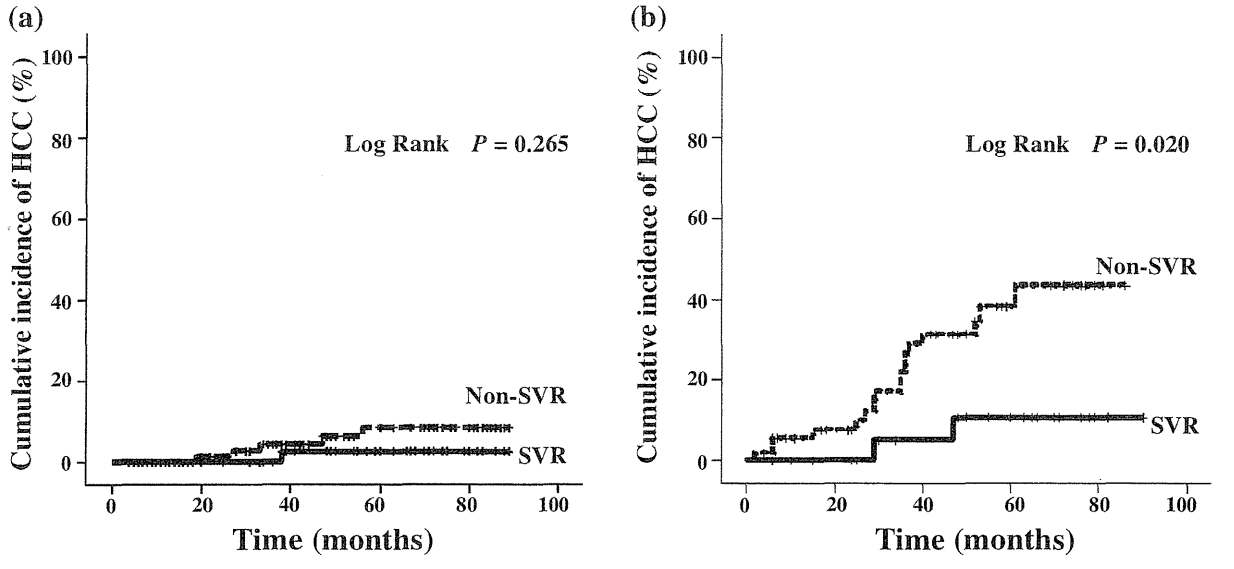

Patients at risk	0	20	40	60	80	HCC events
Non-SVR	95	76	54	40	20	5
SVR	67	45	34	21	6	1

Patients at risk	0	20	40	60	80	HCC events
Non-SVR	57	41	29	12	3	17
SVR	25	22	17	12	2	2

Figure 2 Cumulative incidence of HCC after peginterferon alfa-2b and ribavirin in older patients who achieved SVR (solid line) or did not achieve SVR (dashed line), among those with GGT < 44 IU/L (a) and GGT ≥ 44 IU/L (b). HCC, hepatocellular carcinoma; GGT, gamma-glutamyltranspeptidase; SVR, sustained virological response.

related compounds. It has been observed that the intake of certain xenobiotics, including carcinogens and drugs, induces hepatic expression of this enzyme.[40] GGT was one of the significant factors associated with non-SVR in CH-C patients treated with IFN-based therapy.[41–44] In terms of mechanism, a previous report showed that GGT levels may act as a surrogate marker of tumor necrosis factor-alpha expression in the liver, and this may explain the importance of serum GGT levels in predicting treatment outcome.[45] However, the mechanisms through which GGT significantly affects HCC development among older patients remain unclear.

Platelet counts correlate with advanced fibrosis in the histological examination of the liver in patients with CH-C. Decreased platelet counts and fibrosis were reported to be factors associated with the development of HCC.[17–22]

SVR, a treatment factor, is also reported to be one of the most important factors in reducing the risk of HCC in patients with CH-C.[17–19] Factors associated with development of HCC in older patients are similar to those in previous reports on IFN-based treatment;[20–22] however, this is the first report demonstrating factors associated with development of HCC in CH-C patients 65 years or older treated with only combination therapy between peginterferon alfa-2b and ribavirin therapy.

We examined which CH-C patients at high risk for HCC may especially benefit from combination therapy. We determined factors associated with SVR using a univariate analysis, followed by a multivariate analysis of factors associated with SVR in older patients who underwent combination therapy. In addition, we compared the cumulative incidence of HCC between older patients who did and did not achieve SVR. In older patients, low HCV-RNA, male gender, and genotype 2 were associated with SVR. These were the same factors identified in our previous report,[15] and gender was reported to be a factor associated with SVR in older patients in another report.[46]

This study has several limitations. This is a retrospective cohort study, so selection bias cannot be excluded. We cannot exclude the possibility that older patients selected for treatment in the outpatient department were more likely to be better overall candidates than younger patients. However, regardless of potential bias, older patients had low WBC counts and hemoglobin levels, and more advanced fibrosis. Especially on older patients, as life expectancy is shorter than with younger patients, the health economics evaluation of costs of treatment *versus* savings of reducing HCC incidence would be extremely valuable, so further investigation, including cost–benefit, would be needed.

In conclusion, older patients who received combination peginterferon alfa-2b and ribavirin therapy had low hemoglobin levels, low WBC and platelet counts, and advanced fibrosis. Older patients had higher treatment discontinuation rates, lower SVR rates, and higher rate of HCC development than younger patients. However, older patients who achieved SVR had a marked reduction in the development of HCC compared with older patients who did not achieve SVR, especially among older patients with GGT > 44 IU/L. Low HCV-RNA, male gender, and genotype 2

Table 4 Factors associated with SVR in older patients

Univariate analysis			
Variable	SVR (n = 97)	Non-SVR (n = 157)	P-value
Sex (male/female)	59/38	64/93	0.0017
Age (years)	67.6 ± 2.5	68.3 ± 2.9	0.0473
AST (IU/L)	60.1 ± 39.7	58.4 ± 41.5	0.7807
ALT (IU/L)	63.1 ± 45.7	57.9 ± 45.5	0.3714
GGT (IU/L)	50.2 ± 70.0	46.5 ± 36.8	0.5911
WBC (/μL)	4856.4 ± 1268.6	4909.6 ± 1344.7	0.7550
Hemoglobin (g/dL)	13.8 ± 1.1	13.6 ± 1.4	0.2345
Platelets (× 10^4/μL)	15.8 ± 4.4	15.1 ± 4.8	0.2646
HCV-RNA (logIU/mL)	5.8 ± 0.9	6.2 ± 0.6	< 0.0001
Genotype (1/2)	55/42	122/35	0.0003
Liver activity (A0/A1/A2/A3)	6/38/24/4	4/59/53/6	0.3123
Fibrosis (F0–1/F2/F3/F4)	42/20/9/0	52/36/27/5	0.0560

Multivariate analysis			
Variable	Category	Odds ratio (95% CI)	P-value
HCV-RNA	≥ 5.8	1	0.002
	< 5.8	2.604 (1.408–4808)	
Gender	Female	1	0.004
	Male	2.312 (1.309–4.085)	
Genotype	1	1	0.011
	2	2.203 (1.202–4.038)	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltranspeptidase; HCV-RNA, hepatitis C virus RNA; SVR, sustained virological response; WBC, white blood cell.

were factors associated with SVR in older patients. Older patients who have these factors should be considered for treatment in hopes of achieving SVR to prevent HCC.

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—Rinsho Byori—

Original

The NAFLD Index: A Simple and Accurate Screening Tool for the Prediction of Non-Alcoholic Fatty Liver Disease

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Objective: Non-alcoholic fatty liver disease (NAFLD) is a common debilitating condition in many industrialized countries that increases the risk of cardiovascular disease. The aim of this study was to derive a simple and accurate screening tool for the prediction of NAFLD in the Japanese population.

Methods: A total of 945 participants, 279 men and 666 women living in Hokkaido, Japan, were enrolled among residents who attended a health check-up program from 2010 to 2014. Participants with an alcohol consumption >20 g/day and/or a chronic liver disease, such as chronic hepatitis B, chronic hepatitis C or autoimmune hepatitis, were excluded from this study. Clinical and laboratory data were examined to identify predictive markers of NAFLD.

Results: A new predictive index for NAFLD, the NAFLD index, was constructed for men and for women. The NAFLD index for men = −15.5693 + 0.3264 [BMI] + 0.0134 [triglycerides (mg/dl)], and for women = −31.4686 + 0.3683 [BMI] + 2.5699 [albumin (g/dl)] + 4.6740 [ALT/AST] − 0.0379 [HDL cholesterol (mg/dl)]. The AUROC of the NAFLD index for men and for women was 0.87 (95% CI 0.88−1.60) and 0.90 (95% CI 0.66−1.02), respectively. The cut-off point of −5.28 for men predicted NAFLD with an accuracy of 82.8%. For women, the cut-off point of −7.65 predicted NAFLD with an accuracy of 87.7%.

