Prognostic value of serum autotaxin in liver cirrhosis and prediction of hepatocellular carcinoma

Nagwa Ramadan Ahmed[a], Ahmed Nabil EL-Mazny[a], Sarah Ahmed Hassan[a], Laila Ahmed Rashed[b]

[a]Department of Internal Medicine, Faculty of Medicine, Kasr Al-Ainy, Cairo University, Cairo, Egypt, [b]Department of Biochemistry, Faculty of Medicine, Kasr Al-Ainy, Cairo University, Cairo, Egypt

Correspondence to Nagwa Ramadan Ahmed, Assisstant Professor of Internal medicine, Faculty of Medicine, Cairo University, 9 Shannan Street-Mostafa Abd Razek-Abdeen, 11613, Egypt. Mob: 01001776947; e-mail: dr_nagwa2001@yahoo.com

Received: 22 April 2019
Accepted: 4 May 2019
Published: 18 August 2020

The Egyptian Journal of Internal Medicine
2019, 31:849–855

Background
Autotaxin is a lysophospholipase D related to liver fibrosis; its clinical role in liver cirrhosis is still unknown or limited. In this study we investigate the relation of autotaxin serum levels and prognosis of liver disease and/or prediction of hepatocellular carcinoma (HCC) in hepatitis C virus (HCV) patients.
Patients and methods
This observational, prospective case–control study included 180 participants, 60 patients with HCV-related liver cirrhosis, 60 HCV noncirrhotic patients, and 60 healthy controls. They were enrolled from inpatients and clinics of a tertiary-care hospital. Baseline characteristics, serum autotaxin, Child–Turcotte–Pugh and model of end-stage liver disease scores were determined. Abdominal ultrasound and upper gastrointestinal endoscopy were done at the beginning of the study. Cirrhotic patients were prospectively followed up for 6 months.
Results
Patients with liver cirrhosis had the highest level of autotaxin (106±24 μg/ml) compared with noncirrhotic HCV patients (81.9±21 μg/ml) and healthy controls (42.5±11 μg/ml) using one-way analysis of variance test (P=0.000). Spearman's correlation analysis showed no significant correlation between autotaxin and Child–Turcotte–Pugh score (r=0.02; P<0.70), and model of end-stage liver disease score (r=0.15; P<0.41). At 6 months of follow-up, patients who developed HCC or encephalopathy had significantly higher baseline autotaxin level (141±55 μg/ml; P=0.02, 117±56.6 μg/ml; P=0.000), respectively, than patients who did not (102±34, 90.7±40 μg/ml). Cutoff values of autotaxin for the prediction of HCC and encephalopathy were 95 and 92 μg/ml, respectively, with 91 and 92% sensitivity.
Conclusion
Autotaxin is a sensitive predictor for the development of HCC and encephalopathy in HCV-related cirrhotic patients. However, it was not related to disease severity.

Keywords:
autotaxin, hepatitis C virus, hepatocellular carcinoma, liver cirrhosis

Egypt J Intern Med 31:849–855

1110-7782

Introduction

Hepatitis C virus (HCV) is still one of the most common causes of cirrhosis in the world [1]. Cirrhosis is a major cause of mortality and it is associated with various complications mainly hepatic encephalopathy, variceal bleeding, and hepatocellular carcinoma (HCC). Many prognostic indicators have been suggested as predictors of outcomes in cirrhotic patients and the work is still running [2,3].

Autotaxin is a lysophospholipase D that produces phospholipid lysophosphatidic acid (LPA) from lysophosphatidylcholine. LPA is a fibrosis-stimulating factor promoting fibroblast migration and stimulating connective tissue growth factor [4]. Autotaxin was found to have an important role in different fibroproliferative conditions including liver fibrosis [5,6]. Moreover, autotaxin has been related to tumor aggression and metastasis [7].

Few human studies have investigated autotaxin in relation to different outcomes of liver cirrhosis. These studies included patients with liver cirrhosis of variable causes [8,9]. The aim of this study was to assess serum autotaxin levels in cirrhotic patients and its relation to disease prognosis and development of HCC.

Patients and methods

This prospective, case–control study was conducted from August 2016 till August 2017. All participants

were recruited from our hospital, The Internal Medicine Department and outpatient clinic. The study was approved by the ethics committee and informed written consent was obtained from the participants. The study protocol and procedures conform to the ethical guidelines of the 1975 Declaration of Helsinki.

