Elevated serum levels of diamine oxidase, D-lactate and lipopolysaccharides are associated with metabolic-associated fatty liver disease

Ruike Zhang[a,b,*], Ya-nan Chen[a,b,*], Jixia Zhang[a,b] and Jing Liu[a,b]

Background Studies have suggested an association between metabolic-associated fatty liver disease (MAFLD) and intestinal barrier function. The present study aims to investigate the association between MAFLD and intestinal barrier impairment in humans and identify potential risk factors for MAFLD.

Methods A total of 491 patients were retrospectively enrolled in this study. The serum levels of diamine oxidase, D-lactate and lipopolysaccharide were measured to evaluate intestinal barrier integrity in patients with and without MAFLD. Binary logistic regression and correlational analyses were conducted to verify the association between MAFLD and serum levels of intestinal barrier biomarkers.

Results We enrolled 294 patients with MAFLD and 197 patients without MAFLD in this study. Patients with MAFLD had higher serum levels of diamine oxidase, D-lactate and lipopolysaccharide ($P<0.001$) than those without MAFLD. Multivariate logistic regression analyses showed that BMI [odds ratio (OR) 1.324; $P<0.001$], triglycerides (OR 2.649; $P=0.002$), nonesterified fatty acids (OR 1.002; $P=0.011$), diamine oxidase (OR 1.149; $P=0.011$) and D-lactate (OR 1.221; $P<0.001$) were independent risk factors for MAFLD. Additionally, serum levels of diamine oxidase and D-lactate increase as liver steatosis became more severe. MAFLD patients with ≥2 metabolic abnormalities had higher serum levels of lipopolysaccharide ($P=0.034$).

Conclusions MAFLD is associated with intestinal barrier impairment. Diamine oxidase and D-lactate are potential predictors of MAFLD, and their serum levels are related to liver steatosis. Intestinal barrier impairment is related to metabolic disorders in patients with MAFLD. Eur J Gastroenterol Hepatol 35: 94–101

Introduction

Metabolic-associated fatty liver disease (MAFLD), a new definition of fatty liver, has been proposed for the diagnosis of fatty liver disease with metabolic dysfunction [1]. Unlike the diagnostic criteria for nonalcoholic fatty liver disease (NAFLD), those for MAFLD are based on the evidence of fatty liver with overweight/obesity, metabolic dysregulation and the presence of type II diabetes mellitus (T2DM) instead of a series of exclusion criteria [1]. This new definition has been confirmed to be critical for diagnosis, drug discovery and treatment.

European Journal of Gastroenterology & Hepatology 2023, 35:94–101

Keywords: intestinal barrier dysfunction, metabolic-associated fatty liver disease, metabolic dysregulation, risk factor

[a]Department of Gastroenterology, Zhongnan Hospital of Wuhan University and [b]Hubei Clinical Center and Key Laboratory of Intestinal and Colorectal Diseases, Wuhan, China

Correspondence to Jing Liu, MD, PhD, Department of Gastroenterology, Zhongnan Hospital of Wuhan University, No. 169, Donghu Road, Wuchang District, Wuhan 430071, Hubei Province, China

Tel: +86 18971622466; fax: +86 27 67812892; e-mail: liujing_GI@whu.edu.cn

*Ruike Zhang and Ya-nan Chen contributed equally to the writing of this article.

Received 28 June 2022 **Accepted** 1 September 2022

Intestinal barrier function plays a crucial role in the genesis and development of MAFLD [2]. The theory of the gut–liver axis, which highlights the communication between the gut and liver, suggests that intestinal barrier dysfunction is associated with the pathogenesis of chronic liver diseases [3]. Increased permeability of the intestinal barrier permits the gut microbiota and its metabolites to enter the blood circulation and travel to the liver via the portal vein [4]. Translocation of bacteria and bacterial products can trigger chronic inflammation, hepatocyte injury and metabolic disorders [5]. Hence, for the prevention and promising treatment of MAFLD, it is necessary to verify the relationship between MAFLD and intestinal barrier integrity and explore the effect of intestinal barrier impairment on the metabolic characteristics of MAFLD patients.

Emerging evidence has shown that as convenient and accessible biomarkers, the serum levels of diamine oxidase [6], D-lactate [7] and lipopolysaccharide [8] can reflect the integrity of the intestinal barrier [9]. Diamine oxidase is an intracellular cytoplasmic protein in intestinal epithelial cells. When the intestinal epithelial barrier is disturbed, diamine oxidase is released into the bloodstream, and increased serum levels of diamine oxidase are associated with intestinal barrier impairment [6]. Increased serum concentrations of D-lactate and lipopolysaccharide, which are metabolites of intestinal flora, can be used to verify the translocation of intestinal flora and their related metabolites into the bloodstream as biomarkers [10]. Therefore,

serum levels of diamine oxidase, D-lactate and lipopolysaccharide can be used to assess intestinal barrier dysfunction quickly and with minimal invasiveness.

In this work, we investigated the role of serum biomarkers of intestinal barrier integrity in MAFLD patients to characterize potential risk factors and provide new targets and strategies for the prevention and treatment of MAFLD.

Patients and methods

Study population

This study was designed as a single-centre, retrospective study in Wuhan, China. The present study was performed in accordance with relevant guidelines and regulations and was approved by the ethics committee of the Zhongnan Hospital of Wuhan University. Informed consent was obtained from the research subjects, and we protected personal information during data collection.

