Serum Autotaxin Concentrations Reflect Changes in Liver Stiffness and Fibrosis After Antiviral Therapy in Patients with Chronic Hepatitis C

Wataru Ando,[1] Hiroaki Yokomori,[2] Fumihiko Kaneko,[3] Mana Kaneko,[1] Koji Igarashi,[4] and Hidekazu Suzuki[5]

The purpose of this study was to determine whether serum autotaxin concentrations reflect liver stiffness in patients with chronic hepatitis C virus (HCV) treated with direct-acting antiviral agents. Adult patients with chronic HCV were enrolled from January 2016 to August 2017. Autotaxin concentrations in these patients were compared with those of a control group consisting of healthy individuals. Liver stiffness was determined by transient elastography. The relationship between fibrosis markers and fibrosis scores was evaluated before and after treatment. Data from 155 HCV patients and 56 control subjects were analyzed. Autotaxin concentrations were significantly higher in HCV patients with liver stiffness scores less than or equal to 7.4 kPa versus controls. Autotaxin concentrations at the end of treatment and beyond were significantly lower than those prior to treatment. Pretreatment and posttreatment autotaxin concentrations in male and female patients with liver stiffness scores greater than 14.9 kPa changed significantly ($P < 0.01$ and $P < 0.01$, respectively). From the start of treatment to 6 months following treatment, the fibrosis marker/liver stiffness score ratios changed as follows: autotaxin: 0.189 (95% confidence interval [CI]: 0.169-0.209) to 0.191 (95% CI: 0.166-0.216; $P = 0.88$); *Wisteria floribunda* agglutinin-positive Mac-2-binding protein: 0.294 (95% CI: 0.256-0.332) to 0.223 (95% CI: 0.191-0.255; $P < 0.001$); hyaluronic acid: 19.05 (95% CI: 14.29-23.81) to 13.92 (95% CI: 11.16-16.70; $P = 0.044$); and type IV collagen 7S: 0.560 (95% CI: 0.515-0.604) to 0.546 (95% CI: 0.497-0.895; $P = 0.052$). *Conclusion:* Autotaxin concentrations reflect liver stiffness before and after antiviral treatment in patients with chronic HCV infection. (*Hepatology Communications* 2018; 2:1111-1122)

Liver fibrosis associated with chronic hepatitis C virus (CHC) infection progresses through liver inflammation and increases the risk of liver cirrhosis and hepatocellular carcinoma (HCC).[1] When used to treat CHC, direct-acting antiviral agents (DAAs) achieve high sustained virological response (SVR) rates. However, despite viral elimination, the risk of the liver fibrosis progressing to liver cirrhosis

and carcinogenesis remains.[2,3] Therefore, liver fibrosis must be monitored after DAA treatment.

Invasive and noninvasive methods are used to monitor liver fibrosis. Invasive liver biopsies are difficult to undertake regularly because of the risk of bleeding, the length of hospitalization required to manage these risks, and the associated costs.[4] Transient elastography (TE) and blood sampling to determine the fibrosis
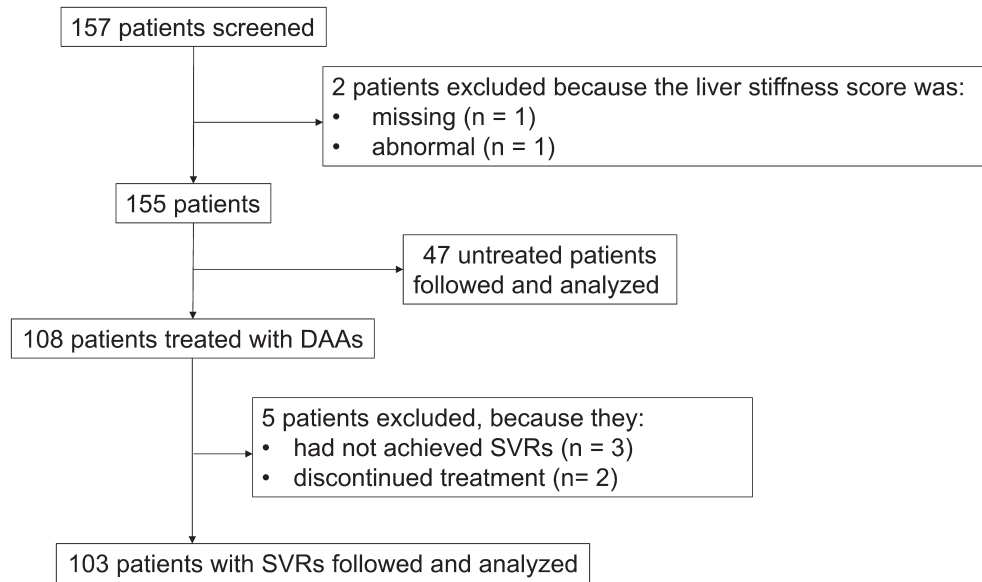
FIG. 1. Flow chart showing the inclusion of the HCV-infected patients in the study.

marker levels are less-invasive liver fibrosis monitoring methods. TE determines the degree of hepatic fibrosis,[5,6] but it requires expensive equipment, recruitment of experienced technicians, and extended consulting hours, and patient throughput is low. Measuring fibrosis marker concentrations in the blood can indicate the stage of liver fibrosis and the related costs are generally lower than those associated with TE. Although previous reports have described reductions in liver fibrosis marker levels in DAA-treated patients with hepatitis C virus (HCV) who had achieved SVRs,[7-9] it is not clear whether the observed reductions in the fibrosis markers correspond to improvements in liver fibrosis. Therefore, a liver fibrosis marker that truly reflects liver fibrosis variations remains to be identified.

