Perfusate Enzymes and Platelets Indicate Early Allograft Dysfunction After Transplantation of Normothermically Preserved Livers

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Background. Normothermic machine perfusion (NMP) has become a clinically established tool to preserve livers in a near-physiological environment. However, little is known about the predictive value of perfusate parameters toward the outcomes after transplantation. **Methods.** Fifty-five consecutive NMP livers between 2018 and 2019 were included. All of the livers were perfused on the OrganOx metra device according to an institutional protocol. Transplant and perfusion data were collected prospectively. **Results.** Forty-five livers were transplanted after NMP. Five livers stem from donors after circulatory death and 31 (68.9%) from extended criteria donors. Mean (SD) cold ischemia time was 6.4 (2.3) h; mean (SD) total preservation time was 21.4 (7.1) h. Early allograft dysfunction (EAD) occurred in 13 of 45 (28.9%) patients. Perfusate aspartate aminotransferase ($P=0.008$), alanine aminotransferase ($P=0.006$), lactate dehydrogenase ($P=0.007$) and their development over time, alkaline phosphatase ($P=0.013$), and sodium ($P=0.016$) correlated with EAD. Number of perfusate platelets correlated with cold ischemia time duration and were indicative for the occurrence of EAD. Moreover, von Willebrand Factor antigen was significantly higher in perfusates of EAD livers ($P<0.001$), and Δ von Willebrand factor antigen correlated with EAD. Although perfusate lactate and glucose had no predictive value, EAD was more likely to occur in livers with lower perfusate pH ($P=0.008$). ΔPerfusate alkaline phosphatase, Δperfusate aspartate aminotransferase, Δperfusate alanine aminotransferase, and Δperfusate lactate dehydrogenase correlated closely with model for early allograft function but not liver graft assessment following transplantation risk score. Bile parameters correlated with extended criteria donor and donor risk index. **Conclusions.** Biomarker assessment during NMP may help to predict EAD after liver transplantation. The increase of transaminases and lactate dehydrogenase over time as well as platelets and vWF antigen are important factors indicative for EAD.

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INTRODUCTION

During the last decade, normothermic machine perfusion (NMP) has emerged as a tool to preserve livers in a near-physiological environment. The ability to monitor and assess livers procured from extended criteria donors

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(ECD) and cardiocirculatory death donors (DCD) is an important objective[1-6] and may help lower the discard rate. However, the predictive value of perfusate biomarkers for the assessment of the outcome after liver transplantation remains to be established.[3,7]

Furthermore prolonged preservation may ease hospital logistics and compliance with working hour regulations and operating room capacity. This is particularly relevant in surgically and immunologically complex recipients.[1] Several international study groups have demonstrated improved graft and patient outcomes after liver transplantation following preservation by combined hypothermic ± oxygenated machine (HMP) and normothermic perfusion.[8-11] The significant advantages of NMP compared with static cold storage (SCS) include expansion of the preservation period, shortening cold ischemia time (CIT), and the ability to assess organ function.[1,3,7,12,13] Currently, both normothermic and hypothermic perfusion are applied at the procurement center (using transportable devices) as well as in a back-to-base concept at the recipient's center. Up to now, only 1 portable liver NMP device is widely available,[14-16] whereas several technologies are in clinical use accommodating the back-to-base procedure.[8-10,17,18] Clinically relevant tests for the assessment of organ quality during NMP include lactate clearance and maintenance of perfusate pH and bile production.[3-5,19-21]

We have previously established a standardized liver-NMP protocol[1] and collected consecutive perfusate analytes during NMP. The aim of our study was to assess the value of repeated measurements of perfusate biomarkers in respect to their predictive value for early liver function.

MATERIAL AND METHODS

At the Medical University of Innsbruck, a standardized NMP protocol for liver allografts, following the back-to-base principle,[1,18] has been implemented. The protocol includes real-time perfusate analyses over a period of up to 24 h, accommodated by the institutional biochemistry and hematology laboratories, to acquire data for organ assessment.[1]

Consecutive liver allografts between February 2018 and December 2019 were included in this study. All organs were accepted with the intent to preserve them normothermically due to certain circumstances, that is, nonstandard donor, complex recipient, and operating room logistics. After transport to the Medical University of Innsbruck (back-to-base approach), livers were perfused using the OrganOx metra device.[16,22] The perfusion protocol and its clinical application were approved by the institutional review board (protocol #1175/2018). Liver recipients included in the study were adults ≥18 y of age, listed for a first transplantation or a retransplantation.

The device, fluid circuit, and the cannulation of the liver graft have been described previously.[12,13,18] The perfusion system is completely closed with a circulating volume of approximately 1250–1340 mL of perfusate, composed of 500 mL gelofusine and 3 units of red blood cells (250–280 mL each). Bolus doses of cefuroxime, heparin, and calcium-gluconate were added before the start of the perfusion, and insulin, heparin, bile salts, and epoprostenol were delivered via syringe pump drivers as published previously.[12,13,18]

Decision Making

The decision to transplant or discard a liver after NMP was based on (1) perfusion hemodynamics (arterial, portal, and venous flow), (2) the perfusate homeostasis represented by a physiological pH of 7.3–7.45, (3) the capability of the liver to process and to metabolize lactate, and (4) bile production. Lactate clearance was considered a prerequisite for all livers (donor after brain death and DCD) and was considered sufficient when a concentration of ≤18 mg/dL was reached within the first 6 h after NMP start. For DCD livers, bile production during NMP was considered a must criterion for transplantation.[8]

Perfusate Parameters and Transplant Characteristics

Perfusate samples were drawn at certain, predefined, time points after the start of NMP: 1, 2, 4, 6, 12, 18, and 24 h; with the last time point coincident with the end of NMP. Alkaline phosphatase (AP), bilirubin, C-reactive protein, gamma-glutamyltransferase (gGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and sodium (all Roche Diagnostics GmbH, Mannheim, Germany) in the perfusate and their development over time were analyzed. To evaluate inflammatory potential of the perfusate and to judge capacity of liver synthesis, leukocyte and platelet counts (both Sysmex Europe GmbH, Norderstedt, Germany), fibrinogen antigen (Liaphen Fibrinogen, Hyphen Biomed, Neuville-sur-Oise, France), von Willebrand factor (vWF) antigen (vWF Ag, Siemens Healthcare Diagnostics GmbH, Marburg, Germany), coagulation factor XIII-A subunit (FXIII-A) antigen (Hexamate Factor XIII, Medical & Biological Laboratories Co., Nagoya, Japan), and procalcitonin (Roche Diagnostics GmbH) were assessed in the perfusion solution.

Donor, recipient, and transplant characteristics/data pretransplant and during the postoperative course were collected and collated. The endpoint for transplanted grafts was the occurrence of early allograft dysfunction (EAD), defined as the presence of 1 or more of (1) bilirubin ≥10 mg/dL on day 7, (2) international normalized ratio ≥1.6 on day 7, and (3) ALT or AST >2000 IU/L within the first 7 d after liver transplantation.[23] In addition, the model for early allograft function (MEAF)[24] and the liver graft assessment following transplantation (liver graft assessment following transplantation [L-GrAFT])[25] scores were evaluated. The follow-up time was 3 mo posttransplant.

Statistical Analyses

Statistical testing was performed using Graph Pad Prism 8 and IBM SPSS Statistics Version 25. Recipient, donor, and transplant factors were analyzed using parametric and nonparametric tests (including Spearman rank correlation). *P* values < 0.05 were considered statistically significant, and all tests were 2-tailed.

To take into account the repeated measurements of the perfusate markers, we considered for each marker (1) the first value (at perfusion start), (2) the last value (at the end of perfusion), (3) the course of all repeated measurements,

calculated with least-square linear regression. This approach hence provided each liver with 3 values for each marker. We then compared the medians of these values in patients with EAD with those without EAD. To do so, we used the Wilcoxon–Mann–Whitney test, adapted for continuous parameters in small population, and we plotted the results of the comparison with boxplots.

RESULTS

Fifty-five consecutive livers were included in this study. The duration of NMP was based mainly on logistics or the need for further liver quality/function assessment.

After perfusing 55 livers normothermically, 45 livers were transplanted, whereas 10 were deemed untransplantable. A flowchart (Figure 1) depicts the decision-making steps and the reasons for discarding 10 livers. In the following paragraphs, 45 livers or transplants, respectively, are referred to as 100%. Baseline donor and transplant characteristics as well as recipient demographics are shown in Table 1. Supplementary **Table S1** (**SDC,** http://links.lww.com/TP/C252) displays donor and transplant characteristics of 10 discarded NMP livers in comparison with 45 transplanted ones. In 15 (33.3%) patients, the underlying liver disease was complicated by liver cancer. The mean balance of risk (BAR) score was 8.2 ± 4.9. Overall CIT was 6.4 ± 2.3 h. The NMP time was 15.1 ± 6.6 h, resulting in an overall preservation time of 21.4 ± 7 h. Forty percent (18/45) of the donor livers were steatotic; 1 moderate and 17 mild according to Flechtenmacher et al.[26] Fourteen transplanted livers had a donor risk index (DRI)[27] higher than 2 (31%). Thirteen patients experienced EAD (13/45 [28.9%]). The proportion of steatotic livers was similar in the EAD (5/13) and no-EAD (13/32) groups ($P = 0.72$). Ischemia times, NMP, and overall preservation times were not significantly different between EAD and no-EAD livers. Nine patients received a second or a third liver allograft (9/45 [20%]); 3 of those developed EAD (3/9 [33.3%]). In the univariate analyses, none of the conventional transplant, recipient, and donor risk factors (age, body mass index, retransplant, CIT, DRI, ECD, steatosis) had a significant impact on the occurrence of EAD. Only donor serum sodium at time of retrieval correlated with EAD (Wald 4.3; $P = 0.04$; odds ratio, 1.12; 95% confidence interval, 1.005-1.248). When adjusted for age, however, donor serum sodium lost its significance. The different reasons/indications for liver NMP (donor, recipient, and logistic factors) did not impact the occurrence of EAD (Supplement **S1: A, SDC,** http://links.lww.com/TP/C252).

