Original Article

Autotaxin: An Early Warning Biomarker for Acute-on-chronic Liver Failure

Caiyun Nie[1,2], Lei Zhang[3], Xiaobing Chen[1,2], Ying Li[4,5,6,7,8,9], Fushuang Ha[5,6,7,8,9], Hua Liu[4,5,6,7,8,9] and Tao Han*[4,5,6,7,8,9]

[1]*Department of Oncology, The Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, Henan, China;* [2]*Henan Cancer Hospital, Zhengzhou, Henan, China;* [3]*Department of Clinical Laboratory, Tianjin Third Central Hospital, Tianjin, China;* [4]*The Third Central Clinical College of Tianjin Medical University, Tianjin, China;* [5]*The Affiliated Hospital of Nankai University, Tianjin, China;* [6]*Department of Hepatology and Gastroenterology, Tianjin Third Central Hospital, Tianjin, China;* [7]*Tianjin Key Laboratory of Extracorporeal Life Support for Critical Diseases, Tianjin, China;* [8]*Artificial Cell Engineering Technology Research Center, Tianjin, China;* [9]*Tianjin Institute of Hepatobiliary Disease, Tianjin, China*

Abstract

Background and Aims: Recent accumulating evidence indicates the biological actions of autotaxin (ATX) in liver disease. However, the relationship between ATX and liver failure has not been reported. The present study aimed to examine alterations of serum ATX in acute-on-chronic liver failure (ACLF) and evaluate whether serum ATX could be useful as an early warning biomarker of ACLF. ***Methods:*** Serum ATX was measured in 50 patients with hepatitis B-related ACLF, 14 patients with alcohol-related ACLF, 11 patients with hepatitis B-related pre-ACLF, 11 patients with alcohol-related Child-Pugh A cirrhosis, 39 patients with hepatitis B-related Child-Pugh A cirrhosis, 26 patients with chronic hepatitis B, and 38 healthy volunteers by enzyme-linked immunosorbent assay. ***Results:*** Serum ATX level was significantly higher in the pre-ACLF group than in the Child-Pugh A cirrhosis and chronic hepatitis B groups but lower than in the ACLF group; furthermore, patients with pre-ACLF deteriorated to ACLF had significantly higher serum ATX levels than pre-ACLF patients that did not progress to ACLF. Serum ATX levels were significantly higher among male ACLF patients with preclinical infection, spontaneous bacterial peritonitis or pneumonia, as compared to patients with ACLF but no spontaneous bacterial peritonitis or pneumonia. Serum ATX levels were well correlated with serum biochemical parameters of liver function and model for end-stage liver disease score. Serum ATX $\geq$ 584.1 ng/mL was a poor prognostic factor for ACLF (hazard ratio of 4.750, 95% confidence interval of 1.106-20.392, p=0.036). ***Conclusions:*** Serum ATX level may be a useful early warning biomarker for ACLF.

Citation of this article: Nie C, Zhang L, Chen X, Li Y, Ha F, Liu H, *et al.* Autotaxin: An early warning biomarker for acute-on-chronic liver failure. J Clin Transl Hepatol 2020;8(3):240–245. doi: 10.14218/JCTH.2020.00045.

Introduction

Acute-on-chronic liver failure (ACLF) encompasses a group of clinical syndromes based on chronic liver disease, acute intrahepatic and/or extrahepatic injury as the inducement, and accompanied by multiple organ failure and early high mortality.[1,2] Although liver transplantation is considered to be the most effective treatment for ACLF, donor organ shortages pose a major obstacle. The prognosis of ACLF depends largely on early detection, as timely intervention can prevent or reverse the process and improve survival. The early warning indicators of ACLF are currently lacking in clinical practice, and the specificity and sensitivity of traditional end-stage liver disease model scores, including the model for end-stage liver disease (MELD) and MELD-Na, and Child-Pugh scores can hardly meet the clinical needs of prognostic assessment.

In recent years, the concept of acute-on-chronic pre-liver failure (pre-ACLF) was proposed.[3–5] Patients with pre-ACLF have high risk of occurrence of ACLF and poor prognosis, and should receive standard medical care for ACLF as soon as possible after diagnosis. The definition of pre-ACLF broadens the concept of ACLF, and research on pre-ACLF contributes to understanding the pathogenesis of ACLF, as it includes searches for early warning biomarkers and intervention strategies for ACLF.

Recent accumulating evidence indicates the biological actions of autotaxin (ATX) in liver disease.[6–9] Previous research suggests that ATX is a valuable biomarker for the prediction of liver fibrosis, and serum ATX is closely related to severity and overall survival for cases of liver injury.[10,11] However, the relationship between ATX level and liver failure has not been reported; in the present study, we examined the alterations of serum ATX in the serum of patients with ACLF, pre-ACLF, Child-Pugh A cirrhosis, and chronic hepatitis B (CHB), and in healthy controls, to explore whether serum ATX level could be useful as an early warning biomarker of ACLF.

