ORIGINAL ARTICLE

Retrospective Study

Serum autotaxin levels are correlated with hepatic fibrosis and ballooning in patients with non-alcoholic fatty liver disease

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Abstract

AIM

To examine the relationship between serum autotaxin (ATX) concentrations and clinicopathological findings in non-alcoholic fatty liver disease (NAFLD) patients.

METHODS

One hundred eighty-six NAFLD patients who had undergone liver biopsy between 2008 and 2017 were retrospectively enrolled. Serum samples were collected at the time of biopsy and ATX was measured by enzyme immunoassays. Sera obtained from 160 healthy, non-obese individuals were used as controls. Histological findings were graded according to an NAFLD scoring system and correlations with serum ATX were calculated by Spearman's test. Diagnostic accuracy was evaluated using the area under the receiver operating characteristic curve (AUC). Cut-off values were identified by the Youden index, and the nearest clinically applicable value to the cutoff was considered the optimal threshold for clinical convenience.

RESULTS

Serum ATX levels were significantly higher in NAFLD patients than in controls (0.86 mg/L *vs* 0.76 mg/L, $P < 0.001$) and correlated significantly with ballooning score and fibrosis stage ($r = 0.36$, $P < 0.001$ and $r = 0.45$, $P < 0.001$, respectively). Such tendencies were stronger in female patients. There were no remarkable relationships between ATX and serum alanine aminotransferase, lipid profiles, or steatosis scores. The AUC values of ATX for predicting the presence of fibrosis ($\geqslant$ F1), significant fibrosis ($\geqslant$ F2), severe fibrosis ($\geqslant$ F3), and cirrhosis (F4), were all more than 0.70 in respective analyses.

CONCLUSION

Serum ATX levels may at least partially reflect histological severity in NAFLD.

Key words: Autotaxin; Non-alcoholic fatty liver disease; Fibrosis; Ballooning

Core tip: Patients with non-alcoholic fatty liver disease (NAFLD) exhibited significantly higher serum levels of autotaxin (ATX) than did healthy subjects. Serum ATX levels correlated significantly with ballooning score and fibrosis stage in NAFLD patients and may therefore reflect histological severity in NAFLD.

INTRODUCTION

The prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing worldwide[1,2]. NAFLD exhibits a wide spectrum, ranging from non-alcoholic fatty liver to non-alcoholic steatohepatitis (NASH) and ensuing cirrhosis and hepatocellular carcinoma[1-3]. Since the concept of NASH was developed using pathological characeristics, *i.e.*, the presence of hepatocyte ballooning and lobular inflammation in addition to macrovesicular steatosis, liver biopsy is currently considered the gold standard for evaluating NAFLD/NASH activity. However, general limitations of liver biopsy are the costs and invasiveness, but also sampling error and inter- and intra-observer variability[4]. So, simple, accurate, non-invasive, quantitative alternatives are needed. Several studies have attempted to estimate histological severity in NAFLD using various serum biomarkers[5-8], but the accuracy of these techniques remains unsatisfactory.

Autotaxin (ATX) was originally discovered in conditioned medium from human melanoma cell cultures[9]. The protein is encoded by ectonucleotide pyrophosphatase/phosphodiesterase family member 2 gene (*ENPP2*) and catalyzes the hydrolysis of lysophosphatidylcholine (LPC) to lysophosphatidic acid (LPA), which functions as a phospholipase[10,11]. Signaling *via* a family of six G-protein-coupled receptors (LPA1-6) regulates the diverse cellular processes of ATX, including proliferation, migration, neurogenesis, angiogenesis, fibrogenesis, glucose homeostasis, insulin action, and cancer progression[12-18]. Disrupted LPC metabolism has been reported in murine NASH models[19,20].

ATX is synthesized by a variety of normal cells and tissues, secreted into the circulation as a glycoprotein, and later degraded by liver sinusoidal endothelial cells[21]. Serum ATX levels are reportedly increased during the progression of pregnancy[22] and in patients with idiopathic pulmonary fibrosis or some kinds of cancers[23-25]. Recently, elevated serum ATX has also been implicated in fibrosis progression in chronic hepatitis C[26,27], for which the retarded degradation of circulating ATX due to liver sinusoidal endothelial cell dysfunction from liver fibrosis was considered a main mechanism[28]. Perisinusoidal fibrosis is more frequently detected in alcoholic and non-alcoholic steatohepatitis than in viral hepatitis, with sinusoidal endothelial dysfunction also being reported in NAFLD[29].

Based on the above reports, we have hypothesized that serum ATX is increased in advanced stage NASH patients, but evidence is scarce on the relationship between circulating ATX concentration and histological severity in NAFLD. Accordingly, we measured serum ATX levels in 186 NAFLD patients who had undergone liver biopsy and examined for associations with clinicopathological findings.

