

Article

Assessing Lactate's Role as a Predictor of Post-Transplant Outcomes During Normothermic Machine Perfusion

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Abstract

Background/Objectives: The use of extended-criteria donors (ECDs) is rapidly increasing, with higher risks for impaired outcomes after liver transplantation (LT). Normothermic machine perfusion (NMP) can enhance graft viability assessment, though lactate clearance as a viability marker lacks consensus in clinical practice. **Methods:** This study analyzed 213 adult liver transplants using NMP from October 2022 to January 2024. Perfusate lactate levels were measured with an Epoc[®] reader and five lactate-based viability criteria were evaluated for predicting post-LT outcomes. **Results:** All livers cleared lactate at 6 h of NMP (median 0.51 mmol/L). However, 33 livers failed at least one lactate viability criterion. Thirteen livers (6.1%) were discarded based on traditional criteria, but discard rates would have been higher using Groningen (14.6%) or Brisbane (11.2%) criteria for lactate. Outcomes for the recipients of livers that failed the lactate criteria were comparable to those of recipients with livers meeting all criteria, including biliary complications ($p = 0.63$), graft survival (>0.99), and 6-month CCI ($p = 0.89$). ROC analysis revealed poor predictive value for graft survival for lactate clearance criteria (AUC 0.50), while lactate was outperformed by MEAF score (AUC 0.67). **Conclusions:** Lactate clearance alone does not predict outcomes, highlighting the need for further validation of alternative viability.

Keywords: liver transplantation; normothermic machine perfusion; lactate; viability assessment; graft survival; post-transplant outcomes



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1. Introduction

Liver transplantation (LT) is the only curative treatment for end-stage liver disease, yet the availability of donor organs remains insufficient to meet the growing demand [1]. To address this gap, extended-criteria donors (ECD) are increasingly utilized, including those donated from donors with advanced age, macro-steatosis, or after circulatory death (DCD), with extended warm and cold ischemia times [2,3]. Although these grafts carry higher post-transplant risk, careful selection and management can yield outcomes comparable

to standard grafts, improving waitlist results [4–6]. Dynamic preservation techniques are increasingly adopted by numerous transplant centers, improving organ preservation and providing an ideal platform to test viability [7]. Two main ex situ liver perfusion techniques are currently employed: hypothermic oxygenated (HOPE) and normothermic machine perfusion (NMP). HOPE is conducted at low temperatures (8–11 °C) using highly oxygenated artificial preservation fluid protecting mitochondria from ischemia–reperfusion injury (IRI) [8,9]. In contrast, NMP technologies aim for near-physiologic perfusion conditions, performed at 37 °C using blood-based perfusate [10]. While such perfusion settings provide the environment for liver quality assessment and therapeutic interventions, it is of relevance to understand the mechanisms of IRI-associated inflammation and how they contribute to perfusate levels of viability parameters [11,12].

In this context, lactate has emerged as the most widely applied biomarker during NMP. Lactate clearance reflects the recovery of hepatocellular oxidative metabolism and mitochondrial function following ischemic injury, whereas impaired clearance may indicate persistent cellular injury and reduced graft viability. However, perfusate lactate levels may also be influenced by factors independent of intrinsic liver quality, including perfusate flow dynamics, oxygen delivery, red blood cell metabolism, and donor-related systemic metabolic conditions, potentially complicating its interpretation as a standalone marker of viability [13].

Various viability criteria have recently been proposed for NMP, most relying on lactate clearance [10,14–20], yet the numerous perfusate lactate thresholds at different timepoints lack consensus [12,19,21]. This lack of standardization introduces variability in clinical decision-making during graft assessment, with potential consequences for graft acceptance, discard rates, and post-transplant outcomes.

Therefore, this study systematically compares the predictive performance of five commonly used lactate-based viability criteria assessed during NMP in relation to post-transplant outcomes. These criteria are further evaluated alongside traditional donor and recipient risk factors as well as markers of mitochondrial injury, with the aim of clarifying their clinical relevance and limitations.

2. Materials and Methods

2.1. Study Design

This retrospective cohort analysis included adult patients (>18 years) undergoing deceased-donor LT with grafts preserved by normothermic machine perfusion (NMP) between October 2022 and January 2024 at Cleveland Clinic, Ohio. Patients were identified from a prospectively maintained institutional database. All patients with 12 months of follow-up from time of transplant were included. Patients with graft loss or death before 12 months were not excluded from the study. The study was approved by the Institutional Review Board (IRB No. 23-1348) and was conducted in accordance with the Declaration of Helsinki.

2.2. Normothermic Machine Perfusion

The OrganOx Metra (OrganOx, Oxford, UK) was used for all ex situ liver perfusions. The criteria for machine perfusion utilization are DCD grafts, ECD DBD livers, open offers, combined organ transplants and logistics, with a final decision by the transplanting surgeon [22]. Extended-criteria donors included DBD donors > 60 years old, macro-steatosis > 20%, high-risk donors (elevated transaminases, HCV-positive), and predicted prolonged cold ischemia time > 8 h. Open offers or out-of-sequence offers define grafts that bypass the traditional allocation system. The detailed inclusion criteria for NMP are listed in Table S1. Grafts underwent NMP as a back-to-base approach, following standard procurement with

cold flush and transport on ice. The hepatic artery, portal vein, inferior vena cava and bile duct were cannulated for NMP as per routine. Livers were perfused at 37 °C using packed red blood cell-based perfusate with the following additives: albumin, heparin, insulin, ceftriaxone, and calcium gluconate [11]. At the end of back-table preparation, livers were connected to the device and perfused until the recipient hepatectomy was completed. During the study period, no additional packed red blood cell transfusions were routinely administered during NMP after the initial perfusate preparation.

2.3. Viability Assessment Criteria and Institutional Protocol

Currently available viability criteria with many supporting proposals were evaluated (Table 1) [19,21,23–25]. Since the primary focus of our study is on perfusate lactate clearance, we evaluated only criteria that included lactate thresholds. Most groups use a combination of different parameters and have repeatedly updated their specific viability criteria, including perfusate lactate thresholds and decision timepoints during perfusion (Table 1). Our center's viability criteria, initially published by Quintini et al., were first utilized with our in-house pump in a non-randomized trial with discarded organs. In addition to a comprehensive macroscopic liver evaluation, liver viability assessment was based on lactate clearance defined as a lowest perfusate lactate of <4.5 mmol/L or a decrease of 60% from the initial peak within the first 4 h of NMP [26]. Since the initiation of clinical OrganOx metra use, our policy involves a more detailed biochemical evaluation of perfusate and bile. This analysis traditionally included perfusate lactate, pH, glucose, and bile flow, bile pH and biochemistry. Biopsies are performed at the accepting surgeon's request. While grafts accepted for transplantation are required to clear lactate sufficiently, our team does not solely rely on single or absolute lactate values. Instead, lactate levels are considered together with other parameters assessed in the context of donor and recipient risk and preservation parameters. During the study period, grafts were not accepted for transplant when lactate was not sufficiently cleared during initial NMP or if it increased again during later perfusion.

Perfusate samples were obtained at priming (0 min), 15 min, 30 min, 1 h and then hourly until the end of NMP. Perfusate lactate samples were obtained directly from the NMP circuit and analyzed immediately after collection using the Epoc[®] Blood Gas Analysis System, without storage or delay between sampling and analysis. The Epoc[®] system is a cartridge-based point-of-care device with automated internal calibration and quality control performed for each measurement according to the manufacturer's specifications, thereby minimizing analytical variability. The Groningen criteria involve decision-making at 2.5 h NMP, a timepoint not routinely served at our center. The median between the 2 h and 3 h lactate values was used for our analysis [23]. Bile samples were collected hourly when bile was produced by the graft during NMP.

Table 1. Proposed traditional viability criteria during NMP involving perfusate lactate.