Autotaxin (ATX) is a secreted enzyme originally discovered in conditioned medium from A2058 human melanoma cell cultures.[10] ATX has an important enzymatic function in converting lysophosphatidylcholine to lysophosphatidic acid (LPA), which has various physiological roles in cell migration, neurogenesis, angiogenesis, smooth-muscle contraction, platelet aggregation, and wound healing.[11-14] LPA also stimulates the proliferation and contractility of hepatic stellate cells.[15] ATX is present in serum and is metabolized by liver sinusoidal endothelial cells. Liver fibrosis reduces the capacity to metabolize ATX, resulting in increases in the ATX level in serum.[16,17] ATX has been shown to be useful as a serum marker for determining the fibrosis stage

ARTICLE INFORMATION:

From the [1]Department of Clinical Pharmacy, School of Pharmacy, Kitasato University, Tokyo, Japan; [2]Department of Gastroenterology, Kitasato University Medical Center, Saitama, Japan; [3]Department of Internal Medicine, Saitama Medical Center, Saitama, Japan; [4]Bioscience Division, Research and Development Management Department, Tosoh Corporation, Kanagawa, Japan; [5]Fellowship Training Center and Medical Education Center, Keio University School of Medicine, Keio University, Tokyo, Japan

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Hidekazu Suzuki, M.D., Ph.D.
Fellowship Training Center and Medical Education Center
Keio University School of Medicine
35 Shinanomachi, Shinjuku-ku

Tokyo 160-8582, Japan
E-mail address: hsuzuki.a6@keio.jp
Tel: +81-3-5363-3914
Fax: +81-3-5363-3967

in CHC patients.[18,19] In addition, ATX is suggested to be useful as an indicator of the severity of liver disease and for determining the prognosis of cirrhotic patients[20] and HCC recurrence in combination with the levels of LPA receptors.[21]

ATX is a new liver fibrosis marker that can be used to effectively evaluate liver fibrosis.[18,22] In this study, we aimed to determine whether ATX concentrations reflect liver stiffness levels assessed by TE in DAA-treated CHC patients and whether ATX has the potential to replace TE.

Patients and Methods

This study was conducted at the Kitasato University Medical Center (Kitamoto, Saitama, Japan) from January 1, 2016, until August 31, 2017. The study protocol was approved by Kitasato University Medical Center's Ethics Committee on December 25, 2015; it conforms to the principles of the 1975 Declaration of Helsinki, as reflected by its prior approval by the institution's human research committee.

STUDY SUBJECTS

Adult patients (≥ 20 years of age) with CHC were enrolled. Patients were excluded if they had Child class B or worse liver failure, were receiving a hepatotoxic drug or any agent that affects cytokines, required hemodialysis, or had complications such as HCC, primary biliary cirrhosis, primary sclerosing cholangitis, pancreatitis, type 1 diabetes, uncontrolled thyroid deficiencies, or severe renal failure.

A control group consisting of heathy volunteers was included in the study. The patient enrollment process is illustrated in Fig. 1. All patients provided written informed consent to participate in the study.

FIBROSIS MARKER ASSAYS AND FIBROSIS SCORING

Serum concentrations of ATX were measured using a two-site enzyme immunoassay and an automated immunoassay analyzer (Tosoh, Tokyo, Japan). Immunoassay kits were used to measure the serum concentrations of *Wisteria floribunda* agglutinin-positive Mac-2-binding protein (WFA(+)-M2BP; HISCL, Sysmex, Hyogo, Japan), hyaluronic acid (HA; Wako

Pure Chemical Industries, Tokyo, Japan), and type IV collagen 7S (IVC7S; Sceti Medical Labo K.K., Tokyo, Japan). Additional surrogate blood indices of liver fibrosis assessed at enrollment were the fibrosis-4 index and the aspartate aminotransferase (AST)-to-platelet ratio, which were calculated as follows: (age [years] × AST [IU/L]/platelet count [10^9/L] × alanine aminotransferase [ALT] [IU/L]$^{1/2}$)[23] and (AST/upper limit of normal; 40 [IU/L]) × (100/platelet count [10^9/L]),[24] respectively.

ASSESSMENT OF LIVER STIFFNESS

Liver stiffness was measured using TE (Fibroscan; Echosens, Paris, France). The fibrosis grades were determined from liver stiffness cutoff values.[25] The fibrosis levels were categorized as follows, where E denotes the liver stiffness score: E ≤ 7.4 kPa, no fibrosis, and pericellular or isolated portal fibrosis; 7.4 kPa < E ≤ 14.9 kPa, combined pericellular and portal fibrosis, and bridging fibrosis; and E >14.9 kPa, cirrhosis.

COMPARISON BETWEEN FIBROSIS GRADES AND FIBROSIS MARKER CONCENTRATIONS

The ATX, WFA(+)-M2BP, HA, and IVC7S serum concentrations were evaluated in the context of the liver stiffness categories.

ASSESSMENT OF FIBROSIS MARKER/LIVER STIFFNESS SCORE RATIO

To confirm the association between variations in ATX concentration and liver stiffness after DAA therapy, the ATX concentration to E value ratios (ATX/E) were determined. Briefly, the ATX/E ratios were calculated at each point of the analysis (i.e., pre, first, second, and third), which were defined as immediately before antiviral treatment, the end of treatment, and 3 months and 6 months after the end of treatment, respectively. The ratio of each ATX/E to the mean value at the pre-analysis point was the reference value. Variations in the ATX/E, WFA(+)-M2BP/E, HA/E, and IVC7S/E ratios were also determined at each time point. Data from untreated patients and treated patients who had achieved SVRs were included in these analyses.