Keywords: Autotaxin (ATX); Liver failure; Serum; Biomarker.

Abbreviations: ACLF, acute-on-chronic liver failure; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATX, autotaxin; CHB, chronic hepatitis B; Cr, creatinine; GGT, gamma-glutamyl endopeptidase; INR, international normalized ratio; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; lysoPLD, lysophospholipase D; MELD, model for end-stage liver disease; pre-ACLF, acute-on-chronic pre liver failure; PT, prothrombin time; SBP, spontaneous bacterial peritonitis; TBA, total bile acid; TBIL, total bilirubin.

Received: 12 May 2020; Revised: 9 June 2020; Accepted: 30 June 2020

***Correspondence to:** Tao Han, Department of Hepatology and Gastroenterology, Tianjin Institute of Hepatobiliary Disease, Tianjin Key Laboratory of Artificial Cells, Tianjin Third Central Hospital, 83 Jintang Road, Tianjin 300170, China. Tel: +86-22-84112298, Fax: +86-22-84112208, E-mail: hantaomedical@126.com

Methods

Patients

Study population: The study included 50 patients with hepatitis B-related ACLF (males/females: 39/11), 14 patients with alcohol-related ACLF (males: 14), 11 patients with hepatitis B-related pre-ACLF (males: 11), 11 patients with alcoholic Child-Pugh A cirrhosis (males: 11), 39 patients with hepatitis B-related Child-Pugh A cirrhosis (LC) (males/females: 28/11), 26 patients with chronic hepatitis B (males/females: 16/10), and 38 healthy volunteers (HS) (males/females: 25/13). All samples were obtained from in-patients and out-patients who attended the Tianjin Third Central Hospital in Tianjin, China. Clinical data are shown in Table 1 and Table 2.

Ethical approval

This study was carried out in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethics committee of the Tianjin Third Central Hospital. Informed consent was obtained from the patients for use of their serum samples.

Disease definition

ACLF was defined as an acute hepatic insult manifesting as jaundice (serum bilirubin ≥5 mg/dL) and coagulopathy [international normalized ratio (INR) ≥1.5 or prothrombin activity <40%] complicated within 4 weeks by clinical ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease/cirrhosis. It is associated with a high 28-day mortality.[1]

The definition for pre-ACLF included the following features: (1) acute worsening of chronic liver disease; (2) extreme fatigue with severe digestive symptoms, such as obvious anorexia, abdominal distension, nausea and vomiting; (3) progressively worsening jaundice within a short period, with 5 mg/dL≤serum total bilirubin (TBIL)<10 mg/dL or a daily elevation of ≥1 mg/dL; (4) 1.28≤INR<1.50 or 40%<prothrombin activity (PTA)≤60%.[3–5]

Exclusion criteria were: 1) coinfection with hepatitis C virus, hepatitis D virus, hepatitis E virus, and/or human immunodeficiency virus; 2) autoimmune liver disease; 3) metabolic diseases, such as diabetes, hyperthyroidism, or hypothyroidism; or 4) any type of cancer.

Sample collection

All serum samples were collected in the morning. One hour after collection, the anticoagulant-free blood was centrifuged at 1600×*g* for 10 m at 4°C, then transferred to a test tube and kept at -80°C for further experiments. For all enrolled patients, it is difficult to ensure that the enrollment date always coincides with the onset time of the disease. However, in the present study, all serum samples were collected at the 1st day since their first admission in our hospital.

Measurement of serum ATX

Human serum ATX was measured by the commercially available "Human ENPP-2/Autotaxin Quantikine Enzyme-Linked Immunosorbent Assay Kit" (R&D Systems Inc., Minneapolis, MN, USA). Serum ATX levels were higher among females than among males; therefore, the serum ATX was evaluated separately in men and women.

Measurement of serum biochemical parameters

Serum biochemical parameters were tested in clinical laboratory and included creatinine (Cr), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl endopeptidase (GGT), TBIL, total bile acid (TBA), INR, and prothrombin time (PT). MELD scores were calculated on admission.