Center	Author, Year	Perfusate					Bile	Decision Timepoint	Criteria for Decision
		Lactate	pH	Glucose	ALT	PV/HA Flow	Bile Flow and Composition		
Cambridge	Watson 2018 [14]	Peak lactate fall $\geq$ 4.4 mmol/L/kg/h	Maintain perfusate pH > 7.2 with $\leq$ 30 mmol bicarbonate supplementation	Falling glucose beyond 2 h OR perfusate glucose < 10 mmol/L with subsequent fall during challenge with 2.5 g glucose	<6000 iU/L	-	pH peak > 7.5 Glucose: $\geq$ 10 mmol less bile than perfusate glucose or, if perfusate glucose < 11 mmol/L, a bile glucose of $\leq$ 3 mmol/L	2 h	2 h ALT, 2 h lactate, requirement for supplementary bicarbonate
	Watson 2022 [19]	<2.8 mmol/L	Continuing requirement for bicarbonate to maintain pH > 7.2 beyond 2 h	When glucose < 10 mmol/L at 30 min $\rightarrow$ 5 g dextrose challenge, glucose rate should fall $\geq$ 1 mmol/L/h at 4 h NMP	<6000 iU/L	-	Flow Yes or No (DCD) pH Peak > 7.5 Glucose: $\geq$ 10 mmol less than in perfusate or, if perfusate glucose < 11 mmol/L, a bile glucose of $\leq$ 2 mmol/L	2 h	2 h ALT, 2 h lactate, highest bile pH, requirement for supplementary bicarbonate in first 4 h, bile production in DCD
Birmingham	Mergental 2016 [15]	<2.5 mmol/L	>7.3	Yes or No	-	PV: >500 mL/min HA: >150 mL/min	Flow Yes or No	4 h	Lactate and 2 of the other criteria
	Mergental 2020 [21]	<2.5 mmol/L	>7.3	Yes or No	-	PV: >500 mL/min HA: >150 mL/min	Yes or No pH > 7.3	4 h	Lactate and 2 of the other criteria
Groningen	Van Leeuwen 2019 [16]	$\leq$ 1.7 mmol/L	7.35–7.45	-	-	-	Flow > 10 mL pH > 7.45	2.5 h	Above-mentioned markers and thresholds
	Van Leeuwen 2022 [23]	Green: <1.7 mmol/L; orange: 1.7–4 mmol/L; red: >4 mmol/L	Green: 7.35–7.45, Orange: 7.25–7.35, Red: <7.25	-	-	-	Flow: Green: $\geq$ 10 mL, $\geq$ 4 mL/last hour; orange: 5–10 mL; red: <5 mL pH: Green: $\geq$ 7.45; orange: 7.40–7.45; red: <7.40 Δ pH: Green: >5; orange: 3.0–5.0; red: <3 Δ glucose: Green: <−5; orange: −3 to −5; red: >−3 Δ HCO ₃ [−] : Green: >0.1; orange: 0.05–0.01; red: <0.05	2.5 h	Parameter in the orange zone could be accepted if other criteria are green, red zone declined

Table 1. Cont.

		Perfusate					Bile	Decision Timepoint	Criteria for Decision
Center	Author, Year	Lactate	pH	Glucose	ALT	PV/HA Flow	Bile Flow and Composition		
Münster	Vogel 2024 [25]	Old: <3.5 mmol/L	-	-	-	HA flow > 150 mL/min	Yes or No (viscous bile) -	4 h	Above-mentioned markers and thresholds
	Vogel 2024 [25]	MVP: <2.0 mmol/L	-	-	-	-	-	6 h	Above-mentioned markers and thresholds
Brisbane	Reiling 2024 [24]	<2 mmol/L within 2 h	Maintenance of physiological pH without the need of ongoing administration of sodium bicarbonate	Decreasing trend by 4 h	-	Stable flows	Flow Yes or No	4 h	Above-mentioned markers and thresholds and homogeneous graft perfusion with soft consistency of parenchyma
Innsbruck	Cardini 2020 [12]	Prompt decline of perfusate lactate levels ≤ 2.5 mmol/L	Maintenance of physiological pH without the need of ongoing administration of sodium bicarbonate		AST > 20,000 U/L ALT > 20,000 U/L LDH > 20,000 U/L	-	-	-	Above-mentioned markers and thresholds and excessively high IL-6 levels
	Weissenbacher 2022 [27]	<18 mg/dL (pprox. < 2.5 mmol/L)	7.3–7.45			Stable flows	Yes or No	6 h	Above-mentioned markers and thresholds

Colors used to differentiate current available Lactate criteria during NMP correspond to the colors used for the same groups in Figure 1c. Abbreviations: ALT: Alanine aminotransferase, AST: aspartate aminotransferase, NMP: normothermic machine perfusion, DCD: donation after circulatory death, IL-6: interleukin 6, MVP: Münster viability protocol.

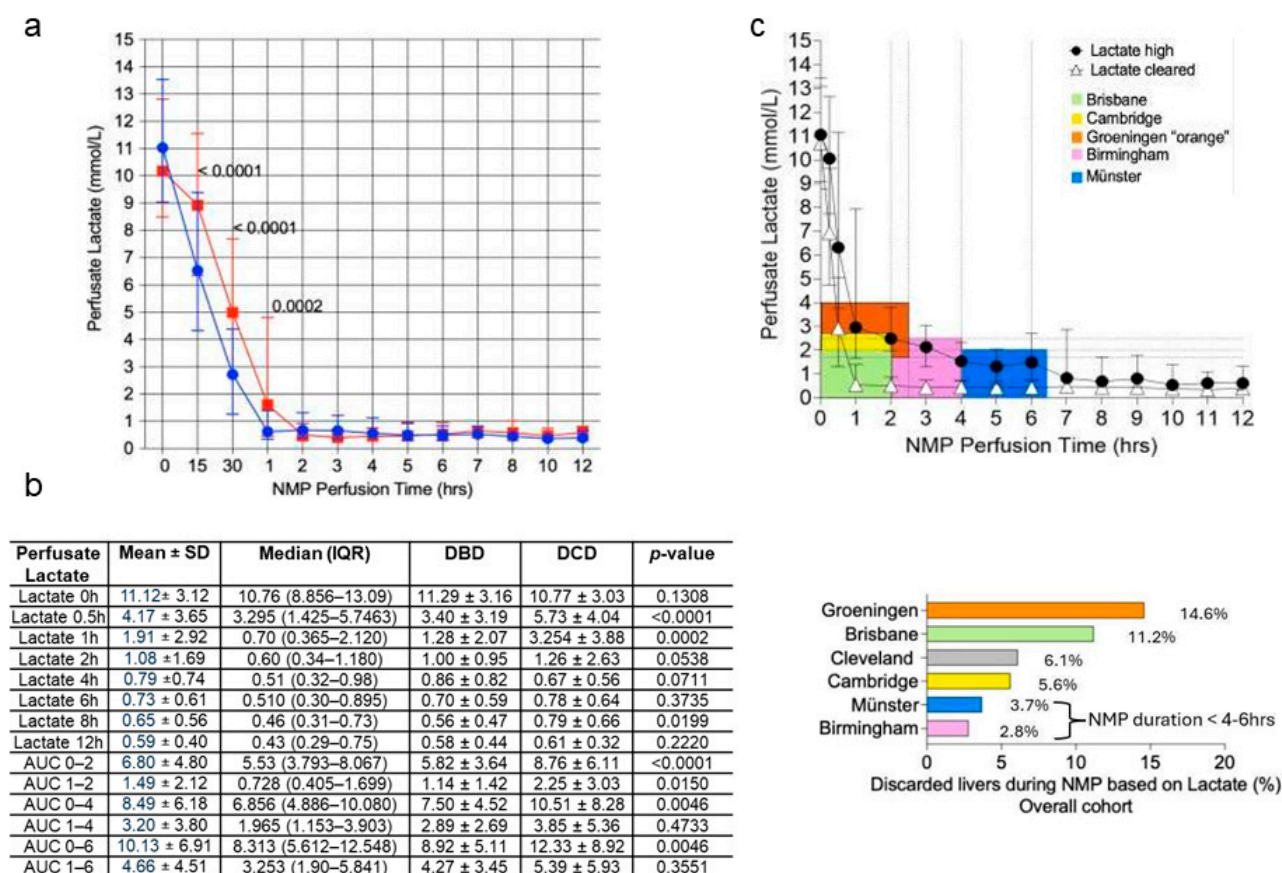


Figure 1. Lactate clearance along with criteria proposed by different groups. (a,b) Lactate values and clearance in DBD (blue) and DCD (red) overall cohorts; trend of high lactate (black dots) and cleared lactate (white triangles) and current lactate thresholds for viability (Brisbane, Cambridge, Groningen, Birmingham and Münster) with respective discard rates when applied to our overall cohort (c). Münster and Birmingham criteria are applied only to a restrict cohort because of thresholds involving longer NMP durations. In template b, mean and median are shown for visualization purposes.

Based on available criteria, the following lactate thresholds and timepoints were used in the analysis: <2 mmol/L at 2 h NMP (Brisbane criteria) [24], <2.8 mmol/L at 2 h (Cambridge criteria) [19], <1.7 mmol/L at 2.5 h (Groningen criteria) [23], <2.5 mmol/L at 4 h (Birmingham criteria) [21], and <2 mmol/L at 6 h (Münster criteria) [25]. Additionally, the Innsbruck group suggested that livers with a prompt perfusate Lactate decline to ≤ 2.5 mmol/L should be accepted. The authors do not provide an exact timepoint for decision-making. Such Innsbruck lactate criteria are however well covered by the above-mentioned values and criteria proposed by others, in that livers that would not meet the “Innsbruck criteria” also do not meet other criteria [12,27,28]. Perfusate lactate was quantified at 2, 2.5, 4, and 6 h, covering the above-mentioned criteria.

The Groningen group developed an “Ample” system with three categories for all viability parameters. Livers with perfusate lactate > 4 mmol/L at 2.5 h of NMP would be declined immediately (“red group”). Perfusate lactate levels between 1.7 mmol/L and 4 mmol/L would allocate livers to the “orange group” (Table 1). Two to three viability parameters would be required to fall in the orange category to decline a liver, as per personal communication by the Groningen team [23]. Livers with lactate in the orange group were therefore allocated to the “lactate-high” group in our analysis. At our center, decisions regarding liver acceptance or not are made within the first 4–6 h of NMP.

