Viability assessment of livers donated after circulatory determination of death during normothermic regional perfusion

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MAIN TEXT

Viability assessment of livers donated after circulatory determination of death during normothermic regional perfusion

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Abstract

Background: Abdominal normothermic regional perfusion (A-NRP) allows in-situ reperfusion and recovery of abdominal organs metabolism in donors after circulatory death (DCD). Besides improving liver transplantation outcomes, liver injury and function can be assessed during A-NRP.

Methods: To refine liver viability assessment during A-NRP, prospectively collected data of controlled DCD donors managed at our Institution between October 2019 and May 2022 were retrospectively analyzed. Baseline characteristics, procedural variables and A-NRP parameters of donors whose liver was successfully transplanted were compared to those of donors whose liver was discarded.

Results: Twenty-seven donors were included and in 20 (74%) the liver was accepted (positive outcome). No differences between study groups were observed concerning baseline characteristics and warm ischemia times (WIT). Initial lactate levels were positively correlated with functional WIT ($r^2 = 0.4$, $p = 0.04$), whereas transaminase levels were not. Blood flow during A-NRP was comparable, whereas oxygen consumption (VO_2) was significantly higher in the positive outcome group after 1 h. Time courses of lactate, AST and ALT were significantly different between study groups ($p < 0.001$). Donors whose liver was accepted showed faster lactate clearance, a difference which was amplified by normalizing lactate clearance to oxygen delivery (DO_2) and VO_2. Lactate clearance was correlated to transaminase levels and DO_2-normalized lactate clearance was the parameter best discriminating between study groups.

Conclusions: DO_2-normalized lactate clearance may represent an element of liver viability assessment during A-NRP.

Irene Steinberg and Damiano Patrono share first co-authorship.

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KEYWORDS
donation after circulatory death, ex-situ machine perfusion, hypothermic oxygenated machine perfusion, lactate clearance, liver viability assessment, normothermic machine perfusion, normothermic regional perfusion, oxygen delivery

1 | INTRODUCTION

Liver transplantation (LT) is a highly successful treatment for end-stage liver disease and hepatocellular cancer, but it is limited by the number of available grafts. Utilization of grafts from donors whose death has been determined by circulatory criteria (DCD) is one mean to expand donor pool, but it has been associated with inferior results as compared to LT using grafts from donors after brain death (DBD), mainly due to the detrimental effects of initial warm ischemia.[1–3] Abdominal normothermic regional perfusion (A-NRP), a technique that uses extracorporeal membrane oxygenation (ECMO) for the sole perfusion of abdominal organs, was introduced in DCD LT to re-establish metabolism before cold storage and to improve outcomes.[4,5] In Italy, given the 20-min no-touch period, use of A-NRP is mandatory.[6]

By providing in-situ perfusion with warm oxygenated blood, A-NRP allows ATP regeneration, prevents the accumulation of toxic metabolites and activates molecular pathways linked to ischemic preconditioning.[7] Additionally, objective data reflecting liver injury and function (so-called "viability assessment") can be obtained during A-NRP. However, liver viability assessment criteria during NRP are highly heterogeneous.[8]

Recovery of liver metabolism during A-NRP depends on the adequacy of in-situ perfusion, which consequently influences the reliability of viability assessment during A-NRP. Several parameters, including lactate clearance, pH, blood flow, base excess and venous oxygen saturation (SvO_2) have been considered to assess the adequacy of A-NRP.[9–12]

To refine liver viability assessment during A-NRP, we analyzed our experience of DCD LT performed using of A-NRP followed by ex-situ machine perfusion. The aim of this study was analyzing the predictive value of parameters normally used to assess liver viability during A-NRP (transaminase level, lactate clearance) in relation to parameters indicative of the adequacy of in-situ perfusion during A-NRP (blood flow, oxygen delivery [DO_2] and consumption [VO_2]), to provide a deeper insight into the difficult process of graft selection during A-NRP.

2 | PATIENTS AND METHODS

This was an observational prospective cohort study on consecutive Maastricht category III liver donors managed at our Institution between October 2019 and May 2022. 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 0094631, approved on 16/09/2021). Recipients were informed of the possibility to receive a liver from a DCD donor at waitlisting and confirmed consent when the organ became available.

Manuscript preparation followed the strengthening the reporting of observational studies in epidemiology (STROBE) guidelines.

2.1 | Study design

Only category III DCD donors (cDCD) were included in this study. Data concerning donor anthropometric characteristics, comorbidities and cause of death were recorded, as well as the timing of events and monitoring parameters during the donation process. We analyzed the association between these data and the primary endpoint, defined as liver procurement and transplantation with the recipient not developing primary non-function (PNF). A negative outcome was defined as the liver not being procured or LT followed by PNF. Data concerning postoperative course and liver function after LT were also collected and analyzed. All transplanted grafts had a minimum follow-up of 6 months.

