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The authors declare no funding or conflicts of interest.

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Abstract

Background.
Liver graft viability assessment has long been considered a limit of hypothermic oxygenated machine perfusion (HOPE). Aim of this study was assessing correlations of easily available perfusate parameters (PP) (aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, glucose, lactate, and pH) with graft features and outcome.

Methods.
In the period October 2018–February 2020, perfusate samples were obtained every 30 minutes during 50 dual-HOPE (D-HOPE) procedures. Correlations of PP with graft factors, 90-day graft loss, early allograft dysfunction (EAD), L-GrAFT score, acute kidney injury, and comprehensive complication index were analyzed using Pearson coefficient, receiver-operating characteristics analysis and by univariable and multivariable regression.

Results.
Median D-HOPE time was 122 minutes. All parameters were normalized to liver weight. Only macrovesicular steatosis (MaS) significantly impacted PP levels and slope. Grafts with ≥30% MaS exhibited significantly different PP values and slope. Graft loss and EAD rate were 2% (n = 1) and 26% (n = 13). All PP except lactate correlated with EAD, 90-minute alanine aminotransferase showing the highest area under the receiver-operating characteristics curve (0.84). However, at multivariable analysis, the only factor independently associated with EAD was MaS (odds ratio, 5.44; confidence interval, 1.05-28.21; P = 0.04). Ninety minutes lactate dehydrogenase had the strongest correlation with L-GrAFT (R = 0.70; P < 0.001). PP correlated poorly with comprehensive complication index and grades 2–3 acute kidney injury rate.

Conclusions.
PP were predictive of graft function after transplant, but their association with graft survival and clinical outcomes requires further evaluation. MaS influenced levels of PP and was the only independent predictor of EAD.

INTRODUCTION

Hypothermic oxygenated machine perfusion (HOPE) is gaining increasing widespread acceptance and has been associated with reduced ischemia-reperfusion injury and improved clinical outcomes in liver transplantation (LT) with both donors after circulatory death (DCD) and after brain death (DBD).1-5 HOPE can be performed by exclusive perfusion of the portal vein6 or, as initially proposed by the Groningen group, by simultaneous perfusion of both portal vein and hepatic artery (so-called dual-HOPE [D-HOPE]).3 Whichever the technique, among the desired goals of any machine perfusion technique, the possibility of assessing graft viability before transplantation has been identified as one of the most relevant. Several studies explored the value of perfusate analysis in predicting graft survival and function after kidney transplantation, whereas findings in the field of LT are much more limited. After the initial studies by Guarrera el al7,8 linking perfusate levels of transaminase and lactate dehydrogenase (LDH) with their equivalent in the recipient, more recently promising results came from a study by the Zurich group,9 evaluating the correlation between perfusate levels of flavin mononucleotide (FMN) and 3-month graft survival.

However, initial studies lacked numerosity and were not focused on perfusate analysis, whereas determination of FMN is still not widely available.

Furthermore, available studies did not analyze correlation between graft characteristics and perfusate parameters (PP), nor between PP and more recently introduced measures of post-LT graft function.

The working hypothesis of this study was that analyzing the level and trend of widely available PP (aspartate aminotransferase [AST], alanine aminotransferase [ALT], LDH, lactate, glucose, and pH) could

provide an estimate of graft quality and be informative of postoperative graft function and patient course. The possibility of having such an estimate would have major implications, not only concerning the decision as to whether to perform the transplant or not but also for patient management, including immunosuppression protocol.

Hence, the aim of this study was double. First, we investigated whether parameters of perfusate samples collected at fixed time points during D-HOPE are indicative of donor or graft features. Second, we analyzed their correlation with graft survival, function, and patient clinical course.

MATERIALS AND METHODS

Study Design

This is a single-center retrospective observational study on 50 consecutive patients who underwent LT in the period October 2018–February 2020 with a DBD graft having been treated with end-ischemic D-HOPE. Cases were identified from our prospectively maintained database, which was retrospectively analyzed. During the study period, 213 adult LT were performed (DBD, n = 209; DCD, n = 4). Twenty-three livers initially accepted (DBD, n = 21; DCD, n = 2) were discarded and not transplanted. D-HOPE, HOPE, and normothermic machine perfusion were used in 53 (24.9%), 3 (1.4%), and 3 (1.4%) cases, respectively. All machine perfusion procedures were performed back-to-base after the organ had been transported to our transplant center. Grafts from DCD donors were excluded from the analysis due to different physiopathology of ischemia-reperfusion injury and constant use of normothermic regional perfusion in this setting.

Perfusate samples were collected at 30 minutes intervals from D-HOPE start until 3 hours of machine perfusion and analyzed to determine levels of AST, ALT, LDH, lactate, glucose, and pH. Lactate, glucose, and pH were determined using a GEM Premier 4000 point-of-care blood gas analyzer (Instrumentation Laboratory, Bedford, MA) and were available during D-HOPE, whereas samples for AST, ALT, and LDH measurement were sent to the biochemistry laboratory at the end of D-HOPE. Transaminases and LDH levels were determined using standard absorption techniques and were available about 2 hours after. In no case, perfusate analysis was used for graft evaluation and all D-HOPE-treated grafts were eventually transplanted. Single-point measurements as well as mean, maximum and minimum level, and slope (mean change per 30-min time point) were calculated for every parameter. We then analyzed intercorrelations between parameters and their correlation with donor and graft variables. Donor variables included age, cause of death, length of intensive care unit stay, transaminases, use of vasopressors, bloodstream infection, cold ischemia time, and donor risk index,[10] whereas graft variables included weight after back table preparation, degree of micro- and macrovesicular steatosis (MaS), necrosis, and histological signs of ischemia-reperfusion injury, including inflammatory changes, graded as mild, moderate, or severe according to Ali et al.[11,12] Primary outcome was incidence of early allograft dysfunction (EAD),[13] whereas secondary outcomes included transaminase peak (as assessed on blood samples obtained 6 h after LT and then every 8 h for the first 48 h after transplant), grades 2–3 acute kidney injury (AKI),[14] liver graft assessment following transplantation risk score (L-GrAFT),[15] and comprehensive complication index (CCI).[16] Based on an estimated EAD incidence between 30% and 40%, sample size = 50 was chosen to allow estimating an 80%–90% sensitivity against a null hypothesis of 50% with a minimum power of 80% and a significance level of 5%.[17]

Minimum follow-up was 3 months. All enrolled patients signed a consent form agreeing to receive a graft treated with machine perfusion. Due to the retrospective observational design, no specific approval was sought from the local Ethics Committee. Study procedures complied with the 2008 version of the Declaration of Helsinki (https://www.wma.net).

Procedural Details

Our D-HOPE and LT protocols have been detailed elsewhere.[1] Briefly, indication for D-HOPE was established on a case-per-case basis taking into account the combination of known risk factors for graft failure, including advanced donor age (≥80 y), high-donor body mass index (>30), graft macroscopic appearance suggesting significant steatosis, altered liver enzymes, and expected ischemia time ≥10 hours. Before aortic cross-clamping, donor was administered 300 IU/kg of heparin. The graft was flushed through an aortic cannula with cold Celsior solution (IGL, Lissieu, France). No heparin was added to perfusion fluid. Upon arrival at our transplant center, the graft was prepared on back table and weighed. Subsequently, portal vein and celiac artery were cannulated, and the liver was connected to the LiverAssist device (OrganAssist, Groningen, The Netherlands) without being previously flushed. Device was primed with 3 L of Belzer MPS solution (Bridge to Life Europe Ltd, Wandsworth, London, United Kingdom), regardless of liver weight. Pressure settings were 3–5 mm Hg and 25 mm Hg for the portal vein and hepatic artery units, respectively. Portal vein pressure was kept as low as possible to achieve a minimum flow allowing circuit temperature to be maintained approxiamtely 10°C (usually ≥80 mL/min). Target perfusate oxygen pressure was 600 mm Hg. Start of transplant operation was scheduled to allow a minimum D-HOPE time of 90 minutes. Thus, all patients had a minimum of 3 perfusate samples collected. At the end of recipient hepatectomy, the graft was disconnected from the device, transferred to the recipient, and flushed with chilled 5% albumin solution during implantation.

