Salvage of declined extended-criteria DCD livers using in situ normothermic regional perfusion

Schurink, I.J.; Goeij, F.H.C. de; Habets, L.J.M.; Leemkolk, F.E.M. van de; Dun, C.A.A. van; Oniscu, G.C.; ... ; Jonge, J. de

OPEN

Salvage of Declined Extended-criteria DCD Livers Using In Situ Normothermic Regional Perfusion

Ivo J. Schurink, BSc, Femke H.C. de Goeij, BSc,* Lex J.M. Habets, BSc,†*
Fenna E.M. van de Leemkolk, MD,† Christian A.A. van Dun, BSc,‡
Gabriel C. Oniscu, MD, PhD,§ Ian P.J. Alwayn, MD, PhD,†
Wojciech G. Polak, MD, PhD, Volkert A.L. Huurman, MD, PhD,†*
and Jeroen de Jonge, MD, PhD✉*

Objective: This study investigates whether liver grafts donated after circulatory death (DCD) that are declined by the entire Eurotransplant region can be salvaged with abdominal normothermic regional perfusion (aNRP).

Background: aNRP is increasingly used for DCD liver grafts because it prevents typical complications. However, it is unclear whether aNRP is capable to rescue pretransplant declined liver grafts by providing the opportunity to test function during donation.

Methods: Donor livers from DCD donors, declined by all centers in the Eurotransplant region, were included for this study. The comparator cohort included standard DCD livers and livers donated after brain death, transplanted in the same time period.

Results: After the withdrawal of life-sustaining treatment, 28 from the 43 donors had a circulatory death within 2 hours, in which case aNRP was initiated. Of these 28 cases, in 3 cases perfusion problems occurred, 5 grafts were declined based on liver assessment, and 20 liver grafts were transplanted. The main differences during aNRP between the transplanted grafts and the assessed nontransplanted grafts were alanine transaminase levels of 53 U/L (34–68 U/L) versus 367 U/L (318–488 U/L) ($P = 0.001$) and bile production in 100% versus 50% of the grafts ($P = 0.024$). The 12-month graft and patient survival were both 95%, similar to the comparator cohort. The incidence of ischemic cholangiopathy was 11%, which was lower than in the standard DCD cohort (18%).

Conclusion: aNRP can safely select and thus is able to rescue DCD liver grafts that were deemed unsuitable for transplantation, while preventing primary nonfunction and minimizing ischemic cholangiopathy.

Keywords: abdominal normothermic regional perfusion, declined organ, donation after circulatory death, extended-criteria donor livers, liver transplantation

(*Ann Surg* 2022;276:e223–e230)

Liver transplantation is the only effective treatment for end-stage liver disease. However, a desperate organ shortage causes many liver transplant candidates to not receive a new liver in time, as they either die or are removed from the waiting list due to deterioration or oncological disease progression. To increase the number of donor livers, many countries resorted to using donors from controlled donation after circulatory death (DCD; Maastricht category III and V).[1] The proportion of DCD donors in the Dutch transplant program has increased over time up to 45%.[2] A drawback of DCD donation is the period of functional warm ischemia (fWIT). As a consequence, DCD grafts are especially at risk for complications such as ischemic cholangiopathy (IC) and early allograft dysfunction (EAD) after transplantation.[3,4] To minimize the risk of these complications, strict selection criteria are applied to DCD grafts. For instance, in The Netherlands, age above 60 years, fWIT > 30 minutes and a body mass index > 30 are contraindications to use DCD grafts for transplantation. Due to these strict selection criteria, the utilization rate of DCD livers is markedly lower compared with liver grafts donated after brain death (DBD) (34% vs 88%).[2]

An effective way to reduce the complication rate after DCD transplantation, is to undertake abdominal normothermic regional perfusion (aNRP). aNRP was initially developed in Spain to transplant uncontrolled DCD grafts (Maastricht category II).[5–7] Subsequently, the protocol has been adopted in the United Kingdom for controlled DCD donation (Maastricht category III).[8] After circulatory arrest, aNRP provides the opportunity to restore the circulation of oxygenated blood to the abdominal organs via cannulas in the aorta and the inferior caval vein using an extracorporeal machine oxygenation system.[9] aNRP has 3 main advantages: (1) the organs are swiftly reoxygenated after the (f)WIT and can therefore potentially

From the *Division of HPB and Transplant Surgery, Department of Surgery, Erasmus MC Transplant Institute, Erasmus University Medical Center, Rotterdam, The Netherlands; †Department of Surgery, LUMC Transplant Center, Leiden University Medical Center, Leiden, The Netherlands; ‡Department of Surgery, Organ Donation Coordinator, Erasmus MC Transplant Institute, Erasmus University Medical Center, Rotterdam, The Netherlands; and §Department of Clinical Surgery, Edinburgh Transplant Centre, University of Edinburgh, Edinburgh, UK.

✉j.dejonge.1@erasmusmc.nl.

Author contributions: I.J.S.: design of the study, patient data collection, statistical analysis of the data, interpretation of the results, preparation of the manuscript, critical revision of the manuscript, and final approval of the version to be published. F.H.C.d.G.: patient data collection, interpretation of the results, critical revision of the manuscript, and final approval of the version to be published. L.J.M.H.: patient data collection, revision of the manuscript, and final approval of the version to be published. F.E.M.v.d.L. and C.C.A.A.v.D.: patient data collection, critical revision of the manuscript, and final approval of the version to be published. G.C.O., I.P.J.A., W.G.P., and V.A.L.H.: design of the study, critical revision of the manuscript, and final approval of the version to be published. J.d.J.: design of the study, supervision of the study, interpretation of the results, preparation of the manuscript, critical revision of the manuscript, and final approval of the version to be published.

The NRP project was financed through the Dutch Ministry of Health, Welfare, and Sport.

The authors report no conflicts of interest.

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website, www.annalsofsurgery.com.

ISSN: 0003-4932/22/27604-e223
DOI: 10.1097/SLA.0000000000005611

recover from this anoxic phase, (2) the liver grafts can be assessed on the potential damage that has occurred during the fWIT, and (3) the hasty procurement during a DCD procedure is transformed in a more DBD-like procedure. Data from the United Kingdom and Spain showed that aNRP has a beneficial effect on complications and graft loss.[10,11]

At the moment, aNRP is routinely undertaken in Spain, the United Kingdom, France, and Italy,[11–14] however, predefined eligibility criteria and acceptance criteria differ slightly between countries and centers. Currently, no aNRP program specifically aimed to rescue donor livers that are declined upfront for transplantation. We investigated if DCD liver grafts initially declined for transplantation by all centers in the Eurotransplant region, could be evaluated during DCD donation and successfully transplanted after aNRP.