2.2 | Liver procurement and preservation

The donation process in controlled DCD in Italy has been described elsewhere.[13] Briefly, after cardiac arrest, death was declared after 20 min of absent electrical activity. Withdrawal of life-sustaining treatment (WLST) was considered in patients having suffered from devastating brain injury not meeting the criteria for brain death, or in patients having been treated with rescue extracorporeal life support (ECLS) for refractory cardiac arrest or cardiogenic shock who developed an extensive post-anoxic brain injury not meeting brain death criteria and in the absence of spontaneous recovery of cardiac activity (Figure 1). Death declaration in patients treated with venoarterial ECMO followed Italian national recommendations.[14]

WLST took place in the operating theater or in the intensive care unit, after guidewires for subsequent femoral

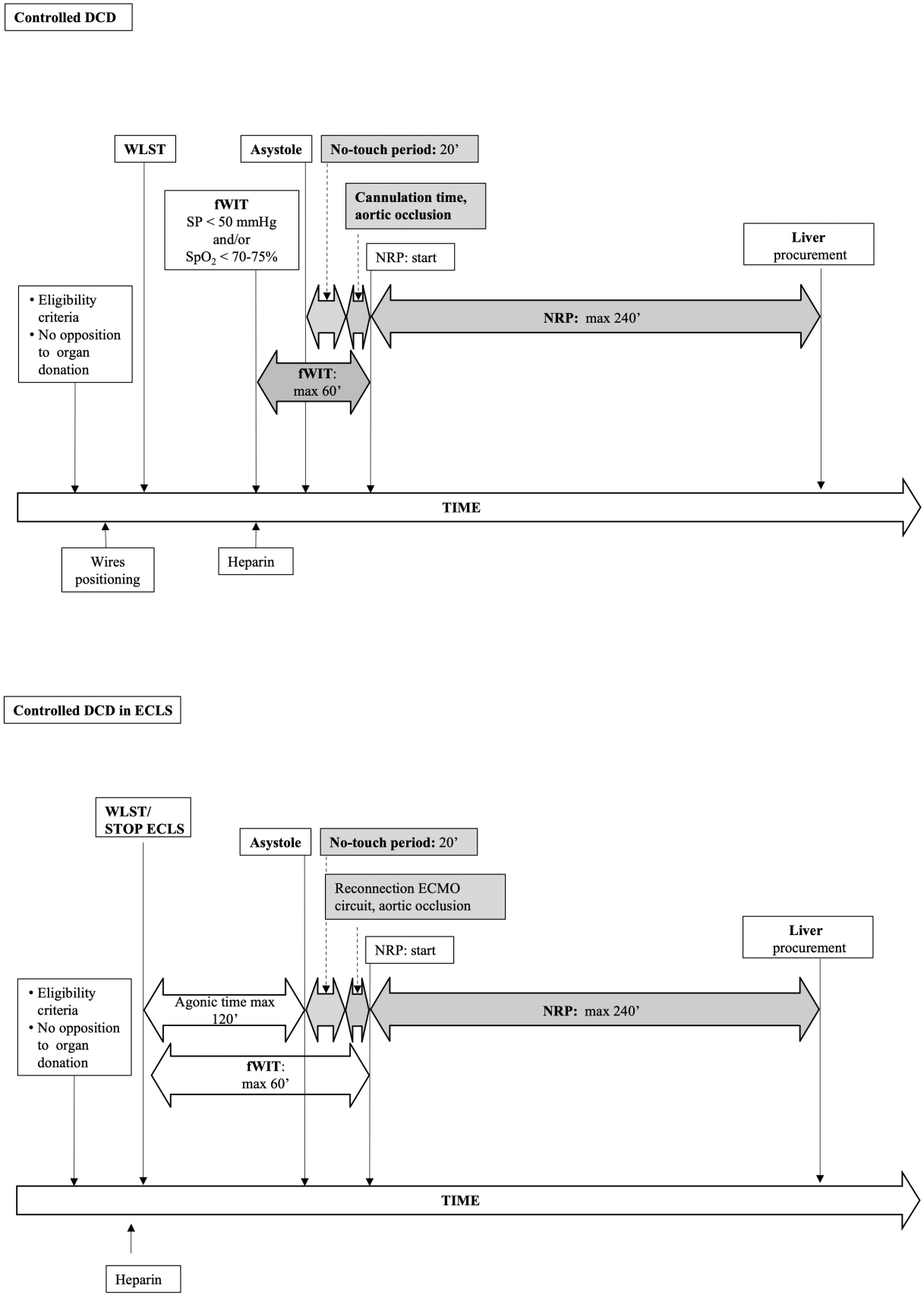

FIGURE 1 Donation process in standard and failed ECLS controlled DCD donors.

vessels cannulation had been inserted percutaneously. Heparin 300 IU/kg was administered at the onset of functional warm ischemia (peripheral O_2 saturation ≤70% or systolic blood pressure ≤ 50 mm Hg). In donors treated with an ECLS protocol heparin was administered before stopping the ECMO circuit targeting an activated clotting time (ACT) ≥ 300 s. In these donors, the onset of functional warm ischemia time (FWIT) coincided with the time the ECMO circuit was stopped. In both scenarios, after 20-min electrical asystole, death was declared, femoral vessels were cannulated and descending aorta was occluded by an endovascular balloon or a surgical clamp, after which A-NRP was started. The Cardiohelp device (Maquet, Wayne, NJ), which includes a heat exchanger, was used for all NRP procedures. Femoral vein was cannulated with a 19–21 Fr cannula, according to body size, whereas a 23 Fr cannula was used for femoral artery. Supra-hepatic inferior vena cava and iliac vessels contralateral to ECMO cannulae were not clamped. During A-NRP, target blood flow was ≥40 mL/kg/min, i.e., ≥50% of theoretical cardiac output. The ECMO gas flow was adjusted to keep $PaCO_2$ between 35 and 45 mm Hg and SaO_2 98%–100%. Hematocrit was maintained >20% by transfusing packed red blood cells, if needed, and heparin boluses were administered to keep ACT ≥300 s. In case of combined lungs and abdominal organs procurement, the lungs were procured with a non-rapid normothermic open-lung technique during NRP.

