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

Riccardo De Carlis, MD,[1] Andrea Schlegel, MD,[2] Samuele Frassoni, MSc,[3] Tiziana Olivieri, MD,[4] Matteo Ravaioli, PhD,[5] Stefania Camagni, MD,[6] Damiano Patrono, PhD,[7] Domenico Bassi, MD,[8] Duilio Pagano, PhD,[9] Stefano Di Sandro, PhD,[4] Andrea Lauterio, MD,[1] Vincenzo Bagnardi, PhD,[3] Salvatore Gruttadauria, MD,[9] Umberto Cillo, MD,[8] Renato Romagnoli, MD,[7] Michele Colledan, MD,[6] Matteo Cescon, MD,[5] Fabrizio Di Benedetto, MD,[4] Paolo Muiesan, MD,[2,10] and Luciano De Carlis, MD[1,11]

Background. Donation after circulatory death (DCD) in Italy, given its 20-min stand-off period, provides a unique bench test for normothermic regional perfusion (NRP) and dual hypothermic oxygenated machine perfusion (D-HOPE). **Methods.** We coordinated a multicenter retrospective Italian cohort study with 44 controlled DCD donors, who underwent NRP, to present transplant characteristics and results. To rank our results according to the high donor risk, we matched and compared a subgroup of 37 controlled DCD livers, preserved with NRP and D-HOPE, with static-preserved controlled DCD transplants from an established European program. **Results.** In the Italian cohort, D-HOPE was used in 84% of cases, and the primary nonfunction rate was 5%. Compared with the matched comparator group, the NRP + D-HOPE group showed a lower incidence of moderate and severe acute kidney injury (stage 2: 8% versus 27% and stage 3: 3% versus 27%; $P = 0.001$). Ischemic cholangiopathy remained low (2-y proportion free: 97% versus 92%; $P = 0.317$), despite the high-risk profile resulting from the longer donor warm ischemia in Italy (40 versus 18 min; $P < 0.001$). **Conclusions.** These data suggest that NRP and D-HOPE yield good results in DCD livers with prolonged warm ischemia.

(*Transplantation* 2021;105: 2385–2396).

INTRODUCTION

Liver transplantation from donation after circulatory death (DCD) donors is an effective means to overcome the donor shortage. However, DCD livers are burdened with a high rate of complications due to the donor warm ischemia time.[1,2] By combining the advantages of organ reconditioning, improved transplant logistics, and viability testing, normothermic regional perfusion (NRP) and machine perfusion (MP) have shown the potential to improve outcomes and reduce the complication rate after DCD liver transplantation, though further research is needed in this field.[3-8]

Received 4 June 2020. Revision received 12 November 2020.

Accepted 13 November 2020.

[1] Department of General Surgery and Transplantation, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy.

[2] Liver Unit, Queen Elizabeth University Hospital Birmingham, Birmingham, United Kingdom.

[3] Department of Statistics and Quantitative Methods, University of Milan-Bicocca, Milan, Italy.

[4] Hepato-Pancreato-Biliary Surgery and Liver Transplantation Unit, University of Modena and Reggio Emilia, Modena, Italy.

[5] UO Chirurgia Generale e dei Trapianti, AOU Sant'Orsola-Malpighi, Alma Mater Studiorum Università di Bologna, Bologna, Italy.

[6] Division of Liver Transplantation, AO Papa Giovanni XXIII, Bergamo, Italy.

[7] General Surgery 2U, Liver Transplant Center, AOU Città della Salute e della Scienza di Torino, University of Turin, Turin, Italy.

[8] Department of Surgery, Oncology and Gastroenterology, Hepatobiliary Surgery and Liver Transplant Unit, Padua University, Padua, Italy.

[9] Abdominal Surgery and Organ Transplantation Unit, Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IRCCS—ISMETT), Palermo, Italy.

[10] Hepatobiliary Unit, Careggi University Hospital, University of Florence, Florence, Italy.

[11] Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy.

The authors declare no funding or conflicts of interest.

R.D.C. and A.S. participated in research design, data analysis, and wrote the article. S.F. and V.B. participated in research design and data analysis. T.O., M.R., S.C., D.Pat., D.B., D.Pag., S.D.S., A.L., S.G., U.C., R.R., M.Co., M.Ce., F.D.B., P.M., and L.D.C. participated in the performance of the research and contributed critically to the intellectual content of the article.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantjournal.com).

Correspondence: Riccardo De Carlis, MD, Department of General Surgery and Transplantation, ASST Grande Ospedale Metropolitano Niguarda, Piazza Ospedale Maggiore 3, Milano 20162, Italy. (riccardo.decarlis@ospedaleniguarda.it).

ISSN: 0041-1337/21/10511-2385

DOI: 10.1097/TP.0000000000003595

<zdoi;10.1097/TP.0000000000003595>

In Italy, circulatory death is legally declared after a stand-off period of 20 min, confirmed by a flat ECG. Based on this legal constraint, the first DCD liver transplant program was launched only in 2015.[9] Unlike in most countries around the world, all DCD livers in Italy are procured with femoral vessels cannulation and NRP in the donor.[10] In the context of the good results from various clinical studies, the majority of DCD livers procured in Italy undergo end-ischemic dual hypothermic oxygenated perfusion (D-HOPE) following NRP and cold storage before implantation, with the aim to mitigate ischemia-reperfusion injury and improve liver function.[3,6] The encouraging results of the first Italian series have promoted the wider use of this protocol and encouraged other Italian centers to implement a DCD liver transplant program despite the high overall donor risk.[10-13]

Based on the exceptionally long donor warm ischemia time in Italy, there is a general reluctance to use such DCD livers for transplantation due to the expected high risk of graft failure. The use of in situ or ex situ MP technology may help to improve graft function and assess liver viability to support the decision making whether to utilize such livers or not.

We present here a multicenter Italian cohort study to evaluate outcomes from controlled DCD (cDCD) liver donors, preserved by a combination of NRP, cold ischemia, and D-HOPE. To rank our results in the context of the high donor risk, we match and compare our cDCD cohort with outcomes from another established transplant center in the United Kingdom.

MATERIALS AND METHODS

This is a multicenter retrospective cohort study. Seven Italian transplant centers participated in this study. Each center provided all consecutive DCD liver transplants performed from September 2015 (start of the activity in Italy) to April 2019. Two investigators from each center completed an electronic data collection form provided by the coordinating center. The primary sources for data collection were donors' and recipients' operative reports, organ quality forms, recipients' medical notes, laboratory tests during hospitalization, and posttransplant follow-up visits. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Donor Management

Two possible cDCD scenarios were included in this study: cDCD donors on mechanical ventilation and cDCD donors offered while on extracorporeal membrane oxygenation (ECMO). All cDCD donors on mechanical ventilation (modified Maastricht classification 2013, category III)[14] were patients with devastating brain injury not fulfilling brain death criteria, where the decision to withdraw treatment was independent of the process of organ donation and based exclusively on futility. For analgesia, we followed the protocol proposed by Kompanje et al.[15] Premortem administration of heparin is allowed in Italy; an intravenous bolus of 300 IU/kg was administered as systolic blood pressure dropped below 50 mm Hg.[16] Death was declared after 20 min of flat ECG, according to the Italian guidelines.[17] cDCD donors on ECMO were patients maintained on systemic arteriovenous ECMO to treat, for example, refractory cardiogenic shock. According to the Italian guidelines, such donors were classified as controlled if the ECMO was maintained for several days with subsequent treatment withdrawal.[16]