Conclusion: A new index for the non-invasive prediction of NAFLD, the NAFLD index, was constructed using available clinical and laboratory data. This index is a simple screening tool to predict the presence of NAFLD. 【Original】

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【**Key Words**】 NAFLD index, non-alcoholic fatty liver disease, screening tool, sensitivity and specificity

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I. Introduction

NAFLD is a growing worldwide medical problem. Large scale surveys in the United States (NHANES III) and Europe (Dionysus study in Italy) have shown that NAFLD is prevalent in at least 20% of the general population[1)~3)], and is as high as 30% (>40% in Hispanic men) if sensitive tools such as proton magnetic resonance spectroscopy are used to detect increased hepatic triglyceride levels[2)]. In the Asia-Pacific area, the prevalence of NAFLD has reached 10% to 29% of the general population according to large surveys in China, Japan, and Korea[4)], values that are very similar to those described in the Western surveys. Two longitudinal studies from Japan indicate that the prevalence of NAFLD is increasing. Over a 12-year period, the prevalence of NAFLD in Japan has more than doubled from ~13% in 1988–9 to ~30% in 2004[5)6)]. Eguchi et al. reported that the prevalence of NAFLD was 29.7% in the general population from 2009 to 2010 in Japan[7)].

NAFLD is characterized by an accumulation of fat in the liver in the absence of chronic alcohol consumption without any other evident cause (such as viral or autoimmune diseases) of chronic liver disease. NAFLD comprises a disease spectrum ranging from simple steatosis to steatohepatitis (NASH), with varying degrees of inflammation and fibrosis, progressing to end-stage liver disease with cirrhosis and hepatocellular carcinoma[8)]. Furthermore, concomitant type 2 diabetes mellitus among patients with NAFLD is associated with more severe liver inflammation and fibrosis and with more rapid fibrosis progression. In patients with type 2 diabetes mellitus, NAFLD increases the risk of cirrhosis-related complications such as hepatocellular carcinoma and has been shown to increase mortality[9)10)]. Furthermore, NAFLD increases cardiovascular risk, referred to collectively as metabolic syndrome[11)].

Most patients with NAFLD are asymptomatic, and NAFLD is often discovered incidentally when a laboratory examination shows elevated liver enzyme levels. The present detection methods use ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI). A simple, non-invasive test that is a valid screening tool is needed to identify patients at high risk for NAFLD, because it is reasonable that patients with a high risk for NAFLD are examined using those imaging modalities for mass screening. Some previous studies have attempted to estimate a screening tool for fatty liver disease, and those tools can be used to predict the presence of NAFLD, such as the NAFLD liver fat score[12)], SteatoTest[13)], fatty liver index (FLI)[13)], the fatty liver disease (FLD) index[15)] and hepatic steatosis index (HSI)[16)]. The NAFLD liver fat score, SteatoTest and FLI were developed for the European population. The FLD index and HSI were developed for the Chinese and Korean populations, respectively. A screening tool for NAFLD in the Japanese population still does not exist.

The purpose of this study was to derive a simple screening tool based on standard laboratory tests and anthropometric data that could be used for the prediction of NAFLD in the Japanese population.

II. Methods

A. Subjects

A total of 945 participants, 279 men and 666 women living in Yakumo Town, a rural area in Hokkaido, Japan, were enrolled in this study from subjects who had attended at least one of five summer health check-up programs from 2010 to 2014. All subjects were over 40 years of age. Of attendees at health check-up programs from 2011 to 2013, 673 subjects (estimation group) were used to develop the NAFLD index. Of attendees at health check-up programs from 2010 and 2014, 272 subjects (validation group) were used to validate the predictive performance of the NAFLD index. Characteristics of the study population are shown in **Table 1.**

This study was conducted with written informed consent obtained from each subject prior to the start of the study, and with the approval of the Fujita Health University Ethics Committee.

B. Participants and laboratory data

Health and life style, such as alcohol and drug consumption and chronic liver disease, were assessed via a

Table 1 Characteristics of the estimation group and validation group

	Estimation Group (n=673)	Validation Group (n=272)	*P* value
Sex (men, %)	28.5	32.0	NS [b]
Age (years) [a]	63.6 ± 10.5	64.3 ± 11.8	NS [b]
BMI [c]	23.61 ± 3.60	24.0 ± 3.5	NS [b]
Abdominal circumference (cm) [a]	79.16 ± 11.00	81.01 ± 10.32	NS [b]
Fasting plasma glucose (mg/dl) [c]	91 (84 - 97)	90 (82 - 99)	NS [d]
HbA1c (NGSP) (%) [c]	5.8 (5.5 - 6.1)	5.6 (5.4 - 6.0)	NS [d]
Total Protein (g/dl) [a]	7.39 ± 0.44	7.37 ± 0.42	NS [b]
Albumin (g/dl) [a]	4.37 ± 0.26	4.41 ± 0.26	NS [b]
ALP (IU/l) [c]	215 (179 - 258)	211 (179 - 250)	NS [d]
AST (IU/l) [c]	22 (19 - 27)	21 (18 - 25)	NS [d]
ALT (IU/l) [c]	20 (16 - 28)	19 (15 - 26)	NS [d]
ALT/AST [c]	0.94 (0.76 - 1.15)	0.94 (0.75 - 1.13)	NS [d]
γ-GTP (IU/l) [c]	21 (14 - 33)	18 (13 - 30)	NS [d]
Triglyceride (mg/dl) [c]	91 (67 - 125)	85 (64 - 115)	NS [d]
Total Cholesterol (mg/dl) [a]	214.5 ± 33.7	208.2 ± 32.2	NS [b]
HDL Cholesterol (mg/dl) [a]	59.4 ± 14.2	59.3 ± 15.0	NS [d]
LDL Cholesterol (mg/dl) [a]	127.0 ± 29.7	124.8 ± 28.4	NS [b]

BMI; body mass index, HbA1c; hemoglobin A1c, ALP; alkaline phosphatase, AST; aspartate aminotransferase, ALT; alanine aminotransferase, γ-GTP; γ-glutamyltransferase, HDL; high-density lipoprotein, LDL; low density lipoprotein, NS; non significant

a Data are expressed as mean value ± standard deviation. b *t*-test.

c Data are expressed as geometric mean values and 25th–75th percentiles in parentheses. d Wilcoxon test.

questionnaire by the nursing staff. Participants with an alcohol consumption >20 g/day and/or a chronic liver disease, such as chronic hepatitis B, chronic hepatitis C or autoimmune hepatitis, were excluded from this study. Body height and weight were measured and the body mass index (BMI) was calculated from the body weight (Kg)/height (m)^2, and abdominal circumference was measured by the nursing staff. Hemoglobin A1c (HbA1c) was measured using high-performance liquid chromatography, and other biochemical analyses were performed using an auto-analyzer (JCS-BM1650, Nihon Denshi Co Ltd., Tokyo, Japan) at the laboratory of the Yakumo Town Hospital. Assessment of seropositivity for hepatitis B surface antigen (HBs Ag) and hepatitis C virus antibody (HCV Ab) were performed using the reversed passive hemagglutination method and the Particle agglutination method, respectively.

C. Assessment of hepatic steatosis

The presence of intrahepatic steatosis was assessed by ultrasound using a ProSound α7 with a UST-9130 convex probe (Hitachi-Aloka Medical, Ltd. Tokyo, Japan) operated by three registered medical sonographers who are authorized by the Japan Society of Ultrasonics in Medicine. Hepatic steatosis was graded as normal, mild, moderate or severe[17]. Mild steatosis was defined as a slight increase in liver echogenicity and hepatorenal echo contrast. In moderate steatosis, visualization of intrahepatic vessels and the diaphragm was slightly impaired, and increased liver echogenicity was present. Severe steatosis was defined as a definite or marked increase in hepatic echogenicity and a poor or no visualization of hepatic vessels and diaphragm. When the grade differed among the three observers, the grade which corresponded to two of the three observers was adopted.

Participants with NAFLD were divided into three groups based on the degree of liver steatosis: mild ≤ (men: n=57, women: n=76), moderate ≤ (men: n=34, women: n=37) and severe (men: n=15, women: n=9) in the estimation group, and mild ≤ (men: n=22, women: n=34), moderate ≤ (men: n=14, women: n=16) and severe (men: n=5, women: n=10) in the validation group.

D. Statistical analysis

All statistical analyses were performed using JMP® ver. 9.0 (SAS Institute, Cary, NC, USA). Since levels of fasting plasma glucose, HbA1c, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), ALT/AST ratio, γ-glutamyltransferase (γ-GTP) and triglyceride were not in the normal distribution, we used the Wilcoxon signed-rank test to analyze those data. The *t*-test was used to analyze other variables. Fasting plasma glucose, HbA1c, ALP, AST, ALT, ALT/AST ratio, γ-GTP and triglyceride are reported as median values and 25th-75th percentile ranges. Other variables are reported as means ± standard deviation. Independent predictors of NAFLD were assessed by multiple regression analysis (ordinal logistic regression). A predictive index was constructed by modeling the values of the independent variables and their coefficient of regression. The optimal discriminate cut-off values of the predictive index and assessment of graded hepatic steatosis were assessed from the areas under the receiver operating characteristics (ROC) curves (AUROC). The optimal discriminating cut-off values were determined at the maximum total of sensitivity and specificity.