Blood samples were obtained at baseline and hourly to monitor biochemical (lactates, ALT, AST, bilirubin, urea, creatinine, glucose, potassium and sodium) and blood gas parameters (pH, PaO_2, $PaCO_2$, base excess). Functional parameters of NRP (gas flow, blood flow, FiO_2, DO_2, VO_2, fluid and transfusion requirement) were also recorded every hour. Although the Cardiohelp device allows for automatically calculating DO_2, for the purpose of the study DO_2 was manually calculated according to the following formula: $DO_2 = CO * CaO$, where CO is the cardiac output, which corresponds to the ECMO blood flow during A-NRP, and CaO is the arterial oxygen content calculated as $CaO = (Hb * 1.39 * SaO_2) + (0.0031 * PaO_2)$, where 1.39 is the oxygen binding capacity of hemoglobin (Hb), SaO_2 is arterial blood oxygen saturation and 0.0031 is the amount of dissolved oxygen in the blood for every mm Hg of arterial oxygen partial pressure (PaO_2). VO_2 was calculated as $VO_2 = CO * (CaO - CvO)$, where the venous oxygen content (CvO) is calculated based on SvO_2, the venous blood oxygen saturation, and PvO_2, the venous oxygen partial pressure.

Target A-NRP time was 4 h, after which the liver was flushed with cold Celsior (IGL, Lissieux, France) solution through both the arterial cannula and a vein tributary of the portal system. The choice for 4 h as target A-NRP time was based on available experimental studies suggesting optimal A-NRP duration between 2 and 4 h,[15] and dictated mainly by practical reasons, in particular the time necessary to get the results of second hour blood sample analyses. Additionally, slightly prolonging A-NRP sometimes facilitates transplant logistic by buying time to prepare the recipient. As a rule, the liver was transported at the transplanting center under static cold storage and connected to the LiverAssist device (XVIVO, Goteborg, Sweden) for dual hypothermic oxygenated machine perfusion (D-HOPE), which lasted for a minimum of 2 h until the end of recipient hepatectomy. Normothermic machine perfusion using the OrganOx Metra device (OrganOx, Oxford, UK) was considered in some cases, mainly to prolong preservation time.

2.3 | Graft selection and allocation

During A-NRP, liver viability assessment was based on a modified version of the criteria proposed by De Carlis et al.,[6] including a blood flow >1.7 lt/min/m2, transaminase level <1000 IU/L at 2 h of A-NRP, downward lactate trend, absence of significant (≥15%) macrovesicular steatosis or Ishak >1 fibrosis, and good liver and abdominal viscera perfusion. A liver biopsy was systematically obtained to assess necrosis and steatosis.

As a rule, DCD grafts were allocated to size-matched low-MELD recipients, most frequently undergoing LT for HCC. However, sicker recipients were considered in the latter part of our experience.

2.4 | Statistical analysis

Normal distribution of variables was tested using Shapiro-Wil normality test. Variables were described as mean (standard deviation) or median (interquartile range) and compared with t-test or Wilcoxon non-parametric tests, as appropriate. Dichotomous variables were compared with chi-square or Fisher's exact tests as appropriate. The correlation between non-normally distributed variables was tested with spearman's R while the time course comparisons between groups were performed with a linear mixed-effects model. Two tailed p values <0.05 were considered statistically significant. Statistical analysis was performed with R software (R Foundation for Statistical Computing, Vienna, Austria).

3 | RESULTS

Thirty cDCD donors were included. In all cases, A-NRP was successfully established. Three donors were excluded due to an unexpected finding of colon cancer in one case

and liver cirrhosis in the other two. In 20 of the 27 included donors, the liver was recovered and successfully transplanted, whereas it was discarded in 7 cases.

In all but one case, livers were discarded during A-NRP, whereas in one case characterized by conflicting findings during A-NRP (transaminases >2000 IU/L but good lactate clearance) the liver was procured and subsequently confirmed as non-viable during ex-situ normothermic machine perfusion due to poor arterial flow, lack of bile production and inhomogeneous perfusion. In 6 (85.7%) cases elevated (>1000 IU/L) 2-h transaminases during A-NRP was the main reason dictating graft discard. Despite elevated transaminases all grafts showed some degree of lactate metabolism during A-NRP. In one case, the decision to discard the graft was based on elevated transaminases (AST 517 UI/L and ALT 630 IU/L at 3 h of A-NRP), very slow lactate clearance (14 mmol/L at the beginning of A-NRP and 11.8 mmol/L at 4 h) and refractory hypoglycemia. No liver was discarded based on histological findings.