MATERIALS AND METHODS

Patients and clinical examinations

This retrospective, cross-sectional study was approved by the Committee for Medical Ethics of Shinshu University School of Medicine (ID number: 3244) and performed

in accordance with the Helsinki declaration of 1975, 1983 revision. Informed consent was obtained from all patients. We enrolled 186 biopsy-proven Japanese NAFLD patients who were admitted to Shinshu University Hospital (Matsumoto, Japan) between November 2008 and May 2017. NAFLD was suspected based on the following criteria: (1) the presence of hepatorenal contrast and increased hepatic echogenicity on abdominal ultrasonography; (2) An average daily consumption of < 20 g/d of ethanol; and (3) the absence of other causes of liver dysfunction, such as viral hepatitis, drug-induced liver injury, autoimmune liver disease, primary sclerosing cholangitis, Wilson's disease, hereditary hemochromatosis, and citrin deficiency[30,31]. The diagnosis of NAFLD/NASH was confirmed with the histological findings of biopsied specimens. Body weight and height were measured before liver biopsy in a fasting state. All laboratory data were obtained in a fasting state on the day of liver biopsy. Homeostasis model assessment for insulin resistance (HOMA-IR), fibrosis-4 index (FIB-4), and aspartate aminotransferase (AST) to platelet ratio index (APRI) were calculated according to the following formulae: HOMA-IR = [fasting blood glucose (mg/dL) × fasting insulin (μU/mL)]/405[32,33], FIB-4 = [age (years) × AST (IU/L)]/[platelet count (10^9/L) × alanine aminotransferase (ALT) (IU/L)$^{1/2}$][34], and APRI = [AST/upper limit of normal; 28 (IU/L)] × [100/platelet count (10^9/L)][35]. One hundred sixty subjects (80 male and 80 female) whose liver function tests and body mass index (BMI) were within normal levels and having no past medical history of NAFLD were selected as healthy controls, with equal age distribution among the male and female individuals (twenties: 20 subjects, thirties: 20 subjects, forties: 20 subjects, fifties: 20 subjects). These healthy controls were same as our previous report[26]. Sera were obtained after overnight fasting on the day of the liver biopsy and stored at -80 ℃ until testing.

Measurement of ATX

Serum ATX concentrations were determined with a specific two-site enzyme immunoassay using the automated immunoassay analyzer AIA-2000 system (Tosoh Co., Tokyo, Japan), as described previously[36]. To prepare the 2-site immunoassay, R10.23 was digested with pepsin and the purified F(ab)$_2$ form using phenyl-5PW (Tosoh Co.) hydrophobic column chromatography in order to avoid the nonspecific binding of human antibodies against various animal IgG in human specimens, like human anti-mouse antibodies. Magnetic beads were coated with R10.23 F(ab)$_2$ and placed in the reaction cup, and 35 ng of alkaline phosphatase-labeled R10.21 in assay buffer (5% BSA, 5% sucrose, 10 mmol/L Tris-HCl, 10 mmol/L MgCl$_2$, pH 7.4) was added to the reaction cup. ATX assay reagent was prepared by immediate freeze-dry procedure of the reaction cup.

The ATX assay reagent thus prepared can be used with AIA-system.

Histological findings

Liver specimens of at least 1.5 cm in length were obtained from segment 5 or 8 using 14-gauge needles, as described previously, and immediately fixed in 10% neutral formalin. Sections of 4 μm in thickness were cut and stained by means of the hematoxylin and eosin and Azan-Mallory methods. The histological activity of NAFLD was assessed by an independent expert pathologist (KS) in a blinded manner according to the NAFLD scoring system proposed by Kleiner *et al*[37]. Steatosis was graded as 0 to 3 based on the rate of steatotic hepatocytes (< 5%, 5%-33%, > 33-66%, and > 66%, respectively). Lobular inflammation was graded as 0 to 3 based on the overall assessment of all inflammatory foci (no foci, < 2 foci/200 × field, 2-4 foci/200× field, and > 4 foci/200× field, respectively). Ballooning grade was scored as 0-2 by the frequency of ballooned hepatocytes (none, few, and many, respectively). NAFLD activity score (NAS) was calculated as the sum of steatosis, lobular inflammation, and ballooning scores, and NASH was defined as the presence of macrovesicular steatosis (> 5% of hepatocytes affected) and hepatocyte ballooning with or without lobular inflammation and fibrosis. Fibrosis stage was scored as follows: F0, none; F1, perisinusoidal or periportal; F2, perisinusoidal and portal/periportal; F3, bridging fibrosis; and F4, cirrhosis.

Statistical analysis

Clinical data are expressed as the number (percentage) or median (interquartile range). Statistical analyses were performed using StatFlex Ver. 6.0 (Artech Co., Ltd., Osaka, Japan) and SPSS 24.0 (IBM, Chicago, IL, United States) software. The Mann-Whitney *U* test was used for comparisons between two groups. Bonferroni's correction test was performed for multiple comparisons. Correlation analysis was conducted by Spearman's test. Diagnostic accuracy was evaluated using the area under the receiver operating characteristic (ROC) curve (AUC). Cut-off values were identified by the Youden index, with the nearest clinically applicable value to the cutoff being considered as the optimal threshold for clinical convenience. All statistical tests were two-sided and evaluated at the 0.05 level of significance.

