Am J Transl Res 2021;13(1):372-382
www.ajtr.org /ISSN:1943-8141/AJTR0120230

Original Article

A panel of biomarkers in the prediction for early allograft dysfunction and mortality after living donor liver transplantation

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Received August 13, 2020; Accepted October 22, 2020; Epub January 15, 2021; Published January 30, 2021

Abstract: Early allograft dysfunction (EAD) is associated with graft failure and mortality after living donor liver transplantation (LDLT). In this study, we report biomarkers superior to other conventional clinical markers in the prediction of EAD and all-cause in-hospital mortality in LDLT patient cohort. Blood samples of living donor liver transplant recipients were collected on postoperative day 1 and analyzed by liquid chromatography coupled with mass spectrometry (LC-MS). Significant metabolites associated with the prediction of EAD were identified using orthogonal projection to latent structures-discriminant analysis (OPLS-DA). A few lipids, more specifically, lysoPC (16:0), PC (18:0/20:5), betaine and palmitic acid (C16:0) were found to effectively differentiate EAD from non-EAD on postoperative day 1. A combination of these four metabolites showed an AUC of 0.821, which was further improved to 0.846 by the addition of a clinical parameter, total bilirubin. The panel exhibits a high prognostic accuracy in prediction of all-cause in-hospital mortality and mortality within 7 postoperative days with AUCs of 0.843 and 0.954. These results show the combination of metabolomics-derived biomarkers and clinical parameters demonstrates the power of panels in diagnostic and prognostic evaluation of LDLT.

Keywords: Lipidomics, early allograft dysfunction, phosphatidylcholines, lysophosphatidylcholines, betaine, living donor liver transplantation

Introduction

Liver transplantation is a life-saving treatment for patients with end stage liver disease and an alternative therapy for patients with hepatocellular carcinoma (HCC); however, a shortage of liver donors has always been a major limitation for liver transplantation. The concept of living donor liver transplantation (LDLT) has recently become widely accepted with a one-year survival rate approximately 90%, better than that of deceased donor liver transplantation (DDLT) [1, 2]. That said, primary graft dysfunction, a syndrome encompassing the milder, reversible form of early allograft dysfunction (EAD) to the more severe, irreversible form of primary non-function (PNF) can occur post-transplantation [3, 4]. Although no consensus in EAD definition is reached, clinically, EAD is recognized frequently based upon deranged liver function, such as elevated serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin levels and the prolonged international normalized ratio (INR) with-

in the first seven days postoperatively [5]. EAD develops at an incidence ranging from 5.2% to 36.3% [4] with an EAD-associated mortality reaching 18.8% [5, 6]. EAD is attributed by a number of factors including hepatic steatosis in donors [7], diagnosis of hepatocellular carcinoma and dialysis at transplant in recipients [8], intraoperative blood loss requiring blood transfusion [9] and long ischemia time [3]. Although age has long been recognized as a risk factor for the development of EAD, recent studies have shown more liberal results for both older donors and recipients [10, 11]. The literature has shown that disturbed lipid homeostasis may be related to the functional status of liver [12]. In the setting of EAD, our team has previously utilized ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectroscopy and liquid chromatography coupled with mass spectrometry (LC-MS) in identifying lipidomic profiles in association with EAD. Previously, we have demonstrated that on postoperative day 7, the changes in lipids serve as prognostic factors for short term outcomes [13]. In this study, we aim to identify novel metabolites that may predict the event of EAD on postoperative day 1 in the hope to initiate timely treatment after LDLT.

Materials and methods

Patients

The study received prior approval from the Institutional Review Board of Chang Gung Memorial Hospital (IRB 1805230007) and registered under The Australian New Zealand Clinical Trials Registry (ACTRN12619000386134). All subjects gave their informed consent for inclusion before they participated in the study. After applying exclusion criteria, including concurrent septic shock status, a preoperative measured pulmonary wedge pressure greater than 35 mmHg, or refusal to give informed consent, 74 recipients undergoing LDLT between May 2015 and February 2018 were recruited to the study.

General anesthesia was conducted for LDLT [14]. Within 7 days postoperatively, the primary allograft function was assessed based on Olthoff's criteria: international normalized ratio (INR) ≥ 1.6 or bilirubin ≥ 10 mg/dL on postoperative day 7 or aspartate (AST) or alanine (ALT) aminotransferaess >2000 IU/mL within the first 7 postoperative days [5, 15]. Grafts meeting one or more of the aforementioned criteria was characterized as EAD. The short-term mortality was also assessed as secondary outcomes.

