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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 $F \geqslant 2$, 1.44 m/s for $F \geqslant 3$, 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 *F*0 in 14 patients, *F*1 in 17, *F*2 in 32, *F*3 in 31 and *F*4 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 *F*1 and *F*2 ($P = 0.0010$), between *F*2

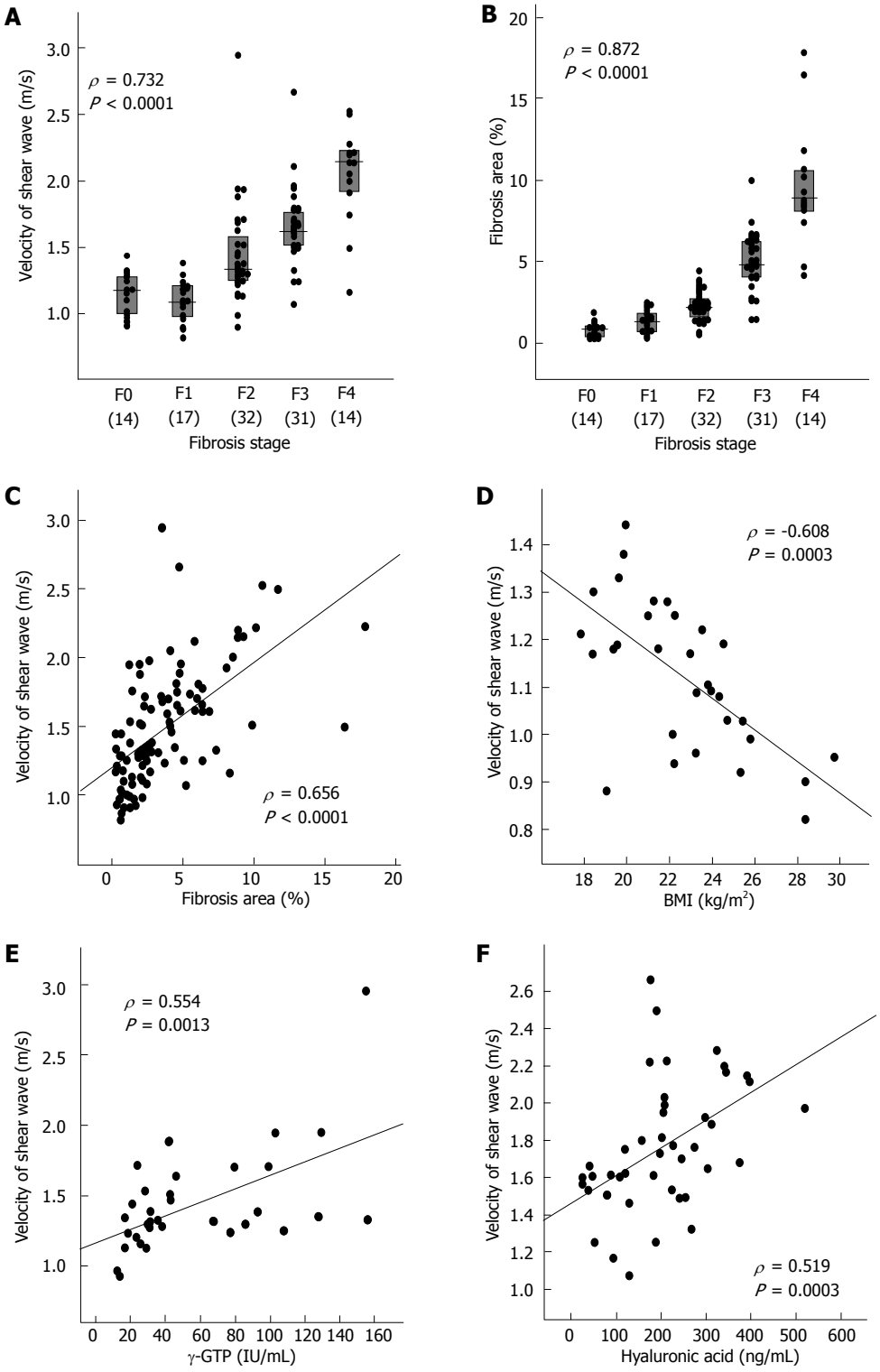

Figure 1 Correlation. A: Between acoustic radiation force impulse (ARFI) and fibrosis stages. The velocity of the sheer wave measured by ARFI was significantly correlated with fibrosis stage in all 108 patients as assessed by the Metavir system (ρ = 0.732, P < 0.0001). Vertical lines and boxes indicate 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 $F \geqslant 1$,

