Liver Grafts From Donors After Circulatory Death on Regional Perfusion With Extended Warm Ischemia Compared With Donors After Brain Death

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Donation after circulatory death (DCD) in Italy constitutes a relatively unique population because of the requirement of a no-touch period of 20 minutes. The first aim of this study was to compare liver transplantations from donors who were maintained on normothermic regional perfusion after circulatory death and suffered extended warm ischemia (DCD group, n = 20) with those from donors who were maintained on extracorporeal membrane oxygenation (ECMO) and succumbed to brain death (ECMO group, n = 17) and those from standard donors after brain death (donation after brain death [DBD] group, n = 52). Second, we conducted an explorative analysis on the DCD group to identify relationships between the donor characteristics and the transplant outcomes. The 1-year patient survival for the DCD group (95%) was not significantly different from that of the ECMO group (87%; $P = 0.47$) or the DBD group (94%; $P = 0.94$). Graft survival was slightly inferior in the DCD group (85%) because of a high rate of primary nonfunction (10%) and retransplantation (15%) but was not significantly different from the ECMO group (87%; $P = 0.76$) or the DBD group (91%; $P = 0.20$). Although ischemic cholangiopathy was more frequent in the DCD group (10%), this issue did not adversely impact graft survival because none of the recipients underwent retransplantation due to biliary complications. Moreover, the DCD recipients were more likely to develop posttransplant renal dysfunction with the need for renal replacement therapy. Further analysis of the DCD group showed that warm ischemia >125 minutes and an Ishak fibrosis score of 1 at liver biopsy negatively impacted serum creatinine and alanine transaminase levels in the first posttransplant week, respectively. In conclusion, our findings encourage the use of liver grafts from DCD donors maintained by regional perfusion after proper selection.

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Abbreviations: AKI, acute kidney injury; ALT, alanine transaminase; ASST, Azienda Socio-Sanitaria Territoriale; BAR, balance of risk; BMI, body mass index; CA, cardiac arrest; CI, confidence interval; CIT, cold ischemia time; CPR, cardiopulmonary resuscitation; CRRT, continuous renal replacement therapy; DBD, donation after brain death; DCD, donation after circulatory death; DRI, donor risk index; EAD, early allograft dysfunction; ECMO, extracorporeal membrane oxygenation; HCC, hepatocellular carcinoma; HMP, hypothermic machine perfusion; IC, ischemic cholangiopathy; ICU, intensive care unit; IGF, immediate graft function; IQR, interquartile range; LT, liver transplantation; MELD, Model for End-Stage Liver Disease; MRCP, magnetic resonance cholangiopancreatography; NRP, normothermic regional perfusion; PNF, primary nonfunction; SCS, static cold storage; SOFT, survival outcomes following liver transplantation; WIT, warm ischemia time.

Different strategies have been proposed to overcome the shortage of organs for transplantation. One such strategy involves the acceptance of organs from donation after circulatory death (DCD). However, liver grafts that have experienced warm ischemia are associated with an increase in primary nonfunction (PNF) and ischemic cholangiopathy (IC), emphasizing the importance of proper donor management and graft selection.[1-3]

Extracorporeal membrane oxygenation (ECMO) is increasingly used for support of patients with overwhelming cardiac failure, and some investigators have reported successful liver transplantations (LTs) from donation after brain death (DBD) during ECMO.[4-7] In DCD, normothermic regional perfusion (NRP) based

on ECMO technology has proven effective for improving transplant outcomes and allowing use of uncontrolled donors.[8,9] In addition to its reconditioning properties, NRP also offers the possibility to assess graft function after warm ischemia. However, the proposed selection criteria during NRP are virtually arbitrary, and no clinical study has investigated the role of these variables in predicting transplant outcomes. Additionally, ex situ machine perfusion has offered advantages in graft preservation, limiting ischemia/reperfusion injury.[10,11]

In 2015, we initiated the first DCD LT program using NRP and hypothermic machine perfusion (HMP) to overcome the legally obligatory no-touch period of 20 minutes in Italy.[12,13] The first aim of this study was to compare the results of these DCD LTs that often have exceptionally prolonged warm ischemia time (WIT) with those from ECMO-assisted and standard DBD donors, as no previous studies have compared these types of donors on mechanical circulatory support. Second, to improve the selection of the DCD grafts, we investigated the impact of the main donor variables on transplant outcomes.

Patients and Methods

STUDY DESIGN

Among the LTs performed at our center, we retrospectively compared those from DCD donors on NRP (DCD group) with those from DBD donors on ECMO support (ECMO group). Moreover, we used a nearest-neighbor matching algorithm[14] to pair each patient in the DCD group with 1 to 3 randomly selected patients from transplants performed in the same period from non-ECMO-supported DBD donors (DBD group). We excluded super-urgent cases, retransplants, split livers, and multiple-organ transplants because these recipients were not likely to receive a DCD graft. Matching variables were donor risk index (DRI) excluding the points for DCD (caliper ± 0.4 points), balance of risk (BAR) score (caliper ± 2 points), donor sex (exact matching), and the presence of hepatocellular carcinoma (HCC; exact matching).[15,16] The primary endpoints were overall patient survival and graft survival from the date of transplant. The secondary endpoints were functional recovery, postoperative renal function, hospital stay, and biliary complications. Moreover, we conducted an explorative analysis on the DCD group to investigate possible relationships between donor variables and secondary outcomes in addition to levels of serum alanine transaminase (ALT) and creatinine after transplant. We considered the following donor variables: Maastricht type, WIT, duration of NRP, cold ischemia time (CIT), levels of serum lactate and ALT during NRP, and Ishak fibrosis score at liver biopsy.

The Italian National Transplant Center (Ministry of Health) issued specific authorization for the transplants from DCD and ECMO-supported DBD donors included in this study.

