Advanced Perfusion Techniques Level Liver Transplantation Outcomes With Different Donor Types: A Propensity Score-matched Analysis

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Background. Advanced perfusion techniques have been shown to improve liver transplantation (LT) outcomes in donation after determination of death by both circulatory (DCD) and neurological (DBD) criteria, but allocation strategies are still controversial. **Methods.** This study compared the outcomes of controlled DCD LT with normothermic regional perfusion and subsequent ex situ machine perfusion to those of DBD LT with static cold storage and extended criteria DBD (ECD) LT with dual hypothermic oxygenated perfusion (DHOPE), selected by propensity score matching. **Results.** Three comparable cohorts were selected from transplants performed between January 2016 and June 2024: 61 DCD (DHOPE, n = 50; normothermic machine perfusion, n = 11), 122 DBD-static cold storage, and 122 ECD-DHOPE. Median functional warm ischemia time in DCD donors was 44 (39–48) min. Livers were assessed and accepted for LT based on normothermic regional perfusion parameters. All considered outcomes were comparable between groups and in line with benchmark values. One-year graft and patient survival exceeded 90% in all groups, whereas 3-y graft survival was 91.8%, 93.4%, and 88% in the DCD, DBD-static cold storage, and ECD-DHOPE groups, respectively. In the same groups, incidence of ischemic cholangiopathy was 3.3%, 4.9%, and 3.3%. **Conclusions.** Tailored application of advanced perfusion techniques allows achieving optimal outcomes in both DCD with prolonged warm ischemia time and ECD-DBD LT.

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INTRODUCTION

Increasing utilization of organs from so-called "extended criteria donors" (ECDs)[1] is a widely adopted strategy to expand donor pool and reduce the gap between organ demand and supply in liver transplantation (LT), although their use has been associated with inferior recipient outcomes.[2] In particular, livers from donors whose death has been determined by circulatory criteria (DCD) have been historically considered ECD grafts, given the higher risk of primary nonfunction (PNF), early allograft dysfunction (EAD), and ischemic cholangiopathy (IC) associated with their use, which leads to inferior graft survival.[3-5] The introduction of advanced in situ and ex situ perfusion techniques, including normothermic regional perfusion (NRP), hypothermic oxygenated machine perfusion (HOPE), and normothermic machine perfusion (NMP), has greatly mitigated the risk associated with ECD use,[6-8] with several studies showing improved outcomes for livers from both DCD donors and donors whose death was determined by neurological criteria (DBD).[8-18] However, the choice of a particular technique in different clinical scenarios is still matter of debate.

Because of regulations imposing a 20-min flat ECG for death determination, DCD LT has long been considered unfeasible in Italy, given the prohibitive risk of graft failure and IC associated with the unavoidably prolonged warm ischemia time (WIT).[19] However, as adversity often gives rise to the most innovative ideas, Italy has also represented an ideal setting to test novel procurement and preservation strategies to enhance outcomes of DCD LT. By combining NRP with ex situ machine perfusion (MP),[20] Italian groups

have reported outcomes in line with a proposed benchmark in DCD LT.[21-25] Remarkably, despite considerably longer WIT, the rate of IC has been comparable to that of European series using NRP alone, suggesting improved organ preservation by a combination of in situ and ex situ techniques. Recent studies have opened to the possibility to avoid the use of ex situ MP in selected cases and to consider livers from elderly (≥70-y-old) donors.[22,25] As Italian DCD LT programs started in 2015–2016 and the number of cases was initially low, progressively increasing only since 2021–2022 (source: https://trapianti.sanita.it/statistiche/attivita), most published series are of low numerosity and suffers from limited follow-up.

By comparing LT outcomes of DCD livers procured by NRP and subsequently treated by ex situ MP with those of standard donors preserved by static cold storage (SCS) and those of ECD-DBD treated by dual HOPE (DHOPE), this study reported a more consolidated experience from our institution, analyzing donor selection process, as well as early and long-term outcomes. The aim was to help transplant professionals in the choice of optimal procurement and preservation techniques to improve LT outcomes.

MATERIALS AND METHODS

This single-center retrospective study analyzed prospectively collected data on adult (≥18-y-old) LT performed at our institution between January 2016 and June 2024, comparing outcomes of LT with Maastricht category 3 DCD donors with those of LT with DBD donors preserved by SCS and ECD-DBD donors treated with end-ischemic DHOPE. Minimum follow-up was 6 mo. Inclusion criteria were adult recipients undergoing LT within the defined time frame. Exclusion criteria were recipient of a combined transplant or a partial graft, retransplant, on-table death, and acute liver failure as an indication for LT. To limit confounding, also recipients of livers from Maastricht category 2 DCD donors and cases in which NMP was used for viability testing were excluded.

The study was conducted according to the principles of the Istanbul and Helsinki declarations and was approved by the ethics committee of our Institution (protocol 506/2021).

Procurement and MP were performed as previously described.[16,24,26,27] Briefly, all DCD livers were procured using NRP with pump flow targeting ~50% of optimal pump flow for therapeutic extracorporeal membrane oxygenation. Guidewires for femoral vessels cannulation were placed premortem and 300 IU/kg of heparin were administered at the onset of functional WIT (peripheral O_2 saturation ≤70% or systolic blood pressure ≤50 mm Hg, whichever occurred first). After death declaration, femoral vessels were cannulated and the descending aorta was occluded by either an endoclamp or a surgical clamp applied through a sternotomy, after which NRP was initiated. During NRP, liver assessment was based on the criteria proposed by De Carlis et al.[21] A liver biopsy was obtained in all DCD donors as part of the evaluation process. In all recipients, a postreperfusion biopsy (so-called "time 0" biopsy) was obtained at the end of LT operation to assess histological signs of ischemia–reperfusion injury and steatosis.[28] At the end of NRP, livers were flushed with Celsior solution (IGL, Lissieux, France) and cold stored for transport. Upon arrival at the transplant center and after backtable preparation, livers underwent MP until the end of recipient hepatectomy. Although DHOPE was the preferred MP technique after NRP, NMP was considered in the case of difficult logistics (simultaneous transplants, issues with recipient preoperative work-up or transport to the hospital) or contingent factors (eg, unavailability of the DHOPE device). Notably, 12 DCD livers were allocated to either DHOPE (n = 6) or NMP (n = 6) in the setting of a randomized controlled trial comparing DHOPE versus NMP after NRP in DCD LT (DCDNet study, NCT04744389). In this cohort, NMP was not used for the purpose of assessing liver viability and all the livers were accepted for LT during NRP. The LiverAssist (XVIVO, Goteborg, Sweden) device was used for DHOPE and part of NMP procedures (n = 7).[29] In the remaining cases (n = 4), the OrganOx Metra (OrganOx, Oxford, United Kingdom) was used.[30] According to the Italian allocation scheme, DCD livers were preferentially allocated to low model for end-stage liver disease (MELD) recipients with hepatocellular carcinoma.[31]

ECD-DBD grafts[1] underwent end-ischemic DHOPE as previously described.[16,32] The decision to use DHOPE was based on donor characteristics and expected cold ischemia time, also considering recipient features.[16] All DHOPE-treated livers were from donors meeting the ECD criteria.