Three groups of participants number totaled to 180: 60 patients with liver cirrhosis secondary to HCV infection (group 1), 60 patients with HCV infection without liver cirrhosis (group 2), and 60 healthy participants selected as controls (group 3). Patients excluded were those with any malignant tumor, HCC at the beginning of the study, or liver cirrhosis of causes other than HCV.

Initially, the patients were thoroughly evaluated for determination of Child–Turcotte–Pugh (CTP), model of end-stage liver disease (MELD) score (as a prognostic score for assessment of severity of liver cirrhosis), and presence of complications of cirrhosis such as hepatic encephalopathy, spontaneous bacterial peritonitis (SBP), hepatorenal syndrome (HRS), and gastrointestinal tract (GIT) bleeding.

Serum autotaxin measurement, liver function tests (alanine transaminase, aspartate transaminase, alkaline phosphatase, gamma-glutamyltransferase, total protein, albumin, prothrombin time (PT), prothrombin concentration (PC), international normalized ratio, bilirubin total, and direct), alphafetoprotein (AFP), complete blood count, urea, creatinine, hepatitis B surface antigen and HCV antibody, abdominal ultrasound, computed tomography or MRI abdomen, and upper GIT endoscopy were done. Patients with liver cirrhosis were followed up for 6 months to detect correlation between serum autotoxin and the occurrence of complications or development of HCC.

Autotaxin sample collection and assay principle

Samples were assembled in serum separator tubes and were allowed to clot for 30 min. Centrifugation was done for 15 min at ~1000*g* and grossly hemolyzed samples were excluded. Serum was separated and stored at −80°C. Quantikine ELISA kits (R&D Systems Inc., USA) were used to quantify serum autotaxin level in μg/ml that employs the quantitative sandwich enzyme immunoassay technique using monoclonal antibody specific to ectonucleotide pyrophosphatase/phosphodiesterase 2. The test was performed according to manufacturers' instructions.

Statistical analyses

Data was analyzed through the Statistical Package of Social Science Software program, version 16 (SPSS, Chicago, IL, USA) as follows: quantitative variables were described using mean, SD, and range; and qualitative variables as using frequency percentage. c^2 test was used to compare qualitative variables between groups. Unpaired *t* test was used to compare quantitative variables for parametric data, while Mann–Whitney test was used for nonparametric data. One-way analysis of variance test was used to compare more than two groups of quantitative variables. Spearman's correlation was used to test for linear relations between variables. A *P* value of 0.05 or less was considered significant. Receiver-operating characteristic curve analysis was used to find out the best cutoff points and validity of certain variables.

Results

Baseline demographic, laboratory, and radiologic data of cirrhotic patients compared with other groups.

There was no statistically significant difference between the studied groups as regards age and sex. The patients in group 1 had advanced stage of liver cirrhosis; 46.7% of them were of CTP stage B and 53.3% were of stage C. Moreover, the MELD score of this group was 14.4±4. Patients with liver cirrhosis suffered most of the complications of cirrhosis; 80% of them had GIT bleeding, 73% had ascites, and 30% had a history of SBP. However, none of the cirrhotic patients had HRS (Table 1).

Baseline autotaxin levels among the different groups

Liver cirrhosis group had the highest level of autotaxin (106+24 ng/ml) compared with the HCV noncirrhotic group (81.9±21 μg/ml) and the control group (42.5 ±11 μg/ml). Using one-way analysis of variance the difference was statistically significant (*P*=0.000) (Fig. 1).