Blood samples

A peripherally indwelling arterial catheter was inserted in the recipient perioperatively, from which the blood samples were collected on postoperative day 1. The blood was centrifuged immediately to obtain plasma. The biochemistry was sent to the central clinical laboratory for analysis.

LC-MS-lipidomic analysis

The plasma sample and precooling isopropanol (IPA) was mixed. The mixture was vortexed for 60 seconds and then stand on ice for 30 min. After incubation, the mixture was centrifuged at 12,000 rpm at 4°C for 30 min. The supernatant was transferred to glass tube. Finally, the clear supernatant was collected for LC-MS analysis [16].

Statistical analysis

The continuous variables data were presented as the mean ± standard deviation (SD). The categorical data were presented as frequencies and compared using the chi-square test or the Fisher's exact test. To identify an independent predictor of postoperative EAD, linear logistic regression analysis was performed. A receiver operator characteristic curve (ROC) was created to compare the predictive accuracy of the identified variables. A p-value <0.05 was considered to be significantly different. Analyses were performed in SAS 9.4 and R 3.3.2 [17]. Several software programs were utilized to analyze metabolomic data. The orthogonal projection to latent structures-discriminant analysis (OPLS-DA) model was performed. Each variable in the model was given a variable importance in the projection (VIP) value.

Results

Patient characteristics

74 patients received LDLT and 22 developed EAD on postoperative day 7 whereas the other 51 had uneventful recovery. **Table 1** summarized the preoperative demographics, intraoperative parameters and postoperative second-

Table 1. A summary of the clinical parameters of LDLT recipients

	EAD (N = 23)	Non-EAD (N = 51)	*p*-value
PRE-OPERATIVE PARAMETERS			
Age	52.48 ± 10.20	56.45 ± 7.10	0.1004
Height	1.65 ± 0.09	1.62 ± 0.08	0.1383
Weight	68.74 ± 13.59	66.77 ± 11.34	0.5178
BMI	25.18 ± 3.77	25.39 ± 3.33	0.8153
MELD	21.22 ± 11.19	16.41 ± 8.37	0.0438
Gender (F/M)	6/17	25/26	0.0657
Gender Mismatch	16	23	0.0520
Female to Male	11	15	0.1281
Blood Type			0.9577
A	8	18	
B	5	11	
O	9	20	
AB	1	2	
ABO Incompatibility	3	8	0.7712
Etiology			
HBV	11	23	0.8303
HCV	6	18	0.4405
Alcoholism	7	14	0.7956
HCC	6	23	0.1244
INTRA-OPERATIVE PARAMETERS			
Blood Loss (mL)	2547.83 ± 1806.71	1712.16 ± 1552.97	0.0455
RBC (units)	11.61 ± 7.83	7.57 ± 7.16	0.0324
FFP (units)	15.48 ± 10.72	10.82 ± 10.34	0.0806
Platelets (units)	9.39 ± 8.83	8.24 ± 9.14	0.6124
Graft Size (mg)	626.78 ± 127.62	637.25 ± 140.63	0.7614
GRWR (%)	0.94 ± 0.18	0.98 ± 0.29	0.4050
CIT (minutes)	43.39 ± 30.07	51.49 ± 32.82	0.3171
WIT (minutes)	45.09 ± 26.66	50.02 ± 39.31	0.5307
POST-OPERATIVE OUTCOMES			
Mortality within 7 days	2	0	N/A
Mortality within 30 days	5	1	0.0380
Mortality within 60 days	7	1	0.0090
Mortality within 90 days	8	2	0.0070
In hospital Mortality	8	3	0.0117

Abbreviations: EAD, early allograft dysfunction; BMI, body mass index; MELD, model for end-stage liver disease; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; PRBC, packed red blood cell; FFP, fresh frozen plasma; GRWR, graft recipient weight ratio; CIT, cold ischemia time; WIT, warm ischemia time; ICU, intensive care unit.