III. Results

A. Development of the NAFLD index in the estimation group

In all participants of the estimation group, all variables except total cholesterol were significantly different between NAFLD (mild ≤ hepatic steatosis) and non-NAFLD subjects (**Table 2**). Among those variables, BMI ($P<0.0001$), albumin ($P=0.0059$), ALT/AST ($P=0.0214$), triglyceride ($P=0.0333$) and HDL cholesterol ($P=0.0365$) were identified as independent predictors of NAFLD by multiple regression analysis (**Table 3**). By multiple regression analysis, the estimated values of BMI, albumin, ALT/AST, triglyceride and HDL cholesterol were calculated as 0.2991, 1.8494, 3.1685, 0.0055 and −0.0256, respectively. The optimum intercept was calculated as −25.1494. Thus, the NAFLD index by all subjects was constructed with these 5 variables: NAFLD Index = −25.1494 + 0.2991 [BMI] + 1.8494 [albumin (g/dl)] + 3.1685 [ALT/AST] + 0.0055 [triglycerides (mg/dl)] − 0.0256 [HDL cholesterol (mg/dl)].

Between the sexes, age, BMI, abdominal circumference, fasting plasma glucose, albumin, ALT, ALT/AST, γ-GTP, triglycerides, total cholesterol and HDL cholesterol were significantly different in the estimation group (**Table 2**). Therefore, the NAFLD index for men and for women were also constructed compared with the NAFLD index constructed by all subjects. For men, 12 variables were significantly different between NAFLD and non-NAFLD subjects (**Table 2**). Among those variables except abdominal circumference, BMI ($P<0.0001$) and triglyceride ($P=0.015$) were identified as independent predictors of NAFLD by multiple regression analysis (**Table 3**). For the reason except abdominal circumference, only triglyceride was identified as an independent predictor of NAFLD by multiple regression analysis. By multiple regression analysis, the estimated values of BMI and triglycerides were calculated as 0.3264 and 0.0134, respectively. The optimum intercept was calculated as −15.5693. Thus, the NAFLD index for men was constructed with those 2 variables: NAFLD index for men = −15.5693 + 0.3264 [BMI] + 0.0134 [triglycerides (mg/dl)].

On the other hand, for women, 14 variables were significantly different between NAFLD and non-NAFLD subjects (**Table 2**). Among those variables, BMI ($P<0.0001$), albumin ($P=0.0046$), ALT/AST ($P=0.0046$) and HDL cholesterol ($P=0.0263$) were identified as independent predictors of NAFLD by multiple regression analysis (**Table 3**). By multiple regression analysis, the estimated values of BMI, albumin, ALT/AST and HDL cholesterol were calculated as 0.3683, 2.5699, 4.6740 and −0.0379, respectively. The optimum intercept was calculated as −31.4686. Thus, the NAFLD index for women was constructed with these 4 variables: NAFLD index for women = −31.4686 + 0.3683 [BMI] + 2.5699 [albumin (g/dl)] + 4.6740 [ALT/AST] − 0.0379 [HDL cholesterol (mg/dl)].

Table 2 Characteristics of all subjects, men and women in the estimation group

	All subjects			Men			Women			Men vs Women
	non-NAFLD	NAFLD	*P* value	non-NAFLD	NAFLD	*P* value	non-NAFLD	NAFLD	*P* value	*P* value
Number	540	133		135	57		405	76		
Age (years) [a]	64.0 ± 10.7	62.0 ± 9.3	<0.05 [b]	67.1 ± 10.9	61.6 ± 10.0	<0.005 [b]	63.0 ± 10.4	62.3 ± 8.8	NS [b]	<0.005 [b]
BMI [a]	22.73 ± 3.00	27.18 ± 3.60	<0.0001 [b]	23.19 ± 2.78	27.24 ± 4.14	<0.0001 [b]	22.58 ± 3.06	27.14 ± 3.18	<0.0001 [b]	<0.001 [b]
Abdominal circumference (cm) [a]	76.81 ± 10.04	88.71 ± 9.38	<0.0001 [b]	81.60 ± 8.10	92.31 ± 9.33	<0.0001 [b]	75.22 ± 10.12	85.99 ± 8.50	<0.0001 [b]	<0.0001 [b]
Fasting plasma glucose (mg/dl) [c]	90 (83 - 97)	94 (88 - 101)	<0.0001 [d]	92 (83 - 100)	93 (86 - 103)	NS [d]	90 (83 - 96)	94 (88 - 99)	0.0001 [d]	0.0288 [d]
HbA1c (NGSP) (%) [c]	5.7 (5.5 - 6.0)	6.0 (5.7 - 6.3)	<0.0001 [d]	5.7 (5.5 - 6.0)	5.9 (5.5 - 6.2)	NS [d]	5.7 (5.5 - 6.0)	6.0 (5.7 - 6.3)	<0.0001 [d]	NS [d]
Total Protein (g/dl) [a]	7.35 ± 0.44	7.53 ± 0.42	<0.0001 [b]	7.28 ± 0.49	7.51 ± 0.45	<0.05 [b]	7.38 ± 0.42	7.54 ± 0.40	<0.05 [b]	NS [b]
Albumin (g/dl) [a]	4.35 ± 0.26	4.46 ± 0.26	<0.0001 [b]	4.28 ± 0.30	4.47 ± 0.30	<0.0001 [b]	4.38 ± 0.24	4.44 ± 0.23	<0.05 [b]	<0.05 [b]
ALP (IU/l) [c]	212 (178 - 254)	228 (185 - 273)	0.0217 [d]	214 (175 - 255)	209 (173 - 247)	NS [d]	212 (178 - 253)	237 (198 - 300)	0.0007 [d]	NS [d]
AST (IU/l) [c]	22 (19 - 26)	25 (21 - 31)	<0.0001 [d]	23 (19 - 26)	27 (21 - 33)	0.0004 [d]	22 (19 - 26)	24 (21 - 30)	0.0022 [d]	NS [d]
ALT (IU/l) [c]	19 (15 - 25)	29 (23 - 42)	<0.0001 [d]	22 (17 - 29)	36 (23 - 49)	<0.0001 [d]	18 (15 - 24)	27 (21 - 37)	<0.0001 [d]	<0.0001 [d]
ALT/AST [c]	0.89 (0.74 - 1.06)	1.21 (1.00 - 1.48)	<0.0001 [d]	1.00 (0.82 - 1.21)	1.37 (1.10 - 1.56)	<0.0001 [d]	0.84 (0.72 - 1.00)	1.15 (0.95 - 1.32)	<0.0001 [d]	<0.0001 [d]
γ-GTP (IU/l) [c]	19 (13 - 30)	28 (20 - 43)	<0.0001 [d]	28 (18 - 39)	35 (25 - 51)	<0.004 [d]	17 (13 - 26)	24 (18 - 38)	<0.0001 [d]	<0.0001 [d]
Triglyceride (mg/dl) [c]	84 (63 - 113)	124 (95 - 157)	<0.0001 [d]	90 (73 - 125)	142 (112 - 221)	<0.0001 [d]	82 (61 - 113)	118 (87 - 139)	<0.0001 [d]	<0.0001 [d]
Total Cholesterol (mg/dl) [a]	213.7 ± 33.3	217.8 ± 35.0	NS [b]	205.1 ± 31.4	212.2 ± 33.0	NS [b]	216.6 ± 33.5	222.1 ± 36.0	NS [b]	<0.0005 [b]
HDL Cholesterol (mg/dl) [a]	61.2 ± 14.3	52.1 ± 11.1	<0.0001 [b]	53.5 ± 12.4	46.4 ± 10.3	<0.0001 [b]	63.7 ± 14.0	56.3 ± 9.8	<0.0001 [b]	<0.0001 [d]
LDL Cholesterol (mg/dl) [a]	125.5 ± 28.8	133.2 ± 32.3	<0.01 [b]	124.0 ± 26.4	129.0 ± 29.2	NS [b]	126.0 ± 29.6	136.3 ± 34.4	<0.01 [b]	NS [b]

BMI; body mass index, HbA1c; hemoglobin A1c, ALP; alkaline phosphatase, AST; aspartate aminotransferase, ALT; alanine aminotransferase, γ-GTP; γ-glutamyltransferase, HDL; high-density lipoprotein, LDL; low density lipoprotein, NS; non significant

a Data are expressed as mean value±standard deviation.

b *t*-test.

c Data are expressed as geometric mean values and 25th–75th percentiles in parentheses.

d Wilcoxon test.