RESULTS

Serum ATX levels were higher in NAFLD patients

The clinicopathological features of the 186 NAFLD patients enrolled in this study are summarized in Table 1. Eighty (43%) were male, and median age was 56 years. The number of patients according to fibrosis stage F0, F1, F2, F3, and F4 was 35, 89, 19, 34, and 9, respectively. Comparisons between genders revealed

Table 1 Clinicopathological features of 186 patients with non-alcoholic fatty liver disease

| | All (*n* = 186) | Male (*n* = 80) | Female (*n* = 106) | *P* value[1] |
	Median (IQR)/*n*	Median (IQR)/*n*	Median (IQR)/*n*	
Age (yr)	56 (46-65)	50 (38-59)	61 (54-66)	< 0.001
BMI (kg/m^2)	26.2 (23.8-29.6)	26.1 (24.3-29.4)	26.5 (23.6-29.7)	NS
Laboratory data				
Albumin (g/dL)	4.5 (4.3-4.7)	4.6 (4.4-4.8)	4.4 (4.2-4.7)	< 0.001
T-bil (mg/dL)	0.87 (0.69-1.17)	0.94 (0.74-1.26)	0.81 (0.67-1.07)	< 0.05
AST (IU/L)	41 (30-65)	39 (30-62)	42 (30-69)	NS
ALT (IU/L)	63 (38-97)	68 (43-103)	53 (33-89)	NS
γ-GT (IU/L)	54 (35-92)	64 (43-99)	50 (32-81)	< 0.05
TG (mg/dL)	122 (92-159)	122 (91-159)	121 (95-159)	NS
LDL-C (mg/dL)	130 (107-151)	132 (105-154)	130 (109-149)	NS
HDL-C (mg/dL)	51 (44-60)	48 (44-56)	55 (47-63)	
Plt (× 10^4/μL)	23.1 (18.5-26.8)	23.0 (19.6-26.7)	23.3 (17.6-26.9)	NS
HbA1c (%)	5.9 (5.7-6.6)	5.9 (5.6-6.5)	5.9 (5.7-6.6)	NS
FBG (mg/dL)	108 (98-121)	108 (98-121)	108 (97-121)	NS
IRI (mU/L)	11.2 (7.2-16.7)	10.5 (6.8-16.3)	11.5 (7.4-17.2)	NS
HOMA-IR	3.0 (1.9-4.6)	2.9 (1.8-4.5)	3.2 (2.0-4.7)	NS
Fe (μg/dL)	111 (90-137)	120 (92-146)	104 (88-129)	< 0.05
Ferritin (ng/mL)	146 (79-274)	172 (126-293)	113 (58-236)	< 0.001
AFP (ng/mL)	3.2 (2.2-4.8)	2.8 (2.1-4.0)	3.4 (2.6-5.2)	< 0.01
Fibrosis markers				
HA (ng/mL)	51 (28-91)	41 (25-62)	63 (34-118)	< 0.001
4C7S (ng/mL)	4.6 (3.8-5.7)	4.5 (3.8-5.5)	4.7 (3.8-6.6)	NS
FIB-4	1.35 (0.94-2.18)	1.12 (0.77-1.88)	1.53 (1.13-2.51)	< 0.001
APRI	0.69 (0.46-1.13)	0.66 (0.44-1.03)	0.71 (0.46-1.25)	NS
Histological findings				
Steatosis (1/2/3)	57/90/39	24/41/15	33/49/24	NS
Lobular inflammation (0/1/2/3)	9/101/69/7	6/48/23/3	3/53/46/4	< 0.05
Ballooning (0/1/2)	43/98/45	22/44/14	21/54/31	NS
Fibrosis (0/1/2/3/4)	35/89/19/34/9	16/43/8/13/0	19/46/11/21/9	NS

[1]Comparison between male and female subjects. IQR: Interquartile range; BMI: Body mass index; T-bil: Total bilirubin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γ-GT: Gamma-glutamyltransferase; TG: Triglyceride; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; Plt: Platelet; FBG: Fasting blood glucose; IRI: Immunoreactive insulin; HOMA-IR: Homeostasis model assessment of insulin resistance; AFP: Alpha-fetoprotein; HA: Hyaluronic acid; 4C7S: Type 4 collagen•7S; FIB-4: Fibrosis-4 index; APRI: AST to platelet ratio; NS: Not significant.

significant differences in fibrosis-related parameters, such as age, albumin, hyaluronic acid (HA), and FIB-4, but fibrosis stage distribution was comparable.

Median serum ATX levels were significantly higher in NAFLD patients than in healthy controls (0.86 *vs* 0.76 mg/L, *P* < 0.001) (Figure 1A). In agreement with a previous report demonstrating a gender difference in serum ATX levels[26], serum ATX levels were higher in female patients and controls than in their male counterparts (Figure 1B). The degree of a serum ATX concentration increase was significant in female NAFLD patients (Figure 1B).

Relationship between serum ATX levels and clinicopathological features in NAFLD patients
We observed significant but weak correlations between ATX and glucose metabolism, BMI, and iron status, but none with lipid profiles. ATX was significantly and positively correlated to the factors of age, AST, HA, type 4 collagen 7S (4C7S), FIB-4, and APRI and was significantly and negatively correlated to platelet count (Table 2), which supported an association with fibrosis stage in NAFLD[38]. Indeed, ATX was significantly and positively correlated with ballooning grade (*r* =

0.36, *P* < 0.001) and fibrosis stage (*r* = 0.45, *P* < 0.001) overall, with no significant relationships for steatosis grades (Table 2, Figure 2). These correlations were stronger for women than for men, as were the correlation coefficients for ballooning score and fibrosis stage (Table 2, Figure 3).