STATISTICAL ANALYSES

Statistical analyses were conducted using EZR software, version 1.32 (Saitama Medical Center, Jichi Medical University, Shimotsuke, Japan) and the R statistical package, version 2.2.0 (the R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/).[26] Spearman's rank correlation coefficient was used to determine correlations between fibrosis marker concentrations and liver stiffness. Baseline continuous data are expressed as the median and the first-to-third quartile, or a mean value and the SDs. Trends in serum ATX concentrations were assessed in the context of the fibrosis stages using a Jonckheere-Terpstra test. The categorical variables are reported as frequencies and percentages. The groups were compared with respect to the continuous variables using the Mann-Whitney U test. Changes in values in relation to treatment were analyzed using Friedman's test. The sensitivities and specificities of the serum fibrosis markers and the fibrosis staging were calculated and assessed using receiver operating characteristic (ROC) curves. The diagnostic performances of the scoring systems were assessed by analyzing the ROC curves. An area under the curve (AUC)

TABLE 1. CLINICAL CHARACTERISTICS OF PATIENTS WITH CHC INFECTION AND HEALTHY CONTROL INDIVIDUALS

Patients With CHC infections	All		Male		Female		P Value*†
n	155		67		88		
	Median	(IQR)	Median	(IQR)	Median	(IQR)	
Age, years	71	(61-77)	70	(61-75)	72	(63-78)	0.273
BMI	22.3	(20.4-24.3)	23.1	(20.7-24.2)	21.76	(20.5-24.3)	0.437
HCV genotype (1/2/3), n	114/30/1		49/15/0		70/15/1		0.216
HCV-RNA, log IU/mL	6.3	(5.80-6.60)	6.2	(5.5-6.5)	6.3	(5.9-6.6)	0.264
T-Bil, mg/L	0.9	(0.7-1.1)	0.85	(0.7-1.2)	0.9	(0.7-1.1)	0.707
AST, IU/L	36	(27-50)	40	(30-50)	34	(26-50)	0.11
ALT, IU/L	30	(21.5-45)	33.5	(24-52)	27	(19-43)	0.020
Alb, g/L	4.0	(3.8-4.3)	4.1	(3.8-4.5)	4.0	(3.8-4.3)	0.147
Plt, 10^9/L	151	(108-190)	154.5	(111.2-192.5)	143.0	(102.0-187.0)	0.663
ATX, mg/L	1.53	(1.06-2.23)	1.3	(0.92-1.69)	1.82	(1.28-2.40)	< 0.001
M2BPGi, COI	2.03	(1.09-3.73)	1.98	(1.04-3.80)	2.13	(1.15-3.68)	0.462
HA, µg/L	102	(48-225)	93.8	(52.9-270.5)	103	(46.9-211)	0.549
IVC7S, µg/L	4.65	(3.90-6.47)	4.8	(4.10-6.60)	4.55	(3.73-6.30)	0.314
APRI†	0.64	(0.39-1.07)	0.62	(0.42-1.21)	0.65	(0.38-3.95)	0.42
Fib-4 index†	3.18	(2.05-5.15)	3.03	(0.85-25.37)	3.57	(0.41-13.11)	0.45
Healthy control individuals	All		Male		Female		
n	56		40		16		P Value
Age, years	45	(37-50)	46.5	(36.25-52.25)	44.5	(39.75-46.75)	0.374
BMI	21.8	(19.8-23.7)	22.75	(20.88-23.80)	19.45	(18.60-20.73)	0.001
T-Bil, mg/L	0.8	(0.6-0.9)	0.8	(0.60-0.90)	0.8	(0.70-1.02)	0.442
AST, IU/L	19	(17-22)	20	(17.00-23.00)	18.5	(13.75-21.00)	0.067
ALT, IU/L	17	(13-20)	19	(14.75-22.00)	14	(11.00-17.25)	0.004
Alb, g/L	21.8	(19.8-23.7)	4.5	(4.40-4.70)	4.4	(4.38-4.62)	0.3
Plt, 10^9/L	223	(193-248)	219.5	(186.0-249.8)	225.5	(196.8-243.5)	0.765
ATX, mg/L	0.74	(0.67-0.83)	0.72	(0.65-0.76)	0.91	(0.81-1.00)	< 0.001
M2BPGi, COI	0.34	(0.30-0.46)	0.34	(0.30-0.44)	0.35	(0.32-0.53)	0.336
HA, µg/L	13	(8-23)	16	(11.75-26.50)	9	(6.00-12.25)	0.002
IVC7S, µg/L	3.3	(2.8-3.6)	3.3	(2.70-3.60)	3.2	(2.80-3.65)	0.61
APRI	0.22	(0.19-0.25)	0.23	(0.20-0.27)	0.19	(0.18-0.22)	0.009
FIB-4 index	0.92	(0.70-1.19)	0.95	(0.74-1.29)	0.89	(0.67-1.02)	0.355

*Comparison between male and female subjects using the Mann-Whitney U test.
†The APRI and the FIB-4 index were determined in 113 samples from CHC patients.
Abbreviations: Plt, platelet; Alb, albumin; T-Bil, total-bilirubin; M2BPGi, Mac-2-binding protein glycosylation isomer; COI, cutoff index; APRI, AST-to-platelet ratio; FIB, fibrosis.

that was close to 1.0 was defined as reflecting a high level of diagnostic accuracy. The sensitivities, specificities, positive predictive values, and negative predictive values were calculated at the cutoff values identified using Youden's index. A value of $P < 0.05$ was considered significant.