Perfusate Parameters and Their Impact on EAD, MEAF, and L-GrAFT

Overall, 267 repeated perfusate measurements during liver NMP were assessed, which represent 5.93 measurements per liver on average. Perfusate parameters and values that were not normally distributed were normalized (**Figure S1, SDC,** http://links.lww.com/TP/C252) and the longitudinal assessment performed. Boxplots for each single measured perfusate parameter stratified for EAD

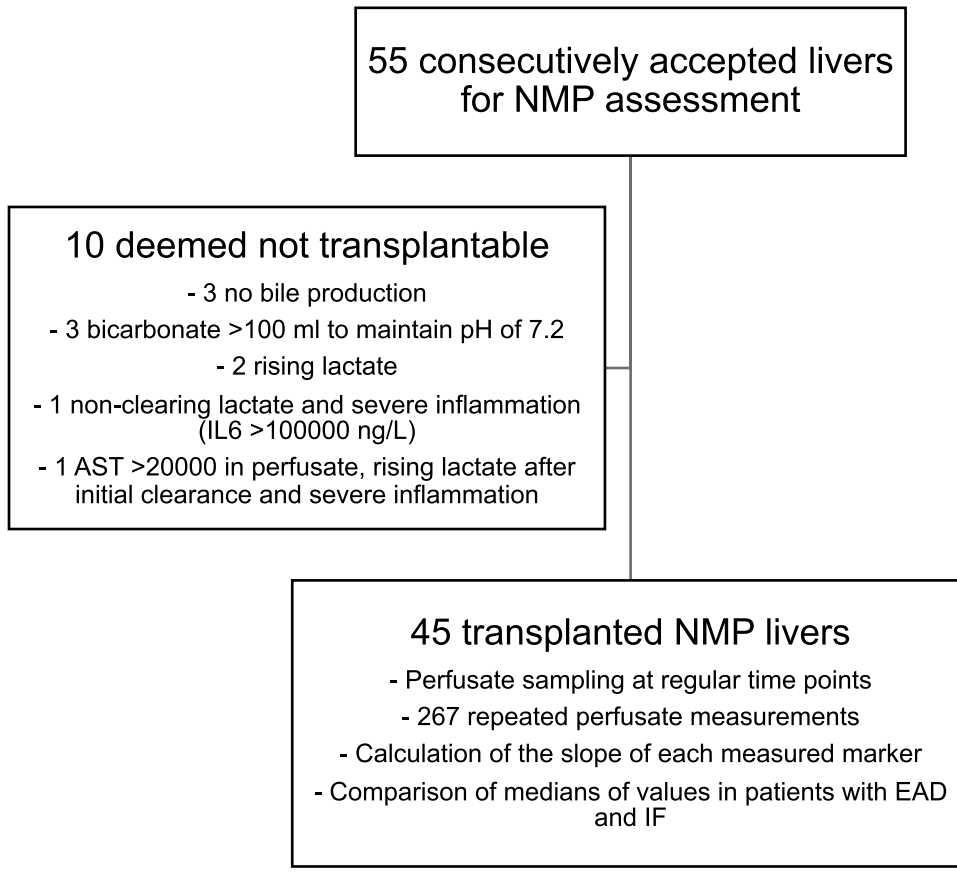

FIGURE 1. Flowchart illustrating reasons for transplanting and discarding livers after normothermic machine perfusion. AST, aspartate aminotransferase; EAD, early allograft dysfunction; IF, initial function; IL6, interleukin 6; NMP, normothermic machine perfusion.

TABLE 1.

Demographics and transplant factors of NMP liver transplant recipients

Characteristics	Overall cohort (N = 45)
Recipient age (y), median (min–max)	60 (23–73)
Recipient BMI (kg/m^2), mean±SD	25.7 ± 4.3
Recipient male gender, n (%)	40 (88.8%)
Prior Tx, n (%)	9 (20%)
BAR score, median (min–max)	7 (4–23)
BAR score >8, n (%)	19 (42.2%)
Donor age (y), median (min–max)	56 (16–80)
Donor BMI (kg/m^2), mean±SD	26.8 ± 3.5
Donor male gender, n (%)	23 (51.1%)
ECD, n (%)	31 (68.9%)
Donation after determination of death by circulatory criteria, n (%)	5 (11.1%)
Liver DRI, mean (SD)	1.9 ± 0.5
Liver DRI >1.2, n (%)	42 (93.3%)
Liver DRI >2, n (%)	14 (31.1%)
Cause of end-stage liver disease, n (%)	
Nonalcoholic steatohepatitis	5 (11.1%)
Alcoholic steatohepatitis	17 (37.8%)
Primary sclerosing cholangitis	2 (4.4%)
Secondary sclerosing cholangitis	1 (2.2%)
Hepatitis B	3 (6.7%)
Hepatitis C	2 (4.4%)
Hemochromatosis	1 (2.2%)
Ischemic cholangiopathy	5 (11.1%)
Others	9 (20%)
Cold ischemia time (h) (mean, SD)	6.4 ± 2.3
Normothermic machine perfusion time (h) (mean, SD)	15.1 ± 6.6
Overall preservation time (h) (mean, SD)	21.4 ± 7.0
Outcome	
EAD, n (%)	13 (28.9%)
90-d mortality, n (%)	4 (8.9%)
With functioning graft, sepsis (aspergillosis, candidiasis, necrotizing fasciitis), n (%)	3 (6.7%)
With dysfunctional graft, sepsis (aspergillosis), n (%)	1 (2.2%)

BAR, balance of risk; BMI, body mass index; DRI, donor risk index; EAD, early allograft dysfunction; ECD, extended criteria donor; ERCP, endoscopic retrograde cholangiopancreaticography; ITBL, ischemic type bile duct lesion; NMP, normothermic machine perfusion; POD, postoperative d.

are shown in Figure 2A–D. For repeated measurements, significant differences in means between livers without EAD and with EAD could be observed for AP ($P = 0.007$), AST ($P < 0.001$), ALT ($P < 0.001$), gGT ($P = 0.045$), and LDH ($P < 0.001$). Results of the univariate logistic regression analysis for EAD are displayed in Table 2. The overall amount of bile was 5–17 mL/h. Bile parameters (pH, bicarbonate, glucose. and lactate) as assessed in 15 transplanted livers revealed that bile pH and bicarbonate correlate significantly with ECD (Spearman's ρ 0.659, $P = 0.008$, Spearman's ρ 0.604, $P = 0.017$) and liver DRI (Spearman's ρ 0.678, $P = 0.005$, Spearman's ρ 0.647, $P = 0.009$). Bile lactate correlated significantly with perfusate gGT (Spearman's ρ 0.625, $P = 0.013$) and perfusate pH (Spearman's ρ 0.669, $P = 0.006$). Bile parameters, however, did neither correlate with the occurrence of EAD nor with the MEAF and L-GrAFT scores (see Supplement **S1: B, SDC,** http://links.lww.com/TP/C252 for details).

Livers implanted in recipients who developed EAD received an insignificantly higher amount of sodium bicarbonate 8.4% during the NMP period compared with no-EAD livers; median (interquartile range [IQR]) 28 (10–37.5) mL in EAD versus 20 (0–25) mL in no-EAD, $P = 0.1$. The last perfusate pH, before the liver was taken off the NMP-device, was significantly higher in no-EAD livers; median (IQR) 7.34 (7.26–7.39) in no-EAD versus 7.26 (7.22–7.31) in EAD, $P = 0.01$. The last measured perfusate glucose levels in median (IQR) (84 [54–202] mg/dL in EAD versus 136 [80–167] mg/dL in no-EAD; $P = 0.6$) before liver transplantation as well as the Δglucose levels over time in median (IQR) (–425 [–533.8 to –259] mg/dL in EAD versus –335.5 [–508.8 to –199.5] mg/dL in no-EAD; $P = 0.4$) were not significantly different between EAD and no-EAD recipients. The last measured perfusate lactate levels in median (IQR) (2 [1–5] mg/dL in EAD versus 5 [2–7.8] mg/dL in no-EAD; $P = 0.07$) before liver transplantation as well as the Δlactate levels over time in median (IQR) (–54.5 [–67 to –43.8] mg/dL in EAD versus –63 [–85 to –34.8] mg/dL in no-EAD; $P = 0.88$) were not significantly different between EAD and no-EAD recipients. **Figure S2** (**SDC,** http://links.lww.com/TP/C252) shows lactate over time in EAD and no-EAD livers.