MANAGEMENT OF DONORS ON NRP OR ECMO

In uncontrolled DCD (Fig. 1A), potential donors who suffered out-of-hospital cardiac arrest (CA) underwent cardiopulmonary resuscitation (CPR) onsite and were transported to the hospital under mechanical chest compressions (LUCAS Chest Compression System, Jolife AB/Physio-Control, Lund, Sweden). Death was declared at the end of 20 minutes of flatline electrocardiogram, according to Italian law.[13] Once the family authorized organ donation, the femoral vessels were cannulated, and a Fogarty balloon was inflated in the supraceliac aorta to start NRP. The NRP circuit consisted of a pump for cardiopulmonary bypass and a membrane oxygenator. WIT was defined as the time from out-of-hospital CA to the start of NRP.

In controlled DCD (Fig. 1B), the sequence after the withdrawal of support and CA was similar. Because these donors were monitored, WIT was defined as the time from systolic blood pressure falling below 50 mm Hg (or oxygen saturation below 70%) to the start of NRP.

Potential uncontrolled and controlled donors were considered eligible for the DCD program in the absence of absolute infective or neoplastic contraindications to donation if they were younger than 65 years old, if the CA was witnessed, and if WIT was ≤160 minutes. Graft acceptance required at least 3 of the following criteria:

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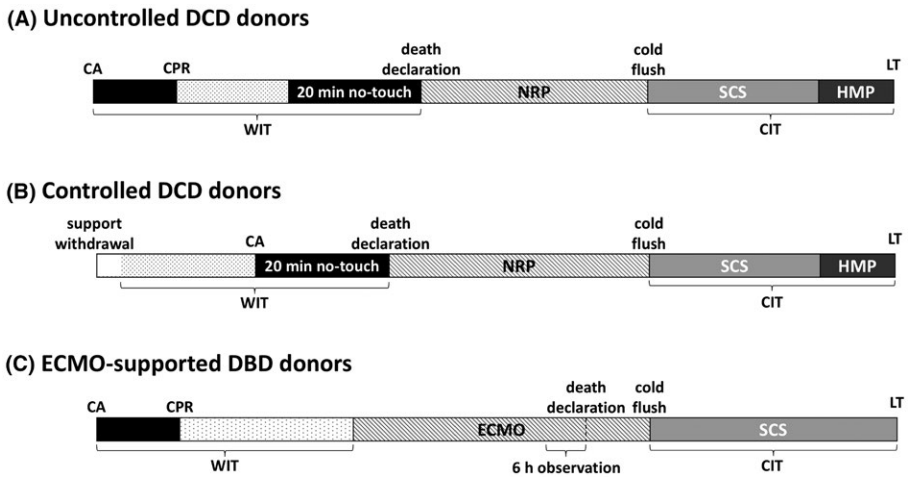

FIG. 1. Management of (A) uncontrolled DCD donors, (B) controlled DCD donors, and (C) ECMO-supported DBD donors. The uncontrolled DCD donors were transported to the hospital, declared dead after 20 minutes of flatline electrocardiogram, and then NRP was started. In controlled DCD, the sequence after the withdrawal of support and CA was similar. In ECMO-supported DBD, patients on arteriovenous ECMO for CA were proposed for organ donation after progression to brain death. The dimension of squares is arbitrary and not in proportion.

ALT ≤ 1000 IU/L, a downward trend in serum lactate, good macroscopic aspect, macrosteatosis ≤30%, and a fibrosis Ishak score ≤1. Serum lactate and ALT were measured hourly during NRP until the transport to the operating room. Frozen section evaluation was requested during the procurement before the cold perfusion.

In ECMO-supported DBD (Fig. 1C), patients on arteriovenous ECMO for CA who progressed to brain death were proposed for organ donation. In this group, WIT was defined as the time from out-of-hospital CA to the start of ECMO. Graft acceptance was based on the same criteria adopted for the DCD group. ALT was always measured after declaration of brain death. Periarrest measurements of lactate and ALT were also considered, if available. Frozen section evaluation was requested only in cases of poor macroscopic aspect.

GRAFT PROCUREMENT, PRESERVATION, AND TRANSPLANT

In DCD and ECMO-supported donors, cold Celsior solution was flushed directly through the arterial cannula used for NRP or ECMO. After back-table preparation, the DCD livers were connected to HMP (LiverAssist; OrganAssist, Groningen, the Netherlands) until the recipient hepatectomy was completed. HMP followed the protocol of the Zurich group.[11] The liver grafts were allocated according to the Italian allocation policy.[17-19] All the recipients in the DCD and ECMO group signed a specific written informed consent at the inclusion on the waiting list, and this consent was confirmed at the time of transplant. We used the piggyback technique without venovenous bypass for all transplants, and arterial anastomosis always followed portal reperfusion. In the DCD transplants, a T-tube was always used. The immunosuppressive protocol included basiliximab, steroids, and tacrolimus.

FOLLOW-UP

The follow-up was carried out monthly for the first 6 months and then annually. All data were collected prospectively from our general transplant database. PNF was defined as graft failure without secondary causes, leading either to retransplantation or death within the first posttransplant week. Early allograft dysfunction (EAD) was defined according to Olthoff et al.[20] Postoperative acute kidney injury (AKI) was defined according to the Risk, Injury, Failure, Loss and End-Stage Kidney Disease criteria, and the need for continuous renal replacement therapy (CRRT) was also considered.[21-23] Biliary complications included anastomotic and nonanastomotic biliary strictures, bile leaks, cholangitis, biliary casts, and stones requiring intervention or surgery.[3] IC was defined according to

O'Neill et al. as strictures, irregularities, or dilatations of the intrahepatic or extrahepatic bile ducts.[3] Isolated anastomotic biliary strictures were not included in the definition of IC. The diagnosis of IC was confirmed with at least 1 adequate imaging study of the biliary tree, and concomitant hepatic artery thrombosis was excluded by Doppler ultrasound or computed tomography. All DCD recipients underwent routine T-tube cholangiography on postoperative day 8 and 3 months posttransplant (at the time of T-tube removal). A magnetic resonance cholangiopancreatography (MRCP) was then performed yearly. MRCP or endoscopic retrograde cholangiopancreatography was also performed in cases of clinical or biochemical evidence of biliary issues during follow-up.

