

Dynamic Preservation of Donation After Circulatory Death Liver Grafts From Donors Aged 60 y and Older

Joris Blondeel, MD, PhD,^{1,2} Otto B. van Leeuwen, MD, PhD,³ Ivo J. Schurink, MD, PhD,⁴ Veerle A. Lantinga, BSc,³ Nicholas Gilbo, MD, PhD,^{1,5} Femke H.C. de Goeij, BSc,⁴ Jacques Pirenne, MD, PhD,^{1,2} Volkert A.L. Huurman, MD, PhD,⁶ Vincent E. de Meijer, MD, PhD,³ Jeroen de Jonge, MD, PhD,⁴ Robert J. Porte, MD, PhD,⁴ and Diethard Monbaliu, MD, PhD^{1,2}

Background. Donor livers from older donation after circulatory death (DCD) donors are frequently discarded for transplantation because of the high risk of graft failure. It is unknown whether DCD livers from older donors benefit from dynamic preservation. **Methods.** In a multicenter study, we retrospectively compared graft and patient outcomes after transplantation of livers from DCD donors older than 60 y, preserved with either static cold storage (SCS), ex situ sequential dual hypothermic perfusion, controlled oxygenated rewarming, and normothermic perfusion (DHOPE-COR-NMP), or in situ abdominal normothermic regional perfusion (aNRP). **Results.** Fifty-six liver transplants were included in the SCS cohort, 33 in the DHOPE-COR-NMP cohort, and 27 in the aNRP cohort. Donor warm ischemia time was significantly shorter in the SCS group than in DHOPE-COR-NMP ($P < 0.001$) and aNRP ($P < 0.001$) groups. Cold ischemia times were similar. One-year incidence of nonanastomotic biliary strictures was lower after DHOPE-COR-NMP (3%, $P = 0.03$) or aNRP (7%, $P = 0.13$), compared with SCS alone (21%). Anastomotic strictures were less frequent in aNRP (19%) compared with DHOPE-COR-NMP (52%; $P = 0.015$). One-year graft and patient survival were similar. **Conclusions.** Dynamic preservation allows safe transplantation of livers from DCD donors aged 60 y or older. The risk of nonanastomotic strictures was significantly lower after DHOPE-COR-NMP than after SCS, despite longer donor warm ischemia times.

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INTRODUCTION

Treatment of end-stage liver diseases with liver transplantation (LT) is very successful. However, the shortage of suitable donor organs limits the treatment options for patients, and many transplant candidates do not receive a liver in time.¹ The acceptance criteria for suitable donor livers have been liberalized to expand the donor pool. As a result, the utilization of donation after circulatory death (DCD) donors has gradually increased, in some

countries, up to 49%.^{2,4} Unfortunately, DCD liver grafts are more susceptible to ischemia/reperfusion injury than donation after brain death liver grafts, resulting in higher rates of posttransplant complications such as early allograft dysfunction (EAD) and nonanastomotic biliary strictures (NAS).^{5,6}

In recent years, several dynamic preservation strategies have been developed to enhance and assess the quality of DCD liver grafts. Ex situ dual hypothermic oxygenated

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¹ Laboratory of Abdominal Transplantation, Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium.

² Department of Abdominal Transplantation Surgery and Coordination, University Hospitals Leuven, Leuven, Belgium.

³ Section of Hepatobiliary Surgery and Liver Transplantation, Department of Surgery, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands.

⁴ Division of HPB and Transplant Surgery, Department of Surgery, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, the Netherlands.

⁵ Department of Abdominal Surgery and Transplantation, CHU Liege, Liege, Belgium.

⁶ Department of Surgery, Leiden University Medical Center Transplant Center, Leiden University Medical Center, Leiden, the Netherlands.

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J.B., O.B.v.L., and I.J.S. contributed equally to this study.

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The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Correspondence: Diethard Monbaliu, MD, PhD, Department of Abdominal Transplantation Surgery and Coordination, University Hospitals Leuven, Herestraat 49, 3000 Leuven, Belgium. (diethard.monbaliu@uzleuven.be).

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machine perfusion (DHOPE) significantly reduces NAS in DCD livers in comparison with conventional static cold storage (SCS).⁷ Alternatively, abdominal normothermic regional perfusion (aNRP) is an in situ perfusion strategy in which the isolated abdominal compartment of the DCD donor is perfused, allowing in situ reconditioning. A recent meta-analysis showed that aNRP reduced NAS rates by 75%, although donor demographics showed considerable heterogeneity between the included studies.⁸ Whether DHOPE or aNRP also offers superior protection in high-risk DCD grafts, specifically from older donors, compared with SCS is currently unknown.

