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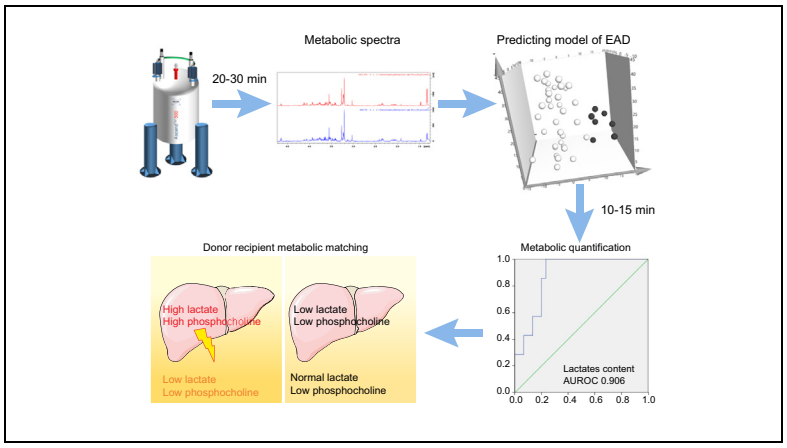
Impact of real-time metabolomics in liver transplantation: Graft evaluation and donor-recipient matching

Graphical abstract



Highlights

- Real-time metabolomics by HR-MAS-NMR accurately predicts early allograft dysfunction.
- Lactate and phosphocholine content are highly predictive biomarkers of early allograft dysfunction.
- Livers from patients with sarcopenia and cirrhosis have low lactate and phosphocholine content.
- Metabolic adaptation in these patients may lead to poor short-term outcomes.

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Lay summary

Real-time metabolomic profiles of human grafts during back-table can accurately predict graft dysfunction. High lactate and phosphocholine content are highly predictive of graft dysfunction whereas low lactate and phosphocholine content characterize patients with sarcopenia. In these patients, the cost of metabolic adaptation may explain the poor outcomes.

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Impact of real-time metabolomics in liver transplantation: Graft evaluation and donor-recipient matching

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Background & Aims: There is an emerging need to assess the metabolic state of liver allografts especially in the novel setting of machine perfusion preservation and donor in cardiac death (DCD) grafts. High-resolution magic-angle-spinning nuclear magnetic resonance (HR-MAS-NMR) could be a useful tool in this setting as it can extemporaneously provide untargeted metabolic profiling. The purpose of this study was to evaluate the potential value of HR-MAS-NMR metabolomic analysis of back-table biopsies for the prediction of early allograft dysfunction (EAD) and donor-recipient matching.

Method: The metabolic profiles of back-table biopsies obtained by HR-MAS-NMR, were compared according to the presence of EAD using partial least squares discriminant analysis. Network analysis was used to identify metabolites which changed significantly. The profiles were compared to native livers to identify metabolites for donor-recipient matching.

Results: The metabolic profiles were significantly different in grafts that caused EAD compared to those that did not. The constructed model can be used to predict the graft outcome with excellent accuracy. The metabolites showing the most significant differences were lactate level >8.3 mmol/g and phosphocholine content >0.646 mmol/g, which were significantly associated with graft dysfunction with an excellent accuracy ($AUROC_{lactates}$ = 0.906; $AUROC_{phosphocholine}$ = 0.816). Native livers from patients with sarcopenia had low lactate and glycerophosphocholine content. In patients with sarcopenia, the risk of EAD was significantly higher when transplanting a graft with a high-risk graft metabolic score.

Conclusion: This study underlines the cost of metabolic adaptation, identifying lactate and choline-derived metabolites as predictors of poor graft function in both native livers and liver grafts. HR-MAS-NMR seems a valid technique to evaluate graft quality and the consequences of cold ischemia on the graft. It could be used to assess the efficiency of graft resuscitation on machine perfusion in future studies.

Lay summary: Real-time metabolomic profiles of human grafts during back-table can accurately predict graft dysfunction. High lactate and phosphocholine content are highly predictive of graft dysfunction whereas low lactate and phosphocholine content characterize patients with sarcopenia. In these patients, the cost of metabolic adaptation may explain the poor outcomes.

Introduction

Liver transplantation is a life-saving procedure for patients with end-stage liver disease and a potentially curative treatment for hepatocellular carcinoma. The major limitation for liver transplantation is the current organ shortage caused by an increasing discrepancy between indications and a stable donor pool. In an attempt to answer to the issue of the increasing number of patients on waiting lists, many teams have extended the criteria for acceptance of liver grafts. Although there is hardly a wide consensus on its definition, extended criteria donors (ECD) represent a growing proportion of the donors. Many donor factors have been reported as influencing the outcome of liver transplantation mainly age,[1] steatosis[2,3] and cold ischemia time.[4] While ECD, particularly DCD, has increased the pool of donors, it may be associated with higher graft loss[5] or increased risk of vascular and biliary complications.[6,7] Indeed the tolerance of allograft to cold ischemia-reperfusion injury is altered in ECD allografts.[8,9]

Donor-recipient matching is of utmost importance in this setting. Model for end-stage liver disease (MELD) score and life-support therapies have been identified as significant recipient factors that alter results, specifically when using extended criteria donor. The balance of risk (BAR) score is an example of application of such donor-recipient matching. Additionally, the addition of graft steatosis in the score further enhances the accuracy of the BAR score.[10]

Among significant factors impacting early outcomes after liver transplantation, sarcopenia and portal hypertension have been increasingly studied in the last years. Both are probably linked, as ascites is often associated with malnutrition, and portal hypertension may be a surrogate of long-term evolving cirrhosis. Sarcopenia may be a marker of significant metabolic shift and there are currently no clear data on differentially

Keywords: Liver transplantation; Metabolomics; Lactate; Early allograft dysfunction.
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expressed metabolites in the liver of patients with cirrhosis and sarcopenia.