STATISTICAL ANALYSIS

Continuous data are reported as median and interquartile ranges (IQRs). Categorical data are reported as counts and percentages. Comparisons between the DCD group and the unmatched ECMO group were performed using the Wilcoxon test for continuous variables and Fisher's exact test for categorical variables. Comparisons between the DCD group and the matched DBD group were performed using the Wilcoxon paired signed rank test for continuous outcomes and conditional logistic regression for categorical outcomes. Graft survival was reported as death-censored. The overall survival and graft survival functions were estimated using the Kaplan-Meier method, and the log-rank test was performed to evaluate differences between groups. When comparing survival between the DCD group and the matched DBD group, the DBD survival functions were estimated using a weighted version of the Kaplan-Meier estimator to consider the different number of DBD control patients for each DCD patient, with weights as the inverse of the number of controls. A statistical test, based on the bootstrap method, was used to compare the 1-year survival between the 2 groups.[24] A P value of <0.05 was considered statistically significant for all survival analyses. Because of the limited number of DCD patients and the multiple outcomes evaluated, the associations between donor characteristics and transplant secondary outcomes were explored only graphically. All analyses were performed with the statistical software SAS, version 9.4 (SAS Institute, Cary, NC).

Results

GENERAL CHARACTERISTICS

Between 2015 and 2017, we evaluated 25 DCD livers for transplantation. We discarded 5 livers (20%; see characteristics in Table 1) due most frequently to the macroscopic aspect (congested and poorly perfused

TABLE 1. Individual Characteristics of the Discarded DCD Donors

Number	Age, years	Sex	BMI, kg/m^2	Type of DCD Donor	WIT, minutes	Duration of NRP, minutes	ALT Peak During NRP, IU/L	Lactate Peak During NRP, mmol/L	Lactate Average Variation During NRP, mmol/L	Macrosteatosis, %	Ishak Score	Main Reasons for Discard
1	58	Male	33	Uncontrolled	150	390	583	23	–1.8	40	2	Gross aspect, fibrosis
2	59	Male	31	Uncontrolled	117	315	297	23	0.3	25	2	Gross aspect, fibrosis
3	49	Male	26	Uncontrolled	150	435	8354	23	0.7	35	0	Gross aspect, ALT peak
4	48	Male	30	Uncontrolled	163	354	833	20	–1.9	60	0	Gross aspect, steatosis
5	41	Male	28	Uncontrolled	143	447	1420	24	–3.0	40	2	Gross aspect, fibrosis

liver) in conjunction with the biopsy findings. The general characteristics of the DCD, ECMO, and DBD groups are listed in Table 2. The DCD group included 20 transplants, of which 14 (70%) were uncontrolled and 6 (30%) were controlled. The donors had a median DRI of 2.58, which decreased to 1.71 if the points for DCD were excluded. The median WIT and duration of NRP were 125 and 352 minutes, respectively. The median survival outcomes following liver transplantation (SOFT) score[25] was 3. The majority (85%) of the recipients had HCC as the main indication for transplant. In the same period, we performed 212 LTs from standard DBD, and 52 of these were included in the matched DBD group. After matching, the DBD group and the DCD group did not differ significantly in any of the characteristics considered. The ECMO group included 17 consecutive transplants that were performed between 2011 and 2017. The median DRI was 1.54, the median WIT was 74 minutes, and ECMO support was maintained for a median of 2.7 days before procurement. HCC was the main indication for transplant in 53% of the recipients in the ECMO group, and the median SOFT score was 4.

COMPARISON BETWEEN GROUPS

In the DCD group, 2 patients underwent early retransplantation due to PNF, and 1 underwent retransplantation because of stenosis of the hepatic venous outflow. The only death in the DCD group resulted from a documented myocardial infarction, and this patient maintained good function of the liver graft during the entire follow-up. None of the recipients in the ECMO group were retransplanted, but 1 developed PNF and died. The median follow-up was 14 months (IQR, 8-26 months) for the DCD group, 20 months (IQR, 7-29 months) for

TABLE 2. Patient Characteristics

	DCD (n = 20)	DBD (n = 52)	*P* Value*	ECMO (n = 17)	*P* Value†
Recipients' characteristics					
Age, years	56 (54-63)	54 (51-59)	0.16	57 (52-63)	0.98
BMI, kg/m^2	26 (22-28)	24 (22-27)	0.57	29 (25-30)	0.03
MELD score	10 (8-13)	10 (8-12)	0.94	13 (10-21)	0.02
Sex			>0.99		0.49
Women	2 (10)	5 (10)		0 (0)	
Men	18 (90)	47 (90)		17 (100)	
HCC	17 (85)	43 (83)	>0.99	9 (53)	0.07
SOFT score	3 (1-7)	4 (2-8)	0.68	4 (2-8)	0.55
Donors' characteristics					
Age, years	51 (46-61)	56 (47-64)	0.18	52 (49-59)	0.51
BMI, kg/m^2	26 (25-27)	25 (23-28)	0.20	28 (26-30)	0.11
DRI score without DCD points	1.71 (1.48-1.94)	1.60 (1.41-1.85)	0.52	1.54 (1.35-1.60)	0.09
BAR score	3 (2-5)	3 (2-3)	0.84	5 (3-8)	0.008
Sex			>0.99		0.23
Women	3 (15)	9 (17)		0 (0)	
Men	17 (85)	43 (83)		17 (100)	
Type of DCD donor					
Uncontrolled	14 (70)	—		—	
Controlled	6 (30)	—		—	
Donors' time parameters					
WIT, minutes	125 (72-143)	—		74 (70-83)	0.048
Missing	0	—		3	
Duration of NRP or ECMO, minutes	352 (308-434)	—		3890 (2760-5206)	<0.001
Missing	0	—		1	
CIT, hours	8 (6-9)	7 (6-10)	0.82	10 (8-11)	0.07

NOTE: Data are given as n (%) or median (IQR).
*Conditional logistic model *P* value for categorical variables; signed rank *P* value for continuous variables.
†Fisher's exact *P* value for categorical variables; Wilcoxon *P* value for continuous variables.