Correlation between autotaxin and different parameters in hepatitis C virus patients (with or without liver cirrhosis)

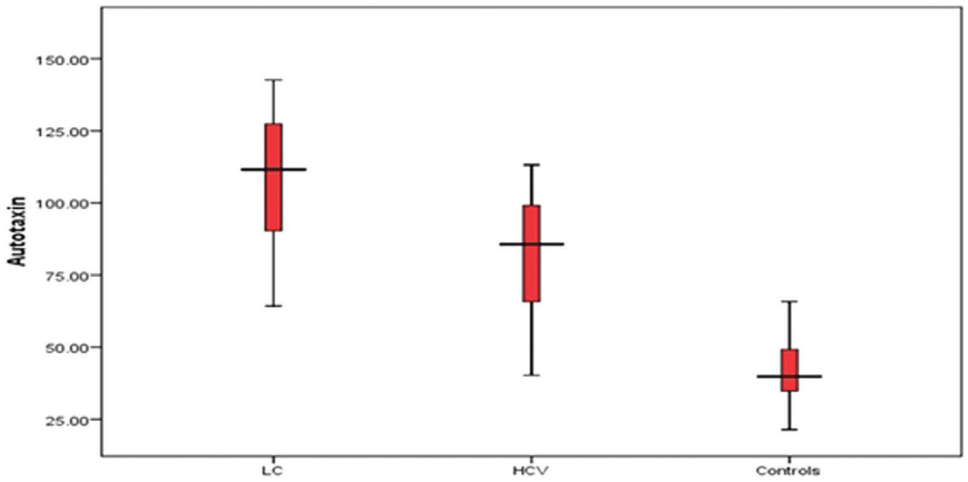
In patients with liver cirrhosis, Spearman's correlation analysis showed no significant correlation between autotaxin and CTP score (*r*=0.02; *P*=0.70) nor MELD score (*r*=0.15; *P*=0.41). In HCV patients, the only significant correlation was between autotaxin and platelet count (*r*=−0.46; *P*=0.02) and total protein (*r*=−0.6; *P*=0.002). Table 2 shows autotaxin correlations with different parameters in groups 1 and 2.

Table 1 Baseline demographic, laboratory, and radiologic data of the studied groups

Variables	Liver cirrhosis group (*N*=60)	HCV group (*N*=60)	Controls (*N*=60)	*P* value
Male/female (*n*/*N*)	36/24	32/28	34/26	0.39
Age (years) (mean±SD)	57.9±8	52±6	55±3	0.16
GIT bleeding (*n*)	24	0	0	0.000
Hepatic encephalopathy (*n*)	13	0	0	0.000
Ascites (*n*)	22	0	0	0.000
Spontaneous bacterial peritonitis (*n*)	9	0	0	0.001
Hepatorenal syndrome (*n*)	0	0	0	–
Child–Pugh score (*n*)				
A	0	60	–	0.000
B	28	–	–	
C	32	–	–	
MELD score (mean±SD)	14.4±4	10.5±3	–	0.001
Splenomegaly (*n*)	17	1	0	0.000
ALT (mean±SD) (U/l)	64.9±40	39±25	2.2	0.05
AST (mean±SD) (U/l)	115±100	39±20	2.1	0.04
Albumin (mean±SD) (mg/dl)	2.1±0.8	3.5±0.6	11	0.000
Bilirubin (mean±SD) (mg/dl)	2.5±0.7	1.3±0.6	1.7	0.22
INR (mean±SD) (mg/dl)	1.3±0.2	1.1±0.09	5	0.000
Platelets (mean±SD) (nl/1)	139±40	169±50	1.6	0.45
Creatinine (mean±SD) (mg/dl)	1.2±0.4	1.3±0.1	1.2	0.60

ALT, alanine transaminase; AST, aspartate transaminase; GIT, gastrointestinal tract; HCV, hepatitis C virus; INR, international normalized ratio; MELD, model of end-stage liver disease.

Figure 1



Comparison of mean autotaxin level in different groups using one-way ANOVA. ANOVA, analysis of variance.

Clinical characteristics of cirrhotic patients at 6 months compared with that at baseline

During the 6 months follow-up, 34 (56.7%) patients had GIT bleeding, 34 (56.7%) had hepatic encephalopathy, 56 (93.3%) of cirrhotic group had ascites, 12 (20%) had SBP, and six (10%) had HRS. Their CTP score became worse, 76.7% had Child C and MELD score became 20.5±8. Table 3 shows a comparison between history data taken at baseline and after 6 months among patients with liver cirrhosis.

Autotaxin level in relation to different complications in cirrhotic patients at baseline and at 6 months follow up.

At the beginning of the study, using unpaired *t* test, autotaxin level was not significantly different between patients with and without hepatic encephalopathy, SBP, ascites, splenomegaly, or esophageal varices (Table 4).