Results

PATIENT CHARACTERISTICS

Of the 157 patients with CHC infection enrolled into the study, 2 were excluded because the liver stiffness score was not available in 1 patient and was abnormal in another (Fig. 1). Therefore, data from 155 patients were included in the analysis. Of these, 67 were male and 88 were female, with a median age of 71 years (interquartile range [IQR]: 61-77). The patients had HCV genotypes 1 (n = 119), 2 (n = 30), 3 (n = 1), and indeterminate (n = 4), with HCV-ribonucleic acid levels greater than 1.2 log/mL. The control group consisted of 56 subjects (40 male, 16 female) with a median age of 45 years (IQR: 37-51). Liver function test results in this group were within the normal parameters. The clinical characteristics of the study participants are summarized in Table 1.

Of the patients with HCV, 108 received antiviral therapy: ombitasvir/paritaprevir/ritonavir (n = 51), sofosbuvir/ledipasvir (n = 24), sofosbuvir/ribavirin (n = 22), daclatasvir/asunaprevir (n = 8), ombitasvir/paritaprevir/ritonavir combined (n = 1), pegylated interferon and ribavirin combined with vaniprevir (n = 1), and simeprevir (n = 1). The SVR rates were 97.6% (83 of 85) for genotype 1 and 95.7% (22 of 23) for genotype 2.

SERUM FIBROSIS MARKER CONCENTRATIONS

Table 1 presents the participants' fibrosis marker concentrations. The median serum ATX concentration in all of the HCV-infected patients (1.53 [IQR: 1.06-2.11] mg/L) before treatment was significantly higher than that in the control individuals (0.74 [IQR: 0.67-0.83] mg/L; $P < 0.001$). The median serum ATX concentration in the female HCV-infected patients (1.80 [IQR: 1.27-2.38] mg/L) was significantly higher than that seen in the male HCV-infected patients (1.30 [IQR: 0.92-1.79] mg/L; $P < 0.001$). The serum WFA(+)-M2BP levels, but not the HA or IVC7S levels, tended to be higher in the female HCV-infected patients compared with those in the male HCV-infected patients.

CORRELATION BETWEEN SERUM AUTOTAXIN CONCENTRATIONS AND LIVER FIBROSIS STAGE

Compared with the control group, the serum ATX concentrations in the E ≤ 7.4 kPa and E ≤ 14.9 kPa groups were significantly higher in all of the patients and in both sexes (Fig. 2). The serum ATX concentrations correlated with the liver stiffness stage in the male ($r = 0.663$, $P < 0.001$) and female ($r = 0.745$, $P < 0.001$) HCV-infected patients.
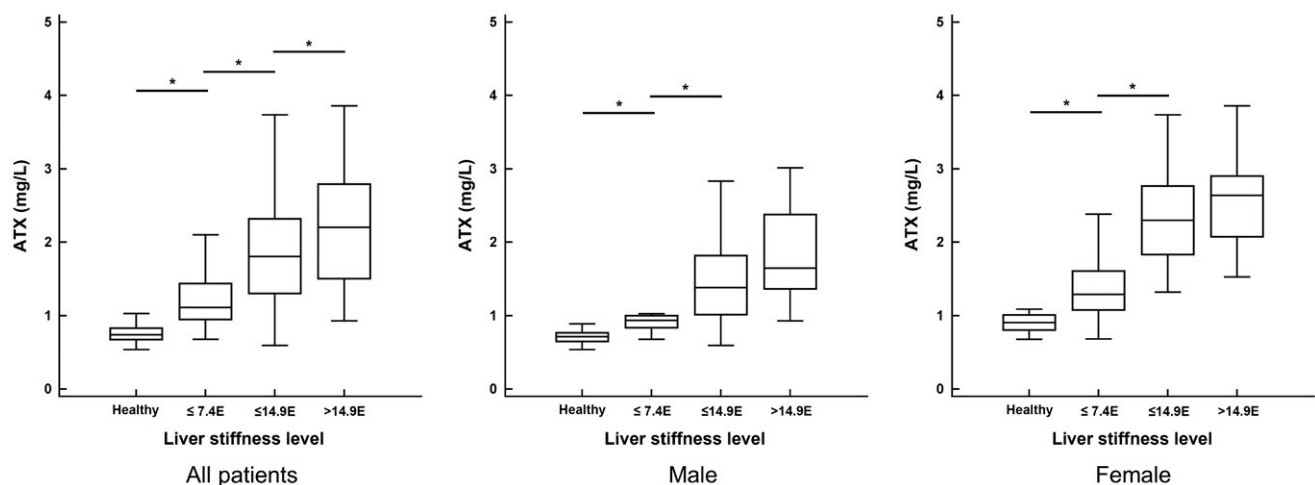

FIG. 2. Correlation between ATX concentration and liver fibrosis stage categorized according to the liver stiffness value.

DIAGNOSTIC PERFORMANCE OF AUTOTAXIN

Table 2 lists the AUCs for ATX in controls versus HCV-infected patients grouped according to liver stiffness score (E ≤ 7.4 kPa, 7.4 kPa < E ≤ 14.9 kPa, and E < 14.9 kPa). The cutoff values for the control group and the HCV group were 0.83 mg/L and 1.09 mg/L, respectively. High AUC, sensitivity, and specificity values were determined. The AUC for ATX in the female participants tended to be higher than that for the male participants. The cutoff value for E ≤ 7.4 kPa was 1.02 mg/L for the male participants (AUC: 0.788; 95% confidence interval [CI]: 0.677-0.880) and 1.69 mg/L for the female participants (AUC: 0.913; 95% CI: 0.833-0.963).

COMPARISON OF THE AUC FOR FIBROSIS MARKERS AND THE CALCULATED FIBROSIS SCORES

The AUCs indicated that the diagnostic performance of ATX was similar to the diagnostic performances of WFA(+)-M2BP, HA, and IVC7S (Table 3). The fibrosis-4 index and the AST-to-platelet ratio showed high AUCs. The AUCs for E ≤ 7.4 kPa and 7.4 kPa < E ≤ 14.9 kPa were similar for male and female subjects. The AUC for the male subjects was lower than that for the healthy individuals versus the CHC-infected patient groups.