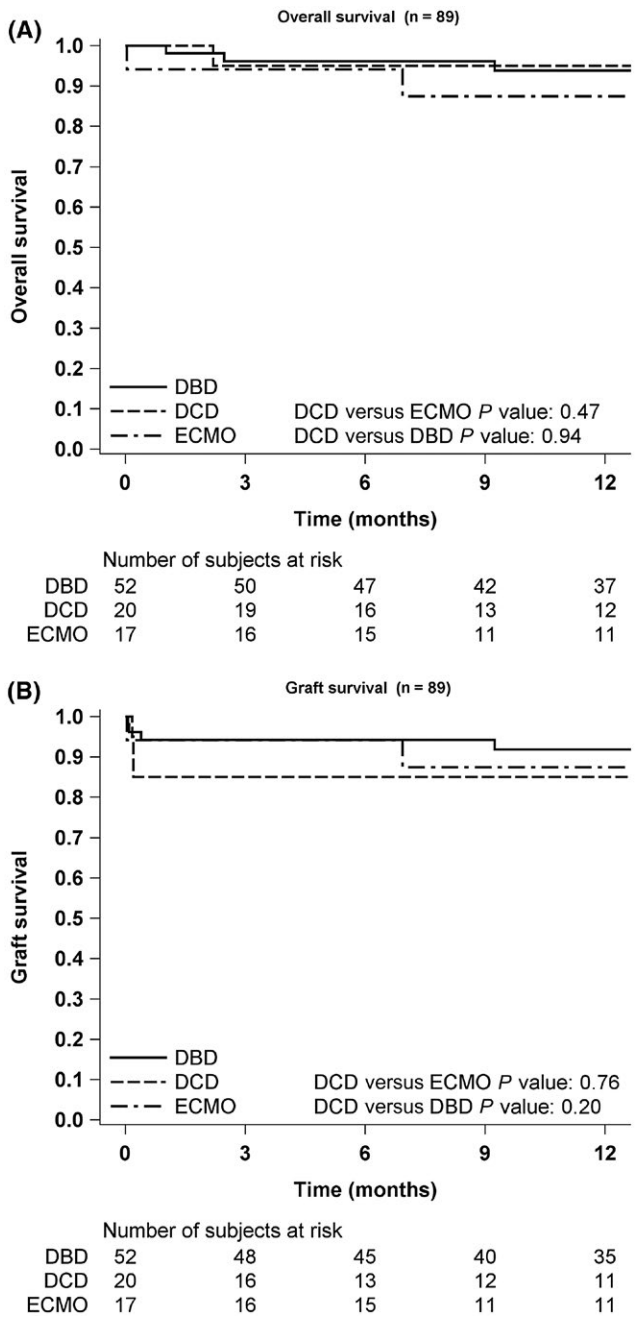

FIG. 2. Kaplan-Meier curves for (A) overall survival and (B) death-censored graft survival for DCD, matched DBD, and ECMO transplants. The curves of the DCD group did not differ significantly from the matched DBD group or the ECMO group at 1 year.

the ECMO group, and 17 months (IQR, 11-23 months) for the DBD group. One-year patient survival was 95% (95% confidence interval [CI], 69%-99%) for the DCD group, 87% (95% CI, 58%-97%) for the ECMO group, and 94% (95% CI, 82%-98%) for the matched DBD group. The overall survival curve (Fig. 2A) of the DCD group did not differ significantly from that of the ECMO group ($P = 0.47$) or the matched DBD group at 1 year ($P = 0.94$). The 1-year death-censored graft survival was 85% (95% CI, 60%-95%) for the DCD group, 87% (95% CI, 58%-97%) for the ECMO group, and 91% (95% CI, 80%-97%) for the matched DBD group. The graft survival curve (Fig. 2B) of the DCD group did not significantly differ from the ECMO group ($P = 0.76$) or the matched DBD group at 1 year ($P = 0.20$).

Compared with the ECMO group (Table 3), the DCD group had fewer EAD (24% versus 44%; $P = 0.28$) but more PNF (10% versus 6%; $P > 0.99$) and retransplants (15% versus 0%; $P = 0.23$). Although the frequency of AKI was similar in the DCD and the ECMO group (30% versus 37%; $P = 0.73$), the need for CRRT was higher in the DCD group (25% versus 6%; $P = 0.20$). There were 2 (10%) patients in the DCD group who had IC, whereas none in the ECMO group did; however, the 2 recipients who developed IC in the DCD group were successfully managed with endoscopic stenting with no need for other measures during follow-up of 15 and 14 months, respectively. Compared with the matched DBD group, the DCD group had more PNF (10% versus 4%; $P = 0.58$) and retransplants (15% versus 6%; $P = 0.35$), a significantly higher need for CRRT (25% versus 6%; $P = 0.04$), and a higher frequency of IC (10% versus 4%; $P = 0.52$).