In Belgium, there is currently no formal age limit for DCD LT and liver grafts from DCD donors older than 60 y are routinely accepted for transplantation and preserved with SCS. In the Netherlands, however, DCD donors older than 60 y are currently considered too high risk for clinical LT unless pretransplant graft assessment can be performed.⁹ To this end, the Groningen group developed a protocol of sequential DHOPE, controlled oxygenated rewarming (COR), and normothermic machine perfusion (NMP): the DHOPE-COR-NMP protocol,¹⁰ whereas the Rotterdam and Leiden groups implemented an aNRP program.¹¹ Results after DHOPE-COR-NMP as well as aNRP in DCD donors are promising, with a lower incidence of NAS and improvement in graft survival.¹⁰⁻¹⁶ It is, however, unknown how these strategies perform relative to the standard SCS preservation in high-risk older DCD donors.

The aim of this multicenter study in Belgium and the Netherlands was to compare the graft and patient outcomes between patients who received a DCD liver graft from donors older than 60 y that was preserved with SCS to those who received such grafts preserved and assessed with DHOPE-COR-NMP or with aNRP.

MATERIALS AND METHODS

Study Design

This was an international multicenter retrospective study of 3 cohorts from 4 LT centers, including (1) a SCS cohort from the University Hospitals of Leuven, Belgium; (2) a DHOPE-COR-NMP cohort from University Medical Center Groningen, the Netherlands; and (3) an aNRP cohort from Erasmus Medical Center Rotterdam and Leiden University Medical Center in The Netherlands. The study periods started in January 2016 for the SCS cohort, in January 2017 for the DHOPE-COR-NMP cohort, and in January 2018 for the aNRP cohort, and ended in January 2022 for all groups. All consecutive patients older than 18 y who received an LT from a DCD donor older than 60 y and who had a minimum of 1-y follow-up were included, with the exception of patients who received a combined organ transplantation. This study was approved by the Research Ethics Committee KU Leuven (protocol S66415). Informed written consent was obtained from the legally authorized representative for each participant in accordance with the Declaration of Helsinki.

Study Endpoints and Definitions

The primary endpoint was the incidence of NAS at 1 y after LT. Secondary endpoints were EAD as defined by

Olthoff et al,¹⁷ peak aspartate aminotransferase (AST) within the first 7 d posttransplantation, incidence of symptomatic anastomotic biliary strictures (AS), 1-y graft- and patient survival, and cumulative incidence of NAS-related interventions. NAS was defined as strictures of the intrahepatic or extrahepatic donor bile ducts, excluding the anastomotic site, in combination with either clinical symptoms or biochemical abnormalities and requiring intervention, in the absence of hepatic artery thrombosis, within 1 y of transplantation. In the SCS cohort, a routine magnetic retrograde cholangiopancreatography is performed 1 y after LT, whereas no routine imaging is performed in the other cohorts. Donor warm ischemia time (WIT) was defined as the time from withdrawal of life-sustaining therapy to initiation of cold flush or the initiation of aNRP. Donor hepatectomy time was defined as the time between initiation of cold flush and immersion in ice-cold preservation solution. Cold ischemia time (CIT) was defined as the time between the initiation of cold flush and the start of DHOPE, or the start of implantation in the recipient. For the DHOPE-COR-NMP cohort, the time between the end of NMP and implantation was added to the CIT. The Donor Risk Index (DRI) was calculated according to Feng et al.¹⁸ Organ utilization rates for the DHOPE-COR-NMP and aNRP cohorts are also reported. In all 3 cohorts, the Milan criteria were applied for the selection of patients with hepatocellular carcinoma as an indication for LT.

Donation and Preservation Procedures

Donation Procedures

In Belgium, withdrawal of life-sustaining therapy in a potential DCD donor is usually performed in the operating theater, and pre-mortem administration of heparin is allowed. After donor asystole, a “stand-off” period of 5 min is to be respected before death is declared by 3 independent physicians who are not members of the transplant team. As a preservation method, SCS is used.

In the Netherlands, DCD procedures differ slightly from those in Belgium in 2 aspects. First, withdrawal of life-sustaining therapy is performed in the intensive care unit, and second, heparin is not administered pre-mortem. At University Medical Center Groningen, the DHOPE-COR-NMP protocol was used. At the Erasmus Medical Center Rotterdam and the Leiden University Medical Center, an aNRP protocol was implemented. The donation procedures in the Netherlands were performed by specialist regional stand-alone organ procurement teams, whereas in Belgium, the transplant center performs the donor procedure.

Static Cold Storage

Within minutes of death, if technically feasible, a cold flush with regular static cold preservation solution (Institute George Lopez-1 solution) was initiated via rapid cannulation of the abdominal aorta followed by portal flush via the inferior mesenteric vein. Donor hepatectomy time was kept as short as possible, and after explantation, the graft was immediately immersed in ice-cold preservation solution and subjected to an additional cold flush via the portal vein. The gallbladder was flushed in situ and the common bile duct ex situ after hepatectomy. After

packaging, the liver was transferred into an icebox and kept at low temperatures (0–4 °C) until the back-table procedure and implantation.