However, six patients who developed HCC after 6 months had significantly higher autotaxin at baseline than those who did not (141±55 vs.102±34 ng/ml; *P*=0.02) (Fig. 2).

Also, serum autotaxin was higher in patients who developed encephalopathy after 6 months than those

Table 2 Correlations between autotaxin and different parameters in hepatitis C virus patients (with and without) liver cirrhosis

	Autotaxin			
	Liver cirrhosis (group 1)		HCV patients (group 2)	
Variables	*r*	*P* value	*r*	*P* value
ALT	0.13	0.22	0.17	0.12
AST	–0.09	0.60	0.04	0.80
ALP	0.02	0.78	0.13	0.78
GGT	0.14	0.35	0.17	0.31
Total protein	–0.02	0.89	–0.60	0.002
Albumin	0.13	0.24	0.10	0.28
Total bilirubin	0.04	0.67	0.02	0.68
INR	0.25	0.11	–0.20	0.40
Total leukocytic count	–0.08	0.70	–0.03	0.70
Platelets	0.17	0.77	–0.46	0.02
Creatinine	0.05	0.90	0.02	0.91
Na	0.17	0.56	0.14	0.66
MELD score	0.15	0.41	0.11	0.21
CTP score	0.02	0.70	–	–
Esophageal varices	0.05	0.83	–	–

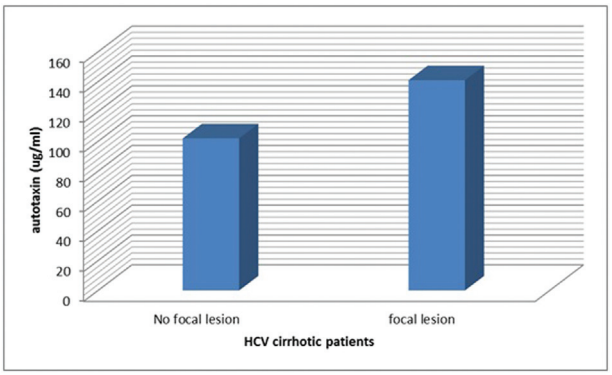
ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; CTP score, Child–Turcotte–Pugh; GGT, gamma-glutamyltransferase; HCV, hepatitis C virus; INR, international normalized ratio; MELD, model of end-stage liver disease.

who did n0t develop (117±56.6 vs. 90.7±40 ng/ml; *P*=0.000) (Fig. 3) (Table 4).

Validity of autotaxin as a predictor of development of hepatic focal lesion and encephalopathy in patients with liver cirrhosis

When autotaxin level 95 ng/ml was used as a cutoff value for the prediction of hepatic focal lesion, area

Figure 2



Mean autotaxin level in cirrhotic patients with and without focal lesions.

Table 3 Comparison between history data taken at baseline and after 6 months among patients with liver cirrhosis

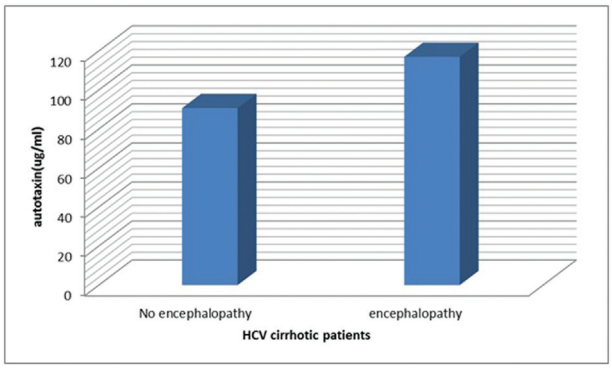
Variables	Baseline [*n* (%)]	After 6months [*n* (%)]	*P* value
GIT bleeding	48 (80)	34 (56.7)	0.000
Hepatic encephalopathy	26 (43.3)	34 (56.7)	0.11
Ascites	44 (73.3)	56 (93.3)	0.02
Spontaneous bacterial peritonitis	18 (30)	12 (20)	0.17
Jaundice	28 (46.7)	44 (73.3)	0.04
Hepatorenal syndrome	0	6 (10)	0.19
CPS			
A	0	0	0.000
B	28 (46.7)	14 (23.3)	
C	32 (53.3)	46 (76.7)	
Edema	34 (56.7)	46 (76.7)	0.03
MELD score	14.4±4	20.5±8	0.001

GIT, gastrointestinal tract; MELD, model of end-stage liver disease.