There is currently a lack of effective tools and biomarkers to evaluate the liver grafts before implantation. Liver biopsies with fibrosis and steatosis assessment may be informative,[11] but do not take into consideration metabolic insults caused by static cold storage. Interpretation of frozen section biopsies can be confusing and can mislead clinicians who have to decide whether to use marginal grafts.[12] In order to further extend and enhance the quality of the grafts, machine perfusion is currently under intense evaluation for liver grafts.[13–15] The benefit of dynamic cold storage has been demonstrated in kidney transplantation[16] and a growing set of data support its use in liver transplantation. The data available tend to show efficient "resuscitation" of ECD grafts especially steatotic grafts.[17,18]

Metabolomics is an emerging area in the omics field consisting of the simultaneous evaluation of cellular metabolic products on liquid or solid phase. Nuclear magnetic resonance (NMR) spectroscopy metabolomics has already been applied in the field of liver transplantation,[19] but only mass spectroscopy has been shown to be predictive of allograft dysfunction.[20] Whereas many metabolomic methods are not relevant for clinical practice because they need complex sample handling and long treatment time, 1H high-resolution magic-angle-spinning NMR (1H HR-MAS NMR) spectroscopy is an attractive solution. Metabolomic profile of solid fresh frozen biopsy can be obtained in a short period of time without destruction of the sample, thus enabling further classical histopathological evaluation.

The goal of this study was to evaluate the potential value of 1H HR-MAS NMR metabolomics in a clinical setting and eventually identify biomarkers in the graft and in the native liver to predict early outcomes after liver transplantation. The main hypothesis was that the liver allograft exerts significant metabolic derangements that depend on each allograft capacity to cope with cold ischemia. At the early stages of reperfusion, the allograft is exposed to significant changes in metabolism, depending on the metabolic state of the recipient. Metabolic donor-recipient matching may be a novel way of looking at early graft function.

Material and methods

This study included patients transplanted between December 2014 and December 2016 who responded to the following criteria: first liver transplantation, absence of early vascular complications, available snap-frozen biopsy within less than 5 min after realization of the biopsy, available histopathological analysis of the specimen, available biological and clinical data and informed consent from the patient. The Ethics Committee affiliated to Strasbourg University (Comité de Protection des Personnes "Est IV") approved the study (Registration number: 09/39 a).

Design of the study

The value of metabolomic profiling using HR-MAS-NMR was evaluated on a set of 42 liver grafts at the time of *ex vivo* preparation and 36 native livers. To check whether the studied population was representative, donor and recipient characteristics of the population transplanted during the same period of time but not included (n = 92) were compared to the patients included.

In a first step, a multivariate non-targeted analysis was used to compare metabolic profiles according to the occurrence of early allograft dysfunction (EAD). Network analysis was conducted to identify differentially expressed metabolites between EAD and non EAD patients as well as between recipients with or without sarcopenia. Optimal cut-off values for the most accurate biomarkers were then defined. A liver graft metabolic score (GMS) using these parameters was calculated. In a second step, this score was evaluated for prediction of EAD and one-year graft loss. Predictive factors for high metabolite levels were searched to define ECD using available clinical data from the donor chart.

Surgical technique and sample procurement

Back-table biopsies are routinely performed at the beginning of the back-table in our center, without selection criteria according to the donor characteristics, but only biopsies that were rapidly snap-frozen in nitrogen were analyzed in order to avoid metabolic changes due to ischemia. A biopsy from the native liver was also procured just after portal clamping.

All donors were brain dead donors procured according to a classical technique.[21] Portal cannulation with washing was always performed using Ringer-lactate before clamping and dual arterial and portal washing was achieved with various conservation liquids.

Histopathological analysis was performed on the sample analyzed in spectroscopy to take into consideration zonal variations of necrosis, fibrosis and steatosis.

ECD were defined according to the European Association for the Study of the Liver (EASL) guidelines[22] as the donor-risk index is not adapted to the liver transplantation organization in France.

Liver recipients were listed on the French national list after complete pre-transplantation evaluation. Indication, presence of hepatocellular carcinoma and lab-MELD at listing and at transplantation are reported.

Endpoint definition

EAD was defined according to the Olthoff's criteria,[23] namely bilirubin ≥170 μmol/L at day seven, INR ≥1.6 at day seven and peak aminotransferases ≥2,000 within the first seven postoperative days, in the absence of technical causes.

Sarcopenia was defined as low psoas surface as defined by Golse *et al.* according to the psoas surface.[24]

Metabolomic study

Samples were prepared in a −20 °C environment. A total of 15 to 20 mg of tissue were punched-biopsied from the specimen. Deuterium oxide (8μl) with 0.75 weight percent 2,2,3,3-D4-3-(trimethylsilyl) propionic acid was added for chemical shift reference for NMR spectrometer.