NRP Management

In Italy, super-rapid recovery is not allowed in DCD donors, and the use of NRP is mandatory in all cases.[16] For the initiation of NRP, the femoral vessels are percutaneously cannulated on one side. Premortem insertion of Seldinger wires, but not complete vessel cannulation, is allowed.[10] An occlusion balloon is inserted through the contralateral femoral artery and inflated at the level of the supraceliac aorta to exclude perfusion of the donor's heart and brain. Proper positioning of the balloon is confirmed by chest x-ray. In cDCD donors on ECMO, systemic perfusion is stopped for death declaration and then restarted and regionalized as NRP. To establish a proper NRP, the circuit is primed with 800–1000 mL of Ringer's acetate solution and pump flow rates are kept at 1.7–3 L/min/m^2 with an arterial Po_2 at 80–90 mm Hg, with an arterial Pco_2 at 35–40 mm Hg, and a perfusion temperature of 37 °C. The maintenance of NRP relied on a membrane oxygenator, heat exchanger, and cardiopulmonary bypass pump—either Rotaflow Console or Cardiohelp System (Maquet, Rastatt, Germany). Alanine transaminase, full arterial gas profile, and lactate are routinely measured every hour during NRP. We aimed at maintaining NRP for 4 h (minimum 1 h) before cold perfusion. Provided that pump flow was stable, we extended perfusion in case of suboptimal trend of parameters or for logistical reasons. A liver biopsy of the left lateral segment is performed before cold flush and examined on a frozen section to assess macrosteatosis and fibrosis, as well as any evidence of extensive necrosis. At the end of NRP, the cold preservation solution (6000–8000 mL Celsior; 4 °C) is flushed directly through the arterial cannula.

Donor and Liver Selection

Potential donors were enrolled in the cDCD program if younger than 75 y and if donor warm ischemia time (DWIT) was ≤60 min. DWIT was defined as the time from oxygen saturation falling below 70% or systolic blood pressure below 50 mm Hg to the start of NRP.[1,18] Donors were excluded if significant infection or neoplastic contraindications to donation were evident. The following criteria were considered as relative contraindications for liver recovery: serum alanine transaminase of >1000 IU/L at the end of NRP, an upward trend in serum lactate during NRP, suboptimal macroscopic aspect (eg, poorly perfused or congested livers), macrosteatosis of >30%, or a fibrosis Ishak score >2.[11]

Allocation

The DCD livers were allocated according to the Italian allocation policy, primarily on a regional basis.[19] Superurgent patients and candidates with a model for end-stage liver disease (MELD) score ≥30 points were excluded. Most centers preferably selected low-risk recipients and prioritized candidates, for example, with hepatocellular carcinoma (HCC).

Machine Perfusion

Six centers regularly used end-ischemic MP in all DCD liver grafts after transport and back-table surgery. Only 1 transplant center reserved MP exclusively for liver grafts presenting with one of the above-mentioned relative contraindications for liver recovery or if a prolonged cold ischemia time (CIT) was expected. For D-HOPE, the livers were connected to an extracorporeal organ perfusion system or Liver Assist device (Organ Assist, Groningen, The Netherlands) and perfused through the portal vein (5 mm Hg, continuous) and the hepatic artery (25 mm Hg, pulsatile) with recirculated, cooled (10 °C), and oxygenated (Po_2 = 60–80 kPa) Belzer MP solution (Bridge to Life, London, United Kingdom). Normothermic MP was used in only 1 case, following the protocol reported by op den Dries.[20]

Study Design

We presented the main characteristics and the transplant results of the cDCD Italian series. In the next step, the cDCD cases with NRP and sequential D-HOPE in the Italian cohort (NRP + D-HOPE group) were matched with an external comparator group, transplanted at the Queen Elizabeth Hospital in Birmingham, United Kingdom. The DCD program represents here a relatively low-risk donor cohort of type III DCD donors, with a 5-min stand-off period, followed by super-rapid donor cannulation, cold flush (UW solution), and recovery with subsequent static cold storage. DCD livers with in situ or ex situ MP were not considered for the matching process.

We performed a 1:1 case-control computer matching analysis without replacement to correct for differences in baseline donor and recipient characteristics in the groups. Based on the overall number of DCD transplantations performed at the center in Birmingham between January 2009 and April 2019 (total n = 347), the matching process involved the correction for potential key confounders as follows: donor age (±1 y), donor body mass index (±0.5 kg/m^2), CIT(±1 h), and recipient age (±1 y). For each NRP + D-HOPE–treated DCD liver in Italy, 1 appropriate untreated DCD liver (simply cold stored) from Birmingham was matched according to previously defined parameters. We accepted a difference of 4–5 MELD points to keep the perfect match in other relevant donor risk parameters, provided that all the recipients in the 2 groups remained low MELD. To limit further biases potentially associated with the shorter follow-up of the Italian series, early and mid-term outcomes (up to 24 mo posttransplant) were considered in the comparison.

Definitions

CIT was defined as the time from cold flush (or end of NRP) to removal of the organ from cold storage solution or the start of ex situ D-HOPE perfusion. The MELD score was calculated as the laboratory MELD without the inclusion of exception points for HCC at the time of liver transplantation.[21] Primary nonfunction (PNF) was defined as graft failure with no identifiable secondary cause resulting in either retransplantation or death within the first week. Acute kidney injury (AKI) was defined according to Kidney Disease: Improving Global Outcomes criteria and classified into 3 stages.[22,23] Patients' morbidity during hospitalization was assessed by the Clavien-Dindo score.[24] The diagnosis of ischemic cholangiopathy (IC) was based on cholangiographic evidence of diffuse intrahepatic, hilar, or extrahepatic biliary strictures in the presence of a patent hepatic artery. Isolated anastomotic strictures were excluded from IC.[1] Patients with T-tube underwent cholangiography on postoperative day 7 and 3 mo posttransplant. Magnetic resonance or endoscopic retrograde cholangiopancreatography was undertaken when clinically indicated by elevated liver enzymes or symptoms of cholestasis. Non-IC biliary complications included bile leaks and anastomotic strictures. Patients who died or required retransplantation within the first week for nonbiliary causes were excluded from the subgroup analyses focusing on IC.

Ethical Approval and Informed Consent

The application of NRP and D-HOPE in DCD donors was established in an ad hoc protocol by the Italian Ministry of Health (National Transplant Center).[16] Local ethical committees review of the protocol deemed that formal approval was not required owing to the retrospective, observational, and anonymous nature of this study. The possibility of organ donation was discussed with the donor families, and a signed authorization was obtained before the procedure. Liver transplant candidates were informed of the possibility to receive a DCD liver and signed a consent form at the time of listing. This consent was confirmed by the recipient at the time of organ offer.[25]

Statistical Analysis

Continuous data were reported as median and range; categorical data were reported as counts and percentages. Comparisons between the NRP + D-HOPE group and the matched comparator group were performed using the Wilcoxon signed-rank test for continuous variables and the Fisher exact test for categorical variables. Overall survival, graft survival, and proportions free from vascular complications, artery thrombosis, biliary complications, and IC were estimated using the Kaplan-Meier method; the log-rank test was used to assess differences among groups. A $P < 0.05$ was considered statistically significant in all the analyses. All the analyses were performed using the statistical software SAS V.9.4 (SAS Institute, Cary, NC).