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

	$F \geqslant 1$	$F \geqslant 2$	$F \geqslant 3$	*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

	$F \geqslant 1$	$F \geqslant 2$	$F \geqslant 3$	*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 $F \geqslant 2$, 1.44 m/s for $F \geqslant 3$, and 1.73 m/s for *F*4 (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 *F*0 and *F*1 ($P = 0.0111$), *F*1 and *F*2 ($P = 0.0022$), *F*2 and *F*3 ($P < 0.0001$), and between *F*3 and *F*4 ($P < 0.0001$) (Table 1).

The fibrosis area cutoff values for the different fibrosis stages determined by ROC analysis were 1.17% for $F \geqslant 1$, 1.80% for $F \geqslant 2$, 3.71% for $F \geqslant 3$, and 7.32% for *F*4 (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 *F*0 or *F*1, those with *F*2, and those with *F*3 or *F*4 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 *F*0 or *F*1 (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 *F*2 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 *F*3 or *F*4, 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 $F \geqslant 1$, 1.28 m/s for $F \geqslant 2$, 1.44 m/s for $F \geqslant 3$ and 1.73 m/s for *F*4. This result suggests that distinguishing between *F*0 and *F*1 is impossible, as the cutoff

Table 4 Factors correlating with velocity of shear wave in 108 patients with chronic hepatitis C virus infection

	All patients (n = 108)				Patients with $F0$ or $F1$ (n = 31)				Patients with $F2$ (n = 32)				Patients with $F3$ or $F4$ (n = 45)			
	Spearman's rank correlation test		Multiple regression analysis		Spearman's rank correlation test		Multiple regression analysis		Spearman's rank correlation test		Multiple regression analysis		Spearman's rank correlation test		Multiple regression analysis	
	ρ	*P* value	β	*P* value	ρ	*P* value	β	*P* value	ρ	*P* value	β	*P* value	ρ	*P* value	β	*P* value
Age (yr)		NS				NS				NS				NS		
Gender (female/male)[1]		NS				NS				NS				NS		
BMI		NS			-0.608	0.0003	-0.033	0.0001		NS				NS		
Fibrosis stage	0.732	< 0.0001	0.187	0.0001		NS							0.505	0.0004	0.292	0.0044
Inflammatory grade	0.612	< 0.0001		NS		NS				NS				NS		
Steatosis grade		NS				NS				NS				NS		
AST (IU/L)	0.430	< 0.0001		NS		NS				NS				NS		
ALT (IU/L)	0.318	0.0008		NS	0.343	0.0593		NS		NS				NS		
γ-GTP (IU/L)	0.407	< 0.0001		NS	0.340	0.0614		NS	0.544	0.0013	0.005	0.0012		NS		
Platelet count ($\times 10^4$/μL)	-0.441	< 0.0001		NS		NS				NS			-0.425	0.0036		NS
Prothrombin time (INR)	0.344	0.0003		NS		NS				NS			0.390	0.0080		NS
Albumin (g/dL)	-0.347	0.0002		NS		NS				NS			-0.459	0.0015		NS
Total cholesterol (mg/mL)	-0.337	0.0004		NS		NS				NS				NS		
γ-globulin (g/dL)	0.252	0.0087		NS		NS			-0.344	0.0581		NS		NS		
Hyaluronic acid (ng/mL)	0.576	< 0.0001	8.00E-4	0.0039		NS				NS			0.519	0.0003	0.001	0.0025
HCV genotype (1/2)[1]		0.0728		NS		NS				NS				NS		
HCV RNA (logIU/mL)		NS				NS				NS				NS		
Fibrosis area (%)	0.656	< 0.0001		NS		NS				NS			0.296	0.0481		NS
R			0.707				0.645				0.546				0.634	
Adjusted R			0.490				0.396				0.275				0.373	
F			51.800				20.700				12.700				14.100	
P value			< 0.0001				0.0001				0.0012				< 0.0001	

[1]Difference of frequency of gender or genotype was assessed by Mann-Whitney *U* test. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γ-GTP: γ-glutamyltranspeptidase.

value for $F \geqslant 1$ and that for $F \geqslant 2$ are the same. However, Sporea *et al*[26] reported that the cutoff value is 1.19 m/s for $F \geqslant 1$, 1.33 m/s for $F \geqslant 2$, 1.43 m/s for $F \geqslant 3$, and 1.55 m/s for $F4$[26]. Rizzo *et al*[13] reported that the cutoff value is 1.3 m/s for $F \geqslant 2$, 1.7 m/s for $F \geqslant 3$ and 2.0 m/s for $F4$[13]. Thus, discrepancies are apparent among the cutoff values reported in different studies. The discrepancies are probably attributed to the difference in the population studied. Further studies should be conducted to establish standard ARFI cutoff values for staging fibrosis.