We reviewed the medical data of all inpatients treated in the Department of Gastroenterology at Zhongnan Hospital of Wuhan University from January 2017 to January 2022. The inclusion criteria were as follows: patients (a) between 18 and 75 years old; (b) who had undergone abdominal ultrasonography; and (c) who had undergone measurement of the three serum biomarkers. Participants were excluded if they met the following criteria: (a) carcinoma; (b) severe heart, lung, liver, or kidney disease; (c) nonsteroidal anti-inflammatory drug use in the previous month; (d) intestinal diseases; (e) history of gastrointestinal surgery; and (f) bacterial infection, except *Helicobacter pylori*. Patients were recorded only once during the study period. A total of 523 participants who had abdominal ultrasonography and measurements of serum levels of diamine oxidase, D-lactate and lipopolysaccharide as part of their clinical review were enrolled. Patients lacking biochemical data ($n=41$) were then excluded. Finally, 491 patients were included in the present study. In this study, 197 participants without MAFLD and 294 participants with MAFLD were included.

Data collection

All data were collected at the time of hospitalization. Patients with several hospitalizations during the study were documented only once. We recorded the following clinical information in the computerized hospital database (HIS): age, sex, height, weight, blood pressure, comorbidities, medication and current alcohol intake. BMI and the presence/absence of T2DM, hypertension and dyslipidaemia were obtained by clinical review according to standard criteria [1,11,12]. We collected the following laboratory parameters from the computerized hospital database at Zhongnan Hospital: full blood count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, γ-glutamyl transpeptidase (GGT), total protein, albumin, globulin, total bilirubin, direct bilirubin, total bile acid (TBA), total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol (LDL), lipoprotein(a) [Lp(a)], nonesterified fatty acid (NEFA), blood urea nitrogen, creatinine, uric acid, electrolytes, fasting glucose, diamine oxidase, D-lactate and lipopolysaccharide.

Metabolic-associated fatty liver disease definition

MAFLD was diagnosed according to the criteria including evidence of fatty liver (hereby, ultrasonography and computed tomography), in addition to one of the following: overweight/obesity defined as BMI ≥ 23 kg/m^2 in this Asian cohort, presence of T2DM, or lean/normal weight with evidence of metabolic dysregulation [1]. Metabolic dysregulation was defined as the presence of at least two metabolic risk abnormalities: (a) waist circumference ≥90 cm in men and ≥80 cm in women, (b) blood pressure ≥130 mm Hg or specific drug treatment, (c) plasma triglycerides ≥ 150 mg/dL or specific drug treatment, (d) plasma HDL-cholesterol <40 mg/dL for men and <50 mg/dL for women or specific drug treatment, (e) fasting glucose ≥100 mg/dL, (f) homeostasis model assessment of insulin resistance score ≥2.5, and (g) high-sensitivity C-reactive protein level >2 ml/L (1). Although the homeostasis model assessment-insulin resistance score and plasma high-sensitivity C-reactive protein level are metabolic risk abnormalities, these were not available in our dataset.

Qualitative ultrasonographic evaluations of metabolic-associated fatty liver disease patients

Skilled technicians, who were unaware of the objective of this study, graded each US examination based on the presence and severity of liver steatosis. Then, 294 patients with MAFLD were classified into the following three classes: mild steatosis, moderate steatosis and severe steatosis. The classification criteria were based on [13,14].

Measurement of gut barrier biomarkers

All blood collection was performed after patients were hospitalized for 12 h. Serum was isolated by centrifugation at 2500 ×g for 10 min. The serum levels of gut mucosal barrier function parameters [endotoxin (lipopolysaccharide), D-lactate and diamine oxidase] were measured by a dry chemical method using the Intestinal Mucosal Barrier Biochemical Index Analysis System (JY-DLT; Beijing Zhongsheng Jinyu Diagnostic Technology Co., Ltd., Beijing, China) according to the manufacturer's instructions. The experiments were performed within 4 h after blood collection.

Statistical analysis

The data were analysed using IBM SPSS 26.0 and tabulated with Microsoft Office software. Categorical data were compared by the chi-square test. Continuous variables were examined by the Mann–Whitney *U* test. Frequencies and proportions are used to represent categorical variables. Medians and interquartile ranges are used to represent continuous values. The correlations were performed by Spearman's rank test and Pearson's correlation analysis. We analysed 28 potentially related factors using univariate analysis. Those risk factors with *P* values less than 0.05 in the univariate analysis were substituted into the binary logistic regression model to verify important risk factors for MAFLD. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. The results were expressed as ORs with corresponding 95% CIs.

Results

Patient characteristics

The study group comprised 231 (47.0%) females and 260 (53.0%) males. The clinical and biochemical characteristics of patients without and with MAFLD are depicted in Table 1. There were no significant differences in the age distribution; however, MAFLD patients tended to be male and had a higher BMI. There were higher frequencies of hypertension and T2DM and a higher serum level of uric acid in MAFLD patients than in those without MAFLD. Moreover, patients with MAFLD had higher serum liver enzyme (AST, ALT and GGT) levels and higher serum levels of total cholesterol, triglycerides, HDL cholesterol, LDL and NEFAs ($P < 0.001$) (Table 1).

Increased serum levels of diamine oxidase, D-lactate and lipopolysaccharide were associated with metabolic-associated fatty liver disease

The serum levels of D-lactate, diamine oxidase and lipopolysaccharide were significantly higher in patients with MAFLD than in those without MAFLD, as shown in Table 1. Binary logistic regression analysis was performed to verify the risk factors for MAFLD. We evaluated 28 variables by univariate logistic regression analysis. Those factors with a P value less than 0.05 were included in the multivariate logistic regression analysis (Table 2). Multivariate logistic regression analysis revealed that BMI (OR = 1.324; 95% CI, 1.156–1.517; $P < 0.001$) and triglycerides (OR = 2.649; 95% CI, 1.437–4.931; $P = 0.002$), NEFA (OR 1.002; 95% CI, 1.000–1.004; $P = 0.011$), diamine oxidase (OR 1.149; 95% CI, 1.055–1.251; $P = 0.011$) and D-lactate (OR 1.221; 95% CI, 1.139–1.308; $P < 0.001$) levels were independently associated with the presence of MAFLD (Fig. 1).