Table 4 baseline autotaxin level in relation to different complications in LC at baseline and after 6 months

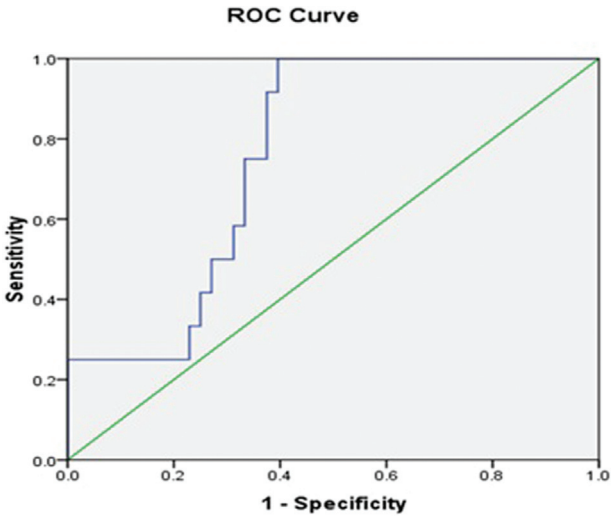
Variables	Negative	Baseline autotaxin level (μg/ml) (mean±SD) Positive	*P* value
At baseline			
Hepatic encephalopathy	106±45	106±50	0.98
Spontaneous bacterial peritonitis	111±55	94.6±30	0.09
Ascites	108±45.8	104.7±30	0.80
Splenomegaly	112.5±46.8	102±50	0.32
Esophageal varices	108±50	100±44.6	0.40
At 6 months follow up			
hepatic focal lesion	102±34	141±55	0.02
Hepatic encephalopathy	90.7±40	117±56.6	0.000
Ascites	70±30	107±50.6	0.22

Figure 3



Mean autotaxin level at baseline in cirrhotic patients who developed hepatic encephalopathy after 6 months compared with those who did not develop.

Figure 4



Autotaxin value for predicting hepatic focal lesion.

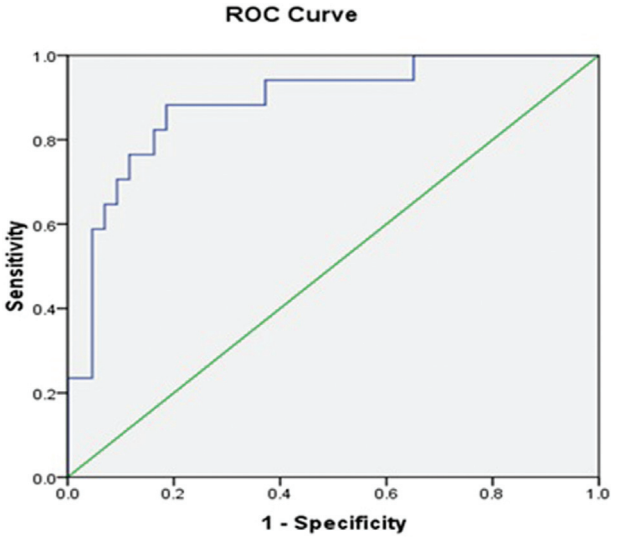
under the curve of the receiver-operating characteristic curve was 0.76, sensitivity was 91%, specificity was 65%, and it had 70% positive predictive value and 93% negative predictive value (Fig. 4).

For the prediction of encephalopathy, we used autotaxin level 92 ng/ml as a cutoff value. Area under the curve was 0.88, sensitivity was 92%, specificity was 64%, and it had 69% positive predictive value and 94% negative predictive value (Fig. 5).

Discussion

LPA stimulates hepatic stellate cell proliferation which is the main cellular mediator of hepatic fibrosis and cirrhosis. It also promotes its transformation into myofibroblasts [10,11]. Autotaxin via LPA plays an

Figure 5



Autotaxin value for predicting hepatic encephalopathy.

important role in the progression of cirrhosis and it has been linked to the fibrosis stage [12,13]. Furthermore, inhibition of autotaxin resulted in direct inhibition of fibrosis, without affecting the hepatocyte inflammation [14].