ary outcomes. EAD and non-EAD groups had a mean age of 53.33 ± 9.03 and 56.45 ± 7.10 years, respectively. The incidence of EAD was 29.7%. Of the pre-operative parameters, no statistical significance was observed for patient age, height, weight, body mass index (BMI), gender and the etiologies of liver transplantations except for the preoperative MELD scores, which were statistically higher in the EAD group (21.22 ± 11.19 versus 16.41 ± 8.37, *p*-value 0.0438). Although donor-to-recipient gender mismatch and ABO incompatibility appeared to be risk factors for poor graft survival after liver transplantation [18, 19], no statistical significance of either in the event of EAD was recognized in our study. Intraoperatively, the EAD group, compared to non-EAD group, had slightly shorter cold ischemia time (43.39 ± 30.07 ver-

sus 51.49 ± 32.82 minutes, respectively) and warm ischemia time (45.09 ± 26.66 versus 50.02 ± 39.31 minutes, respectively) with no statistical significance. Clinically, the EAD group appeared to endure more blood loss than non-EAD group (2547.83 ± 1806.71 versus 1712.16 ± 1552.97 mL, respectively, *p*-value 0.0455) and required more red blood cell transfusions intraoperatively. Among the 74 recipients, the difference in mortality within 7 postoperative days between EAD and non-EAD groups within 7 days was not computed as no mortality was observed in the non-EAD group. More mortality was observed in the EAD group within 30 days, 60 days and 90 days postoperatively with statistical significance (*p*-value 0.0380, 0.0090 and 0.0070, respectively). Eighteen patients had a graft recipient weight ratio (GRWR) less than 0.8%, satisfying the definition for small for graft syndrome (SFGS) [20, 21].

Preoperatively, the majority of lab data showed no difference except for glomerular filtration rate (GFR), total protein and bilirubin levels (**Table 2**). On postoperative day 1 (T2), EAD group showed significantly worse coagulation, renal and liver function and electrolyte imbalance. Similarly, on postoperative day 7 (T3), EAD group demonstrated significantly poorer coagulation profiles and liver function than non-EAD group, in support of the diagnosis of EAD.

Circulatory lipid profiles in recipients using LC-MS

Ultra performance liquid chromatography-time of flight mass spectrometry (UPLC-TOFMS) was performed with the plasma samples collected from EAD and non-EAD recipients on postoperative day 1. **Figure 1A** shows the OPLS-DA score plot for EAD (red) and non-EAD (blue) datasets collected in electrospray positive ion mode endowed with $R_2X = 0.793$, $R_2Y = 0.843$, $Q_2 = 0.664$, representing the explanation, fitness and prediction power of the model. Additionally, **Figure 1B** shows OPLS-DA score plot for EAD (red) and non-EAD (blue) datasets collected in electrospray negative ion mode endowed with $R_2X = 0.666$, $R_2Y = 0.735$ and $Q_2 = 0.529$. Heatmap analysis (**Figure 1C**) and OPLS-DA demonstrated a separation of metabolites distinguishing EAD from non-EAD. Twenty-nine metabolites were selected and shown in **Table 3**. They included betaine, free fatty acids, such as palmitic acid, linoleic acid, oleic acid, lysophosphatidylcholines (lysoPC (16:0)), and phosphatidylcholines (PCs). The levels of betaine, free fatty acids and PC (32:0 and 32:1) appeared to be elevated whereas other PC species were lowered in the EAD group as compared to non-EAD group.

Discriminative abilities of betaine, LysoPC (16:0), PC (38:5) and palmitic acid (C16:0) as predictors for EAD and short-term mortality

Betaine, palmitic acid, lysoPC (16:0) and PC (18:0/20:5) were tested for their discriminative ability as potential biomarkers for the early prediction of EAD. The area under the curve (AUC) of ROC curve of the 4 selected metabolites and five other parameters including lactate dehydrogenase (LDH), ALT, AST, bilirubin, and INR were calculated (**Table 4**). Individual metabolites betaine, palmitic acid, lysoPC (16:0) and PC (18:0/20:5) *per se* showed AUCs of 0.6855, 0.6406, 0.6806 and 0.6304, respectively. The AUC for a combination of the aforementioned metabolites increased to 0.8210 (**Figure 2A**), suggesting a potential of these lipidomics-derived biomarkers in predicting EAD after LDLT as early as postoperative day 1. As a panel to predict EAD, four metabolites in addition to total bilirubin further augmented AUC to 0.846. Models for other secondary outcomes were similarly constructed as shown in **Figure 2B** and **2C**.