Overall median (IQR) MEAF score was 4.2 (3.5–4.9). The MEAF score was significantly higher in the EAD group; 4.6 (4.2–5.5) in EAD versus 3.9 (3.2–4.6) in no-EAD, $P = 0.03$. The MEAF score also correlated significantly with EAD; Spearman's ρ 0.326, $P = 0.03$. ΔPerfusate AP (Spearman's ρ 0.31, $P = 0.04$), Δperfusate AST (Spearman's ρ 0.38, $P = 0.01$), Δperfusate ALT (Spearman's ρ 0.5, $P = 0.001$), and Δperfusate LDH (Spearman's ρ 0.42, $P = 0.005$) correlated significantly with the MEAF score; the higher the increase of mentioned perfusate parameters over time, the higher the MEAF score. The overall median (IQR) L-GrAFT score was –1.3 (–1.9 to –0.9) and was comparable between EAD and no-EAD recipients; –1.2 (–1.8 to –1.0) versus –1.3 (–1.9 to –0.8), $P = 0.6$. None of the measured perfusate parameters correlated significantly with the L-GrAFT score.

Perfusate Parameters of Discarded Livers

Figure 3A–D displays individual trajectories of 10 discarded NMP livers and the comparison of perfusate parameters over time for transplanted and discarded NMP livers. Slight differences of perfusate parameters between transplanted and discarded NMP livers were observed for AP (first value $P = 0.022$, last value $P = 0.255$, slope $P = 0.387$), bilirubin (first value $P = 0.150$, last value $P = 0.009$, slope $P = 0.015$), interleukin (IL)-6 (first value $P = 0.850$, last value $P = 0.005$, slope $P = 0.018$), gGT (first value 0.069, last value $P = 0.031$, slope $P = 0.017$), and sodium (first value $P = 0.069$, last value $P = 0.907$, slope $P = 0.031$). Important differences were observed for perfusate AST (first, last value, and slope $P < 0.001$), ALT (first and last values $P < 0.001$, slope $P = 0.275$), and LDH (first and last values $P < 0.001$, slope $P = 0.294$), which were significantly higher in the perfusates of discarded livers. Median (IQR) total given sodium bicarbonate 8.4% was significantly higher in the discarded livers (60 [35–87.5]

mL) compared with all transplanted livers (20 [2.5–30] mL; $P < 0.001$) and the livers developing EAD (28 [10–37.5] mL; $P = 0.006$).

Leukocytes, Platelets, and Liver Synthesis

In addition to liver function parameters, we investigated factors associated with liver synthesis and inflammation in the perfusion solution. Median (IQR) leukocyte count over time was 1.8 (1–2.9) g/L in EAD versus 1.7 (1–2.7) g/L in no-EAD livers, $P = 0.6$. Median (IQR) platelet count over time was significantly higher with 9 (4–15) g/L in EAD versus 5 (3–7) g/L in no-EAD livers, $P < 0.01$. Median (IQR) vWF antigen was significantly higher over time in perfusates of livers, which experienced EAD after transplantation; 14.5% (12–19) in EAD (n = 11/13) versus 12% (9–14) in no-EAD livers (n = 27/32), $P < 0.01$. Median (IQR) fibrinogen antigen over time was 72 (39–162.5) mg/dL in EAD versus 60 (34–138) mg/dL in no-EAD livers, $P = 0.4$.

Median (IQR) FXIII-A antigen was significantly higher in EAD livers (n = 11/13) 11% (9–15) versus 10% (9–13) in no-EAD livers (n = 28/32), $P = 0.04$. Median (IQR) procalcitonin levels over time were 3.1 (0.5–15.8) µg/L in EAD (n = 10/13) versus 2.8 (0.3–19.9) µg/L in no-EAD livers (n = 17/32), $P = 0.9$. Median (IQR) IL-6 levels over time were 105.7 (41.6–763) ng/L in EAD (n = 11/13) versus 174.9 (45.9–511.6) ng/L in no-EAD livers (n = 28/32), $P = 0.9$. The values over time of leukocytes, platelets, vWF antigen, fibrinogen antigen, FXIII-A antigen and procalcitonin, stratified for EAD, and no-EAD are shown in Figure 4A–F. Table 3 displays correlations of perfusate parameters (platelets, vWF antigen, leukocytes), transplant factors, and the event of EAD.

EAD and Preservation Times

In addition to the analyses of longitudinal repeated measurements, we calculated the impact of CIT stratified for more or less than 6 h, NMP stratified for more or less than 6 and 12 h and total preservation time more or less than 18 h on the occurrence of EAD. The occurrence of EAD was comparable between the CIT-groups; 7 EAD in 27 livers with ≥6 h versus 6 EAD in 18 livers with <6 h CIT, $P = 0.7$. Also, duration of NMP did not impact development of EAD in the postoperative course; 9 EAD in 26 livers with ≥12 h versus 4 EAD in 19 livers with <12 h NMP, $P = 0.5$. Furthermore, overall preservation time was not a factor influencing liver function postoperatively; 8 EAD in 26 livers with ≥18 h versus 5 EAD in 19 livers with <18 h CIT + NMP, $P > 0.9$.

To address the heterogeneity of the perfusate analyses, mainly caused by the variable end of NMP, we compared perfusate parameters measured at hour 6 after NMP-start

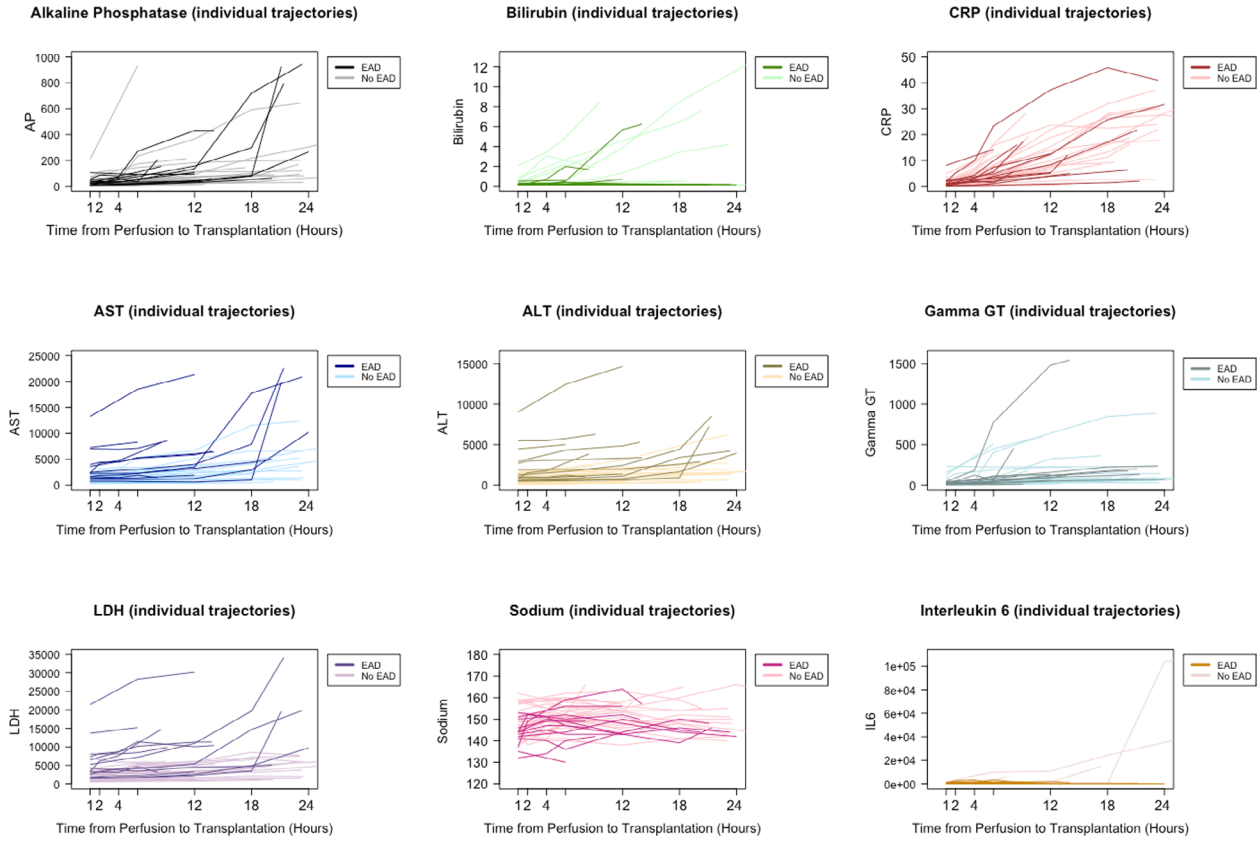

FIGURE 2. A, Individual trajectories of repetitively measured perfusate parameters of 10 discarded NMP livers. B, Boxplot stratified by transplanted NMP livers (n = 45) vs discarded NMP livers (n = 10); illustrating AP, bilirubin, CRP; 0 = transplanted, 1 = discarded. C, Boxplot stratified by transplanted NMP livers (n = 45) vs discarded NMP livers (n = 10); illustrating AST, ALT, gGT; 0 = transplanted, 1 = discarded. D, Boxplot stratified by transplanted NMP livers (n = 45) vs discarded NMP livers (n = 10); illustrating LDH, sodium, IL6; 0 = transplanted, 1 = discarded. ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CRP, C-reactive protein; gGT, gamma-glutamyltransferase; IL6, interleukin 6; LDH, lactate dehydrogenase; NMP, normothermic machine perfusion.