Performance of ATX for diagnosing fibrosis status
To assess the significance of ATX as a predictor of fibrosis stage, ROC analysis was performed. Cut off values, sensitivities, specificities, positive predictive values, negative predictive values, and accuracies for predicting the presence of fibrosis (≥ F1), significant fibrosis (≥ F2), severe fibrosis (≥ F3), and cirrhosis (F4) in overall, male, and female NAFLD patients are shown in Table 3, and these ROC curves are shown in Figure 4. The AUC values of ATX for predicting the presence of fibrosis (≥ F1), significant fibrosis (≥ F2), severe fibrosis (≥ F3), and cirrhosis (F4), were all more than 0.70 in respective analyses.

For comparison, ROC analysis of serum ATX and conventional fibrosis indicators (HA, 4C7S, APRI, and FIB-4) for determination of severe fibrosis (≥ F3) were performed (Table 4). Although sensitivity of ATX

Table 2 Correlation between autotaxin and clinicopathological findings

	All (*n* = 186)		Male (*n* = 80)		Female (*n* = 106)	
	r	*P* value	*r*	*P* value	*r*	*P* value
Age (yr)	0.48	< 0.001	0.45	< 0.001	0.28	< 0.01
BMI (kg/m^2)	0.18	< 0.05	0.06	NS	0.31	< 0.01
Platelet (× 10^4/μL)	-0.32	< 0.001	-0.28	< 0.05	-0.43	< 0.001
Albumin (g/dL)	-0.32	< 0.001	-0.10	NS	-0.31	< 0.01
AST (IU/L)	0.31	< 0.001	0.34	< 0.01	0.40	< 0.001
ALT (IU/L)	0.06	NS	0.14	NS	0.24	< 0.05
TG (mg/dL)	-0.09	NS	-0.14	NS	-0.08	NS
LDL-C (mg/dL)	-0.04	NS	-0.01	NS	-0.06	NS
HDL-C (mg/dL)	0.13	NS	-0.04	NS	-0.04	< 0.001
FBG (mg/dL)	0.22	< 0.01	0.36	0.001	0.21	< 0.05
IRI (mU/L)	0.20	< 0.01	0.15	NS	0.31	0.002
HOMA-IR	0.22	< 0.01	0.22	< 0.05	0.31	0.001
Fe (μg/dL)	0.09	NS	0.12	NS	0.35	< 0.001
Ferritin (ng/mL)	0.04	NS	0.22	NS	0.31	0.002
HA (ng/mL)	0.49	< 0.001	0.47	< 0.001	0.46	< 0.001
4C7S (ng/mL)	0.40	< 0.001	0.30	< 0.01	0.50	< 0.001
FIB-4	0.58	< 0.001	0.51	< 0.001	0.60	< 0.001
APRI	0.43	< 0.001	0.45	< 0.001	0.55	< 0.001
Histological findings						
Steatosis score	0.02	NS	0.12	NS	-0.03	NS
Lobular inflammation score	0.22	< 0.01	0.06	NS	0.25	< 0.01
Ballooning score	0.36	< 0.001	0.34	< 0.01	0.38	< 0.001
NAS	0.27	< 0.001	0.27	< 0.05	0.26	< 0.01
Fibrosis stage	0.45	< 0.001	0.44	< 0.001	0.53	< 0.001

Correlations were calculated using Spearman's test. ATX: Autotaxin; BMI: Body mass index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TG: Triglyceride; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; FBG: Fasting blood glucose; IRI: Immunoreactive insulin; HOMA-IR: Homeostasis model assessment of insulin resistance; HA: Hyaluronic acid; 4C7S: Type 4 collagen•7S; FIB-4: Fibrosis-4 index; APRI: AST to platelet ratio; NAS: NAFLD activity score; NS: Not significant.
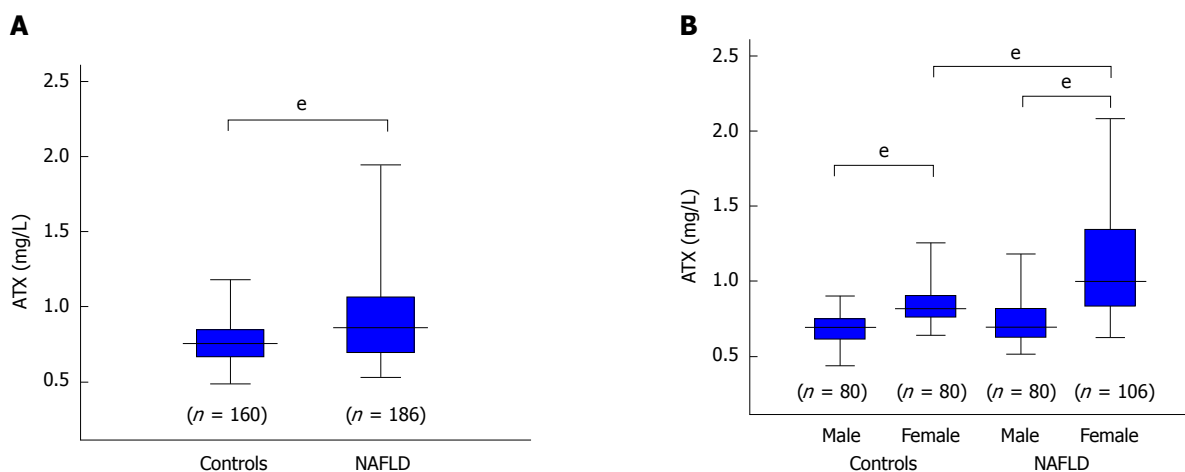

Figure 1 Comparison of autotaxin levels between controls and all patients with non-alcoholic fatty liver disease (A) and according to gender (B). The box plot shows the interquartile range, 95% confidence interval, and median. The difference between each group was tested with the Mann Whitney *U* test. [e]*P* < 0.001. ATX: Autotaxin; NAFLD: Non-alcoholic fatty liver disease.

is lower than those of HA, 4C7S, APRI, and FIB-4, specificity of ATX was highest (91%) compared to others.