All grafts were implanted using piggyback technique and reperfused through portal vein first. Graft histology, including the degree of macro- and microvesicular steatosis and inflammatory changes related to ischemia-reperfusion injury, was assessed on hematoxylin and eosin-stained biopsies obtained 1–2 hours after graft reperfusion into the recipient (so-called time-zero biopsies). As a rule, graft histology was obtained after D-HOPE and subsequent reperfusion into the recipient and in no case was taken into account in evaluating the indication for machine perfusion.

Standard immunosuppression included basiliximab (20 mg intraoperatively and on postoperative d [POD] 4), tacrolimus, steroids (methylprednisolone 1000 mg intraoperatively; prednisone 20 mg from POD 1) and mycophenolate mofetil.

Statistical Analysis

Data are expressed as median (interquartile range [IQR]) or number (percentage). Slopes were defined as mean variation per time point. Variables were compared using nonparametric Wilcoxon, Kruskal-Wallis, Chi-squared, or Fisher's exact tests, as appropriate. Correlations between study variables were analyzed using Pearson correlation coefficient (R) and linear regression. Association between PP and outcome measures were analyzed using linear regression, Pearson coefficient, as well as univariable and multivariable logistic regression using least absolute shrinkage and selection operator (Lasso) method.[18] Receiver-operating characteristics analyses with area under the curve (AUC) calculation were performed to assess predictive value of PP with regards to binary outcomes. Cutoff values were calculated using Youden method. All analyses were performed with R version 3.6.1. (R Foundation for Statistical Computing, Vienna, Austria: https://www.R-project.org/).

RESULTS

Donor and recipient characteristics are summarized in Table 1. Median (IQR) model for end-stage liver disease and balance of risk score19 were 13 (9–17) and 5 (3–19), respectively. Thirty (60%) patients were transplanted for hepatocellular carcinoma. Median (IQR) D-HOPE time was 122 (103–176) minutes. Given the minimum perfusion time of 90 minutes, all patients had at least 3 perfusate samples collected 30, 60, and 90 minutes after D-HOPE start available for analysis, whereas the percentage of missing values was 28%, 50%, and 74% at the 120, 150, and 180 minutes time points, respectively. Levels measured at 90 minutes were therefore included in subsequent analyses, as they were available for all patients and reflected different perfusate trends throughout perfusion.

Thirteen (26%) patients developed EAD, of whom only 6 (12%) due to elevated bilirubin or international normalized ratio on POD 7, whereas incidence of grades 2–3 AKI was 26% (Table 2). According to the L-GrAFT score, the majority of patients (82%) classified as low-risk based on postoperative liver function tests. Only 1 (2%) graft was lost at 3-month follow-up.

Intercorrelations Between Perfusate Parameters

Absolute values of PP showed strong intercorrelations. In particular, AST, ALT, LDH, glucose, and lactate levels correlated positively between them and negatively with perfusate pH (Figure 1A). However, as expected, all parameters also correlated with liver weight (Figure 1B). Thus, all subsequent analyses were carried out on values normalized to liver weight. After normalization, correlations between PP were weaker. In particular, glucose and lactate levels lost their correlation with transaminases and LDH, whereas pH still was negatively correlated with AST, ALT, LDH, and lactate levels (Figure 1C).

Correlation of Perfusate Parameters With Donor and Graft Features

Among the analyzed donor and graft features, the degree of MaS was the only variable significantly influencing levels and slope of normalized PP (Figure 2). Other donor factors, including age, cause of death, length of intensive care unit stay, bloodstream infection, use of vasopressors, cold ischemia time, and donor risk index did not appear to impact significantly on PP (Table 3; Figure 2; Figures S1–S3, SDC, https://links.lww.com/TP/B977). The degree of necrosis and of microvesicular steatosis was not associated with levels of perfusate biomarkers. Mean levels of PP were not associated with the severity of ischemia-reperfusion injury as assessed on time-zero biopsies, with the exception that grafts showing signs of severe ischemia-reperfusion injury had higher levels of perfusate LDH (Table S1 and Figure S4, SDC, https://links.lww.com/TP/B977). In grafts with moderate (≥30%) MaS (Figure 3), levels of PP were different at most time points (Table 4) and exhibited a different trend as compared to grafts with MaS 15%–30% or <15% (Figure 4). Use of normalized values appeared to be important also in these analyses. For example, absolute perfusate glucose level positively correlated with MaS, but this trend was actually inversed after normalization of glucose values to liver weight (Figure 5), meaning that higher the degree of MaS, the lower the perfusate glucose level per weight unit.

Correlation of Perfusate Parameters With Outcomes

Transaminase peak after LT was positively correlated with transaminases and LDH levels and negatively correlated with pH (Figure S5, SDC, https://links.lww.com/TP/B977).

Values and trend of PP were different in cases characterized by subsequent development of EAD (Figure 6). Except lactate, PP were all predictive of the development of EAD (Table 5). The best predictor of EAD was ALT level at 90 minutes, showing an AUC of 0.84 (Confidence interval, 0.69-0.97) (Figure 7). The normalized ALT cutoff value of 537 IU/L, calculated using Youden method, had 0.84 sensitivity, 0.81 specificity, 0.61 positive predictive value, and 0.93 negative predictive value for EAD development. These observations were confirmed at univariable logistic regression, in which other variables potentially associated with EAD were included. Given the low number of events (n = 13) and the intercorrelations between different PP (in particular between AST, ALT, LDH, and pH), multivariable Lasso regression was performed by including in the model MaS, 90-minute ALT (parameter with highest AUC) and 90-minute glucose (parameter not correlated with others). In this model, the only independent predictor of EAD was graft MaS (odds ratio, 5.44; 95% CI, 1.05-28.21; P = 0.04) (Table 6).

The performance of PP in predicting EAD caused by international normalized ratio ≥ 1.7 and total bilirubin ≥10 mg/dL on POD 7 (ie, excluding transaminase peak from EAD definition) was lower, with 90-minute ALT and LDH level showing the highest AUC of 0.68. The same 90-minute ALT cutoff value of 537 IU/L had 0.66

sensitivity, 0.68 specificity, 0.22 positive predictive value, and 0.93 negative predictive value in predicting this outcome.

Concerning other analyzed outcomes, 90-minute LDH level had the higher correlation with L-GrAFT score (R = 0.70; *P* < 0.001). Correlation with in-hospital CCI and the development of grades 2–3 AKI was generally poor (Table 5).

As we observed, only 1 case of 3-month graft failure, no analysis was possible concerning this outcome. In that case, donor was a 63-year-old DBD with body mass index 29 and graft had 40% MaS and 30% microvesicular steatosis. Perfusate level at 90 minutes of D-HOPE was AST 5676 IU/L, ALT 4320 IU/L, LDH 94 685 IU/L, lactate 10.9 mmol/L, glucose 500 mg/dL, and pH 6.93. Postoperative course was characterized by a markedly elevated transaminase peak (AST > 12 000 IU/L), hemoperitoneum requiring relaparotomy on POD 1, grade 3 AKI requiring renal replacement therapy and persistently elevated bilirubin compatible with delayed nonfunction. Patient was retransplanted on POD 31 and made a good recovery afterwards.