INFLUENCE OF DONOR VARIABLES ON TRANSPLANT OUTCOMES IN THE DCD GROUP

In the DCD group, median CIT was higher among recipients who developed EAD (Fig. 3). Immediate graft function (IGF) was more frequent in controlled than uncontrolled cases (83% versus 57%), and 80% of patients with an average decrease ≤–1.11 mmol/L in serum lactate during NRP had an immediate functional recovery, as opposed to only 50% of those with an average decrease >–1.11 mmol/L (Fig. 4). Moreover, IGF was more frequent in the absence of fibrosis at liver biopsy (73% versus 40%). Upon further analysis (Fig. 5A), ALT levels during the first posttransplant week were higher for livers with an Ishak fibrosis score of 1 compared with ALT levels for livers with an Ishak fibrosis score of 0. Despite the minimal fibrotic changes at biopsy, these grafts had a good macroscopic aspect, and the donors

TABLE 3. Posttransplant Outcomes Among DCD, DBD, and ECMO Transplant Recipients (n = 89)

	DCD (n = 20)	DBD (n = 52)	*P* Value*	ECMO (n = 17)†	*P* Value‡
ICU stay, days	4 (3-7)	4 (3-4)	0.20	4 (3-4)	0.86
Hospital stay, days	17 (14-24)	14 (12-20)	0.62	16 (14-19)	>0.99
EAD§			>0.99		0.28
No	13 (76)	36 (73)		9 (56)	
Yes	4 (24)	13 (27)		7 (44)	
PNF			0.58		>0.99
No	18 (90)	50 (96)		16 (94)	
Yes	2 (10)	2 (4)		1 (6)	
Retransplant			0.35		0.23
No	17 (85)	49 (94)		17 (100)	
Yes	3 (15)	3 (6)		0 (0)	
AKI			0.19		0.73
No	14 (70)	45 (87)		10 (63)	
Yes	6 (30)	7 (13)		6 (37)	
Need for CRRT			0.04		0.20
No	15 (75)	49 (94)		15 (94)	
Yes	5 (25)	3 (6)		1 (6)	
Overall biliary complications			0.65		0.35
No	16 (80)	45 (87)		15 (94)	
Yes	4 (20)	7 (13)		1 (6)	
IC			0.52		0.49
No	18 (90)	50 (96)		16 (100)	
Yes	2 (10)	2 (4)		0 (0)	

NOTE: Data are given as n (%) or median (IQR).
*Conditional logistic model *P* value for categorical variables; signed rank *P* value for continuous variables.
†The percentages of ICU stay, hospital stay, EAD, AKI, need for CRRT, overall biliary complications, and IC were calculated for 16 ECMO transplant recipients because 1 ECMO transplant recipient died from PNF soon after LT.
‡Fisher's exact *P* value for categorical variables; Wilcoxon *P* value for continuous variables.
§The percentage of EAD was calculated excluding the retransplanted recipients.

had no medical history or signs of hepatopathy. CRRT was necessary in all but 1 patient with AKI and only in recipients of uncontrolled donors. The median WIT was 157 minutes (138-164 minutes) for patients needing CRRT compared with 114 minutes (59-133 minutes) for patients not needing CRRT (Fig. 3). CRRT was also indicated 4-fold more frequently in cases with an average decrease >–1.11 mmol/L in serum lactate during NRP (40% versus 10%). Furthermore, serum creatinine during the first posttransplant week was higher for patients with WIT > 124.5 minutes than for those with WIT ≤ 124.5 minutes (Fig. 5B). Biliary complications were not affected by donor time variables but were 3-fold more frequent in cases with an average ALT increase >34.5 IU/L during NRP (30% versus 10%) and 4-fold more frequent in livers with an Ishak fibrosis score of 1 at biopsy (40% versus 13%).

Discussion

In this study, the transplants from DCD donors on NRP did not differ significantly from the matched DBD group or the ECMO group with respect to 1-year patient survival. Graft survival was slightly inferior in the DCD group due to a higher rate of PNF and retransplantation, although this difference was not statistically significant. We presented death-censored graft survival because the only death in the DCD group was because of an extrahepatic cause and the patient maintained normal graft function until death. The DCD recipients