Table 1. Clinical characteristics of male subjects with liver disease and healthy controls

	Hepatitis B-related liver disease				Alcohol-related liver disease		Healthy controls
	CHB, *n*=16	LC, *n*=28	pre-ACLF, *n*=11	ACLF, *n*=39	LC, *n*=11	ACLF, *n*=14	*n*=25
ALB (g/L)	49.33±3.19	37.48±4.42	33.28±3.25	30.14±4.16	36.85±4.72	27.13±3.68	46.84±4.54
ALT (U/L)	47.92±38.20	35.66±28.49	841.69±624.22	423.08±372.66	29.73±10.54	56.21±47.95	22.29±10.33
AST (U/L)	13.72±10.35	37.31±31.88	573.92±444.10	381.47±290.40	31.09±10.34	116.57±57.47	18.88±6.39
ALP (U/L)	75.42±46.18	71.22±17.85	146.22±45.14	132.10±49.14	91.18±38.21	105.43±27.85	59.19±12.82
GGT (U/L)	32.11±28.31	58.78±57.30	131.08±95.12	93.06±60.02	87.27±33.54	175.21±156.19	30.50±17.30
TBIL (μmol/L)	13.33±5.67	20.18±12.95	135.85±36.40	275.04±114.38	19.95±6.35	341.41±143.41	12.25±4.33
TBA (μmol/L)	–	10.36±12.82	261.41±83.59	272.83±135.70	10.20±8.56	203.16±108.98	–
INR	–	1.20±0.12	1.38±0.10	2.51±0.99	1.13±0.06	2.72±1.78	–
Cr (μmol/L)	74.81±9.68	62.34±10.71	62.33±13.74	68.40±25.73	61.82±10.75	101.43±77.02	71.65±15.57
MELD	–	4.97±2.62	14.15±3.47	23.37±5.58	4.83±2.88	25.15±10.12	–

Abbreviations: CHB, chronic hepatitis B; LC, liver cirrhosis; pre-ACLF, acute on chronic pre-liver failure; ACLF, acute-on-chronic liver failure; ALB, albumin, ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TBIL, total bilirubin; TBA, total bile acid; INR, international normalized ratio; Cr, creatinine; MELD, model for end-stage liver disease.

Table 2. Clinical characteristics of female subjects with liver disease and healthy controls

	CHB, *n*=10	LC, *n*=11	ACLF, *n*=11	HS, *n*=13
ALB in g/L	47.45±2.23	37.82±3.89	29.19±2.25	44.82±2.17
ALT in U/L	54.80±46.34	37.10±11.54	422.18±331.96	17.00±6.49
AST in U/L	12.30±7.67	41.91±21.43	400.27±253.77	15.08±4.13
ALP in U/L	78.30±63.13	72.00±8.75	125.18±47.13	51.85±10.20
GGT in U/L	38.50±67.05	38.82±13.39	57.55±50.53	22.62±8.31
TBIL in μmol/L	13.21±5.89	20.33±6.97	217.65±33.15	11.83±3.84
TBA in μmol/L	–		251.55±140.19	–
INR	–	1.11±0.05	2.50±1.05	–
Cr in μmol/L	57.10±8.37	57.27±8.22	58.64±18.00	53.54±9.58
MELD	–	3.74±1.43	21.28±5.03	–

Abbreviations: CHB, chronic hepatitis B; LC, liver cirrhosis; ACLF, acute-on-chronic liver failure; HS, healthy controls; ALB, albumin, ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TBIL, total bilirubin; TBA, total bile acid; INR, international normalized ratio; Cr, creatinine; MELD, model for end-stage liver disease.

Statistical analysis

All data were expressed as the mean±standard deviation (SD). Correlations between serum ATX levels and clinical biochemical parameters were obtained using linear regression analysis. The statistical significance of differences between two groups was determined by Mann-Whitney *U* test. Kruskal-Wallis test was used to evaluate the statistical significance of differences of the variances for three or more groups. The cut-off value of serum ATX for predicting the ACLF patients' mortality was determined by receiver operating characteristic analysis. Logistic regression was employed to analyze the risk factors of prognosis. All the statistical analyses were performed with SPSS 17.0 (SPSS, Chicago, IL, USA) software. A p-value of <0.05 was considered to be statistically significant.

Results

Serum ATX levels in male patients with hepatitis B-related liver disease and healthy controls

Serum ATX levels were measured in 39 male patients with hepatitis B-related ACLF, 11 male patients with hepatitis B-related pre-ACLF, 28 male patients with LC, 16 male patients with CHB, and 25 male HS. The serum ATX levels of ACLF, pre-ACLF, LC, CHB and healthy control groups were found to be 662.39±142.25, 539.74±158.92, 362.45±62.75, 257.39 ±58.36, and 182.31±40.02 ng/mL respectively (Fig. 1A).

Dynamic tracking analysis demonstrated that among the 11 male cases of pre-ACLF, 5 cases deteriorated to ACLF, while the other 6 gradually restored and did not progress to ACLF. Patients of pre-ACLF deteriorated to ACLF showed significantly higher serum ATX levels than patients that did not progress to ACLF (641.9 vs. 454.6 ng/mL, p<0.01).