SERUM AUTOTAXIN CONCENTRATIONS AND ANTIVIRAL THERAPY

The ATX concentrations at the end of antiviral therapy (first point) and beyond were considerably lower than the pretreatment levels in the male and female subjects (Fig. 3). The ATX concentration decreased at the highest rate at the end of treatment. The ATX/E ratio did not change between the end of treatment and 6 months after treatment (Fig. 4). The ATX concentrations changed dramatically from pretreatment to the end of treatment in the CHC-infected male and female patients in the E > 14.9 kPa group ($P < 0.01$ and $P < 0.01$, respectively). The E ≤ 7.4 kPa and E ≤ 14.9 kPa groups did not show any changes between these points.

VARIATION IN FIBROSIS MARKER/LIVER STIFFNESS SCORE RATIOS IN TREATED/UNTREATED PATIENTS

The variations in the ATX/E ratio over time were slight (Fig. 4). The HA/E and IVC7S/E ratios were low and the WFA(+)-M2BP/E ratio differed considerably between the DAA-treated and DAA-untreated patients. The WFA(+)-M2BP/E ratio declined by approximately 20% after DAA therapy compared with that in the DAA-untreated patients. To analyze the

TABLE 2. DIAGNOSTIC PERFORMANCE OF ATX IN PATIENTS WITH CHC INFECTIONS

	Cutoff Value	AUC	(95% CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
All patients							
E ≤ 7.4 kPa	1.44	0.787	0.713-0.849	74.7	76.6	81.2	69.0
7.4 kPa < E ≤ 14.9 kPa	1.91	0.762	0.686-0.827	70.0	72.7	38.9	90.7
E > 14.9 kPa	1.91	0.755	0.664-0.845	68.8	73.8	51.0	94.5
Male patients							
E ≤ 7.4 kPa	1.02	0.788	0.677-0.880	82.2	84.2	92.5	66.7
7.4 kPa < E ≤ 14.9 kPa	1.30	0.789	0.669-0.881	86.7	63.3	41.9	93.9
E > 14.9 kPa	1.35	0.760	0.635-0.884	81.2	62.7	59.4	98.2
Female patients							
E ≤ 7.4 kPa	1.69	0.913	0.833-0.963	90.5	80.0	80.9	90.0
7.4 kPa < E ≤ 14.9 kPa	1.91	0.806	0.708-0.883	93.3	63.9	35.0	97.9
E > 14.9 kPa	1.91	0.819	0.723-0.916	93.8	65.3	54.2	99.9

Note: Cutoff values were determined using Youden's index; the nearest clinically applicable value to the cutoff value was considered the optimal threshold for clinical convenience. E ≤ 7.4 kPa, 7.4 kPa < E ≤ 14.9 kPa, and E > 14.9 kPa were evaluated in patients with CHC infections only.
Abbreviations: PPV, positive predictive value; NPV, negative predictive value; E, liver stiffness.

TABLE 3. COMPARISON OF THE AUC FOR FIBROSIS MARKERS AND FIBROSIS STAGE

	ATX	WFA(+)-M2BP	HA	IVC7S
All patients				
Healthy vs CHC	0.934	0.971*	0.957	0.846*
E ≤ 7.4 kPa	0.787	0.835	0.851	0.827
7.4 kPa < E ≤ 14.9 kPa	0.762	0.822	0.816	0.812
E > 14.9 kPa	0.755	0.827	0.870	0.826
Male patients				
Healthy vs CHC	0.939	0.987*	0.968	0.885
E ≤ 7.4 kPa	0.788	0.763	0.822	0.785
7.4 kPa < E ≤ 14.9 kPa	0.789	0.801	0.813	0.761
E > 14.9 kPa	0.760	0.756	0.785	0.746
Female patients				
Healthy vs CHC	0.925	0.954	0.978*	0.810*
E ≤ 7.4 kPa	0.913	0.907	0.887	0.864
7.4 kPa < E ≤ 14.9 kPa	0.806	0.844	0.819	0.853
E > 14.9 kPa	0.819	0.854	0.836	0.870

*$P < 0.05$ versus ATX at a nondirectional two-tailed significance level.

Abbreviation: E, liver stiffness.

relationship between the decrease in the liver stiffness measurement (LSM) value at the end of DAA treatment and reduced inflammation, the upper limit of normal (ULN) before treatment was used as a reference to group patients into a high ALT group (> 2 × ULN) and a low ALT group (≤ 2 × ULN). The high ALT group consisted of 17 patients and the low ALT group consisted of 85. When the high ALT and low ALT group were compared, the ATX/E ratio was constant from pretreatment to the third point of the analysis (Fig. 5). WFA(+)-M2BP, HA, and IVC7S serum concentration showed large changes in their E ratios at the end of treatment (first point).

Discussion

Predicting the development of inflammation and fibrosis in HCV-infected patients assists clinicians in treatment decision making, and blood tests for liver fibrosis markers can promote better practice. In this study, AUC analysis revealed that assessment of ATX concentration showed a high degree of diagnostic accuracy in the E ≤ 7.4 kPa group prior to DAA therapy. Furthermore, the ATX concentration identified patients with E ≤ 14.9 and E > 14.9 kPa. The similarity of the ATX AUC to those of WFA(+)-M2BP, HA, and IVC7S suggests that ATX is not inferior to other liver fibrosis markers. The AUC of ATX was equivalent to that of competing fibrosis markers, and it could show

noninferiority. The advantage of ATX is that its level depends on a mechanism that is different from those of other fibrosis markers. Compared with WFA(+)-M2BP, whose production and metabolic mechanisms are unclear, ATX metabolism has been extensively investigated and clarified. ATX is specifically metabolized in sinusoidal endothelial cells of the liver, and the serum level of ATX might increase as sinusoidal endothelial cells start to show basement membrane deposition and loss of open fenestrations. This mechanism is different from that of the existing liver fibrosis markers HA and IVC7S; thus, ATX levels can evaluate pathologies from a different perspective and contribute to multilateral analyses in clinical practice.