DISCUSSION

In this study, we assessed perfusate levels and trend of classic markers of hepatocyte (AST, ALT) or cellular (LDH) injury, metabolic substrates (lactate and glucose) and pH, measured at fixed intervals during D-HOPE, analyzing their intercorrelations and their association with graft features and posttransplant course.

In kidney transplantation, several studies have explored the possibility of assessing graft viability and quality by analyzing levels of different substances in the perfusate collected during hypothermic machine perfusion.[20-27] In this realm, elevated LDH perfusate levels have been frequently associated with delayed graft function (DGF)[22,25,26,28,29] or primary nonfunction.[22,29] Fewer and generally older studies have shown the association between elevated perfusate lactate levels and DGF.[23,25] A recent study from Guy et al[20] using nuclear magnetic resonance spectroscopy showed lower perfusate glucose levels at 45 minutes and 4 hours of perfusion in kidneys subsequently developing DGF. Other studies have focused on the predictive value of renal vascular resistance during machine perfusion,[27,30-32] which has been associated with DGF and 1-year graft survival, albeit with a low predictive value. However, as also highlighted by recent reviews,[21,33-35] results of these studies are far from conclusive and, as the ability of single parameters to predict kidney viability appears to be limited, discarding a graft based solely on perfusate analysis or vascular resistance parameters is generally not recommended.

In LT, graft viability assessment has been the focus of many studies using normothermic machine perfusion, whereas data about hypothermic perfusion are much more limited. In their 2010 study presenting the first clinical series of patients transplanted with a graft treated with hypothermic machine perfusion, Guarrera et al[7] observed a strong correlation between 2 hours levels of perfusate AST, ALT, and LDH with recipient transaminase peak. These findings were confirmed in a later study from the same group,[8] in which elevated perfusate levels of liver enzymes (>4500 IU/L), and higher portal pressure were observed in 2 grafts characterized by 40%–50% MaS and 30%–40% necrosis. Notably, the recipient of the graft with 40%–50% MaS developed primary nonfunction. Authors suggested that elevated enzyme levels and high portal pressure were sign of impending severe ischemia-reperfusion injury and could be considered as surrogate markers of liver viability. More recently, Muller et al[9] prospectively analyzed perfusate collected during 54 HOPE procedures at a single center and proposed FMN as a reliable predictor of postoperative graft function, graft loss, and clinical outcome, given its correlation with peak creatinine level, length of stay, and CCI at discharge. Although promising, these findings still need external validation.

The choice of perfusate biomarkers to be evaluated in this study, besides their wide availability, was based on the rationale of assessing different aspects of liver graft damage. AST and ALT are abundant in hepatocyte cytosol and are released in case of damage to cellular membrane or hepatocyte death.[36] Similarly, LDH is present at high concentrations in almost all tissues, including liver, but also in mitochondria, and serves as a marker of cellular and mitochondrial injury.[37] The observed intercorrelation between AST, ALT, and LDH in our study is therefore not surprising. On the other hand, raised glucose level in perfusate during D-HOPE is mainly due to glycogen breakdown during cold storage and its levels may be indicative of baseline hepatocyte glycogen stores as well as of duration of cold ischemia.[38] Finally, lactate accumulates during cold storage as an end product of anaerobic glycolysis, and it is released in perfusate

determining a reduction of pH, a finding confirmed by negative correlation between lactate and pH (Figure 1C). As the beneficial effect of hypothermic-oxygenated perfusion is mainly exerted through a restoration of mitochondrial respiration,[39] levels of these last 2 parameters were particularly interesting to investigate, particularly in relation to their trend (slope) during D-HOPE.

The first message from our data is that normalization to liver weight is of paramount importance in this kind of analyses. This is logical, as heavier grafts tend to release larger amounts of any biomarker in a fixed volume (3 Lt) of perfusate, independently from graft quality or ischemic injury suffered during static cold storage (SCS). Normalization allowed a more meaningful interpretation of the intercorrelations between single parameters and of their prognostic value. After normalization, lactate and glucose levels lost their correlation with markers of hepatocyte and cellular injury (Figure 1C), in keeping with their association with different mechanisms of injury. Normalization was particularly important in interpreting the correlation between glucose levels and graft steatosis. Indeed, before normalization, it appeared that steatotic grafts released more glucose in the perfusate, but this correlation was inversed after glucose levels were normalized to liver weight (Figure 5), suggesting that actually steatotic graft release less glucose per weight unit during D-HOPE, which can be explained by the impaired energy balance characteristic of hepatic steatosis[40] or by increased rate of glucose consumption during initial cold storage.

MaS was also the graft feature most significantly influencing PP, whereas other donor and graft factors, including donor age and duration of cold ischemia, did not. The severity of histological features of ischemia-reperfusion was also correlated with perfusate LDH levels, but not with those of other parameters. Concerning cold ischemia time, it is likely that its relatively short duration (median 5 h 54 min) before D-HOPE prevented this variable from influencing PP.

Data on the impact of HOPE on the preservation of steatotic grafts are limited. In an isolated-reperfused rat liver model of grafts with methionine-choline deficient diet-induced steatosis, Bessems et al[41] observed decreased signs of liver damage and improved markers of liver function after 24-hour D-HOPE, as compared to SCS. In a similar model of diet-induced hepatic steatosis, Kron et al[42] observed that 1-hour HOPE following 12 hours of SCS was associated with markedly decreased signs of reperfusion injury after transplant. In the same study, clinical outcome of 6 recipients of steatotic HOPE-treated grafts was compared with that of 12 patients receiving steatotic grafts preserved by conventional cold storage. HOPE treatment was associated with lower ALT peak, lower need for renal replacement therapy, shorter intensive therapy unit stay, and increased graft survival. No data about perfusate biomarkers were available for this patient series.

Despite having observed equally favorable clinical results in our series, the strong association between PP and steatosis in our study remains concerning. MaS was associated with higher levels of injury biomarkers, lower pH, and lower glucose, suggesting a more severe degree of cellular injury suffered by fatty grafts and an impaired energy balance even after a short period of cold storage. Furthermore, AST, ALT, LDH, and lactate levels progressively increased throughout perfusion, whereas pH progressively decreased, raising a concern about possible ongoing injury and incomplete restoration of mitochondrial respiration during D-HOPE in fatty grafts. Our findings mirror those from Monbaliu et al,[43] who applied D-HOPE to discarded human grafts and observed higher levels and a similar increasing trend of perfusate AST and LDH in livers that were a posteriori classified as nontransplantable, as compared to those classified as transplantable in retrospect. Notably, in 8 out of 11 livers, the reason for being deemed nonstransplantable was severe steatosis, which was in turn, highly correlated with perfusate AST level. Overall, these findings are in keeping with the higher susceptibility of steatotic grafts toward ischemic injury[44] and suggest that D-HOPE at 10°C, although superior to SCS, may be suboptimal for fatty grafts. It appears that even a short period of cold ischemia before D-HOPE is detrimental in steatotic grafts. In this perspective, starting machine perfusion at donor hospital as soon as possible after retrieval and continuing machine perfusion throughout preservation using a transportable device, as opposed to an end-ischemic approach, may yield better results in this particular subset. Furthermore, steatotic livers are characterized by narrower sinusoids[45] and may be particularly susceptible to vasoconstriction induced by machine perfusion in hypothermic conditions,[46] which has pushed other research groups to explore subnormothermic[47,48] or normothermic machine perfusion for preservation of fatty liver grafts.[49]

Finally, the importance of MaS was apparent also in the analysis of the predictive ability of PP toward postoperative outcome. Levels of all parameters except lactate were associated with EAD development. Higher AST, ALT, LDH levels, and lower glucose levels and pH were all good predictors of EAD, the higher AUC (0.84) being that of normalized 90-minute ALT level (Table 5). However, at multivariable analysis, MaS was the only variable significantly associated with EAD development. LDH levels showed good correlation (90-min LDH, R = 0.70; $P < 0.001$) with graft functional recovery, quantified using L-GrAFT score, whereas predictive ability of PP for EAD was lower when transaminase peak was excluded from EAD definition or for other clinical outcomes, including grades 2–3 AKI and CCI. More importantly, all but 1 graft were functioning at 3-month follow-up, suggesting that long-term graft survival could be achieved despite elevated PP and initial EAD.