Table 3 The multiple regression predicting NAFLD in the estimation group

		Estimated Value	Standard Error	$\chi 2$	P value
All participants	Age (year)	-0.0191142	0.0142323	1.80	0.1793
	BMI	0.2991212	0.0565963	27.93	< 0.0001
	Abdominal circumference (cm)	0.0360046	0.0209648	2.95	0.0859
	Fasting plasma glucose (mg/dl)	0.0009124	0.0100574	0.01	0.9277
	HbA1c (NGSP) (%)	0.1589392	0.2952409	0.29	0.5903
	Total Protein (g/dl)	0.2253899	0.4066308	0.31	0.5794
	Albumin (g/dl)	1.8494490	0.6711655	7.59	0.0059
	ALP (IU/l)	0.0002640	0.0020839	0.02	0.8992
	AST (IU/l)	0.0778303	0.0609725	1.63	0.2018
	ALT (IU/l)	-0.0425274	0.0496706	0.73	0.3919
	ALT/AST	3.1684633	1.3765717	5.30	0.0214
	γ-GTP (IU/l)	-0.0057039	0.0044923	1.61	0.2042
	Triglyceride (mg/dl)	0.0054844	0.0025770	4.53	0.0333
	HDL Cholesterol (mg/dl)	-0.0255746	0.0122303	4.37	0.0365
	LDL Cholesterol (mg/dl)	0.0034737	0.0042980	0.65	0.4190
Men	Age (year)	-0.0112108	0.0238543	0.22	0.6384
	BMI	0.3264417	0.0815099	16.04	<0.0001
	Total Protein (g/dl)	0.2217686	0.6861280	0.10	0.7465
	Albumin (g/dl)	1.0503587	1.0832097	0.94	0.3322
	AST (IU/l)	-0.0778169	0.1223918	0.40	0.5249
	ALT (IU/l)	0.0942836	0.1022881	0.85	0.3567
	ALT/AST	-0.5833548	2.6951008	0.05	0.8286
	γ-GTP (IU/l)	-0.0086165	0.0059311	2.11	0.1463
	Triglyceride (mg/dl)	0.0133632	0.0042218	10.02	0.0015
	HDL Cholesterol (mg/dl)	-0.0112164	0.0200952	0.31	0.5767
Women	BMI	0.3682900	0.0710365	26.88	< 0.0001
	Abdominal circumference (cm)	0.0211599	0.0249965	0.72	0.3973
	Fasting plasma glucose (mg/dl)	-0.0116459	0.0140082	0.69	0.4058
	HbA1c (NGSP) (%)	0.5774762	0.4208209	1.88	0.1700
	Total Protein (g/dl)	0.1435939	0.5245140	0.07	0.7843
	Albumin (g/dl)	2.5698968	0.9070345	8.03	0.0046
	ALP (IU/l)	0.0013360	0.0025025	0.28	0.5934
	AST (IU/l)	0.1223129	0.0735538	2.77	0.0963
	ALT (IU/l)	-0.0885379	0.0578792	2.34	0.1261
	ALT/AST	4.6740262	1.6491393	8.03	0.0046
	γ-GTP (IU/l)	-0.0029338	0.0082430	0.13	0.7219
	Triglyceride (mg/dl)	0.0001029	0.0034176	0.00	0.9760
	HDL Cholesterol (mg/dl)	-0.0379169	0.0170661	4.94	0.0263
	LDL Cholesterol (mg/dl)	0.0051631	0.0051129	1.02	0.3126

BMI; body mass index, HbA1c; hemoglobin A1c, ALP; alkaline phosphatase, AST; aspartate aminotransferase, ALT; alanine aminotransferase, γ-GTP; γ-glutamyltransferase, HDL; high-density lipoprotein, LDL; low density lipoprotein

B. Predictive performance of the NAFLD index in the estimation group

To demonstrate the particular predictive performance of the NAFLD index, ROC analysis of the NAFLD index was performed to discriminate between NAFLD and non-NAFLD subjects in the estimation group (**Fig. 1**). For the detection of NAFLD (mild ≤), the AUROC for the NAFLD index for all subjects, for men and for women was 0.90 (95% CI 0.81–1.14), 0.87 (95% CI 0.88–1.60) and 0.90 (95% CI 0.66–1.02), respectively (**Table 4**). The optimal discriminating cut-off point of the NAFLD index was determined by ROC analysis.

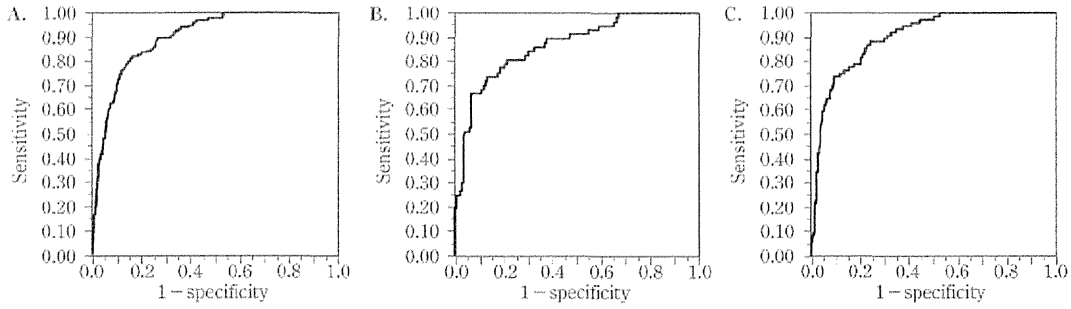

Figure 1

A) ROC curve of the NAFLD index by all subjects in the estimation group. The AUROC is 0.90; the optimal cut-off point is −6.86< with a sensitivity of 81.2% and a specificity of 84.4%.

B) ROC curve of the NAFLD index for men. The AUROC is 0.87; the optimal cut-off point is −5.28< with a sensitivity of 73.7% and a specificity of 86.7%.

C) ROC curve of the NAFLD index for women. The AUROC is 0.90; the optimal cut-off point is −7.65< with a sensitivity of 73.7% and a specificity of 86.7%.

The optimal cut-off point of −6.86 by all subjects predicted NAFLD with an accuracy of 83.8%. For men and women, NAFLD was predicted with an accuracy of 77.6% and 86.2%, respectively (**Table 4**). The optimal cut-off point of the −5.28 NAFLD index for men predicted NAFLD with an accuracy of 82.8%. Furthermore, the optimal cut-off point of −7.65 NAFLD index for women predicted NAFLD with an accuracy of 87.7% (**Table 4**). The accuracy of the NAFLD index for men and for women was higher than the accuracy of the NAFLD index for all subjects.

C. Comparison of the NAFLD index with the FLD index and HSI in the estimation group

The predictive performance of the NAFLD index was compared with the FLD index and HSI which were developed for the Korean and Chinese populations, respectively. To compare those three predictors of NAFLD, the optimal discriminating cut-off point of the FLD index and HSI was determined by ROC analysis in the estimation group, respectively. The optimal cut-off point of the 29.0 FLD index predicted NAFLD with an accuracy of 78.5%, and the optimal cut-off point of the 36.5 HSI predicted NAFLD with an accuracy of 81.3% (**Table 4**). The accuracy of the NAFLD index for men and for women was higher than the FLD index and HSI.

D. Assessment of hepatic steatosis grade in NAFLD by the NAFLD index in the estimation group

To assess hepatic steatosis grade in NAFLD, the optimal cut-off point of the NAFLD index was determined by ROC analysis in the estimation group. For assessment of moderate ≤ hepatic steatosis in NAFLD, the cut-off points of −4.86 for men and −7.53 for women predicted moderate ≤ hepatic steatosis with an accuracy of 82.8% and 85.7%, respectively. Furthermore, the cut-off points of −4.24 for men and −7.13 for women predicted severe hepatic steatosis with an accuracy of 89.6% and 85.7%, respectively (**Table 4**). The accuracy of the NAFLD index for men and for women was higher than the NAFLD index for all subjects to assess moderate ≤ and severe hepatic steatosis in NAFLD. Furthermore, the accuracy of the NAFLD index for men and for women was higher than the FLD index and HSI to assess moderate ≤ hepatic steatosis in NAFLD. To assess severe hepatic steatosis in NAFLD, the accuracy of the NAFLD index for men was the highest, but the accuracy of the NAFLD index for women (85.7%) was less than the FLD index (87.4%).

E. Validation of performance of the NAFLD index

The results in the estimation group were validated in the validation group of 272 participants. The accuracy of the NAFLD index for men and for women was 79.3% and 84.3%, respectively, similar to the values in the estimation group, and was the highest among the other methods (**Table 5**). Furthermore, to assess moderate ≤ and severe hepatic steatosis in NAFLD, the accuracy of the NAFLD index for men and for women

Table 4 The predictive performance and assessment of hepatic steatosis grade by NAFLD index in the estimation group

			AUROC (95% CI)	Cut-off point		Sensitivity (%)	Specificity (%)	Accuracy (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Positive Likelihood ratio	Negative Likelihood ratio
NAFLD Index by all subjects	Mild ≤		0.90 (0.81 - 1.14)		-6.86	81.2	84.4	83.8	56.3	94.8	5.21	4.49
		using in Men				87.7	73.7	77.6	58.1	93.4	3.33	6.00
		using in Women				76.3	88.1	86.2	54.7	95.2	6.41	3.72
	Moderate ≤		0.89 (0.68 - 1.02)		-6.60	80.3	80.4	80.4	32.6	97.2	4.10	4.08
		using in Men				85.3	67.7	70.8	36.3	95.5	2.64	4.61
		using in Women				75.7	84.9	84.2	29.5	97.7	5.01	3.49
	Severe		0.93 (0.62 - 1.12)		-6.30	91.7	80.0	80.4	14.5	99.6	4.59	9.64
		using in Men				93.3	65.5	67.7	18.7	99.1	2.70	9.78
		using in Women				88.9	85.4	85.4	10.4	99.8	6.09	7.69
NAFLD Index for Men				Low	-6.96	94.7	40.7	56.8	40.3	94.8	1.60	7.68
	Mild ≤		0.87 (0.88 - 1.60)	Optimal	-5.28	73.7	86.7	82.8	70.0	88.6	5.54	3.30
				high	-4.60	50.9	94.8	81.8	80.6	82.1	9.79	1.93
	Moderate ≤		0.84 (0.66 - 1.36)		-4.86	70.6	85.4	82.8	51.1	93.1	4.84	2.90
	Severe		0.87 (0.54 - 1.33)		-4.24	73.3	91.0	89.6	40.7	97.6	8.14	3.41
NAFLD Index for Women				Low	-9.61	94.7	62.2	67.4	32.0	98.4	2.51	11.74
	Mild ≤		0.90 (0.66 - 1.02)	Optimal	-7.65	73.7	90.4	87.7	58.9	94.8	7.68	3.41
				high	-7.05	59.2	95.1	91.3	30.8	94.8	12.08	2.33
	Moderate ≤		0.88 (0.45 - 0.81)		-7.53	70.3	86.9	85.7	31.0	97.2	5.37	2.93
	Severe		0.91 (0.35 - 0.94)		-7.30	88.9	85.6	85.7	11.1	99.8	6.17	7.71
Fatty liver disease index (FLD index)	Mild ≤		0.88 (0.35 - 0.51)		29.0	85.0	76.9	78.5	47.5	95.4	3.68	5.13
	Moderate ≤		0.86 (0.28 - 0.43)		30.3	70.4	80.2	79.2	29.6	95.8	3.56	2.71
	Severe		0.90 (0.27 - 0.50)		32.3	79.2	87.7	87.4	11.9	99.4	6.44	4.22
Hepatic steatosis index (HSI)	Mild ≤		0.87 (0.28 - 0.40)		35.6	77.4	82.2	81.3	51.8	93.7	6.56	3.64
	Moderate ≤		0.85 (0.22 - 0.34)		35.8	77.5	78.1	78.0	29.4	96.7	3.54	3.47
	Severe		0.88 (0.20 - 0.38)		36.0	87.5	76.0	76.4	11.9	99.4	3.65	6.08