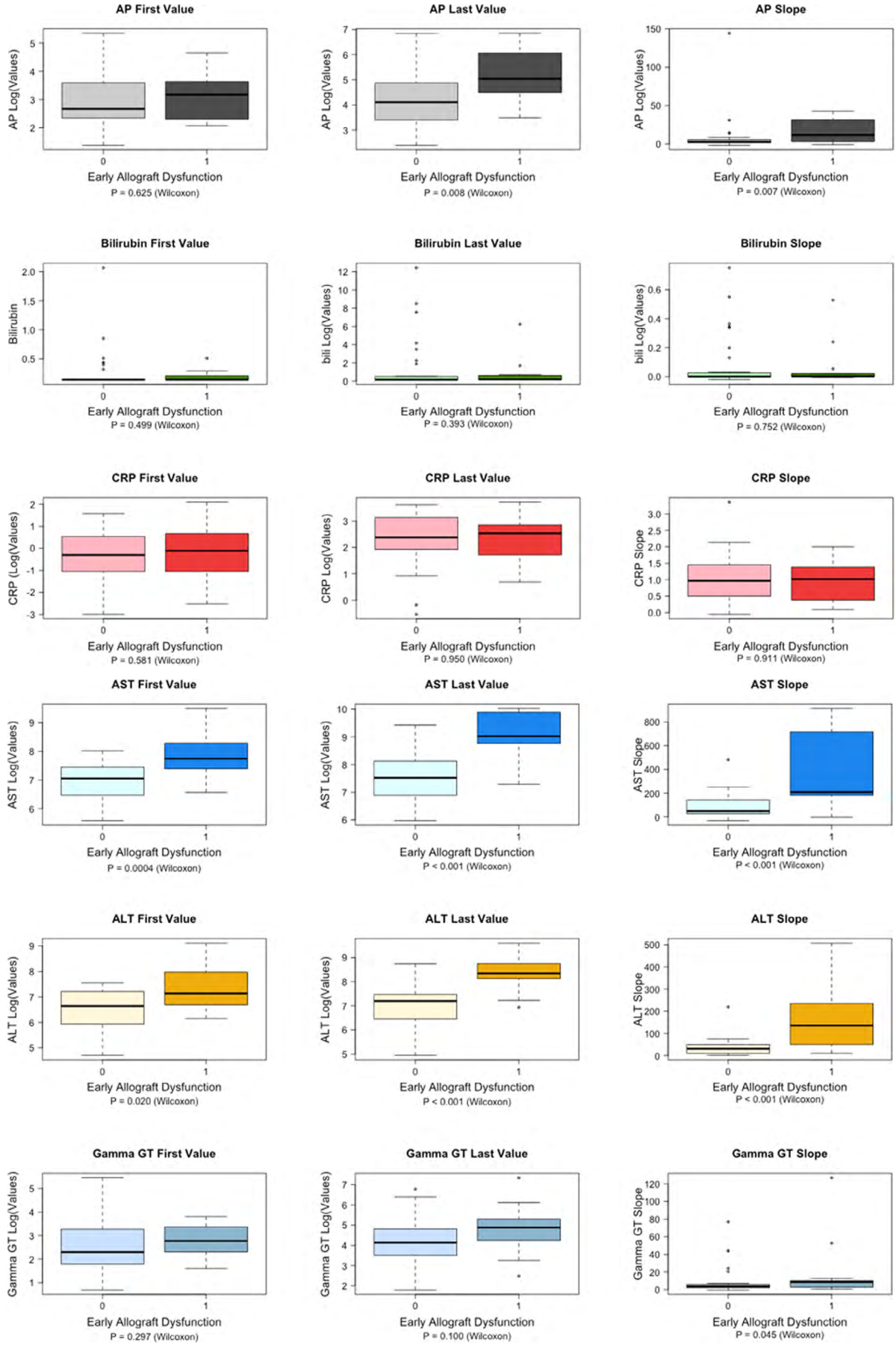

FIGURE 2. Continued.

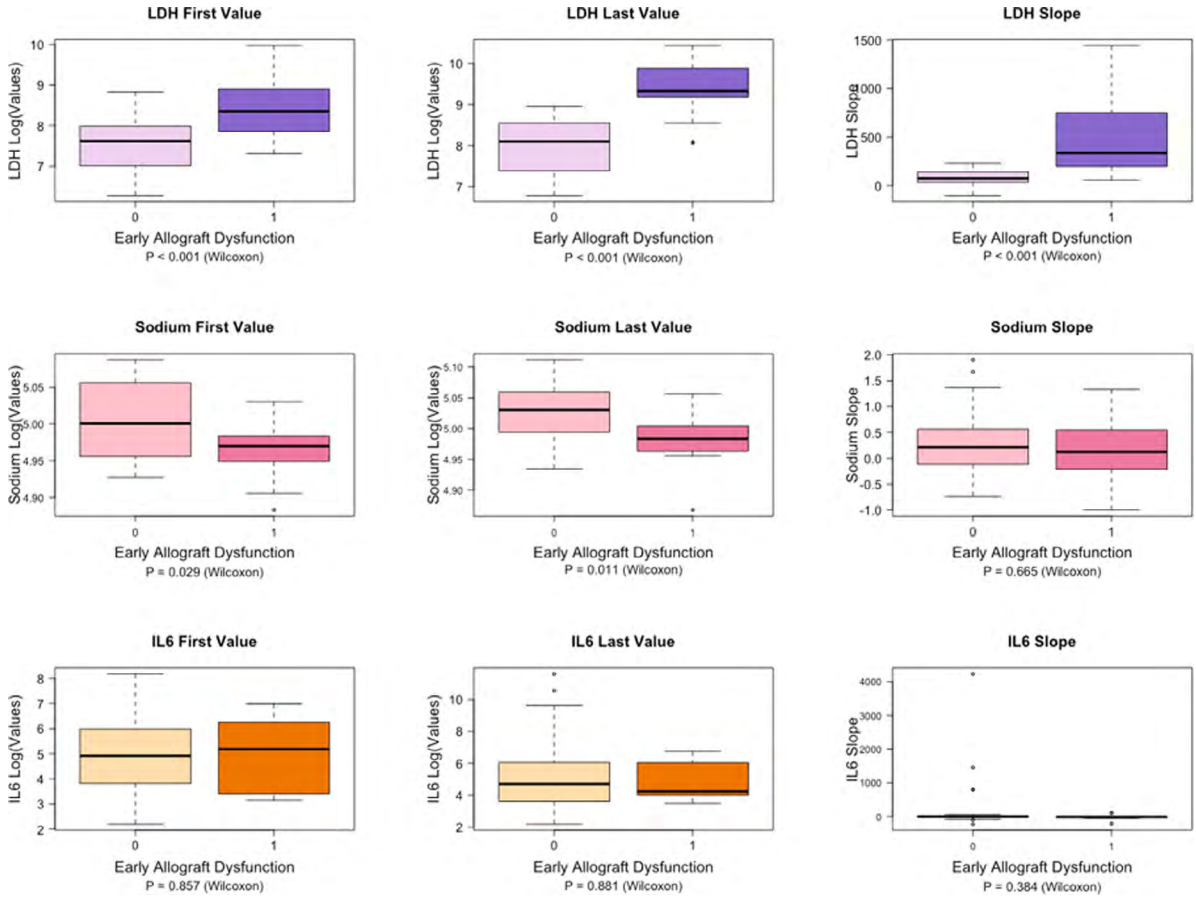

FIGURE 2. Continued.

TABLE 2.