Discussion

As LDLT has become widely accepted, the early identification of poor function of liver allograft is of imminent importance for the transplant surgeons as EAD is often associated with allograft loss or mortality [22]. Previously, we have identified biomarkers that may be associated with early allograft dysfunction on postoperative day 7. In the present study, we have evaluated the metablomics using LC-MS between EAD and non-EAD recipients as early as postoperative day 1. Lipidomic analysis may provide clinicians information on the function and cellular state of a graft. The ROC analysis revealed that a combination of these plasma lipid molecules and clinically available parameters may serve as an excellent early predictor panel

Table 2. Biochemical data preoperatively (T1) on postoperative day 1 (T2) and day 7 (T3)

	T1			T2			T3		
	EAD (N = 23)	Non-EAD (N = 51)	*p*-value	EAD (N = 23)	Non-EAD (N = 51)	*p*-value	EAD (N = 23)	Non-EAD (N = 51)	*p-value*
Hemoglobin (g/dL)	9.72 ± 2.33	10.73 ± 2.40	0.0971	10.88 ± 3.02	10.91 ± 3.17	0.9661	11.1 ± 2.2	10.3 ± 1.3	0.1065
Hematocrit (%)	28.91 ± 6.90	31.64 ± 6.75	0.1142	31.35 ± 8.42	31.14 ± 6.59	0.9067	32.1 ± 5.7	30.0 ± 3.9	0.14603
Platelet (1000/dL)	77.35 ± 58.34	84.44 ± 43.94	0.5644	53.96 ± 37.78	68.06 ± 38.60	0.1476	47.5 ± 30.3	70.0 ± 37.0	0.0163
INR (seconds)	1.79 ± 0.65	1.63 ± 0.68	0.3448	2.11 ± 0.59	1.71 ± 0.31	0.0053	1.5 ± 0.4	1.2 ± 0.1	0.0016
BUN (mg/dL)	32.88 ± 33.86	17.80 ± 17.41	0.0535	34.75 ± 25.33	24.63 ± 15.29	0.0861	56.7 ± 44.1	23.8 ± 13.4	0.0029
Creatinine (mg/dL)	1.69 ± 1.75	1.02 ± 1.33	0.0732	1.95 ± 1.23	1.13 ± 0.76	0.0056	1.9 ± 1.4	0.8 ± 0.5	0.0008
GFR (mL/mL/m/1.73 min/1.73 m^2)	79.74 ± 51.46	112.25 ± 51.34	0.0140	47.36 ± 35.01	86.43 ± 49.78	0.0011	64.6 ± 64.3	116.7 ± 45.3	0.0021
Total protein (g/dL)	6.11 ± 1.17	6.62 ± 0.88	0.0444	4.17 ± 0.85	4.74 ± 0.65	0.0026	4.1 ± 0.7	4.4 ± 0.5	0.0476
Albumin (g/dL)	3.03 ± 0.64	3.09 ± 0.71	0.7544	2.36 ± 0.38	2.55 ± 0.41	0.0579	2.5 ± 0.5	2.7 ± 0.4	0.2575
Na (mEq/L)	140.22 ± 5.66	138.43 ± 3.92	0.1794	142.17 ± 5.90	139.00 ± 4.31	0.0111	130.6 ± 27.5	137.4 ± 3.4	0.2698
K (mEq/L)	3.58 ± 0.54	3.75 ± 0.55	0.2230	3.71 ± 0.56	3.71 ± 0.57	0.9842	3.8 ± 0.7	3.6 ± 0.5	0.2033
Ca (mEq/L)	8.26 ± 0.51	8.27 ± 0.70	0.9678	7.38 ± 0.65	7.48 ± 0.70	0.5628	7.9 ± 0.7	7.4 ± 0.9	0.0284
Sugar	129.70 ± 42.22	138.59 ± 52.99	0.4807	214.83 ± 76.18	282.67 ± 102.53	0.0059	184.1 ± 72.1	178.5 ± 55.9	0.7300
Bilirubin total (mg/dL)	13.17 ± 15.68	4.61 ± 6.43	0.0183	12.34 ± 9.25	4.55 ± 3.42	0.0006	12.1 ± 7.8	2.7 ± 2.1	<0.0001
Bilirubin direct (mg/dL)	7.65 ± 9.81	2.17 ± 3.24	0.0152	6.00 ± 5.29	1.99 ± 1.77	0.0016	7.0 ± 4.8	1.5 ± 1.4	<0.0001
AST (U/L)	103.78 ± 141.52	68.75 ± 51.87	0.2600	720.35 ± 923.72	295.69 ± 220.09	0.0399	147.2 ± 191.9	110.4 ± 77.0	0.40391119
ALT (U/L)	54.70 ± 34.37	38.82 ± 22.45	0.0513	764.17 ± 1059.86	304.41 ± 228.64	0.0510	273.7 ± 289.6	201.3 ± 162.9	0.2916