Serum levels of intestinal barrier biomarkers in patients with different grades of liver steatosis

Fatty infiltration of the liver in patients with MAFLD was assessed and diagnosed by ultrasonography. Patients with MAFLD were divided into three groups: mild steatosis ($n = 154$), moderate steatosis ($n = 110$) and severe steatosis ($n = 30$). The serum levels of diamine oxidase, D-lactate and lipopolysaccharide in these three groups were analysed (Fig. 2). The results showed that the serum level of diamine oxidase was significantly higher in the moderate

Table 1. Comparisons of clinical and biochemical characteristics between patients without and with metabolic-associated fatty liver disease

	Patients without MAFLD ($n = 197$)	Patients with MAFLD ($n = 294$)	P value
Age	55 (45–64)	53 (44–60)	0.068
Sex (female/male)	56.9%/43.1% (112/85)	40.3%/59.7% (119/175)	<0.001
BMI (kg/m^2)	22.21 (19.60–23.70)	25.95 (23.94–28.09)	<0.001
Type 2 diabetes mellitus (presence/absence)	4.1% (8/189)	15.6% (46/248)	<0.001
Hypertension (presence/absence)	18.8% (37/160)	32.3% (95/199)	0.001
Alcohol intake habit (yes/none)	6.6% (13/184)	11.9% (35/259)	0.063
Smoking (yes/none)	11.3% (20/177)	23.8% (70/224)	<0.001
White blood cell count (10^9/L)	5.10 (4.40–6.10)	6.21 (5.15–7.36)	<0.001
Red blood cell count (×10^{12}/L)	4.30 (4.00–4.62)	4.57 (4.22–4.92)	<0.001
Hemoglobin (g/L)	131.9 (123.0–141.5)	140.8 (130.1–151.4)	<0.001
Platelet count (×10^9/L)	195 (166–236)	213 (175–255)	0.005
AST (U/L)	14 (11–21)	24 (19–35)	<0.001
ALT (U/L)	19 (16–24)	28 (19–47)	<0.001
ALP (U/L)	77 (64–92)	83 (68–103)	0.004
GGT (U/L)	16 (12–23)	36 (22–63)	<0.001
Total protein (g/L)	66.9 (63.4–72.5)	70.0 (65.2–73.9)	0.002
Globulin(g/L)	26.5 (24.4–29.1)	28.2 (25.2–31.1)	<0.001
Albumin (g/L)	40.5 (38.1–43.3)	41.3 (38.7–44.3)	0.107
Total bilirubin (μmol/L)	13.9 (10.9–17.9)	14.3 (10.7–18.0)	0.585
Direct bilirubin (μmol/L)	2.6 (2.0–3.3)	2.4 (1.9–3.4)	0.288
Indirect bilirubin (μmol/L)	11.3 (8.8–14.6)	11.8 (8.7–14.8)	0.452
TBA (μmol/L)	2.5 (1.4–4.6)	2.7 (1.4–5.1)	0.373
FPG (mmol/L)	4.78 (4.45–5.30)	5.2 (4.7–6.2)	<0.001
Triglycerides (mg/dL)	0.97 (0.78–1.34)	2.00 (1.46–3.04)	<0.001
Total cholesterol (mg/dL)	4.44 (3.85–4.97)	4.99 (4.29–5.79)	<0.001
HDL cholesterol (mg/dL)	1.28 (1.07–1.53)	1.00 (0.84–1.19)	<0.001
LDL cholesterol (mg/dL)	2.65 (2.22–3.07)	2.99 (2.38–3.66)	<0.001
Lp(a) (mg/L)	106.4 (49.0–196.4)	101.5 (39.0–201.6)	0.514
NEFA (μmol/L)	430.4 (268.4–565.9)	516.6 (398.4–664.7)	<0.001
BUN (mmol/L)	5.07 (4.21–6.20)	5.18 (4.34–6.12)	0.375
Creatinine (μmol/L)	62.7 (54.0–72.5)	68.1 (54.3–78.3)	0.020
Uric acid (μmol/L)	305.2 (250.2–369.9)	377.7 (314.6–438.5)	<0.001
Potassium (mmol/L)	3.95 (3.69–4.14)	3.92 (3.72–4.18)	0.895
Sodium (mmol/L)	141.6 (139.8–143.0)	141.0 (139.4–142.7)	0.035
Chloride (mmol/L)	105.1 (103.7–106.8)	104.7 (101.8–106.2)	0.001
Diamine oxidase (U/L)	9.70 (7.53–12.21)	15.00 (11.02–20.81)	<0.001
D-lactate (mg/L)	13.72 (7.88–17.15)	23.16 (17.87–35.37)	<0.001
Lipopolysaccharide (U/L)	13.20 (5.69–15.77)	14.04 (9.105–17.16)	0.006

Qualitative variables were reported as frequency (percentage). Quantitative variables were presented median (interquartile range). The Wilcoxon–Mann–Whitney test or the chi-squared test was utilized to compare data between patients without and with MAFLD.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; FPG, fasting plasma glucose; GGT, γ-glutamyl transpeptidase; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); MAFLD, metabolic-associated fatty liver disease; NEFA, nonesterified fatty acid; TBA, total bile acid.