HR-MAS analysis was achieved on a Brucker Avance III 500 spectrometer operating at a proton frequency of 500.13 MHz and equipped with a 4 mm triple resonance gradient HR-MAS probe. The analysis was conducted at −80 °C after placing the insert in a 4 mm ZrO_2 rotor.

A one-dimensional (1 D) proton spectrum using Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence and 128 transients was acquired for each tissue sample. Free induction decay was multiplied by an exponential window function of 0.3 Hz prior to Fourier transformation and the result was corrected for phase and baseline distortion using TopSpin 3.2 (Brucker GmbH, Germany). The chemical shift was calibrated to the peak of the methyl proton of L-lactate at 1.33 ppm.

In order to confirm resonance assignments, two-dimensional (2D) heteronuclear experiments were recorded immediately after the end of 1 D spectra acquisition for four representative samples. Spectra were referenced by setting lactate doublet chemical shift to 1.33 ppm in proton dimension and 22.70 ppm in carbon dimension. Metabolites were assigned using standard metabolite chemical shift tables available in the literature.

Metabolite quantification was performed using an external reference standard of lactate (3 µmol), scanned under the same analytical conditions. Spectra were normalized according to sample weight. Peaks of interest were automatically defined by an in-house program using Matlab 7.0 (Mathwork, Natik, USA). Peak integration was then compared to the one obtained with the lactate reference and corrected according to the number of protons. Only well-defined peaks with no overlapping in the 1 D CPMG spectra were selected for quantification.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation. Student's *t* test or Mann-Whitney U test were used to compare the means between groups as appropriate. Categorical variables are expressed as number and percentage. Chi-square test was used to compare the distribution of categorical variables between groups. Spearman's test was performed to determine correlation between variables. These statistical analyses were performed using Statview software (USA).

Receiving Operating Characteristics (ROC) curves were constructed to evaluate the value of identified metabolic biomarkers and to identify the best cut-off for these variables. SPSS software was used for this analysis.

Statistical analysis for metabolomics study

Principal component analysis (PCA) was performed to evaluate whether metabolic profile differentiated groups of patients. The two measurements of model quality were $R^2\gamma$ and Q^2 representing the accuracy of fit (*i.e.* data variation) and accuracy of prediction respectively. Q^2 ≥0.5 was considered a good predictor.

Network analysis

Algorithm to determine expected metabolite level alterations using mutual information (ADEMA) was applied on metabolite quantification values. This method enables strong and valid comparison of very small samples (2 *vs.* 2 as demonstrated in the original article).[25] The network was constructed using Kyoto Encyclopedia of Genes and Genomes and Selway's work[26] using the following pathways: glucose/lactate; glucose/ascorbate/glutathione/glutamate; glucose/alanine/valine/isoleucine; choline/glycerophosphocholine/phosphocholine/total choline.

For further details regarding the materials used, please refer to the CTAT table.

Results

Population

There were 42 available biopsies for the analysis. As shown (Table 1) 69% of donors were ECD according to EASL. Median donor age was 56.5 (21–81). Regarding recipients, there were 33% (n = 14) with very high MELD (>35) and 15% were transplanted while in the ICU. The main indication was alcohol cirrhosis (62%) followed by HCV and metabolic cirrhosis.

The incidence of EAD was 17% in the whole population and 21% in the ECD. The 90-day mortality was 4%.

The mean acquisition time was 12 min and the total analysis including sample preparation and metabolite quantification was 30 min.

Metabolomic profile from ex vivo liver graft predicts EAD

Metabolomic profiles were significantly different between *ex vivo* biopsies of liver grafts presenting EAD *vs.* no EAD (Q^2 = 0.573, $R^2\gamma$ = 0.697) (Fig. 1). Of note cold ischemia time >6 h was not associated with a significantly different metabolic profile.

Metabolomic study identifies potential biomarkers of EAD

The network analysis showed higher lactate, glutamate, glutamine, alanine, valine, isoleucine and choline derivatives concentration in EAD grafts. There was no change in glucose, ascorbate or GSH levels (Fig. 2).

Table 1. Population characteristics and comparison to non-selected population during the same period of time.