Accepted grafts were transplanted preferentially to low-MELD (11.5 [8 – 18-2]) patients with HCC in 13 (65%) cases (Table 1). After procurement, grafts were cold stored and subsequently treated by either D-HOPE ($n=17$, 85%) or normothermic machine perfusion ($n=3$, 15%). With the exception of the aforementioned case, the choice to utilize normothermic machine perfusion in three cases in this series was dictated by logistical constraints. All grafts exhibited primary function, with respectively 5 (25%) and 3 (15%) recipients presenting with early allograft dysfunction and stage 2–3 acute kidney injury, while median estimated risk of early graft failure as assessed by the L-GrAFT[16] and EASE[17] scores of 13% and 1.5%, respectively. There was one case of early allograft failure in a patient who was retransplanted following primary non-function of a previous DBD graft. This patient, who was retransplanted with MELD 37 and already on renal replacement therapy, went on to develop multiorgan failure despite good function of the second graft and died on day 7th after retransplant. Incidence of biliary complications was low, with 1 (5%) and 1 (5%) patient presenting with biliary anastomotic stricture and a mild form of ischemic cholangiopathy,[18] respectively.

When comparing the positive and negative outcome groups, no significant differences were observed in terms of donor age, gender, BMI, cause of brain injury and graft histology (Table 2). Similarly, no differences were found with regards to FWIT (47 vs. 44 min, $p=0.53$) and asystolic time (30 vs. 30 min, $p=1$). Mean blood flow, as well as fluids and blood transfusions administered hourly to maintain an adequate DO_2 during NRP did not differ between groups, although the positive outcome group showed a trend towards and increased fluid demand to maintain the target flow (250 vs. 1500 mL/h, $p=0.06$).

TABLE 1 Recipient characteristics, operational variables and outcome.

n	20
Rec. age	62.6 [60.2, 67.3]
Gender	
F	3 (15.0)
M	17 (85.0)
BMI	26.2 [22.6, 28.4]
Rank of LT	
1	19 (95.0)
2	1 (5.0)
MELD	11.5 [8.0, 18.2]
HCC	13 (65.0)
Status pre-LT	
Home	16 (80.0)
Hospital	3 (15.0)
ICU	1 (5.0)
Cold ischemia time (min)	226.5 [200.5, 246.2]
Ex-situ perfusion type	
D-HOPE	17 (85.0)
NMP	3 (15.0)
Machine perfusion time (min)	240.0 [183.0, 297.8]
Tot. pres. time (min)	510.5 [416.8, 544.8]
Operation time (min)	353.0 [300.0, 388.0]
PRBC transfused (units)	1.5 [0.0, 6.5]
Lactate end of LT (mmol/L)	1.6 [1.0, 3.4]
Severe PRS	5 (25.0)
AST peak (IU/L)	827.0 [661.5, 1862.2]
ALT peak (IU/L)	499.0 [290.5, 770.8]
EAD	5 (25.0)
Stage 2–3 AKI	3 (15.0)
RRT	1 (5.0)
L-GrAFT (risk %)	13.0 [10.5, 18.9]
EASE (risk %)	1.5 [1.1, 5.6]
Reoperation	4 (20.0)
Clavien-Dindo ≥3	7 (35.0)
CCI at discharge	21.8 [0.0, 41.5]
Early graft failure	1 (5.0)
ITU stay (days)	3.5 [2.0, 5.2]
Hospital stay (days)	13.0 [8.8, 19.2]
Biliary complications	
Anastomotic	1 (5.0)
ITBL	1 (5.0)

Note: Values are presented as counts (percentage) and median (interquartile range).

Abbreviations: AKI, acute kidney injury; BMI, body mass index; CCI, comprehensive complication index; EAD, early allograft dysfunction; EASE, early allograft failure simplified estimation; HCC, hepatocellular carcinoma; ITU, intensive therapy unit; LGrAFT, liver graft assessment following transplantation score; LT, liver transplantation; MELD, model for end-stage liver disease; PRBC, packed red blood cells; RRT, renal replacement therapy.

TABLE 2 Donor and A-NRP characteristics.

	Discarded (negative outcome) ($n=7$)	Transplanted (positive outcome) ($n=20$)	p
Age	55 [37.5; 59.5]	56.5 [53.8; 63.5]	0.23
Sex female (%)	2 (28)	6 (30)	1
BMI	27.8 [25.2; 28.0]	26.2 [23.1; 27.9]	0.51
Macrosteatosis (%)	0.0 [0.0; 0.0]	1.0 [0.0; 5.0]	0.02
0%–15%	7 (100)	18 (90)	0.975
15%–30%	0 (0)	0 (0)	
>30%	0 (0)	2 (10)	
Microsteatosis (%)	0.0 [0.0; 7.5]	6.5 [0.0; 20.0]	0.40
Necrosis (%)	5.0 [5.0; 5.0]	5.0 [2.8; 6.2]	0.68
Cause of death			
Anoxic brain injury	6 (86)	17 (85)	0.96
Stroke	1 (14)	3 (15)	0.96
FWIT (min)	47 [44; 55]	44 [39; 49.8]	0.53
Asystolic time (min)	30 [28; 33]	30 [28.25; 31]	1
Mean blood flow (L/min)	3.09 [2.87; 4.05]	3.00 [2.64; 3.27]	0.24
Fluids (mL/h)	250 [165; 1000]	1500 [1125; 1875]	0.06
PRBC units (units/h)	1.67 [1; 1.75]	1.5 [1; 2]	0.78
Hb start A-NRP (g/dL)	6.8 [5.9; 8.6]	8.2 [6.65; 9.22]	0.45
Hb 2 h A-NRP (g/dL)	7.2 [6.6; 8.3]	7.5 [6.6; 8.6]	0.93
A-NRP time (min)	220 [205; 275]	233 [200; 249]	0.73

Note: Values are presented as counts (percentage) and median (interquartile range).