DISCUSSION

Rachakonda *et al*[39] recently reported increased serum ATX levels in NAFLD patients. In severely obese and non-diabetic women, serum ATX was higher in those with NAFLD compared with those without NAFLD and positively correlated with insulin resistance. However, they did not assess liver pathology in their cohort of female subjects only. In this study, we compared serum ATX levels with clinicopathological background factors in biopsy-proven NAFLD patients and found that serum ATX levels were significantly related to hepatic fibrosis stage and ballooning score, implicating at least a partial reflection of histological severity in NAFLD.

Table 3 Diagnostic performance of autotaxin for predicting liver fibrosis stage in patients with non-alcoholic fatty liver disease

	Cut off	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
All patients							
≥ F1	0.73	0.71	77	57	89	36	73
≥ F2	1.19	0.75	45	94	80	77	78
≥ F3	1.19	0.75	51	91	63	86	82
F4	1.20	0.87	78	85	21	99	84
Male							
≥ F1	0.70	0.73	58	94	97	36	65
≥ F2	0.71	0.75	81	68	47	91	71
F3	0.82	0.74	62	82	40	92	79
Female							
≥ F1	1.03	0.76	53	95	98	31	60
≥ F2	1.19	0.80	66	91	82	81	81
≥ F3	1.19	0.78	73	86	67	89	82
F4	1.20	0.78	78	74	22	97	75

ATX: Autotaxin; AUC: Area under the receiver operating characteristic curve; PPV: Positive predictive value; NPV: Negative predictive value.
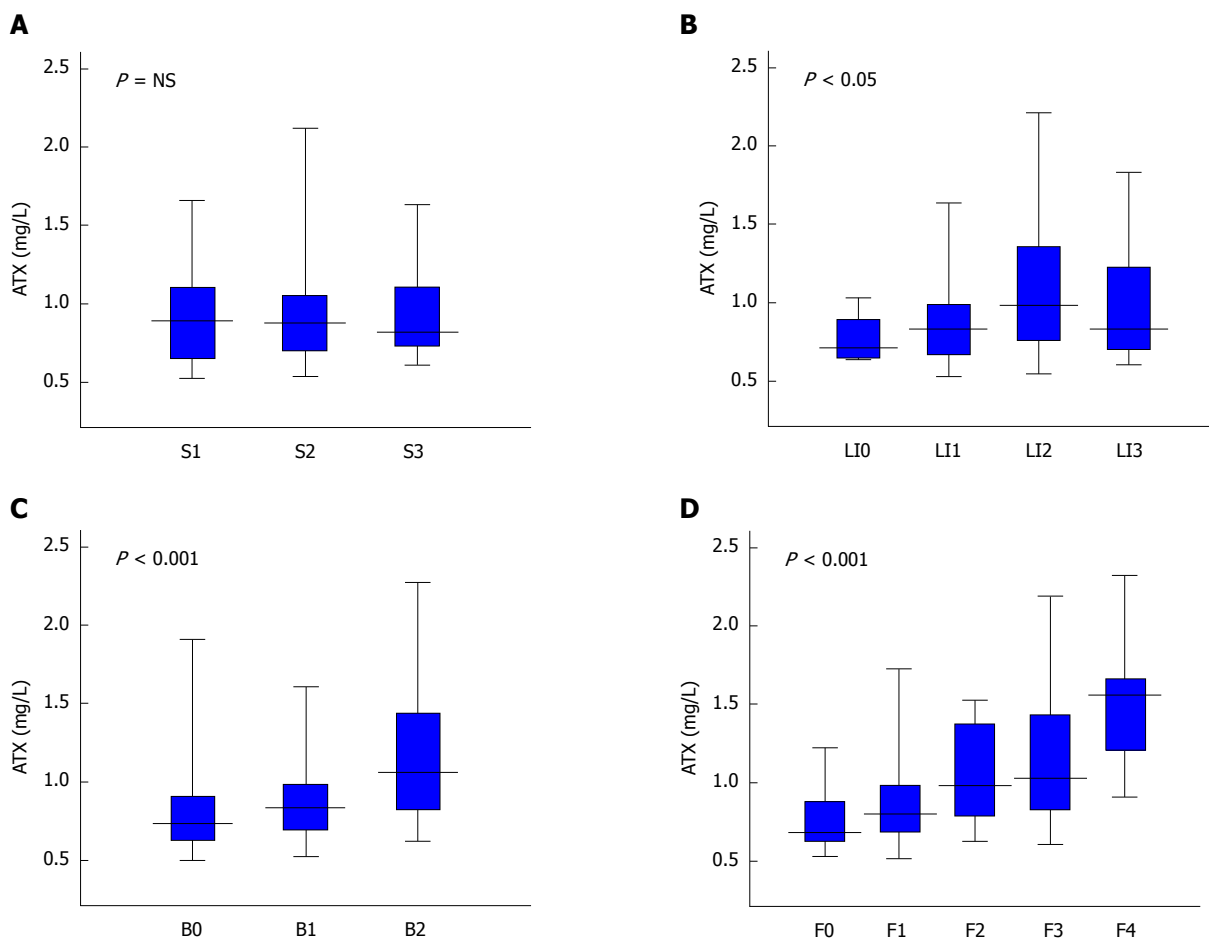