	n = 42	n = 92	*p* value
Recipient's characteristics			
Age	53 ± 12	54 ± 11	0.646
Male gender	30 (71%)	63 (68%)	0.731
Indication for transplantation			0.166
Alcoholic	26 (62%)	46 (50%)	
HCV	5 (12%)	11 (12%)	
HBV	2 (5%)	0	
Metabolic	4 (10%)	8 (9%)	
Fulminant hepatitis	1 (2%)	6 (7%)	
Other	7 (17%)	22 (24%)	
Hepatocellular carcinoma	12 (29%)	26 (28%)	0.971
Lab-MELD at listing	26.8 ± 12	24.8 ± 11	0.371
Lab-MELD at LT	27.1 ± 14	22.2 ± 9	0.087
Bilirubin at LT (µmol/L)	192 ± 33	137 ± 21	0.114
Creatinine at LT (µmol/L)	83 ± 8	88 ± 7	0.634
INR at LT	2.92 ± 2	2.37 ± 1	0.064
Sarcopenia	14 (33%)	32 (35%)	0.87
ACLF	19 (45%)	27 (29%)	0.072
Donor's characteristics			
Age	57.7 ± 18	57.8 ± 17	0.972
BMI	26.4 ± 6	26.5 ± 5	0.891
Diabetes	7 (17%)	12 (13%)	0.594
Statin use	9 (21%)	25 (27%)	0.458
Metabolic syndrome	12 (29%)	16 (28%)	0.956
AST/ALT (IU)	74 ± 14/72 ± 20	63 ± 8/49 ± 6	0.507
Bilirubin (µmol/L)	14 ± 11	12 ± 6	0.472
GGT (IU)	59 ± 9	50 ± 5	0.354
Lactate at procurement (mmol/L)	1.8 ± 1.3	1.7 ± 1.3	0.819
Graft steatosis			
Overall degree of steatosis (%)	2% (0–65)	7% (0–25)	0.749
Macrovascular steatosis >30%	2 (6%)	0 (0%)	
Extended criteria donor*	29 (69%)	55 (58%)	0.304
Operative data			
Cold ischemia time	453 ± 91	462 ± 91	0.588
Reperfusion syndrome	20 (48%)	28 (30%)	0.212
Red blood cell transfusion (units)	7 ± 1	7 ± 1	0.973
Fresh frozen plasma (units)	9.5 ± 2	10 ± 1	0.68
Platelets (units)	1.3 ± 2	1.5 ± 2	0.713

ACLF, acute-on-chronic liver failure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HBV, hepatitis B virus; HCV, hepatitis C virus; INR, international normalized ratio; LT, liver transplant; MELD, model for end-stage liver disease.
*According to the European Association for the Study of the Liver definition.

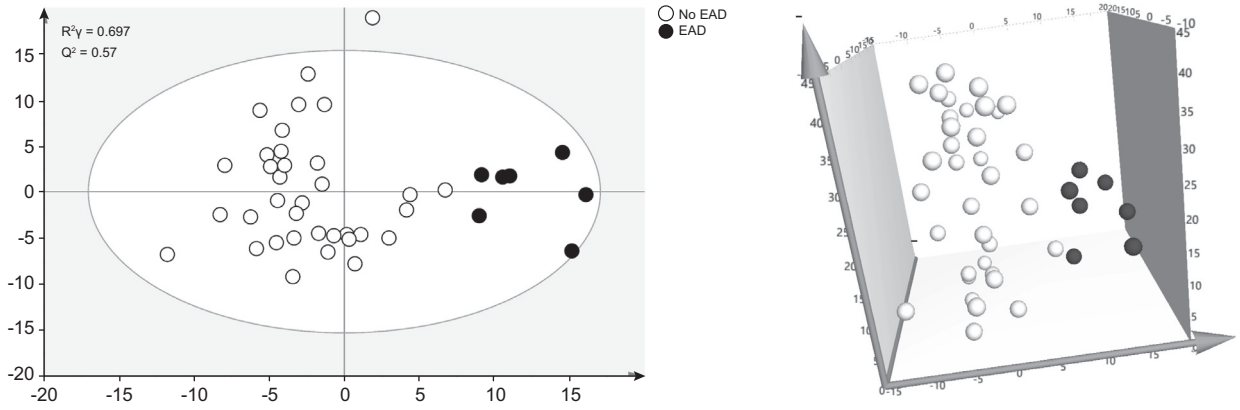

Fig. 1. PCA analysis comparing metabolic profiles of back-table liver allograft biopsies in patients experiencing early allograft dysfunction (black dots; n = 7) or not (white dots; n = 35). This analysis shows a clear distinction of metabolic profiles between the two groups (Q^2 >0,5). EAD, early allograft dysfunction; PCA, principal component analysis. PCA analysis with 1D and 3D representation.