This study has limitations, including its retrospective, single-center nature, and limited sample size. In particular, no correlation between perfusate biomarkers and graft survival could be ascertained given that only 1 graft was lost at 3-month follow-up. Additionally, study population was heterogeneous as grafts were treated with machine perfusion for different indications. Finally, due to the low number of grafts with moderate MaS (n = 5), our findings concerning its impact on PP level and EAD need confirmation in larger series.

In conclusion, levels and trends of perfusate transaminases, LDH, glucose, and pH are influenced mainly by graft MaS and are associated with the onset of EAD after transplant. However, in our series, EAD did not have a major impact on 3-month graft survival and even grafts with high levels of perfusate biomarkers had good functional recovery. Thus, information gathered by perfusate analysis should be interpreted with caution and taking into account the whole picture of other known risk factors of graft dysfunction.

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TABLE 1. Recipient and donor characteristics

	Overall (n = 50)
Recipient age (y)	60.6 (56.2–63.2)
Sex (male)	38 (76%)
MELD	13 (9–17)
HCC	30 (60%)
Retransplant	0 (0%)
Donor age (y)	71.98 (60.8–82.7)
Donor BMI	27.2 (23.5–30.0)
DCD	(0%)
Cause of death	
Cerebrovascular	34 (68%)
Anoxia	10 (20%)
Trauma	5 (10%)
Other	1 (2%)
ICU d (donor)	3 (2–4)
Vasopressors	44 (88%)
Donor diabetes	9 (18%)
Donor GGT (IU/L)	36 (18.00, 64.00)
Graft weight (gr)	1365 (1125–1790)
Macrovesicular steatosis (%)	5 (1–15)
Macrovesicular steatosis ≥15%	14 (28%)
Macrovesicular steatosis ≥30%	5 (10%)
Microvesicular steatosis (%)	20 (6–40)
Necrosis (%)	10 (5–14)
Preservation injury	
Mild	18 (36%)
Moderate	21 (42%)
Severe	11 (22%)
DRI	2.08 (1.48–2.44)
D-MELD	895 (653–1184)
BAR	5 (3–19)
Cold ischemia time (min)[a]	354 (299–390)
D-HOPE time (min)	122 (103–176)
Anastomoses time (portal vein)[b]	23 (21–27)

[a]Refers to cold ischemia time before machine perfusion.
[b]Time elapsed from the start of vascular anastomosis to portal vein reperfusion. Data are expressed as number (%) or median (interquartile range).
BAR, balance of risk score; BMI, body mass index; DCD, donation after circulatory death; D-HOPE, dual hypothermic oxygenated machine perfusion; D-MELD, donor age*recipient MELD; DRI, donor risk index; GGT, gamma glutamyl transferase; HCC, hepatocellular carcinoma; ICU, intensive care unit; MELD, model for end-stage liver disease.

TRANSPLANTATION

TABLE 2. Clinical outcomes

	Overall (n = 50)
AST peak (IU/L)	991 (565–1623)
ALT peak (IU/L)	491 (279–945)
EAD	13 (26%)
EAD excluding transaminase peak	6 (12%)
Grade 2–3 AKI	13 (26%)
L-GrAFT score	−1.90 (−2.34, −1.38)
Low risk	41 (82%)
Moderate risk	5 (10%)
Moderate-to-high risk	3 (6%)
High risk	1 (2%)
In-hospital CCI	20.9 (8.7–29.6)
Graft failure	1 (2%)

AKI, acute kidney injury; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCI, comprehensive complication index; EAD, early allograft dysfunction; L-GrAFT, liver graft assessment following transplantation risk score.

FIGURE 1. Correlograms showing intercorrelations between mean perfusate parameters before normalization (A), with graft weight (B), and after normalization to graft weight (C). Numbers in squares represent Pearson correlation coefficients for each couple of variables. P for correlation coefficients for (B) are reported in Table 3. Positive correlations are displayed in blue and negative correlations in red color. Color intensity is proportional to the correlation coefficients as indicated by the sidebar. ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.