This study demonstrated higher levels of autotaxin in HCV cirrhotic patients than noncirrhotic HCV patients. Previous studies in chronic hepatitis C patients had reported elevated levels of autotaxin and demonstrated its association with markers and degree of liver fibrosis [12,13].

The precise mechanism of elevation of autotaxin in liver fibrosis is not clear. Some investigators have suggested increased production [11,15,16]; others proposed decreased clearance of autotaxin by the injured liver [17,18].

In a study on mice, the increase in autotaxin level after liver insult was related to the degree of hepatic injury. A previous report in cirrhotic patients linked elevated autotaxin levels to higher mortality and disease severity as assessed by CTP score and MELD score [9]. However, in our study we could not identify significant association between any of these scores and serum autotaxin. This contradiction may be related to the etiology of cirrhosis; while there were multiple causes of cirrhosis in their study. HCV was the only cause in ours. In addition, most of their patients were of Child A or Child B, while more than half of our patients were of Child C. In rats with carbon tetrachloride-induced liver fibrosis autotaxin was not increased after fibrosis induction [17], while in HCV-infected patients autotaxin was elevated and

normalized with HCV treatment [19], which may indicate that the production of autotaxin expression could be dependent on the cause of damage.

Immune-mediated thrombocytopenia is one of the possible mechanisms of thrombocytopenia in HCV patients. Kostadinova *et al.* [19] suggested that autotaxin may induce immune activation during HCV infection. This may explain our findings of negative correlation of autotaxin and platelet count in HCV patients; the same finding was reported by Watanabe *et al.* [12].

Data from our study showed an association between high autotaxin levels and the possible development of HCC and hepatic encephalopathy in HCV cirrhotic patients.

Autotaxin and LPA are recognized as tumor-promoting factors; they stimulate cancer cell proliferation, invasion, and metastasis [20]. Autotaxin has a key role in inflammation-provoked hepatic malignancies and it was found to be produced by HCC cells in one study [21], and by the surrounding fibrous tissue in another [8]. The highest expression has been found in HCV-related HCC [22]. This goes with our findings of higher autotaxin level among patients who developed HCC compared with those who did not. Pleli and colleagues did not find significant difference in autotaxin level between patients with HCC and those without, but the most common cause of cirrhosis in their study was alcoholic hepatitis.

A previous study found no correlation between autotaxin and leukocyte count, and autotaxin level was not affected by infection [9]. We achieved the same result; moreover autotaxin levels were not significantly different between patients with and without SBP.Autotaxin is cleared by hepatic sinusoidal endothelial cells, so endothelial dysfunction such that occurs in portal hypertension may cause elevation of autotaxin level [9]. Yet, little is known about the relation between autotaxin and the presence of esophageal varices in cirrhotic patients. In a cohort of cirrhotic patients, most of them were alcoholics, patients with esophageal varices had significantly higher autotaxin level [9]. However, this study did not demonstrate significant association between autotaxin level and the presence of esophageal varices. Alcohol-induced impairment of the hepatic sinusoidal endothelial cell function has been previously reported [23,24]. This may explain the conflict between the two studies.

Portosystemic shunt is a major contributing factor in the development of hepatic encephalopathy [25]. In this study, most of our patients had esophageal varices which reflect the presence of portal hypertension and portosystemic shunts. This may explain the higher levels of autotaxin in patients who developed encephalopathy at 6 months. Same finding was reported by Pleli *et al.* [9].

Conclusion

This study reported elevated levels of autotaxin in HCV-related cirrhotic patients. Higher serum autotaxin level was related to the development of HCC and hepatic encephalopathy and it was a sensitive predictor of both complications. However, autotaxin was not associated with the presence of esophageal varices.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet 2014; 383:1749–1761.

2 Poca M, Puente A, Graupera I, Villanueva C. Prognostic markers in patients with cirrhosis and portal hypertension who have not bled. Dis Markers 2011; 31:147–154.

3 de Franchis R, Dell'Era A. Invasive and noninvasive methods to diagnose portal hypertension and esophageal varices. Clin Liver Dis 2014; 18:293–302.

4 Watterson K, Lanning D, Diegelmann R, Spiegel S. Regulation of fibroblast functions by lysophospholipid mediators: potential roles in wound healing. Wound Repair Regen 2007; 15:607–616.