METHODS

Preceding the start of the clinical Dutch aNRP program, the organ perfusionists received extensive training consisting of instruction of the manufacturer, a training visit to a large aNRP center in the United Kingdom, and hands-on training on animal cadavers. In addition, the surgical team received theoretical instructions and training on animal cadavers.

This study uses prospective data collection from all aNRP procedures between October 1, 2018, and March 31, 2021. All patients provided informed consent to the use of extended-criteria DCD livers, and the use of the aNRP data and patient data was approved by the Medical Ethical Committee of the Erasmus Medical Center (MEC 2019-0370).

aNRP was performed by the stand-alone organ retrieval teams of the Erasmus Medical Center and LUMC. aNRP was considered in DCD donors (Maastricht III and V), when the donor liver was deemed unsuitable for transplantation by all 12 centers in Eurotransplant region that accept DCD grafts in the normal allocation procedure. Thereafter, livers were offered to the aNRP performing center as a center offer. Time between the withdrawal of life-sustaining treatment (WLST) and declaration of death was maximized at 2 hours, and the fWIT was maximized at 60 minutes. The start of fWIT was determined as an oxygen saturation <80% and/or the mean arterial blood pressure <50 mm Hg. WLST and declaration of death were performed at the intensive care unit (ICU), after which the donor was transferred to the operating room.

Abdominal Normothermic Regional Perfusion

After a rapid median thoracolaparotomy, the abdominal aorta and inferior caval vein were cannulated and the thoracic aorta was cross clamped. Subsequently, aNRP was started. When cold in situ perfusion of the lungs was performed simultaneously, the inferior caval vein was clamped just below the right atrium to separate the thoracic and abdominal compartments and the azygos vein was ligated. After stabilization of the perfusion, the hepatoduodenal ligament was dissected and the common bile duct was cannulated with a soft 12 Fr silicone tube to collect bile samples.

Two different extracorporeal membrane oxygenation systems were used; the Donor Assist (Organ Assist, Groningen, The Netherlands) and the CardioHelp (Getinge AB, Göteborg, Sweden). The circuit composed of a reservoir, membrane oxygenator, centrifugal pump, and leukocyte filter. The prime solution and volume boluses are described in the Supplementary Data (Supplemental Table 1 and 2, Supplemental Digital Content 1, http://links.lww.com/SLA/E65).

The target flow was >1.7 L/min and the arterial target partial pressure of oxygen was between 100 and 200 mm Hg. The pH was actively corrected to a range of 7.25 to 7.45. During aNRP, every 30 minutes, bile production was measured and blood gas and laboratory analyses were performed on portable analyzers (ePOC; Siemens, Erlangen, Germany and Piccolo Xpress, Abbott, IL). The liver was considered suitable for transplantation if assessment could be done over 60 minutes of perfusion, showing stable alanine transaminase (ALT) levels <200 U/L, a plasma glucose peak >10 mmol/L, and decreasing lactate level, preferably <5 mmol/L. Also, sufficient bile quality was assessed, defined as a pH >7.45 and glucose <3.0 mmol/L. Macroscopy of the liver did not influence the decision whether or not to transplant the liver, only to rule out cirrhosis or severe fibrosis. At the end of the aNRP procedure, 6 to 8 L of cold Belzer UW (Bridge to life, Columbia, SC) solution was infused and the liver was retrieved using standard rapid retrieval techniques and transported to the accepting center on melting ice.

Comparator Cohort

The comparator cohort includes all standard DCD and DBD livers transplanted in the Erasmus MC between October 2018 and April 2021. Recipients that received retransplantation, or were transplanted because of acute liver failure, or received a combined liver-kidney transplantation were excluded from the analysis.

Outcomes and Definitions

Well-known graft and recipients characteristics of the normothermic regional perfusion and comparator cohorts were collected. Patient and graft survival were calculated at 12 months. Graft survival was defined as the time from transplantation to retransplantation or patient death.

All relevant outcome measures including primary nonfunction (PNF), incidence of IC, postoperative EAD scores, length of ICU and hospital stay, and hepatic artery thrombosis (HAT) were collected. PNF was defined as early allograft failure resulting in either recipient death or retransplantation within 72 hours postoperatively, in the absence of any vascular complication. IC was defined as symptomatic radiologically proven nonanastomotic strictures in the biliary tree of the donor graft without the presence of a HAT. The cholangiographic imagining was performed based on clinical indication. The following risk scores were used: ET-DRI, UK-DCD-Risk-Score, discard risk index, EAD following the Olthoff criteria, and the model of early allograft function.[15–19] HAT was defined as occlusion of the hepatic artery observed on routine ultrasonography on days 0, 1, and 7 or on computed tomography angiography.

Statistical Analysis

Continuous variables were presented as medians with their interquartile range and comparisons between groups were done using the Mann-Whitney *U* test. Categorical variables were presented as numbers and percentages and were compared using the χ^2 or Fisher exact test. Graft and patient survival were determined using a Kaplan-Meier curve and differences between groups were assessed with the log-rank test. Two-sided *P* values <0.05 were considered statistically significant. All statistical analysis were performed in IBM SPSS Statistics 25 (IBM SPSS Statistics for Windows, Version 25.0, Released 2017; IBM Corp., Armonk, NY).

RESULTS

aNRP Donors

Between October 2018 and March 2021, 45 donor livers were offered to be rescued by aNRP after Eurotransplant region-wide decline for regular acceptance by all centers (Fig. 1). From these 45 donors, 1 donor had a premature cardiac arrest before the perfusion team was available and in 1 donor a malignancy was discovered during procurement. After WLST, 15 donors did not have circulatory death within 2 hours. Thus, in 28 donors, aNRP was initiated. In 3 cases, aNRP was prematurely terminated due to technical failure. In 1 case, insufficient blood flow could be due to massive aortailiac calcification. In the other 2 cases, hemostasis could not be achieved sufficiently due to a combination of the aNRP with rapid lung procurement. Finally, in 25 donors, the donor liver was evaluated for transplantation during aNRP.