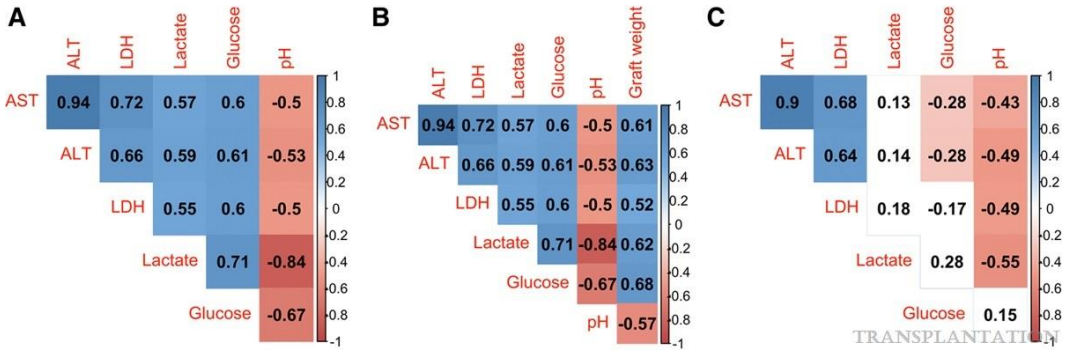


TABLE 3. Correlation between perfusate parameters and graft features

	Graft weight (gr)	Macrovesicular steatosis (%)	Donor age (y)	Donor risk index	Cold ischemia time (min)
AST (IU/L)					
Mean level	0.61 (<0.001)	0.83 (<0.001)	−0.21 (0.15)	−0.30 (0.03)	0.17 (0.24)
Normalized mean level		0.86 (<0.001)	−0.20 (0.16)	−0.29 (0.04)	0.17 (0.24)
Maximum level	0.60 (<0.001)	0.84 (<0.001)	−0.20 (0.17)	−0.30 (0.03)	0.18 (0.22)
Normalized maximum level		0.87 (<0.001)	−0.19 (0.18)	−0.29 (0.04)	0.18 (0.22)
Slope	0.15 (0.31)	0.51 (<0.001)	−0.16 (0.27)	−0.13 (0.37)	−0.08 (0.58)
Normalized Slope		0.56 (<0.001)	−0.20 (0.17)	−0.15 (0.29)	−0.02 (0.91)
ALT (IU/L)					
Mean level	0.63 (<0.001)	0.75 (<0.001)	−0.16 (0.28)	−0.27 (0.06)	0.14 (0.32)
Normalized mean level		0.79 (<0.001)	−0.16 (0.28)	−0.26 (0.07)	0.16 (0.27)
Maximum level	0.60 (<0.001)	0.75 (<0.001)	−0.15 (0.29)	−0.26 (0.07)	0.12 (0.41)
Normalized maximum level		0.79 (<0.001)	−0.15 (0.29)	−0.25 (0.07)	0.13 (0.36)
Slope	0.20 (0.16)	0.53 (<0.001)	−0.11 (0.46)	−0.09 (0.54)	−0.11 (0.43)
Normalized Slope		0.58 (<0.001)	−0.14 (0.32)	−0.11 (0.44)	−0.06 (0.69)
LDH (IU/L)					
Mean level	0.52 (<0.001)	0.58 (<0.001)	−0.10 (0.47)	−0.23 (0.10)	0.29 (0.04)
Normalized mean level		0.61 (<0.001)	−0.09 (0.53)	−0.23 (0.11)	0.29 (0.04)
Maximum level	0.54 (<0.001)	0.62 (<0.001)	−0.11 (0.44)	−0.24 (0.09)	0.28 (0.05)
Normalized maximum level		0.64 (<0.001)	−0.10 (0.45)	−0.24 (0.09)	0.28 (0.05)
Slope	0.23 (0.10)	0.55 (<0.001)	−0.14 (0.33)	−0.14 (0.34)	−0.05 (0.72)
Normalized Slope		0.58 (<0.001)	−0.14 (0.32)	−0.11 (0.44)	−0.06 (0.69)
Lactate (mmol/l)					
Mean level	0.62 (<0.001)	0.46 (<0.001)	−0.16 (0.28)	0.43 (<0.01)	0.10 (0.47)
Normalized mean level		0.06 (0.66)	0.03 (0.83)	−0.17 (0.23)	−0.16 (0.28)
Maximum level	0.61 (<0.001)	0.48 (<0.001)	0.13 (0.36)	−0.39 (<0.01)	0.06 (0.68)
Normalized maximum level		0.09 (0.49)	0.01 (0.97)	−0.13 (0.38)	−0.19 (0.18)
Slope	0.31 (0.03)	0.38 (<0.01)	−0.09 (0.45)	−0.11 (0.44)	0.10 (0.47)
Normalized Slope		0.27 (0.05)	−0.15 (0.30)	−0.11 (0.44)	0.12 (0.39)
Glucose					
Mean level	0.68 (<0.001)	0.44 (<0.01)	0.50 (0.75)	−0.24 (0.09)	0.09 (0.53)
Normalized mean level		−0.36 (0.01)	0.29 (0.04)	0.43 (<0.01)	−0.33 (0.02)
Maximum level	0.67 (<0.001)	0.45 (<0.01)	0.07 (0.71)	−0.23 (0.11)	0.05 (0.73)
Normalized maximum level		−0.33 (0.02)	0.33 (0.02)	0.43 (<0.01)	−0.37 (<0.01)
Slope	0.45 (0.001)	0.31 (0.03)	−0.04 (0.80)	−0.20 (0.17)	0.09 (0.51)
Normalized Slope		0.04 (0.76)	0.08 (0.59)	0.01 (0.95)	0.03 (0.81)
Ph					
Mean	−0.57 (<0.001)	−0.38 (<0.01)	0.04 (0.76)	0.31 (0.03)	−0.16 (0.27)
Minimum	−0.58 (<0.001)	−0.41 (<0.01)	0.03 (0.86)	0.27 (0.06)	−0.15 (0.29)
Slope	−0.22 (0.13)	−0.16 (0.26)	0.11 (0.44)	0.05 (0.73)	0.04 (0.74)

Data are expressed as Pearson R correlation coeeficient (p).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.

TABLE 4. Levels of normalized perfusate parameters according to macrovesicular steatosis of the graft

	<15% (n = 36)	15%–30% (n = 9)	≥30% (n = 5)	*P*
AST (IU/L) (min)				
30	363 (235–508)	1192 (825–1511)	1504 (1015–1878)	<0.001
60	330 (239–552)	1121 (750–1832)	2915 (1413–3099)	<0.001
90	342 (241–526)	1023 (858–1763)	2365 (1404–5319)	<0.001
120	359 (270–491)	1304 (1012–1566)	2220 (1171–5422)	<0.001
150	416 (289–584)	1084 (1011–1557)	3541 (2340–4742)	0.002
180	430 (342–721)	1096 (1056–1351)	5779 (5779–5779)	0.028
ALT (IU/L) (min)				
30	227 (149–402)	925 (777–1318)	1299 (952–1781)	<0.001
60	261 (134–427)	923 (680–1402)	1885 (1264–1896)	<0.001
90	265 (132–441)	850 (683–1298)	1800 (1267–2062)	<0.001
120	253 (144–388)	1020 (710–1256)	1784 (1178–2148)	<0.001
150	258 (148–372)	954 (668–1031)	3271 (2189–4354)	0.002
180	347 (225–396)	971 (85–1312)	5448 (5448–5448)	0.028
LDH (IU/L) (min)				
30	1763 (970–2493)	6231 (3670–7352)	4962 (3345–7517)	<0.001
60	1678 (1070–2818)	4662 (3518–8808)	10 485 (6081–11 081)	<0.001
90	1671 (1125–2732)	4430 (3703–8395)	10 940 (6109–18 285)	<0.001
120	1778 (1426–2481)	6981 (3909–9837)	11 249 (5899–20 140)	<0.001
150	1809 (1537–2797)	4842 (3742–8928)	13 042 (9360–16 724)	0.003
180	1565 (1230–3390)	4836 (4231–7185)	19 450 (19 450–19 450)	0.055
Glucose (mg/dL) (min)				
30	192 (163–219)	133 (120–173)	138 (117–159)	0.014
60	210 (171–248)	176 (136–182)	161 (140–180)	0.028
90	219 (188–265)	180 (154–188)	186 (166–202)	0.024
120	227 (199–272)	187 (170–194)	194 (168–207)	0.173
150	250 (223–283)	189 (168–203)	207 (172–213)	0.069
180	259 (232–292)	197 (179–202)	226 (226–226)	0.034
Lactate (mmol/L) (min)				
30	2.36 (1.61, 3.45)	2.75 (1.83, 3.89)	3.06 (1.43, 3.24)	0.958
60	2.69 (2.17, 3.20)	2.91 (2.52, 3.90)	3.08 (2.47, 3.29)	0.488
90	2.78 (2.38, 3.36)	3.15 (2.83, 3.40)	3.29 (3.16, 4.45)	0.149
120	2.74 (2.50, 3.57)	2.99 (2.93, 3.42)	3.41 (3.39, 4.20)	0.311
150	2.78 (2.51, 3.71)	3.17 (2.92, 3.29)	3.47 (3.30, 4.39)	0.542
180	2.91 (2.54, 3.67)	3.28 (3.15, 3.38)	5.47 (5.47, 5.47)	0.267
pH (min)				
30	7.15 (7.10, 7.23)	7.09 (7.08, 7.15)	6.98 (6.83, 7.17)	0.161
60	7.15 (7.09, 7.16)	7.09 (7.01, 7.13)	7.04 (6.82, 7.09)	0.022
90	7.14 (7.10, 7.17)	7.08 (7.01, 7.13)	6.93 (6.86, 7.06)	0.011
120	7.13 (7.11, 7.19)	7.10 (7.03, 7.15)	6.90 (6.86, 6.99)	0.037
150	7.16 (7.12, 7.19)	7.11 (7.08, 7.18)	6.93 (6.87, 6.99)	0.043
180	7.16 (7.10, 7.24)	7.07 (7.03, 7.12)	6.80 (6.80, 6.80)	0.099

ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.

FIGURE 2. Correlograms showing correlations between perfusate parameters and graft factors. Numbers in squares represent Pearson correlation coefficients for each couple of variables. Positive correlations are displayed in blue and negative correlations in red color. Color intensity is proportional to the correlation coefficients as indicated by the sidebar. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ICU, intensive care unit; LDH, lactate dehydrogenase.