5 Georas S. Lysophosphatidic acid and autotaxin: emerging roles in innate and adaptive immunity. Immunol Res 2009; 45:229–238.

6 Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. J Clin Invest 2007; 117:524–529.

7 Ikeda H, Watanabe N, Nakamura K, Kume Y, Nakai Y, Fujishiro M, *et al.* Significance of serum autotaxin activity in gastrointestinal disease. Rinsho Byori 2009; 57:445–449.

8 Kondo M, Ishizawa T, Enooku K, *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.

9 Pleli T, Martin D, Kronenberger B, Brunner F, Köberle V, Grammatikos G, *et al.* Serum autotaxin is a parameter for the severity of liver cirrhosis and overall survival in patients with liver cirrhosis-a prospective cohort study. PLoS One 2014; 9:e103532.

10 Yamaoka K, Nouchi T, Marumo F, Satoc C. Alpha-smooth-muscle actin expression in normal and fibrotic human livers. Dig Dis Sci 1993; 38:1473–1479.

11 Ikeda H, Nagashima K, Yanase M, Tomiya T, Arai M, Inoue Y, *et al.* Involvement of Rho/Rho kinase pathway in regulation of apoptosis in rat hepatic stellate cells. Am J Physiol Gastrointest Liver Physiol 2003; 285: G880–G886.

12 Watanabe N, Ikeda H, Nakamura K, *et al.* Both plasma lysophosphatidic acid and serum autotaxin levels are increased in chronic hepatitis C. J Clin Gastroenterol 2007; 41:616–623.

13 Nakagawa H, Ikeda H, Nakamura K, *et al.* Autotaxin as a novel serum marker of liver fibrosis. Clin Chim Acta 2011; 412:1201–1206.

14 Bain G, Shannon KE, Huang F, *et al.* Selective inhibition of autotaxin is efficacious in mouse models of liver fibrosis. J Pharmacol Exp Ther 2017; 360:1–13.

15 Yanase M, Ikeda H, Ogata I, *et al.* Functional diversity between Rho-kinase and MLCK-mediated cytoskeletal actions in a myofibroblast like hepatic stellate cell line. Biochem Biophys Res Commun 2003; 305:223–228.

16 Ikeda H, Yatomi Y, Yanase M, *et al.* Effects of lysophosphatidic acid on proliferation of stellate cells and hepatocytes in culture. Biochem Biophys Res Commun 1998; 248:436–440.

17 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.

18 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.

19 Kostadinova L, Shive CL, Judge C, Zebrowski E, Compan A, Rife K, *et al.* During hepatitis c virus (HCV) infection and HCV-HIV coinfection, an elevated plasma level of autotaxin is associated with lysophosphatidic acid and markers of immune activation that normalize during interferon-free HCV therapy. J Infect Dis 2016; 214:1438–1448.

20 Xia Q, Deng AM, Wu SS, Zheng M. Cholera toxin inhibits human hepatocarcinoma cell proliferation in vitro via suppressing ATX/LPA axis. Acta Pharmacol Sin 2011; 32:1055–1062.

21 Wu JM, Xu Y, Skill NJ, Sheng H, Zhao Z, Yu M, *et al.* Autotaxin expression and its connection with the TNF-alpha-NF-kappaB axis in human hepatocellular carcinoma. Mol Cancer 2010; 9:71.

22 Cooper AB, Wu J, Lu D, Maluccio MA. Is autotaxin (ENPP2) the link between hepatitis C and hepatocellular cancer? J Gastrointest Surg 2008; 11:1628–1634.

23 Auroux J, Lamarque D, Roudot-Thoraval F, Deforges L, Chaumette MT, Richardet JP, Delchier JC. Gastroduodenal ulcer and erosions are related to portal hypertensive gastropathy and recent alcohol intake in cirrhotic patients. Dig Dis Sci 2003; 48:1118–1123.

24 Seo W, Jeong WI. Hepatic non parenchymal cells: master regulators of alcoholics liver disease?. World J Gastroenterol 2016; 22:1348–1356.

25 Ciecko-Michalska I, Szczepanek M, Słowik A, Mach T. Pathogenesis of hepatic encephalopathy. Gastroenterol Res Pract 2012; 2012:642108.