DHOPE-COR-NMP

DHOPE-COR-NMP was performed “back to base” in the transplant center after SCS during transportation, as previously described.⁹ Machine perfusion procedures were performed with the Liver Assist device (XVIVO, Gothenburg, Sweden). Before machine perfusion, an additional portal vein and hepatic artery flush were performed with 2 L of the University of Wisconsin (UW) machine perfusion solution (CarnaMedica, Warsaw, Poland). All livers underwent 1 h of DHOPE (8–12 °C) with portal vein pressure of ≤ 5 and hepatic artery pressures of ≤ 25 mmHg. During DHOPE, 1 L/min of 100% O₂ was administered, resulting in a PaO₂ of >80 kPa. After 1 h of DHOPE, the liver was shortly removed from the device to allow a fluid change into a red blood cell-based perfusion solution and COR was started at a temperature of 20 °C with a stepwise increase of 3 °C per 7.5 min, up to 37 °C (controlled rewarming phase). In addition, the portal vein and hepatic artery pressures were slowly increased to ≤ 11 and ≤ 70 mmHg, respectively. When the perfusate temperature reached 37 °C, the NMP phase started, which allowed for the assessment of viability. During NMP, an air/oxygen mixture was used for ventilation, aiming at a hepatic venous oxygen saturation of 55%–75%. During the first 2.5 h of NMP, hepatobiliary function was tested using the following previously published viability criteria: lactate <1.7 mmol/L, perfusate pH 7.35–7.45, bile production >10 mL, biliary pH >7.45 , difference biliary and perfusate bicarbonate >5 mmol/L, and glucose <-5 mmol/L.^{19,20} Livers were accepted for transplantation on the condition that all viability criteria were met. Viable donor livers remained on NMP until the recipient hepatectomy was completed and implantation could be initiated. After NMP, livers were flushed out with 2 L of the cold UW cold storage solution to remove the machine perfusion solution and stored on ice until implantation.

Abdominal Normothermic Regional Perfusion

In situ preservation with aNRP was performed using the Donor Assist (XVIVO, Gothenburg, Sweden) or the CardioHelp (Getinge AB, Gothenburg, Sweden) machine, as previously described.¹¹ Briefly, after thoracoabdominal laparotomy, the abdominal aorta and inferior caval vein were cannulated and the thoracic aorta was cross-clamped. Subsequently, aNRP was initiated. The target flow was >1.7 L/min, the target arterial PO₂ was between 100 and 200 mmHg, and the target perfusion duration was 120 min. The liver was considered for transplantation if aNRP flow was adequate, the alanine transaminase blood level was stable below 200 U/L, and the lactate level showed a progressive decrease. Biliary viability was considered acceptable if bile pH >7.45 and bile glucose level <3.0 mmol/L. At the end of the aNRP procedure, 6–8 L of cold preservation in the UW cold storage solution was infused, the liver was obtained using standard recovery techniques and transported to the accepting center using SCS. For logistic reasons (ie, difficult hepatectomy or no immediate

availability of operation room), some grafts underwent subsequent DHOPE perfusion to avoid prolonged cold ischemia, according to the protocol of Bruggenwirth et al.¹² Grafts undergoing DHOPE after aNRP were included in the analyses.

LT Procedure

In the SCS cohort, LT was performed using the classic cava replacement, whereas in the DHOPE-COR-NMP and aNRP cohorts, the cava preserving technique (piggyback) was routinely used. Bile duct anastomoses were performed in an end-to-end manner with absorbable sutures and without a stent or T-tube. In all cases, sequential portal and arterial reperfusion was performed. Standard immunosuppression regimens consisted of induction with basiliximab on days 0–4, tacrolimus, mycophenolate mofetil, and steroid with a tapering schedule.

Statistical Analysis

Continuous variables are presented as median (interquartile range) and compared using a Kruskal-Wallis 1-way ANOVA with pairwise comparisons. Categorical variables are presented as number (percentage) and compared with a pairwise Fisher exact or chi-square test, as appropriate. Survival curves of NAS within 1 y are plotted and compared with a log-rank test. All tests are 2-sided and *P* values of <0.05 are considered statistically significant. Unadjusted *P* values are reported. Data were analyzed using IBM SPSS Statistics, Version 28.0 (IBM Corp, Armonk, NY).

RESULTS

Donor and Recipient Demographics

A total of 116 patients were included, of which 56 livers were included in the SCS cohort, 33 in the DHOPE-COR-NMP cohort, and 27 patients in the aNRP cohort. In the aNRP cohort, 37 aNRP procedures were performed in total, in which 6 livers did not meet the viability criteria and 4 technical failures occurred where perfusion time was too short to assess the livers, resulting in an organ utilization rate of 73%. Eight livers in this cohort underwent DHOPE after aNRP (117 [100–210] min). In the DHOPE-COR-NMP cohort, 50 livers were evaluated, of which 17 did not meet the viability criteria, resulting in an organ utilization rate of 66% (Figure 1).