Abbreviations: EAD, early allograft dysfunction; INR, internationalized ratio; BUN, blood urea nitrogen; GFR, glomerular filtration rate; Na, sodium; K, potassium; Ca, calcium; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

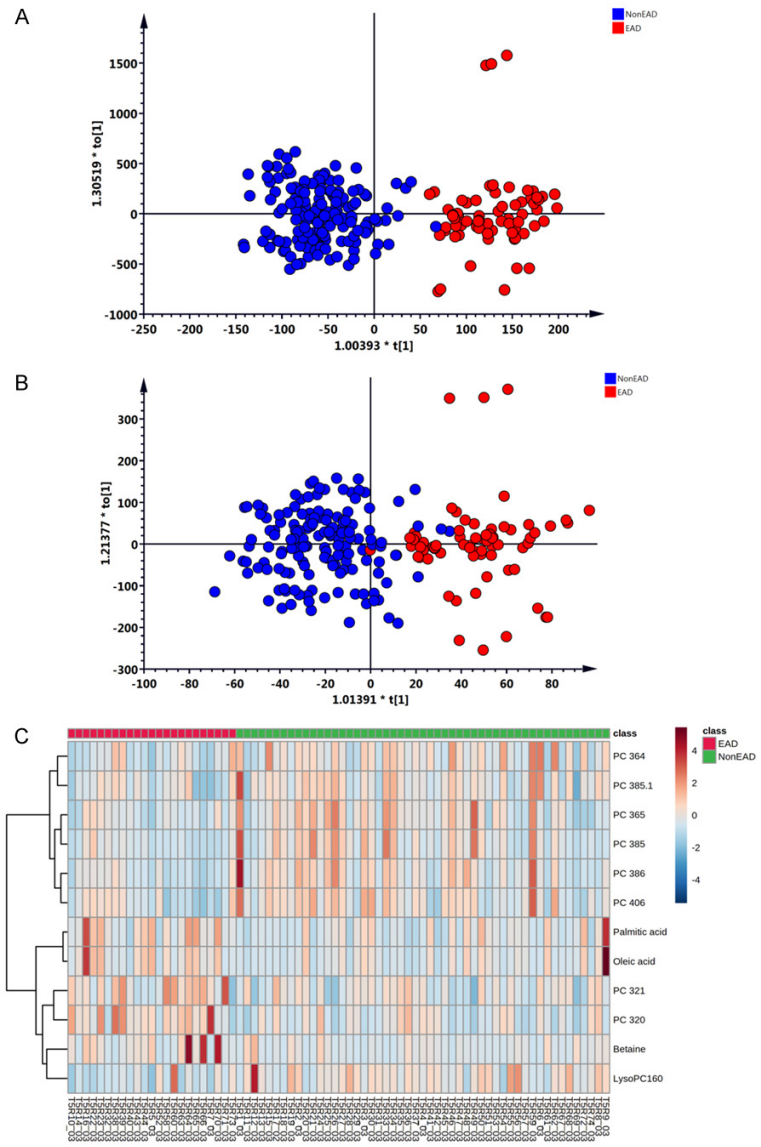

Figure 1. Metabolomic analysis of the plasma samples from EAD and non-EAD recipients. A. The OPLS-DA score plot for EAD (red) and Non-EAD (blue) datasets collected in electrospray positive ion mode (with parameters R_2X = 0.793, R_2Y = 0.843, Q_2 = 0.664). B. The OPLS-DA score plot for EAD (red) and Non-EAD (blue) datasets collected in electrospray negative ion mode (with parameters R_2X = 0.666, R_2Y = 0.735, Q_2 = 0.529). C. Heatmap analysis distinguishing EAD from non-EAD recipients. Abbreviations: EAD, early allograft dysfunction; OPLS-DA, orthogonal projection to latent structures-discriminant analysis.

for EAD and early mortality, which may become applicable to clinical practice.