The primary endpoint of the study was graft survival. Secondary endpoints included incidence of postreperfusion syndrome (PRS), indicators of graft function (PNF, EAD,[33] liver graft assessment following transplantation,[34] and early allograft failure simplified estimation[35] scores) rate of acute kidney injury (AKI), requirement for renal replacement therapy, biopsy-proven acute rejection, postoperative complications,[36] and patient survival. Biliary complications were diagnosed based on the 3-mo cholangiogram obtained before removing the t-tube, if present, or by magnetic resonance cholangiopancreatography, which was performed if clinically indicated. IC cases were defined according to the criteria proposed by Esser et al.[37] Importantly, patients with concomitant anastomotic and nonanastomotic strictures were classified as having IC.

Propensity score matching was used to control for selection bias and create comparable groups. Propensity scores were calculated based on covariates reflecting general recipient features (age and sex), the severity of liver disease (MELD-Na), the indication for LT (with hepatocellular carcinoma as the main discriminant) and donor characteristics (age and body mass index). The number of packed red blood cells units transfused during LT was also included in the model as an additional indicator of the severity of liver disease, pre-LT anemia, and surgical complexity to enhance comparability between study groups. By 1:2 propensity score matching, 2 comparator groups of the DCD cohort were selected: one group of DBD LT performed using SCS (DBD-SCS) and the second group of LT performed with grafts from ECD-DBD treated by end-ischemic DHOPE (ECD-DHOPE). Outcomes of the DCD and ECD-DHOPE cohorts were then compared with those of benchmark values proposed by Schlegel et al[5] and Muller et al.[38]

Variables are presented as number (percentage) of median (interquartile range) and compared using Fisher, chi-square, and Mann-Whitney tests as appropriate.

Patient and graft survival were analyzed using Kaplan–Meier curves. Statistical analysis was performed using R: a language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria). The function matchit (method = "nearest," distance = "glm," ratio = 2) from the MatchIt package was used for propensity score matching.

RESULTS

During the study period, 1280 LTs were performed at our center, of which 74 using organs proceeding from a DCD donor. After applying inclusion and exclusion criteria, 61 recipients of category 3 DCD grafts treated with NRP followed by ex situ MP were included in the analysis (DCD group). Of these, 50 grafts underwent DHOPE, whereas 11 were treated by NMP. Outcomes in this group were compared with those of 2 matched groups of recipients of DBD grafts preserved using SCS (DBD-SCS group, n = 122) and DHOPE-treated ECD-DBD grafts (ECD-DHOPE group, n = 122) (Figure 1). By the effect of matching process, all absolute mean differences in the model covariates were <0.10 between the DCD and DBD-SCS groups. However, because of the preferential use of DHOPE for DBD livers from elderly donors in the ECD-DHOPE group, absolute mean difference in donor age between DCD and ECD-DHOPE groups (Figure 2) was >0.10 even after matching. DBD-SCS and ECD-DHOPE groups were also well balanced, except for donor age

which was higher in ECD-DHOPE group (**Table S1, SDC,** https://links.lww.com/TP/D311).

Donor and Recipient Characteristics

Recipient characteristics, including the indication for LT, MELD, MELD-Na, and baseline creatinine at LT, were comparable between study groups (Table 1). Because of selection bias, donors in the ECD-DHOPE group were older (66.8 versus 60.5, $P = 0.01$), whereas other variables were comparable. In the DCD group, total (withdrawal of life-sustaining therapy—NRP start) and functional WIT was 44 (39–48) and 44 (36–47) min, respectively. NRP time was 240 (218–271) min. Because of the fact that most DCD donors were procured in our hospital, SCS time was shorter in this group (260 versus 426 versus 362 min, $P < 0.01$ each), whereas MP time was longer (240 versus 137 min, $P < 0.01$), mainly because of the use of NMP in a subset of livers in this group.

Viability Assessment During NRP

All livers fulfilled viability criteria[21] during NRP (Figure 3). In particular, all donors had a downward trend of blood lactate during NRP and transaminases never exceeded the 1000 IU/L threshold, with most donors showing levels <250 IU/L. Notably, there were no significant differences in NRP parameters between livers that were subsequently treated by DHOPE or NMP. Pump flow was maintained according to our protocol in all but
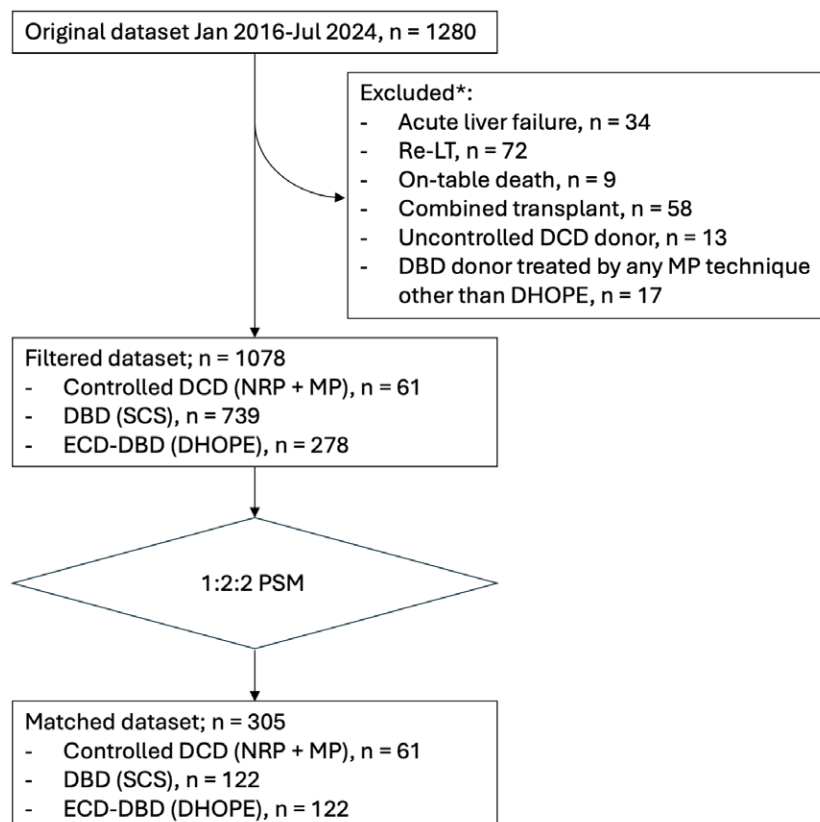
FIGURE 1. Patient selection process. *The same patient may meet more than one exclusion criterion. DBD, donation after determination of death by neurologic criteria; DCD, donation after determination of death by circulatory criteria; DHOPE, dual hypothermic oxygenated machine perfusion; ECD, extended criteria donor; LT, liver transplantation; MP, machine perfusion; PSM, propensity score matching; SCS, static cold storage.
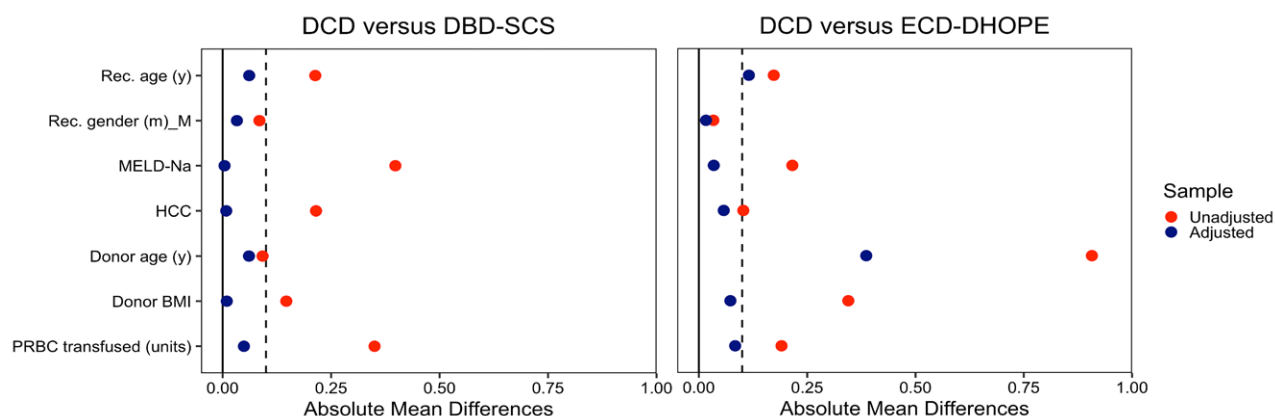
FIGURE 2. Balance plots showing absolute standardized mean differences of covariates used to calculate propensity scores before and after adjustment. BMI, body mass index; DBD, donation after determination of death by neurologic criteria; DCD, donation after determination of death by circulatory criteria; DHOPE, dual hypothermic oxygenated machine perfusion; ECD, extended criteria donor; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease; PRBC, packed red blood cells; SCS, static cold storage.