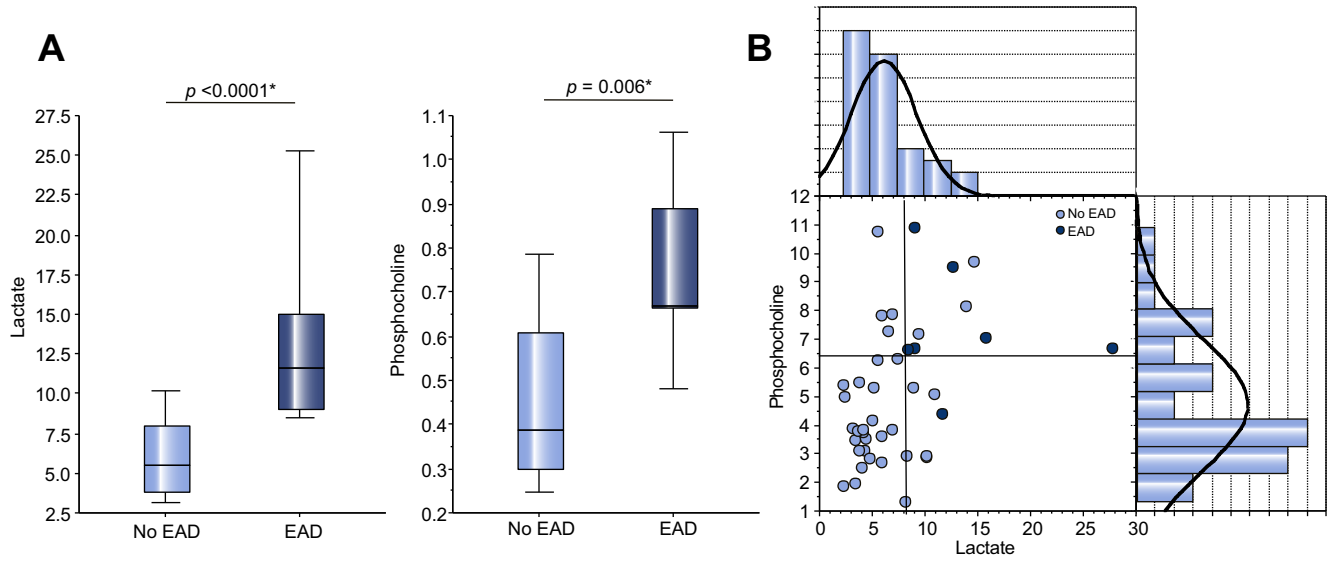

Fig. 2. Box plot figures and bivariate analysis of lactate and phosphocholine content. (A) Box plot figure shows the significant difference in lactate and phosphocholine content according to the occurrence of EAD or not (*paired Student's *t* test). (B) Bivariate analysis of these variables according to the occurrence of EAD (blue dots) shows the individual values and the diagnostic value of phosphocholine and lactate. EAD, early allograft dysfunction.

Quantification showed that grafts presenting EAD had significantly higher lactate level (p <0.0001), phosphocholine (p = 0.006) and taurine levels (p = 0.011).

Among the identified metabolites, only lactate and phosphocholine showed high accuracy for predicting EAD. ROC curve analysis showed an excellent predicting value of intragraft lactate level (AUROC = 0.906; 95% CI 0.813–0.999) (Fig. 3a). The optimal threshold was 8.3 mmol/g predicting EAD with a 100% sensitivity and 80% specificity. Similarly, AUROC for phosphocholine was 0.816 (95% CI 0.679–0.954) and a threshold of 0.65 mmol/g gave an 86% sensitivity and 80% specificity (Fig. 3b).

Predicting factors and prognostic value of MD-ECD

A metabolomic-defined extended criteria donor (MD-ECD) could be defined by the association of high lactate and high phosphocholine levels at the time of *ex vivo* preparation. None of the donor characteristics predicted MD-ECD, even the degree of macroscopic steatosis. Neither cold ischemia time nor preservation solution were associated with MD-ECD.

MD-ECD were associated with a 63% risk of EAD. Most interestingly, MD-ECD recipients had a significantly higher risk of one-year graft loss and/or patient death (n = 3/8; 38%) than non MD-ECD (n = 3/34; 10%) (p = 0.037). In the MD-ECD, one graft was lost due to the only case of primary non-function (PNF), there were two deaths, one due to pulmonary sepsis leading to multiorgan failure at day 25, and one due to overwhelming biliary infection in the setting of ischemic cholangitis at 10 months post-transplantation. At reperfusion, MD-ECDs had higher levels of IL6 (1,309 ± 333 *vs.* 575 ± 108; p = 0.014).

Predicting factors of EAD

Patients presenting EAD had a significantly higher MELD (37 ± 7 *vs.* 25 ± 13; p = 0.014), with higher bilirubin and INR

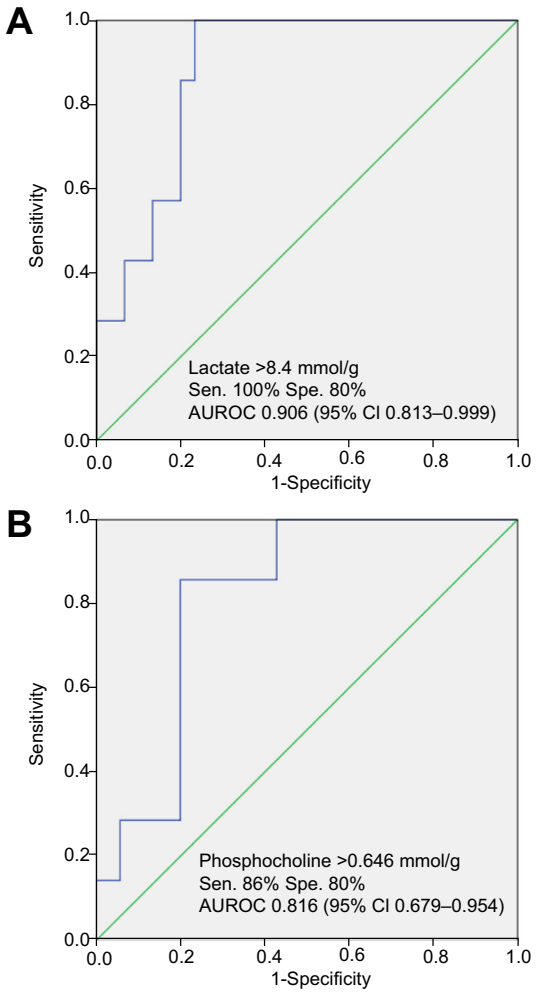

Fig. 3. ROC curve analysis showing the sensitivity and specificity of intragraft lactate and phosphocholine content quantified by ^{1}H-HR-MAS-NMR. AUROC was of 0.906 for lactate content and 0.816 for phosphocholine content. AUROC, area under the ROC curve; ROC, receiver operating characteristic; Sen, sensitivity; Spe, specificity.

levels, less frequently HCC. Sarcopenia was associated with EAD ($p = 0.019$). In multivariate analysis, none of the donor characteristics predicted EAD. Cold ischemia time was not associated with EAD in this population ($p = 0.57$) (Table 2).