Betaine is a stable and natural substance existing in biological systems. The original betaine trimethylglycine, a derivative of choline, is involved in transmethylation reactions, and is mainly present in kidneys and liver. In kidneys, betaine serves as an osmolyte that balances the high extracellular osmolarity and maintains normal cell volume. In liver, via betaine-homocysteine methytransferase (BHMT), betaine transfers a methyl group to homocysteine to form methionine, which in turn can form S-adenosylmethionine (SAM), another methylating agent critical to maintenance of the integrity of the liver. A schematic illustration of metabolomic disturbances associated with liver function after LDLT is depicted in **Figure 3**. SAM converts phosphatidylethanolamine (PE) to phosphatidylcholine (PC), which is an integral component of lipoproteins. Betaine increases the transmethylation rate of methionine, homocysteine re-methylation and oxidation of methionine in healthy adults [23]. In humans, decreased betaine levels were associated with non-alcoholic steatohepatitis [24]. On the other hand, increased betaine levels secondary to a diminished SAM level has been observed in individuals with chronic alcohol abuse, as the hepatocytes cannot replenish SAM via the BHMT pathway [25]. Dietary betaine treatment appears to alleviate the fatty liver in such patients [26]. Impairment of BHMT pathways may increase homocysteine, which contributes to the development of liver steatosis and injury, suggesting the importance of the betaine/BHMT system in maintenance of the homeostasis of homocysteine and methionine [27]. In animal studies, *bhmt*$^{-/-}$ mice had an elevated plasma homocysteine concentration and a reduced hepatic methionine to homocysteine ratio. Deletion of *bhmt* gene also led to an accumulation of betaine and diminishment of choline, phosphocholine and phosphatidylcholines in tissues. Additionally, histopathological analysis of these *bhmt*$^{-/-}$ mice developed hepatocellu-

Table 3. A summary of metabolites distinguishing the EAD from the non-EAD group on postoperative day 1 (VIP >1 and *p*-value <0.05)

ID	Adduct	EAD (N = 23)	Non-EAD (N = 51)	*p*-value	VIP
Betaine	M+H	7286.22 ± 5287.27	4738.75 ± 1738.33	0.0002	4.57
Palmitic acid (C16:0)	M-H	458.96 ± 297.70	297.00 ± 231.01	0.0001	2.18
Oleic acid (C18:1)	M-H	2470.17 ± 1959.52	1555.71 ± 1783.92	0.0007	4.60
LysoPC (16:0)	M+H	861.00 ± 683.70	1454.67 ± 721.06	<0.0001	2.28
PC (16:0/16:1)	M+H	4029.76 ± 1719.00	2850.05 ± 1237.37	<0.0001	3.22
PC (16:0/16:0)	M+H	10269.97 ± 4303.20	6959.97 ± 2268.48	<0.0001	5.99
PC (16:0/20:5)	M+H	1903.41 ± 980.02	2906.62 ± 1996.07	<0.0001	2.50
PC (16:0/20:4)	M+H	43859.61 ± 13800.01	52715.85 ± 19138.16	0.0001	6.96
PC (16:0/22:6)	M+H	19410.38 ± 5771.54	27480.17 ± 12769.92	<0.0001	7.90
PC (18:0/20:5)	M+H	499.05 ± 424.38	1065.36 ± 997.00	<0.0001	2.00
PC (16:0/22:5)	M+H	5017.37 ± 1245.70	5773.87 ± 1391.83	0.0001	2.15
PC (18:0/22:6)	M+H	5048.24 ± 1735.41	6283.42 ± 2299.50	<0.0001	2.78

Abbreviations: EAD, early allograft dysfunction; lysoPC, lysophosphatidylcholines; PC, phosphatidylcholine.