Abbreviations: A-NRP, Abdominal normothermic regional perfusion; BMI, body mass index; FWIT, functional warm ischemia time; PRBC, packed red blood cells.

Values and trend of lactate, AST, ALT, blood flow, VO_2 and DO_2 are reported and depicted in Table 3 and Figure 2. Lactate levels was comparable between groups at the beginning of A-NRP (T0) but diverged at 1 (T1), 2 (T2) and 3 (T3) hours of A-NRP. AST and ALT levels were significantly different between the two groups at T0, T1, T2 and T3. Overall, the time course of lactate, AST and ALT (Figure 2, panel A, B and C) was significantly different between the two groups over time ($p<0.001$). DO_2 was comparable at each time point but T3, whereas VO_2 was significantly higher in the positive outcome group at T1; however, the time course of both DO_2 and VO_2 (Figure 2, panel D and E) was significantly different ($p=0.03$ and $p=0.009$, respectively).

Lactate levels at T0 were significantly correlated with FWIT ($r^2=0.4$, $p=0.04$), whereas neither AST nor ALT values were (Figure 3). As compared to discarded grafts, accepted grafts showed faster lactate clearance during A-NRP (Figure 4). This was analyzed both in terms of absolute values (Figure S1) and after normalizing lactate clearance to DO_2 and VO_2. Lactate clearance was not directly correlated with DO_2 (Figure S2). However, the time course of percentage, VO_2-normalized and DO_2-normalized lactate percentage decrease was significantly different between study groups ($p<0.001$, $p<0.001$ and $p=0.03$, respectively). In particular, DO_2-normalized lactate clearance best discriminated between accepted and discarded grafts (Figure 4). The discriminatory ability of DO_2-normalized lactate clearance after 2 h of NRP was confirmed at ROC analysis (AUC 0.89, confidence interval 0.77–0.1), with a DO_2-normalized lactate reduction of 0.086% per mL of DO_2 from the baseline at 2 h of A-NRP showing 100% specificity and 75% sensitivity in identifying outcome group. Both absolute and DO_2-normalized lactate clearance were positively correlated with transaminase levels (Figure 5).

4 | DISCUSSION

Our results stress the importance of adequate oxygen delivery during A-NRP, highlight the importance of lactate metabolism as a viability parameter also in this setting, and confirm that the sequential use of A-NRP followed by ex-situ machine perfusion is associated with good results in DCD LT with prolonged warm ischemia time.

TABLE 3 A-NRP parameters.

	T0	1 h	2 h	3 h	4 h
Lactate (mmol/L)					
Accepted	10.0 [8.85; 11.7]	7.6 [6.25; 8.93]	5.2 [3.8; 8.27]	4.6 [2.65; 7.15]	6.4 [2.7; 8.2]
Discarded	11.8 [10.4; 13]	10.2 [9.8; 12.6]	10.7 [8.25; 12.6]	8.35 [7.12; 10.6]	9.05 [5.3; 12.8]
p	0.19	0.02	0.03	0.04	0.23
AST (U/I)					
Accepted	81 [59; 168]	152 [80; 212]	139 [81; 232]	148 [73; 253]	424 [110; 632]
Discarded	762 [526; 1739]	1127 [1074; 1736]	1164 [945; 1785]	1783 [1317; 2365]	1670 [1670; 1670]
p	0.006	0.003	0.003	<0.001	0.15
ALT (U/I)					
Accepted	61 [41; 121]	77 [58; 212]	78 [58; 268]	107 [54; 324]	342 [73; 381]
Discarded	422 [218; 879]	665 [465; 989]	904 [368; 1242]	949 [652; 1476]	971 [971; 971]
p	0.006	0.003	0.003	<0.001	0.15
Blood flow (L/min)					
Accepted	3.1 [2.58; 3.66]	3.35 [2.58; 3.70]	2.95 [2.5; 3.3]	2.95 [2.54; 3.2]	2.5 [2; 2.8]
Discarded	3.3 [2.65; 3.75]	3.15 [2.95; 4.05]	3.9 [3.15; 4.04]	3.66 [3.23; 4.33]	3.66 [3.03; 4.15]
p	0.87	0.56	0.10	0.04	0.04
DO_2 (mL/min)					
Accepted	326 [265; 420]	365 [297; 431]	287 [243; 350]	267 [206; 311]	247 [149; 295]
Discarded	316 [250; 442]	329 [276; 434]	369 [335; 422]	365 [365; 453]	379 [351; 408]
p	0.85	0.88	0.13	0.003	0.12
VO_2 (mL/min)					
Accepted	148 [109; 270]	94 [80; 138]	105 [93; 135]	81 [70; 97]	69 [63; 118]
Discarded	87 [65; 110]	51 [50; 59]	80 [76; 87]	68 [61; 112]	69 [69; 69]
p	0.06	0.006	0.08	0.68	1

Note: Values are presented as median (interquartile range).