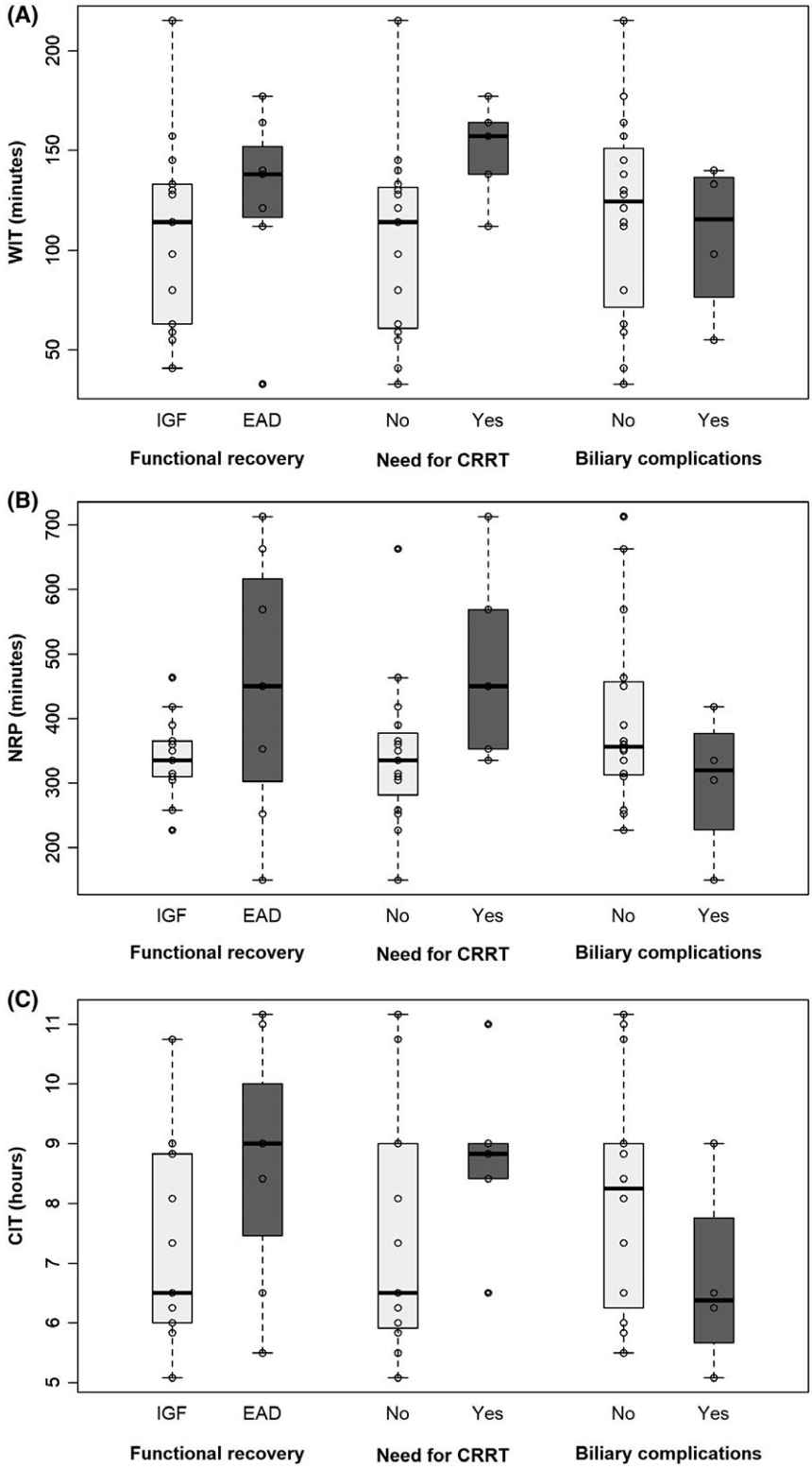

FIG. 3. Distributions of (A) WIT, (B) NRP duration, and (C) CIT according to functional recovery, need for CRRT, and biliary complications among DCD transplants. Median WIT was 157 minutes (138-164 minutes) for patients needing CRRT and 114 minutes (59-133 minutes) for patients not needing CRRT. Median CIT was higher among the recipients who developed EAD.

were also more likely to develop posttransplant AKI with a need for CRRT. Further analysis showed that WIT and an Ishak fibrosis score of 1 at liver biopsy negatively impacted renal function and liver enzyme levels, respectively, in the first posttransplant week.

Our DCD series is unique for its prolonged WIT that results from the legal obligation of a no-touch period of at least 20 minutes, which far exceeds the commonly accepted recommendations for DCD transplants.[26] We addressed this problem by using NRP to interrupt the detrimental effects of the association between warm and cold ischemia on functional recovery.[8,27,28] Moreover, we added HMP before implantation to improve organ preservation and limit the ischemia/reperfusion injury as previously demonstrated by Dutkowski et al.[10,11]

The use of liver grafts from donors suffering CA is expanding as a result of extracorporeal perfusion systems. Fondevila et al. and Savier et al. have successfully used NRP in 34 and 13 uncontrolled DCD LTs, respectively, with a no-touch period of 5 minutes.[9,27] On the other hand, Carter et al. have reported 15 LTs from ECMO-supported DBD donors with a 1-year graft survival of 93%, and several other studies report similar results.[4-7] Although some authors have hypothesized that ECMO-supported DBD may display the same physiology as DCD on NRP,[5,7] these 2 methods differ considerably from each other in 3 main areas. First, circulatory death precedes NRP, whereas brain death is assessed during ECMO. Second, NRP is limited to the abdominal organs, whereas ECMO is systemic. Third, NRP is usually maintained for hours, whereas ECMO may last days before progression to brain death. Accordingly, the shorter