RESULTS

A total of 78 DCD livers treated with NRP were evaluated for transplant throughout the study period, 64 of them were transplanted (45 from cDCD, 19 from uncontrolled DCD [uDCD]), and 44 transplants from cDCD were finally included in the analysis (Figure 1). The utilization rate was calculated from initiation of NRP to acceptance for transplantation and was found to be 86.5% and 73.1% for cDCD and uDCD, respectively. The number of overall DCD donors (both controlled and uncontrolled) initially offered for NRP was not available for this study period. No technical failure nor complications were observed during NRP. The use of sequential D-HOPE depended on the local protocol adopted by each center. Normothermic MP was used in 1 cDCD case, which displayed immediate graft function and no complications over a 12-mo follow-up; for its uniqueness, it was excluded from the analysis. The median follow-up of the Italian cohort was 17 mo, and

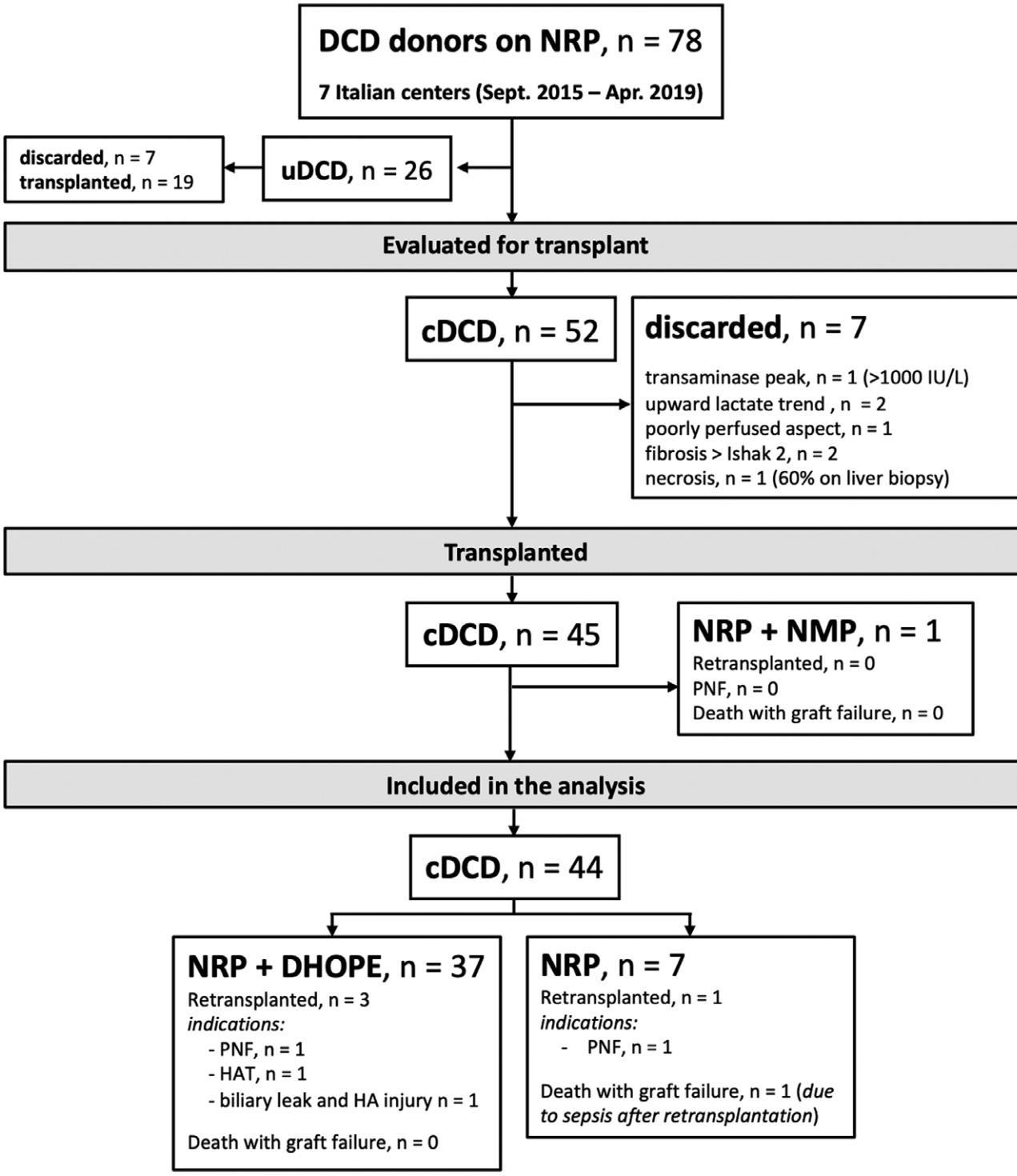

FIGURE 1. Composition of the Italian DCD cohort according to donor type and management. NRP was used in all cases. The utilization rate was 86.5% and 73.1% for controlled and uncontrolled donors, respectively. The use of sequential D-HOPE or normothermic MP depended on the local protocol adopted by each center. No livers were declined during D-HOPE. Overall, 44 controlled liver transplants were included in the analysis. cDCD, controlled donation after circulatory death; DCD, donation after circulatory death; D-HOPE, dual hypothermic oxygenated perfusion; HA, hepatic artery; HAT, hepatic artery thrombosis; MP, machine perfusion; NRP, normothermic regional perfusion; PNF, primary nonfunction; uDCD, uncontrolled donation after circulatory death.

all transplants have a follow-up of at least 1 y. Within the same period, overall, 867 liver transplantations were performed in Birmingham. Following exclusion of all DBD livers, split procedures, combined and domino transplants, and machine perfused grafts, a pool of 181 transplanted DCD livers was used for the computerized match.

Characteristics and Outcomes of the Italian cDCD Cohort

Table 1 provides patient characteristics and early post-transplant outcomes of Italian cases. D-HOPE was used in 84% of cases, and the PNF rate was 5%. In the 2 livers, which developed PNF, pretransplant biopsy showed

TABLE 1.