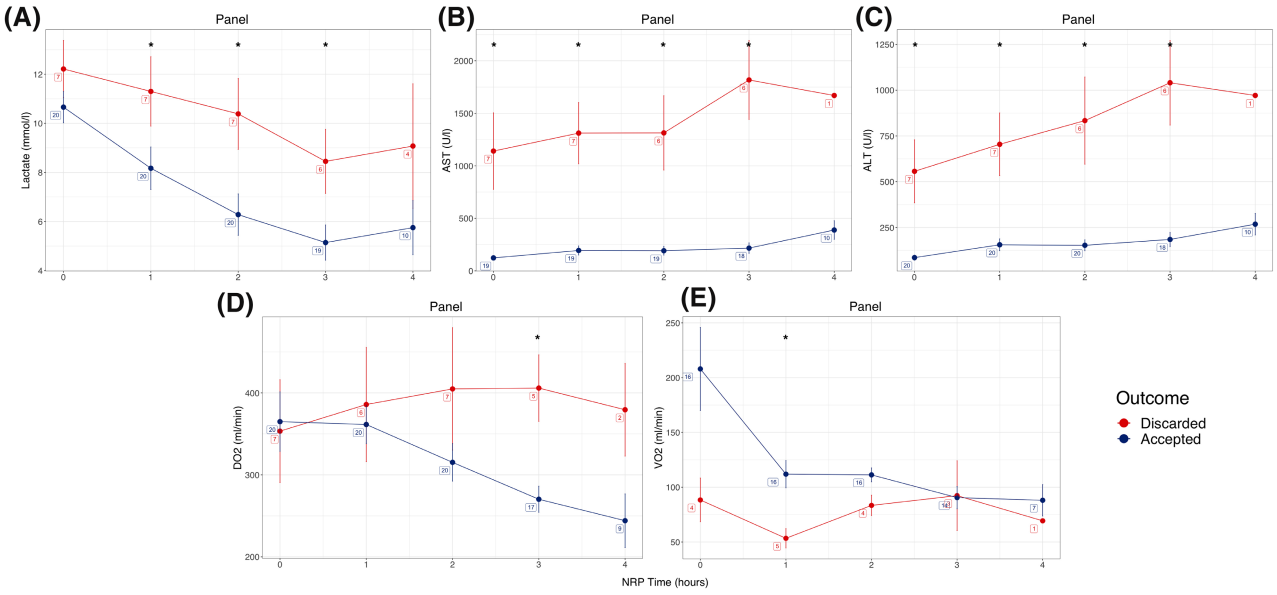

FIGURE 2 Time course of transaminase, lactate, DO_2 and VO_2 during A-NRP. [Color figure can be viewed at wileyonlinelibrary.com]

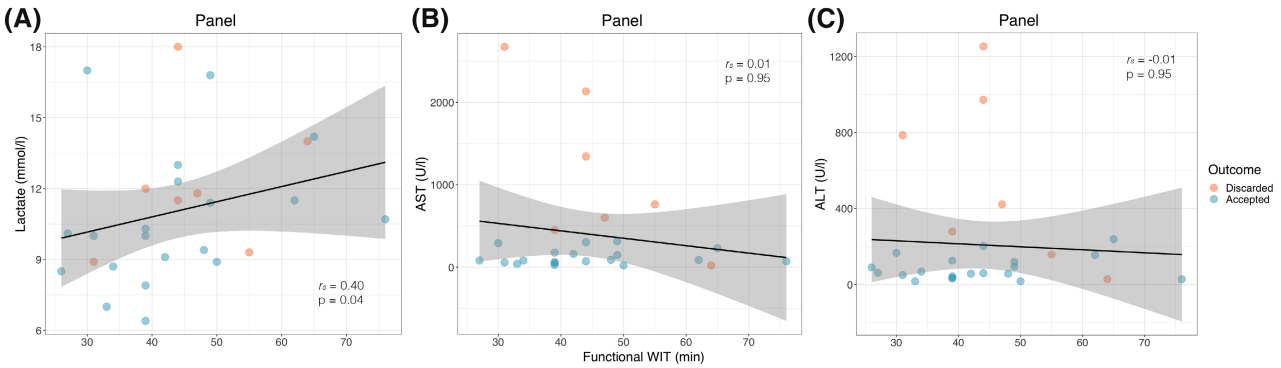

FIGURE 3 Scatterplots with regression lines depicting the correlation between functional warm ischemia time and lactate and transaminase level at the beginning of A-NRP. [Color figure can be viewed at wileyonlinelibrary.com]