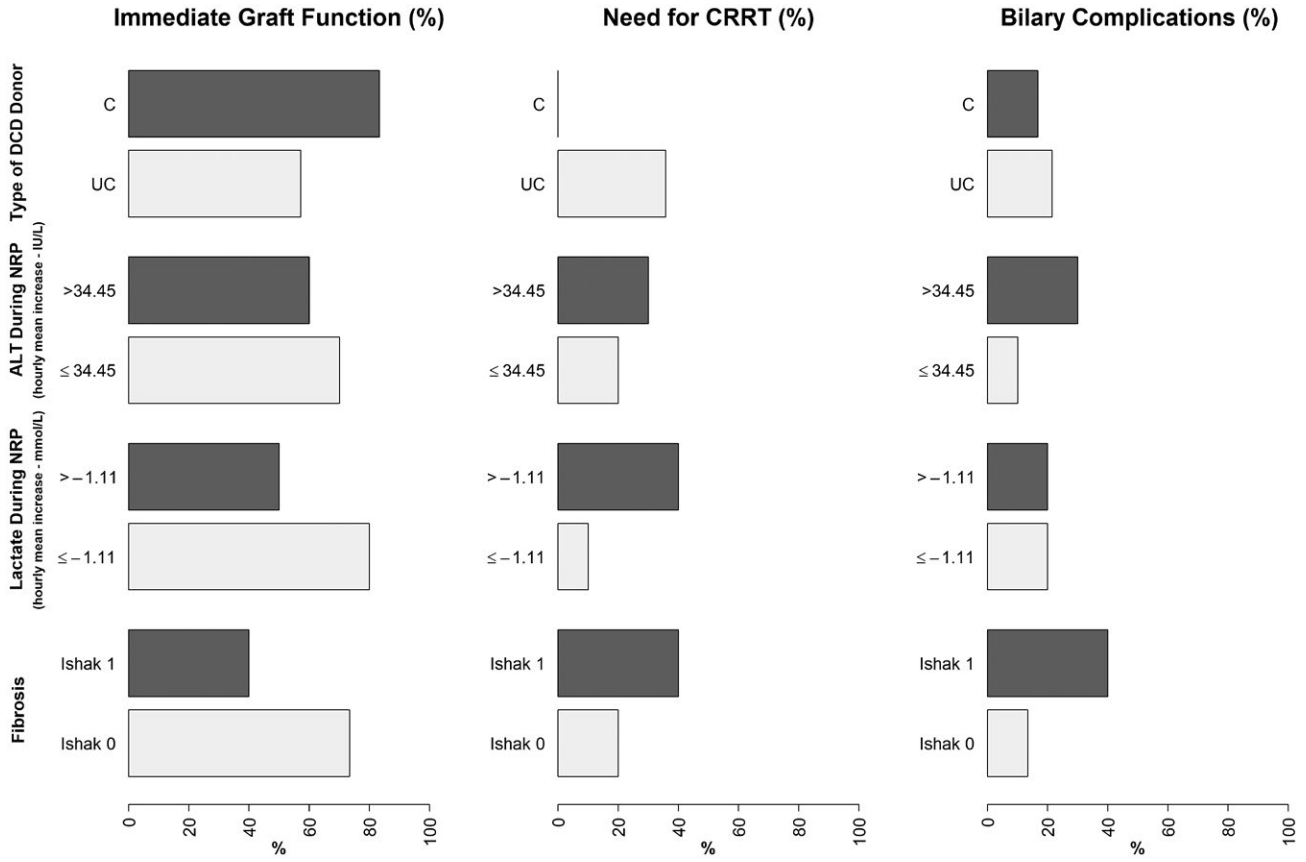

FIG. 4. Distributions of (A) immediate functional recovery, (B) need for CRRT, and (C) biliary complications according to type of donor, ALT during NRP, lactate during NRP, and fibrosis (Ishak score) at liver biopsy among the DCD transplants. IGF was more frequent in controlled patients (83% versus 57%) in patients with an average decrease ≤–1.11 mmol/L in serum lactate during NRP (80% versus 50%) and if fibrosis was absent at liver biopsy (73% versus 40%). CRRT was 4-fold more frequent in patients with an average decrease >–1.11 mmol/L in serum lactate during NRP (40% versus 10%).

WIT and the greater distance from the cardiac event in the ECMO group suggest a lower graft injury at the time of transplant, which was confirmed by the absence of IC and decreased need for CRRT in our study. The 1 death from PNF in the ECMO group may be a concern. However, neither the donor nor the perfusion parameters differed from the other transplants in this group. Thus, the presence of massive portosystemic shunting leading to insufficient portal flow after reperfusion seems to have been the main determinant of this event.