Figure 2 Relationship between autotaxin and histological grade in non-alcoholic fatty liver disease patients for steatosis (A), lobular inflammation (B), ballooning (C), and fibrosis (D). Table 1 presents the number of subjects for each histological stage. The Kruskal-Wallis test was used for multi-group simultaneous comparisons. *P* values are displayed in the upper left of each graph. ATX: Autotaxin; NAFLD: Non-alcoholic fatty liver disease; NS: Not significant.

The correlation between serum ATX levels and the severity of hepatic fibrosis has been explained by a mechanism of impaired circulating ATX degradation in damaged or impaired sinusoidal endothelial cells[28]. However, a recent study documented that ATX expression in hepatocytes activated hepatic stellate cells and amplified the fibrotic process, suggesting direct fibrosis-promoting properties of ATX[40]. Since ATX is a novel biomarker for hepatic fibrosis in chronic hepatitis C patients[26,27], we presumed similar results in NAFLD patients, but the correlation between ATX and fibrosis stage was comparatively weaker.

Table 4 Diagnostic performance of autotaxin and conventional fibrosis indicators for predicting severe fibrosis (≥ F3) in patients with non-alcoholic fatty liver disease

	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
All patients						
ATX	0.75	51	91	63	86	82
HA	0.82	93	63	44	96	70
4C7S	0.87	75	88	64	92	85
APRI	0.82	60	89	62	88	82
FIB-4	0.85	79	74	48	92	75
Male						
ATX	0.74	62	82	40	92	79
HA	0.76	85	72	41	95	75
4C7S	0.81	69	89	56	94	86
APRI	0.74	77	64	29	93	66
FIB-4	0.81	92	75	41	98	78
Female						
ATX	0.78	73	86	67	89	82
HA	0.86	78	86	68	91	83
4C7S	0.89	78	90	75	92	87
APRI	0.86	63	95	83	87	86
FIB-4	0.85	80	75	56	90	76

AUC: Area under the receiver operating characteristic curve; PPV: Positive predictive value; NPV: Negative predictive value; ATX: Autotaxin; HA: Hyaluronic acid; 4C7S: Type 4 collagen•7S; APRI: AST to platelet ratio; FIB-4: Fibrosis-4 index.
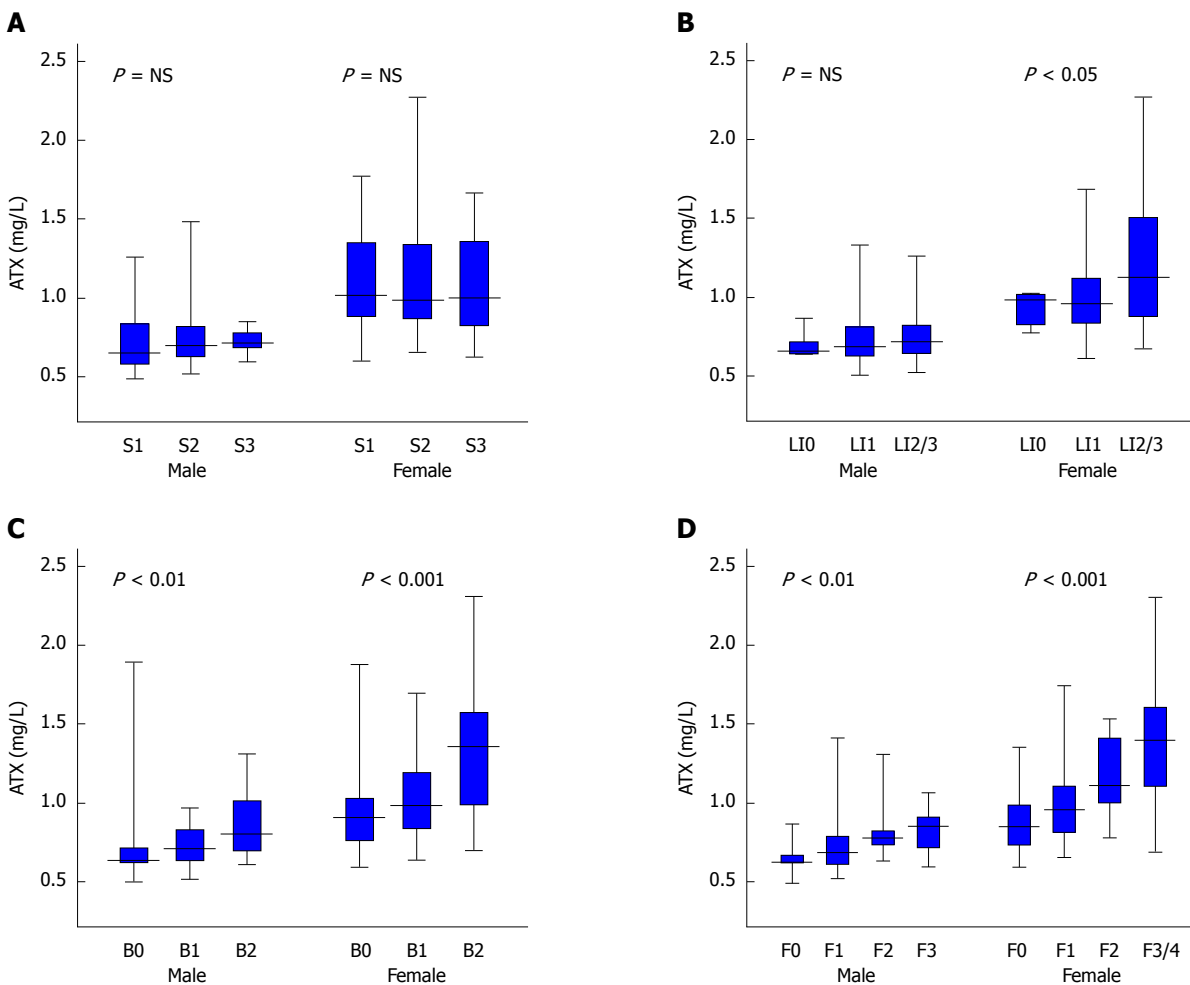