Perfusate parameters associated with EAD-univariate logistic regression analysis

Repeated measurement	Time points considered	N	OR (95% CI)	*P*
AST (U/L)	Value at perfusion time (log)	45	8.32 (2.48-50.0)	0.004
	Last value before transplantation (log)	45	7.00 (2.59-30.1)	0.001
	Slope	45	1.008 (1.003-1.016)	0.008
ALT (U/L)	Value at perfusion time (log)	45	3.93 (1.58-13.6)	0.012
	Last value before transplantation (log)	45	14.14 (3.70-101.1)	0.001
	Slope	45	1.02 (1.01-1.04)	0.006
	Last value before transplantation (log)	45	1.54 (0.88-2.89)	0.146
	Slope	45	1.02 (0.99-1.05)	0.224
AP (U/L)	Value at perfusion time (log)	45	1.28 (0.60-2.76)	0.512
	Last value before transplantation (log)	45	2.28 (1.28-4.72)	0.013
	Slope	45	1.01 (0.98-1.05)	0.384
LDH (U/L)	Value at perfusion time (log)	45	6.83 (2.21-33.0)	0.004
	Last value before transplantation (log)	45	21.96 (4.65-261.2)	0.002
	Slope	45	1.01 (1.01-1.03)	0.007
Sodium (mmol/L)	Value at perfusion time (log)	45	0.87 (0.77-0.97)	0.021
	Last value before transplantation (log)	45	0.87 (0.77-0.96)	0.016
	Slope	45	0.78 (0.25-2.21)	0.645
CRP (mg/dL)	Value at perfusion time (log)	45	1.17 (0.65-2.19)	0.603
	Last value before transplantation (log)	45	1.07 (0.55-2.25)	0.848
	Slope	45	0.90 (0.32-2.33)	0.835
IL6 (ng/dL)	Value at perfusion time (log)	37	1.07 (0.67-1.72)	0.786
	Last value before transplantation (log)	37	0.92 (0.62-1.28)	0.645
	Slope	37	1.00 (0.99-1.00)	0.363
Bilirubin (mg/dL)	Distribution not suitable for logistic regression	45	NA	NA

ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CI, confidence interval; CRP, C-reactive protein; gGT, gamma-glutamyltransferase; IL6, interleukin 6; LDH, lactate dehydrogenase; NA, not available; OR, odds ratio.

between EAD and no-EAD livers: AP (89 [61.5–122.5] IU/L EAD versus 30 [18.5–42] IU/L no EAD, $P < 0.001$), AST (4000 [2192–6486] IU/L EAD versus 1288 [601.8–1875] IU/L no EAD, $P < 0.001$), ALT (1770 [793–5008] IU/L EAD versus 983 [465.5–1597] IU/L no EAD, $P = 0.008$), LDH (7399 [3404–11072] IU/L EAD versus 2244 [1211–3617] IU/L no EAD, $P = 0.0001$), and platelets (10 [8.3–17.5] g/L EAD versus 5.5 [2–8.5] g/L no EAD, $P = 0.01$) were significantly higher in EAD. Number of leukocytes was not significantly different (2.5 [1.2–4] g/L EAD versus 2 [1.1–2.4] g/L no EAD, $P = 0.2$). Values are in median (IQR).

Postoperative Course, Graft, and Patient Survival

Length of stay in intensive care unit was similar for EAD and no-EAD recipients; median (IQR) overall intensive care unit days 4 (4–8.5), EAD 4 (4–5) versus no-EAD 4 (3.3–9.9), $P = 0.8$. Renal replacement therapy was necessary in 21 of 45 (46.7%) liver transplant recipients overall. All patients recovered fully from acute kidney injury and were discharged with normal kidney function parameters. There was no significant difference between EAD (6/13, 46.1%) and no-EAD recipients (15/32, 46.9%). Perfusate parameters over time did not correlate with the necessity of renal replacement therapy posttransplant. Postoperative complications are listed in Table 4.

Median (IQR) recipient gGT on postoperative day (POD) 7 was significantly higher in liver transplant recipients developing EAD; 378 (183–612) IU/L in EAD versus 204 (146.5–348.5) in no EAD, $P = 0.013$. Median (IQR) recipient platelets on POD 7 were significantly higher in EAD than in no-EAD recipients; 125 (44–178) g/L versus 72 (47.5–103) g/L, $P = 0.04$. Figure 5A–G illustrates postoperative parameters for EAD and no-EAD recipients until day 10, including AST, ALT, bilirubin, international normalized ratio, gGT, platelets, and lactate (until day 7). The MEAF score correlated significantly with recipients' lactate on POD 1; Spearman's ρ 0.43, $P = 0.004$. Also, the higher the decrease of recipients' lactate between POD 1 and POD 7 was, the lower the MEAF score; Spearman's ρ –0.4, $P = 0.007$.

Four of the 45 (8.9%) NMP-liver transplant recipients died within the first 3 mo after transplantation, no one thereafter. All of them were male individuals. One was a retransplant patient (BAR sore 11, EAD, functioning graft) with a sudden onset of severe pain in his left leg diagnosed as necrotizing fasciitis on POD 34 and died 1 wk later. Two

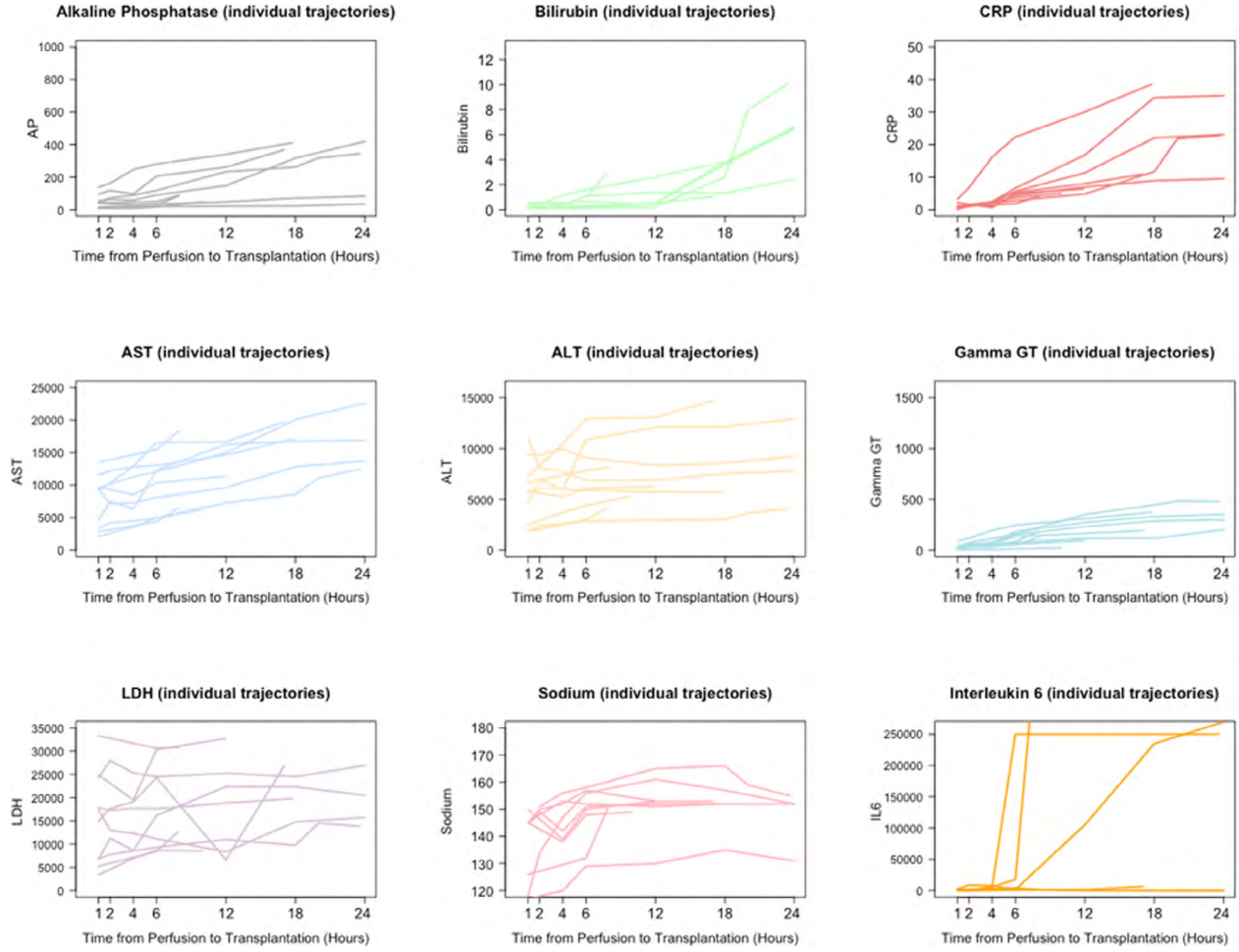

FIGURE 3. A, Individual trajectories of repetitively measured perfusate parameters of 45 transplanted NMP livers; color-coded EAD (n = 13) and no-EAD (n = 32) livers. B, Boxplot stratified by EAD (in 13 of 45 [28.9%]) transplanted NMP livers (n = 45); illustrating AP, bilirubin, CRP; 0 = initial function, 1 = early allograft dysfunction. C, Boxplot stratified by EAD (in 13 of 45 [28.9%]) transplanted NMP livers (n = 45); illustrating AST, ALT, gGT; 0 = initial function, 1 = early allograft dysfunction. D, Boxplot stratified by EAD (in 13 of 45 [28.9%]) transplanted NMP livers (n = 45); illustrating LDH, sodium, IL6; 0 = initial function, 1 = early allograft dysfunction. ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CRP, C-reactive protein; EAD, early allograft dysfunction; gGT, gamma-glutamyltransferase; IL6, interleukin 6; LDH, lactate dehydrogenase; NMP, normothermic machine perfusion.