The donor and donation characteristics of the 3 cohorts are summarized in Table 1. No differences in donor age were found between the different cohorts. There was a significant difference in DRI between the SCS cohort and the DHOPE-COR-NMP cohort (3.2 [3.0–3.6] versus 2.9 [2.8–3.2], *P* <0.001). No differences were found between other cohorts. The WIT was significantly shorter in the SCS cohort (20 [16–24] min) compared with both the DHOPE-COR-NMP cohort (32 [26–35] min, *P* <0.001) and aNRP cohort (36 [31–40] min, *P* <0.001), whereas the WIT was not significantly different between the DHOPE-COR-NMP and the aNRP cohort. Donor hepatectomy time was longer in DHOPE-COR-NMP (39 [33–49] min) when compared with both SCS (29 [25–35] min, *P* <0.001) and aNRP (25 [21–31] min, *P* <0.001). Total CIT was similar between all 3 cohorts.

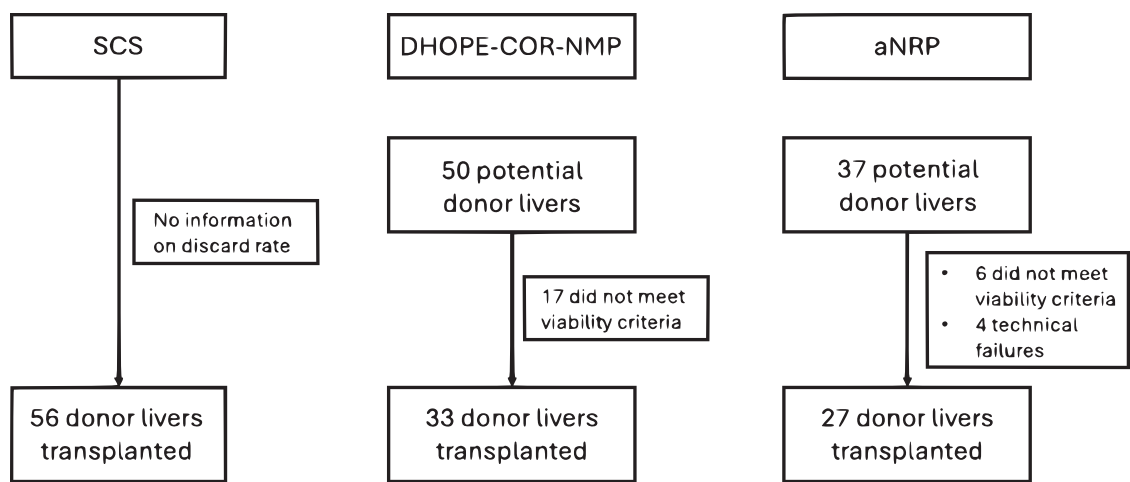


FIGURE 1. Flow diagram of the organ utilization rates. Viability criteria in the DHOPE-COR-NMP cohort: the first 2.5 h of NMP, hepatobiliary function was tested using the following, previously published viability criteria: lactate <1.7 mmol/L, perfusate pH 7.35–7.45, bile production >10 mL, biliary pH >7.45, difference biliary and perfusate bicarbonate >5 mmol/L, and glucose <–5 mmol/L. In the aNRP cohort, the liver was considered for transplantation if aNRP flow was adequate, ALT blood level was stable below 200 U/L and lactate level showed a progressive decrease. Biliary viability was considered acceptable if bile pH >7.45 and bile glucose level <3.0 mmol/L. ALT, alanine transaminase; aNRP, abdominal normothermic regional perfusion; COR, controlled oxygenated rewarming; DHOPE, dual hypothermic oxygenated perfusion; NMP, normothermic machine perfusion; SCS, static cold storage.

TABLE 1.
Donor and preservation characteristics

	SCS (N = 56)	DHOPE-COR-NMP (N = 33)	aNRP (N = 27)	P ₁	P ₂	P ₃
Age, y	68 (65–73)	66 (63–71)	67 (64–71)	0.13	0.13	0.93
Sex (male)	36 (64%)	23 (70%)	14 (52%)	0.65	0.34	0.19
BMI, kg/m ²	25 (23–29)	25 (24–27)	25 (21–29)	0.78	0.27	0.45
DRI	3.2 (3.0–3.6)	2.9 (2.8–3.2)	3.1 (2.9–3.3)	<0.001	0.026	0.21
Total WIT, min	20 (16–24)	32 (26–35)	36 (31–40)	<0.001	<0.001	0.07
Hepatectomy time, min	29 (25–35)	39 (33–49)	25 (21–31)	<0.001	0.13	<0.001
Total CIT, min	306 (271–341)	319 (278–352)	339 (287–389)	0.99	0.16	0.20
DHOPE, min	–	66 (60–87)	–			
NMP, min	–	495 (428–548)	–			
aNRP, min	–	–	120 (110–127)			

Continuous variables are presented as median (interquartile range) and compared using a Kruskal-Wallis 1-way ANOVA with pairwise comparisons. Categorical variables are presented as number (percentage) and compared with a pairwise Fisher exact or chi-square test, as appropriate. Bold indicates statistical significance ($P < 0.05$). aNRP, abdominal normothermic regional perfusion; BMI, body mass index; CIT, cold ischemia time; COR, controlled oxygenated rewarming; DHOPE, dual hypothermic oxygenated perfusion; DRI, donor risk index; NMP, normothermic machine perfusion; P₁, SCS vs DHOPE-COR-NMP; P₂, SCS vs aNRP; P₃, DHOPE-COR-NMP vs aNRP; SCS, static cold storage; WIT, warm ischemia time.