Table 2. Factors associated with metabolic-associated fatty liver disease using binaryl logistic regression analysis

	Univariate		Multivariable	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Sex	1.938 (1.344–2.793)	<0.001		
BMI (kg/m^2)	1.561 (1.432–1.702)	<0.001	1.324 (1.156–1.517)	<0.001
Type 2 diabetes mellitus	4.382 (2.020–9.505)	<0.001		
Hypertension	2.064 (1.339–3.183)	0.001		
Alcohol intake	1.913 (0.985–3.716)	0.056		
Smoke	0.362 (0.212–0.061)	<0.001		
White blood cell count (10^9/L)	1.623 (1.412–1.866)	<0.001		
Red blood cell count (×10^{12}/L)	2.483 (1.733–3.558)	<0.001		
Hemoglobin (g/L)	1.029 (1.018–1.041)	<0.001		
Platelet count (×10^9/L)	1.005 (1.001–1.008)	0.006		
ALT (U/L)	1.089 (1.067–1.111)	<0.001		
AST (U/L)	1.096 (1.067–1.126)	<0.001		
GGT (U/L)	1.053 (1.038–1.068)	<0.001		
ALP (U/L)	1.012 (1.005–1.020)	0.007		
Total protein (g/L)	1.026 (1.001–1.051)	0.043		
Globulin (g/L)	1.067 (1.023–1.113)	0.002		
FPG (mmol/L)	1.008 (0.986–1.031)	0.486		
Triglycerides (mg/dL)	7.004 (4.649–10.552)	<0.001	2.661 (1.437–4.931)	0.002
Total cholesterol (mg/dL)	1.861 (1.527–2.267)	<0.001		
HDL (mg/dL)	0.057 (0.028–0.113)	<0.001		
LDL (mg/dL)	1.413 (1.140–1.750)	0.002		
NEFA (μmol/L)	1.002 (1.001–1.002)	<0.001	1.002 (1.000–1.004)	0.011
UA (μmol/L)	1.008 (1.005–1.010)	<0.001		
Creatinine (μmol/L)	1.011 (1.001–1.022)	0.040		
Diamine oxidase (U/L)	1.190 (1.149–1.233)	<0.001	1.149 (1.055–1.251)	0.001
D-lactate (mg/L)	1.171 (1.130–1.213)	<0.001	1.221 (1.139–1.308)	<0.001
Lipopolysaccharide (U/L)	1.052 (1.024–1.081)	0.004		
Sodium (mmol/L)	0.899 (0.845–0.956)	0.001		

Data are expressed as odds ratio and 95% confidence interval.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; FPG, fasting plasma glucose; GGT, γ-glutamyl transpeptidase; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); NEFA, nonestesterified fatty acid; TBA, total bile acid; UA, uric acid.

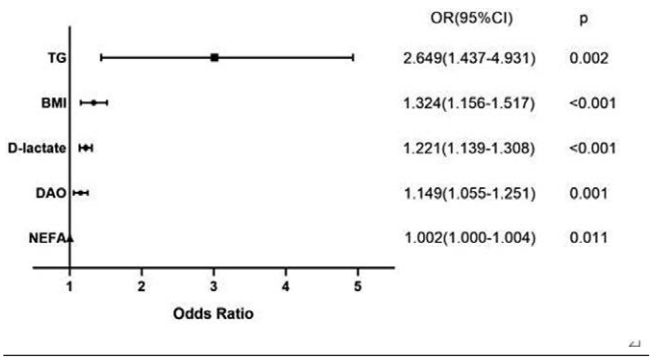

Fig. 1. Forest plot of serum intestinal barrier biomarkers associated with MAFLD risk. MAFLD, metabolic-associated fatty liver disease.

steatosis group ($P=0.005$) and the severe steatosis group ($P=0.009$) than in the mild steatosis group ($P=0.002$). The serum D-lactate level was also higher in the moderate steatosis group ($P<0.001$) and severe steatosis group ($P=0.003$) than in the mild steatosis group. Finally, the serum level of lipopolysaccharide was significantly higher in the moderate steatosis group than in the mild steatosis group ($P=0.022$).

Correlations between laboratory parameters and serum levels of intestinal barrier biomarkers

We analysed the correlations between the three biomarkers and laboratory parameters to verify the association between intestinal barrier impairment and MAFLD. The serum level of diamine oxidase was positively correlated with ALT and GGT ($r=0.303$, $P<0.0001$; $r=0.266$; $P<0.001$; Fig. 3a and b) levels. Serum ALT, GGT, total cholesterol and triglycerides levels were positively correlated with D-lactate levels ($r=0.366$, $P<0.001$; $r=0.348$, $P=0.031$; $r=0.238$, $P<0.001$; $r=0.373$, $P<0.001$; Fig. 3c–f). Furthermore, no association was found between serum lipopolysaccharide levels and laboratory parameters.

Increased serum lipopolysaccharide levels in metabolic-associated fatty liver disease patients with multiple metabolic abnormalities

According to the increase in the number of metabolic abnormalities, patients were classified into two groups: <2 metabolic abnormalities and ≥2 metabolic abnormalities [15]. The serum levels of D-lactate, diamine oxidase and lipopolysaccharide in patients with different numbers of metabolic abnormalities are shown in Table 3. The serum level of lipopolysaccharide was higher in MAFLD patients with ≥2 metabolic abnormalities than in those with <2 metabolic abnormalities ($P=0.034$). However, there was no significant difference in the serum levels of diamine oxidase and D-lactate in patients with different numbers of metabolic abnormalities.