one donor, in which NRP was complicated by iliac artery dissection that required repositioning of the inflow cannula into the abdominal aorta by open laparotomy. This resulted in additional 65 min of poor pump flow (after 44 min of initial functional WIT), after which pump flow was 1.5–1.8 L/min for the subsequent 252 min of NRP. Considering that liver biopsy showed normal histology, lactate trend was downward, and second-hour aspartate aminotransferase and alanine aminotransferase levels were 674 and 330 IU/L, this liver was accepted for LT.

Primary and Secondary Outcomes

Median follow-up was 22 (13–52), 62 (39–90), and 32 (19–55) mo in the DCD, DBD-SCS, and ECD-DHOPE groups, respectively. No differences were observed in graft and patient survival between groups (Figure 4). One-year graft survival was 91.8% (85.2%–98.9%), 94.2% (90.2%–98.5%), and 91.8% (87%–96.8%) in the DCD, DBD-SCS, and ECD-DHOPE groups, respectively. In the same groups, 3-y graft survival was 91.8% (85.2%–98.9%), 93.4% (89%–97.9%), and 88% (81.9%–94.6%), respectively.

In general, all indicators of early graft function (including EAD, liver graft assessment following transplantation, and early allograft failure simplified estimation scores) were comparable among study groups, as were the severity and incidence of complications (Table 2). The only significant difference was a lower alanine aminotransferase peak in the DCD group as compared with DBD-SCS group (584 versus 847 IU/L; P < 0.01). Median ICU and hospital length of stay were low and identical across all 3 groups (3 and 10 d, respectively). AKI occurred in >20% of LT in each group. However, new-onset renal replacement therapy was required only in 2 (3.3%) cases in the DCD group, 1 (0.8%) case in the DBD-SCS group, and 10 (8.2%) cases in the ECD-DHOPE group. Notably, all considered procedural variables and outcome measures in the DCD group, including comprehensive complication index (CCI) at discharge, 3, 6 and 12-mo follow-up, were within benchmark cutoffs in DCD LT proposed by Schlegel et al.[5] This was true also for the ECD-DHOPE group, with the exception

that the rate of renal replacement therapy (8.2%) slightly exceeded the benchmark value (8%).[38]

The only case of PNF occurring in the DCD group involved the aforementioned liver that had been exposed to prolonged WIT because of iliac artery dissection. As the liver met all viability criteria during NRP, it was accepted for LT and transplanted after DHOPE. Early after LT, the recipient developed severe hemodynamic instability and multiorgan failure requiring emergent graft hepatectomy and placement on a portocaval shunt. Despite being retransplanted in <24 h after the index LT, the recipient died because of the consequences of bowel ischemia and multiorgan failure.

Incidence of biliary complications was similar across study groups. In particular, incidence of IC was 3.3%, 4.9%, and 3.3% in the DCD, DBD-SCS, and ECD-DHOPE groups, respectively. Two grafts were lost in the DCD group as a result of biliary complications, but none was related to IC cases, which were both classified as minor forms[39] and resolved with treatment. Both cases had a concomitant anastomotic stricture, with intrahepatic strictures possibly issuing from cholangitis of sludge accumulation. Only 1 intrahepatic stricture required treatment in the first case, whereas in the second case intrahepatic strictures were left untreated and resolved after treatment of the anastomotic stricture (**Figures S1 and S2, SDC,** https://links.lww.com/TP/D311). On the other hand, both biliary complication-related graft losses resulted from late biliary leaks, presenting with a perihilar biloma >1 mo after LT. In the first case, the attempt to perform a hepaticojejunostomy resulted in irreparable damage to the hepatic artery, requiring re-LT. In the second case, patient developed a carbapenemase-producing *Klebsiella pneumoniae* infection leading to sepsis and patient death.

DCD Outcomes According to Preservation Modality

We compared patients who received a DCD graft preserved with either DHOPE (n = 50) or NMP (n = 11). Donor characteristics and operative parameters were similar between the groups (**Table S2, SDC,** https://links.lww.com/TP/D311). As NMP was utilized in some cases of difficult LT logistics, MP time (295 versus 195 min, P < 0.01)

TABLE 1.