Patients' characteristics and posttransplant outcomes of the Italian cases

Variable	Level	cDCD (N = 44)
Recipient characteristics		
Age (y), median (min–max)		58 (37–70)
BMI (kg/m^2), median (min–max)		25.2 (18.4–34.5)
Sex, n (%)	Women	9 (20)
	Men	35 (80)
Liver disease, n (%)	Alcoholic cirrhosis	12 (27)
	Hepatitis B	5 (11)
	Hepatitis C	16 (36)
	NAFLD	1 (2)
	Primary biliary cirrhosis	4 (9)
	Primary sclerosing cholangitis	1 (2)
	Retransplant	2 (5)
	Other	3 (7)
HCC, n (%)	No	15 (34)
	Yes	29 (66)
Laboratory MELD score, median (min–max)		9 (6–25)
Life support pretransplant, n (%)	No	44 (100)
	Yes	0 (0)
Previous dialysis, n (%)	No	44 (100)
	Yes	0 (0)
Previous admission to hospital, n (%)	No	43 (98)
	Yes	1 (2)
Ascites, n (%)	No	32 (73)
	Yes	12 (27)
Encephalopathy, n (%)	No	39 (89)
	Yes	5 (11)
Donor characteristics		
Age (y), median (min–max)		59 (34–69)
BMI (kg/m^2), median (min–max)		25.7 (16.2–35.2)
Sex, n (%)	Women	15 (34)
	Men	29 (66)
DWIT[a] (min), median (min–max)		40 (20–80)
Liver biopsy		
Macrosteatosis (%), median (min–max)		1.5 (0–20)
Fibrosis (Ishak score), n (%)	0	25 (58)
	1	11 (26)
	2	7 (16)
	Missing	1
Necrosis, n (%)	Absent	31 (72)
	Focal	10 (23)
	Zonal	2 (5)
	Confluent	0 (0)
	Missing	1
Preservation and recipient operation		
Duration of NRP (min), median (min–max)		250 (53–463)
D-HOPE, n (%)	No	7 (16)
	Yes	37 (84)
Duration of D-HOPE (min), median (min–max)[b]		120 (42–380)
CIT (min), median (min–max)		394 (180–660)
Implantation technique, n (%)	Classic (cava replacement)	6 (14)
	Piggy-back	38 (86)

(*Continued next page*)

TABLE 1. (Continued)

Variable	Level	cDCD (N = 44)
Hospitalization		
ALT peak in first 7 d, median (min–max)		897 (120–6832)
INR on postoperative d 7		1.20 (1.00–10.00)
PNF, n (%)	No	42 (95)
	Yes	2 (5)
AKI, n (%)	No AKI	28 (64)
	AKI stage 1	8 (18)
	AKI stage 2	5 (11)
	AKI stage 3	3 (7)
ICU stay (d), median (min–max)		3 (1–22)
Hospital stay (d), median (min–max)[c]		14 (5–63)
Clavien-Dindo highest grade, n (%)	0	21 (47)
	I-II	17 (39)
	IIIa	1 (2)
	IVa	3 (7)
	IVb	1 (2)
	V	1 (2)
Biliary complications[d]		(N = 41)
Biliary complication type, n (%)	No biliary complications	32 (78)
	Anastomotic stenosis	5 (12)
	Biliary leak at anastomosis	1 (2)
	Biliary leak at T-tube site	2 (5)
	IC	1 (2)
T-tube[e] n (%)	No	12 (29)
	Yes	29 (71)
Cholangiography (M/ERCP), n (%)	No	30 (73)
	Yes	11 (27)
Timing of M/ERCP (mo), median (min–max)		11.6 (6.1–40.9)

[a]Systolic blood pressure <50 mm Hg or oxygen saturation <70% to start of NRP.
[b]Three missing.
[c]One missing.
[d]Patients dead or retransplanted within the first week for nonbiliary causes were excluded from the count (insufficient follow-up).
[e]All patients with T-tube underwent cholangiography on postoperative d 7 and 3 mo posttransplant, before tube removal.
AKI, acute kidney injury; ALT, alanine transaminase; BMI, body mass index; cDCD, controlled donation after circulatory death; CIT, cold ischemia time; D-HOPE, dual hypothermic oxygenated perfusion; DWIT, donor warm ischemia time; HCC, hepatocellular carcinoma; IC, ischemic cholangiopathy; ICU, intensive care unit; INR, international normalized ratio; MELD, model for end-stage liver disease; M/ERCP, magnetic resonance/endoscopic retrograde cholangiopancreatography; NAFLD, nonalcoholic fatty liver disease; NRP, normothermic regional perfusion; PNF, primary nonfunction.

zonal and absence of necrosis, respectively, without evidence of steatosis or fibrosis. Only 1 recipient died from multiorgan failure due to bacterial infection. Two-year graft survival was 91% (Figure 2). The proportion of patients free of any biliary complication at 2 y was 77%, as reflected in Figure 2C. Only 1 recipient developed hilar strictures without peripheral involvement of the liver and was diagnosed with IC 212 d posttransplant. Such features were managed conservatively with endoscopic stenting, and no relisting was required during the overall follow-up of 27 mo. Associations between PNF, AKI, and biliary complications with selected pretransplant variables are shown in Tables S1–S3 (SDC, http://links.lww.com/TP/C90).

Comparison Between cDCD With NRP + D-HOPE and the External, Matched Comparator Group With Standard Cold Storage

NRP with sequential D-HOPE was used in 37 cDCD transplantations in Italy. In Table 2, donor and recipient characteristics and results of this Italian group are compared with the matched external comparator group. The MELD score was slightly lower in the NRP + D-HOPE group (9 versus 13, $P = 0.002$) with a higher frequency of HCC (70% versus 35%; $P = 0.005$).

The median functional DWIT in the Italian NRP + D-HOPE group was 40 versus 18 min in the Birmingham cohort ($P < 0.001$). The Italian cohort achieved, therefore, significantly more points in the UK-DCD Risk Score (65% of cases were allocated to the futile group), while all other donor and recipient risk parameters remained comparable.[26] Despite such lower overall risk in the UK cohort, the NRP + D-HOPE–treated high-risk cohort from Italy achieved a lower incidence of AKI (stage 2: 8% versus 27% and stage 3: 3% versus 27%, $P = 0.001$) and recipients experienced fewer grade IIIa and IVa complications. Additionally, the 2-y graft survival was better in Italy (92% versus 81%; $P = 0.227$), and recipients were found with a lower incidence of ischemic cholangiopathies although the difference was not statistically significant (2-y proportion free from IC: 97% versus 92%; $P = 0.317$) (Figures 3 and 4).

DISCUSSION

NRP is routinely used in a few countries, where the recent literature has highlighted good outcomes in the context of DCD liver transplantation with however very