During NMP, graft acceptance at our center is not based on a fixed algorithm but on a comprehensive clinical evaluation incorporating macroscopic appearance, perfusion dy-

namics, biochemical parameters, bile production, and donor–recipient risk profiles. During the early phase of the cohort, decisions were guided by previously published criteria; however, clinical judgment remained central to decision-making. Lactate thresholds proposed by external centers were applied retrospectively for research purposes only and did not influence clinical decision-making in this study.

Our immunosuppression protocol includes a standard combination of steroid induction, tacrolimus and mycophenolate mofetil. Recipients with existing kidney injury, positive crossmatch or redo liver transplantation receive basiliximab induction.

2.4. Study Endpoints and Definitions

The electronic medical record was reviewed to collect donor and recipient information, with additional data supplemented from the United Network for Organ Sharing (UNOS) and Scientific Registry of Transplant Recipients (SRTR) databases as needed. The overall risk associated with the transplant, including both donor/graft and recipient risk factors, was estimated using various scoring systems: BAR, DRI, MEAF, L-GrAFT7, EASE, and the UK DCD risk score. Routine intra- and post-transplant outcomes were assessed including liver transplant-related, biliary, vascular and overall complications using the Clavien–Dindo classification and the Comprehensive Complication Index (CCI) during hospital stay and at 3 and 6 months [29,30]. Other outcomes included the new requirement for renal replacement therapy (RRT) and rates of acute cellular rejection (ACR), early allograft dysfunction (EAD), and hepatic artery thrombosis (HAT). Overall biliary complications were divided into anastomotic (AS) and non-anastomotic stricture (NAS). Six- and twelve-month graft and recipient survival and death-censored graft loss were analyzed.

2.5. Statistical Analysis

Data are presented as median and interquartile range (IQR), or as *n* and %, or mean and Standard Deviation (SD). Statistical analysis was performed using the non-parametric Mann–Whitney U test or the Chi-square and Fisher tests to account for the sample size. Regression analysis, point-biserial correlation and two-way Anova tests were used to assess correlations between variables. Survival analysis using Kaplan–Meier methods with censoring at last follow-up were performed. Death-censored graft survival was calculated with death treated as a censoring event. Cox proportional hazards regression models and a mixed-effect model were used to assess the association between predictor variables and graft survival. Hazard ratios (HRs) along with 95% CIs were estimated. The amount of missing data was minimal (<5%) and was handled using complete-case analysis.

The statistical analyses were conducted using GraphPad Prism, version 10 (San Diego, CA, USA) and SPSS Version 24.0. A *p*-value of <0.05 was considered statistically significant.

3. Results

3.1. Study Population and Risk Factor Distribution

During the study period, 213 livers underwent NMP for different reasons (Table S1). The donor demographics and perfusion characteristics are summarized in Table 2. Within this cohort, 69 grafts were from DCD donors (32.4%). In total, 200 livers were transplanted and 13 (6.1%) were discarded for various historical criteria including a lack of bile production, low perfusion flows during NMP, “poor biopsy results” or “poor lactate clearance”. Only one liver was discarded because of lactate rebound at 4 h and a value of 5 mmol/L. NRP was used in two cases for DCD liver procurement based on OPO practice. In total, 25 of the transplants were combined transplants (liver–cardiac procedure, liver–kidney, liver–heart, liver–lung transplantation), and seven were retransplants. Median cold ischemia time (CIT) was 6.3 h (5.2–7.3) followed by a median NMP of 8.62 h (5.88–12.80),

resulting in a median total preservation time of 15.4 h (12.0–18.8). EAD occurred in 52 (26%) of recipients. The median MEAF score was 3.50 (2.21–5.46) points. The L-GrAFT score at day 7 was −3.37 (−3.77–2.86) points.

Table 2. Donor and recipient risk factors and post-transplant outcomes.

	Overall Cohort	Lactate Cleared	Lactate High	<i>p</i> Value	Discarded
Donor and Preservation Parameter	(n = 213)	(n = 180)	(n = 33)		(n = 13)
Donor age (y)	51.0 (37.0–61.0)	51.0 (35.3–61.0)	49.0 (40.5–64.5)	0.45 ^a	44.0 (35.0–62.0)
Donor sex, male (n/%)	114 (53.5)	103 (57.2)	11 (33.3)	0.011 ^b	4 (30.8)
Donor BMI (kg/m ²)	27.7 (24.0–32.1)	27.9 (24.1–32.1)	27.4 (23.6–33)	0.75 ^a	29.7 (25.6–32.8)
DRI (points)	1.9 (1.5–2.3)	1.8 (1.5–2.2)	2.0 (1.6–2.4)	0.17 ^a	2.1 (1.4–2.6)
Donor type, DCD (n/%)	69 (32.4)	58 (32.2)	11 (33.3)	0.95 ^b	7 (53.8)
Graft weight (g)	1913.5 (1685.3–2215.3)	1898.5 (1679.0–2179.5)	2187.0 (1725.8–2427.5)	0.053 ^a	1932.0 (1825.0–2430.0)
Total DWIT (min)	23.0 (19.0–28.0)	23.5 (19.8–27.0)	20.0 (19.0–25.0)	0.43 ^a	25.0 (16.0–27.0)
fDWIT (min)	19.0 (16.0–25.0)	19.0 (16.0–23.0)	19.0 (17.5–23.0)	0.83 ^a	18.0 (15.0–22.0)
Cold storage (h)	6.3 (5.2–7.2)	6.2 (5.1–7.2)	6.4 (5.6–7.3)	0.21 ^a	7.3 (5.8–8.0)
NMP duration (h)	8.6 (5.9–12.8)	8.5 (5.9–13)	9.1 (5.3–12.5)	0.79 ^a	7.8 (4.3–12.4)
Total preservation (h)	15.4 (12.0–18.8)	15.3 (12.0–18.8)	16 (11.1–20.3)	0.96 ^a	16.5 (12.9–20.1)
Discarded (n/%)	13 (6.1)	8 (4.5)	5 (15.5)	0.018 ^b	
Recipient Parameter	(n = 200)	(n = 172)	(n = 28)		
Recipient age (y)	58.0 (51.0–65.0)	58.0 (51.0–65.0)	56.5 (49.3–66.0)	0.99 ^a	
Recipient gender, male (n/%)	124 (62.0)	107 (62.2)	17 (60.7)	0.88 ^b	
Recipient BMI (kg/m ²)	28.6 (25.1–34.2)	28.2 (25.0–34.1)	29.6 (24.7–34.9)	0.79 ^a	
Lab MELD (points)	19.0 (13.0–25.0)	19.0 (13.3–46.0)	19.5 (12.3–24.8)	0.95 ^a	
BAR score (points)	8.0 (4.0–10.0)	8.0 (4.0–10.0)	7.5 (4.0–10.0)	0.85 ^a	
UK DCD score (points)	7.0 (5.0–8.0)	7.0 (5.0–8.0)	5.0 (2.5–7.5)	0.13 ^a	
Duration of surgery (h)	7.2 (6.6–8.0)	7.2 (6.5–8.1)	7.4 (6.8–7.9)	0.62 ^a	
Recipient WIT (min)	39.0 (34.0–43.8)	38.0 (34.0–43.0)	40.5 (32.8–46.8)	0.51 ^a	
Outcome Parameter					
AST peak, U/L	1046.0 (622.5–2107.0)	1008 (617.5–1886.75)	1359.5 (664.75–2651.25)	0.23 ^a	
ALT peak, U/L	388.5 (216.3–656.0)	349 (210.25–627.5)	466.5 (298–974.25)	0.048 ^a	
AUC AST day 0–7	1373.0 (826.5–2485)	1130.5 (710.6–2193)	1556 (961.5–2826.5)	0.058 ^a	
AUC ALT day 0–7	1977.0 (1210.0–3906.0)	1029.5 (584–1848.5)	1356 (1044.6–2101)	0.0075 ^a	
Peak INR	1.7 (1.6–2.0)	1.7 (1.6–2.0)	1.8 (1.6–2.2)	0.074 ^a	
Peak creatinine day 0–7 (mg/dL)	1.6 (1.2–2.2)	1.5 (1.1–2.2)	1.8 (1.4–2.7)	0.062 ^a	

Table 2. Cont.