Multivariate analysis for EAD showed that graft lactate content was the only independent predictor of EAD ($p = 0.046$) (Table 2).

Recipient parameters affecting the metabolic profiles

Metabolomic profiles from patients with cirrhosis were compared according to MELD score, acute-on-chronic liver failure and presence of sarcopenia. Metabolomic profiles differed only according to presence or absence of sarcopenia ($Q^2 = 0.528$, $R^2\gamma = 0.783$).

Network analysis showed a lower level of lactate and glycerophosphocholine, but a significantly higher level of choline, neoglucogenic amino acids and ethanolamine. Using ROC curve analysis, only low glycerophosphocholine <0.556 mmol/g was an accurate biomarker (AUROC = 0.812) (Fig. 4).

Table 2. Risk factors of early allograft dysfunction in uni- and multivariate analysis.

	No EAD n = 35	EAD n = 7	*p* univariate	*p* multi
Recipient's characteristics				
Age	53 ± 12	53 ± 15	0.885	
Male gender	25 (71%)	5 (71%)	>0.999	
Indication for transplantation				
Alcoholic	23 (66%)	3 (43%)	0.256	
HCV	5 (14%)	0 (0%)	0.287	
Metabolic	3 (9%)	1 (14%)	0.638	
Hepatocellular carcinoma	12 (34%)	0 (0%)	0.067	
Lab-MELD at listing	24.7 ± 12	37.3 ± 7	0.013	
Lab-MELD at LT (uncapped)	24.3 ± 13	40.7 ± 11	0.003*	0.116
Bilirubin at LT (μmol/L)	162 ± 32	337 ± 103	0.041	
Creatinine at LT (μmol/L)	83 ± 50	81 ± 37	0.905	
INR at LT	2.61 ± 1	4.37 ± 2	0.011	
Sarcopenia	9 (26%)	5 (71%)	0.019*	0.158
Donor's characteristics				
Age	59 ± 17	53 ± 20	0.421	
BMI	26.7 ± 6	24.8 ± 6	0.468	
Diabetes	7 (20%)	0 (0%)	0.195	
Statin use	8 (23%)	1 (14%)	0.614	
Metabolic syndrome	11 (31%)	1 (14%)	0.359	
GGT (IU)	55 ± 9	82 ± 32	0.274	
Lactate at harvesting (mmol/L)	1.78 ± 1	1.93 ± 1	0.786	
Graft steatosis				
Overall steatosis >30%	0 (0%)	2 (28%)	0.002*	0.102
Macrovascular steatosis (%)	5.7 ± 2	11.4 ± 5	0.137	
Extended criteria donor	23 (%)	6 (%)	0.296	
Operative data				
Cold ischemia time (minutes)	453 ± 90	450 ± 104	0.926	
Ischemia time to biopsy (minutes)	309 ± 74	312 ± 38	0.937	
Reperfusion syndrome	16 (46%)	4 (57%)	0.581	
Red blood cell transfusion (units)	5 ± 4	15 ± 13	0.002	
Fresh frozen plasma (units)	7 ± 5	20 ± 8	0.006	
Platelets (units)	1.6 ± 1	1.3 ± 1	0.817	
Metabolomic quantification				
Alanine	1.512 ± 0.2	2.595 ± 1.5	0.175	
Valine	0.21 ± 0.04	0.528 ± 0.4	0.111	
Isoleucine	0.145 ± 0.03	0.325 ± 0.2	0.139	
Glutamate	1.795 ± 1	2.713 ± 2	0.078	
Glutamine	0.76 ± 0.4	1.087 ± 0.7	0.115	
GABA	0.485 ± 0.1	1.059 ± 0.5	0.069	
Glucose	6.697 ± 3	7.191 ± 5	0.762	
Lactate	6.122 ± 3	13.445 ± 7	<0.0001	
Lactate >8.4 mmol/g	7 (%)	6 (%)	0.0006	0.046
Glycerol	4.699 ± 3	6.615 ± 7	0.27	
Ascorbate	0.175 ± 0.02	0.195 ± 0.04	0.702	
Glutathione	0.41 ± 0.07	0.346 ± 0.2	0.725	
Creatine	0.5 ± 0.05	0.7 ± 0.1	0.111	
Choline	1.062 ± 0.1	1.568 ± 0.6	0.17	
Phosphocholine	0.471 ± 0.2	0.741 ± 0.2	0.006	
Phosphocholine >0.65 mmol/g	7 (%)	6 (%)	0.00006*	0.207
Glycerophosphocholine	1.18 ± 0.1	1.564 ± 0.5	0.15	
Taurine	3.48 ± 1	5.031 ± 1	0.011	
Ethanolamine	0.371 ± 0.06	0.633 ± 0.19	0.108	

BMI, body mass index; GABA, gamma-aminobutyric acid; GGT, gamma-glutamyl transferase; HCV, hepatitis C virus; INR, internal normalized ratio; LT, liver transplant; MELD, model for end-stage liver disease.
*According to the European Association for the Study of the Liver definition.