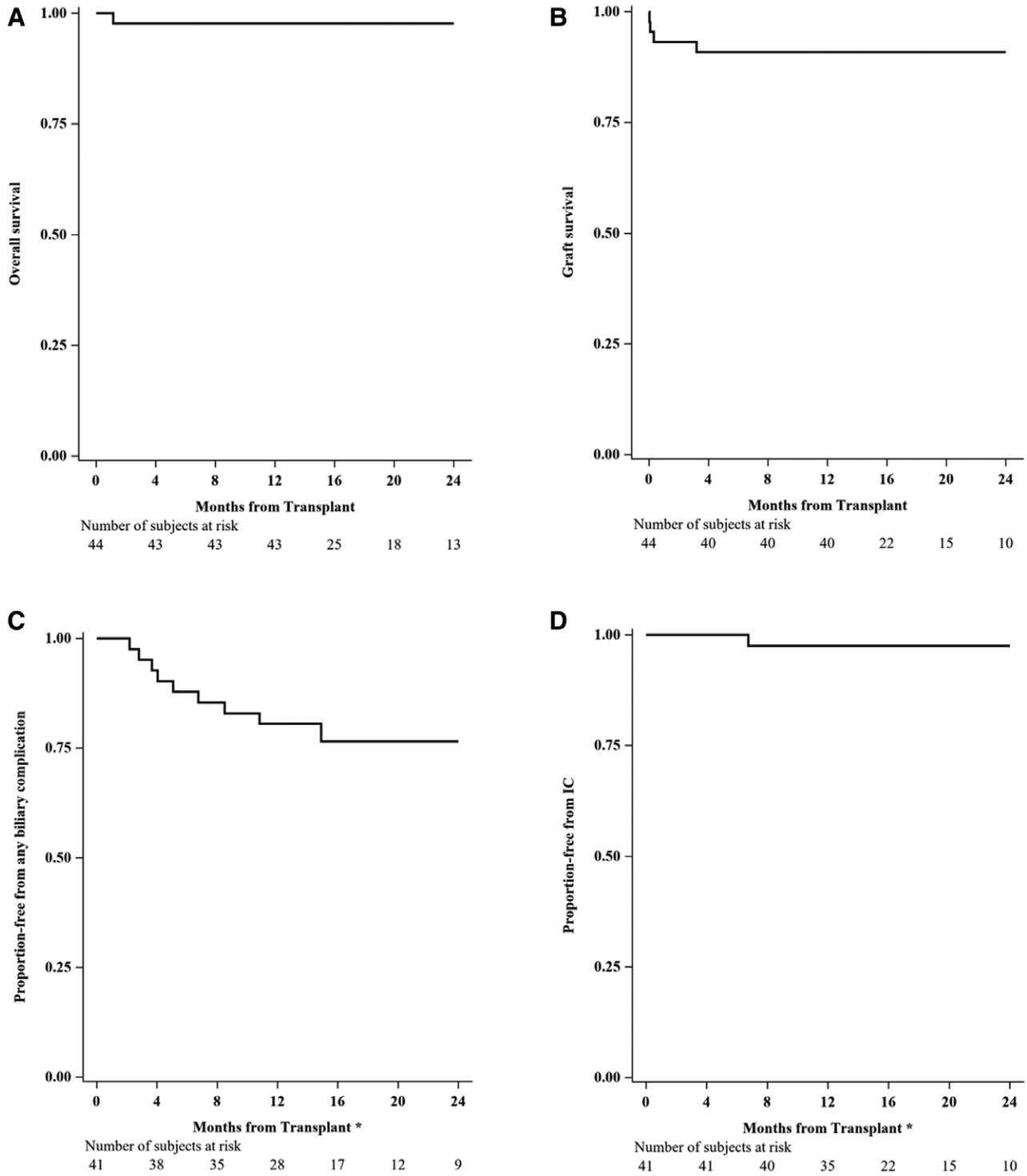

FIGURE 2. Two-y transplant outcomes of the Italian-controlled DCD livers: overall survival (A), graft survival (B), proportion free from any biliary complication* (C), and proportion free from IC* (D). *The observation period started 7 d posttransplant, patients dead or retransplanted within the first week for nonbiliary causes were excluded from the analysis. DCD, donation after circulatory death; IC, ischemic cholangiopathy.

different donor risk profiles.[4,27-30] In the present study, we provide new insight regarding the use of NRP in combination with cold storage and D-HOPE in a high-risk DCD population. Despite the unique prolonged DWIT, we demonstrate here excellent results with the combined protocol of the 2 preservation technologies, NRP and D-HOPE.

In the last few years, we have observed a progressive reduction in the number of uDCD donors in Italy, mainly due to inferior results and logistical challenges of this practice, which was paralleled by an increase in cDCD donors.[31] Given the absence of a cDCD comparator group without NRP in our country, we have compared the controlled transplants, preserved with NRP and D-HOPE, with static-preserved transplants obtained from a large and European DCD program. These data have been used as a comparison also in other international studies

TABLE 2.

Characteristics and posttransplant outcomes of cDCD patients with NRP + D-HOPE and matched comparator group of patients with super-rapid recovery and static cold storage

Variable	Level	cDCD with NRP + D-HOPE (N = 37)	Comparator group (N = 37)	P[a]
Matching variables				
Recipients' age (y), median (min– max)		58 (37–70)	56 (38–66)	0.27
Donors' age (y), median (min–max)		59 (34–69)	55 (36–69)	0.28
Donors' BMI (kg/m^2), median (min–max)		25.7 (16.3–35.2)	24.2 (13.8–38.2)	0.23
CIT (min), median (min–max)		411 (330–660)	390 (240–583)	0.18
Recipient characteristics				
BMI (kg/m^2), median (min–max)		25.0 (18.7–34.5)	25.9 (18.5–48.9)	0.56
Sex, n (%)	Women	8 (22)	11 (30)	0.60
	Men	29 (78)	26 (70)	
Class of disease, n (%)	Alcoholic cirrhosis	12 (32)	10 (27)	0.10
	Autoimmune hepatitis	0 (0)	1 (3)	
	Hepatitis B	3 (8)	1 (3)	
	Hepatitis C	14 (38)	6 (16)	
	NAFLD	1 (3)	1 (3)	
	Primary biliary cirrhosis	3 (8)	7 (19)	
	Primary sclerosing cholangitis	1 (3)	6 (16)	
	Retransplant	0 (0)	0 (0)	
	Other	3 (8)	5 (14)	
HCC, n (%)	No	11 (30)	24 (65)	0.005
	Yes	26 (70)	13 (35)	
Laboratory MELD score, median (min–max)		9 (6–25)	13 (6–19)	0.002
Life support pretransplant, n (%)	No	37 (100)	37 (100)	–
	Yes	0 (0)	0 (0)	
Previous dialysis, n (%)	No	37 (100)	37 (100)	–
	Yes	0 (0)	0 (0)	
Previous admission to hospital, n (%)	No	37 (100)	37 (100)	–
	Yes	0 (0)	0 (0)	
Ascites, n (%)	No	27 (73)	19 (51)	0.093
	Yes	10 (27)	18 (49)	
Encephalopathy, n (%)	No	32 (86)	25 (68)	0.096
	Yes	5 (14)	12 (32)	
Donor characteristics				
Sex, n (%)	Women	14 (38)	19 (51)	0.35
	Men	23 (62)	18 (49)	
DWIT[b] (min), median (min–max)		40 (30–80)	18 (10–44)	<0.001
UK-DCD Risk Score, n (%)	Low risk (0–5 points)	0 (0)	27 (73)	<0.001
	High risk (6–10 points)	13 (35)	6 (16)	
	Futile (>10 points)	24 (65)	4 (11)	
Preservation and recipient operation				
Duration of D-HOPE (min), median (min–max)		120 (42–380)	–	–
Implantation technique, n (%)	Classic (cava replacement)	1 (3)	0 (0)	1.00
	Piggy-back	36 (97)	37 (100)	
Hospitalization				
ALT peak in first 7 d, median (min–max)[c]		831 (120–6441)	1078 (159–3143)	0.35
INR on postoperative d 7[c]		1.25 (0.95–10.00)	1.00 (0.90–2.00)	<0.001
PNF, n (%)	No	36 (97)	36 (97)	1.00
	Yes	1 (3)	1 (3)	
AKI, n (%)	No AKI	27 (73)	13 (35)	0.001
	AKI stage 1	6 (16)	4 (11)	
	AKI stage 2	3 (8)	10 (27)	
	AKI stage 3	1 (3)	10 (27)	
ICU stay (d), median (min–max)[c]		3 (1–22)	3 (1–22)	0.19