Baseline characteristics

	DCD	DBD-SCS	ECD-DHOPE	p1	p2
n	61	122	122		
Rec. age	60.3 [52.6, 65.8]	58.2 [54.0, 62.6]	60.1 [54.7, 66.1]	0.24	0.67
Gender (male)	49 (80.3)	100 (82.0)	103 (84.4)	0.95	0.63
Rec. BMI	25.4 [22.5, 28.3]	24.9 [23.2, 27.7]	25.8 [23.5, 27.8]	0.99	0.61
Indication				0.24	0.56
Viral hepatitis	25 (41.0)	70 (57.9)	66 (54.1)		
ETOH	14 (23.0)	15 (12.4)	19 (15.6)		
Cholestatic	4 (6.6)	8 (6.6)	4 (3.3)		
Autoimmune	2 (3.3)	2 (1.7)	5 (4.1)		
NASH	2 (3.3)	6 (5.0)	4 (3.3)		
Other	14 (23.0)	20 (16.5)	24 (19.7)		
HCC	44 (72.1)	92 (75.4)	94 (77.0)	0.76	0.58
MELD	9.0 [8.0, 14.0]	11.0 [8.0, 15.0]	10.0 [8.0, 14.0]	0.40	0.54
Na-MELD	10.3 [8.0, 16.0]	11.0 [8.0, 15.0]	11.0 [8.0, 15.8]	0.66	0.90
Creatinine (mg/dL)	0.8 [0.7, 0.9]	0.8 [0.7, 1.0]	0.8 [0.7, 1.0]	0.41	0.34
Dialysis pre-LT	1 (1.6)	2 (1.6)	0 (0.0)	1.00	0.72
Donor age	60.5 [52.1, 69.9]	61.9 [52.1, 71.8]	66.8 [58.2, 75.6]	0.54	0.01
Donor BMI	25.5 [23.3, 27.7]	25.4 [23.2, 27.8]	26.1 [23.7, 29.4]	0.94	0.19
Macrosteatosis				1.00	0.21
Mild (<30%)	61 (100.0)	121 (99.2)	116 (95.0)		
Moderate (30%–60%)	0 (0.0)	1 (0.8)	5 (4.2)		
Severe (>60%)	0 (0.0)	0 (0.0)	1 (0.8)		
Graft weight (kg)	1.5 [1.2, 1.7]	1.5 [1.3, 1.8]	1.5 [1.25, 1.8]	0.41	0.95
D-MELD	559 [438, 806]	628 [471, 895]	698 [520, 882]	0.43	0.06
tWIT (min)	44 [39, 48]				
fWIT (min)	44 [36, 47]				
NRP time (min)	240 [218, 271]				
SCS time (min)	280 [233, 335]	426 [366, 476]	362 [316, 417]	<0.01	<0.01
MP time (min)	240 [150, 297]		137 [120, 189]		<0.01
DHOPE	195 [136, 268]		137 [120, 189]		
NMP	305 [278, 430]				
Tot. pres.time (min)	525 [445, 585]	426 [366, 476]	514 [475, 576]	<0.01	0.82
PRBC units (n)	1 [0, 4][a]	1 [0, 4]	1 [0, 4][a]	0.56	0.81

Data are presented as counts (percentage) or median (interquartile range) as appropriate. p1: DCD versus DBD-SCS; p2: DCD versus ECD-DHOPE.

[a]This variable was within benchmark cutoffs proposed by Schlegel et al[5] and Muller et al.[38]

BMI, recipient body mass index; DBD, donation after determination of death by neurologic criteria; DCD, donation after determination of death by circulatory criteria; DHOPE, dual hypothermic oxygenated perfusion; ECD, extended criteria donors; ETOH, ethanol; fWIT, functional warm ischemia time; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease; MP, machine perfusion; NMP, normothermic machine perfusion; NRP, normothermic regional perfusion; PRBC, packed red blood cells; SCS, static cold storage; tWIT, total warm ischemia time.

and total preservation time (605 versus 516 min, $P = 0.01$) were longer in the NMP group.

Graft and patient survival were comparable between NMP and DHOPE groups (**Figure S3, SDC,** https://links.lww.com/TP/D311). In general, outcome measures were comparable, with the exception that incidence of severe PRS was significantly higher in the NMP group (45.5 versus 6.1%, $P = 0.01$), whereas recipients of a DHOPE-treated graft had higher CCI scores, with the difference being significant at 12-mo follow-up (34.8 versus 20.9, $P = 0.03$).

All cases of severe PRS (n = 5) after NRP + NMP were observed in recipients of grafts treated with the LiverAssist device. To investigate whether NMP parameters were associated with severe PRS, we analyzed median values of perfusate oxygen tension obtained from blood gas analyses performed during NMP. Median oxygen tension was 244 (242–247) mm Hg in patients developing severe PRS versus 107 (92–214) mm Hg in those who did not, although

this difference did not achieve statistical significance ($P = 0.14$).

DISCUSSION

The results of our study confirm that, by synergistically applying advanced perfusion techniques, favorable early and long-term outcomes can be achieved with different donor types, including DCD donors characterized by prolonged WIT. In our study, outcomes of DCD grafts procured using NRP followed by ex situ MP were comparable to those of 2 matched groups of standard DBD livers preserved by SCS and ECD-DBD livers treated by end-ischemic DHOPE, which is standard treatment for ECD-DBD livers at our center.[16,32] Besides the fact that parameters of graft function and postoperative outcomes were similar, graft and patient survival were excellent across all study groups. Furthermore, all considered outcome measures, including incidence of IC, were within benchmark values in DCD
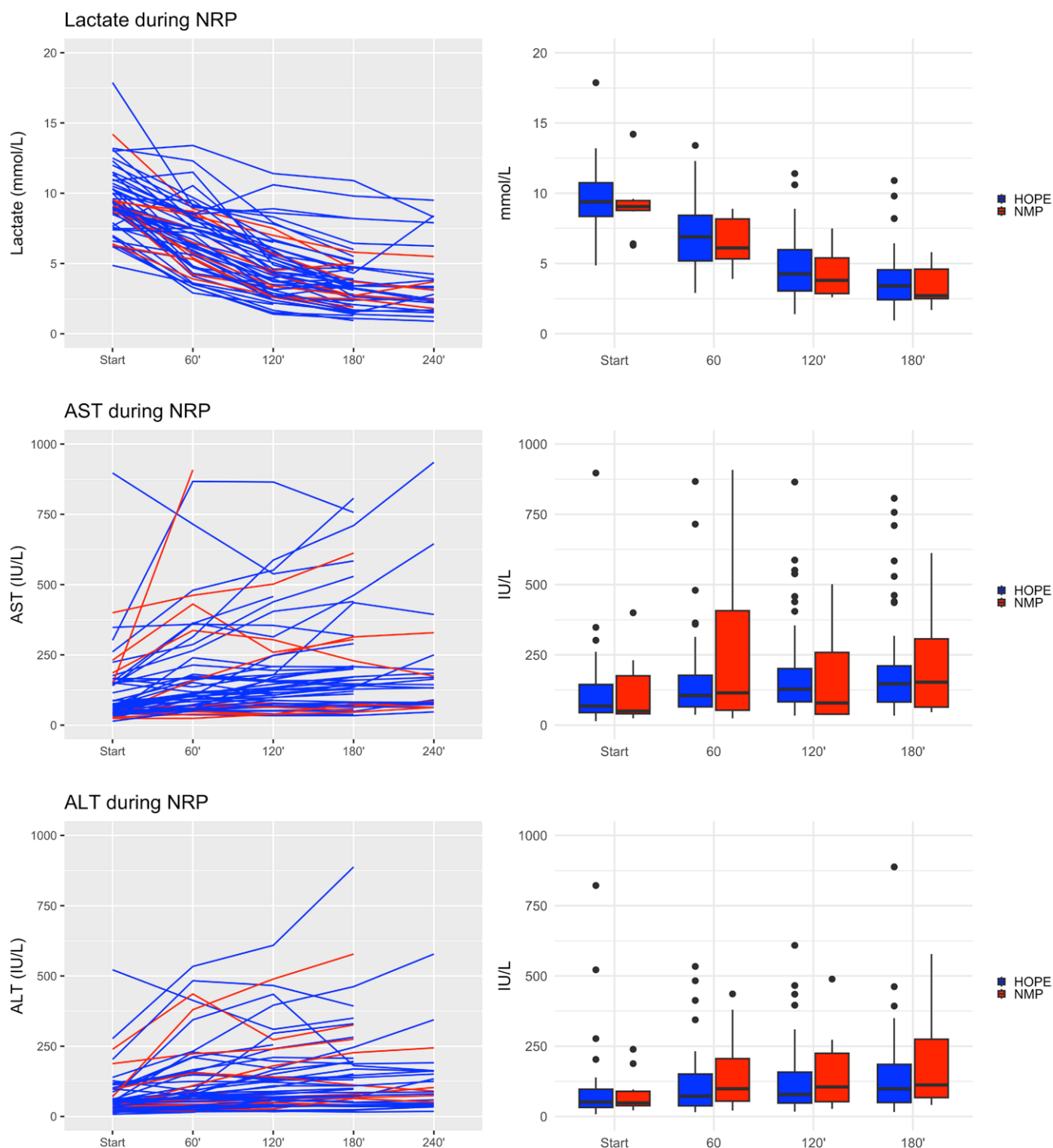
FIGURE 3. Individual (left column) and aggregate (right column) trends of lactate, AST and ALT during NRP in the DCD group by ex situ MP technique used after NRP. ALT, alanine aminotransferase; AST, aspartate aminotransferase; DHOPE, dual hypothermic oxygenated machine perfusion; NMP, normothermic machine perfusion; NRP, normothermic regional perfusion.