Recipients in the SCS cohort (66 [62–70] y) were older than in the DHOPE-COR-NMP cohort (63 [59–67] y, $P = 0.039$) and aNRP cohort (59 [51–64] y, $P < 0.001$; Table 2), whereas there was no age difference between patients in the latter cohorts. The laboratory model for end-stage liver disease (labMELD) score was higher in the SCS cohort, compared with the aNRP cohort (14 [10–19] versus 12 [8–15], $P = 0.045$). The LabMELD score did not differ between the DHOPE-COR-NMP cohort (14 [9–17]) and the other cohorts. There were no significant differences regarding the indications for LT. Implantation times were shorter in the aNRP cohort (27 [25–34] min) compared with the other cohorts ($P < 0.001$ for both), with no difference between the SCS cohort (38 [34–43] min) and DHOPE-COR-NMP cohort (42 [35–47] min). Perioperative transfusion of packed red blood cells was less frequent in the aNRP cohort compared with the DHOPE-COR-NMP cohort (56% versus 85%, $P = 0.002$) but not in the SCS cohort (68%).

Incidence of NAS

The 1-y incidence of NAS was 21% in the SCS cohort, 3% in the DHOPE-COR-NMP cohort, and 7% in the aNRP cohort (Table 3). Comparison of Kaplan-Meier curves with a log-rank test revealed a significant difference between the 3 cohorts ($P = 0.024$; Figure 2). Pairwise comparisons showed that 1-y NAS incidence was significantly lower in the DHOPE-COR-NMP cohort compared with the SCS cohort (3% versus 21%, $P = 0.027$). There was no significant difference when the aNRP cohort was compared with other cohorts ($P = 0.13$ versus SCS; $P = 0.58$ versus DHOPE-COR-NMP). Patients with NAS had considerable morbidity in the first year after transplantation as shown in Table 4. Six patients were listed for retransplant, whereas 3 of these patients died.

Secondary Endpoints

DHOPE-COR-NMP resulted in a lower peak AST compared with SCS (510 [333–844] U/L versus 759

TABLE 2.
Recipient and transplantation characteristics

	SCS (N = 56)	DHOPE-COR-NMP (N = 33)	aNRP (N = 27)	P ₁	P ₂	P ₃
Age, y	66 (62–70)	63 (59–67)	59 (51–64)	0.039	<0.001	0.08
Sex (male)	42 (75%)	22 (67%)	18 (67%)	0.47	0.44	>0.99
BMI	27 (23–30)	28 (25–31)	27 (24–30)	0.22	0.27	0.97
labMELD	14 (10–19)	14 (9–17)	12 (8–15)	0.13	0.045	0.61
Pretransplant PVT	8 (14%)	2 (6%)	3 (11%)	0.31	>0.99	0.65
Indication				0.06	0.28	0.15
Alcoholic cirrhosis	16 (29%)	4 (12%)	2 (7%)			
HCC	23 (41%)	10 (30%)	13 (48%)			
NASH	7 (13%)	7 (21%)	3 (11%)			
HBV/HCV cirrhosis	3 (5%)	0	5 (19%)			
Other	7 (22%)	9 (37%)	4 (14%)			
Implantation time, min	38 (34–43)	42 (35–47)	27 (25–34)	0.47	<0.001	<0.001
Transfusion (yes)	38 (68%)	28 (85%)	15 (56%)	0.086	0.33	0.02
No. of PC, units	3 (0–6)	5 (1–8)	2 (0–4)			

Continuous variables are presented as median (interquartile range) and compared using a Kruskal-Wallis 1-way ANOVA with pairwise comparisons. Categorical variables are presented as number (percentage) and compared with a pairwise Fisher exact or chi-square test, as appropriate. Bold indicates statistical significance ($P < 0.05$). aNRP, abdominal normothermic regional perfusion; BMI, body mass index; COR, controlled oxygenated rewarming; DHOPE, dual hypothermic oxygenated perfusion; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; labMELD, laboratory model for end-stage liver disease score; NASH, nonalcoholic steatohepatitis; NMP, normothermic machine perfusion; P_1 , SCS vs DHOPE-COR-NMP; P_2 , SCS vs aNRP; P_3 , DHOPE-COR-NMP vs aNRP; PC, packed cells; PVT, portal vein thrombosis; SCS, static cold storage.