	Overall Cohort	Lactate Cleared	Lactate High	p Value	Discarded
MEAF (points)	3.50 (2.21–5.46)	3.46 (2.16–5.35)	4.74 (2.40–6.29)	0.057 ^a	
L-GrAFT7 (points)	−3.37 (−3.77–−2.86)	−3.36 (−3.79–−2.81)	−3.45 (−3.75–−3.00)	0.71 ^a	
EASE (points)	−3.35 (−4.01–−2.62)	−3.35 (−4.02–−2.61)	−3.30 (−4.00–−2.66)	0.91 ^a	
Early allograft dysfunction	52 (26.0)	43 (25.0)	9 (32.1)	0.49 ^b	
Primary non-unction (n./%)	2 (1.0)	2 (1.2)	0 (0.0)	>0.99 ^b	
New need for RRT at 7 days (n/%)	11 (5.5)	10 (5.8)	1 (3.6)		
Overall new need for RRT (n/%)	20 (10.0)	18 (10.5)	2 (7.1)	0.75 ^b	
Early graft loss (≤15 days) (n/%)	5 ^{1a} (2.5)	4 (2.3)	1 (3.6)	0.53 ^b	
ICU length of stay (days) ²	5.00 (3.00–9.00)	5.00 (3.00–9.00)	4.50 (4.00–7.00)	0.63 ^b	
Hospital length of stay (days) ²	12.00 (9.00–20.75)	12.00 (9.00–21.00)	12.00 (9.00–19.00)	0.79 ^b	
Acute cellular rejection (n/%) ²	29 (14.9)	25 (14.9)	4 (14.8)	>0.99 ^b	
Biliary complications (n/%) ²	47 (24.1)	40 (23.8)	7 (25.9)	0.81 ^b	
Anastomotic stricture (n/%) ²	40 (20.5)	34 (20.2)	6 (22.2)	0.80 ^b	
Non-anastomotic stricture/ischemic cholangiopathy (n/%) ²	18 (9.2)	14 (8.3)	4 (14.8)	0.28 ^b	
Bile leaks (n/%) ²	6 (3.1)	6 (4.0)	0 (0.0)	>0.99 ^b	
CCI (points)					
-In-hospital	28.94 (12.55–49.62)	29.26 (12.25–48.67)	26.59 (20.92–51.75)	0.87 ^a	
-3-month	41.38 (24.24–57.87)	41.00 (24.24–59.35)	42.57 (24.24–51.48)	0.92 ^a	
-6-month	45.96 (30.82–63.15)	45.62 (30.82–63.02)	47.43 (30.82–65.38))	0.91 ^a	
-12-month	55.23 (38.44–77.96)	55.68 (39.29–77.68)	49.74 (35.27–88.12)	0.73 ^a	
Graft-related CCI (points)					
-In-hospital	0.00 (0.00–20.92)	0.00 (0.00–20.92)	0.00 (0.00–20.92)	0.33 ^a	
-3-month	20.92 (0.00–26.22)	20.92 (0.00–26.22)	20.92 (0.00–26.22)	0.67 ^a	
-6-month	20.92 (0.00–26.22)	20.92 (0.00–26.22)	20.92 (0.00–34.37)	0.68 ^a	
-12-month	20.92 (0.00–33.54)	20.92 (0.00–33.54)	20.92 (0.00–37.08)	0.94 ^a	
Hepatic artery thrombosis	3 (1.5)	3 (1.7)	0 (0.0)	>0.99 ^b	
Death-censored graft loss (n/%)	9 (4.5)	8 (4.7)	1 (3.6)	>0.99 ^b	

Table 2. Cont.

	Overall Cohort	Lactate Cleared	Lactate High	p Value	Discarded
Overall graft loss (n/%)	22 (11.0)	19 (11.1)	3 (10.7)	>0.99 ^b	
Mortality (n/%)	14 (7.0)	12 (7.0)	2 (7.1)	>0.99 ^b	

^a: Calculated using Mann–Whitney U test, ^b: calculated with Fisher’s exact test, ^{1a}: 2 intracerebral hemorrhages, 2 primary non-functions, 1 retransplant for antibody-mediated rejection, ²: early-graft-loss cases were excluded for calculation of length of stay, acute cellular rejection, and biliary complications. Abbreviations: BMI: Body mass index, DCD: donation after circulatory death, DRI: donor risk index, fDWIT: functional donor warm ischemia time, NMP: normothermic machine perfusion, MELD: model for end-stage liver disease, CCI: comprehensive complication index, ICU: intensive care unit, BAR: balance of risk, MEAF: model of early allograft function, EASE: early allograft failure simplified estimation, L-GrAFT7: liver graft assessment following transplantation (at 7 days).

3.2. Perfusate Lactate as Viability Test During NMP

All livers showed high lactate levels at the start of NMP with a median lactate value at the priming of 10.8 mmol/L (8.9–13.1) in a range from 5.4 mmol/L to >20 mmol/L. Lactate decreased to 3.3 (1.4–5.7), 0.7 (0.4–2.1) and 0.6 (0.3–1.2) mmol/L after 30 min and 1 h and 2 h, respectively. During the subsequent course, the lactate levels decreased steadily further to reach 0.5 (0.3–1.0) mmol/L at 4 h and 0.5 (0.3–0.9) mmol/L at 6 h of perfusion. (Figure 1a,b).

AUCs were calculated, including different decision timepoints (NMP durations) and repeated with exclusion of priming lactate (Figure 1b).

The subgroup analysis of DBD and DCD perfusion was performed, showing significant differences in perfusate lactate at 30 min, 1 h and 8 h of NMP ($p < 0.0001$, $p = 0.0002$, $p = 0.020$). Significance was also seen comparing AUCs (Figure 1b).

Thirty-three perfused livers did not meet the current lactate viability criteria, including 28 of the accepted grafts. Of these, seven livers would have been categorized as “orange” according to the Groningen criteria, but these would have also been discarded based on other viability parameter within the Traffic Light system classified as “orange” or “red”, as discussed above.

Based on current lactate criteria, the highest discard rate would have been seen using Groningen and Brisbane criteria, of 14.6% and 11.2%, respectively. Additionally, Birmingham and Münster criteria discard rates are likely false lows because of a moderate number of cases that did not reach an NMP duration of 4 or 6 h in our cohort. The total discard rate would have been 15.5% (Figure 1c). The analysis was repeated in the primary-liver-transplant-only cohort showing no substantial differences (Figure S1).

Non-lactate-clearing livers were allocated to the “lactate-high” group (Table S2). Recipients of such livers did not experience clinically relevant complications.

The differences after transplantation between the two groups were significant only for ALT peak ($p = 0.048$) and ALT AUC in the first 7 days ($p = 0.008$) (Table 2, Figure 2). No significant differences were observed between the “lactate-high” and “lactate-cleared” groups concerning donor and recipient risk factors (Table 2). Perfusate lactate values were also compared for their prediction of clinically relevant complications (Figure 3). Lactate clearance was mainly linked with EAD ($p = 0.003$), while it did not correlate with graft loss, biliary complications, RRT and death-censored graft loss at 12 months (Table 2).

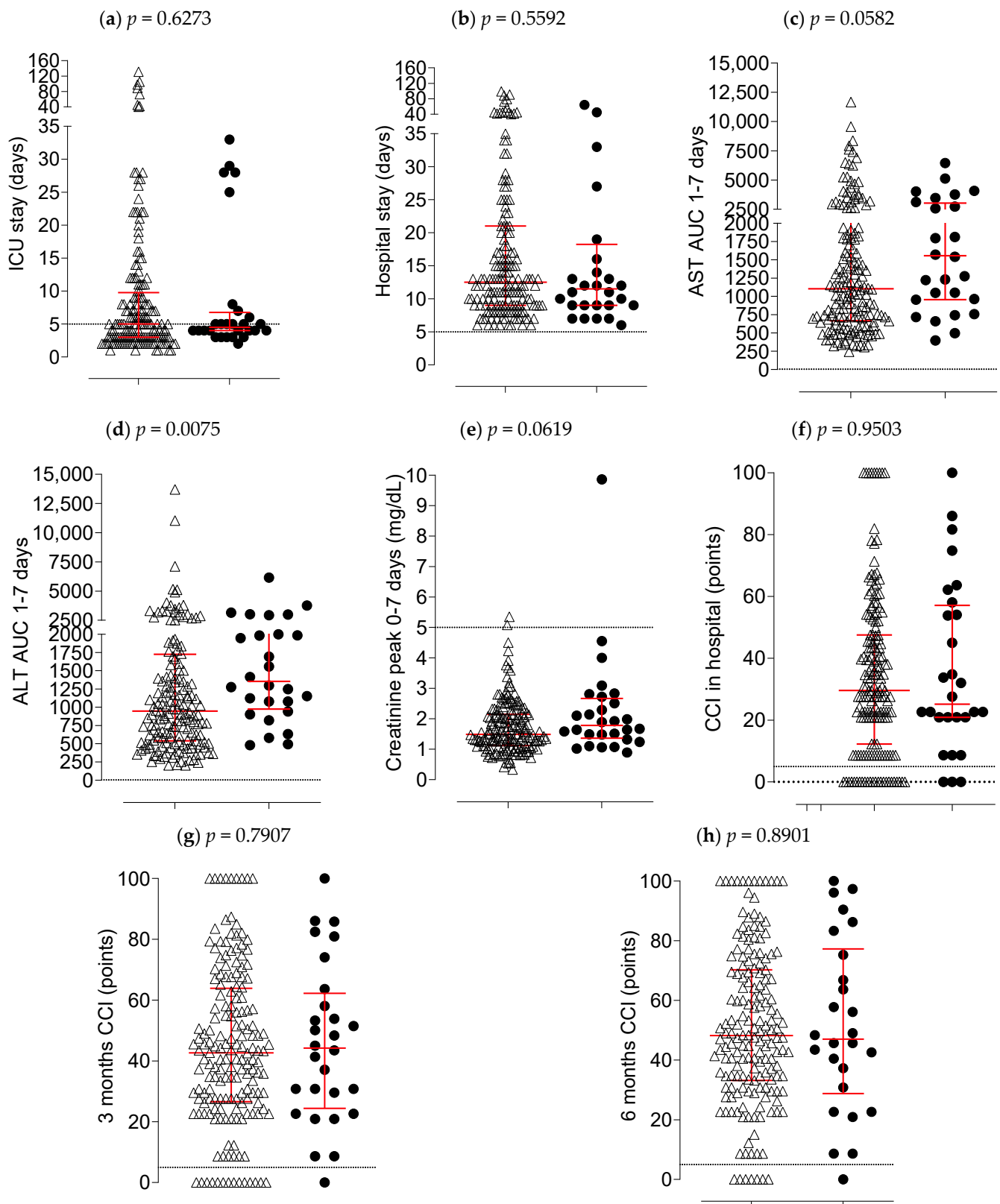


Figure 2. Lactate clearance and clinically relevant outcomes after liver transplantation: (a) ICU stay; (b) hospital stay; (c) AST area under the curve day 1–7; (d) ALT area under the curve day 1–7; (e) creatinine peak; (f) CCI in-hospital; (g) 3-month CCI; (h) 6-month CCI.