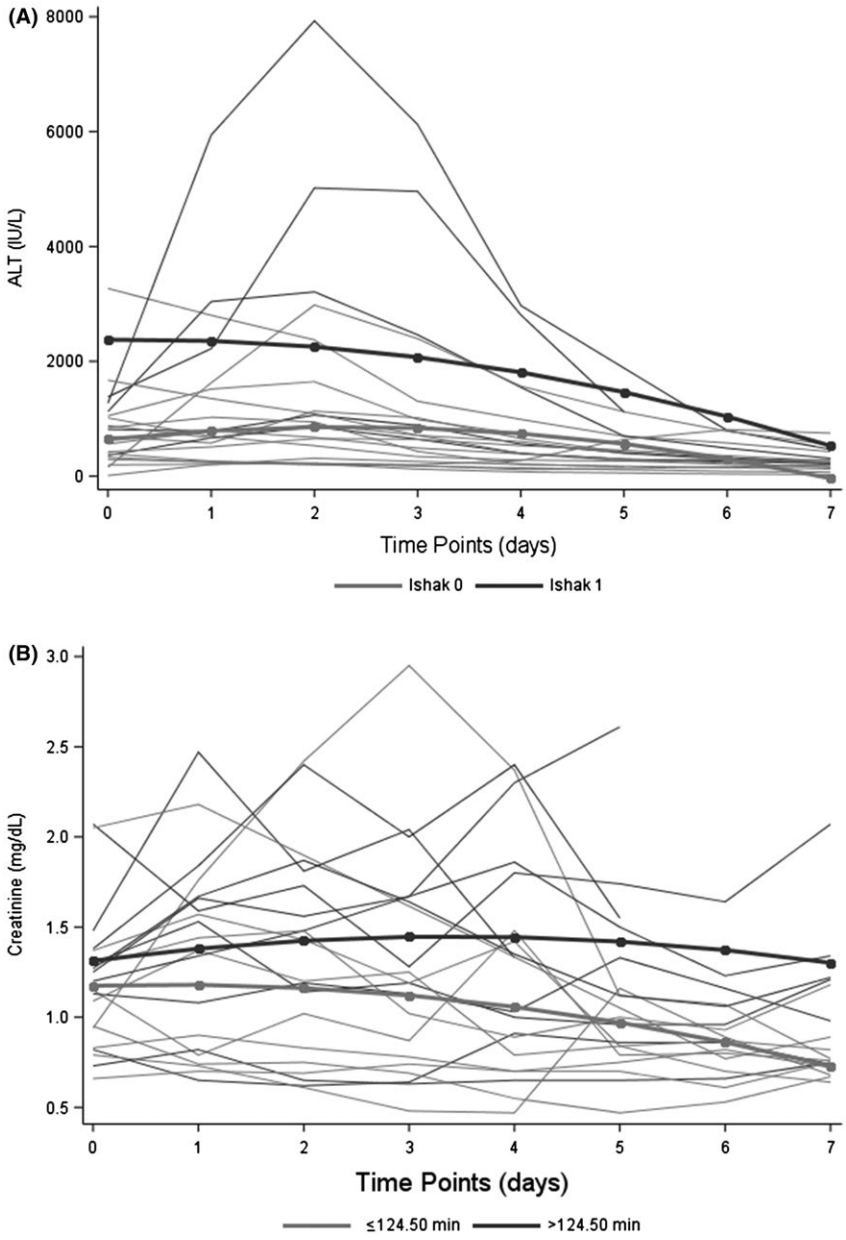

FIG. 5. Trend of posttransplant ALT according to (A) Ishak score and (B) trend of posttransplant serum creatinine according to WIT among the DCD transplants. ALT levels during the first posttransplant week were higher and exceeded the cutoff for EAD (2000 IU/L) for livers with an Ishak fibrosis score of 1 compared with ALT levels for livers with an Ishak fibrosis score of 0. Serum creatinine during the first posttransplant week was higher for patients with WIT >124.5 minutes than for those with WIT ≤124.5 minutes. To account for nonlinearity, trends were estimated with a mixed model for repeated measures considering time as both linear and quadratic.

Leithead et al. have reported an increased incidence of AKI among DCD LTs.[22,29] Accordingly, in our study, AKI was more frequent in the DCD group. We also noted an increased need for CRRT, probably due to the prolonged WIT in this group. These findings add further support to the hypothesis that DCD livers, once reperfused, trigger a systemic inflammatory response that ultimately causes renal dysfunction.[30] In these transplants, it thus seems advisable to opt for immunosuppression regimens that offer reduced nephrotoxicity and to explore strategies to limit the inflammatory response.

IC is traditionally considered to be a serious weakness of DCD donors, and 2 meta-analyses have demonstrated a significant increase in biliary complications with a consequent decrease in graft survival in these patients.[1-3] In our study, all DCD recipients had a minimum follow-up of >3 months, as required for IC diagnosis.[2,3] Although IC was more frequent among DCD transplants, this complication did not seem to adversely impact graft survival, as neither of the recipients with IC underwent retransplantation after a follow-up of more than 1 year.

Selection criteria for DCD donors on NRP represent a challenging issue. For ALT, a cutoff of 4-fold of the upper limit of normal has been adopted by Fondevila et al., whereas Savier et al. have defined this limit at 200 IU/L.[8,9] Although serum lactate is considered to be a marker of adequate perfusion and hepatic clearance, these groups have placed little importance on this factor, probably because its concentration during NRP may be altered by reflux from extra-abdominal regions.[8,9,31] Recent studies on normothermic machine perfusion have used serum lactate levels for ex situ evaluation.[32-34] In our study, although levels exceeded the above-mentioned limits, ALT did not exhibit a clear relationship to the transplant outcomes. Conversely, the higher frequency of immediate functional recovery and preserved renal function that we observed among transplants with a faster lactate clearance seems to confirm the use of lactate in the selection of these grafts.

Not all authors recommend routine microscopic evaluation of DCD livers maintained on NRP.[8] We did not consider steatosis in our analyses because all transplanted DCD livers in our study had ≤30% macrosteatosis and were thus low risk based on this variable.[35] The decision to use some DCD grafts with an Ishak fibrosis score of 1 was based on the observed good macroscopic aspect and the absence of clinical evidence of hepatopathy in the donors. Upon subsequent analysis, however, even minimal fibrotic changes (as in an Ishak score of 1) seemed to negatively impact functional recovery, emphasizing the importance of liver biopsy during DCD graft evaluation. We did not use the parameters of HMP for graft selection because no clear evidence of graft viability can be observed in hypothermic conditions. Moreover, postponing the decision to accept the graft until this point in time would considerably increase total CIT.[36]

The majority of the recipients in the DCD group had a low Model for End-Stage Liver Disease (MELD) score and HCC as the main indication for transplant. The presence of HCC was thus included in the matching criteria for the DBD control group. The allocation of DCD and ECMO grafts followed the Italian center-based allocation policy,[17-19] although these organs were excluded for super-urgent patients and retransplants. Accordingly, we allocated these organs to patients on the waiting list of our center, possibly with a preference for patients with a low biological MELD score and a priority for transplant dictated by their HCC status. These patients are likely most suitable to receive a DCD graft, as suggested by other studies.[37] It is possible then that patients with a higher biological MELD score would not have tolerated these marginal DCD grafts.

The main limits of this study are its retrospective nature and the small number of patients, resulting in low statistical power. The decreased graft survival observed for the DCD group compared with the DBD group, although not statistically significant, remains clinically relevant. However, as the patient survival was similar, we believe that the difference in graft survival is still acceptable in terms of the balance between posttransplant survival and dropout or mortality on the waiting list. Furthermore, most of our DCD donors were uncontrolled. With uncontrolled DCD donors only, Fondevila et al. and Savier et al. have reported 1-year graft survival of approximately 70%, which was slightly inferior to that of our DCD group.[9,27] Another limitation is the lack of comparison with a group from standard DCD without NRP, as this option is not allowed in Italy because of the requirement of a no-touch period of 20 minutes. This limitation impacts the generalizability of these results to the use of standard DCD donors.

In conclusion, the results of our study suggest that NRP and HMP, in line with the emerging acceptance of extended criteria for DCD donors,[38] may be useful for salvaging some DCD organs that may have been discarded in many other countries. Thus, these technologies have offered the opportunity for 20 additional LTs in our center, ultimately representing an advantage for the patients on the waiting list. Further studies are needed to investigate the longterm outcomes and define reliable

predictors of functional recovery and complications for DCD organ transplants.

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