and DBD LT.[5,38] The relatively high incidence of IC in the DBD-SCS group was possibly linked to median donor age older than 60 y in this group. Overall, our findings support the use of DHOPE in ECD-DBD and of sequential NRP and MP in controlled DCD LT with prolonged WIT, even outside the Italian setting.

A rapidly growing body of literature shows that the individual use of NRP, HOPE/DHOPE and NMP improves use rate and outcomes in DCD LT,[5-7,14,18,30,40-42] with some groups having proposed the combined use of

different MP techniques (eg, DHOPE-controlled oxygenated rewarming-NMP) to take advantage of the benefits of each technique.[43-45] Recently, Puttappa et al[7] showed that both NRP and NMP were associated with a significant reduction in PRS, AKI, and EAD compared with SCS alone, even when used in high-risk settings (eg, longer WIT, higher recipient serum creatinine, and retransplants), suggesting that combining NRP and NMP techniques could further minimize ischemia-related injury in DCD LT. In the United States, where its use is still not
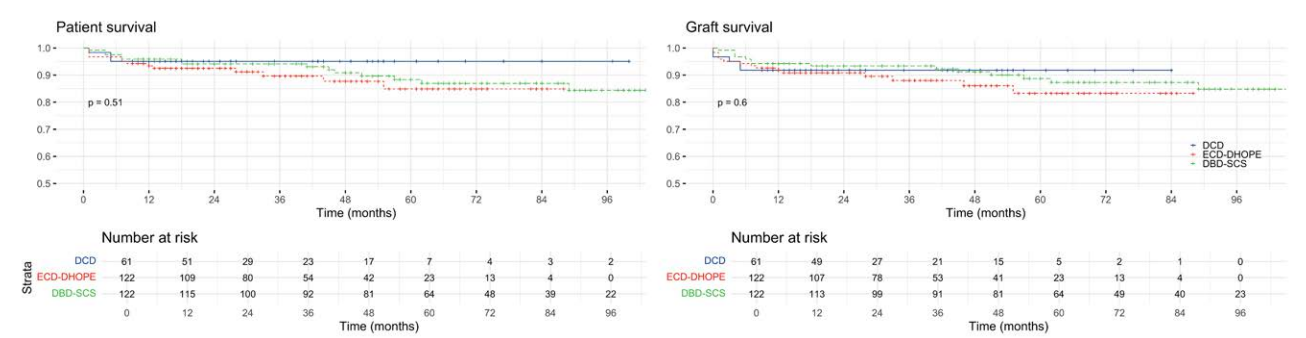
FIGURE 4. Patient and graft survival. DBD, donation after determination of death by neurologic criteria; DCD, donation after determination of death by circulatory criteria; DHOPE, dual hypothermic oxygenated machine perfusion; ECD, extended criteria donor; SCS, static cold storage.

TABLE 2.

Outcomes

	DCD	DBD-SCS	ECD-DHOPE	p1[a]	p2[b]
n	61	122	122		
Severe PRS	8 (13.3)	9 (7.4)	13 (10.7)	0.30	0.78
AST peak (IU/L)	1030 [571, 1883]	1376 [782, 2183]	998 [593, 2164]	0.06	0.55
ALT peak (IU/L)	584 [354, 977]	847 [474, 1563]	632 [337, 1337]	<0.01	0.31
EAD	17 (27.9)	43 (35.2)	45 (36.9)	0.40	0.29
PNF	1 (1.6)[a]	0 (0.0)	0 (0.0)	0.72	0.72
L-GrAFT (score)	−1.9 [−2.2, −1.5]	−2.1 [−2.5, −1.6]	−1.9 [−2.3, −1.3]	0.17	0.75
L-GrAFT (estimate)	13.1 [10.1, 17.9]	11.3 [7.8, 16.8]	13.3 [9.0, 22.0]	0.17	0.75
EASE (score)	−3.9 [−4.5, −3.5]	−3.9 [−4.5, −3.4]	−3.8 [−4.4, −3.0]	0.54	0.10
EASE (estimate)	2.0 [1.1, 3.0]	2.0 [1.1, 3.3]	2.2 [1.3, 4.6]	0.54	0.10
Severe AKI	15 (24.6)	25 (20.5)	40 (32.8)	0.66	0.33
New-onset RRT	2 (3.3)[a]	1 (0.8)	10 (8.2)[b]	0.54	0.34
Early rejection	4 (6.6)	11 (9.0)	7 (5.8)	0.78	1.00
Grade ≥ 3 comp.	10 (17.2)[a]	16 (13.1)	16 (13.1)	0.61	0.61
ICU stay	3.0 [2.0, 4.0][a]	3.0 [2.0, 4.0]	3.0 [2.0, 5.0][b]	0.50	0.70
Hospital stay	10.0 [8.0, 16.5][a]	10.0 [8.0, 16.0]	10.0 [8.0, 16.8][b]	0.73	0.80
CCI at discharge	12.2 [0.0, 24.2][a]	20.9 [8.7, 24.2]	20.9 [8.7, 24.2][b]	0.24	0.37
CCI at 3 mo	22.6 [8.7, 41.8][a]	22.6 [12.2, 39.7]	20.9 [8.7, 36.2][b]	0.71	0.55
CCI at 6 mo	29.6 [8.7, 47.7][a]	29.6 [20.9, 44.9]	22.6 [20.9, 41.5][b]	0.58	0.58
CCI at 1 y	30.8 [12.2, 51.7][a]	30.8 [20.9, 52.1]	30.8 [20.9, 51.0][b]	0.68	0.86
Early mortality	1 (1.6)[a]	0 (0.0)	1 (0.8)[b]	0.72	1.00
EAF	1 (1.7)[a]	1 (0.8)	6 (4.9)[b]	1.00	0.51
Biliary comp. (all)	11 (18.0)	22 (18.0)	17 (13.9)[b]	1.00	0.61
Fistula	2 (3.3)[a]	1 (0.8)	1 (0.8)	0.54	0.54
Stricture	7 (11.5)[a]	15 (12.3)	13 (10.7)	1.00	1.00
IC	2 (3.3)[a]	6 (4.9)	4 (3.3)	0.90	1.00
Biliary-related graft loss	2 (3.3)	2 (1.6)	0 (0.0)	0.86	0.21
Biliary-related mortality	1 (1.6)	1 (0.8)	0 (0.0)	1.00	0.72