Evaluation of the Donor Liver During aNRP

Of the 25 liver livers evaluated, 20 (80%) were accepted for liver transplantation (Fig. 2A–E, Table 1), although not always all predefined criteria were met according to protocol. One liver (5%) had a final ALT level of 588 U/L. Three livers (15%) had a biliary pH never exceeding the threshold of 7.45 and although all livers showed decreasing lactate levels, actually none reached the predefined protocol level <5 mmol/L. During the evaluation, 5 liver grafts (20%) were declined due to rising ALT levels. The main differences in the upfront donor characteristics between the 20 transplanted and 5 nontransplanted livers included significantly higher ALT and aspartate aminotransferase (AST) values before WLST in the nontransplanted group (Table 1). At the beginning of aNRP, these preexisting differences persisted with significantly higher ALT and AST levels in nontransplanted livers [ALT: 53 U/L (34–68 U/L) vs 367 U/L (318–488 U/L); $P = 0.001$; AST: 71 U/L (41–137 U/L) vs 363 U/L (349–486 U/L); $P < 0.001$].

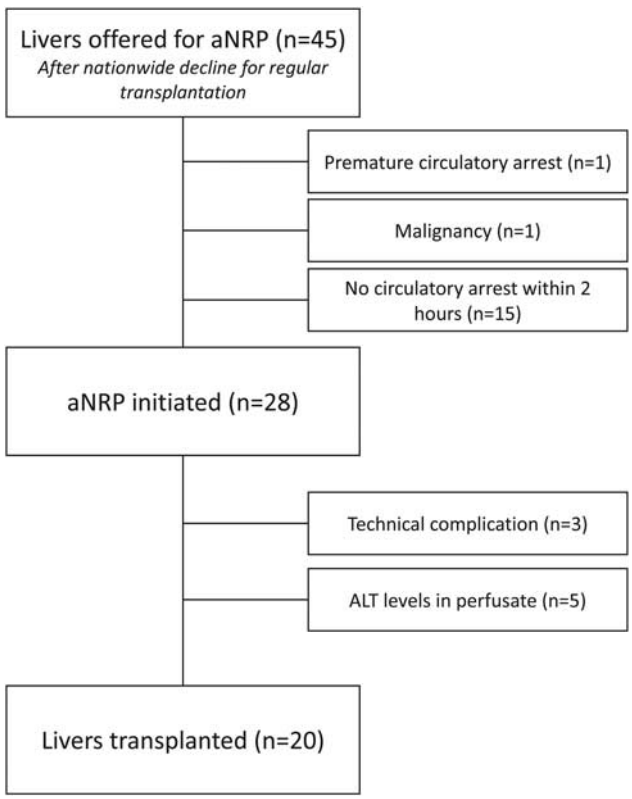

FIGURE 1. Flowchart of livers offered for aNRP.

Furthermore, all 20 (100%) transplanted grafts demonstrated production of bile. In 4 of the 5 nontransplanted grafts, cannulation of the bile duct was successful, with bile production in 2 of 4 liver grafts (50%; $P = 0.024$). However, the total amount of bile production was not different between groups. Also, no significant difference between groups was found regarding bile quality (Table 1). At the end of aNRP, in the transplanted group, biliary pH was 7.57 (7.37–7.71), bicarbonate level was 25.3 mmol/L (16.2–34.5 mmol/L). The delta pH between bile and perfusate was 0.19 (0.02–0.43) and the delta bicarbonate level was 10 mmol/L (2–17 mmol/L). In 18 of the 20 cases (90%) glucose in bile was <1.1 mmol/L, which is the lower detection limit of the blood gas analyzer. In the other 2 cases, the glucose in bile was 1.2 and 1.9 mmol/L. The delta between bile and perfusate glucose level was −11 mmol/L (−14 to −10 mmol/L), and the ratio glucose was 0.09 (0.07–0.10).

Of the 20 accepted aNRP liver grafts, retrospectively, the grade of steatosis was assessed. Eleven grafts had a minimal amount of steatosis (< 5%), 7 grafts had moderate amount of steatosis (5%–30%), and 2 livers had a sever amount of steatosis (40% and 50%).

Noticeably, donors that underwent aNRP were older than standard DCD donors (67 vs 48 years, $P < 0.001$) and DBD donors (67 vs 58 years, $P = 0.025$). Compared with the standard DCD cohort, the fWIT was 5 minutes longer in aNRP (29 vs 24 minutes, $P < 0.001$), while the hepatectomy time was 9 minutes shorter in aNRP (25 vs 36 minutes; $P < 0.001$). Calculated donor risk was higher in the aNRP cohort, with a ET-DRI of 3.10 versus 2.19 ($P < 0.001$) and an UK-DCD-Risk-Score of 9 versus 6 ($P = 0.004$).

Recipients of aNRP Liver Grafts

The laboratory-MELD (Model for End-Stage Liver Disease) score of aNRP recipient was 11 (8–15), which was comparable to the DCD cohort [14 (9–19); Table 2]. In contrast, the laboratory-MELD score of the DBD cohort was significant higher [15 (10–25); $P = 0.015$]. The age, body mass index, and indication for transplantation was comparable between the aNRP cohort and the DCD and DBD cohort.

Clinical Outcomes of aNRP Salvaged Liver Grafts

18 of the 20 aNRP grafts (90%) were transplanted in the Erasmus MC and the other 2 in the LUMC. For logistic reasons, 5 of 20 aNRP liver grafts (25%) received additional consecutive dual hypothermic machine perfusion (DHOPE) to bridge anticipated longer cold ischemia times due to recipient hepatectomy difficulties. There were no differences in postoperative AST and ALT levels, or any meaningful patient outcomes between the grafts that did or did not receive additional DHOPE (Supplementary Table 3, Supplemental Digital Content 1, http://links.lww.com/SLA/E65). Within the DCD cohort, DHOPE was undertaken in 40% of the cases, while in the DBD cohort this was 4%. The median follow-up of the aNRP cohort was 23 (14–28) months. In the comparator DCD and DBD cohorts, median follow-up time was 25 (15–33) months and 26 (18–34) months, respectively. The maximum ALT and AST levels in the first 7 days in the aNRP cohort were 546 U/L (431–837 U/L) and 783 U/L (575–1767 U/L), respectively, which was significantly lower compared with the standard DCD cohort [ALT: 1079 U/L (718–1682 U/L), $P = 0.001$ and AST: 1814 U/L

TABLE 1. Donor and aNRP Characteristics of Transplanted and Nontransplanted Livers