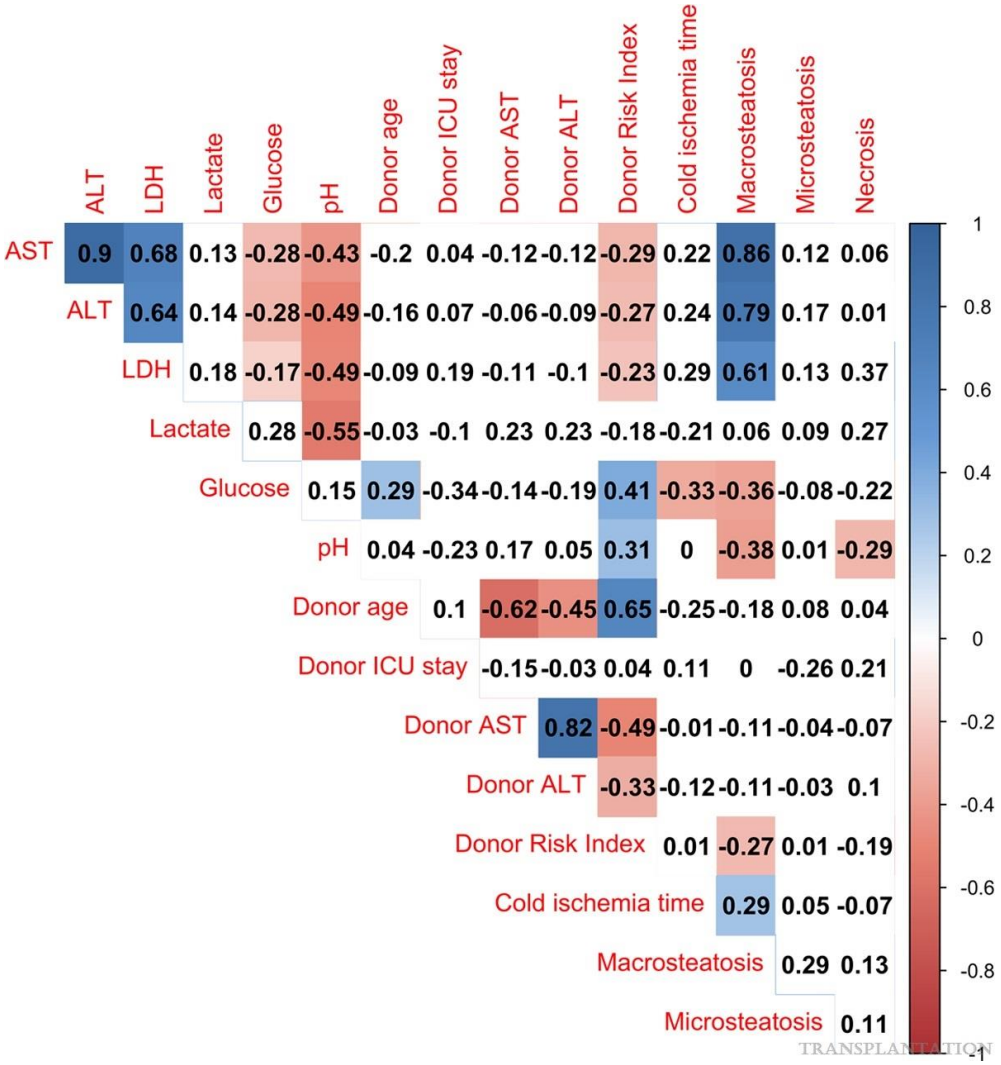


FIGURE 3. Picture of grafts with moderate ≥30% macrovesicular steatosis (left column) with correspondent histology (right column) (hematoxylin and eosin staining, ×100 original magnification). Percentage of MaS, MiS, Ne, and grade of histological PI, as assessed on time-zero biopsies, is indicated. The graft marked with an asterisk developed delayed nonfunction and the recipient required retransplantation. MaS, macrovesicular steatosis; MiS, microvesicular steatosis; Ne, necrosis; PI, preservation injury.

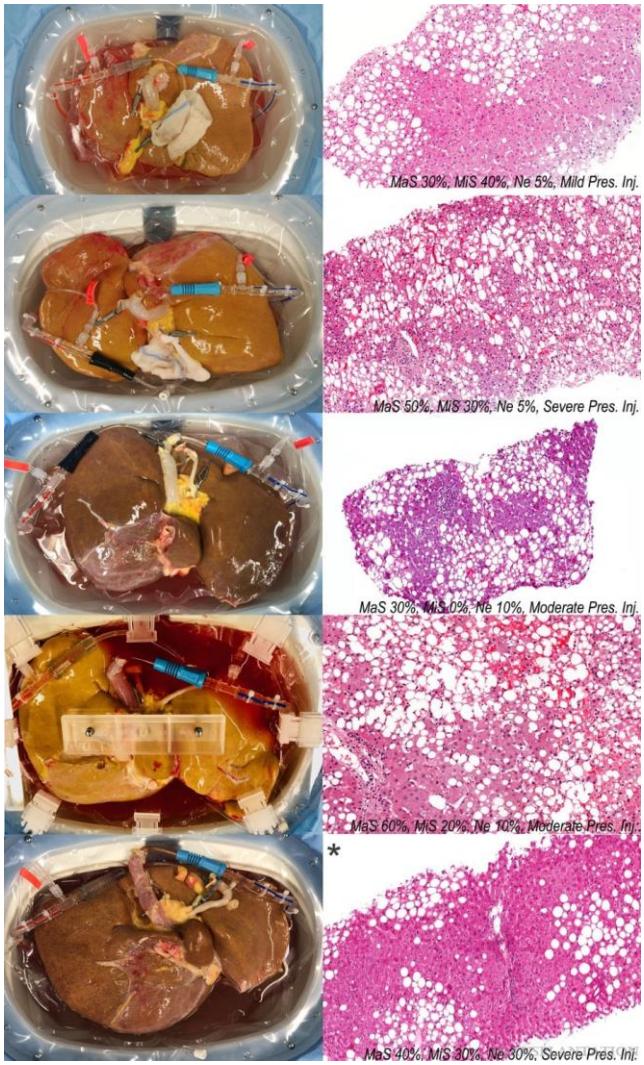
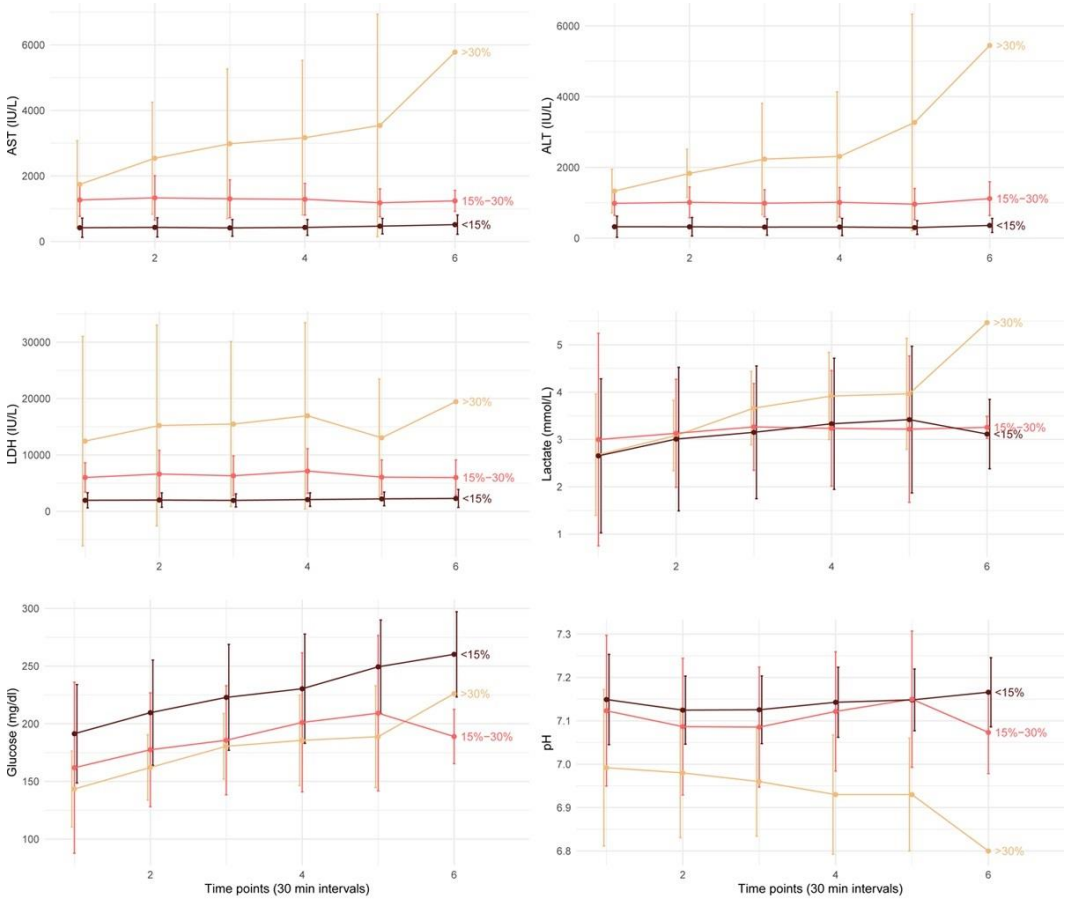