Figure 3 Relationship between autotaxin and histological grade in non-alcoholic fatty liver disease patients by gender for steatosis (A), lobular inflammation (B), ballooning (C), and fibrosis (D). Table 1 presents the number of subjects for each histological stage. The Kruskal-Wallis test was used for multi-group simultaneous comparisons. *P* values are displayed in the upper left of each graph. ATX: Autotaxin; NAFLD: Non-alcoholic fatty liver disease; NS: Not significant.
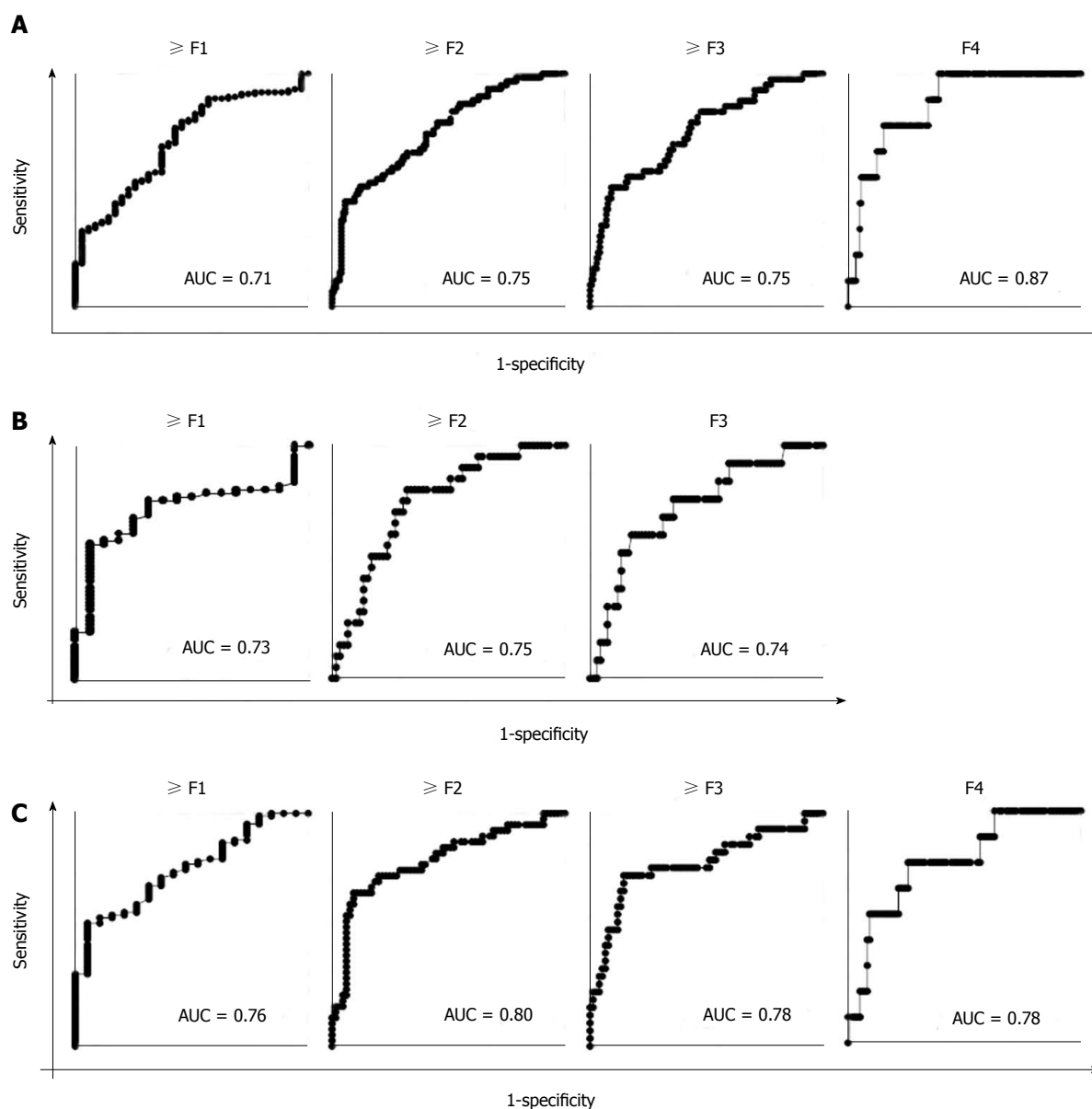
Figure 4 Receiver operating characteristic analysis of autotaxin for the estimation of the presence of fibrosis (≥ F1), significant fibrosis (≥ F2), severe fibrosis (≥ F3), and cirrhosis (F4) in all (A), male (B), and female (C) patients. The areas under the receiver operating characteristic curve are displayed in the lower right of each graph. AUC: Receiver operating characteristic curve; F: Fibrosis.