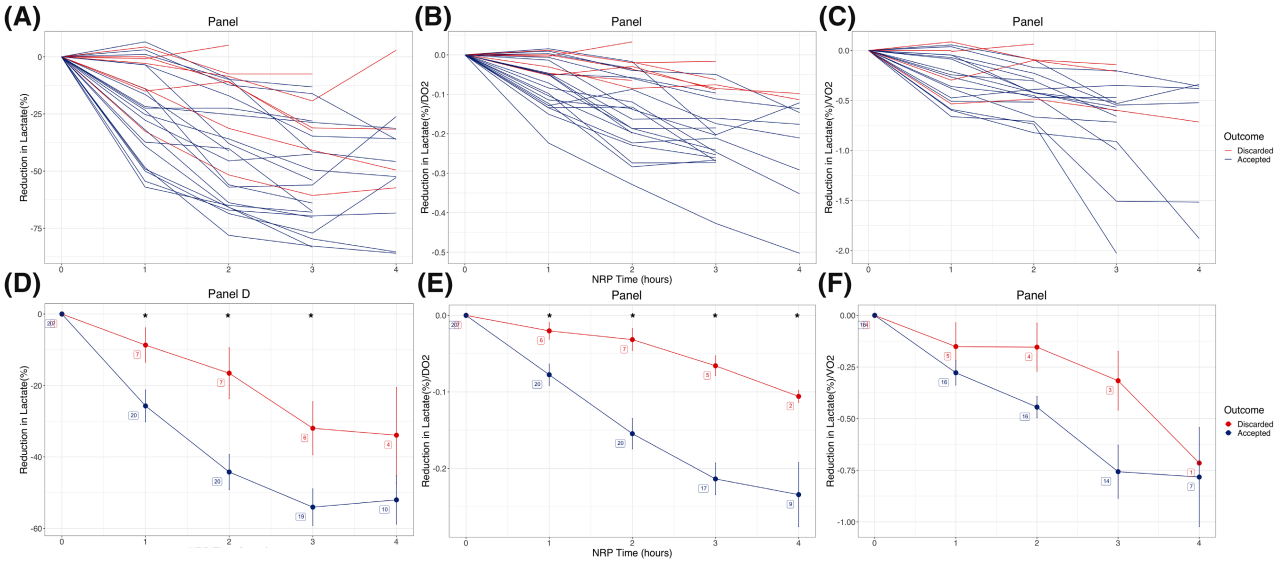

FIGURE 4 Time course of absolute and normalized lactate clearance during A-NRP. [Color figure can be viewed at wileyonlinelibrary.com]

Besides improving outcomes[11,19–24] and utilization rate,[25,26] one advantage of A-NRP is obtaining data about liver function and damage. However, viability criteria are not clearly defined and lactate clearance is considered in only ~50% of protocols,[8] due to the interference of continuous lactate release from ischemic tissue (limbs and thorax) into the circuit. This is in steep contrast with normothermic machine perfusion, during which lactate clearance is considered an important indicator of viability.[27–29]

In our series, donor characteristics and warm ischemia times did not differ between accepted and discarded grafts, whereas A-NRP parameters dictated graft acceptance. Although lactate levels at the beginning of A-NRP correlated with FWIT, they did not predict graft utilization. In contrast, transaminase and lactate time course were significantly different between groups. Overall, our data suggest that, while transaminase levels was the main decisional element, lactate clearance normalized to DO_2 could equally serve as a viability parameter. Noteworthily, lactate clearance and transaminase levels after 2 h of A-NRP were positively correlated, suggesting concordance between liver damage and function. The practical implication of our results is that adequate oxygen delivery during A-NRP is a necessary prerequisite to reliably assess liver viability. If for technical reasons this cannot be achieved, viability assessment should better be postponed during ex-situ normothermic machine perfusion.[30]

It should be noted that no donor in our series experienced important blood losses during A-NRP, as reflected by the low hourly PRBC requirements in both groups (Table 2). Gradual volume loss is frequently observed during A-NRP and PRBC transfusions are frequently needed, as an effect of the need for volume expansion and the blood losses during cannulation and surgical maneuvers in a fully anticoagulated patient.[31] As PRBC can have high lactate levels, significant blood losses (e.g., combined lung procurement) can jeopardize the ability to meaningfully evaluate lactate clearance.