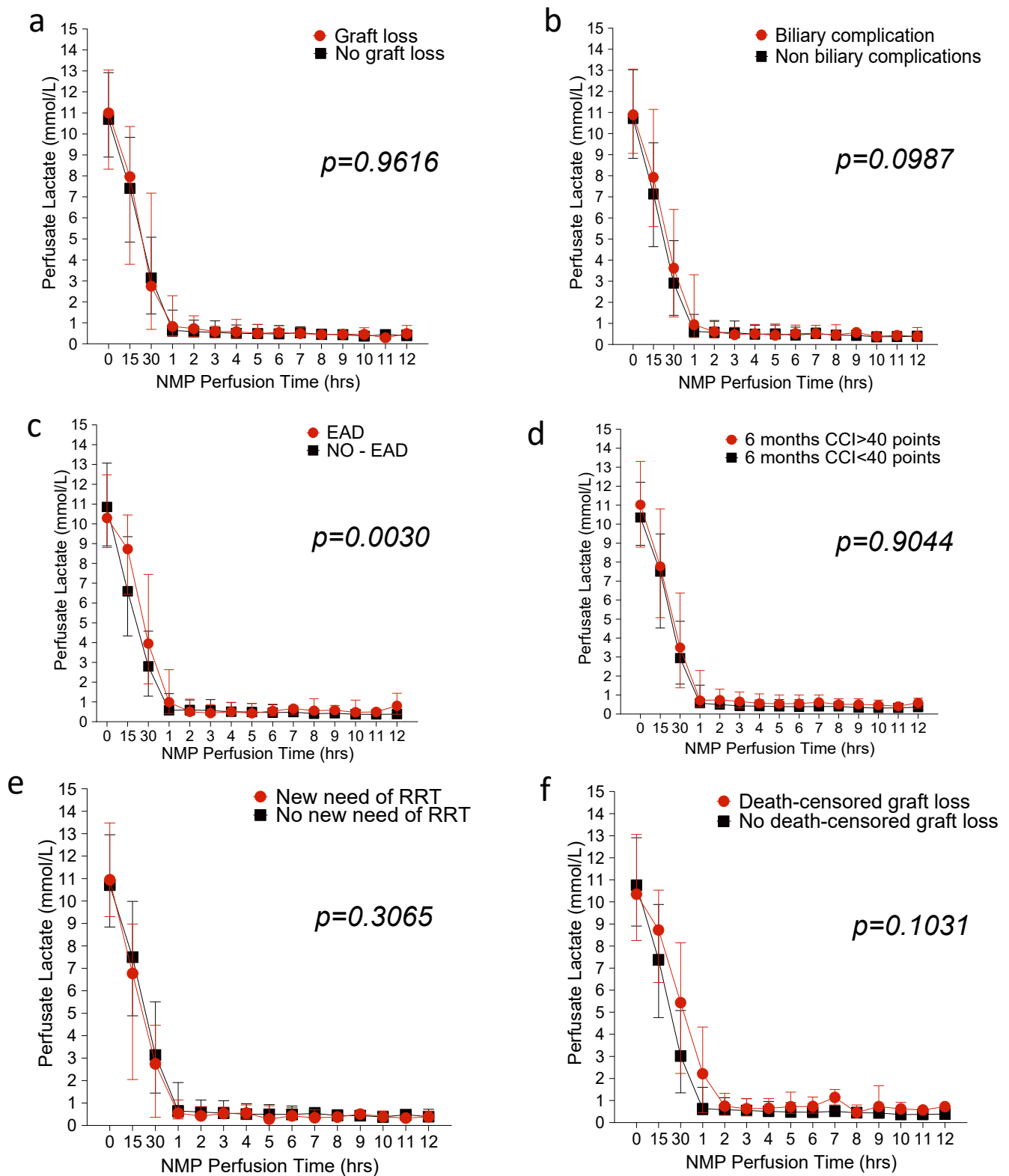


Figure 3. Lactate clearance and clinically relevant outcomes after liver transplantation. Lactate trend in patients that experienced (a) graft loss; (b) biliary complications; (c) early allograft dysfunction (EAD); (d) 6-month CCI; (e) new need of renal replacement therapy (RRT); (f) death-censored graft loss. Two-way Anova test.

4. Predictive Value of Perfusate Lactate Compared to Other Risk Factors

The potential correlation between lactate levels (individual levels at different NMP timepoints and AUCs) and outcome-related scores, such as MEAF and L-GrAFT, was

investigated (Table 3). The results revealed a good correlation between lactate AUC and MEAF at every timepoint, confirming the findings of Hofmann et al. [14]. In contrast, no correlations were identified with L-GrAFT. Interestingly, significant correlations were found with EAD and lactate levels at 30 min, 1, 8 and 12 h of NMP. Such results were even more pronounced when AUCs were adjusted for liver weight with significant correlations at every timepoint.

Next, the impact of lactate on graft survival, death-censored graft survival, and recipient survival was assessed, showing no significant differences between the two groups (Figure 4a–c and Figure S2a–c). Interestingly, no significant differences were found in post-operative outcomes or complication rates, including EAD, PNF, 3-month, 6-month and 12-month CCI and biliary complications (Table 2).

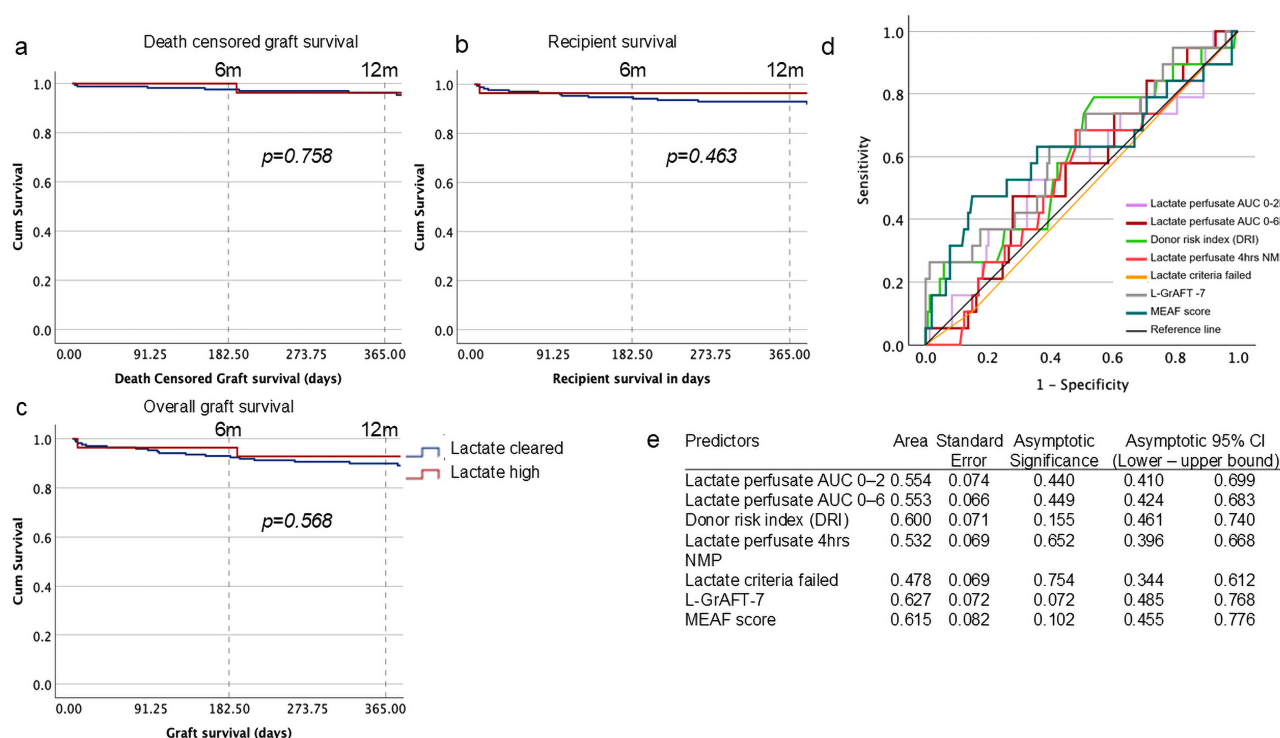


Figure 4. Survival rates and prediction through risk factors and viability parameters: Survival rates of lactate-cleared (blue) and lactate-high (red) cohorts (a–c). ROC curve for 12-month graft loss prediction graph (d) for lactate criteria and comprehensive viability assessment criteria (e) with associated table (e) showing the scarce predictability of lactate, as single value at 4 h, 0–2 and 0–6 AUC.