Data are presented as counts (percentage) or median (interquartile range) as appropriate. p1: DCD versus DBD-SCS; p2: DCD versus ECD-DHOPE.
[a]Variables within benchmark cutoffs proposed by Schlegel et al.[5]
[a]Variables within or without benchmark cutoffs proposed by Muller et al.[38]
AKI, acute kidney injury; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCI, comprehensive complication index; DBD, donation after determination of death by neurologic criteria; DCD, donation after determination of death by circulatory criteria; DHOPE, dual hypothermic oxygenated perfusion; EAF, early allograft failure; EAD, early allograft dysfunction; EASE, early allograft failure simplified estimation; ECD, extended criteria donors; IC, ischemic cholangiopathy; ICU, intensive care unit; L-GrAFT, liver graft assessment following transplantation; PNF, primary non-function; PRS, postreperfusion syndrome; RRT, renal replacement therapy; SCS, static cold storage.

widespread, NRP has shown the potential to improve liver and kidney transplantation outcomes from DCD donors and reduce waitlist mortality, despite having been used in donors with worse characteristics (eg, advanced age, high kidney donor profile index).[46] Croome et al[47] showed lower rates of IC and improved graft survival with NRP alone or NRP+NMP as compared with SCS when using liver grafts from DCD donors. In their experience, the use of sequential NRP + NMP was considered in cases with prolonged travel distances, complicated recipients, or when it was felt there was a need for additional liver recovery in "marginal" cases.

It is worth noticing that, although WIT >30 min are rather the norm in our setting, these are rarely reported in the international literature. Furthermore, a frequently overlooked issue is represented by pulseless electric activity preceding the cessation of electric activity in death declaration. As Italian law mandates a 20-min flat ECG for death declaration, a variable period (usually 5–10 min) of pulseless electric activity precedes the 20-min no-touch time, significantly prolonging the time during which oxygen delivery is actually nihil. This strengthens the relevance of our findings that, along with those of previous Italian series,[21,23-25,48,49] support a broader application of NRP + ex situ MP in DCD donors with prolonged WIT.

Notably, our study was not designed to compare results of NRP alone versus NRP + ex situ MP. In most European series,[6,9,10,50] use of NRP alone has led to an improvement in DCD LT outcomes, so the benefit of additional ex situ MP is so far not supported by strong evidence. According to a recent survey by De Carlis et al,[20] the majority of Italian centers use ex situ MP, mostly HOPE or DHOPE, after NRP. This practice probably reflects the perceived difficulties of the Italian setting and the attempt at maximizing recipient outcomes. HOPE has been the preferred technique at the majority of centers, including our own, given its lower costs, simplicity of use and also because of the fact that liver viability is assessed during NRP, making further viability assessment unnecessary in most cases. Although reported outcomes have been so far very encouraging,[21,23] further studies comparing NRP versus NRP + MP are necessary to ascertain whether the increased costs of ex situ MP are justified.

Although our results are encouraging, the only episode of PNF in our series prompts a reflection. This was a liver that, despite having been exposed to an exceptionally prolonged period of WIT, fulfilled all viability criteria during NRP and was therefore accepted for LT using end-ischemic DHOPE. The observed poor outcome highlights the necessity to refine viability assessment protocols, both during NRP and ex situ MP. In retrospect, this liver would have benefited of additional viability testing during ex situ MP. Although NMP is most frequently employed nowadays to this aim,[51,52] viability biomarkers should arguably be available during any MP technique. The availability of one or more reliable biomarkers during HOPE/DHOPE would allow to select more accurately those livers that are not suitable for direct transplantation and require additional assessment, improving the safety of the procedure. Perfusate flavin mononucleotide, a noncovalently bound cofactor of mitochondrial complex I, has shown promising results as a viability biomarker during HOPE/DHOPE,[53] although its use is still not widely implemented and decisional cutoffs in grafts treated by sequential NRP + HOPE/DHOPE have not been established. Overall, this case highlights the need for further viability assessment when NRP parameters are not conclusive and especially when serious adverse events occur during NRP.

In our series, part of the grafts (n = 11) was treated with end-ischemic NMP before implantation. NMP was not used as a viability assessment tool but rather for logistical reasons or in the setting of a multicenter study comparing DHOPE versus NMP, and the present study was not designed to allow a meaningful comparison between these 2 techniques. Notwithstanding these limitations, NMP use was associated with a higher incidence of severe PRS (45.5% versus 6.1%), which did not result in poorer clinical outcomes, with 12-mo CCI in the NMP group being significantly lower (20.9 versus 34.8) than in the DHOPE group. This last finding should be interpreted cautiously, given the different numerosity between the 2 groups and the occurrence of severe clinical complications not related to preservation injury (eg, 2 graft losses because of late biliary fistulas) in the DHOPE group. Despite the small sample size, the finding of an increased rate of severe PRS after NMP is questioning. NMP was performed using 2 different MP devices and perfusate oxygen tensions tended to be higher in livers developing severe PRS. Watson et al[54] observed that high oxygen tensions (~600 mm Hg) during NMP were associated with PRS and protracted vasoplegia. Although in our series oxygen tensions in livers developing severe PRS were lower (~240 mm Hg), perfusate hyperoxia could have possibly played a role. Alternatively, metabolic alterations occurring during 2 sequential warm reperfusions (NRP and NMP) could have predisposed to severe PRS. Indeed, levels of inflammatory cytokines and biomarkers progressively increase during both NRP and NMP[55,56] and different metabolic profiles have been linked to graft dysfunction after LT.[55-57] It should be noted, however, that despite an increased rate of severe PRS, clinical outcomes of NMP livers were comparable to those of DHOPE group. Overall, our findings suggest that NMP might not be of additional value when graft assessment during NRP is favorable, all the more that prolonging preservation time to facilitate transplant logistics has been demonstrated to be feasible, at least to some extent, also with DHOPE.[58-60] In contrast, NMP should probably be the preferred technique when further viability assessment is needed after NRP and flavin mononucleotide or other biomarkers allowing viability testing during cold perfusion are not available.