TABLE 3.
Posttransplant outcome

	SCS (N = 56)	DHOPE-NMP (N = 33)	aNRP (N = 27)	P ₁	P ₂	P ₃
1-y NAS	12 (21%)	1 (3%)	2 (7%)	0.027	0.13	0.58
Peak AST, U/L	759 (536–1222)	510 (333–844)	682 (540–1153)	0.022	0.92	0.064
EAD	9 (16%)	7 (21%)	5 (19%)	0.58	0.76	>0.99
1-y AS	19 (34%)	17 (52%)	5 (19%)	0.12	0.29	0.015
1-y patient survival	52 (93%)	32 (97%)	26 (96%)	0.65	>0.99	>0.99
1-y graft survival	52 (93%)	31 (94%)	25 (93%)	>0.99	>0.99	>0.99

Continuous variables are presented as median (interquartile range) and compared using a Kruskal-Wallis 1-way ANOVA with pairwise comparisons. Categorical variables are presented as number (percentage) and compared with a pairwise Fisher exact or chi-square test, as appropriate. Bold indicates statistical significance ($P < 0.05$). aNRP, abdominal normothermic regional perfusion; AS, anastomotic biliary stricture; AST, aspartate transaminase; COR, controlled oxygenated rewarming; DHOPE, dual hypothermic oxygenated perfusion; EAD, early allograft dysfunction; NAS, nonanastomotic biliary strictures; NMP, normothermic machine perfusion; P_1 , SCS vs DHOPE-COR-NMP; P_2 , SCS vs aNRP; P_3 , DHOPE-COR-NMP vs aNRP; SCS, static cold storage.

[536–1222] U/L, $P = 0.022$). There were no differences with aNRP (682 [483–1153] U/L). EAD was similar between cohorts. ASs were significantly less frequent in the aNRP cohort compared with DHOPE-COR-NMP (19% versus 52%, $P = 0.015$) but not to SCS (34%). One-year graft- and patient survival were >90% in all cohorts and there were no differences between groups.

DISCUSSION

In this study, we compared graft and patient outcomes after transplantation of livers from DCD donors older than 60 y, whereby livers were either preserved with SCS alone or preserved and assessed with 1 of 2 dynamic preservation strategies: ex situ DHOPE-COR-NMP or in situ aNRP. Our data show that the incidence of NAS at 1 y was markedly lower in the DHOPE-COR-NMP cohort compared with SCS alone. A lower incidence of NAS was also noted in the aNRP cohort, but this did not reach statistical significance, probably due to the small sample size. Notably, the 2 NAS cases in the aNRP

cohort occurred within the first 3 transplantations and could reflect a learning curve. Peak AST level was lower in the DHOPE-COR-NMP cohort compared with the SCS cohort. This potentially indicates reduced hepatocellular injury; alternatively, a wash-out effect during dynamic preservation may also have played a role. Peak AST in the aNRP cohort was not different from the other cohorts. The incidence of AS was lowest in the aNRP cohort and significantly lower than in the DHOPE-COR-NMP cohort. As anastomotic strictures can be a combination of both ischemic damage and surgical technique, some degree of center bias may be present and limit the strength of these findings. No differences in 1-y patient survival or 1-y graft survival were observed; however, a follow-up period of 1 y might have been too short because 4 patients with NAS in the SCS cohort were listed for retransplantation but did not receive a suitable organ offer within 1 y after their first LT. Additionally, dynamic preservation techniques such as DHOPE-COR-NMP and aNRP allow for graft assessment, lowering the possible risk for primary nonfunction after

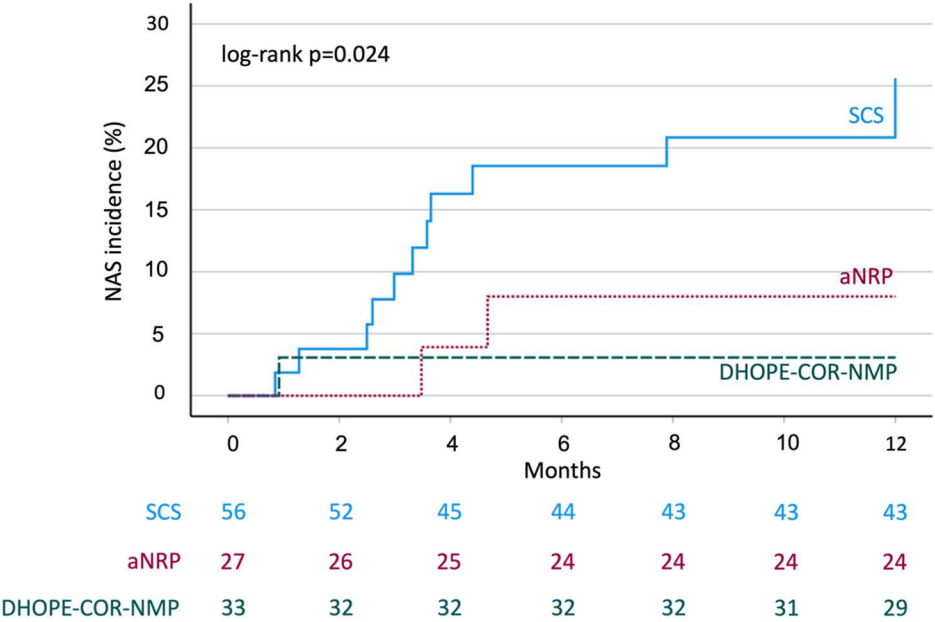


FIGURE 2. Hazard plot of cumulative NAS incidence during the first year after LT, vertical marks on the curves represent censored events due to graft loss unrelated to NAS, numbers at risk are presented below the x axis. aNRP, abdominal normothermic regional perfusion; COR, controlled oxygenated rewarming; DHOPE, dual hypothermic oxygenated perfusion; LT, liver transplantation; NAS, nonanastomotic biliary strictures; NMP, normothermic machine perfusion; SCS, static cold storage.