Discussion

Our research illustrates that MAFLD patients had significantly higher serum levels of diamine oxidase, D-lactate and lipopolysaccharide than those without MAFLD, indicating the association between MAFLD and intestinal barrier dysfunction and translocation of gut bacterial

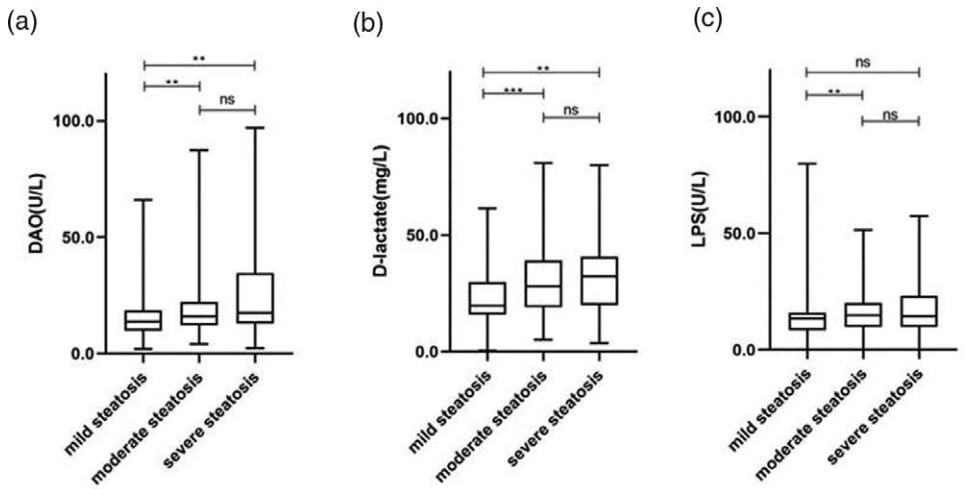

Fig. 2. Serum levels of intestinal barrier biomarkers in different grades of liver fatty infiltration. Comparison of serum levels of diamine oxidase (a), D-lactate (b), and lipopolysaccharide (c). *$P<0.05$; **$P<0.01$; ***$P<0.001$; 'ns' indicates not significant.

metabolites. The serum diamine oxidase, D-lactate and lipopolysaccharide levels increased as the degree of fat infiltration detected by ultrasonography increased. Diamine oxidase and D-lactate are independent risk factors for MAFLD and might be used to improve diagnosis, prevention and identification of potential therapeutic targets for impaired intestinal barrier function in patients with MAFLD. Moreover, our results suggested that diamine oxidase, D-lactate and lipopolysaccharide were related to multiple metabolic abnormalities in MAFLD patients.

Recent studies have evaluated intestinal barrier integrity with several biomarkers, such as 51Cr-ethylene diamine tetraacetate (51Cr-EDTA) and zona occludens-1 (ZO-1) [16]. However, detection of the urinary excretion of 51Cr-EDTA is time-consuming, and duodenal biopsy to obtain specimens for immunohistochemical expression is not easy to perform for the majority of patients with MAFLD [17]. Serum diamine oxidase, D-lactate and lipopolysaccharide are more convenient and better-accepted biomarkers for intestinal barrier impairment [6,9]. Diamine oxidase, a type of oxidative deaminase, is especially active in the intestinal mucosa. Normally, there is a small amount of diamine oxidase in circulation, and its plasma levels are positively correlated with intestinal barrier impairment [18]. D-lactate is produced mostly by intestinal bacteria through the glycolysis pathway, and humans express only L-lactate dehydrogenase but not D-lactate dehydrogenase [19]. D-lactate is released into the circulation when the intestinal barrier is damaged and intestinal mucosal permeability is increased. Therefore, an increased serum level of D-lactate is usually associated with abnormal intestinal permeability [20]. Lipopolysaccharide, a component of the cell wall of gram-negative bacteria, is transferred into the blood when there is gut microbiota dysbiosis and altered intestinal barrier permeability [21]. These three serum biomarkers are used to evaluate intestinal barrier permeability and assess the efficacy of gastrointestinal disease treatment in clinical practice [22]. Hence, it was reasonable to select serum biomarkers to conduct the present study.

In our present study, ultrasonography was used for the diagnosis and qualitative assessment of liver steatosis. We discovered that circulating diamine oxidase, D-lactate and lipopolysaccharide levels showed a corresponding increase with the severity of steatosis, implying that increased intestinal barrier permeability may be linked to deterioration of MAFLD. In addition, our analysis of clinical characteristics showed that elevated diamine oxidase and D-lactate levels are related to ALT and GGT concentrations, while serum D-lactate is correlated with total cholesterol and triglycerides. Several mechanisms could explain why patients with MAFLD have a higher serum level of D-lactate. Recent evidence has shown that microbial D-lactate can promote Kupffer cells to catch and eliminate enterogenic flora from the bloodstream in the portal vein [23]. Activated Kupffer cells play a crucial role in the progression of NAFLD/NASH, as they can cause inflammation and regulate the lipid metabolism of liver cells by secreting cytokines [24]. An elevated level of D-lactate may be related to metabolic dysregulation and may take part in lipid metabolism by activating liver-resident Kupffer cells. On the other hand, metabolites of the intestinal microbiome, including butyrate, bile acids and lipopolysaccharide, have multiple effects on liver cells or macrophages, leading to increased cytokine release and hepatic steatosis [25–27]. Recent evidence has also shown that dysregulation of bile acid homeostasis caused by the gut microbiome is associated with NAFLD severity [26,28]. These studies suggested that metabolites of the intestinal microbiome could have a mutual and complex role in the development of MAFLD and be associated with a worse metabolic state in patients with MAFLD. More research and analyses on gut flora products are required to identify the pathogenesis of and develop treatments for MAFLD.