ATX levels are known to change according to gender, and this was also confirmed in the present study. The mechanism responsible for this difference is unknown. We examined various factors that could contribute to the gender difference but were unable to clarify the relationship between ATX levels and gender. Adipocytes express large amounts of ATX and occupy a larger volume in adipose tissues in women than in men,[27] suggesting that this might be the cause of gender difference; However, there is no correlation between ATX levels and BMI.[28] ATX levels are independent of age in men and women, and the menstrual cycle. ATX levels are higher in people taking oral contraceptives[29] and dose-dependently increase after prednisolone administration.[30] However, these reports do not clarify the gender difference. It is possible that differences in hepatic blood flow between men and women might be responsible, but there is no clear evidence to support this. Future studies should seek to address the reason for the gender difference in ATX levels.

Many noninvasive methods of evaluating liver fibrosis prior to DAA therapy have been described,[23,24,31,32] but approaches to the assessment of liver fibrosis following DAA therapy (particularly noninvasive methods) have not been well evaluated. DAA therapy can achieve extremely high rates of SVR, thereby suppressing inflammation and halting the progress of liver fibrosis. However, the degree of liver fibrosis improvement must be monitored to determine which individuals remain at a high risk of HCC. LSMs are almost as reliable as liver biopsies,[5,6] but their versatility is limited. Although serum liver fibrosis marker tests provide valuable information, few studies have evaluated their value for liver fibrosis staging after DAA therapy. A key finding of our study was that ATX levels reflect liver stiffness changes and indicate the presence of liver
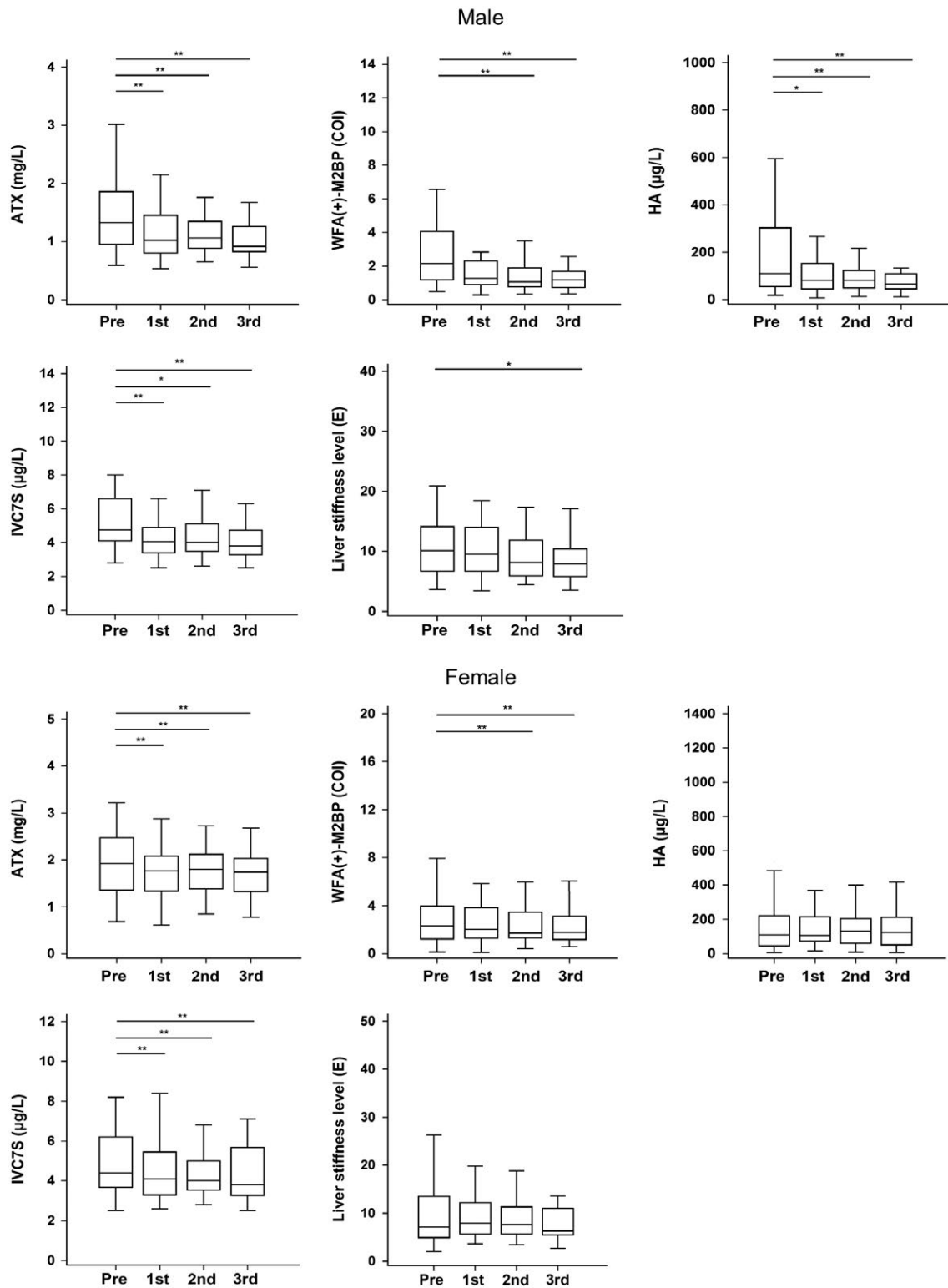
FIG. 3. Variation in serum ATX level as a result of antiviral therapy. Abbreviations: E, liver stiffness level; COI, cutoff index. The definitions of the time points are as follows: "Pre" denotes the time point immediately before treatment; "1st" denotes 3 months after Pre in the group that did not receive antiviral treatment, or immediately after treatment in the antiviral treatment group; and "2nd" and "3rd" denote 3 and 6 months after the first time point, respectively.