Serum ATX levels in female patients with hepatitis B-related liver disease and healthy controls

Serum ATX levels were measured in 11 female hepatitis B-related patients with ACLF, 11 female patients with LC, 10 female patients with CHB, and 13 female HS. For female patients, the alterations of serum ATX levels in hepatitis B-related cirrhosis and ACLF were similar to those in male patients. The mean serum ATX levels in ACLF, LC, CHB and healthy control groups were 702.52±199.22, 453.66±65.55, 342.27±104.48 and 229.83±54.63 ng/mL respectively (Fig. 1B).

Serum ATX levels in male patients with alcohol-related liver disease and healthy controls

Serum ATX levels were measured in 14 male patients with alcohol-related ACLF, 11 male patients with LC, and 25 male HS. The mean serum ATX levels in ACLF, LC and healthy control groups were 612.89±151.73, 359.96±128.16 and 182.31±40.02 ng/mL respectively. Serum ATX levels were significantly higher in ACLF than in LC and HS (Fig. 1C).

Performance of serum ATX levels in male LC and ACLF patients with different etiologies

We compared the serum ATX levels in male Child-Pugh A cirrhosis and ACLF patients with different etiology. Patients with LC and alcohol-related Child-Pugh A cirrhosis did not exhibit different serum ATX levels. Then, we analyzed the alterations of serum ATX levels in male hepatitis B-related ACLF and alcohol-related ACLF; similarly, no significant differences were found.

Performance of serum ATX levels in male ACLF patients with spontaneous bacterial peritonitis or pneumonia

Next, we analyzed the relationship between serum ATX levels and infection in ACLF patients. Among the 53 cases of male ACLF patients, 8 patients had no infection and there were 35 cases of ACLF accompanied by infection [spontaneous bacterial peritonitis (SBP) or pneumonia, at the time of hospital admission]. The other 10 cases of ACLF had no infection at the time of hospital admission, but infection emerged within 2 weeks after admission (defined as 'preclinical infection' in this study). The serum ATX levels were significantly higher among the ACLF patients with preclinical infection, SBP or pneumonia than among the ACLF patients

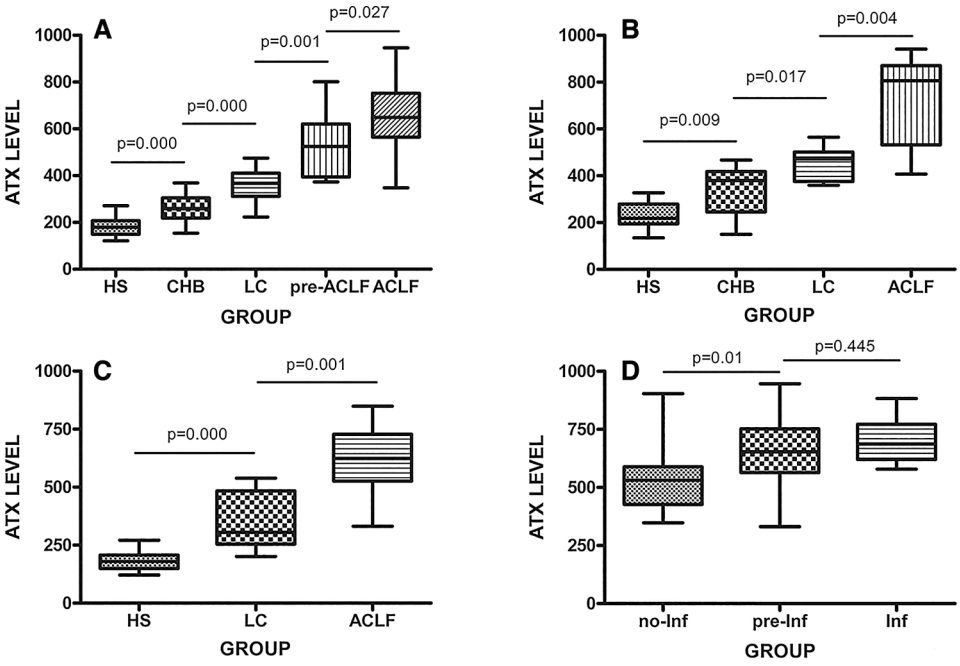

Fig. 1. Alterations of serum ATX levels in patients with liver disease.

(A) Serum ATX levels in male patients with hepatitis B-related liver disease and healthy controls. (B) Serum ATX levels in female patients with hepatitis B-related liver disease and healthy controls. (C) Serum ATX levels in male patients with alcohol-related liver disease and healthy controls. (D) Performances of serum ATX levels in male ACLF patients with SBP or pneumonia.

without SBP or pneumonia. However, no significant difference was found in serum ATX levels among ACLF patients with preclinical infection, SBP or pneumonia (Fig. 1D).