AUROC, Area Under the ROCs; 95% CI, 95% Confidence Interval

Table 5 Validaion of performance of NAFLD index in the validation group

			Sensitivity (%)	Specificity (%)	Accuracy (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Positive Likelihood ratio	Negative Likelihood ratio
NAFLD Index by all subjects	Mild ≤		69.6	77.3	75.7	44.3	90.8	3.07	2.54
		using in Men	77.3	58.5	63.2	38.6	88.4	1.86	2.58
		using in Women	67.6	85.4	82.2	51.1	92.1	4.63	2.64
	Moderate ≤		73.3	77.7	77.2	28.9	95.9	3.29	2.91
		using in Men	71.4	60.3	62.1	25.6	91.7	1.80	2.11
		using in Women	75.0	84.6	83.8	31.6	97.3	4.87	3.38
	Severe		80.0	80.0	80.1	19.0	98.6	4.00	4.00
		using in Men	100	64.6	66.7	14.7	100	2.82	-
		using in Women	70.0	87.4	86.5	24.1	98.1	5.56	2.91
NAFLD Index for Men	Mild ≤		63.6	84.6	79.3	58.3	87.3	4.13	2.32
	Moderate ≤		57.1	86.3	81.6	44.4	91.3	4.17	2.01
	Severe		60.0	85.3	83.9	18.2	96.1	4.08	2.13
NAFLD Index for Women	Mild ≤		61.8	89.4	84.3	56.8	91.2	5.83	2.34
	Moderate ≤		75.0	85.8	84.9	33.3	97.3	5.28	3.43
	Severe		70.0	85.1	84.3	22.6	96.8	4.70	2.84
Fatty liver disease index (FLD index)	Mild <		76.8	68.5	70.2	38.7	91.9	2.44	2.95
	Moderate ≤		80.0	76.4	76.8	29.6	96.9	3.39	3.82
	Severe		80.0	86.4	86.0	25.5	98.7	5.88	4.32
Hepatic steatosis index (HSI)	Mild <		67.9	78.7	76.5	67.9	78.7	3.19	2.45
	Moderate ≤		73.3	76.9	76.5	28.2	95.9	3.17	2.88
	Severe		86.7	76.7	77.2	17.8	99.0	3.72	5.77

AUROC, Area Under the ROCs; 95% CI, 95% Confidence Interval

was 81.6% and 84.9%, 83.9 and 84.3%, respectively, similar to the values in the estimation group (**Table 5**). These findings indicate that the predictive performance of the NAFLD index for the validation group was similar to that for the estimation group.

IV. Discussion

In the present study, we constructed a new index to predict the presence of NAFLD in the Japanese population, the NAFLD index, using easily available clinical and laboratory data, such as BMI and triglyceride levels for men, and BMI, albumin, ALT/AST ratio and HDL cholesterol levels for women. The performance of this index was confirmed in validation studies.

Some non-invasive imaging methods, such as US, CT and MRI, are reasonably accurate, and have been recommended as the standard to diagnose the presence of fatty liver disease. However, a simple, non-invasive test that is a valid screening tool is needed to identify patients at high risk for NAFLD, because it is reasonable that patients with a high risk for NAFLD are not always examined using those imaging modalities for mass screening.

We used US instead of histology as the standard for the diagnosis of steatosis in this study because US has been recommended as the standard and it enables a highly accurate diagnosis of fatty liver disease. In addition, our study used subjects who attended health check-up programs, and liver biopsies in asymptomatic individuals are usually unavailable.

In this study, we constructed two types of NAFLD index in the estimation group. One type was the NAFLD index for all subjects using BMI, albumin, ALT/AST, triglyceride and HDL cholesterol levels, and the other type was the NAFLD index for men and for women using BMI and triglyceride levels for men, and BMI, albumin, ALT/AST ratio and HDL cholesterol levels for women. The reason for using the two types of NAFLD index was to provide an NAFLD index with a higher predictive performance for NAFLD. Therefore, the predictive performance for NAFLD compared the NAFLD index for all subjects with the NAFLD index for men and for women. In the estimation group, for the detection of NAFLD (mild ≤), the AUROC for the NAFLD index for all subjects was 0.90 (95% CI 0.81−1.14), and the optimal cut-off point with an accuracy of 83.8%, namely, the predictive performance of the NAFLD index for all subjects had higher performance. However, the optimal cut-off point was used for men and women, respectively, although it predicted NAFLD with an 86.2% accuracy in women, while for men it was lower at 77.6%. We estimate that the reason for the lower predictive performance for men was caused by the lower ratio of men (28.5%) in the estimation group. Furthermore, BMI, abdominal circumference, fasting plasma glucose, albumin, ALT, ALT/AST, γ-GTP, triglyceride, total cholesterol and HDL cholesterol were significantly different between the sexes. Therefore, the estimated values of factors for the NAFLD index for all subjects were used to calculate the predominant value for women in the estimation group. On the other hand, the NAFLD index for men and women predicted NAFLD with an accuracy of 82.8% and 87.7%, respectively, values that were higher than the values for men and for women using the NAFLD index for all subjects. These results suggest that the NAFLD index for men and for women should be used to accurately predict the presence of NAFLD.

Some previous studies have attempted to estimate a screening tool for fatty liver disease, and those tools can be used to predict the presence of NAFLD, such as the NAFLD liver fat score, SteatoTest, fatty liver index (FLI), the fatty liver disease (FLD) index and hepatic steatosis index (HSI). The NAFLD liver fat score was developed using the presence of metabolic syndrome and type 2 diabetes, fasting serum insulin, AST and AST/ALT ratio in the Finnish population[12]. The SteatoTest for the diagnosis of steatosis was developed to identify patients with grade 2−4 steatosis of different etiologies in the French population. A SteatoTest includes 12 clinical variables such as ALT α2-macroglobulin, apolipoprotein A-I, haptoglobin, total bilirubin, γ-glutamyltransferase, cholesterol, triglycerides, glucose, age, gender and BMI[13]. In a general health-check program, α2-macroglobulin, apolipoprotein A-I and haptoglobin are not usually examined, and our study did not provide them. The FLI was developed in a cohort in which hepatic steatosis was diag-

nosed using ultrasound in an Italian population. The FLI incorporates the results of biochemical tests and anthropometric data, such as BMI, waist circumference, γ-glutamyltransferase and triglycerides[14]. Those three predictors for NAFLD were developed for the European population, and therefore, they may not be suitable for the Japanese population since the different factors used for each index may be attributed to interracial differences of lifestyles which cause NAFLD.

The FLD index and HSI were developed using an Asia-Pacific area population. The FLD index was developed for the Chinese population using biochemical tests and anthropometric data such as BMI, triglycerides, ALT/AST ratio and the presence of hyperglycemia[15], and the HSI, which uses the ALT/AST ratio, BMI, gender and diabetes mellitus, was developed for the Korean population[16]. The NAFLD index for men and for women, and those two predictors of NAFLD incorporate similar biochemical tests and anthropometric data, but are not identical. The FLD index was proposed for Chinese subjects: NAFLD can be ruled out if the FLD index is <28 and NAFLD can be detected if the FLD index is >37 in the Chinese population. Similarly, the HSI was proposed for Korean subjects: NAFLD can be ruled out if the HSI is <30.0 and NAFLD can be detected if the HSI is >36.0 in the Korean population. To compare the predictive performance of the NAFLD index for men and for women with the FLD index and the HSI, the optimal discriminating cut-off point of the FLD index and HSI was determined by ROC analysis in the estimation group. The optimal cut-off point of 29.0 for the FLD index predicted NAFLD with an accuracy of 78.5%, and the optimal cut-off point of 36.5 for the HSI predicted NAFLD with an accuracy of 81.3%. Those predictors have a higher predictive performance than the NAFLD, however, the accuracy of the FLD index and the HSI were lower than the accuracy of the NAFLD index for men and for women. These results suggest that a predictor of NAFLD needs to be designed for each different population even in the Asia-Pacific area population, since the different factors used for each index may be attributed to interracial differences of lifestyles which cause NAFLD.