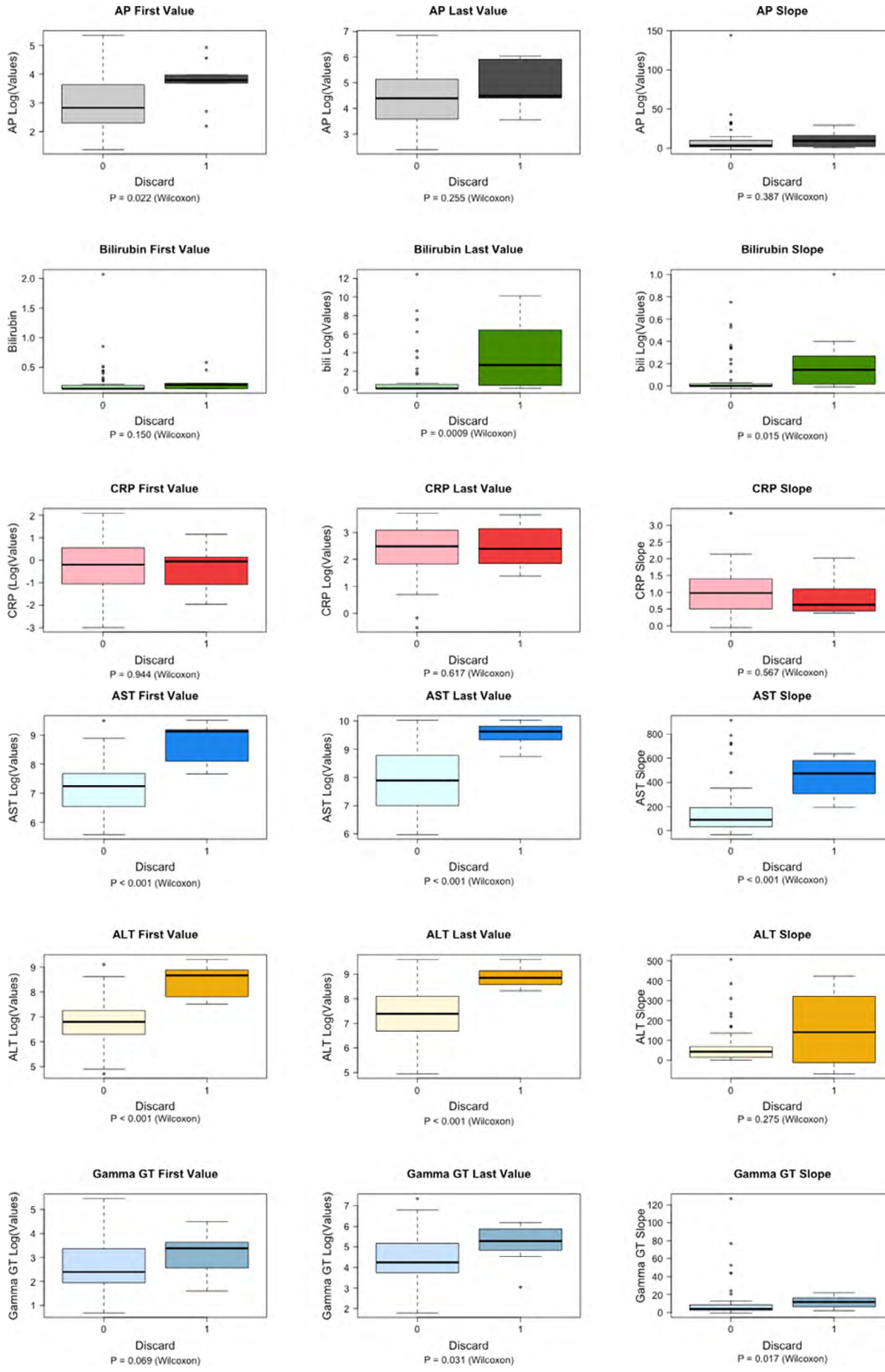

FIGURE 3. Continued.

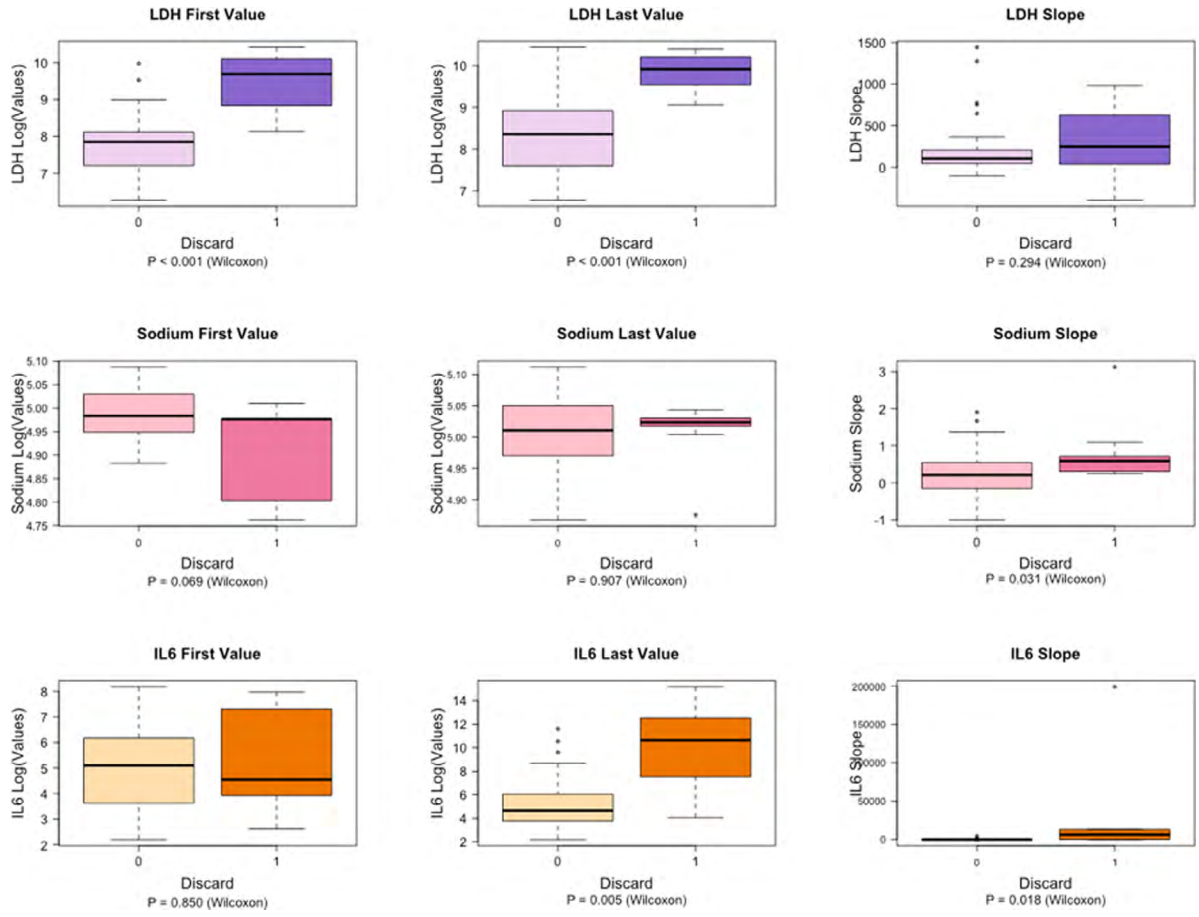

FIGURE 3. Continued.

patients were lost due to severe invasive aspergillosis on POD 48 (BAR sore 15, no EAD, with functioning graft) and POD 15 (BAR sore 5, no EAD, graft deterioration in the second week posttransplant). One patient (BAR sore 8, EAD, outpatient) died on POD 57 due to candidiasis with a functioning graft. Perfusate parameters over time and the L-GrAFT score did not correlate significantly with the event of death (n = 4). The MEAF score was significantly higher in the patients we lost with 6.17 (3.5–7.1) versus 4.2 (3.5–4.7) in the patients alive, $P = 0.02$.

DISCUSSION

We herein studied the value of repeated biomarker measurement in the perfusate during normothermic liver preservation. We found that high perfusate enzymes, their change over time, and the number of platelets in the perfusate are indicative for EAD after liver transplantation. This supports the idea that liver damage as assessed during NMP is similarly effective to the assessment of liver damage in the recipient. These results further endorse the back-to-base liver preservation approach and repeated measurements during NMP.[1,18] Biomarker assessment could augment the current decision-making process based on lactate clearance, hemodynamics, acid–base stability, and bile production. The in-depth perfusate analyses also revealed that it is the trend in biomarker expression over time, rather than single time point parameter assessment, that is meaningful. The slope of the curves of AST, ALT, and LDH were significantly associated with EAD and MEAF after transplantation. Comparing the perfusate biomarkers of transplanted livers with 10 discarded ones showed even higher perfusate enzymes in those and a significantly higher amount of administered sodium bicarbonate 8.4% to maintain a physiological pH. This incredibly high amount of substitution possibly explains the significantly higher perfusate sodium levels over time in the discarded group of livers.