Intestinal barrier impairment could be caused by many factors, including diet, alcohol intake, medication and dysbiosis of the gut microbiome [29,30]. Accumulating evidence has shown that excessive food intake is significantly associated with alterations in the intestinal barrier [31]. Both a high-fat diet and a fructose-rich diet contribute to dysbiosis of the gut microbiome and increased intestinal permeability [32,33]. Alteration of the gut microbiome has been discovered in patients with metabolic dysregulation. Diminished abundances of *Ruminococcaceae*, *Fusobacterium*, *Bifidobacterium*, *Faecalibacterium*

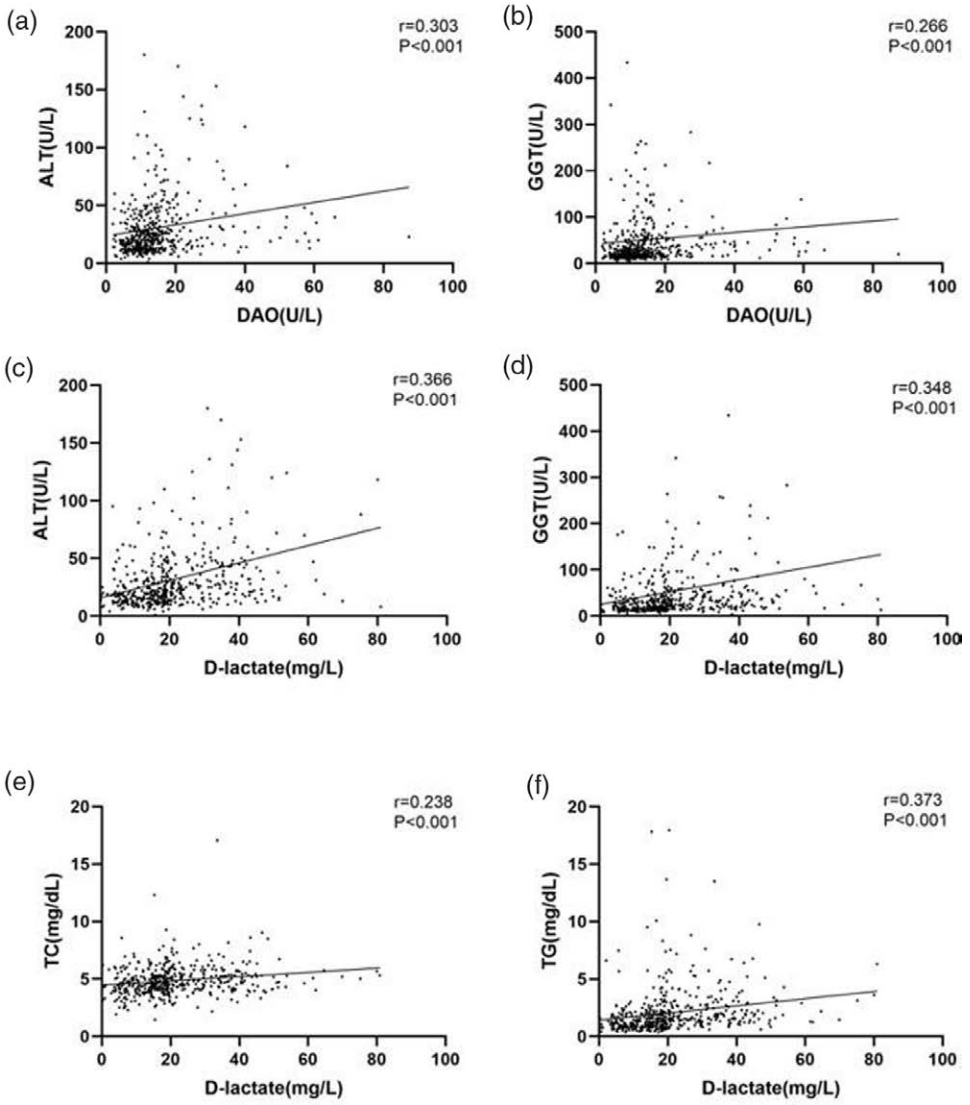

Fig. 3. Correlation between serum levels of intestinal barrier biomarkers and laboratory parameters. (a) Correlation of serum ALT with diamine oxidase; (b) Correlation of serum GGT with diamine oxidase; (c) Correlation of serum ALT with D-lactate; (d) Correlation of GGT with D-lactate; (e) Correlation of serum total cholesterol with D-lactate; and (f) Correlation of serum triglycerides with D-lactate; ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase.

Table 3. Comparisons of serum levels of diamine oxidase, D-lactate and lipopolysaccharide between metabolic-associated fatty liver disease patients with <2 metabolic abnormalities and those with ≥2 metabolic abnormalities

	MAFLD patients with <2 metabolic abnormalities ($n=151$)	MAFLD patients with ≥2 metabolic abnormalities ($n=143$)	*P* value
Diamine oxidase (U/L)	14.30 (10.34–20.22)	15.19 (11.76–20.84)	0.420
D-lactate (mg/L)	23.36 (17.20–34.87)	23.15 (18.53–35.780)	0.323
Lipopolysaccharide (U/L)	13.40 (8.14–16.27)	14.61 (10.02–17.60)	0.034

Quantitative variables were presented median (interquartile range). The Wilcoxon–Mann–Whitney test was utilized to compare data between MAFLD patients with <2 metabolic abnormalities and those with ≥2 metabolic abnormalities.
MAFLD, metabolic-associated fatty liver disease.

prausnitzii and *Bacteroidetes* and higher abundances of *Enterobacteriaceae*, *Porphyromas* and *Fusobacterium* have been detected in patients with NAFLD [34,35]. Xia *et al*. found that *Lactobacillus*, *Bifidobacterium* and *Clostridium cluster I* negatively correlated with D-lactate and endotoxin, while *Lactobacillus* negatively correlated with diamine oxidase. Moreover, *Fusobacterium nucleatum* and *Enterobacteriaceae* correlated positively with D-lactate, diamine oxidase and endotoxin [36]. Hence, intestinal dysbacteriosis may increase the risk of MAFLD as a result of increased intestinal barrier permeability.