	Transplanted (N = 20)	Nontransplanted (N = 5)	*P*
Age (y)	67 (64–71)	67 (62–69)	0.756
BMI	25 (22–29)	24 (23–25)	0.857
Sex (male)	10 (50)	3 (60)	1.000
Cause of death			0.120
Trauma	3 (15)	3 (60)	
Cerebrovascular attack	7 (35)	2 (40)	
Anoxia	2 (10)	0 (0)	
Other	8 (40)	0 (0)	
fWIT (min)	29 (26–33)	39 (30–50)	0.116
Laboratory values before WLST			
AST (U/L)	35 (27–53)	66 (39–195)	0.049
ALT (U/L)	21 (18–46)	41 (40–128)	0.036
GGT (U/L)	30 (19–53)	46 (28–332)	0.187
NRP characteristics			
Perfusion time (min)	120 (110–128)	120 (102–124)	0.755
Perfusion characteristics at end of NRP			
Flow (L/min)	2.1 (1.9–2.4)	2.1 (2.0–2.3)	0.907
Flow (L/min per body surface area)	1.1 (1.0–1.2)	1.1 (1.0–1.2)	0.869
ALT (U/L)	53 (34–68)	367 (318–488)	0.001
AST (U/L)	71 (41–137)	363 (349–486)	< 0.001
GGT (U/L)	9 (9–11)	19 (9–162)	0.162
pH perfusate	7.33 (7.27–7.41)	7.33 (7.30–7.35)	1.000
Bicarbonate perfusate	16.6 (15.1–20.0)	16.0 (14.9–18.6)	0.437
Lactate perfusate	13.0 (11.7–14.2)	18.3 (10.2–18.7)	0.277
Glucose perfusate	12.1 (10.7–15.5)	17.3 (14.1–23.4)	0.069
Bile production	20 (100)	2* (50)	0.024
Total bile production (mL)	20 (13–34)	6 (0–19)	0.137
pH bile	7.57 (7.37–7.71)	7.40 (7.38–7.42)	0.364
Bicarbonate bile	25.3 (16.2–34.5)	16.1 (15.4–16.7)	0.554
Glucose bile	1.0 (1.0–1.0)†	1.6 (1.3–1.9)‡	0.100
ΔGlucose (bile−perfusate)	−11 (−14 to −10)	−20 (−22 to −18)	0.139
Ratio glucose (bile/perfusate)	0.09 (0.07–0.10)	0.07 (0.06–0.08)	0.361
ΔpH (bile−perfusate)	0.19 (0.02–0.43)	0.09 (0.07–0.10)	0.554
ΔHCO^{-3} (bile−perfusate)	10 (2–17)	−1 (−1 to 0)	0.312

Values are represented as n (%) and median (interquartile range).

*Of the 5 procedures, in 4 cases the common bile duct was successfully cannulated.

†The minimum measurement limit of glucose was 1.1 mmol/L, everything <1.1 mmol/L is set on 1.0 mmol/L. Two of the 20 aNRPs was the glucose value <1.1 mmol/L. The glucose values were 1.2 and 1.9 mmol/L.

‡The minimum detection limit of glucose was 1.1 mmol/L, everything <1.1 mmol/L is set on 1.0 mmol/L. One of the 2 aNRPs which produced bile had a glucose value <1.1 mmol/L. The other glucose value was 2.2 mmol/L.

BMI indicates body mass index; GGT, gamma-glutamyl transferase; NRP, normothermic regional perfusion.

(1100–2596 U/L), $P = 0.003$] and comparable to the DBD cohort [ALT: 713 U/L (442–1217 U/L) and AST: 1038 U/L (569–1580 U/L); Table 2]. The amount of EAD was not significantly different between the aNRP cohort (25%), the DCD cohort (45%), and the DBD cohort (31%). The model of early allograft function score was significantly lower in the aNRP cohort [3.7 (3.4–4.6)] compared with the DCD [5.1 (3.6–6.0); $P = 0.025$] and even to the DBD cohort [4.9 (3.5–6.0); $P = 0.032$; Table 2].

In the aNRP cohort the length of ICU stay was 2 (2–4) days, which did not differ from the other cohorts. The length of hospital stay was 13 (10–18) days, comparable to the DCD cohort but lower than the DBD cohort [17 (12–27) days; $P = 0.026$]. None of the patients in the aNRP cohort developed PNF or HAT. In the aNRP cohort at 12-month the incidence of IC was 11%, comparable to that in the DBD cohort (7%), and lower than in the DCD cohort (18%; Fig. 3, Table 2). In patients that did not receive any DHOPE, this difference was even more pronounced; the 12-month incidence of IC was 7% in the aNRP cohort without DHOPE versus 26% in the standard DCD cohort without DHOPE (Supplementary Table 4, and Figure 1 Supplemental Digital Content 1, http://links.lww.com/SLA/E65). Both IC cases in the aNRP cohort were classified as confluence dominant according to the Croome et al[20] classification (Table 3). Of these 2 patients, 1 case of IC could be resolved through progressive stenting and the other patient was retransplanted in the first year.

The 3-, 6-, and 12-month graft survival after aNRP was 95%, 95%, and 90%, respectively (Fig. 3). The 3-, 6-, and 12-month patient survival was 95%. One recipient, suffering from extensive portomesenteric thrombosis before transplantation, deceased in the early postoperative period due to recurrence of mesenteric thrombosis not related to the liver graft.

In the DCD cohort, 3-, 6-, and 12-month graft survival was 92%, 90%, and 82%. In the DBD cohort, 3-, 6-, and 12-month graft survival was 93%, 90%, and 86%. Twelve-month patient survival was 86% in the DCD and 91% in the DBD cohort. There were no significant differences in graft and patient survival of aNRP liver grafts compared with the standard DCD and DBD cohort.