To further examine the impact of lactate on post-transplant complications, the cohort was subdivided into benchmark cases as previously defined [4,5] (Supplementary Material) and non-benchmark cases. Non-benchmark cases had significantly higher length of stay, higher rates of biliary complications, and higher CCI (Table 4). Within this cohort, twenty-four grafts failed the lactate criteria and were found to have similar post-transplant outcomes as grafts that passed the criteria. While only four grafts failed the criteria within the benchmark cohort, recipients of these grafts experienced minimal complications after transplant. Three death-censored graft losses occurred within the benchmark cases at 1 year; two grafts required retransplant due to NAS, and one graft developed re-cirrhosis. All three grafts cleared lactate and passed the criteria.

Table 3. Correlation of lactate with EAD, MEAF and L-GrAFT 7 score.

	Lactate and EAD			Lactate and MEAF			Lactate and L-GrAFT 7			Lactate and Peak ALT			Lactate and Peak AST		
	<i>p</i> -Value	R Squared	Pearson R	<i>p</i> -Value	R Squared	Pearson R	<i>p</i> -Value	R Squared	Pearson R	<i>p</i> -Value	R Squared	Pearson R	<i>p</i> -Value	R Squared	Pearson R
Lactate 0 h	0.37	0.0043	−0.0652	0.69	0.0008	0.0288	0.58	0.0016	0.0395	0.41	0.0035	0.0592	0.83	0.0002	0.0157
Lactate 0.5 h	0.002	0.0456	0.2136	<0.001	0.0576	0.2400	0.26	0.0065	0.0807	0.17	0.0095	0.0974	0.02	0.0294	0.1716
Lactate 1 h	0.009	0.0349	0.1868	0.002	0.0421	0.2051	0.96	<0.0001	0.0040	0.46	0.0029	0.0534	0.08	0.0160	0.1265
Lactate 2 h	0.77	0.0004	0.0210	0.05	0.0194	0.1396	0.46	0.0028	0.0531	0.72	0.0006	0.0255	0.90	<0.0001	0.0093
Lactate 4 h	0.64	0.0012	−0.0352	0.003	0.0486	0.2205	0.93	<0.0001	0.0066	0.64	0.0012	0.0342	0.89	0.0001	0.0103
Lactate 6 h	0.86	0.0002	0.0139	0.02	0.0376	0.1940	0.73	0.0008	0.0282	0.72	0.0008	0.0288	0.76	0.0006	0.0246
Lactate 8 h	<0.001	0.1091	0.3303	<0.001	0.1260	0.3550	0.15	0.0176	0.1325	0.31	0.0088	0.0936	0.01	0.0554	0.2355
Lactate 12 h	<0.001	0.1900	0.4359	<0.001	0.1973	0.4442	0.02	0.0818	0.2860	<0.001	0.1521	0.3900	<0.001	0.2576	0.5075
AUC 0–2	0.003	0.0446	0.2111	<0.001	0.0581	0.2410	0.52	0.0021	0.0461	0.12	0.0120	0.1096	0.02	0.0268	0.1636
AUC 1–2	0.02	0.0261	0.1616	0.002	0.0499	0.2233	0.92	<0.0001	0.0076	0.46	0.0028	0.0532	0.14	0.0112	0.1059
AUC 0–4	0.03	0.0243	0.1558	<0.001	0.0640	0.2530	0.75	0.0005	0.0232	0.15	0.0107	0.1032	0.06	0.0185	0.1359
AUC 1–4	0.24	0.0070	0.0835	<0.001	0.0553	0.2352	0.82	0.0003	0.0159	0.44	0.0031	0.0553	0.38	0.0039	0.0627
AUC 0–6	0.11	0.0151	0.1228	0.002	0.0536	0.2315	0.91	<0.0001	0.0090	0.32	0.0059	0.0767	0.14	0.0126	0.1122
AUC 1–6	0.46	0.0032	0.0569	0.002	0.0544	0.2333	0.92	<0.0001	0.0078	0.68	0.0010	0.0320	0.50	0.0026	0.0515
AUC 0–2/W	<0.001	0.0705	0.2655	<0.001	0.0661	0.2571	0.73	0.0006	0.0242	0.03	0.0235	0.1531	0.003	0.0435	0.2086
AUC 1–2/W	0.003	0.0428	0.2069	<0.001	0.0579	0.2405	0.89	0.0001	0.0102	0.23	0.0074	0.0859	0.05	0.0193	0.1388
AUC 0–4/W	<0.001	0.0604	0.2458	<0.001	0.0754	0.2746	0.78	0.0004	0.0199	0.03	0.0235	0.1532	0.006	0.0371	0.1926
AUC 1–4/W	0.02	0.0258	0.1607	<0.001	0.0643	0.2536	0.82	0.0003	0.0164	0.16	0.0097	0.0987	0.11	0.0131	0.1145
AUC 0–6/W	<0.001	0.0640	0.2530	<0.001	0.0611	0.2471	0.88	0.0001	0.0108	0.05	0.0196	0.1400	0.006	0.0376	0.1938
AUC 1–6/W	0.01	0.0332	0.1821	<0.001	0.0651	0.2552	0.97	<0.0001	0.0030	0.18	0.0092	0.0960	0.06	0.0181	0.1346

Table 4. Post-operative outcomes in benchmark and non-benchmark cases based on lactate criteria.

Outcomes	Benchmark DBD (n = 5202) [4]	Benchmark DCD (n = 1012) [5]	Benchmark Cases (n = 52)	Lactate Criteria Passed (n = 48)	Lactate Criteria Failed (n = 4)	<i>p</i>	Non-Benchmark Cases (=148)	<i>p</i> (Benchmark vs. Non-Benchmark Cases)	Lactate Criteria Passed (n = 124)	Lactate Criteria Failed (n = 24)	<i>p</i>
Early allograft dysfunction	N.A	N.A.	14 (26.9)	12 (25.0)	2 (50.0)	0.29 ^a	38 (25.7)	0.85 ^a	31 (25.0)	7 (29.2)	0.80 ^a
Primary non-function (n./%)	N.A	≤2.5%	0 (0.0)	0 (0.0)	0 (0.0)	>0.99 ^a	2 (1.4)	>0.99 ^a	2 (1.6)	0 (0.0)	>0.99 ^a
New need for RRT at 7-days (n/%)	≤8%	≤9.6%	2 (3.8)	2 (4.2)	0 (0.0)	>0.99 ^a	9 (6.1)	0.73 ^a	8 (6.5)	1 (4.2)	>0.99 ^a
Overall new need for RRT (n/%)	N.A	N.A	4 (7.7)	4 (8.3)	0 (0.0)	>0.99 ^a	16 (10.8)	0.60 ^a	14 (11.3)	2 (8.3)	>0.99 ^a
Early graft loss (≤15 days) (n/%)	N.A	N.A	0 (0.0)	0 (0.0)	0 (0.0)	>0.99 ^a	5 (3.4) ^{1a}	0.33 ^a	4 (3.2)	1 (4.2)	>0.99 ^a
ICU length of stay (days) ²	≤4 d	≤3 d	4.00 (3.00–5.00)	4.00 (3.00–5.00)	3.50 (2.25–4.00)	0.42 ^b	5.00 (3.00–12.00)	0.012 ^b	6.00 (3.00–12.00)	5.00 (4.00–8.00)	0.76 ^b
Hospital length of stay (days) ²	≤18 d	≤16 d	10.00 (8.00–13.00)	10.50 (8.00–13.75)	8.00 (7.00–10.50)	0.14 ^b	13.00 (9.75–25.00)	0.0025 ^b	13.00 (10.00–25.00)	12.00 (9.00–27.00)	0.80 ^b
Acute cellular rejection (n/%) ²	N.A	N.A	6 (11.5)	6 (12.5)	0 (0.0)	>0.99 ^a	22 (15.4)	0.65 ^a	18 (15.0)	4 (17.4)	0.76 ^a
Biliary complications (n/%) ²	≤28%	N.A	6 (11.5)	6 (12.5)	0 (0.0)	>0.99 ^a	41 (28.7)	0.014 ^a	34 (28.3)	7 (30.4)	0.81 ^a
Anastomotic stricture (n/%) ²	N.A	≤28.4%	6 (11.5)	6 (12.5)	0 (0.0)	>0.99 ^a	34 (23.8)	0.072 ^a	28 (23.3)	6 (26.1)	0.79 ^a
Non-anastomotic stricture/ischemic cholangiopathy (n/%) ²	N. A	≤16.8%	2 (3.8)	2 (4.2)	0 (0.0)	>0.99 ^a	16 (11.2)	0.24 ^a	12 (10.0)	4 (17.4)	0.29 ^a
Bile Leaks (n/%) ²	N.A	≤8.3%	0 (0.0)	0 (0.0)	0 (0.0)	>0.99 ^a	6 (4.2)	0.34 ^a	6 (5.0)	0 (0.0)	0.59 ^a
CCI (points)											
-Discharge	≤29.6	≤22.2	22.64 (8.66–35.3)	22.64 (8.66–36.95)	8.66 (8.66–29.08)	0.30 ^b	35.71 (22.64–55.79)	0.0002 ^b	37.08 (22.64–55.79)	33.42 (21.35–55.36)	0.81 ^b
-3-month	≤34.5	≤30.8	32.78 (22.64–45.34)	33.54 (24.24–45.34)	15.65 (8.66–42.5)	0.16 ^b	49.88 (30.82–70.00)	<0.0001 ^b	51.6 (30.82–70.00)	48.46 (31.12–77.69)	0.81 ^b
-6-month	≤37.2	≤36.4	39.61 (25.74–48.6)	40.23 (25.74–48.6)	16.45 (8.66–42.9)	0.10 ^b	55 (39.21–75.99)	<0.0001 ^b	56.01 (40.47–75.66)	52.88 (37.74–87.39)	0.89 ^b
-12-month	≤42.1	≤38.9	41.08 (33.26–56.46)	41.91 (34.64–56.46)	16.45 (8.66–60.92)	0.084 ^b	60.52 (43.3–83.22)	0.0002 ^b	61.64 (44.3–81.09)	52.88 (39.56–88.79)	0.70 ^b

Table 4. Cont.