Serum lipopolysaccharide elevation in NALFD/NASH has been reported in several studies [37]. Alteration of intestinal flora and increased intestinal mucosal permeability lead to a higher level of lipopolysaccharide in circulation and favour a proinflammatory state [38]. Lipopolysaccharide can stimulate TLR4 expression in the membranes of hepatocytes and Kupffer cells, leading to the secretion of inflammatory cytokines [39]. In the subgroup study, patients with MAFLD were classified into two groups, those with <2 metabolic abnormalities and those with ≥2 metabolic abnormalities, and serum levels of intestinal barrier biomarkers were compared. The serum lipopolysaccharide levels were higher in MAFLD patients with multiple metabolic abnormalities, indicating

that endotoxin translocation was related to complex metabolic disorders in MAFLD. On the other hand, low or normal lipopolysaccharide levels were observed in MAFLD patients who had endotoxin hyperresponsiveness, resulting in a state similar to that of elevated lipopolysaccharide levels in this population [37]. The status of endotoxin hyperresponsiveness can also explain the normal lipopolysaccharide levels of some MAFLD patients in this study. There is an interaction between metabolic dysregulation and intestinal barrier function. Disrupted intestinal homeostasis can lead to metabolic abnormalities and subsequent metabolic illnesses, such as hypertension, T2DM and MAFLD [40]. In return, intestinal epithelial integrity is affected by hyperglycaemia and hyperlipemia, causing an abnormal influx of gut microbial products into the circulation and chronic inflammation [41]. Thus, the restoration of intestinal barrier homeostasis and metabolic regulation will require more attention in future studies and therapy.

While these results are novel, there are several limitations to our current investigation. First, our data were gathered retrospectively from a single centre. More participants need to be enrolled for us to create subgroups and confirm our conclusion. Second, more biomarkers are needed in the evaluation of intestinal barrier impairment and translocation of bacterial metabolites. Finally, the cause of intestinal barrier impairment was not clear in our study, and the association of MAFLD with the intestinal microbiome and their related metabolites requires further studies *in vivo* and *in vitro*.

In summary, our study was designed to verify the potential risk factors based on the association between MAFLD and increased intestinal barrier permeability. Overweight/obesity and higher plasma levels of diamine oxidase, D-lactate, triglycerides and NEFA were associated with a higher risk of MAFLD. Additionally, the study suggested that the translocation of endotoxins and damage to the intestinal barrier were related to multiple metabolic abnormalities. More research *in vitro* is required to confirm the potential mechanism underlying the increase in intestinal barrier permeability, the gut microbiota and their products in MAFLD.

Acknowledgements

The authors thank all staff in the Department of Gastroenterology, Zhongnan Hospital of Wuhan University (Wuhan, China) and Hubei Clinical Center and Key Laboratory of Intestinal and Colorectal Diseases (Wuhan, China) for their technical support.

This work was supported by a research grant from the National Natural Science Foundation of China (JL, grant no. 81472735); Wuhan University (JL, 2042019kf0206); and the National Basic Research Program of China (973 program, 2015CB932600).

R.Z., Y.-n.C. and J.Z. designed the study and carried out the data collection. J.L. and Q.Z. designed the study. All authors wrote the article, read and approved the final article.

All datasets generated for this study are included in the article/Supplementary Material.

This study was performed in accordance with the Declaration of Helsinki and with the approval of the Ethical Committee of the Zhongnan Hospital of Wuhan University.

Conflicts of interest

There are no conflicts of interest.

References

1 Eslam M, Sanyal AJ, George J; International Consensus Panel. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. *Gastroenterology* 2020; 158:1999–2014.e1.
2 Albillos A, de Gottardi A, Rescigno M. The gut-liver axis in liver disease: pathophysiological basis for therapy. *J Hepatol* 2020; 72:558–577.
3 Szabo G. Gut-liver axis in alcoholic liver disease. *Gastroenterology* 2015; 148:30–36.
4 An L, Wirth U, Koch D, Schirren M, Drefs M, Koliogiannis D, *et al*. The role of gut-derived lipopolysaccharides and the intestinal barrier in fatty liver diseases. *J Gastrointest Surg* 2021; 26:671–683.
5 Tripathi A, Debelius J, Brenner DA, Karin M, Loomba R, Schnabl B, *et al*. The gut-liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol* 2018; 15:397–411.
6 Luk GD, Bayless TM, Baylin SB. Diamine oxidase (histaminase) – a circulating marker for rat intestinal mucosal maturation and integrity. *J Clin Investig* 1980; 66:66–70.
7 Zhou X, Li J, Guo JL, Geng B, Ji W, Zhao Q, *et al*. Gut-dependent microbial translocation induces inflammation and cardiovascular events after ST-elevation myocardial infarction. *Microbiome* 2018; 6:66.
8 Tulkens J, Vergauwen G, Van Deun J, Geeurickx E, Dhondt B, Lippens L, *et al*. Increased levels of systemic LPS-positive bacterial extracellular vesicles in patients with intestinal barrier dysfunction. *Gut* 2020; 69:191–193.
9 Kong WC, Wang J, Ping XC, Shen J, Ni X, Liu F, *et al*. Biomarkers for assessing mucosal barrier dysfunction induced by chemotherapy: identifying a rapid and simple biomarker. *Clin Lab* 2015; 61:371–378.
10 Camara-Lemarroy CR, Silva C, Greenfield J, Liu WQ, Metz LM, Yong VW. Biomarkers of intestinal barrier function in multiple sclerosis are associated with disease activity. *Mult Scler J* 2020; 26:1340–1350.
11 Cosentino F, Grant PJ, Aboyans V, Bailey CJ, Ceriello A, Delgado V, *et al*. 2019 ESC guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. *Eur Heart J* 2020; 41:255–323.
12 Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, *et al*. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *Eur Heart J* 2020; 41:111–188.
13 EASL., EASD., EASO. EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. *Obesity Facts* 2016; 9:65–90.
14 Charatcharoenwitthaya P, Lindor KD. Role of radiologic modalities in the management of non-alcoholic steatohepatitis. *Clin Liver Dis* 2007; 11:37–54.
15 Yamamura S, Eslam M, Kawaguchi T, Tsutsumi T, Nakano D, Yoshinaga S, *et al*. MAFLD identifies patients with significant hepatic fibrosis better than NAFLD. *Liver Int* 2020; 40:3018–3030.
16 Miele L, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, *et al*. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 2009; 49:1877–1887.
17 Jenkins RT, Lock CJL, Rooney PJ. CR-51-EDTA in studies of intestinal permeability. *Lancet* 1984; 2:1342.
18 Wolvekamp MCJ, Debruin RWF. Diamine oxidase - an overview of historical, biochemical and functional-aspects. *Dig Dis* 1994; 12:2–14.
19 Smith SM, Eng RHK, Buccini F. Use of D-lactic acid measurements in the diagnosis of bacterial-infections. *J Infect Dis* 1986; 154:658–664.
20 Nielsen C, Mortensen FV, Erlandsen EJ, Lindholt JS. L- and D-lactate as biomarkers of arterial-induced intestinal ischemia: an experimental study in pigs. *Int J Surg* 2012; 10:296–300.
21 Stevens BR, Goel R, Seungbum K, Richards EM, Holbert RC, Pepine CJ, Raizada MK. Increased human intestinal barrier permeability plasma biomarkers zonulin and FABP2 correlated with plasma LPS