FIGURE 4. Line plot with error bars (mean ± SD) showing different trends of perfusate parameters in graft with moderate (≥30%), 15%–30%, or <15% macrovesicular steatosis. The attrition table indicates a number of patients in each group at each time point. ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.



	T1	T2	T3	T4	T5	T6
< 15%	36	36	36	25	18	9
15% – 30%	9	9	9	6	5	3
> 30%	5	5	5	5	2	1

FIGURE 5. Scatterplots with linear regression lines showing correlation between macrovesicular steatosis and perfusate glucose level before (left panels) and after (right panels) normalization of glucose value to graft liver weight. Normalization inverts the sign of the correlations. Pearson correlation coefficients (R) with P are indicated. ALT, alanine aminotransferase; AST, aspartate aminotransferase; EAD, early allograft dysfunction; LDH, lactate dehydrogenase.

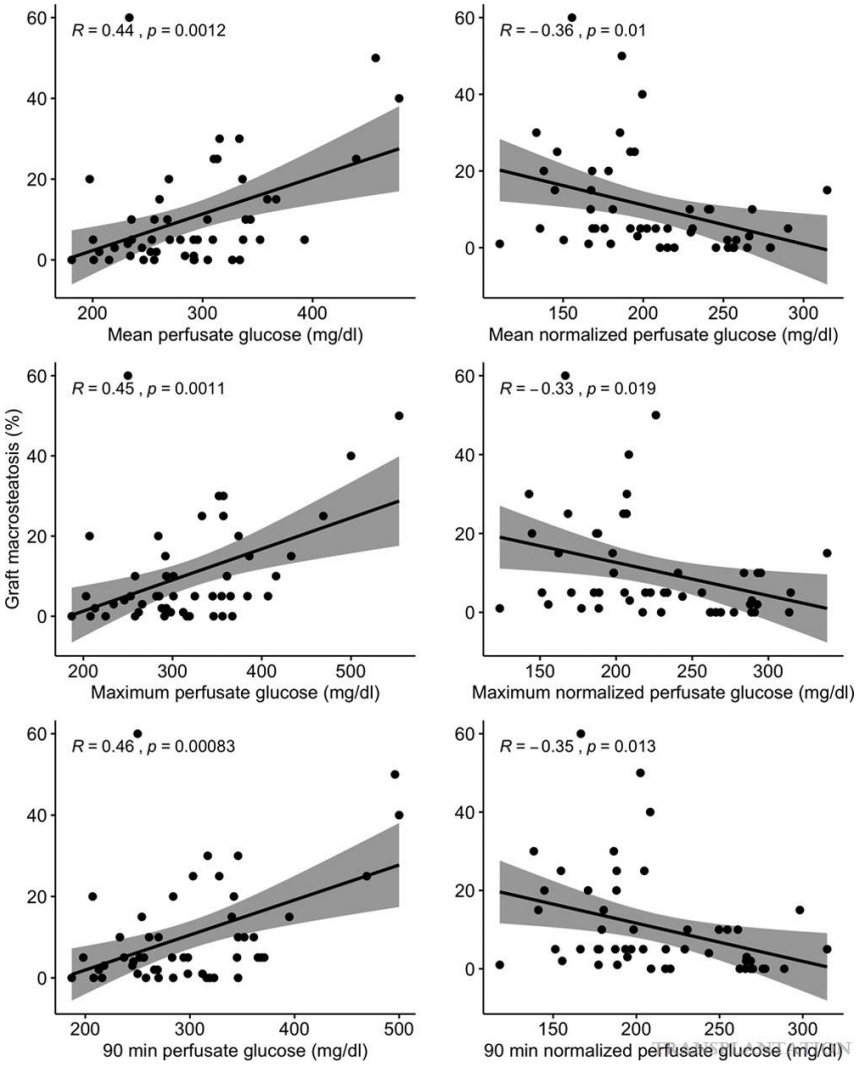


TABLE 5. Correlation between perfusate parameters and outcome

	EAD AUC (CI)	EAD[a] AUC (CI)	Grade 2–3 AKI AUC (CI)	L-GRaFT R (p)	CCI R (p)
AST level (IU/L)					
Mean	0.77 (0.60-0.90)	0.62 (0.32-0.91)	0.61 (0.44-0.78)	0.45 (<0.01)	0.29 (0.04)
Maximum	0.78 (0.92-0.93)	0.60 (0.31-0.90)	0.63 (0.46-0.80)	0.47 (<0.001)	0.33 (0.02)
Slope	0.66 (0.47-0.85)	0.55 (0.27-0.83)	0.54 (0.32-0.75)	-0.08 (0.56)	0.03 (0.82)
90-min level	0.78 (0.62-0.94)	0.62 (0.34-0.91)	0.61 (0.44-0.77)	0.39 (<0.01)	0.25 (0.07)
ALT level (IU/L)					
Mean	0.83 (0.69-0.97)	0.67 (0.41-0.93)	0.61 (0.43-0.78)	0.51 (<0.001)	0.33 (0.02)
Maximum	0.80 (0.66-0.95)	0.64 (0.37-0.91)	0.61 (0.44-0.79)	0.49 (<0.001)	0.33 (0.02)
Slope	0.66 (0.47-0.85)	0.55 (0.27-0.83)	0.54 (0.32-0.75)	0.23 (0.11)	0.13 (0.38)
90-min level	0.84 (0.71-0.98)	0.68 (0.43-0.94)	0.61 (0.44-0.79)	0.51 (<0.001)	0.32 (0.02)
LDH level (IU/L)					
Mean	0.79 (0.64-0.94)	0.65 (0.37-0.94)	0.62 (0.45-0.78)	0.70 (<0.001)	0.44 (<0.01)
Maximum	0.77 (0.62-0.93)	0.63 (0.35-0.91)	0.62 (0.46-0.78)	0.70 (<0.001)	0.44 (<0.01)
Slope	0.72 (0.53-0.91)	0.60 (0.32-0.87)	0.58 (0.37-0.78)	0.03 (0.86)	0.04 (0.80)
90-min level	0.80 (0.66-0.95)	0.68 (0.39-0.94)	0.63 (0.47-0.79)	0.70 (<0.001)	0.44 (<0.01)
Lactate level (mmol/l)					
Mean	0.45 (0.25-0.64)	0.47 (0.15-0.78)	0.51 (0.30-0.72)	0.17 (0.23)	0.27 (0.06)
Maximum	0.46 (0.28-0.65)	0.48 (0.18-0.77)	0.50 (0.29-0.70)	0.19 (0.18)	0.24 (0.09)
Slope	0.68 (0.52-0.84)	0.73 (0.53-0.93)	0.47 (0.29-0.65)	0.26 (0.07)	0.001 (0.99)
90-min level	0.52 (0.34-0.71)	0.53 (0.24-0.83)	0.56 (0.36-0.76)	0.23 (0.11)	0.28 (0.05)
Glucose level (mg/dL)					
Mean	0.77 (0.59-0.89)	0.42 (0.19-0.64)	0.40 (0.21-0.60)	-0.08 (0.59)	0.03 (0.85)
Maximum	0.73 (0.56-0.87)	0.44 (0.20-0.68)	0.40 (0.22-0.59)	-0.05 (0.72)	0.01 (0.96)
Slope	0.45 (0.62-0.79)	0.67 (0.46-0.88)	0.42 (0.21-0.62)	0.08 (0.56)	-0.12 (0.42)
90' level	0.73 (0.55-0.86)	0.45 (0.22-0.69)	0.42 (0.23-0.61)	0.04 (0.77)	0.03 (0.81)
Ph					
Mean	0.66 (0.47-0.81)	0.56 (0.34-0.80)	0.45 (0.25-0.65)	-0.41 (<0.01)	-0.36 (<0.01)
Maximum	0.65 (0.45-0.82)	0.56 (0.35-0.82)	0.45 (0.24-0.65)	-0.44 (<0.01)	-0.37 (<0.01)
Slope	0.65 (0.45-0.82)	0.60 (0.31-0.83)	0.57 (0.37-0.77)	-0.18 (0.21)	0.05 (0.71)
90-min level	0.68 (0.48-0.84)	0.60 (0.36-0.83)	0.46 (0.26-0.66)	-0.43 (<0.01)	-0.34 (0.02)