The Cambridge group—Watson et al[7]—previously reported the importance of ALT and composition of biomarkers associated with transplantation in a series of NMP livers. In their experience, ALT levels <6000 IU/L 2 h after perfusion start are crucial to achieve a favorable outcome. When we applied the Cambridge criteria in our data set, all but 1 of our transplanted livers were inside the recommended range. This 44-y-old donor after brain death liver started with a perfusate ALT of 9044 IU/L. The recipient developed EAD; however, the clinical course was good, and he is in good shape with normal serum transaminases (AST 28 IU/L, ALT 23 IU/L, gGT 47 IU/L, LDH 221 IU/L), normal serum bilirubin (0.58 mg/dL), and with a pristine ultrasound without signs indicative for cholestasis or bile duct irregularities during the last outpatient clinic. In this analysis, perfusate lactate was not the focus as livers were only considered transplantable when lactate was cleared and in normal range after 6 h of NMP. Similar to the Cambridge criteria (proposing a peak lactate fall >4.4 mmol/L/kg/h, equivalent to 39.64 mg/dL/kg/h, as a sign for

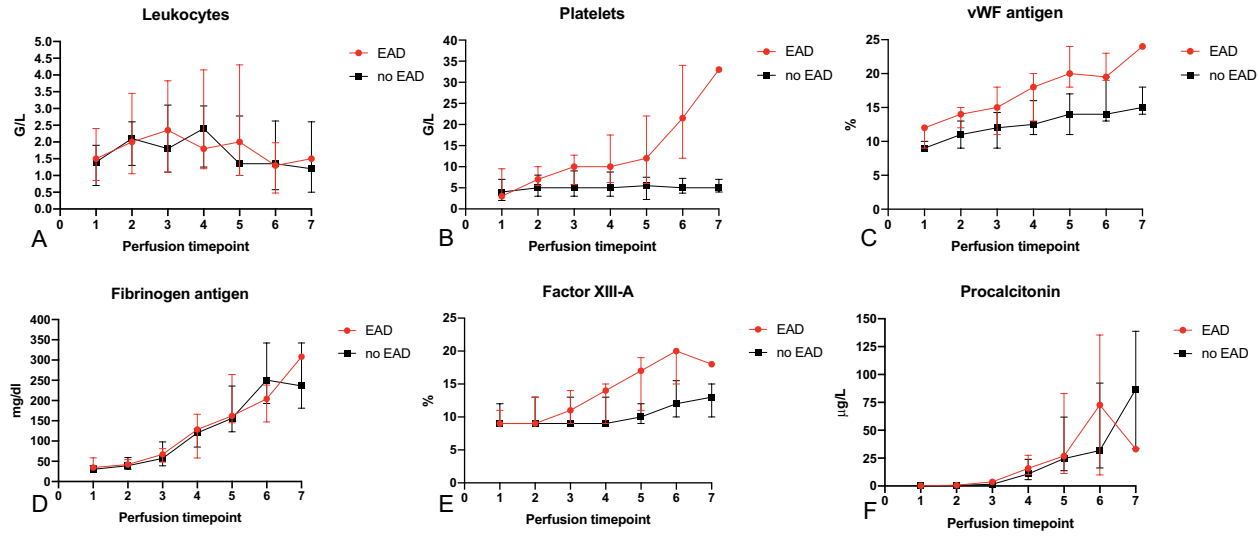

FIGURE 4. Illustration of longitudinal perfusate values of leukocytes (A), platelets (B), vWF antigen (C), fibrinogen antigen (D), factor XIII-A subunit antigen (E), and procalcitonin (F) over time at 7 perfusion time points (1, 2, 4, 6, 12, 18, 24 h after NMP start/end of NMP, respectively) stratified for EAD and no-EAD in 45 NMP livers. Graphs were created on the basis of median (interquartile ranges) for each value per perfusion time point per group (EAD, no-EAD). EAD, early allograft dysfunction; NMP, normothermic machine perfusion; vWF, von Willebrand factor.

allograft viability), we found a slope of lactate decrease exceeding the suggested benchmark (min 40 mg/dL, max 119.02 mg/dL) in almost all livers. No differences between EAD and no-EAD livers could be detected in this series. The same applies to the postoperative course of lactate in the recipients in which no differences between EAD and no-EAD livers could be detected.

Another basic function of the liver and kidney[28] is the ability to maintain a physiological acid–base balance. All of the livers in this series were able to sustain an intended perfusate pH of >7.2 without much need for bicarbonate.[7] However, it seems that even slight deviations from the physiological pH range of 7.3–7.45 make a significant difference in the outcome after transplantation and reveals that perfusate pH might be still underestimated as an important factor with strong predictive value.

In addition to "classical" perfusate parameters and their predictive capacity for the early outcome after liver transplantation, we also investigated markers characterizing the inflammatory state and the synthetic or regenerative

TABLE 3.

Correlation of perfusate parameters, transplant factors, and the event of EAD

Characteristic 1	Characteristic 2	Spearman's ρ	*P*
ΔPerfusate thrombocytes	ΔPerfusate vWF antigen	0.403	0.011
	CIT	–0.36	0.015
	NMP	0.054	0.727
	Overall preservation time	–0.077	0.62
	EAD	0.28	0.063
	Recipient thrombocytes POD 3	0.433	0.003
	Recipient thrombocytes POD 7	0.375	0.011
	Recipient thrombocytes POD 10	0.296	0.049
ΔPerfusate vWF antigen	EAD	0.315	0.051
	Recipient thrombocytes POD 1	0.274	0.091
	Recipient thrombocytes POD 3	0.273	0.093
	Recipient thrombocytes POD 7	0.311	0.054
	Recipient thrombocytes POD 10	0.214	0.19
ΔPerfusate leukocytes	CIT	0.209	0.168
	NMP	–0.418	0.005
	Overall preservation time	–0.314	0.038
	EAD	–0.125	0.412
EAD	Recipient thrombocytes POD 1	0.291	0.053
	Recipient thrombocytes POD 3	0.21	0.167
	Recipient thrombocytes POD 7	0.338	0.023
	Recipient thrombocytes POD 10	0.315	0.035

CIT, cold ischemia time; EAD, early allograft dysfunction; NMP, normothermic machine perfusion; POD, postoperative d; vWF, von Willebrand factor.

TABLE 4.

Postoperative complications within the first 90 d posttransplant

Complication	N
Relaparotomy overall	8/45 (17.8%)
Relaparotomy for bleeding/hematoma	3 1 POD 3 (no EAD), 1 POD 4 (EAD), 1 POD 7 (EAD)
Relaparotomy for bile duct leakage	5 2 POD 2 (EAD and no-EAD), 2 POD 7 (no-EAD), 1 POD 12 (no-EAD)
Bile duct strictures overall	8/45 (17.8%)
Anastomotic stricture POD <30	4 (2 EAD, 2 no-EAD); treated with ERCP
Anastomotic stricture POD 30–90	3 (no-EAD); treated with ERCP
ITBL POD 61	1 (EAD); stable, not listed for retransplant

EAD, early allograft dysfunction; ERCP, endoscopic retrograde cholangio-pancreaticography; ITBL, ischemic type bile duct lesion; POD, postoperative d.

capacity of the ex situ perfused liver. Angelico et al[29] reported better immediate postreperfusion hemodynamics with a less severe drop of mean arterial pressure in recipients receiving normothermically preserved livers compared with SCS. The NMP phase may serve as a third party, representing the "intermediate recipient" as Lechler and Batchelor[30] described it in their important work about allorecognition and the importance of donor-derived leukocytes. We detected an increase of leukocytes in the perfusate over time (not significantly different between EAD and no-EAD livers). Leukocytes are shed as residential cells from the liver into the perfusate or might get stuck on components of the circuit. The perfusate itself, comprising 3 units of red blood cells, had a very low count of leukocytes <0.1 G/L—before the liver was connected to the circuit. Jassem et al[31] described in their work on inflammatory responses and regeneration during NMP that the parenchyma of such preserved livers was strikingly less infiltrated by neutrophils compared with SCS liver tissue. However, the livers described by the King's College group have a different grade of injury, and their study protocol was also different to ours. To develop the investigation of perfusate leukocytes further, an approach published by Pagano et al in 2019[32] could be of interest. This research group investigated white blood cells in the perfusate of SCS livers, phenotyped them, and correlated the results with donor factors and postoperative outcomes. Even more noticeable was the influx of platelets in the perfusate over time—in particular in NMP livers whose recipients developed EAD. Before the livers were placed on the