Outcomes	Benchmark DBD (n = 5202) [4]	Benchmark DCD (n = 1012) [5]	Benchmark Cases (n = 52)	Lactate Criteria Passed (n = 48)	Lactate Criteria Failed (n = 4)	<i>p</i>	Non-Benchmark Cases (=148)	<i>p</i> (Benchmark vs. Non-Benchmark Cases)	Lactate Criteria Passed (n = 124)	Lactate Criteria Failed (n = 24)	<i>p</i>
Graft-related CCI (points)											
-Discharge	N.A	N.A	0.00 (0.00–20.92)	0.00 (0.00–20.92)	0.00 (0.00–25.30)	0.98 ^b	0.00 (0.00–26.22)	0.12 ^b	0 (0.00–26.22)	0.00 (0.00–20.92)	0.21 ^b
-3-month			0.00 (0.00–20.92)	0.00 (0.00–20.92)	10.46 (0.00–37.27)	0.63 ^b	20.92 (0.00–26.22)	0.016 ^b	20.92 (0.00–33.54)	20.92 (0.00–26.22)	0.87 ^b
-6-month			0.00 (0.00–20.92)	0.00 (0.00–20.92)	10.46 (0.00–37.27)	0.85 ^b	20.92 (0.00–33.54)	0.027 ^b	20.92 (0.00–33.54)	20.92 (0.00–34.37)	0.99 ^b
-12-month			10.46 (0.00–25.32)	10.46 (0–25.32)	10.46 (0.00–37.27)	0.94 ^b	20.92 (0.00–33.73)	0.029 ^b	26.22 (0.00–33.73)	20.92 (0.00–37.08)	0.81 ^b
Hepatic artery thrombosis (n/%)	≤4.4% (HAT)	≤4.5% (HAT)	0 (0.0)	0 (0.0)	0 (0.0)	>0.99 ^a	3 (2.0)	0.57 ^a	3 (2.5)	0 (0.0)	>0.99 ^a
Death-censored graft loss (n/%)	≤4% (retp rate)	≤6.9% (retp rate)	3 (5.8)	3 (6.3)	0 (0.0)	>0.99 ^a	6 (4.1)	0.70 ^a	5 (4.2)	1 (4.4)	>0.99 ^a
Overall graft loss (n/%)	≤11%	≤14.4%	5 (9.6)	5 (10.4)	0 (0.0)	>0.99 ^a	17 (11.5)	0.80 ^a	14 (11.7)	3 (13.0)	0.74 ^a
Mortality (n/%)	≤9%	≤9.6	2 (3.9)	2 (4.2)	0 (0.0)	>0.99 ^a	12 (8.1)	0.53 ^a	10 (8.1)	2 (8.3)	>0.99 ^a

^a: calculated with Fisher's exact test, ^b: calculated using Mann-Whitney U test, ^{1a}: 2 intracerebral hemorrhage, 2 primary non function, 1 retransplant for antibody-mediated rejection, ²: early graft loss cases were excluded for calculation of length of stay, acute cellular rejection, and biliary complications. Abbreviations: RRT: Renal Replacement Therapy, ICU: Intensive Care Unit, CCI: comprehensive complications index.

The lactate clearance criteria demonstrated an AUC of 0.50 at ROC analysis for 1-year graft survival, while AUC 0–2, AUC 0–6, and lactate at 4 h had slightly increased AUCs, though not significant. In contrast, the MEAF score had a higher value of 0.67 ($p = 0.01$). Combining lactate with other parameters proposed by the Cambridge, Birmingham and Groningen groups (Table 1) showed slight improvement in AUC, although it did not reach statistical significance (Figure 4d,e).

Survival analysis, using the Cox proportional hazards regression and mixed-effects models, was conducted to evaluate the association between predictor variables and graft survival. Hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated. A Cox proportional hazards model assessed the association between lactate and death-censored graft survival. Failure of lactate criteria not significantly associated with graft loss (HR: 0.52, 95% CI: 0.047–5.73, $p = 0.59$, (Table S3a) A mixed-effects model was performed for repeated measures of death-censored graft survival over 6 months, with multiple variables (Table S3b) as fixed effects and binary lactate criteria (failed) as a random effect. The mixed-effects model showed that both conditional and marginal pseudo- R^2 were 0.125, indicating that 12.5% of the variation in 6-month graft survival was explained by known parameters. This indicated no additional predictive correlation between the fulfillment of lactate criteria and graft survival.

5. Discussion

This study represents one of the largest single-center cohorts of NMP to date, providing a comprehensive analysis of current viability criteria during NMP with a particular focus on lactate clearance. The findings clearly demonstrate the limitations of lactate as a reliable liver viability parameter, showing challenges associated with its use in clinical practice. We first analyzed lactate levels in our NMP cohort, finding significant early differences between DBD and DCD livers, though both groups showed similar lactate clearance trends over time. Subgroup analysis revealed no relevant correlation between lactate clearance and post-transplant outcomes, including PNF, biliary complications and graft survival, underlining its limitations as a predictor. Strict adherence to current lactate-based viability criteria would have elevated our discard rate from 6.1% to 15.5%, without impacting post-transplant outcomes. Finally, perfusate lactate showed poor predictability for long-term graft survival despite relative equivalence of other traditional graft-, donor- and recipient-related risk factors.

As NMP is increasingly adopted for liver preservation, the need for reliable biomarkers to guide decision-making is key to unlock the potential of currently available organ donors. While various parameters have been proposed and tested in clinical trials, lactate clearance during NMP is often considered a key biomarker by many [19,21,23–25,31]. Data about long-term NMP from the Zurich group showed lactate clearance by every liver within the first 24 h of NMP. In contrast, “bad livers”, defined by a comprehensive assessment of other parameters, stopped clearing lactate after continuous NMP of 1 day and beyond.

The same group also highlighted the similar lactate trend in bile, comparable to perfusate [32,33]. In contrast, perfusate flavin mononucleotide (FMN) levels were recently validated internationally HOPE, and no other parameter has demonstrated such strong predictive value for graft survival and complications [34].

Lactate is produced during anaerobic glycolysis when glucose is converted to pyruvate. Instead of entering the mitochondria for further metabolism, pyruvate is reduced to lactate by lactate dehydrogenase (LDH). This process compensates for the lack of ATP during hypoxia, and subsequent tricarboxylic acid (TCA) cycle inhibition. Lactate metabolism involves two key pathways: oxidation to pyruvate for mitochondrial metabolism or conversion into glucose through gluconeogenesis [13]. Clinically, lactate plays a critical role

in diagnosing and prognosing a variety of diseases. Elevated lactate levels, particularly after ischemic injuries like cardiac, cerebral, or intestinal ischemia, are routinely used as indicators of tissue hypoperfusion. In liver transplantation, major hepatectomies and acute liver failures, lactate monitoring is widely applied, providing valuable insight into early graft function and perfusion. Quick lactate clearance after transplant frequently correlates with a good early liver function and many studies have demonstrated the prognostic value of lactate, showing that persistent hyperlactatemia is associated with higher post-transplant mortality, both in the short and longer term [35,36].

Transferring lactate from such clinical scenarios to isolated organs during NMP remains challenging. As previously discussed by Watson et al., only a small number of viable hepatocytes are needed to clear lactate in a rather small-volume normothermic perfusion circuit, not representing the functional challenges such livers are faced when implanted in an entire human body [37,38]. Although complete failure to clear lactate can indicate extensive pan-lobular damage or areas of hypoperfusion, rapid lactate clearance may not fully reflect appropriate liver function, or that the liver will recover from transplant-induced injury [37]. Based on these findings, even a damaged or suboptimal liver can clear lactate if few healthy hepatocytes are present. For this reason, relying solely on lactate clearance could lead to an overestimation of liver viability.