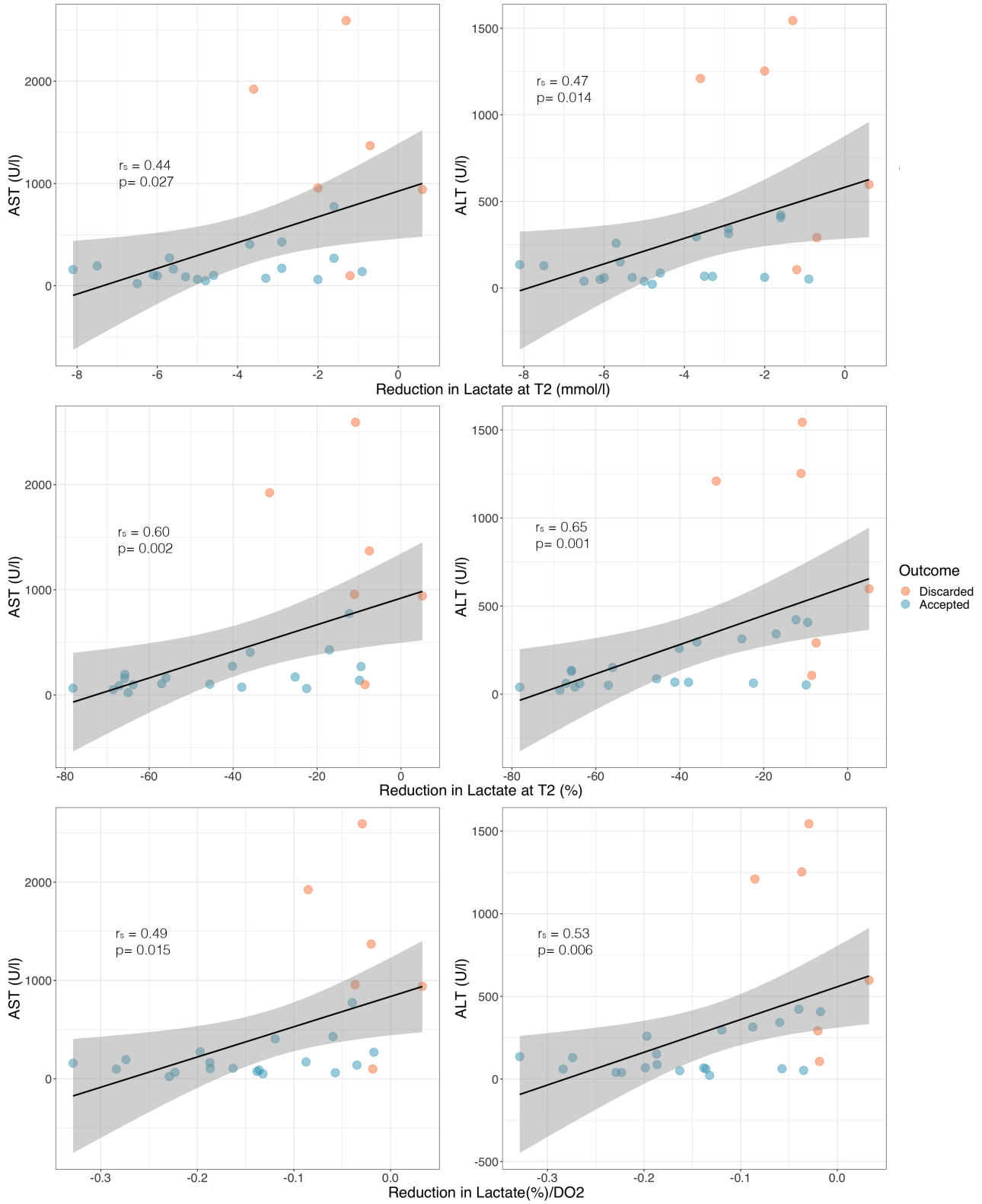

FIGURE 5 Correlation between lactate clearance and second-hour transaminase. [Color figure can be viewed at wileyonlinelibrary.com]

Our data also highlight the importance of perfusion parameters during A-NRP. Although a blood flow ≥1.7 L/min/m^2 has been frequently indicated as the threshold to provide adequate organ perfusion,[4,32] target blood flow in our series was ≥40 mL/kg/min,[12] corresponding to ≥50% of the target blood flow during clinical veno-arterial ECMO. This was effective in providing adequate oxygenation, as demonstrated by the DO_2 largely

exceeding VO_2 at any timepoint. Accepted and discarded graft appear to utilize oxygen differently: while accepted grafts avidly consumed oxygen during the first 2 h of A-NRP, oxygen utilization by discarded grafts was lower (Figure 2, Panel E), suggesting quicker metabolic recovery in the positive outcome group.

Finally, our favorable results, in keeping with previous series,[6,33–36] confirms that by combining A-NRP with ex-situ machine perfusion, good clinical outcomes can be achieved also in DCD donors with extremely prolonged warm ischemia time. In settings characterized by shorter warm ischemia times, use of A-NRP alone has been associated with superior outcomes in DCD LT,[11,19,20,24] with results comparable to those of DBD LT.[21–23] Similarly, both hypothermic oxygenated[37,38] and normothermic[39,40] machine perfusion have been shown to improve outcomes of DCD LT. To cope with prolonged FWIT, most Italian centers have adopted a sequential approach of A-NRP followed by ex-situ machine perfusion. Our data are in keeping with a recent consensus statement from the European Society for Organ Transplantation and confirm that prolonged warm ischemia time should not constitute per se a contraindication to graft acceptance.[5]

Limitations of our study include its retrospective single-center design and low numbers. The choice of graft acceptance as primary outcome, in particular, was forcedly suboptimal, as arguably some grafts discarded during A-NRP could have been further evaluated during ex-situ machine perfusion and possibly transplanted.[30] Given the inherent difficulties of our setting, the early phase of our experience and the concerns for patient safety, this approach was not systematically explored and will be matter of further studies. In the only case in this series in which normothermic MP was used as a tool for further viability assessment, it did not change the initial decision to discard the graft. Additionally, viability assessment during normothermic MP is still an imperfect science and criteria are constantly evolving. However, also given the results of this study, we are increasingly considering ex-situ normothermic MP as an additional possibility of viability assessment, especially in those cases in which, for any reason, pump flow and DO_2 are suboptimal.

In conclusion, this study adds further insight into the complex task of assessing liver viability during A-NRP and suggest that, if adequate perfusion and oxygen delivery are provided, the trend of lactate clearance, especially if normalized to oxygen delivery, could represent a precious element to assess liver viability. Further studies are necessary to define lactate clearance threshold for graft acceptance and explore alternative approaches to further increase organ utilization.

AUTHOR CONTRIBUTIONS

Data collection, analysis and interpretation, drafting article: Irene Steinberg and Damiano Patrono; data collection and critical revision of article: Enrico De Cesaris, Michele Lucà, Giorgia Catalano, Matteo Marro, Giorgia Rizza, Erika Simonato; critical revision and approval of article: Luca Brazzi and Renato Romagnoli; conception/design, article drafting and revision: Marinella Zanierato.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest with the contents of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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