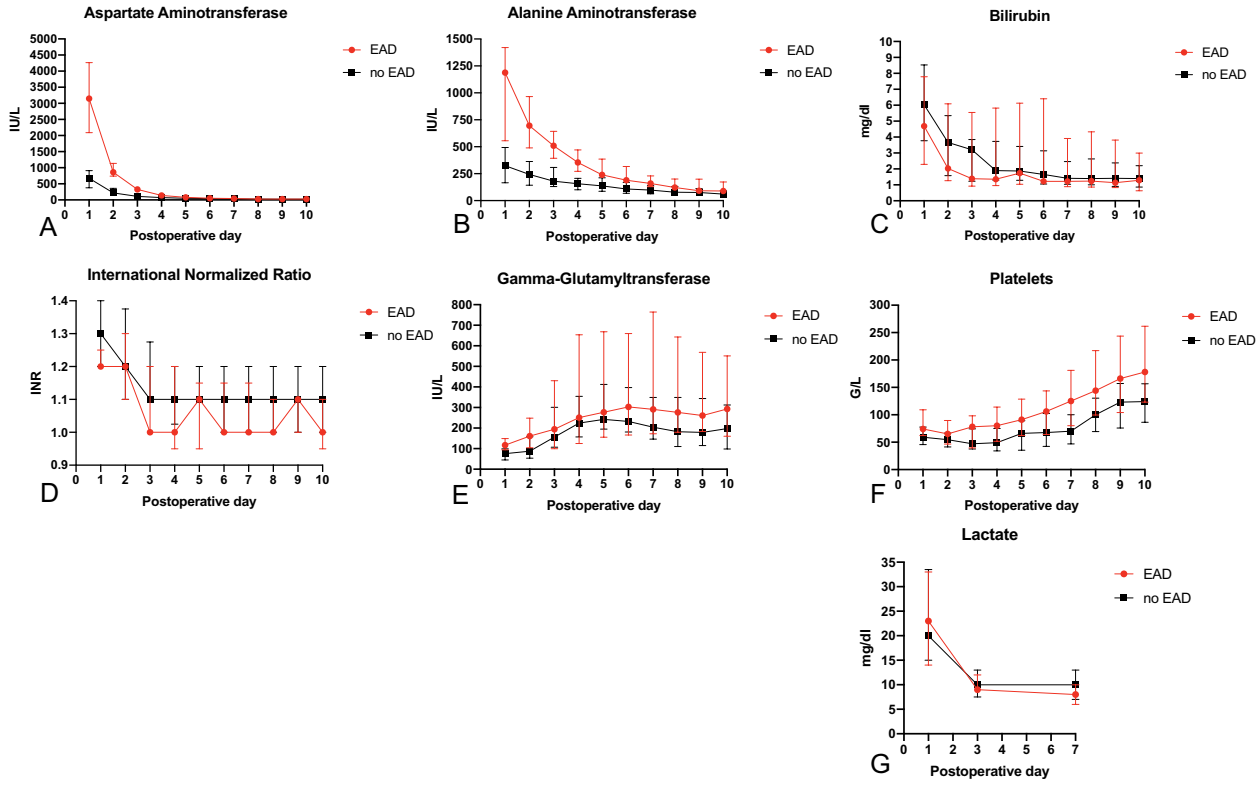

FIGURE 5. Presentation of longitudinal recipients' values measured from postoperative d 1 until 10 (7 for lactate) stratified for patients with EAD and no-EAD. Graphs were created on the basis of median (interquartile ranges) for each value per postoperative d per group (EAD, no-EAD). A, aspartate aminotransferase; (B) alanine aminotransferase; (C) bilirubin; (D) INR; (E) gamma-glutamyltransferase; (F) platelets; (G) lactate. EAD, early allograft dysfunction; INR, international normalized ratio.

NMP device, the perfusates contained 1 (min) to 3 (max) g/L platelets. This influx of platelets was accompanied by an increase of vWF antigen and FXIII-A antigen in the perfusate. The vWF antigen, tested to measure vWF quantitatively, increased in parallel with the platelets over time. The vWF antigen can either derive from the platelets or directly from the endothelial cells of the liver, as Weibel–Palade bodies can be found in all arteries and veins.[33] Moreover, the change of perfusate platelets correlated inversely with the duration of CIT; the longer the SCS period, the lower the platelet count in the perfusate. Both perfusate platelet count and perfusate vWF antigen correlated significantly with the occurrence of EAD and with the platelet count in the recipients up to the 10th POD, respectively. Recipients with EAD had an insignificant higher platelet count postoperatively compared with no-EAD patients. Kollmann et al[34] addressed the intrahepatic platelet sequestration after liver transplantation in an experimental porcine model. This impressive work on porcine liver transplantation revealed that liver NMP reduced platelet aggregation in the graft and thrombocytopenia in the recipient postoperatively effectively compared with SCS liver transplants. The effect of NMP on platelets was even more pronounced compared with clopidogrel-pretreated liver donors and recipients.[34] This experimental work demonstrates once more that liver NMP could be clearly beneficial in terms of reduced ischemia-reperfusion injury posttransplant via an inflammatory pathway involving platelets. Interestingly enough, the efflux of leukocytes from the liver into the perfusate became less with duration of preservation time. This may be indicative of the perfusate influx of leukocytes occurring in the earlier half of NMP and confirming that even shorter NMP periods are helpful to avoid a reperfusion syndrome.[13,18,29] Moreover, perfusate leukocytes did not correlate with initial liver function. Platelets and vWF seemed to play an important role in liver regeneration and postoperative outcome and were significantly associated with a dysfunctional liver in the early postoperative period in our analysis. Data generated by Starlinger et al,[35] in a nontransplant and nonpreservation setting, may support this hypothesis. They observed that an initial vWF burst is important for sufficient liver regeneration after liver resection. In addition to the aspect that a missing vWF burst is a consequence of dysfunctional hepatic endothelial cells, preoperative vWF antigen levels were also predictive for the outcome after liver resection.[35] Several mechanisms are described for the crosstalk between liver and platelets. Platelets play a role in improving fibrosis in terms of liver regeneration through joint effects with Kupffer cells, hepatocytes, and liver sinusoidal endothelial cells triggered by signaling cascades mediating these effects via tumor necrosis factor-alpha, nuclear factor-kappa B, and IL-6/signal transducer.[36] We believe that there is a clear link between platelets and vWF antigen (and FXIII-A antigen) in the perfusate and the early outcome after transplantation of NMP livers and the platelet counts in the recipients; however, no strong conclusions can be drawn at this point. The findings for fibrinogen antigen, FXIII-A antigen, and procalcitonin indicate that an isolated liver and its residential platelets take up their synthetic function when preserved under nearly physiological conditions. In perfusates of both EAD and no-EAD livers, fibrinogen antigen increased over time without showing significant differences. Fibrinogen is a protein made in the liver, and low plasma fibrinogen levels were identified as an important risk factor for liver dysfunction and mortality in patients undergoing liver resection.[37] To establish fibrinogen as a marker for liver regeneration and postoperative outcomes, it will require further studies and a higher number of livers and transplanted patients. The same applies to factor XIII and procalcitonin. Factor XIII, circulating as a heterotetramer, might be of interest because factor XIII-A has been significantly elevated in EAD livers. The A-chain of factor XIII, which includes the catalytic site of factor XIII, is produced by platelets and other bone marrow cells, whereas the B-chain is secreted into the blood by hepatocytes.[38] This very nature of factor XIII could be helpful to either partly explain the platelet function or define liver function in an ex situ model. The increasing levels of procalcitonin over time have generated interest and deserve further investigation because this could signal severe inflammation.[39] Under physiological circumstances, however, the liver is not the origin of procalcitonin, and therefore, observing procalcitonin alterations over time during liver NMP might have a different meaning.

We applied the Olthoff classification[23] to define EAD, which was characterized by elevated AST levels in the vast majority of our patients. It is highly unlikely for any score based on just a few variables to predict outcome in the most impeccable way, but the continuity of the score is beneficial and practicable. Thirteen (28.9%) of our NMP liver recipients had poor primary function. This is comparable with 31.8% EAD rate in the VITTAL study[40] on resuscitated discarded livers, published recently by the Birmingham group. Our cohort is characterized by 2 key factors that are similar to livers in the VITTAL study: (1) 17 (37.8%) of our recipients had a BAR score >9 compared with 2 (9.1%) in the VITTAL study; (2) our median CIT before NMP start was 6.35 (2.2–12.5) h compared with median 7.53 (6.48–10) h in the Birmingham cohort.

Overall, despite the still imperfect methods to predict short- and long-term outcomes after liver transplantation, the trend of certain perfusate parameters, measured during NMP, can help predict the initial postoperative function. These parameters, especially the standard transaminases and pH, provide additional information on prognosis that can be put to good use when informing patients, nursing staff, and caregivers about expected posttransplant outcomes. Using biomarkers during normothermic preservation of the liver could aid decision making and improve transplant outcomes. With growing knowledge and evidence that livers show a diverse performance during dynamic preservation, the biomarkers assessed here could be considered to be used for developing a score with predictive value. For the development of adjusted scores, multicenter preservation studies are desirable. The limitations of our study include the fact that EAD is mostly defined by AST raise. EAD converts graft function into a binary condition when the clinical reality is that organ function is a continuous outcome measure, and most livers meet the EAD definition based on peak AST. However, the MEAF score, which is known as a score stratifying graft function over time,[24] correlated with the occurrence of EAD and with measured perfusate parameters over time significantly in our cohort. Additional histological analyses, especially

immunohistochemistry studies, could potentially clarify and explain the impact of NMP on short- and long-term liver transplant outcomes further.

Although NMP has made significant technical advancements, we need to better define the clinical impact. Viability of livers during NMP preservation has the potential to improve utilization, especially in conservative territories, while maintaining good clinical outcomes. Further analyses of the correlation of perfusate and outcome markers will greatly improve the efficacy of this new approach.

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