Relationship between serum ATX levels and serum biochemical parameters in hepatitis B-related LC and ACLF patients

A correlation analysis was made between serum ATX levels and serum biochemical parameters of liver and renal function, including Cr, ALB, ALT, AST, ALP, GGT, TBIL, TBA, INR, PT and MELD score in hepatitis B-related male and female LC and ACLF patients respectively. Correlation analysis demonstrated that there was a strong correlation between serum ATX levels and serum biochemical parameters of liver function, both in male and female LC and ACLF patients. For male LC and ACLF patients, serum ATX levels were well correlated with ALB (r= −0.663, p=0.000), ALT (r=0.524, p=0.000), AST (r=0.661, p=0.000), ALP (r=0.525, p=0.000), TBIL (r=0.606, p=0.000), TBA (r=0.513, p=0.000), INR (r=0.704, p=0.000), and MELD score (r=0.620, p=0.000) (Fig. 2). Similarly, a good correlation was also observed between serum ATX levels and biochemical parameters and MELD score in female LC and ACLF patients.

Performance of serum ATX in predicting prognosis of male ACLF patients

Serum ATX levels were further analyzed in 39 male hepatitis B-related ACLF patients with different prognoses (16 patients survived, and 23 patients died). The best cut-off value of serum ATX for predicting the ACLF patients' mortality was

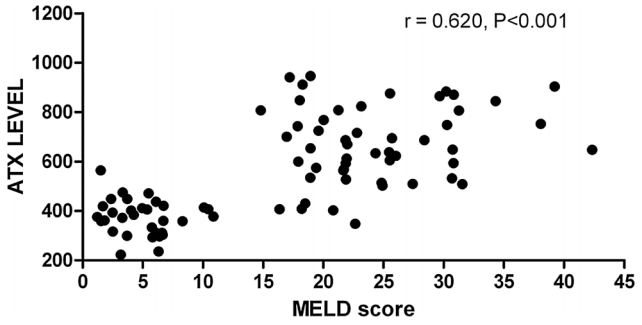

Fig. 2. Relationship between serum ATX levels and MELD score in hepatitis B-related LC and ACLF patients.

determined to be 584.1 ng/mL, using SPSS software. The patients were divided into two groups, namely a high-serum ATX group (serum ATX ≥584.1 ng/mL, n=27) and low-serum ATX group (serum ATX <584.1 ng/mL, n=12). Serum ATX ≥584.1 ng/mL was a prognostic factor for poor outcome of ACLF (hazard ratio of 4.750, 95% confidence interval of 1.106-20.392, p=0.036) (Table 3).

Discussion

In our previous metabolomics analysis study, ultra-performance liquid chromatography-mass spectrometry was used to analyze metabolite profiling for hepatitis B-related ACLF. Among the characteristic metabolites, which were significantly different between hepatitis B-related ACLF and Child-Pugh A cirrhosis, lysophosphatidylcholine (LPC) and

Table 3. Logistic regression analysis of risk factors for hepatitis B-related ACLF

Variable	HR	95% CI	*P* value
Age	1.034	0.957-1.117	0.395
PLT	0.998	0.986-1.010	0.791
ALB	1.016	0.882-1.171	0.825
ALT	1.000	0.998-1.001	0.607
AST	0.999	0.996-1.001	0.237
SBP/pneumonia, Yes vs. No	1.125	0.298-4.241	0.862
MELD	1.220	1.013-1.469	0.036
ATX, ≥584.1 vs. <584.1 ng/mL	4.750	1.106-20.392	0.036

Abbreviations: PLT, platelets; ALB, albumin, ALT, alanine aminotransferase; AST, aspartate aminotransferase; SBP, spontaneous bacterial peritonitis; MELD, model for end-stage liver disease.

lysophosphatidic acid (LPA) comprised the highest proportion. In hepatitis B-related ACLF, the level of LPA (P-16:0e/0:0) and LPA (18:0e/0:0) were significantly elevated, and the levels of 10 LPCs were reduced significantly in the previous study.[12] The alterations of LPC and LPA in ACLF highlight the role of ATX in ACLF. In the present study, serum ATX in ACLF was further analyzed.

We evaluated serum ATX levels in LC and ACLF. Serum ATX levels were significantly higher in hepatitis B-related ACLF than in Child-Pugh A cirrhosis and chronic hepatitis B groups. Then, we analyzed the alterations of serum ATX levels in alcohol-related Child-Pugh A cirrhosis and alcohol-related ACLF; similar changes in serum ATX levels were observed. For female patients, the alterations of serum ATX levels in hepatitis B-related cirrhosis and ACLF were similar to that in male patients. There was also a close correlation between serum ATX levels and serum biochemical parameters of liver function.