The NAFLD index for men and for women is not only an accurate predictor for NAFLD but is also an accurate estimation tool for hepatic steatosis in NAFLD. Regarding its accuracy, the NAFLD index for men and for women was moderate $\leq$ hepatic steatosis in NAFLD had an accuracy of 82.8% and 85.7%, respectively. Severe hepatic steatosis in NAFLD had an accuracy of 89.6% in men and 85.7% in women, respectively. The NAFLD index for men and for women has a higher predictive performance in all hepatic steatosis grades in NAFLD.

These findings suggest that the NAFLD index for men and for women enables an effective prediction of the presence of NAFLD as a simple and accurate screening tool for NAFLD. The cut-off points of -6.96 in the NAFLD index for men and of -9.61 in the NAFLD index for women predict NAFLD with a sensitivity of 94.7%. The cut-off points of -4.60 in the NAFLD index for men and of -7.05 in the NAFLD index for women predict NAFLD with specificities of 94.8% and 95.1%, respectively (**Table 4**). If a male or female subject has an NALFD index of <-6.96 or <-9.61, respectively, NAFLD can be ruled out. On the other hand, if the male or female subject has an NALFD index of $-4.60<$ or $<-7.05<$, respectively, he or she has a high possibility of NAFLD, and should undergo US screening for a diagnosis of NAFLD.

However, there are limitations to using the NAFLD index to predict NAFLD, because this index uses only clinical and laboratory data. We hope to further improve the NAFLD index to more accurately predict NAFLD. We are investigating the early detection of NAFLD by another approach, using serum microRNAs (miRNAs). We have already reported that circulating microRNAs, such as miR-21, miR-34a, miR-122 and miR-451, are associated with NAFLD[18]. We hope that NAFLD can be detected accurately and at an earlier stage in the near future by combining, for example, the NAFLD index with miRNA data.

V. Conclusion

In conclusion, a new index for the non-invasive prediction of NAFLD for the Japanese population, the NAFLD index, was constructed for men and for women, using easily available clinical and laboratory data.

The NAFLD index is a simple, non-invasive screening tool to predict the presence of NAFLD, and this index has a higher assessment performance for hepatic steatosis grade in patients with NAFLD.

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ORIGINAL ARTICLE

Factors correlating with acoustic radiation force impulse elastography in chronic hepatitis C

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Abstract

AIM: To investigate the factors other than fibrosis stage correlating with acoustic radiation force impulse (ARFI) elastograpy in chronic hepatitis C.

METHODS: ARFI elastograpy was performed in 108 consecutive patients with chronic hepatitis C who underwent a liver biopsy. The proportion of fibrosis area in the biopsy specimens was measured by computer-assisted morphometric image analysis.

RESULTS: ARFI correlated significantly with fibrosis stage (β = 0.1865, $P < 0.0001$) and hyaluronic acid levels (β = 0.0008, P = 0.0039) in all patients by multiple regression analysis. Fibrosis area correlated significantly with ARFI by Spearman's rank correlation test but not by multiple regression analysis. ARFI correlated significantly with body mass index (BMI) (β = -0.0334, P = 0.0001) in $F0$ or $F1$, with γ-glutamyltranspeptidase levels (β = 0.0048, P = 0.0012) in $F2$, and with fibrosis stage (β = 0.2921, P = 0.0044) and hyaluronic acid levels (β = 0.0012, P = 0.0025) in $F3$ or $F4$. The ARFI cutoff value was 1.28 m/s for $F \geq 2$, 1.44 m/s for $F \geq 3$, and 1.73 m/s for $F4$.

CONCLUSION: ARFI correlated with fibrosis stage and hyaluronic acid but not with inflammation. ARFI was affected by BMI, γ-glutamyltranspeptidase, and hyaluronic acid in each fibrosis stage.

Key words: Acoustic radiation force impulse; Body mass index; Chronic hepatitis C; Computer-assisted morphometric image analysis; Fibrosis stage; Hyaluronic acid; Liver stiffness measurement; Transient elastography; Velocity of shear wave

Core tip: The assessment of liver fibrosis stage is important to estimate prognosis and to identify the patients requiring antiviral treatment in chronic hepatitis C. Liver biopsy is a gold standard for assessing fibrosis, but is invasive. Thus methods for noninvasively assessing fibrosis have been developed. Liver stiffness measurement (LSM) by Fibroscan and acoustic radiation force impulse correlate with fibrosis stage. However, LSM may be affected by factors other than fibrosis, such as edema, steatosis, and inflammation.

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INTRODUCTION

The assessment of liver fibrosis stage is important to estimate prognosis and to identify the patients requiring antiviral treatment in chronic hepatitis C.

Methods for noninvasively assessing liver fibrosis have been developed. Liver stiffness measurement (LSM) by transient elastography (TE) with Fibroscan[1-3] and velocity of shear wave (Vs) measured by acoustic radiation force impulse (ARFI)[4-6] correlate with liver fibrosis stage in various liver diseases. However, LSM is affected by factors other than liver fibrosis, such as edema, steatosis, inflammation and necrosis. In particular, inflammation affects LSM; acute or chronic inflammation can result in a high LSM, indicating the presence of falsely higher fibrosis stage than the actual fibrosis stage by both TE[7-9] and ARFI[10-12]. However, Rizzo *et al*[13] reported that ARFI is not correlated with alanine aminotransferase (ALT) levels[13].

Liver fibrosis is usually semi-quantitatively assessed by the numerical systems of Scheuer[14], the Metavir group[15] or Ishak *et al*[16]. Direct measurements of the amount of fibrosis in a biopsy specimen by computer-assisted morphometric image analysis has been reported, in which morphometric collagen content is measured quantitatively; it has been shown to correlate well with liver biopsy assessment numerical systems scores[17-19]. Isgro *et al*[20] reported that fibrosis area has a better relationship with TE than Ishak stage[20], whereas our previous study demonstrated a better correlation of TE with fibrosis stage than with fibrosis area in patients with chronic hepatitis C[21].

In the present study, factors other than fibrosis stage that affect ARFI were investigated in patients with chronic hepatitis C. The proportion of fibrosis area was quantitatively measured by image analysis software in liver biopsy specimens and the correlation with ARFI was assessed.

MATERIALS AND METHODS

Ethical statement

This study was performed in strict accordance with the ethical guidelines of the Declaration of Helsinki and was approved by the Fujita Health University ethics committee. All study participants provided written informed consent.

Patients

A total of 108 consecutive patients with chronic hepatitis C virus infection who underwent a liver biopsy before treatment with interferon at Fujita Health University Hospital from October 2009 to October 2012 were included (Table 1). Liver biopsy was performed using a 14G disposable true-cut needle under ultrasonographic guidance. Sections were stained with hematoxylin-eosin and azan stain. Liver specimens of at least 1.5 cm length with more than 8 portal tracts were assessed. Liver biopsy specimens were assessed by two hepatologists (Yoshioka K and Nakaoka K) blinded to the clinical data according to Metavir score[15]. Fibrosis was staged as follows: *F*0, no fibrosis; *F*1, portal fibrosis without septa; *F*2, portal fibrosis and few septa; *F*3, numerous septa without cirrhosis; and *F*4, cirrhosis. Activity was graded as follows: A0, none; A1, mild; A2, moderate; and A3, severe activity. Steatosis was graded according to the nonalcoholic fatty liver disease activity score as follows: grade 0; < 5% of hepatocytes involved, grade 1, 5%-33%; grade 2, >33%-66%; and grade 3, > 66%[22]. When fibrosis stage, activity grade, or steatosis grade evaluated by the two hepatologists differed, the higher fibrosis stage, activity grade, or steatosis grade was adopted.

ARFI measurement

Vs measurement by ARFI was performed with a Siemens ACUSON S2000 (Mochida Siemens Medical Systems Co., Ltd., Tokyo, Japan) within 1 wk of liver biopsy[4]. A region in liver to be examined for elastic properties is targeted with a region-of-interest (ROI) cursor while performing B-mode imaging. Tissue at the ROI is mechanically excited using acoustic push pulses to generate localized tissue displacements. The displacements result in propagation of shear-wave away from the region of excitation which is tracked using ultrasonic correlation-based methods. The maximal displacement is estimated for many ultrasound tracking beams laterally adjacent to the single push-beam. By measuring the time to peak displacement at each lateral location, the shear wave propagation velocity can be reconstructed. The examination was performed on the right lobe of the liver. A measurement depth of 2-3 cm below the liver capsule was chosen. Ten successful acquisitions at different locations were performed on each patient, and the results are expressed in meters/second (m/s), and the median value was calculated. The shear wave propagation velocity is considered to be proportional to the square root of tissue elasticity.

The procedures were performed by two investigators (Nishikawa T and Hashimoto S) who were blind to clinical, serological and histological data. The correlation in Vs measurement between two operators was good (r = 0.934).