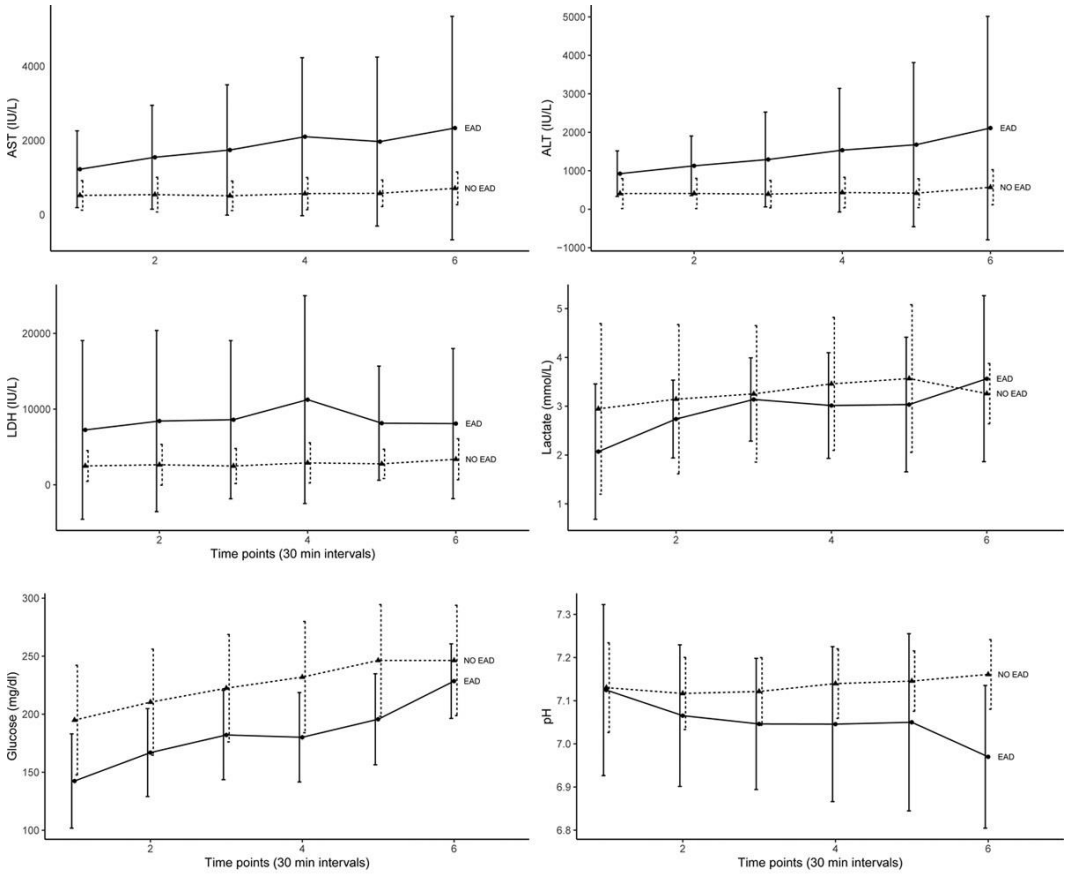
[a]EAD due to INR ≥ 1.7 or total bilirubin ≥ 10 mg/dL on postoperative d 7 (ie, excluding transaminase peak from EAD definition). All values except pH are normalized to graft weight.
AKI, acute kidney injury; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the curve; CCI, comprehensive complication index; CI, confidence interval; EAD, early allograft dysfunction; INR, international normalized ratio; LDH, lactate dehydrogenase; L-GrAFT, liver graft assessment following transplantation risk score; R, Pearson correlation coefficient.

TABLE 6. Results of univariable logistic regression and Lasso regression

	Univariable regression				Lasso regression			
		CI				CI		
	OR	Lower 0.95	Upper 0.95	*P*	OR	Lower 0.95	Upper 0.95	*P*
Donor age (y)	0.80	0.38	1.64	0.52				
Donor BMI	1.28	0.82	1.99	0.27				
Donor diabetes	2.84	0.63	12.86	0.17				
Donor GGT (IU/L)	1.39	0.95	2.03	0.09				
Macro steatosis (%)	7.35	2.28	24.27	0.001	5.44	1.05	28.21	0.04
MELD	1.59	0.59	4.27	0.36				
DRI	0.51	0.16	1.62	0.25				
D-MELD	1.26	0.55	2.88	0.59				
Cold ischemia time (min)	2.71	0.89	8.27	0.08				
PRBC units	1.62	0.86	3.04	0.13				
90-min AST (IU/L)	2.97	1.31	6.70	0.01				
90-min ALT (IU/L)	3.93	1.64	9.44	0.002	1.34	0.34	5.27	0.67
90-min LDH (IU/L)	1.93	1.14	3.28	0.01				
90-min lactate (mmol/L)	0.93	0.56	1.53	0.78				
90-min glucose (mg/dL)	0.16	0.04	0.70	0.015	0.92	0.38	2.23	0.86
90-min Ph	0.49	0.25	0.96	0.04				

Results are expressed as odds ratio and confidence interval.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; DRI, donor risk index; GGT, gamma glutamyl transferase; LDH, lactate dehydrogenase; MELD, model for end-stage liver disease.

FIGURE 6. Line plot with error bars (mean ± SD) showing different trends of perfusate parameters according to subsequent development of early allograft dysfunction. The attrition table indicates a number of patients in each group at each time point. ALT, alanine aminotransferase; AST, aspartate aminotransferase; EAD, early allograft dysfunction; LDH, lactate dehydrogenase.



	T1	T2	T3	T4	T5	T6
EAD	13	13	13	9	5	3
NO EAD	37	37	37	27	20	10

FIGURE 7. Receiver-operating characteristics curves of the predictive value of normalized 90-min perfusate parameters levels for early allograft dysfunction. ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the curve; LDH, lactate dehydrogenase.