(*Continued next page*)

TABLE 2. (Continued)

Variable	Level	cDCD with NRP + D-HOPE (N = 37)	Comparator group (N = 37)	P[a]
Hospital stay (d), median (min–max)[d]		13 (5–63)	9 (5–59)	0.041
Clavien-Dindo highest grade, n (%)	0	18 (49)	9 (24)	0.17
	I-II	15 (41)	16 (43)	
	IIIa	1 (3)	4 (11)	
	IIIb	0 (0)	1 (3)	
	IVa	2 (5)	5 (14)	
	IVb	1 (3)	2 (5)	
	V	0 (0)	0 (0)	
Biliary complications[e]		(N = 35)	(N = 36)	
Biliary complication type, n (%)	No biliary complications	27 (77)	25 (69)	0.58
	Anastomotic stenosis	4 (11)	6 (17)	
	Biliary leak at anastomosis	1 (3)	1 (3)	
	Biliary leak at T-tube site	2 (6)	0 (0)	
	IC	1 (3)	3 (8)	
	Other	0 (0)	1 (3)	

[a]Wilcoxon signed-rank test for continuous variables, Fisher exact test for categorical variables.
[b]Systolic blood pressure <50 mm Hg (or oxygen saturation <70%) to start of NRP or cold flush.
[c]One missing: comparator group.
[d]Two missing: 1 "cDCD with NRP + D-HOPE group" and 1 "comparator group."
[e]Patients dead or retransplanted within the first week for nonbiliary causes were excluded from the count (insufficient follow-up).
AKI, acute kidney injury; ALT, alanine transaminase; BMI, body mass index; cDCD, controlled donation after circulatory death; CIT, cold ischemia time; D-HOPE, dual hypothermic oxygenated perfusion; DWIT, donor warm ischemia time; HCC, hepatocellular carcinoma; IC, ischemic cholangiopathy; ICU, intensive care unit; INR, international normalized ratio; MELD, model for end-stage liver disease; NAFLD, nonalcoholic fatty liver disease; NRP, normothermic regional perfusion; PNF, primary nonfunction; UK-DCD, United Kingdom donation after circulatory death.

and served here as a reference for DCD transplantation in Europe.[32] Despite the potential limitations resulting from a matched analysis between 2 different countries, the donor risk parameters, including DWIT, were defined and measured with the same methods in both cohorts. We considered a difference of 4–5 MELD points clinically neglectable, provided that recipients in both groups presented with a low laboratory MELD score. Other than DWIT, where matching was impossible, the selected risk parameters appeared homogeneous between the groups.[26] Premortem administration of heparin in the Italian cohort may constitute an advantage compared with the United Kingdom.[33] Nevertheless, the higher UK-DCD Risk Score, resulting from the longer DWIT, would have advised

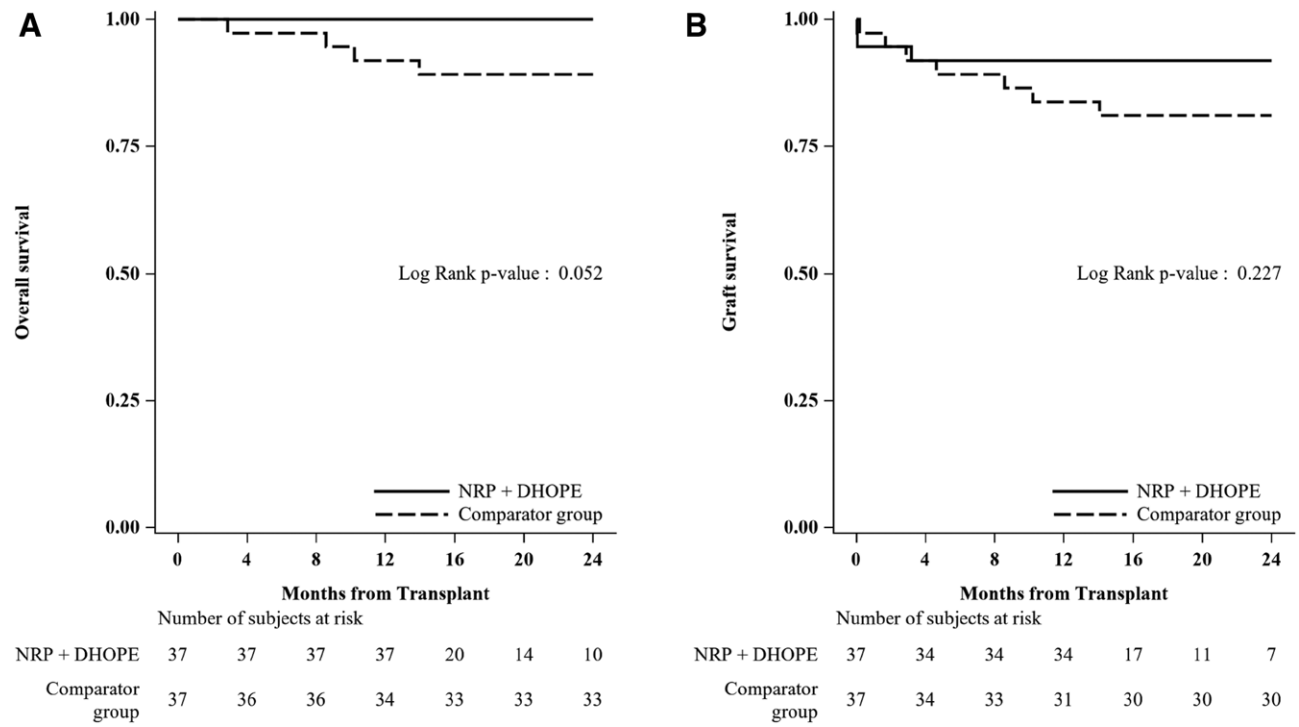

FIGURE 3. Two-y transplant outcome divided by cDCD transplants with NRP + D-HOPE and the matched external comparator group: overall survival (A) and graft survival (B). cDCD, controlled donation after circulatory death; D-HOPE, dual hypothermic oxygenated perfusion; NRP, normothermic regional perfusion.