Table 4. Receiver operating characteristic (ROC) curve analysis for individual metabolites in the prediction of EAD

	AUC	Standard error
Betaine	0.686	0.0394
PC (18:0/20:5)	0.630	0.0392
LysoPC (16:0)	0.681	0.0404
Palmitic acid (C16:0)	0.641	0.0401
Combination of betaine, PC (18:0/20:5), palmitic acid and lysoPC (16:0)	0.821	0.0336
Total bilirubin	0.754	0.0438
Combination of metabolites and total bilirubin	0.846	0.0314

Abbreviations: EAD, early allograft dysfunction; PC, phosphatidylcholine; lysoPC, lysophosphatidylcholine; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, internationalized ratio.

lar carcinoma or carcinoma precursors [28]. In this study, betaine level is elevated in the EAD group. Further investigation is needed to elucidate whether this betaine level is related to abnormal BHMT expression owing to recipients' liver status.

In our study, a few lipids, more specifically, palmitic acid (C16:0), lysoPC (16:0) and PC (18:0/20:5), were found to effectively differentiate EAD from non-EAD on postoperative day 1. Free fatty acids (FFA), derived from catabolism of triglyceride, play important roles in the synthesis of signalling molecules and complex lipids [29]. Serum FFA levels are elevated in obese patients. Palmitic acid (PA) is more hepato-toxic than other saturated and unsaturated FFA, and is implicated in the pathogenesis of non-alcoholic fatty liver disease and liver fibrosis [30-32]. Lysophosphatidylcholine is another species of lipids that are associated with the progression of liver disease [33] and inflammation status [34]. A relationship between the decrease in abundance of long chain lysoPC species and the progression of HBV-associated liver disease has also been established [35]. Consistent with such findings, lysoPC (16:0) and (18:0) may have a role in signalling liver tissue damage and early allograft dysfunction in patients undergoing liver transplantation [36].

Phosphatidycholine is synthesized by the two pathways, namely the CDP-choline pathway and the conversion from phosphatidylethanolamine (PE). In CDP-choline pathway, choline entering the cell is rapidly phosphorylated to phosphocholine via choline kinase, followed by the conversion of phosphocholine to CDP-choline via CTP: phosphocholine cytidylyltransferase (CT). Phosphocholine is transferred from CDP-choline to diacylglycerol (DAG) by CDP-choline: 1,2-diacylglycerol cholinephosph-