DISCUSSION

We introduced aNRP in our Dutch transplant program to increase the donor liver utilization rate in DCD donors and at the same time, reduce the complication rate in the recipient. In the literature, the liver acceptance rate during aNRP in controlled DCD ranges from 55% to 84%.[8,11,21] This is accompanied by low complication rates: the incidence of PNF is between 0% and 2%, the reported incidence of IC is between 0% and 5%, the 1-year graft survival is 88% to 100%, and 1-year patient survival ranges between 93% and 100%.[10,11,14,22]

We now demonstrate that aNRP is also a safe way to evaluate extended controlled DCD liver grafts that are primarily deemed unsuitable for transplantation. This is the first study that exclusively used these declined liver grafts and transplanted them after positive evaluation criteria to explore the full potential of aNRP to increase organ utilization. The posttransplantation results are comparable to the comparative cohort of "standard" DCD and even to DBD liver transplantation. Our acceptance and complication rates in this high-risk donor cohort were comparable to the previously published results on aNRP, and the posttransplant results almost resemble those of DBD liver transplantation in the same period in our institution. Especially, the difference in IC between the aNRP and control DCD cohort is remarkable, although IC in the DCD cohort was relatively high. In the DCD cohort, this was 26% which can probably be attributed to extended fWIT or extended hepatectomy times in the whole history. It is, however, in line with the findings in the recent randomized DHOPE study where the incidence of IC was 18% at 6 months.[23]

To safeguard recipients in this study from PNF or biliary complications, we assessed donor livers during aNRP on both hepatocellular and cholangiocellular function. Primarily, only 3 hepatocellular function parameters were defined in our protocol, reflecting the original experience in the UK[8,10]: decreasing trend in lactate, glucose output peak level and

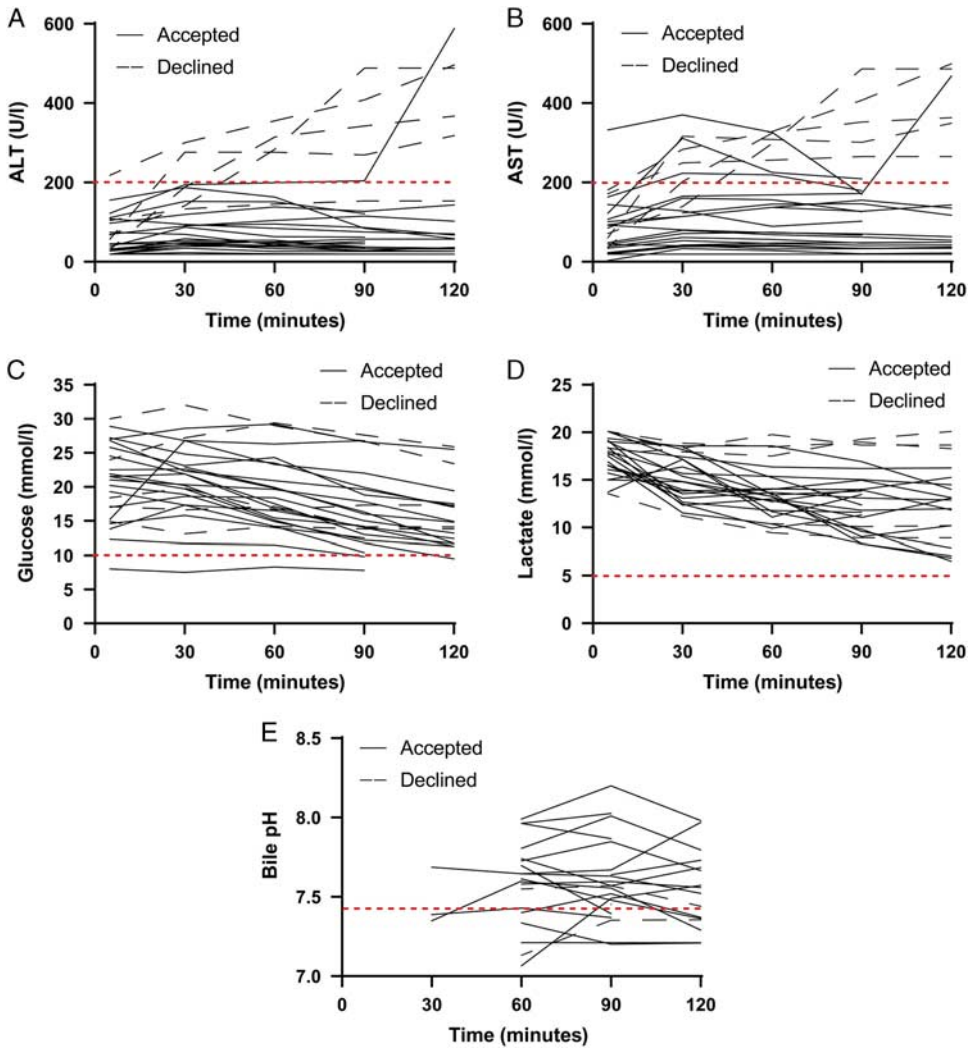

FIGURE 2. Flowchart of the acceptance parameters during aNRP. A, ALT levels. B, AST levels. C, Glucose levels. D, Lactate levels. E, pH levels in the bile.

stable ALT levels <200 U/L during perfusion. The first 2 criteria are actually liver function assessment criteria, while ALT levels reflect the ischemic injury sustained during the functional warm ischemic period. In clinical practice, however, lactate remained rather high due to leakage of lactate-rich blood entering into our circuit (eg, via supplemented packed red blood cells or from nonperfused thorax, arms and head, returning to the azygos/right atrium), and the value of <5 mmol/L was never achieved. Also, progressive knowledge from normothermic machine perfusion (NMP) indicates that the presence of lactate clearance may not be an ideal measure of liver function. Watson et al,[12] reported PNF after reperfusion in the recipient of a liver that cleared lactate during NMP. Glucose levels never turned out to be a disqualifier for transplantation, but all livers that were declined for transplantation in this series failed on ALT levels >200 U/L. In one aNRP procedure the last level of transaminases was not available at the time of decision to accept due to technical issues with the laboratory. This was not clearly communicated and the decision was made to accept the organ. The sample was retrospectively analyzed in the hospital and to our surprise the level of transaminases was unexpectedly high and not within our range of acceptance (ALT 588 U/L). The liver was uneventfully transplanted, the patient who received this graft did well in the postoperative course. Peak transaminases were AST 1738 U/L and ALT 1138 U/L. This patient had an ICU stay of 2 days and total hospital stay of 8 days. This patient was transplanted 16 months ago and is still doing well. This indicates that a cutoff level of transaminases of <200 U/L might be to narrow and probably eliminates possible functioning grafts. This is also the main limitation of the study; that livers that did not pass the test criteria during aNRP, were not transplanted, which seriously limits the information about the negative predictive value of these criteria.