TABLE 4.
Details on NAS cases in all cohorts

Cohort	Case	Days from LT to NAS diagnosis	Biochemical parameters that represent cholestasis at time of diagnosis			NAS-related treatment or interventions within 1 y (number of events)				
			GGT, U/L	AP, U/L	Bilirubin, mg/dL	Antibiotics for cholangitis	Endoscopic or percutaneous intervention	Readmission	Listed for re-LT	Deceased
SCS	1	109	486	380	1.18	0	2	1	Yes	Died on the waitlist
	2	240	90	212	3.20	1	3	3	No	—
	3	158	454	289	1.26	1	3	3	No	—
	4	111	219	555	12.59	3	5	3	Yes	—
	5	134	398	684	0.96	2	7	1	No	Died 10 mo after LT
	6	39	217	363	0.51	1	4	4	No	—
	7	76	240	495	0.49	1	2	2	No	—
	8	111	251	283	1.16	2	3	2	Yes	Died on the waitlist
	9	26	166	313	3.17	1	3	3	No	—
	10	91	366	550	0.47	0	1	1	No	—
	11	101	209	333	1.42	2	4	5	Yes	—
	12	79	427	300	0.88	0	3	3	No	—
DHOPE-COR-NMP	1	28	294	297	1.55	1	0	5	Yes	—
aNRP	1	73	54	139	0.76	0	7	1	No	—
	2	200	293	496	3.74	3	2	3	Yes	—

aNRP, abdominal normothermic regional perfusion; AP, alkaline phosphatase; COR, controlled oxygenated rewarming; DHOPE, dual hypothermic oxygenated perfusion; GGT, gamma-glutamyltransferase; LT, liver transplantation; NAS, nonanastomotic biliary strictures; NMP, normothermic machine perfusion; Re-LT, redo liver transplantation; SCS, static cold storage.

transplantation of older DCD livers. Primary nonfunction was not observed in our SCS cohort due to stringent selection. However, the lack of assessment possibilities

with this technique may also lead to the underutilization of livers of sufficient quality that with SCS are not even considered for transplantation.

The high incidence of NAS in the SCS cohort (21%) underlines that SCS alone does not offer sufficient protection against biliary ischemia/reperfusion injury in DCD livers. The oxidative stress resulting from a combination of warm ischemia during withdrawal of life-sustaining therapy in the donor, followed by several hours of cold ischemia and sudden normothermic reperfusion after implantation, exceeds the regenerative capacity of the liver and, more specifically, of the biliary tract.^{5,21} Preclinical evidence has shown that DHOPE primes the liver to better withstand reperfusion injury through the replenishment of adenosine triphosphate stores and the reversal of intramitochondrial succinate accumulation, thereby preventing catastrophic production of reactive oxygen species on reperfusion.^{22–24} The clinical benefit was clearly demonstrated by van Rijn et al⁷ who showed almost 70% risk reduction of NAS incidence at 6 mo posttransplantation of DCD livers. As one of the inclusion criteria for this randomized trial was donor age younger than 60 y, it is unclear whether this benefit of dynamic organ preservation would also apply to older donor livers. As DCD donor livers older than 60 y are routinely declined in the Netherlands, the DHOPE-COR-NMP protocol was implemented to assess these livers before transplantation.¹⁰ This protocol allowed for the successful transplantation of 66% of declined DCD livers with significantly lower incidence of NAS compared with the SCS cohort, despite experiencing longer WITs and donor hepatectomy times, both risk factors for NAS.^{25,26} These observations support the reconditioning effect of DHOPE, whereas the strict viability criteria applied during the NMP phase might also have prevented the transplantation of livers at higher risk of developing NAS. Viability criteria included perfusate lactate and pH, bile production, as well as absolute values of bile pH, biliary bicarbonate and glucose, and the difference (delta) between bile and perfusate pH, bicarbonate and glucose to assess alkalization of the bile and glucose reabsorption by the biliary epithelium. However, because livers that did not meet the predefined viability criteria were not transplanted, it remains possible that some degree of unnecessary discard is still present.