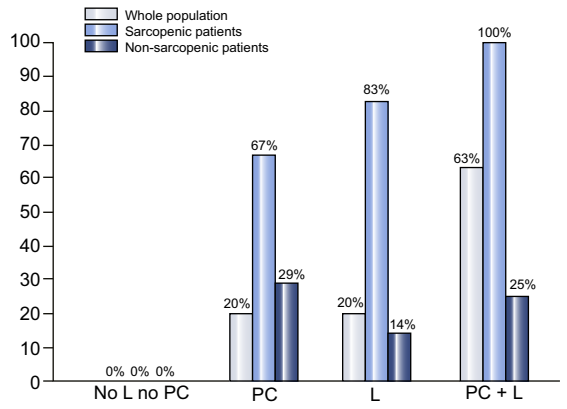

Fig. 4. Rate of EAD according to the metabolic profile of the graft. The population was evaluated as a whole (n = 42), or according to the presence (light blue) or not (dark blue) of sarcopenia. This graph shows the significant impact of metabolic donor-recipient matching as use of lactate and/or phosphocholine rich-liver grafts in patients with sarcopenia, who exert low lactate and glycerophosphocholine content, is associated to a growing risk of EAD. EAD, early allograft dysfunction; L, lactate; PC, phosphocholine.

Metabolomic profile from native liver do not predict EAD

Metabolomic profiles from native liver were not significantly different between patients presenting with EAD *vs.* no EAD ($Q^2 = 0.296$, $R^2\gamma = 0.629$).

Donor-recipient matching according to MD-ECD

Given its significant impact on liver metabolic profile, sarcopenia was used as a major determinant for donor-recipient matching. Use of a MD-ECD in a patient with sarcopenia was associated with a 100% risk of EAD, whereas use of a non MD-ECD in a patient without sarcopenia was associated with no risk of EAD. Most interestingly, EAD was more common in recipients with sarcopenia, receiving grafts from phosphocholine-poor donors underlining the metabolic cost of graft adaptation (Fig. 4).

Discussion

Evaluation of graft quality is a highly complex daily task for transplant teams, as many factors must be considered. Metabolic biomarkers have been evaluated, but they may provide the unique advantage of informing clinicians on the cellular state and function of a graft. This study demonstrates that metabolomics using HR-MAS-NMR is efficient in predicting EAD and is potentially applicable to daily clinical practice.

The predictive value of metabolomic study of liver grafts has already been shown in a previous study using mass spectroscopy.[16] HR-MAS-NMR presents the advantage of being easily applicable in the clinical setting and the data from this study support its use in a clinical setting

One of the main findings of this study was to show that lactate content is highly predictive of EAD, even when measured as early as back-table preparation. Given the 0.906 AUROC, lactate content is one of the most powerful reported biomarkers for prediction of early graft outcome. Indeed, even established scores using both donor and recipient parameters do not achieve such accuracy. For instance, the BAR score achieves a c-statistic of 0.7.[27] Although adding the degree of macrovascular steatosis increases the accuracy, the simple measure of lactate content, available at the time of back-table preparation, is at least as powerful as the modified BAR score.

The high level of lactate is mainly due to anaerobic metabolism that takes place during cold ischemia. It may be a marker of graft tolerance to static cold storage and underlines the impact of mitochondrial dysfunction during this phase. Recent works have shown the beneficial impact of oxygenation on mitochondrial function in liver perfused grafts.[28] In mammalian cells, lactate represents the end product of anaerobic glycolysis, explaining its significant impact as a predictive biomarker for liver dysfunction. High lactate levels induce local acidosis and may act as damage-associated molecular patterns, leading to exacerbated inflammatory response at reperfusion.[29] Furthermore intracellular acidosis and cold storage may lead to imbalance in the protein turnover[30,31] that lead to vital protein destruction and enzymatic inhibition that will block the reconstitution of the ATP reserve after reperfusion. Systemic lactate clearance within the 24 h after liver transplantation is a marker of graft function.[32] The lactate content in the graft should be distinguished from excreted lactates which are measured in the effluent. Another metabolomic study on machine perfused grafts has shown that lactate effluent may be low even in cases of high intragraft content.