Proportion of fibrosis area in the liver biopsy specimens

The proportion of fibrosis area in the biopsy specimens was measured by computer-assisted morphometric image analysis. Liver biopsy specimens were stained with azan stain. Microscopic images of the entire biopsy specimen were obtained with a digital microscope (BZ-9000, Keyence, Tokyo, Japan). Fibrosis area, which was stained blue with azan, was marked and measured with Image Pro

Table 1 Characteristics of 108 patients with chronic hepatitis C virus infection

	All patients (n = 108)	F0 (n = 14)	P values of Mann-Whitney U test between F0 and F1	F1 (n = 17)	P values of Mann-Whitney U test between F0-1 and F2	F2 (n = 32)	P values of Mann-Whitney U test between F2 and F3	F3 (n = 31)	P values of Mann-Whitney U test between F3 and F4	F4 (n = 14)
Age (yr)[1]	59.5 (49.0-66.0)	48.0 (41.0-60.0)	NS	51.0 (41.8-65.5)	NS	61.5 (51.5-66.5)	NS	61.0 (52.0-67.0)	NS	60.5 (54.0-66.0)
Gender (female/male)[2]	52/56	8/6	NS	8/9	NS	15/17	NS	13/18	NS	8/6
BMI	22.5 (20.5-24.6)	22.0 (20.0-23.2)	NS	23.5 (19.8-25.4)	NS	23.0 (20.7-24.4)	NS	23.2 (21.1-26.1)	NS	21.9 (19.0-23.4)
Fibrosis stage (F0/F1/F2/F3/F4)	14/17/32/31/14	-		-		-		-		-
Inflammatory grade (A0/A1/A2/A3)	12/32/53/11	9/5/0/0	0.0261	2/15/0/0	0.0001	1/9/22/0	0.0060	0/2/22/7	NS	0/1/9/4
Steatosis grade (S0/S1/S/2/S3)	42/42/14/10	8/5/0/1	NS	7/9/0/1	NS	10/11/6/5	NS	9/14/5/3	NS	8/3/3/0
AST (IU/L)[1]	44.0 (31.5-82.0)	28.5 (24.0-38.0)	NS	36.0 (23.0-41.3)	0.0033	48.5 (36.5-101.5)	NS	48.0 (42.5-85.3)	NS	65.5 (37.0-88.0)
ALT (IU/L)[1]	55.0 (35.0-91.5)	37.5 (22.0-59.0)	NS	39.0 (24.8-52.0)	0.0095	65.0 (41.0-153.0)	NS	70.0 (41.3-109.0)	NS	64.0 (36.0-91.0)
γ-GTP (IU/L)[1]	33.0 (23.5-75.0)	23.0 (14.0-27.0)	0.0802	28.0 (19.5-71.3)	NS	39.5 (24.5-89.5)	NS	41.0 (28.0-96.8)	0.0329	30.5 (27.0-38.0)
Platelet count ($\times 10^4/\mu L$)[1]	14.3 (11.3-17.6)	14.6 (11.7-20.2)	NS	18.2 (16.6-21.2)	0.0107	16.1 (14.0-17.4)	0.0080	12.2 (11.3-14.3)	0.0078	10.1 (7.1-11.6)
Prothrombin time (INR)[1]	1.00 (0.96-1.06)	0.95 (0.90-0.99)	NS	0.96 (0.93-1.02)	NS	1.00 (0.95-1.03)	0.0144	1.03 (1.00-1.08)	0.0229	1.10 (1.03-1.12)
Albumin (g/dL)[1]	4.2 (4.0-4.5)	4.4 (4.1-4.6)	NS	4.4 (4.2-4.5)	NS	4.3 (4.0-4.5)	0.0524	4.1 (3.8-4.3)	NS	4.0 (3.8-4.2)
Total cholesterol (mg/dL)[1]	170 (150-188)	193 (177-207)	0.0619	169 (155-193)	NS	172 (156-189)	0.0615	159 (141-177)	NS	160 (144-183)
γ-globulin (g/dL)[1]	1.51 (1.33-1.79)	1.28 (1.14-1.40)	0.0262	1.40 (1.28-1.70)	NS	1.44 (1.34-1.64)	0.0067	1.63 (1.51-2.11)	NS	1.66 (1.43-1.97)
Hyaluronic acid (ng/mL)[1]	89 (49-206)	39 (30-64)	NS	49 (26-77)	0.0041	89 (66-185)	0.0601	184 (82-245)	0.0291	232 (191-338)
HCV genotype (1/2)	81/26	10/4	NS	12/5	NS	22/9	NS	5/26	NS	11/3
HCV RNA (logIU/mL)[1]	6.6 (5.8-7.0)	6.5 (6.0-6.9)	NS	6.6 (5.4-7.0)	NS	6.6 (5.8-7.1)	NS	6.7 (5.9-7.1)	NS	6.6 (6.3-6.8)
Fibrosis area (%)[1]	2.63% (1.35-4.95)	0.85% (0.41-1.04)	0.0111	1.37% (0.73-1.85)	0.0022	2.20% (1.62-2.74)	< 0.0001	4.83% (4.03-6.24)	< 0.0001	8.87% (8.04-10.52)
Velocity of shear wave (m/s)[1]	1.38 (1.19-1.71)	1.2 (1.0-1.3)	NS	1.1 (1.0 -1.2)	0.0010	1.3 (1.2-1.6)	0.0014	1.6 (1.5-1.8)	0.0008	2.1 (1.9-2.2)

[1]Data are shown as median (interquartile range); [2]Difference of frequency of gender was assessed by χ^2 test. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γ-GTP: γ-glutamyltranspeptidase.

Plus 4.0 imaging software (Nippon Roper Co., Ltd., Tokyo, Japan).

Statistical analysis

Patients were categorized according to fibrosis stage. The groups were compared with the χ^2 test and Mann-Whitney U test. Factors correlated with ARFI were estimated by Spearman's rank correlation test. Factors independently correlated with ARFI were assessed by multiple regression analysis. The diagnostic performance of ARFI and fibrosis area was determined in terms of sensitivity, specificity, positive and negative predictive value, positive likelihood ratio, diagnostic accuracy, and area under the receiver operating characteristics (ROC) curve. Optimal cutoff values between fibrosis categories were determined at maximum sum of sensitivity and specificity. Data were analyzed using StatFlex version 5.0 for Windows (StatFlex, Osaka, Japan). A two-sided P value of < 0.05 indicated statistical significance.

RESULTS

Semiquantitative histological assessment using the Metavir system

The liver biopsies of the 108 patients were assessed by the Metavir system. Fibrosis stage was $F0$ in 14 patients, $F1$ in 17, $F2$ in 32, $F3$ in 31 and $F4$ in 14 (Table 1).

ARFI measurement

ARFI was significantly correlated with fibrosis stage ($\rho = 0.732$, $P < 0.0001$) (Figure 1A). ARFI values differed significantly between stages $F1$ and $F2$ ($P = 0.0010$), between $F2$