ATX was originally discovered in conditioned medium from A2058 human melanoma cell cultures and understanding of ATX action increased significantly with the discovery of its lysophospholipase D (known as lysoPLD) activity, which can hydrolyze LPC to LPA.[13,14] The ATX-LPA signaling axis acts on a series of G protein-coupled receptors, leading to diverse biological features, including liver injury and liver fibrosis.[6,10,15–18] Research studies of ATX in ACLF have not been reported, but our previous metabolomics analysis and the present enzyme-linked immunosorbent assay study of ATX provide evidence of alterations of ATX in ACLF. First, elevated level of ATX in ACLF may be related with the impaired liver function in liver failure. It has been reported that serum ATX activity is increased in various liver injuries and in relation to severity and that ATX is degraded in liver sinusoidal endothelial cells.[19,20] Second, biological functions of ATX can be largely attributed to its lysoPLD activity to produce LPA; as a lipid mediator, LPA is capable of exerting multiple biological actions. Our present study provided new evidence of biological functions of ATX-LPA signaling axis in liver failure.

ACLF occurs on the basis of chronic liver disease, especially for CHB. ACLF is a static diagnosis for liver function status, which is diagnosed until the appearance of acute deterioration of liver function with the progression of disease.[21] With the dynamic development of disease, severe CHB patients may not develop liver failure and instead experience a gradually restoration of function, but the status may also may further aggravate to liver failure and lead to diagnosis of ACLF. Thus, in the early phase of acute exacerbation of CHB, prediction of the risk of the occurrence of ACLF, timely prevention, intervention and standardized treatment to prevent its progression to ACLF will no doubt improve the prognosis of patients. However, until now there is no definition or predictive models for evaluating the risk of occurrence of ACLF. Understanding the concept of ACLF contributes to understanding of its pathogenesis, facilitating searches for early warning biomarkers and intervention strategies.[4,5,22]

The course of ACLF is a dynamic process, and there are no established diagnostic criteria for pre-ACLF. However, the definition for pre-ACLF in the present study is suitable and accepted in clinical practice. Our present study demonstrated that serum ATX levels were significantly higher in the pre-ACLF group than in the Child-Pugh A cirrhosis and CHB groups but lower than in the ACLF group. The performances of serum ATX levels in pre-ACLF highlight the role of ATX in ACLF. ATX may serve as an early warning biomarker for ACLF. Further dynamic tracking analysis demonstrated that among the 11 cases of male pre-ACLF, 5 deteriorated to ACLF, while 6 gradually restored and did not progress to ACLF. This observation suggests that patients with pre-ACLF should receive the standard medical therapy for ACLF as soon as possible after diagnosis. However, based on the current concept of pre-ACLF, not all of the pre-ACLF patients progressed to ACLF and serum ATX level was further compared between the two groups. Our results demonstrated that patients with pre-ACLF who deteriorated to ACLF had significantly higher serum ATX levels than patients who did not progress to ACLF. ATX is, thus, valuable in predicting the risk of occurrence of ACLF through the pre-ACLF model used in this study.

Systemic inflammation is an important feature of ACLF, and increased levels of various inflammatory cytokines have been described in ACLF, including TNF-α, sTNF-αR1, sTNF-αR2, IL-2, IL-2R, IL-6, IL-8, IL-10, and IFN-γ.[23,24] In the present study, 10 cases of ACLF had no infection at the time of hospital admission, but infection emerged within 2 weeks after admission (called 'preclinical infection' in this study). Serum ATX levels were significantly higher among male ACLF patients with preclinical infection, SBP or pneumonia than in ACLF patients without SBP and pneumonia. The ATX–LPA signaling axis has been linked to proinflammatory cytokine production, including that of IL-6, IL-8 and VEGF.[25,26] Meanwhile, elevated LPA production induced by ATX has also been observed in chronic hepatitis.[27] The results of our present study highlight the role of ATX in ACLF patients with infection; furthermore, alterations of ATX occur in the early stage of infection and no significant difference was found in serum ATX levels among the groups of ACLF patients with preclinical infection and infection. This indicates that, first, alterations of ATX may be used as early biomarkers of infection for ACLF and ATX can predict the occurrence of infection at an early stage, helping in timely prevention and intervention strategy. Second, studies on ATX contribute to future investigations on the pathogenesis of inflammation in ACLF.

We compared the serum ATX levels in male Child-Pugh A cirrhosis and ACLF with different etiology. Hepatitis B-related cirrhosis and alcoholic cirrhosis did not exhibit different serum ATX level. Similarly, no significant differences were found in male hepatitis B-related ACLF and alcohol-related ACLF.