To protect recipients from IC, we added cholangiocellular function assessment. To our knowledge, this is the first study describing the biochemical composition of bile during aNRP. We used cholangiocellular viability criteria, previously suggested for NMP.[12,24] These studies showed that low pH of bile (< 7.45–7.5) and high glucose levels in bile (> 3 or ≤ 10 mmol/L difference

TABLE 2. Donor, Recipient, and Transplantation Characteristics and Posttransplant Outcomes of the Transplanted aNRP Livers and the Comparator Cohorts

	NRP (N = 20)	DCD (N = 49)	DBD (N = 81)	*P* (aNRP vs DCD)	*P* (aNRP vs DBD)
Donor characteristics					
Age (y)	67 (64–71)	48 (34–55)	58 (47–70)	< 0.001	0.025
BMI	25 (22–29)	25 (23–28)	25 (23–28)	0.677	0.542
Sex (male)	10 (50)	21 (43)	36 (44)	0.605	1.000
Cause of death				0.605	0.943
Trauma	3 (15)	12 (24)	10 (12)		
Cerebrovascular accident	7 (35)	12 (24)	34 (42)		
Anoxia	2 (10)	9 (18)	9 (11)		
Other	8 (40)	16 (33)	28 (35)		
fWIT (min)	29 (26–33)	24 (19–28)	—	< 0.001	—
Last sodium (mmol/L)	142 (140–146)	145 (141–150)	147 (143–152)	0.141	0.002
Last AST (U/L)	38 (27–64)	55 (28–72)	38 (27–76)	0.345	0.779
Last ALT (U/L)	21 (18–41)	33 (21–73)	30 (16–61)	0.043	0.165
Last GGT (U/L)	30 (19–53)	36 (23–95)	28 (17–50)	0.121	0.952
Last ALP (U/L)	67 (55–72)	71 (53–95)	66 (55–78)	0.161	0.412
ICU stay (d)	2 (1–4)	3 (1–5)	2 (1–3)	0.196	0.842
Hepatectomy time (min)	25 (21–31)	36 (29–46)	36 (28–46)	< 0.001	0.003
Discard risk index	46 (36–52)	26 (21–33)	2 (2–4)	< 0.001	< 0.001
Recipient					
Age (y)	60 (52–64)	60 (51–66)	57 (49–63)	0.711	0.500
Sex (male)	14 (70)	30 (61)	55 (68)	0.587	1.000
BMI	26 (24–30)	25 (23–27)	26 (23–30)	0.368	0.615
Laboratory-MELD score	11 (8–15)	14 (9–19)	15 (10–25)	0.368	0.015
ICU stay before LTX	1 (5)	2 (4)	9 (11)	1.000	0.682
Transplantation indication				0.801	0.285
HCC	11 (55)	25 (51)	27 (33)		
Cirrhosis	4 (20)	10 (20)	19 (23)		
Biliary disease	3 (15)	12 (24)	21 (26)		
Other	2 (10)	2 (4)	14 (17)		
Transplantation					
Static cold storage	342 (294–387)	359 (300–392)	349 (283–428)	0.643	0.720
DHOPE performed	5 (25)	19 (39)	3 (4)	0.404	0.007
Anastomosis time	27 (25–30)	29 (23–33)	27 (23–34)	0.681	0.838
Estimated blood loss	3000 (1975–5500)	3500 (1500–5000)	2900 (2000–5500)	0.648	0.983
Prognostic scores					
ET-DRI	3.10 (2.97–3.24)	2.19 (1.90–2.42)	1.69 (1.49–2.01)	< 0.001	< 0.001
UK-DCD-Risk Index	9 (8–12)	6 (5–9)	—	0.004	—
EAD	5 (25)	22 (45)	25 (31)	0.176	0.786
MEAF	3.7 (3.4–4.6)	5.1 (3.6–6.0)	4.9 (3.5–6.0)	0.023	0.032
Postoperative results					
Graft survival 3 mo (%)	95	92	93		
Graft survival 6 mo (%)	95	90	90		
Graft survival 12 mo (%)	90	82	86	0.643	0.895
Peak ALT	546 (431–837)	1079 (718–1682)	713 (442–1217)	0.001	0.273
Peak AST	783 (575–1767)	1814 (1100–2596)	1038 (569–1580)	0.002	0.487
Bilirubin day 7	21 (13–46)	28 (16–63)	38 (15–94)	0.375	0.182
INR day 7	1.2 (1.1–1.2)	1.2 (1.1–1.5)	1.2 (1.1–1.3)	0.380	0.578
Length of IC stay (d)	2 (2–4)	2 (2–3)	2 (2–4)	0.809	0.602
Length of hospital stay (d)	13 (10–18)	14 (11–20)	17 (12–27)	0.195	0.026
IC incidence 12 mo (%)	11	18	7	0.371	0.990
PNF	0 (0)	1 (2)	0 (0)	1.000	—
HAT	0 (0)	1 (2)	4 (5)	1.000	0.582

Values are represented as n (%) and median (interquartile range).

BMI indicates body mass index; GGT, gamma-glutamyl transferase; HCC, hepatocellular carcinoma; INR, international normalized ratio; LTx, lung transplantation; MEAF, model of early allograft function; NRP, normothermic regional perfusion.

with perfusate glucose levels), are associated with biliary injury.[12,24] In hindsight, 3 of 20 (15%) transplanted grafts in our cohort failed the pH criteria (> 7.45), while none of these grafts developed IC. Three distinct patterns were observed: acidic bile in the first sample ameliorating over time (n = 2), alkaline bile becoming more acidic during aNRP (n = 2), and unchanged pH (n = 16). While the first observation probably reflects ischemic injury restored through aNRP, the second observation is maybe more worrisome, and these grafts require further surveillance to ensure that the bile chemistry is not reflecting a nonrecoverable injury translating in IC. In our aNRP cohort 2 patients developed IC, and one of these patients was eventually retransplanted. The fWIT (25 and 29 minutes) and the hepatectomy time (22 and 29 minutes) of these 2 grafts were comparable to the other aNRP grafts. Comparing these fWITs and hepatectomy times to literature, these values cannot be considered high risk.[17,25,26] A

TABLE 3. IC in the Normothermic Regional Perfusion Cohort

	IC#1	IC#2
Time from transplantation to first signs of IC (d)	73	200
Cholestatic laboratory tests at time of detection of IC		
GGT	54	293
Alkaline phosphatase	139	496
Bilirubin (μmol/L)	13	64
Clinical symptoms at time of detection of IC	No	Yes
Radiologic classification of IC	Confluence dominant	Confluence dominant
No. ERCP/PTC in year 1 posttransplant due to IC	7	2
No. stents ERCP/PTC in year 1 posttransplant due to IC	4	1
Re-LT within 1 y posttransplant	No	Yes
Other biliary complications		
Anastomotic stricture	Yes	Yes
Leakage/biloma	No	Yes