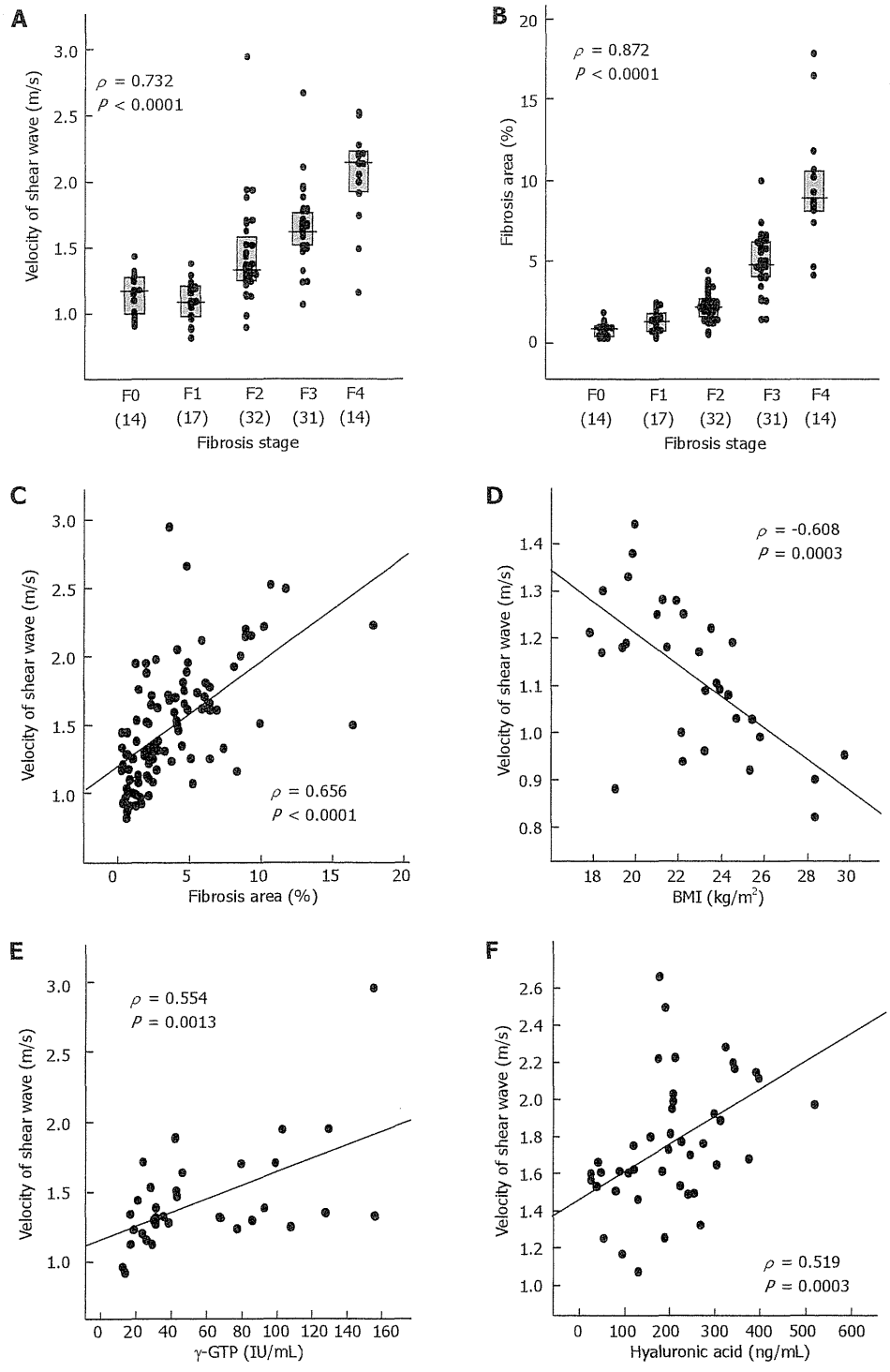

Figure 1 Correlation. A: Between acoustic radiation force impulse (ARFI) and fibrosis stages. The velocity of the sheer wave measured by ARFI was significantly correlated with fibrosis stage in all 108 patients as assessed by the Metavir system (ρ = 0.732, P < 0.0001). Vertical lines and boxes indicate median values and interquartile ranges, respectively; B: Between proportion of fibrosis area and fibrosis stage. The proportion of fibrosis area was significantly correlated with fibrosis stage in all 108 patients as assessed by the Metavir system (ρ = 0.872, P < 0.0001). Vertical lines and boxes indicate median values and interquartile ranges, respectively; C: Between ARFI and proportion of fibrosis area. The velocity of the sheer wave measured by ARFI significantly correlated with the proportion of fibrosis area in all 108 patients (ρ = 0.656, P < 0.0001); D: Between ARFI and body mass index (BMI). The velocity of the sheer wave measured by ARFI significantly negatively correlated with BMI in patients with stage F0 or F1 (ρ = −0.608, P = 0.0003); E: Between ARFI and γ-glutamyltranspeptidase (γ-GTP) levels. The velocity of the sheer wave measured by ARFI significantly correlated with γ-GTP levels in patients with stage F2 (ρ = 0.544, P = 0.0013); F: Between ARFI and hyaluronic acid levels. The velocity of the sheer wave as measured by ARFI significantly correlated with hyaluronic acid levels in patients with stage F3 or F4 (ρ = 0.519, P = 0.0003).

and *F*3 (P = 0.0014), and between *F*3 and *F*4 (P = 0.0008) (Table 1).

The ARFI cutoff values for different fibrosis stages determined by ROC analysis were 1.28 m/s for $F \geq 1$,

Table 2 Optimal cutoff value of velocity of shear wave for each fibrosis stage was determined at maximum sum of sensitivity and specificity

	$F \geq 1$	$F \geq 2$	$F \geq 3$	$F4$
Cutoff value (m/s)	1.28	1.28	1.44	1.73
Positive predictive value	97.0%	94.0%	78.4%	48.0%
Negative predictive value	29.3%	65.9%	91.2%	97.6%
Sensitivity	69.1%	81.8%	88.9%	85.7%
Specificity	85.7%	87.1%	82.5%	86.2%
Positive likelihood ratio	4.8	6.3	5.1	6.2
Diagnostic accuracy	71.3%	83.3%	85.2%	86.1%
AUROC	0.810	0.909	0.869	0.885
Standard error of AUROC	0.046	0.027	0.036	0.058

AUROC: Area under receiver operating characteristic curve

Table 3 Optimal cutoff value of fibrosis area for each fibrosis stage was determined at maximum sum of sensitivity and specificity

	$F \geq 1$	$F \geq 2$	$F \geq 3$	$F4$
Cutoff value	1.17%	1.8%	3.71%	7.32%
Positive predictive value	97.7%	94.3%	93.0%	92.3%
Negative predictive value	60.0%	71.1%	92.3%	97.9%
Sensitivity	91.5%	85.7%	88.9%	85.7%
Specificity	85.7%	87.1%	95.2%	98.9%
Positive likelihood ratio	6.4	6.6	18.7	80.6
Diagnostic accuracy	90.7%	86.1%	92.6%	97.20%
AUROC	0.935	0.927	0.963	0.962
Standard error of AUROC	0.025	0.024	0.018	0.023

AUROC: Area under receiver operating characteristic curve

1.28 m/s for $F \geq 2$, 1.44 m/s for $F \geq 3$, and 1.73 m/s for $F4$ (Table 2).

Fibrosis area in liver biopsy specimens

The proportion of fibrosis area was significantly correlated with fibrosis stage as assessed by the Metavir system ($\rho = 0.872$, $P < 0.0001$) (Figure 1B). The fibrosis area values differed significantly between stages $F0$ and $F1$ ($P = 0.0111$), $F1$ and $F2$ ($P = 0.0022$), $F2$ and $F3$ ($P < 0.0001$), and between $F3$ and $F4$ ($P < 0.0001$) (Table 1).

The fibrosis area cutoff values for the different fibrosis stages determined by ROC analysis were 1.17% for $F \geq 1$, 1.80% for $F \geq 2$, 3.71% for $F \geq 3$, and 7.32% for $F4$ (Table 3).

Factors correlating with ARFI in all 108 patients

ARFI was significantly correlated with fibrosis stage ($P < 0.0001$) (Figure 1A), inflammatory grade ($P < 0.0001$), aspartate aminotransferase (AST) levels ($P < 0.0001$), ALT levels ($P = 0.0008$), γ-glutamyltranspeptidase (γ-GTP) levels ($P < 0.0001$), platelet count ($P < 0.0001$), prothrombin time (INR) ($P = 0.0003$), albumin levels ($P = 0.0002$), total cholesterol levels ($P = 0.0004$), γ-globulin levels ($P = 0.0087$), hyaluronic acid levels ($P < 0.0001$), and fibrosis area ($P < 0.0001$) (Figure 1) by Spearman's rank correlation test (Table 4). ARFI tended to be higher in genotype 1 [median, 1.49 (interquartile range, 1.22-1.75) m/s] than in genotype 2 [1.30 (1.17-1.46)] ($P = 0.0728$). The multiple regression analysis selected fibrosis stage ($\beta = 0.1865$, $P < 0.0001$) and hyaluronic acid levels ($\beta = 0.0008$, $P = 0.0039$) as factors that independently correlated with ARFI, whereas inflammatory grade, AST, ALT and fibrosis area were not selected (Table 4).

Factors correlating with ARFI in stage F0 or F1 patients

To elucidate the factors affecting ARFI other than fibrosis stage, patients with stage $F0$ or $F1$, those with $F2$, and those with $F3$ or $F4$ were analyzed separately.

Body mass index (BMI) was significantly correlated with ARFI ($P = 0.0003$) (Figure 1D) and ALT levels ($P = 0.0593$) and γ-GTP levels ($P = 0.0614$) tended to be correlated with ARFI by Spearman's rank correlation test in the 31 patients with stage $F0$ or $F1$ (Table 4). Only BMI was correlated with ARFI by multiple regression analysis ($\beta = -0.0334$, $P = 0.0001$).

Factors correlating with ARFI in the stage F2 patients

γ-GTP levels were significantly correlated with ARFI ($P = 0.0013$) (Figure 1E) and γ-globulin levels ($P = 0.0581$) tended to be correlated with ARFI in the 32 patients with stage $F2$ by Spearman's rank correlation test (Table 4). The multiple regression analysis only selected γ-GTP levels as a factor correlating with ARFI ($\beta = 0.0048$, $P = 0.0012$).

Factors correlating with ARFI in the stage F3 or F4 patients

In the patients with stage $F3$ or $F4$, fibrosis stage ($P = 0.0004$), platelet count ($P = 0.0036$), prothrombin time (INR) ($P = 0.0080$), albumin levels ($P = 0.0015$), hyaluronic acid levels ($P = 0.0003$) (Figure 1F), and fibrosis area ($P = 0.0481$) were significantly correlated with ARFI by Spearman's rank correlation test (Table 4). The multiple regression analysis selected fibrosis stage ($\beta = 0.2921$, $P = 0.0044$) and hyaluronic acid levels ($\beta = 0.0012$, $P = 0.0025$) as factors correlating with ARFI.

DISCUSSION

The assessment of fibrosis stage is important to estimate prognosis and to identify the patients requiring antiviral treatment in chronic hepatitis C. A lot of noninvasive methods to assess liver fibrosis stage other than liver biopsy are available, for example, ARFI, TE, real-time elastography[23], and algorithm of serum fibrosis markers such as FibroTest[24] and APRI[25]. They provide good performances in estimation of fibrosis stage, while there are problems such as influence of inflammation. In the present study, factors other than fibrosis stage that affect ARFI were investigated in patients with chronic hepatitis C.

The present study confirmed findings reported previously that ARFI correlates with fibrosis stage[10-13,26,27]. The ARFI cutoff values for different fibrosis stages were 1.28 m/s for $F \geq 1$, 1.28 m/s for $F \geq 2$, 1.44 m/s for $F \geq 3$ and 1.73 m/s for $F4$. This result suggests that distinguishing between $F0$ and $F1$ is impossible, as the cutoff