Performances of serum ATX in predicting prognosis of male ACLF patients were analyzed, and serum ATX ≥584.1 ng/mL was found to be a prognostic factor for poor outcome of ACLF. Further research is needed to verify the prognostic value of ATX, however.

Conclusions

The current study indicates that serum ATX level was significantly higher in pre-ACLF than in Child-Pugh A cirrhosis and CHB but lower than in the ACLF group. Furthermore, patients of pre-ACLF who deteriorated to ACLF showed significantly higher serum ATX levels than patients that did not progress to ACLF. Serum ATX levels were significantly higher among male ACLF patients with preclinical infection, SBP or pneumonia than ACLF without SBP and pneumonia. Alterations of ATX occur in the early stage of infection. Serum ATX ≥584.1 ng/mL was a prognostic factor for poor outcome of ACLF. Ultimately, based on these findings, serum ATX level may be a useful early warning biomarker for ACLF.

Funding

This work was supported by the National 13th 5-Year Plan for Hepatitis Research (No. 2017ZX10203201) and the National Natural Science Foundation of China (No. 81870429).

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Contributed to methodology (CN, LZ), data curation (CN, LZ, FH), formal analysis and drafting of the original article (CN, TH), funding acquisition and supervision (TH), investigation (CN, HL, YL), writing, review and editing of the article (CN, XC, TH).

References

[1] Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, *et al*. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update. Version 2. Hepatol Int 2019;13:353–390. doi: 10.1007/s12072-019-09946-3.

[2] Stål P, Oksanen A. Acute-on-chronic liver failure (ACLF) is a separate clinical entity. Lakartidningen 2016;113:DLUL.

[3] Xia Q, Dai X, Zhang Y, Guo Y, Xu X, Yang Q, *et al*. A modified MELD model for Chinese pre-ACLF and ACLF patients and it reveals poor prognosis in pre-ACLF patients. PLoS One 2013;8:e64379. doi: 10.1371/journal.pone.0064379.

[4] Chen F, Shi Y, Liu X, Lei L, Xu J. Corticosteroid improves liver function but does not curb the clinical progression of hepatitis B virus-related acute-on-chronic pre-liver failure. Expert Rev Gastroenterol Hepatol 2019;13:1129–1135. doi: 10.1080/17474124.2019.

[5] Li C, Zhu B, Lyu S, You SL. Discussion and evaluation of diagnostic criteria for hepatitis B virus-related acute-on-chronic pre-liver failure. Zhonghua Gan Zang Bing Za Zhi 2018;26:130–135. doi: 10.3760/cma.j.issn.1007-3418.2018.02.011.

[6] Fujimori N, Umemura T, Kimura T, Tanaka N, Sugiura A, Yamazaki T, *et al*. Serum autotaxin levels are correlated with hepatic fibrosis and ballooning in patients with non-alcoholic fatty liver disease. World J Gastroenterol 2018;24:1239–1249. doi: 10.3748/wjg.v24.i11.1239.

[7] Fujino H, Tanaka M, Imamura M, Morio K, Ono A, Nakahara T, *et al*. Pruritus in patients with chronic liver disease and serum autotaxin levels in patients with primary biliary cholangitis. BMC Gastroenterol 2019;19:169. doi: 10.1186/s12876-019-1092-z.

[8] Joshita S, Ichikawa Y, Umemura T, Usami Y, Sugiura A, Shibata S, *et al*. Serum autotaxin is a useful liver fibrosis marker in patients with chronic hepatitis B virus infection. Hepatol Res 2018;48:275–285. doi: 10.1111/hepr.12997.

[9] Kondo M, Ishizawa T, Enooku K, Tokuhara Y, Ohkawa R, Uranbileg B, *et al*. Increased serum autotaxin levels in hepatocellular carcinoma patients were caused by background liver fibrosis but not by carcinoma. Clin Chim Acta 2014;433:128–134. doi: 10.1016/j.cca.2014.03.006.

[10] Honda Y, Imajo K, Kobayashi T, Kessoku T, Ogawa Y, Tomeno W, *et al*. Autotaxin is a valuable biomarker for the prediction of liver fibrosis in patients with non-alcoholic fatty liver disease. Hepatol Res 2019;49:1136–1146. doi: 10.1111/hepr.13382.

[11] Wunsch E, Krawczyk M, Milkiewicz M, Trottier J, Barbier O, Neurath MF, *et al*. Serum autotaxin is a marker of the severity of liver injury and overall survival in patients with cholestatic liver diseases. Sci Rep 2016;6:30847. doi: 10.1038/srep30847.