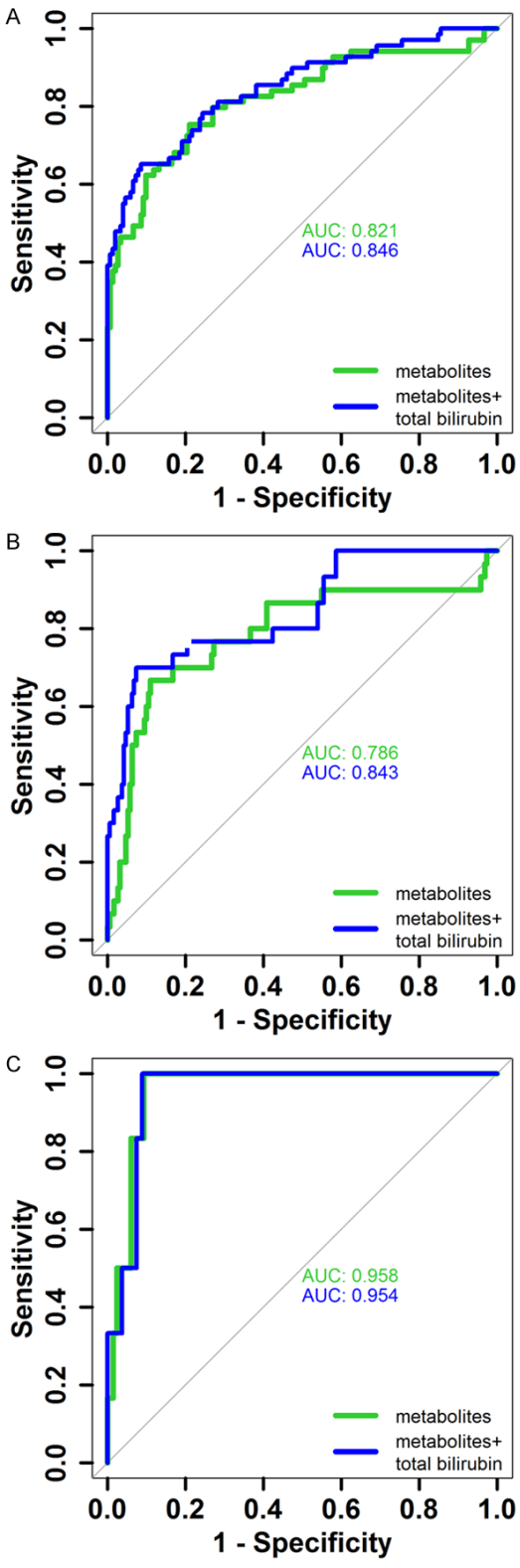

Figure 2. Prediction of (A) EAD, (B) the all-cause in-hospital mortality and (C) the mortality within 7 days after LDLT. (A) A combination of betaine, PC (18:0), lysoPC (16:0) and palmitic acid (C16:0) gives an AUC of 0.821 in the prediction of EAD. A panel consisting of the metabolites and total bilirubin gives an AUC of 0.846. (B) A combination of betaine, PC (18:0), lysoPC (16:0) and palmitic acid (C16:0) gives an AUC of 0.786 in the prediction of in-hospital mortality. A panel consisting of the metabolites and total bilirubin gives an AUC of 0.843. (C) A combination of betaine, PC (18:0), lysoPC (16:0) and palmitic acid (C16:0) gives an AUC of 0.958 in the prediction of 7-day mortality. A panel consisting of the metabolites and total bilirubin gives an AUC of 0.954. Abbreviations: LDLT, living donor liver transplantation; PC, phosphatidylcholine; lysoPC, lysophosphatidylcholines; AUC, the area under the receiver operator characteristic curve.

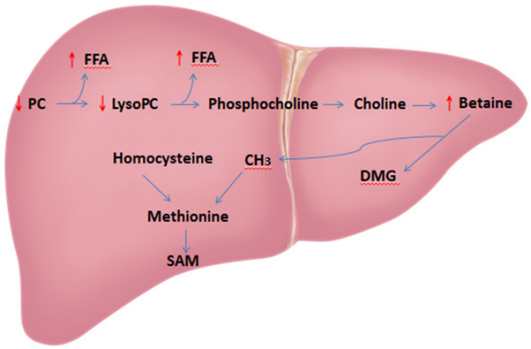

Figure 3. Schematic illustration of metabolomic disturbances associated with liver function after living donor liver transplantation. A decrease in PC and lysoPC species with an increase in FFA and betaine were associated with EAD and mortality. Abreviations: EAD, early allograft dysfunction; PC, phosphatidylcholine; FFA, free fatty acid; DMG, dimethylglycine; SAM, S-adenosylmethionine.

atransferase (CPT) to form phosphatidylcholine. This reaction occurs essentially only in hepatocytes in mammals. PCs have been proposed as a risk and prognostic biomarkers for different liver disease. For example, PC (16:0/16:0) and PC (16:0/18:0) were elevated in liver cirrhosis patients with and without HCC [36]. However, PEMT that preferentially synthesizes long chain polyunsaturated PC is downregulated in expression in HCC patients, leading to a specific decrease in such phospholipid species. Consistent with this, we have found that patients of the EAD group had higher level of palmitic acid and lower levels of lysoPC (16:0) and long chain PC than those of non-EAD group, suggesting that such changes can be used as early predictors for the development of EAD. As previously mentioned, EAD is diagnosed on postoperative day 7 based on live function and coagulation profiles. We have demonstrated that, as early as postoperative day 1, both clinical

parameters and metabolomic biomarkers may be adequate in predicting EAD on postoperative day 7; however, a panel consisting of metabolites and bilirubin performs more superior than either alone. Limitations still apply to the study. The small number of patients can be the potential limitation and validation in a larger data set is warranted. Additional research is also required to pursue the mechanisms of these identified biomarkers in association with the dysfunction and mortality after LDLT.

Conclusions

The combination of abovementioned four metabolites in patients on postoperative day 1 is highly predictive of EAD with AUCs of 0.8210, better than the other clinically available markers. The predictive power of metabolites is further improved when total bilirubin is added to the calculation as a panel. In addition, this panel exhibits a high prognostic accuracy in the prediction of all-cause in-hospital mortality and mortality within 7 postoperative days with AUCs of 0.843 and 0.954. The panel consisting of betaine, palmitic acid, PC and lysoPC species and total bilirubin demonstrates the power in in diagnostic and prognostic evaluation of LDLT.

Acknowledgements

This work was supported by Chang Gung Memorial Hospital (CMRPG3H1191-3).

Disclosure of conflict of interest

None.

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