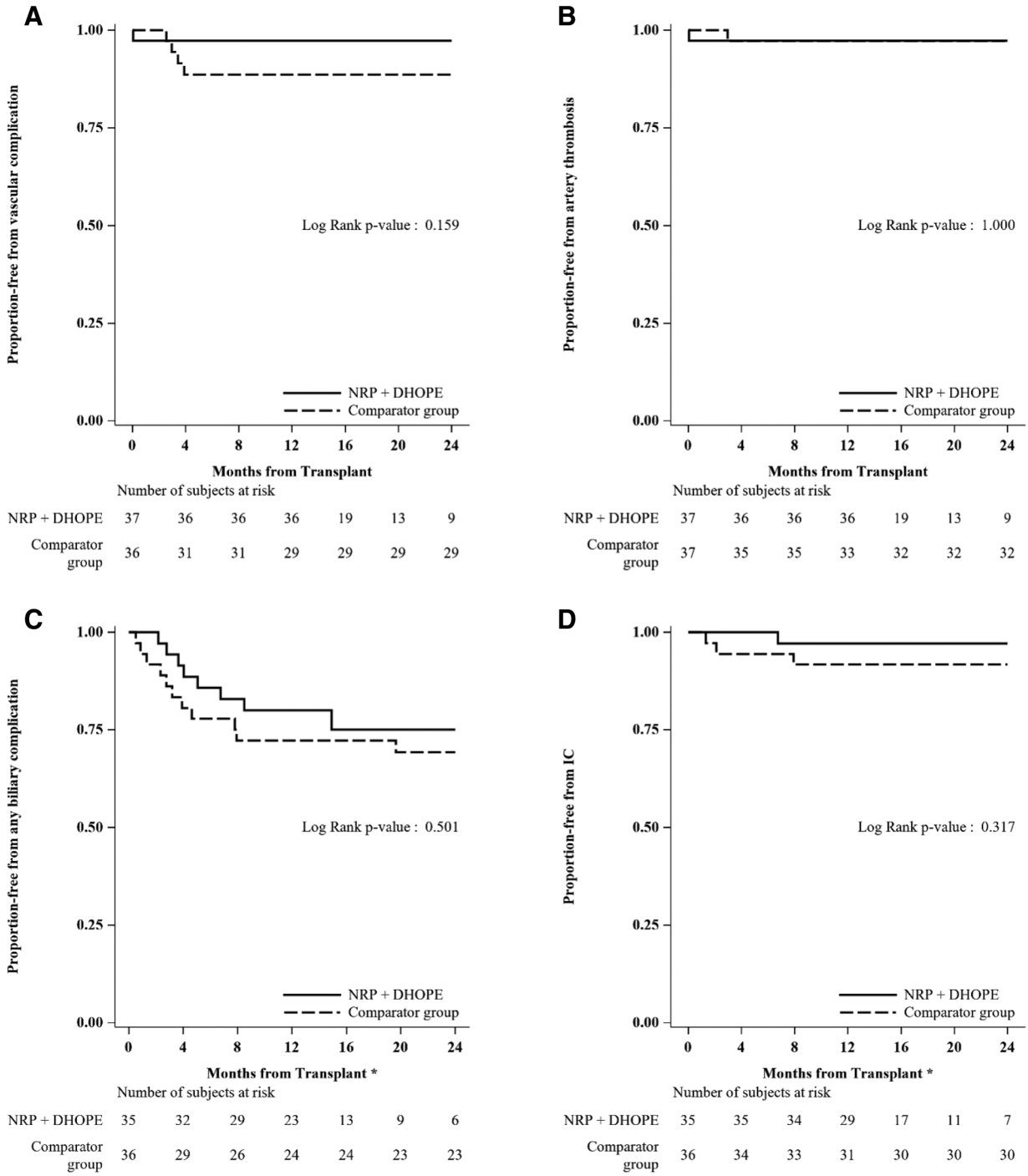

FIGURE 4. Two-y proportion free from complications divided by cDCD transplants with NRP + D-HOPE and the matched external comparator group: vascular complications (A), artery thrombosis (B), any biliary complications* (C), ischemic cholangiopathy* (D). *The observation period started 7 d posttransplant, patients dead or retransplanted within the first week for nonbiliary causes were excluded from the analysis. cDCD, controlled donation after circulatory death; D-HOPE, dual hypothermic oxygenated perfusion; NRP, normothermic regional perfusion.

against transplantation of 65% of the Italian cases when offered in the United Kingdom. Despite such high risk, the NRP + D-HOPE group has achieved similar good results and a lower incidence of posttransplant AKI when compared with the UK comparator group.

A recent national Spanish cohort study has reported superior results in cDCD transplantations using NRP when compared with super-rapid recovery and standard cold storage. However, despite the much lower DWIT of the Spanish cohort (13 min) compared with the Italian one, which has been achieved partly by premortem cannulation, the authors still reported a slightly higher graft loss of 12%.[4] Conversely, a recent UK report has shown a 97.7% 90-d graft survival and no IC with NRP only, but still with a much shorter asystolic period (16 min) compared with Italy.[28]

IC is traditionally considered the Achilles' heel of DCD liver transplantation, leading to higher patient morbidity

and graft loss.[1,2] While prolonged DWIT is a recognized risk factor for IC, several retrospective studies have highlighted the role of NRP or D-HOPE/portal vein HOPE in reducing its incidence.[3,4,28,34] Considering the exceptionally long DWIT, the incidence of IC in the Italian cohort was low, which further supports the role of perfusion technologies. In our cDCD cohort, we identified only 1 case (2.3%) of IC, which constitutes an encouraging result in the context of the available literature.[1,2] Importantly, the occurrence of IC did not impact graft survival, and the recipient, who developed signs of IC maintained fit with good graft function at 27 mo posttransplant.

The combination of NRP and subsequent D-HOPE-treatment provides the advantages of early liver reoxygenation to enable the initial selection of DCD livers with a favorable metabolic situation to achieve successful liver transplantation. This approach may also improve transplant logistics. Although the application of NRP has been found to induce similar pathways as found during ischemic preconditioning with an increase of adenosine levels within the liver, the entire mechanism of protection remains incompletely understood.[35,36] Further analysis of more parameters obtained from donors and perfusates during NRP in DCD donors is needed to fully establish a reliable set of parameters to assess liver viability and increase the utilization rate of DCD livers.

There is evidence that D-HOPE and portal vein HOPE ensure improved and prolonged preservation and protects from reperfusion injury by switching mitochondria into a low-flux electron transfer stage before reperfusion.[3,6,37] These underlying mechanisms could have contributed to the low complication rate observed in our series. The specific contribution of each perfusion approach requires however further investigation because a comparative cohort with the same risk and procurement with NRP and cold ischemia without additional D-HOPE treatment was small and conclusive results are therefore not available. Ongoing and future trials comparing both technologies are required to answer this question.

Despite the high overall donor risk, the utilization rate of the Italian DCD livers on NRP appears higher when compared with most other European series. The majority of reported DCD livers used with NRP ranged between 50% and 60%.[4,38] The Zurich group has reported a 90% utilization rate of HOPE-treated DCD livers classified as extended or futile without previous NRP.[39]

The possibility of predicting graft function based on parameters measured during NRP has not been demonstrated conclusively, as also illustrated in the supplementary data (SDC, http://links.lww.com/TP/C90). A level of transaminases 3 or 4 times higher than normal baseline values during or at the end of NRP are routinely considered as contraindications for liver utilization.[36] Based on such criteria, 55% of the DCD livers in our Italian cohort would have been declined for transplantation. Our results emphasize that such boundaries could be pushed further, and the subsequent D-HOPE treatment has very likely contributed to this higher utilization. The experience of safety of our combined approach has led to a continuation of our DCD program.