and altered gut microbiome in anxiety or depression. *Gut* 2018; 67:1555–1557.
22 Geng S-T, Zhang J-B, Wang Y-X, Xu Y, Lu D, Zhang Z, *et al*. Predigested protein enteral nutritional supplementation enhances recovery of CD4(+) T cells and repair of intestinal barrier in HIV-infected immunological non-responders. *Front Immunol* 2021; 12:757935.
23 McDonald B, Zucoloto AZ, Yu IL, Burkhard R, Brown K, Geuking MB, McCoy KD. Programing of an intravascular immune firewall by the gut microbiota protects against pathogen dissemination during infection. *Cell Host Microbe* 2020; 28:660–668.e4.
24 Li HO, Zhou YJ, Wang HZ, Zhang M, Qiu P, Zhang M, *et al*. Crosstalk between liver macrophages and surrounding cells in nonalcoholic steatohepatitis. *Front Immunol* 2020; 11:1169.
25 Zhao ZH, Wang ZX, Zhou D, Han Y, Ma F, Hu Z, *et al*. Sodium butyrate supplementation inhibits hepatic steatosis by stimulating liver kinase B1 and insulin-induced gene. *Cell Mol Gastroenterol Hepatol* 2021; 12:857–871.
26 Puri P, Daita K, Joyce A, Mirshahi F, Santhekadur PK, Cazanave S, *et al*. The presence and severity of nonalcoholic steatohepatitis is associated with specific changes in circulating bile acids. *Hepatology* 2018; 67:534–548.
27 Carpino G, Del Ben M, Pastori D, Carnevale R, Baratta F, Overi D, *et al*. Increased liver localization of lipopolysaccharides in human and experimental NAFLD. *Hepatology* 2020; 72:470–485.
28 Xue R, Su LY, Lai SY, Wang Y, Zhao D, Fan J, *et al*. Bile acid receptors and the gut-liver axis in nonalcoholic fatty liver disease. *Cells* 2021; 10:2806.
29 Mullin JM, Valenzano MC, Verrecchio JJ, Kothari R. Age- and diet-related increase in transepithelial colon permeability of Fischer 344 rats. *Dig Dis Sci* 2002; 47:2262–2270.
30 Jennison E, Byrne CD. The role of the gut microbiome and diet in the pathogenesis of non-alcoholic fatty liver disease. *Clin Mol Hepatology* 2021; 27:22–43.
31 Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 2008; 57:1470–1481.
32 Nier A, Brandt A, Rajcic D, Bruns T, Bergheim I. Short-term isocaloric intake of a fructose- but not glucose-rich diet affects bacterial endotoxin concentrations and markers of metabolic health in normal weight healthy subjects. *Mol Nutr Food Res* 2019; 63:e1800868.
33 Chopyk DM, Grakoui A. Contribution of the intestinal microbiome and gut barrier to hepatic disorders. *Gastroenterology* 2020; 159:849–863.
34 Aron-Wisnewsky J, Vigliotti C, Witjes J, Le P, Holleboom AG, Verheij J, *et al*. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat Rev Gastroenterol Hepatol* 2020; 17:279–297.
35 Loomba R, Seguritan V, Li WZ, Long T, Klitgord N, Bhatt A, *et al*. Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. *Cell Metab* 2017; 25:1054–1062.e5.
36 Liu X, Cheng YW, Shao L, Ling ZX. Alterations of the predominant fecal microbiota and disruption of the gut mucosal barrier in patients with early-stage colorectal cancer. *Biomed Res Int* 2020; 2020:2948282.
37 Kessoku T, Kobayashi T, Imajo K, Tanaka K, Yamamoto A, Takahashi K, *et al*. Endotoxins and non-alcoholic fatty liver disease. *Front Endocrinol* 2021; 12:770986.
38 Salazar J, Angarita L, Morillo V, Navarro C, Martínez MS, Chacín M, *et al*. Microbiota and diabetes mellitus: role of lipid mediators. *Nutrients* 2020; 12:3039.
39 O'Neill LAJ, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nat Rev Immunol* 2007; 7:353–364.
40 Tilg H, Zmora N, Adolph TE, Elinav E. The intestinal microbiota fuelling metabolic inflammation. *Nat Rev Immunol* 2020; 20:40–54.
41 Thaiss CA, Levy M, Grosheva I, Zheng D, Soffer E, Blacher E, *et al*. Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection. *Science* 2018; 359:1376–1383.