Glutamine is the most important amino acid in the body with major roles in gut integrity and immune system viability, as it can serve as an energy source through the production of α-ketoglutarate, one of the components of the Krebs cycle. It is also the main carrier of ammonium. Glutamate is the intermediate metabolite between glutamine and α-ketoglutarate. Elevation of glutamate has already been reported in metabolomic studies of cirrhotic livers, according to the stage of liver fibrosis, necroinflammatory activity and steatosis,[33] as well as in circulating blood of diabetic patients.[34] In hibernating animals, high glutamine levels are observed during the hypothermic torpor period and are thought to be a metabolic adaptation for the storage of nitrogen. Indeed, during a period of hypothermic ischemia, the urea cycle is inhibited, thus limiting the capacity to eliminate ammonia.[35] Regulation of the glutamine-glutamate axis is not fully described, but data have shown that it is dependent on the response to steroids.[36]

Phospholipid metabolism is highly impaired during ischemia. The formation of "blebs" in ischemic livers is a well described phenomenon preceding membrane rupture and cell destruction due to ATP depletion.[37] The differential concentration of choline derivatives emphasizes the major impact of membrane turnover in liver grafts subjected to ischemia. Phosphocholine is derived from choline and is transformed in endoplasmic reticulum (ER) into glycerophosphocholine (GPC). High phosphocholine may be a marker of ER stress. GPC has already been shown to decrease during the different stages of liver transplantation from procurement to reperfusion.[15] GPC has also been reported as protective against microvascular alterations in rat models of ischemia.[38] Choline deficiency represents a model of liver disease and specifically metabolic and steatotic disease.[39] In mice with choline deficiency, tolerance to hepatectomy is reduced and the inflammatory response has been reported to be increased.[40]

The second highlight of this study is to show the significant cost of metabolic adaptation in high risk patients. Sarcopenia is now a recognized risk factor for poor LT outcomes. Sarcopenia is a marker of long-term evolving cirrhosis and is often associated with portal hypertension and ascites. It is a sign of late

metabolic adaptation in a patient with cirrhosis when the liver metabolism has switched to lactate use from the muscle, by the Cori cycle. It has been well shown that end-stage liver disease is associated with a metabolic shift, whatever the cause of cirrhosis. There are now studies showing the significant difference in metabolic profiles between fibrotic, compensated cirrhotic and decompensated cirrhotic livers. Although the difference in metabolic profiles between patients with and without sarcopenia is not surprising, the striking finding of this study was that lactate and choline derivatives are decreased in patients with sarcopenia, even in cases of low MELD.

When using the GMS, it becomes obvious that donor-recipient matching is particularly important in a cirrhotic population. Although we identify patients with sarcopenia in this study, other clinical parameters may be associated with low GPC and lactate. In this population, the use of MD-ECD leads to a significant need for metabolic adaptation for the liver graft. The cost of metabolic adaptation may explain poor early graft function that is otherwise poorly explained by the routinely used clinical and biological parameters.

The main limitation of this study may be that EAD should not necessarily lead to refusing a liver graft. Although it is associated with long-term results, the life-saving place of liver transplantation may overcome this point. Although the rate of donors exerting extended criteria as defined by EASL was quite high, the rate of severely steatotic livers was low in this series. One could question the significant impact of the two steatotic liver donors on metabolic profiles as they both experienced EAD. An additional analysis confirmed the data, even after exclusion of these two donors, even with an high AUROC for lactate of 0.936. Unfortunately, it is not possible to identify steatotic livers with favorable metabolic profiles. Indeed, the literature has shown a significantly higher rate of EAD in severely steatotic livers but "only" 40% exert EAD and recent studies have confirmed good results with steatotic livers when the recipient is well selected. Another limitation of the study is the size of the population which should lead to cautious interpretation of the data. However, the studied population was representative of the overall population transplanted during the study period (n = 134). Moreover, the statistical analysis used to identify discriminating metabolites, specifically ADEMA, is a highly powerful method enabling strong conclusions even in case of small samples.[25]

In clinical practice, the back-table time seems the most appropriate time for the study, as it takes into consideration the impact of cold ischemia on the graft, but leaves sufficient time to change strategy in case of major metabolic abnormalities on the profile. Future studies should focus on metabolic changes over time. Altogether, the data acquired through this technique, in less than 30 min, may increase the objectivity of graft selection in liver transplantation. Besides, evaluation of DCD grafts may be an excellent application of this technique as significant differences in metabolic profiles and metabolic adaptation between DCD and DBD have already been shown.[41] Identifying metabolic biomarkers in this specific population may enable use of older donors and donors with longer ischemic time. In the field of lung transplantation, HR-MAS-NMR metabolomics has shown that perfusion confers significant metabolic benefit according to different ischemia times.[42]

We advocate the use of HR-MAS-NMR metabolomics to evaluate the quality of liver grafts after cold storage. In the case of favorable metabolic profiles, liver transplantation could be safely performed, even with ECD. In light of this analysis, interpretation of biological results may avoid the question of relisting in cases where PNF is deemed a risk. In case of significant metabolic derangements identified by HR-MA-NMR, the use of machine perfusion could be indicated as there is a significantly higher risk of one-year graft loss. Most importantly the use of lactate- and phosphocholine-rich donor grafts in recipients with sarcopenia and more generally in patients with cirrhosis, exerting advanced stage metabolic derangements, should be avoided as it leads to significant graft failure. Metabolomic profiling may help to evaluate the efficiency of graft resuscitation on machine perfusion and to objectively select DCD grafts.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Study concept and design: IJN, FF. Acquisition of data: FF, SB, ER, PA. Analysis and interpretation of data: IJN, FF, SB, ER. Statistical analysis: FF, SB, ER. Drafting of the manuscript: FF, IJN. Critical revision of the manuscript: FF, SB, CB, ER, BE, MLWJ, PA, PB, IJN.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jhep.2017.11.022.

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