ERCP/PTC indicates endoscopic retrograde cholangiopancreatography/percutaneous transhepatic cholangiography; GGT, gamma-glutamyl transferase; LT, liver transplantation.

combination of donor, donation, and recipient factors may have played a role in the development of the IC in these cases. Furthermore, it is important to take into account that patient who was retransplanted received an aNRP graft which also was treated with DHOPE. This patient also had recipient factors including transplant from ICU status, acute on chronic autoimmune disease and deep sepsis in the postoperative course that may have contributed to the development of IC. During aNRP there was in these 2 patients no sign of biliary injury as the biliary pH of the grafts was 7.60 and 7.98 and the glucose levels were both <1.1 mmol/L. Summarizing, applying NMP cholangiocellular criteria resulted in an acceptable negative predictive value of 88% (pH) and 90% (glucose <3 mmol/L). However, the positive predictive value is zero and therefore in the aNRP setting the NMP cholangiocellular criteria need adaptation, which we will propose below.

With such good results, it raises the question whether acceptance criteria could even be extended to increase the utilization rate, preserving acceptable outcomes. In this study, declining of the liver graft turned out to be exclusively based on ALT levels, reflecting injury occurring during the agonal period. Other studies, such as Watson et al[10] and De Carlis et al,[22] accepted liver grafts in a standard controlled DCD donors with even higher ALT levels: up to 500 or 1000 U/L. We also transplanted one graft with high ALT levels (588 U/L). This graft fared well, without PNF, IC, or early graft lost. ALT level at day 1 posttransplant was 984 U/L, which is still acceptable. Increasing ALT acceptance levels would be the easiest way to increase the utilization rate, as all of the 5 declined grafts that did not meet the acceptance criteria would be accepted with an ALT limit of 500 U/L. With regard to the cholangiocellular acceptance criteria, we propose to extend the protocol limits to different acceptance criteria including bile production being present, glucose <3.0 mmol/L, delta bicarbonate >5 mmol/L,

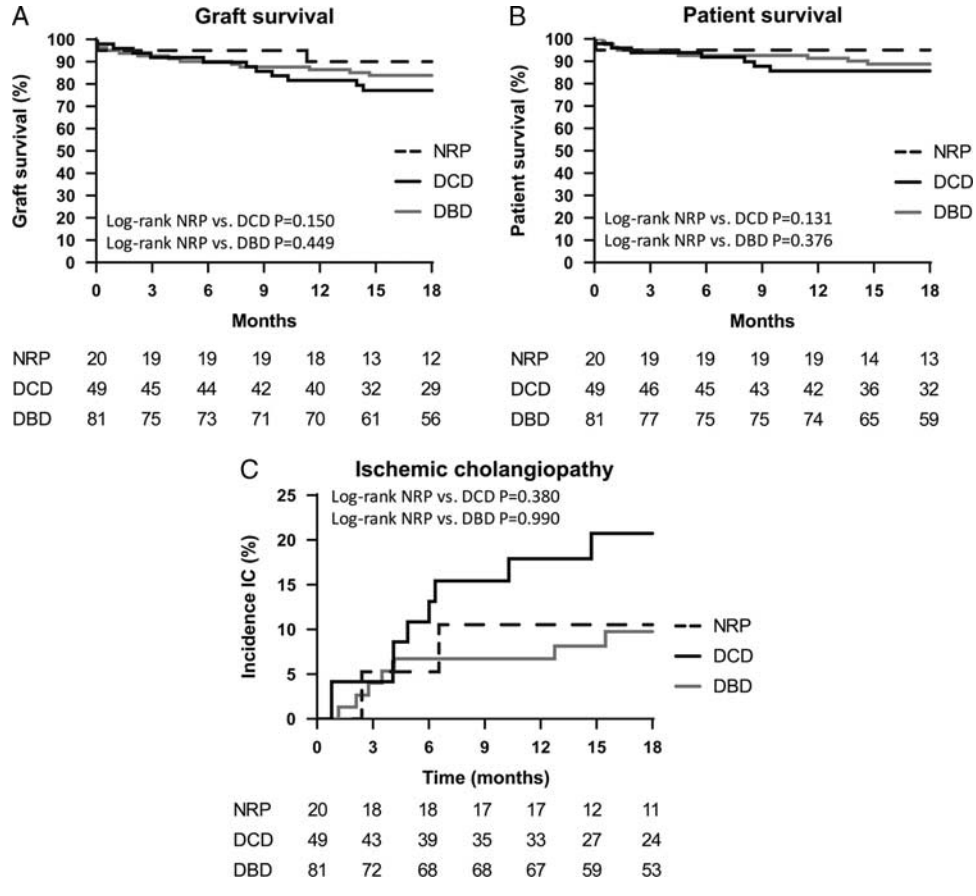

FIGURE 3. Posttransplantation outcome of the aNRP, standard DCD, and DBD cohort. A, The Kaplan-Meier curve of the graft survival. B, The Kaplan-Meier curve of the patient survival. C, The Kaplan-Meier curve of the incidence of IC.

and delta pH bile >0.1. This would result in a positive predictive value and negative predictive value of 33% and 94%. One of the 2 livers which developed IC was not be detected by these proposed cholangiocellular criteria. In this specific case however, the blood flow during normothermic regional perfusion was <1 L/min in the last half out of the perfusion, which might have provoked IC. Because of this additional injury from hypoperfusion occurred at the end of the procedure, this was probably too late to be detected in the composition of the bile samples at the end of the procedure. However, the positive predictive value of the cholangiocellular acceptance criteria is modest. Further research with a larger cohort is required to assess the clinical applicability of these biliary viability criteria to balance optimal donor use with freedom from biliary complications for the recipient. The question remains if additional DHOPE is required for these extended DCD grafts after aNRP. Five of our liver grafts received additional DHOPE because of predicted long cold ischemia times. This combination was described earlier by De Carlis et al.[22] However, in their situation, DHOPE was added because of the mandatory lengthy "no touch" time of 20 minutes, while in most other European countries, this is limited to 5 minutes.[1,22] Although excellent results were achieved with this combination of perfusion techniques, it has not been shown that adding DHOPE is required or cost-effective when the fWIT is limited. However, the question that really stands out, is whether DHOPE or aNRP should be the preferred perfusion method for standard controlled DCD donors to increase organ utilization and minimize postoperative complications. In the light of recent publications, performing controlled DCD transplantation with a "standard rapid retrieval technique," without additional protective organ perfusion should no longer be considered justified because of the high risk of biliary complications. Both aNRP,[10,11] and (D)HOPE[23,27] showed excellent graft survival outcomes, with comparable incidences of IC. A randomized controlled trial would be indicated to compare these techniques head-to-head in organ utilization, meaningful recipient outcomes and cost-effectivity.