In our study, the liver grafts preserved with NRP and subsequent D-HOPE presented a lower incidence of AKI. D-HOPE treatment was associated with a reduction in the incidence of AKI also in the supplementary analysis. Very few other studies have considered the impact of MP on posttransplant renal function, with mixed results. Patrono et al[40] have reported a lower incidence of moderate-to-severe AKI using D-HOPE in grafts from brain-dead donors, while Dutkowski et al[3] have observed no difference between HOPE-treated DCD livers compared with statically preserved controls. The pathogenesis of posttransplant AKI is multifactorial but mostly explained by the release of proinflammatory mediators after reperfusion. The protective effect of D-HOPE toward AKI thus constitutes further evidence that D-HOPE reduces the ischemia-reperfusion injury with subsequent reduced downstream inflammation with less cytokine release and improved early kidney function after liver transplantation.[23,41,42]

The main limitations of this study are its retrospective nature, the absence of randomization, and the relatively low numbers.

In conclusion, DCD liver transplantation is practicable and safe in Italy despite the legal restraint of a 20-min stand-off period. The analysis of this unique population suggests that the use of NRP and sequential D-HOPE has contributed to such excellent results despite the very high-risk profile.

ACKNOWLEDGMENTS

The authors thank Marcella Gambino and Elisa Lodi for their technical support in MP management. They are very grateful to all donors and donor families in both countries and all staff involved in this challenging field of medicine.

REFERENCES

1. 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.
2. Jay CL, Lyuksemburg V, Ladner DP, et al. Ischemic cholangiopathy after controlled donation after cardiac death liver transplantation: a meta-analysis. *Ann Surg.* 2011;253:259–264.
3. 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.
4. 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.
5. Magliocca JF, Magee JC, Rowe SA, et al. Extracorporeal support for organ donation after cardiac death effectively expands the donor pool. *J Trauma.* 2005;58:1095–1101. Discussion 1101.
6. van Rijn R, Karimian N, Matton APM, et al. Dual hypothermic oxygenated machine perfusion in liver transplants donated after circulatory death. *Br J Surg.* 2017;104:907–917.
7. 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.
8. Friend PJ. Strategies in organ preservation—a new golden age. *Transplantation.* 2020;104:1753–1755.
9. De Carlis L, Lauterio A, De Carlis R, et al. Donation after cardiac death liver transplantation after more than 20 minutes of circulatory arrest and normothermic regional perfusion. *Transplantation.* 2016;100:e21–e22.
10. De Carlis L, De Carlis R, Muiesan P. Past, present, and future of donation after circulatory death in Italy. *Updates Surg.* 2019;71:7–9.
11. De Carlis R, Di Sandro S, Lauterio A, et al. Successful donation after cardiac death liver transplants with prolonged warm ischemia time using normothermic regional perfusion. *Liver Transpl.* 2017;23:166–173.
12. 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.

13. 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.
14. 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.
15. Kompanje EJ, van der Hoven B, Bakker J. Anticipation of distress after discontinuation of mechanical ventilation in the ICU at the end of life. *Intensive Care Med.* 2008;34:1593–1599.
16. Italian National Transplant Center. *Position paper—Determination of death based on circulatory criteria. Organ recovery for transplantation from the asystolic donor*. 2016. Available at http://www.salute.gov.it/imgs/C_17_cntPubblicazioni_60_allegato.pdf. Accessed March 26, 2020.
17. Italian Republic. Decree of the Ministry of Health n. 136, 11 April 2008. Gazzetta Ufficiale. 2008. Available at https://www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto/originario?atto.dataPubblicazioneGazzetta=2008-06-12&atto.codiceRedazionale=08A04067&elenco30giorni=false. Accessed March 20, 2020.
18. DeOliveira ML, Jassem W, Valente R, et al. Biliary complications after liver transplantation using grafts from donors after cardiac death: results from a matched control study in a single large volume center. *Ann Surg.* 2011;254:716–722. Discussion 722–723.
19. 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.
20. op den Dries S, Karimian N, Sutton ME, et al. Ex vivo normothermic machine perfusion and viability testing of discarded human donor livers. *Am J Transplant.* 2013;13:1327–1335.
21. Kamath PS, Wiesner RH, Malinchoc M, et al. A model to predict survival in patients with end-stage liver disease. *Hepatology.* 2001;33:464–470.
22. KDIGO AKI Work Group. KDIGO clinical practice guideline for acute kidney injury. *Kidney Int Suppl*. 2012;2:1–138.
23. Kalisvaart M, Schlegel A, Umbro I, et al. The impact of combined warm ischemia time on development of acute kidney injury in donation after circulatory death liver transplantation: stay within the golden hour. *Transplantation.* 2018;102:783–793.
24. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg.* 2004;240:205–213.
25. Mone T, Heldens J, Niemann CU. Deceased organ donor research: the last research frontier? *Liver Transpl.* 2013;19:118–121.
26. 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.
27. Savier E, Dondero F, Vibert E, et al; Donation After Cardiac Death Study Group. First experience of liver transplantation with type 2 donation after cardiac death in France. *Liver Transpl.* 2015;21:631–643.
28. 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.
29. Miñambres E, Suberviola B, Dominguez-Gil B, et al. Improving the outcomes of organs obtained from controlled donation after circulatory death donors using abdominal normothermic regional perfusion. *Am J Transplant.* 2017;17:2165–2172.
30. 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.
31. Italian National Transplant Center. *Annual activity report 2019*. 2020. Available at http://www.trapianti.salute.gov.it/imgs/C_17_cntPubblicazioni_351_allegato.pdf. Accessed March 20, 2020.
32. Dutkowski P, Polak WG, Muiesan P, et al. First comparison of hypothermic oxygenated PErfusion versus static cold storage of human donation after cardiac death liver transplants: an international-matched case analysis. *Ann Surg*. 2015;262:764–770; discussion 770–771.
33. Narvaez JRF, Nie J, Noyes K, et al. Transplant outcomes of donation after circulatory death livers recovered with versus without premortem heparin administration. *Liver Transpl.* 2020;26:247–255.
34. Taner CB, Bulatao IG, Willingham DL, et al. Events in procurement as risk factors for ischemic cholangiopathy in liver transplantation using donation after cardiac death donors. *Liver Transpl.* 2012;18:100–111.
35. Net M, Valero R, Almenara R, et al. The effect of normothermic recirculation is mediated by ischemic preconditioning in NHBD liver transplantation. *Am J Transplant.* 2005;5:2385–2392.
36. Fondevila C, Hessheimer AJ, Flores E, et al. Applicability and results of Maastricht type 2 donation after cardiac death liver transplantation. *Am J Transplant.* 2012;12:162–170.
37. Schlegel A, de Rougemont O, Graf R, et al. Protective mechanisms of end-ischemic cold machine perfusion in DCD liver grafts. *J Hepatol.* 2013;58:278–286.
38. 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.
39. Muller X, Schlegel A, Würdinger M, et al. Can hypothermic oxygenated perfusion (HOPE) rescue futile DCD liver grafts? *HPB (Oxford).* 2019;21:1156–1165.
40. Patrono D, Surra A, Catalano G, et al. Hypothermic oxygenated machine perfusion of liver grafts from brain-dead donors. *Sci Rep*. 2019;9:9337.
41. Umbro I, Tinti F, Scalera I, et al. Acute kidney injury and post-reperfusion syndrome in liver transplantation. *World J Gastroenterol.* 2016;22:9314–9323.
42. Jochmans I, Meurisse N, Neyrinck A, et al. Hepatic ischemia/reperfusion injury associates with acute kidney injury in liver transplantation: prospective cohort study. *Liver Transpl.* 2017;23:634–644.