Our study has several limitations. First, it is a retrospective, single-center analysis, which may reduce the generalizability of the findings to other centers with different practices and patient populations. Despite the design our study was aimed selecting 3 comparable patient cohorts, some significant differences between study groups persisted, notably higher donor age in the ECD-DHOPE group and shorter cold ischemia time in the DCD group. Additionally, propensity score matching cannot rule out residual selection bias and confounding. Finally, the number of grafts treated by NMP in the DCD group was low (n = 11), and the study was not aimed at comparing DHOPE and NMP after NRP in DCD LT.

In conclusion, our findings suggest that a flexible and tailored application of different advanced perfusion techniques allows achieving excellent results in both DCD and ECD-DBD LT, which are comparable to those obtained with standard DBD donors and are in line with benchmark values. In DCD grafts, both HOPE and NMP appear to be viable strategies following NRP, offering similar postoperative outcomes and thus providing flexibility in graft preservation approaches based on clinical and logistical considerations. Further studies are necessary to identify reliable biomarkers and decisional thresholds during NRP and ex situ MP, and investigate the respective benefits of HOPE/DHOPE and NMP after NRP in DCD LT.

REFERENCES

1. Attia M, Silva MA, Mirza DF. The marginal liver donor—an update. *Transpl Int*. 2008;21:713–724.

2. Hessheimer AJ, Hartog H, Marcon F, et al. Deceased donor liver utilisation and assessment: consensus guidelines from the European Liver and Intestine Transplant Association. *J Hepatol*. 2025;82:1089–1109.

3. Hessheimer AJ, Cardenas A, Garcia-Valdecasas JC, et al. Can we prevent ischemic-type biliary lesions in donation after circulatory determination of death liver transplantation? *Liver Transpl*. 2016;22:1025–1033.

4. Ziogas IA, Kakos CD, Esagian SM, et al. Liver transplant after donation from controlled circulatory death versus brain death: a UNOS database analysis and publication bias adjusted meta-analysis. *Clin Transplant*. 2022;36:e14521.

5. Schlegel A, van Reeven M, Croome K, et al; DCD Collaborator Group. A multicentre outcome analysis to define global benchmarks for donation after circulatory death liver transplantation. *J Hepatol*. 2022;76:371–382.

6. Hessheimer AJ, de la Rosa G, Gastaca M, et al. Abdominal normothermic regional perfusion in controlled donation after circulatory determination of death liver transplantation: outcomes and risk factors for graft loss. *Am J Transplant*. 2022;22:1169–1181.

7. Puttappa A, Gaurav R, Kakhandki V, et al. Normothermic regional and ex situ perfusion reduces postreperfusion syndrome in donation after circulatory death liver transplantation: a retrospective comparative study. *Am J Transplant*. 2025;25:1296–1305.

8. van Rijn R, Schurink IJ, de Vries Y, et al; DHOPE-DCD Trial Investigators. Hypothermic machine perfusion in liver transplantation—a randomized trial. *N Engl J Med*. 2021;384:1391–1401.

9. Savier E, Lim C, Rayar M, et al. Favorable outcomes of liver transplantation from controlled circulatory death donors using normothermic regional perfusion compared to brain death donors. *Transplantation*. 2020;104:1943–1951.

10. Ruiz P, Valdivieso A, Palomares I, et al. Similar results in liver transplantation from controlled donation after circulatory death donors with normothermic regional perfusion and donation after brain death donors: a case-matched single-center study. *Liver Transpl*. 2021;27:1747–1757.

11. Blok JJ, Detry O, Putter H, et al; Eurotransplant Liver Intestine Advisory Committee. Longterm results of liver transplantation from donation after circulatory death. *Liver Transpl*. 2016;22:1107–1114.

12. Blondeel J, van Leeuwen OB, Schurink IJ, et al. Dynamic preservation of donation after circulatory death liver grafts from donors aged 60 y and older. *Transplantation*. 2024;109:844–852.

13. Czigany Z, Uluk D, Pavicevic S, et al. Improved outcomes after hypothermic oxygenated machine perfusion in liver transplantation—long-term follow-up of a multicenter randomized controlled trial. *Hepatol Commun*. 2024;8:e0376.

14. Markmann JF, Abouljoud MS, Ghobrial RM, et al. Impact of portable normothermic blood-based machine perfusion on outcomes of liver transplant: the OCS Liver PROTECT Randomized Clinical Trial. *JAMA Surg*. 2022;157:189–198.

15. Morawski M, Zhylko A, Rykowski P, et al. Routine end-ischemic hypothermic machine perfusion in liver transplantation from donors after brain death: results of two-year follow-up of a randomized controlled trial. *Int J Surg*. 2024;110:7003–7010.

16. Patrono D, Cussa D, Sciannameo V, et al. Outcome of liver transplantation with grafts from brain-dead donors treated with dual hypothermic oxygenated machine perfusion, with particular reference to elderly donors. *Am J Transplant*. 2022;22:1382–1395.

17. Ravaioli M, Germinario G, Dajti G, et al. Hypothermic oxygenated perfusion in extended criteria donor liver transplantation—a randomized clinical trial. *Am J Transplant*. 2022;22:2401–2408.

18. Schlegel A, Mueller M, Muller X, et al. A multicenter randomized-controlled trial of hypothermic oxygenated perfusion (HOPE) for human liver grafts before transplantation. *J Hepatol*. 2023;78:783–793.

19. Mateo R, Cho Y, Singh G, et al. Risk factors for graft survival after liver transplantation from donation after cardiac death donors: an analysis of OPTN/UNOS data. *Am J Transplant*. 2006;6:791–796.

20. De Carlis R, Lauterio A, Centonze L, et al; Italian DCD Collaborator Group. Current practice of normothermic regional perfusion and machine perfusion in donation after circulatory death liver transplants in Italy. *Updates Surg*. 2022;74:501–510.

21. 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.

22. Fallani G, Stocco A, Siniscalchi A, et al. Beyond the concepts of elder and marginal in DCD liver transplantation: a prospective observational matched-cohort study in the Italian clinical setting. *Transpl Int*. 2023;36:11697.

23. Ghinolfi D, Patrono D, De Carlis R, et al. Liver transplantation with uncontrolled versus controlled DCD donors using normothermic regional perfusion and ex-situ machine perfusion. *Liver Transpl*. 2024;30:46–60.

24. Patrono D, Zanierato M, Vergano M, et al. Normothermic regional perfusion and hypothermic oxygenated machine perfusion for livers donated after controlled circulatory death with prolonged warm ischemia time: a matched comparison with livers from brain-dead donors. *Transpl Int*. 2022;35:10390.