fibrosis following DAA. Because the ATX concentration showed similar variations to those associated with liver stiffness, it may be comparable with TE. Therefore, ATX may be a useful marker of liver fibrosis and may be compatible with variations in LSM. Analyzing ATX levels by dividing them according to normal, F1, F2, F3, and F4 fibrosis stages was impossible because LSMs, strictly speaking, do not always indicate the liver fibrosis stage determined by liver biopsy. Moreover, the segmentation of each group made statistical analysis difficult. However, if the number of samples could be increased, it might be possible to perform a statistical comparison of ATX levels at different liver fibrosis stages. ATX levels declined following treatment with DAAs, with the greatest decrease occurring during the interval between pretreatment and the end of treatment. The level of decline was similar to that observed in the levels of the other hepatic fibrosis markers. The level of the fibrosis markers, including LSM values and ALT levels, declined rapidly after DAA therapy. As liver fibrosis improvements are not rapid, the rate at which the fibrosis markers declined indicates that this is a reflection of changes in inflammation rather than fibrosis. Hence, fibrosis markers should be evaluated in the context of inflammation. All of the fibrosis markers were higher in the CHC-infected patients who had early fibrosis compared with those in the control subjects. Continuous monitoring from immediately before DAA treatment until 6 months after treatment revealed that liver stiffness decreased gradually between 3 and 6 months after treatment in almost all patients without significant differences. The ratios of ATX levels to liver hardness remained unchanged regardless of the ALT level before treatment. The improvement in inflammation by DAA in the high ALT group did not significantly affect the ATX/E ratio. Long-term analyses of fibrosis markers should reveal associations between liver carcinogenesis and improvements in fibrosis as a consequence of DAA therapy. LSM is a highly reliable measure of liver fibrosis. By contrast, serum markers are highly susceptible to factors other than fibrosis. We investigated fibrosis marker/LSM ratios to determine
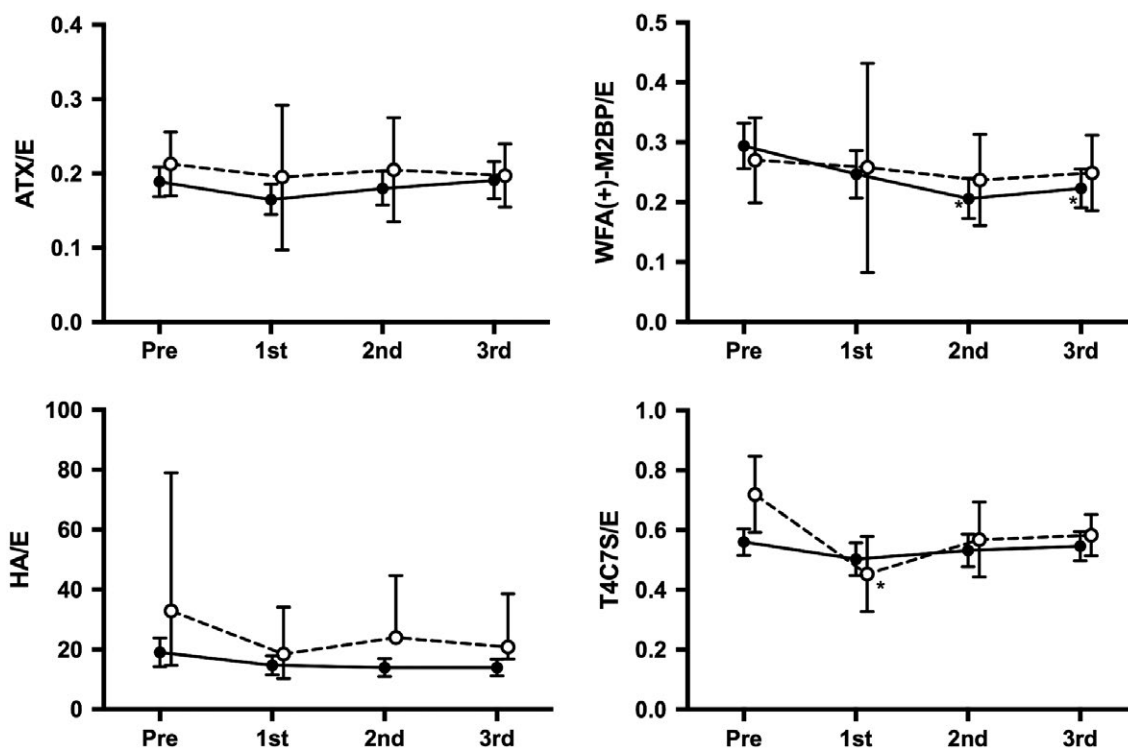