In contrast to DHOPE, the exact protective mechanisms of aNRP are still limitedly understood. It is postulated that reoxygenation of the liver in situ immediately after circulatory arrest in the donor allows organs to recover from metabolic disturbances during donor warm ischemia, restoring cellular energy substrates and increasing the endogenous antioxidant adenosine before subsequent SCS.^{27,28} There is currently no evidence from randomized trials confirming risk reduction of NAS after aNRP in DCD LT compared with other preservation strategies; however, results from large retrospective series are compelling.^{11,14–16,29,30} In a multivariable analysis, Hessheimer et al¹⁵ demonstrated that aNRP was independently associated with lower NAS incidence compared with SCS in controlled DCD (89% relative risk reduction). In a direct comparison between HOPE treatment and aNRP in controlled DCD LT, Muller et al³¹ found no difference regarding NAS incidence. They did find that posttransplant AST values were higher in the HOPE cohort compared with NRP; however, this had no impact on early graft function or long-term graft survival. In our

cohort, there was no difference in posttransplant peak AST between DHOPE-COR-NMP and aNRP.

In addition to a reconditioning effect, aNRP also allows for in situ graft assessment, which in our aNRP cohort resulted in a 73% organ utilization rate of nationwide declined livers, which is similar to the DHOPE-COR-NMP cohort. The decision of liver acceptance in our aNRP cohort was based on similar parameters that were used for the assessment of DHOPE-COR-NMP: alanine transaminase levels, lactate levels, and bile composition. In many aNRP programs, macroscopic liver appearance is the most used parameter to determine suitability for transplantation, and more objective acceptance criteria may further increase liver utilization after aNRP.³² Whether the lower rates of NAS observed in both dynamic preservation cohorts can primarily be attributed to the reconditioning effect of DHOPE or aNRP, or rather better selection through graft assessment, cannot be concluded from these results; however, we believe that it is a combination of both.

Another potential advantage of aNRP is its cost-effectiveness. Because it allows for the reconditioning of multiple organs at once—kidneys, liver, pancreas, and the intestine³³—aNRP could be economically more interesting than separate ex situ perfusion of all individual organs. A recent US article showed a trend toward increased use of the pancreas for transplantation after aNRP from 2% to 8%,³⁴ as was seen in the UK cohort with a 1.6-fold increase in use of the pancreas,³⁵ but cost-effectiveness studies are still lacking. Furthermore, aNRP, compared with DHOPE-COR-NMP, is more time efficient, as aNRP takes on average 1.5–2 h, whereas DHOPE-COR-NMP takes a minimum of 4.5 h. In contrast, some grafts that were perfused with aNRP also received back-to-base DHOPE perfusion of approximately 2 h.

Our study shows that both dynamic preservation strategies provide better protection of high-risk grafts compared with SCS. However, whether 1 dynamic preservation strategy will outperform the other might depend not only on the level of protection but also on the accuracy and reliability of graft assessment criteria and organ utilization rates. To this end, DHOPE-COR-NMP might be better positioned as viability assessment is performed at the end of preservation, whereas in aNRP, it is done before procurement, although there is currently no evidence to support this statement. In our opinion, research focusing on the validation of viability criteria is therefore paramount. Median labMELD scores of the recipients in our study were low, attributed to a high proportion of patients transplanted for hepatocellular carcinoma. However, in our centers, the introduction of dynamic preservation has led to greater confidence in using these high-risk livers for higher-risk recipients, including those undergoing retransplantation.

This study has some limitations. First, aside from its retrospective design, the study may be biased because of a potential center effect, which could not be taken into account due to the small sample size. In the SCS cohort, routine magnetic retrograde cholangiopancreatography was performed after 1 y, in contrast to no routine imaging in the other cohorts. However, this is unlikely to have affected NAS detection rates, as all included NAS cases in the SCS group were diagnosed earlier during follow-up

(Table 4). Additionally, the small sample size, especially of the dynamic preservation groups, did not allow for matching baseline characteristics of the 3 cohorts. DRI was higher in the SCS group; however, most differences in donor- and procedure characteristics between cohorts were in favor of the SCS cohort (shorter WIT and donor hepatectomy time, pre-mortem heparin, and withdrawal of life-sustaining therapy in operating theater); nevertheless, we observed the highest rate of NAS and listing for retransplant in this cohort. Furthermore, although the *P* value for the overall difference in NAS between the 3 groups was significant, note that the significance at the 0.05 level of the DHOPE-COR-NMP versus SCS comparison would not remain significant after applying a Bonferroni-Holm correction for multiple testing. Finally, we could not investigate the organ utilization rate in the SCS cohort because it is impossible to retrospectively determine which livers that were refused would have been accepted if graft assessment was available, and the procuring surgeon did not have to make the decision solely based on donor characteristics, biochemistry, and visual inspection.

In conclusion, our data suggest that dynamic preservation methods are better suited than SCS for the preservation of livers from DCD donors older than 60 y. Combined end-ischemic ex situ DHOPE-COR-NMP allowed for a significant decrease in NAS at 1 y, in comparison with SCS alone. The use of in situ aNRP also resulted in a marked decrease in NAS incidence, compared with SCS, although this did not reach statistical significance. Future research should focus on the validation of viability criteria and cost-effectiveness studies to motivate reimbursement.

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