[12] Nie CY, Han T, Zhang L, Li Y, Liu H, Xiao SX, *et al*. Cross-sectional and dynamic change of serum metabolite profiling for Hepatitis B-related acute-on-chronic liver failure by UPLC/MS. J Viral Hepat 2014;21:53–63. doi: 10.1111/jvh.12122.

[13] Umezu-Goto M, Kishi Y, Taira A, Hama K, Dohmae N, Takio K, *et al*. Autotaxin has lysophospholipase D activity leading to tumor cell growth and motility by lysophosphatidic acid production. J Cell Biol 2002;158:227–233. doi: 10.1083/jcb.200204026.

[14] Tokumura A, Majima E, Kariya Y, Tominaga K, Kogure K, Yasuda K, *et al*. Identification of human plasma lysophospholipase D, a lysophosphatidic acid-producing enzyme, as autotaxin, a multifunctional phosphodiesterase. J Biol Chem 2002;277:39436–39442. doi: 10.1074/jbc.M205623200.

[15] Shao Y, Yu Y, He Y, Chen Q, Liu H. Serum ATX as a novel biomarker for breast cancer. Medicine (Baltimore) 2019;98:e14973. doi: 10.1097/MD.0000000000014973.

[16] Lee D, Suh DS, Lee SC, Tigyi GJ, Kim JH. Role of autotaxin in cancer stem cells. Cancer Metastasis Rev 2018;37:509–518. doi: 10.1007/s10555-018-9745-x.

[17] Mazzocca A, Schönauer LM, De Nola R, Lippolis A, Marrano T, Loverro M, *et al*. Autotaxin is a novel molecular identifier of type I endometrial cancer. Med Oncol 2018;35:157. doi: 10.1007/s12032-018-1222-4.

[18] Shim SJ, Shin E, Lee CS, Koo JS. The expressions of autotaxin-lysophosphatidate signaling-related proteins in metastatic breast cancer. Int J Clin Exp Pathol 2019;12:2920–2930.

[19] Watanabe N, Ikeda H, Nakamura K, Ohkawa R, Kume Y, Tomiya T, *et al*. Plasma lysophosphatidic acid level and serum autotaxin activity are increased in liver injury in rats in relation to its severity. Life Sci 2007;81:1009–1015. doi: 10.1016/j.lfs.2007.08.013.

[20] Jansen S, Andries M, Vekemans K, Vanbilloen H, Verbruggen A, Bollen M. Rapid clearance of the circulating metastatic factor autotaxin by the scavenger receptors of liver sinusoidal endothelial cells. Cancer Lett 2009;284:216–221. doi: 10.1016/j.canlet.2009.04.029.

[21] Ren Y, Liu L, Li Y, Yang F, He Y, Zhu Y, *et al*. Development and validation of a scoring system to predict progression to acute-on-chronic liver failure in patients with acute exacerbation of chronic hepatitis B. Hepatol Res 2018;48:692–700. doi: 10.1111/hepr.13062.

[22] Zhang XQ, Jiang L, You JP, Liu YY, Peng J, Zhang HY, *et al*. Efficacy of short-term dexamethasone therapy in acute-on-chronic pre-liver failure. Hepatol Res 2011;41:46–53. doi: 10.1111/j.1872-034X.2010.00740.x.

[23] Wu W, Yan H, Zhao H, Sun W, Yang Q, Sheng J, *et al*. Characteristics of systemic inflammation in hepatitis B-precipitated ACLF: Differentiate it from No-ACLF. Liver Int 2018;38:248–257. doi: 10.1111/liv.13504.

[24] Moreau R. The Pathogenesis of ACLF: The Inflammatory Response and Immune Function. Semin Liver Dis 2016;36:133–140. doi: 10.1055/s-0036-1583199.

[25] Yu S, Murph MM, Lu Y, Liu S, Hall HS, Liu J, *et al*. Lysophosphatidic acid receptors determine tumorigenicity and aggressiveness of ovarian cancer cells. J Natl Cancer Inst 2008;100:1630–1642. doi: 10.1093/jnci/djn378.

[26] Liu S, Umezu-Goto M, Murph M, Lu Y, Liu W, Zhang F, *et al*. Expression of autotaxin and lysophosphatidic acid receptors increases mammary tumorigenesis, invasion, and metastases. Cancer Cell 2009;15:539–550. doi: 10.1016/j.ccr.2009.03.027.

[27] Watanabe N, Ikeda H, Nakamura K, Ohkawa R, Kume Y, Aoki J, *et al*. Both plasma lysophosphatidic acid and serum autotaxin levels are increased in chronic hepatitis C. J Clin Gastroenterol 2007;41:616–623. doi: 10.1097/01.mcg.0000225642.90898.0e.