25. Torri F, Balzano E, Melandro F, et al; DCDNet Study Group. Sequential normothermic regional perfusion and end-ischemic ex situ machine perfusion allow the safe use of very old dcd donors in liver transplantation. *Transplantation*. 2024;108:1394–1402.

26. Abbas SH, Ceresa CDL, Pollok JM. Steatotic donor transplant livers: preservation strategies to mitigate against ischaemia-reperfusion injury. *Int J Mol Sci* . 2024;25:4648.

27. Patrono D, De Carlis R, Gambella A, et al. Viability assessment and transplantation of fatty liver grafts using end-ischemic normothermic machine perfusion. *Liver Transpl*. 2022;29:508–520.

28. Neil DAH, Minervini M, Smith ML, et al. Banff consensus recommendations for steatosis assessment in donor livers. *Hepatology*. 2021;75:1014–1025.

29. Ghinolfi D, Rreka E, De Tata V, et al. Pilot, open, randomized, prospective trial for normothermic machine perfusion evaluation in liver transplantation from older donors. *Liver Transpl*. 2019;25:436–449.

30. Nasralla D, Coussios CC, Mergental H, et al; Consortium for Organ Preservation in Europe. A randomized trial of normothermic preservation in liver transplantation. *Nature*. 2018;557:50–56.

31. Cillo U, Burra P, Mazzaferro V, et al; I-BELT (Italian Board of Experts in the Field of Liver Transplantation). A multistep, consensus-based approach to organ allocation in liver transplantation: toward a "blended principle model". *Am J Transplant*. 2015;15:2552–2561.

32. Patrono D, Surra A, Catalano G, et al. Hypothermic oxygenated machine perfusion of liver grafts from brain-dead donors. *Sci Rep*. 2019;9:9337.

33. 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.

34. Agopian VG, Harlander-Locke MP, Markovic D, et al. Evaluation of early allograft function using the liver graft assessment following transplantation risk score model. *JAMA Surg*. 2018;153:436–444.

35. Avolio AW, Franco A, Schlegel A, et al. Development and validation of a comprehensive model to estimate early allograft failure among patients requiring early liver retransplant. *JAMA Surg*. 2020;155:e204095.

36. Slankamenac K, Graf R, Barkun J, et al. The comprehensive complication index: a novel continuous scale to measure surgical morbidity. *Ann Surg*. 2013;258:1–7.

37. Esser H, de Jong IEM, Roos FM, et al. Consensus classification of biliary complications after liver transplantation: guidelines from the BileducTx meeting. *Br J Surg*. 2025;112:znae321.

38. Muller X, Marcon F, Sapisochin G, et al. Defining benchmarks in liver transplantation: a multicenter outcome analysis determining best achievable results. *Ann Surg*. 2018;267:419–425.

39. 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.

40. Oniscu GC, Mehew J, Butler AJ, et al. Improved organ utilization and better transplant outcomes with in situ normothermic regional perfusion in controlled donation after circulatory death. *Transplantation*. 2023;107:438–448.

41. 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.

42. Watson CJE, Gaurav R, Butler AJ. Current techniques and indications for machine perfusion and regional perfusion in deceased donor liver transplantation. *J Clin Exp Hepatol*. 2024;14:101309.

43. Blondeel J, van Leeuwen OB, Schurink IJ, et al. Dynamic preservation of donation after circulatory death liver grafts from donors aged 60 y and older. *Transplantation*. 2025;109:844–852.

44. Liu Q, Del Prete L, Ali K, et al. Sequential hypothermic and normothermic perfusion preservation and transplantation of expanded criteria donor livers. *Surgery*. 2023;173:846–854.

45. van Leeuwen OB, Bodewes SB, Lantinga VA, et al. Sequential hypothermic and normothermic machine perfusion enables safe transplantation of high-risk donor livers. *Am J Transplant*. 2022;22:1658–1670.

46. Sellers MT, Grandas J, Warhoover MT, et al. Normothermic regional perfusion performed by a United States organ procurement organization for nonthoracic organ donors. *Am J Transplant*. 2025;25:1677–1684.

47. Croome KP, Subramanian V, Mathur AK, et al. Outcomes of DCD liver transplant using sequential normothermic regional perfusion and normothermic machine perfusion or NRP alone versus static cold storage. *Transplantation*. 2025;109:1184–1190.

48. Dondossola D, Ravaioli M, Lonati C, et al. The role of ex situ hypothermic oxygenated machine perfusion and cold preservation time in extended criteria donation after circulatory death and donation after brain death. *Liver Transpl*. 2021;27:1130–1143.

49. Ghinolfi D, Dondossola D, Rreka E, et al. Sequential use of normothermic regional and ex situ machine perfusion in donation after circulatory death liver transplant. *Liver Transpl*. 2021;27:385–402.

50. 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.

51. Panconesi R, Flores Carvalho M, Mueller M, et al. Viability assessment in liver transplantation—what is the impact of dynamic organ preservation? *Biomedicines*. 2021;9:161.

52. Patrono D, Lonati C, Romagnoli R. Viability testing during liver preservation. *Curr Opin Organ Transplant*. 2022;27:454–465.

53. Eden J, Thorne AM, Bodewes SB, et al. Assessment of liver graft quality during hypothermic oxygenated perfusion: the first international validation study. *J Hepatol*. 2025;82:523–534.

54. Watson CJE, Kosmoliaptsis V, Randle LV, et al. Normothermic perfusion in the assessment and preservation of declined livers before transplantation: hyperoxia and vasoplegia-important lessons from the first 12 cases. *Transplantation*. 2017;101:1084–1098.

55. Basta G, Babboni S, Pezzati D, et al. Perfusate liver arginase 1 levels after end-ischemic machine perfusion are associated with early allograft dysfunction. *Biomedicines*. 2025;13:244.

56. Pezzati D, Torri F, Franzini M, et al. Association of perfusate cytokine concentrations during liver graft ex situ normothermic perfusion to donor type and postoperative outcomes. *Liver Transpl*. 2025;31:877–889.

57. Guo Z, Zhan L, Gao N, et al. Metabolomics differences of the donor livers between in situ and ex situ conditions during ischemia-free liver transplantation. *Transplantation*. 2023;107:e139–e151.

58. Bruggenwirth IMA, Lantinga VA, Lascaris B, et al; DHOPE-PRO Trial Investigators. Prolonged hypothermic machine perfusion enables daytime liver transplantation—an IDEAL stage 2 prospective clinical trial. *EClin Med*. 2024;68:102411.

59. Bruggenwirth IMA, Mueller M, Lantinga VA, et al. Prolonged preservation by hypothermic machine perfusion facilitates logistics in liver transplantation: a European observational cohort study. *Am J Transplant*. 2022;22:1842–1851.

60. De Carlis R, Lauterio A, Schlegel A, et al; Italian HOPE Collaborator Group. Are there any benefits of prolonged hypothermic oxygenated perfusion?: Results from a national retrospective study. *Liver Transpl*. 2024;31:70–84.