In conclusion, this study shows the safety and feasibility of aNRP to test and transplant extended-criteria controlled DCD liver grafts. By evaluating hepatocellular and cholangiocellular function during aNRP, 71% of all livers grafts previously declined for transplantation could be eventually transplanted with results comparable to DBD liver graft transplantation.

ACKNOWLEDGMENTS

The authors thank all Dutch organ donation coordinators for their help to identify suitable extended DCD donor candidates for aNRP. The certified organ retrieval surgical teams of the Erasmus MC and LUMC are thanked for their valued efforts to perform aNRP donations. This implementation trajectory was financed through the Dutch Ministry of Health, Welfare, and Sport.

REFERENCES

1. Thuong M, Ruiz A, Evrard P, et al. New classification of donation after circulatory death donors definitions and terminology. *Transpl Int*. 2016;29:749–759.
2. NTS. Cijferoverszicht 2019; 2020. Available at: https://www.transplantatiestichting.nl/files/2020-06/NTS_cijferbijlage_jaarverslag_2019.pdf. Accessed November 4, 2021.
3. O'Neill S, Roebuck A, Khoo E, et al. A meta-analysis and meta-regression of outcomes including biliary complications in donation after cardiac death liver transplantation. *Transpl Int*. 2014;27:1159–1174.
4. Verhoeven CJ, Farid WRR, de Jonge J, et al. Biomarkers to assess graft quality during conventional and machine preservation in liver transplantation. *J Hepatol*. 2014;61:672–684.
5. Tabet J, Garcia-Valdecasas JC, Rull R, et al. Non-heart-beating donor pigs: the feasibility of liver donation. *Transplantation proceedings*. 1997;29:1374–1375.
6. Valero R, Cabrer C, Oppenheimer F, et al. Normothermic recirculation reduces primary graft dysfunction of kidneys obtained from non-heart-beating donors. *Transpl Int*. 2000;13:303–310.
7. Fondevila C, Hessheimer AJ, Ruiz A, et al. Liver transplant using donors after unexpected cardiac death: novel preservation protocol and acceptance criteria. *Am J Transplant*. 2007;7:1849–1855.
8. Oniscu GC, Randle LV, Muiesan P, et al. In situ normothermic regional perfusion for controlled donation after circulatory death—the United Kingdom experience. *Am J Transplant*. 2014;14:2846–2854.
9. van de Leemkolk FEM, Schurink IJ, Dekkers OM, et al. Abdominal normothermic regional perfusion in donation after circulatory death: a systematic review and critical appraisal. *Transplantation*. 2020;104:1776–1791.
10. 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;19:1745–1758.
11. Hessheimer AJ, Coll E, Torres F, et al. Normothermic regional perfusion vs. super-rapid recovery in controlled donation after circulatory death liver transplantation. *J Hepatol*. 2019;70:658–665.
12. Watson CJE, Kosmoliaptsis V, Pley C, et al. Observations on the ex situ perfusion of livers for transplantation. *Am J Transplant*. 2018;18:2005–2020.
13. De Carlis R, Di Sandro S, Lauterio A, et al. Liver grafts from donors after circulatory death on regional perfusion with extended warm ischemia compared with donors after brain death. *Liver Transpl*. 2018;24:1523–1535.
14. Muller X, Mohkam K, Mueller M, et al. Hypothermic oxygenated perfusion versus normothermic regional perfusion in liver transplantation from controlled donation after circulatory death: first international comparative study. *Ann Surg*. 2020;272:751–758.
15. Olthoff KM, Kulik L, Samstein B, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transpl*. 2010;16:943–949.
16. Pareja E, Cortes M, Hervás D, et al. A score model for the continuous grading of early allograft dysfunction severity. *Liver Transpl*. 2015;21:38–46.
17. Schlegel A, Kalisvaart M, Scalera I, et al. The UK DCD Risk Score: a new proposal to define futility in donation-after-circulatory-death liver transplantation. *J Hepatol*. 2018;68:456–464.
18. Braat AE, Blok JJ, Putter H, et al. The Eurotransplant donor risk index in liver transplantation: ET-DRI. *Am J Transplant*. 2012;12:2789–2796.
19. Rana A, Sigireddi RR, Halazun KJ, et al. Predicting liver allograft discard: the discard risk index. *Transplantation*. 2018;102:1520–1529.
20. Croome KP, Mathur AK, Aqel B, et al. Classification of distinct patterns of ischemic cholangiopathy following DCD liver transplantation: distinct clinical courses and long-term outcomes from a multicenter cohort. *Transplantation*. 2022;106:1206–1214.
21. Ruiz P, Gastaca M, Bustamante FJ, et al. Favorable outcomes after liver transplantation with normothermic regional perfusion from donors after circulatory death: a single-center experience. *Transplantation*. 2019;103:938–943.
22. De Carlis R, Schlegel A, Frassoni S, et al. How to preserve liver grafts from circulatory death with long warm ischemia? A retrospective Italian cohort study with normothermic regional perfusion and hypothermic oxygenated perfusion. *Transplantation*. 2021;105:2385–2396.
23. van Rijn R, Schurink IJ, de Vries Y, et al. Hypothermic machine perfusion in liver transplantation—a randomized trial. *N Engl J Med*. 2021;384:1391–1401.
24. van Leeuwen OB, de Vries Y, Fujiyoshi M, et al. Transplantation of high-risk donor livers after ex situ resuscitation and assessment using combined hypo-and normothermic machine perfusion: a prospective clinical trial. *Ann Surg*. 2019;270:906–914.
25. Jochmans I, Fieuws S, Tieken I, et al. The impact of hepatectomy time of the liver graft on post-transplant outcome: a Eurotransplant cohort study. *Ann Surg*. 2019;269:712–717.
26. Schlegel A, van Reeven M, Croome K, et al. A multicentre outcome analysis to define global benchmarks for donation after circulatory death liver transplantation. *J Hepatol*. 2022;76:371–382.
27. Schlegel A, Muller X, Kalisvaart M, et al. Outcomes of DCD liver transplantation using organs treated by hypothermic oxygenated perfusion before implantation. *J Hepatol*. 2019;70:50–57.