FIG. 4. Variation in the fibrosis marker/liver stiffness ratio in antiviral treated (solid lines)/untreated (dotted lines) CHC patients. Data points represent the mean and 95% CI. $^*P < 0.05$ versus the pretreatment value (Mann-Whitney U test). The definitions of the time points are as follows: "Pre" denotes the time point immediately before treatment; "1st" denotes 3 months after Pre in the group that did not receive antiviral treatment or immediately after treatment in the antiviral treatment group; and "2nd" and "3rd" denote 3 and 6 months after the first time point, respectively.
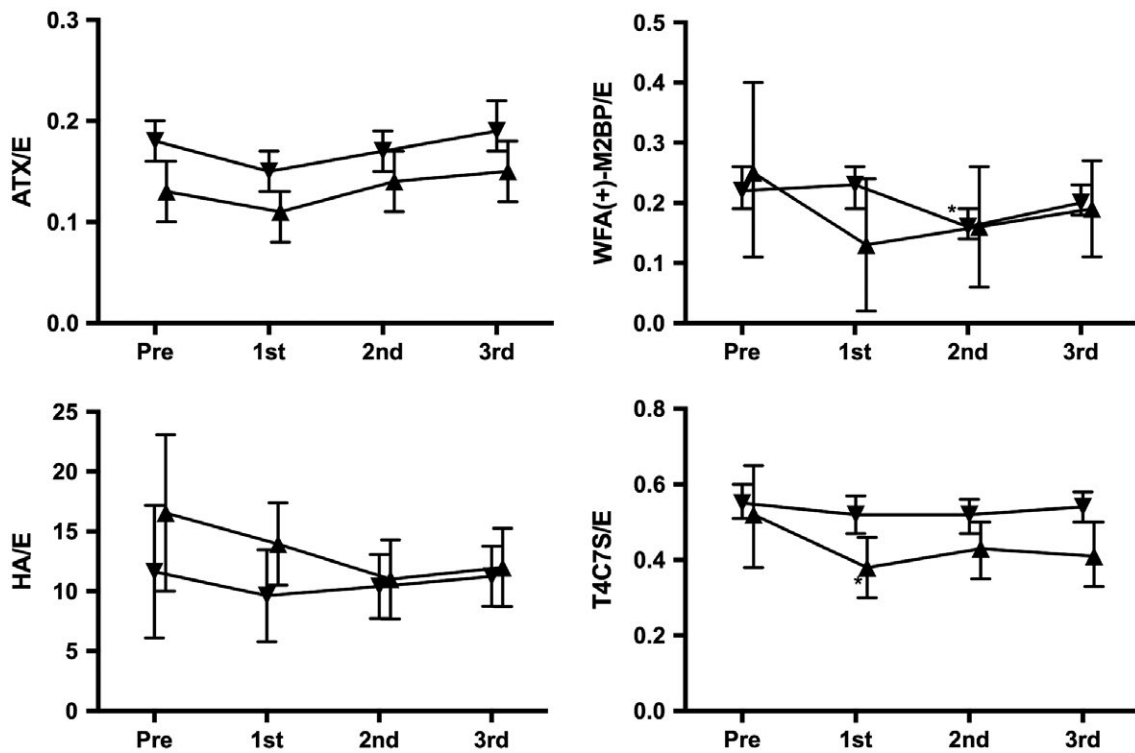
FIG. 5. Variation in the fibrosis marker/liver stiffness ratio in antiviral treated CHC patients with high ALT (> 2 × ULN; up-pointing triangle) and low ALT (≤ 2 × ULN; down-pointing triangle). Data points represent the mean and 95% CI. The definitions of the time points are as follows: "Pre" denotes the time point immediately before treatment; "1st" denotes immediately after treatment in the antiviral treatment group; and "2nd" and "3rd" denote 3 and 6 months after the first time point, respectively.

which marker would provide the same level of confidence as LSM. Because small clinics and institutions cannot introduce LSM, liver fibrosis markers provide an alternative for the convenient, long-term, and cost-effective follow-up of liver cirrhosis. In this respect, ATX could provide an alternative to LSM and existing fibrosis markers.

During chronic liver injury, angiogenesis can be interpreted in the context of two basic phenomena. First, many liver diseases are characterized by inflammation and fibrosis that lead to progressive tissue hypoxia, which stimulates angiogenesis.[33–35] Second, increases in the expression of some cytokines and proangiogenic growth factors characterize wound healing, which is a feature of chronic liver disease. These processes contribute to structural and functional changes in the liver.[33–35] The HCV may activate several pathways and systems that are implicated in angiogenesis. The HCV core E1, NS3, and NS5A proteins cause mitochondrial dysfunction, which generates new reactive oxygen species that induce hypoxia-inducible factor 1 and upregulate vascular endothelial growth factor

and placental growth factor.[36] ATX is involved in cell migration, neurogenesis, angiogenesis, and wound healing.[37] ATX may also be associated with liver fibrosis. Proangiogenic marker levels are higher in the sera of HCV-infected patients and their concentrations diminish after pegylated interferon and ribavirin therapy.[38] Hence, proangiogenic markers may be useful for follow-up assessment of HCV infections and for determining treatment responses.

A limitation of this study is that liver biopsy was not used to stage liver fibrosis. Although liver biopsy is the gold standard method for evaluating liver fibrosis, it was not included in the protocol because many of the subjects were elderly. Moreover, liver biopsies are not essential for the diagnosis and treatment of HCV infections. Even if a patient has undergone a liver biopsy in the past, the results from that biopsy may not be representative of the current state of the patient's liver, and liver biopsy samples taken from the right and left liver lobes may have different histological grading and staging.[4] In addition, repeat liver biopsies may not be undertaken after HCV treatment due to problems

associated with the invasiveness of the procedure. As TE findings show a strong correlation with liver fibrosis stages determined using liver biopsies, TE-based liver fibrosis staging was adopted. Future studies should determine the AUC of ATX with higher accuracy and include larger numbers of patients.

In conclusion, this study suggests that ATX may be a useful marker of patient responses to DAA therapy and disease progression in CHC infections. As a noninvasive technique, assessment of ATX may facilitate routine patient monitoring.

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