Thus, other mechanisms determining circulating ATX concentrations may exist as ATX is present in various tissues, such as white adipose tissue and the nervous system[41-43]. The importance of visceral fat has also been discussed[44], but in this study, we have not been able to examine waist circumference or waist-to-hip ratio, so this point is the limitation of this study.

In this study, we also conducted AUC analysis of ATX for determination of severe fibrosis (≥ F3) compared to conventional fibrosis indicators (HA, 4C7S, APRI, and FIB-4). AUC values and sensitivity of ATX was inferior to those other indicators[41], but specificity of ATX was highest among those other indicators. So ATX might be useful as a biomarker to exclude severe hepatic fibrosis.

Serum ATX levels were significantly associated with hepatocyte ballooning in our cohort, and a correlation was detected between fibrosis stage and ballooning grade ($r = 0.56$, $P < 0.001$). Ballooning degeneration is caused by an impaired intracellular cytoskeleton and resultant protein transport and appears after exposure to oxidative and endoplasmic reticulum stresses and during lipoapoptotic processes[45]. ATX expression was up-regulated by oxidative stress in microglia[46] and by LPC (18:1), an inducer of lipoapoptosis[47], in isolated hepatocytes[42]. Additionally, intravenous injection of LPC (18:1) into mice increased hepatic *Enpp2* mRNA expression and hepatocyte apoptosis[40]. These findings may explain how circulating ATX concentrations are

positively correlated with the prevalence of hepatocytes with ballooning degeneration.

In this study, we examined the relationship between NAFLD activity score as the severity of NAFLD/NASH and ATX, the correlation coefficient was significant but not high (r = 0.27, P < 0.001, Table 2). It seems difficult to predict the histological severity of NAFLD with ATX alone.

In conclusion, serum ATX levels were significantly higher in NAFLD patients over controls and correlated with ballooning score and fibrosis stage, especially in female patients. Further prospective research in larger cohorts is necessary for understanding the metabolism of circulating ATX in NAFLD.

ARTICLE HIGHLIGHTS

Research background
The prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing worldwide. NAFLD exhibits a wide spectrum, ranging from non-alcoholic fatty liver to non-alcoholic steatohepatitis (NASH) and ensuing cirrhosis and hepatocellular carcinoma. Although the evaluation of NAFLD/NASH depends on the histological findings, there is a limitation and an alternative method is required.

Research motivation
Several studies have attempted to estimate histological severity in NAFLD using various serum biomarkers, but the accuracy of these techniques remains unsatisfactory.

Research objectives
Recently, elevated serum autotaxin (ATX) has been implicated in fibrosis progression in chronic liver disease, especially hepatitis C. So, we examine the relationship between serum ATX concentrations and clinicopathological findings in NAFLD patients.

Research methods
One hundred eighty-six NAFLD patients who had undergone liver biopsy between 2008 and 2017 were retrospectively enrolled. Serum samples were collected at the time of biopsy and ATX was measured by enzyme immunoassays. Sera obtained from 160 healthy, non-obese individuals were used as controls. Histological findings were graded according to an NAFLD scoring system and correlations with serum ATX were calculated by Spearman's test. Diagnostic accuracy was evaluated using the area under the receiver operating characteristic curve (AUC). Cut-off values were identified by the Youden index, and the nearest clinically applicable value to the cutoff was considered the optimal threshold for clinical convenience.

Research results
Serum ATX levels were significantly higher in NAFLD patients than in controls (0.86 *vs* 0.76 mg/L, P < 0.001) and correlated significantly with ballooning score and fibrosis stage (r = 0.36, P < 0.001 and r = 0.45, P < 0.001, respectively). Such tendencies were stronger in female patients. There were no remarkable relationships between ATX and serum alanine aminotransferase, lipid profiles, or steatosis scores. The AUC values of ATX for predicting the presence of fibrosis ($\geqslant$ F1), significant fibrosis ($\geqslant$ F2), severe fibrosis ($\geqslant$ F3), and cirrhosis (F4), were all more than 0.70 in respective analyses.

Research conclusions
Serum ATX levels may at least partially reflect histological severity in NAFLD.

Research perspectives
In order to evaluate the severity of NAFLD, it is considered that a method that can simultaneously evaluate activity and fibrosis is necessary.

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