Despite achieving lactate clearance during NMP, complications occurred after transplantation, as shown by many. Importantly, these outcomes were observed despite the clinical application of predefined lactate-based viability thresholds published by the respective centers, reflecting their intended use as reproducible criteria. For instance, the Birmingham cohort experienced 18.2% of NAS and a one-year graft survival rate of 86.4%, similar to cold-stored control livers [21]. Next, the Groningen group reported 38% biliary complications in their sequential perfusion (DHOPE, COR, NMP), despite lactate clearance within proposed “green or orange zones” [23]. Additionally, the Münster group experienced four PNFs despite lactate clearance with no significant differences observed between high- and low-DRI cohorts [25]. In their study, Watson et al. described a NAS rate of 11% and one PNF [19]. These findings support the results presented here. Clinically relevant complications occur despite lactate clearance. Accordingly, our study aimed to systematically apply these published thresholds within an independent cohort to assess their external reproducibility and predictive performance in real-world clinical practice. We further highlight the importance of employing a standardized methodology for data and outcome collection, which has hindered the reliability of nearly all publications on this topic [39,40].

Our study was conducted to assess the role of currently published lactate criteria, although none have yet been validated. In fact, the newest Groningen criteria were independently selected by the group, based on their previous criteria [23]. Similarly, the Birmingham criteria were initially developed based on data from 6 livers on NMP, and later revised in a larger cohort with 31 livers and 22 transplants [15,21]. In 2022, Watson et al. updated their viability criteria but emphasized that such criteria were never intended as strict thresholds to determine whether organs are accepted. Instead, they reflected on their results and described that they may not be applicable to NMP performed with other devices besides the OrganOx Metra® [19]. The strict application of published lactate criteria would have substantially increased graft discard rates in our cohort without improving post-transplant outcomes. Also, fixed lactate-based thresholds assume uniform decision timepoints during NMP, whereas in clinical practice perfusion duration is variable and driven by logistics and the center’s policies, limiting the applicability of such cut-offs across centers. These findings underscore the limitations of static cut-offs at predefined timepoints and support a more contextual, dynamic interpretation of lactate kinetics. The lack of

consensus regarding lactate thresholds and decision timepoints during NMP has important clinical implications, as it may directly influence graft acceptance or discard decisions and therefore impact organ utilization and transplant outcomes across centers.

Although perfusate lactate clearance did not predict graft survival or major post-transplant complications, its association with early hepatocellular injury provides important pathophysiological insight. In our cohort, slower lactate metabolism during NMP was associated with higher rates of EAD and increased peak ALT and ALT AUC, indicating greater early hepatocellular injury after implantation. This could reflect a initial impaired metabolic recovery and increased susceptibility to IRI, resulting in early biochemical injury rather than long-term graft failure. The correlation with MEAF, but not with L-GrAFT7, further supports the concept that lactate primarily reflects early metabolic stress rather than a reliable predictor of long-term outcomes.

Hofmann et al. also investigated the utility of lactate as a biomarker in a large European multi-center cohort. Lactate levels at 6 h NMP strongly correlated with MEAF score, a metric assessing the risk for early allograft dysfunction within 12 months after liver transplantation. Based on their analysis, Hofmann et al. suggested a slightly extended NMP duration of 6 h for decision-making based on lactate clearance. However, no correlation was found between lactate levels and patient or graft survival, further questioning the predictive value of this parameter in the longer term. Additionally, their study showed a correlation between cold ischemia time (CIT) and initial lactate values during NMP, yet it is well-established that initial lactate is released by aged red blood cells, commonly used for NMP [41]. Given the lack of an organ when the first perfusate sample is obtained during perfusion priming (i.e., prior to liver connection), the impact of the CIT duration prior to NMP on the initial priming lactate appears questionable [18].

A recent paper from the same group identified a distinct 7-gene signature in non-transplanted NMP livers (non-benchmarking cases), showing significantly higher CD274 expression, which encodes PD-L1. Additionally, LEAP2 gene expression during NMP was proposed as additional marker to predict early graft function post-transplantation [28]. However, these markers do not seem easily reproducible in real-time settings, despite showing promise in such retrospective analyses.

Interestingly, in a deeper analysis of our cases with graft loss or severe graft-related complications, we found that such livers had excellent lactate clearance during NMP, meeting all criteria and were thus accepted for transplantation. However, the number of such severe complications could possibly have been reduced by measuring perfusate FMN during NMP. Based on our results with the first 200 livers transplanted with NMP, our viability testing concept has changed to explore the role of FMN prospectively. As mentioned earlier, lactate is also considered together with other “traditional” parameters to provide a more comprehensive overview of liver performance during NMP.

Collectively, our results argue against single-parameter decision-making during NMP and support the development of multidimensional viability models integrating lactate with complementary markers. Future approaches should consider indicators of mitochondrial injury, like FMN, biliary tree injury, perfusion parameters, and inflammatory or endothelial stress to better capture graft status. As reported recently by our group, FMN serves as a prospectively validated marker of mitochondrial injury that identifies risks for graft dysfunction and loss while reducing healthcare costs. Incorporating FMN quantification within the first hours of NMP (1–4 h) allows for an early graft acceptance or discard [42]. Additionally, high FMN levels necessitate comparison to traditional parameters, whereas low FMN levels may safely justify the use of grafts with borderline lactate clearance. This multidimensional strategy improves predictive accuracy and supports the economic sustainability of transplant programs by minimizing both post-operative complications and

unnecessary organ discards. In addition, NMP should be increasingly viewed not only as an assessment tool but also as a conditioning and therapeutic platform, potentially in combination with HOPE or other sequential preservation strategies. Finally, standardized risk factor, outcome and viability criteria definitions across centers and devices are essential to enable meaningful external validation and comparison of emerging viability concepts [43].

This study has several limitations due to its retrospective, single-center design. The grouping of grafts into “lactate-high” and “lactate-cleared” categories was based on published thresholds suggested by many, that may not reflect real clinical practice worldwide. As a result, the “lactate-high” group may be larger than typically seen, possibly overestimating the number of high-lactate cases. In practice, some of these livers might have been acceptable, despite slight deviations from the published lactate thresholds. Our cohort had relatively good outcomes, with a 1-year survival rate of 89%, potentially limiting the predictive value of lactate within our cohort for graft survival. Additional biases could have been the exclusion of the discarded graft with high lactate values from the outcome analysis. However, it is important to note that exclusion of grafts based specifically on unfavorable lactate dynamics was limited in our cohort, with most grafts being discarded for other reasons such as inadequate bile production, poor perfusion flows during NMP, or unfavorable histological findings. Therefore, while selection bias cannot be fully excluded, the bias directly attributable to lactate-based viability assessment was limited to a small number of cases and is unlikely to have substantially influenced the overall outcome analysis. A further limitation is the potential selection bias related to graft acceptance. Decisions were not based on a fixed algorithm but on a comprehensive clinical evaluation, and lactate criteria were applied only retrospectively. As a result, grafts with elevated lactate that were transplanted likely had favorable features in other domains, potentially attenuating the observed association between lactate levels and outcomes. Residual confounding related to donor and recipient heterogeneity (including cause of donor death, comorbidities, and ischemia times) cannot be fully excluded and may have influenced the observed association between lactate levels and outcomes.

In summary, this study strongly advocates for a re-evaluation of lactate clearance as a viability marker during NMP, as it may lead to unnecessary liver discards or acceptance of high-risk grafts. Alternative biomarkers, such as molecules related to mitochondrial injury, should be considered further and undergo multi-center validation in future studies to enhance decision-making in liver transplantation.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/livers6040067/s1>, (1) Methods and results, (2) Table S1: Indications for NMP, (3) Table S2: Donor livers with NMP and failures in one or more viability criteria due to impaired lactate clearance, (4) Figure S1: Lactate clearance along with criteria proposed by different groups in primary liver transplants only, (5) Figure S2: Correlation between perfusate lactate and MEAF score, (6) Table S3: Cox regression and mixed-effect model for death-censored graft survival.

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Abbreviations

ATP, Adenosine triphosphate; ACR, acute cellular rejection; ALT, alanine amino transferase; AS, anastomotic stricture; AST, aspartate amino transferase; AUC, area under curve; BAR, balance-of-risk score; BMI, body mass index; CCI, comprehensive complication index; CD274, cluster of differentiation 274; CI, confidence interval; CIT, cold ischemia time; EAD, early allograft dysfunction; EASE, early allograft failure simplified estimation; ECDs, extended-criteria donors; FMN, flavin mononucleotide; DCD, donation after circulatory death; DBD, donation after brain death; DRI, donor risk index; fWIT, functional warm ischemia time; IRB, institutional review board; IRI: ischemia–reperfusion injury; HAT, hepatic artery thrombosis; HOPE, hypothermic oxygenated perfusion; HR, hazard ratio; ICU, intensive care unit; INR, international normalized ratio; IQR, interquartile range; LDH, lactate dehydrogenase; LEAP2, liver enriched antimicrobial peptide 2; L-GrAFT₇, liver graft assessment following transplantation, LT, liver transplantation; MEAF: model of early allograft function; NAS, non-anastomotic stricture; NMP, normothermic machine perfusion; OPO, organ procurement organization; PD-L1, programmed cell death ligand 1; PNF, primary non-function; RBCs, red blood cells; ROC, receiver operating characteristic; RRT, renal replacement therapy; TCA, tricarboxylic acid; UNOS, United Network for Organ Sharing; SRTR, Scientific Registry of Transplant Recipients.

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