conducted the research described; RT and EA performed the statistical analysis; all authors contributed to writing the final manuscript.

DISCLOSURE

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

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Watson CJE, Hunt F, Messer S, et al. In situ normothermic perfusion of livers in controlled circulatory death donation may prevent ischemic cholangiopathy and improve graft survival. *Am J Transplant*. 2019;00:1–14. https://doi.org/10.1111/ajt.15241