In the present study, AST, ALT and inflammatory grade were correlated with ARFI in the univariate analysis that included all patients, but were not selected as factors independently correlating with ARFI in the multiple regression analysis. In addition, inflammatory factors did not correlate with ARFI when patients with different fibrosis stages were analyzed separately. These results suggest that inflammatory activity does not affect ARFI in patients with chronic hepatitis C. Rizzo *et al*[13] also reported that ARFI is not associated with ALT, BMI, Metavir grade, or liver steatosis, whereas TE is significantly correlated with ALT[13]. Bota *et al*[10] reported that discordance of at least two fibrosis stages between ARFI and histologic assessment were associated with female sex, interquartile range interval (IQR) $\geqslant$ 30%, high AST and high ALT in univariate analysis, while, in multivariate analysis, the female gender and IQR $\geqslant$ 30% ($P = 0.004$) were associated with the discordances. In contrast, Yoon *et al*[12] reported that the optimum ARFI cutoff values are 1.13 m/s for $F \geqslant 2$ and 1.98 m/s for $F4$, whereas these values decreased to 1.09 m/s for $F \geqslant 2$ and 1.81 m/s for $F4$ when patients with normal ALT levels were selected. Chen *et al*[11] reported that ALT, ActiTest A score, Metavir activity (A) grade, Metavir F stage, BMI, and platelet count are independently associated with ARFI and suggested that a 100 IU/L increase in serum ALT levels augmented ARFI by approximately 0.155 m/s. In the present study, only 25 patients had ALT levels of 100 IU/L

or higher. The low ALT levels among the patients studied may be a reason why ALT was not correlated with ARFI.

A multiple linear regression analysis in our previous study on TE selected fibrosis area, ALT levels, γ-GTP levels, prothrombin time, and hyaluronic acid levels as factors correlating with TE[21]. Many studies on TE have reported that LSM is affected by ALT levels. Franquelli *et al*[28] reported that TE fibrosis staging is overestimated by necroinflammatory activity and steatosis. Coco *et al*[7] found that LSM is higher in patients with an elevated ALT than in those with either spontaneous biochemical remission or after antiviral therapy. Thus, it is probable that ALT or inflammatory activity affects TE. However, it is still unclear whether they also affect ARFI. Further studies are needed to clarify factors that affect ARFI other than fibrosis stage.

ARFI was significantly correlated with BMI in the 31 patients with stage *F*0 or *F*1; the higher the BMI, the lower the ARFI. However, ARFI was not associated with steatosis grade. Motosugi *et al*[29] reported that fat deposition in the liver does not affect ARFI. Thus, the negative correlation between BMI and ARFI could not be attributed to steatosis, which accompanies higher BMI[30]. Actually, BMI and steatosis grade were not correlated in patients with stage *F*0 or *F*1 in the present study (data not shown). The mechanism of the association between higher BMI and lower ARFI is unclear. Because a higher BMI is associated with lower ARFI, and may cause an underestimation of fibrosis staging, careful attention should be paid to BMI during ARFI staging of fibrosis in patients with stage *F*0 or *F*1 disease.

ARFI significantly correlated with γ-GTP levels in patients with *F*2 and with fibrosis stage and hyaluronic acid levels in patients with stage *F*3 or *F*4. γ-GTP[24,31] and hyaluronic acid[32,33] levels have been regarded as the most informative fibrosis markers. Thus, it is reasonable that γ-GTP and hyaluronic acid levels independently correlated with ARFI.

Isgro *et al*[20] showed that the collagen proportional area has a better relationship with TE and with hepatic venous pressure gradient compared with Ishak stage. In the present study, fibrosis area was correlated significantly with fibrosis stage, but only fibrosis stage and hyaluronic acid levels were selected as factors independently correlating with ARFI. Our previous study demonstrated a better correlation of TE with fibrosis stage than with fibrosis area in patients with chronic hepatitis C[21]. The Metavir stages represent categories of increasing fibrosis severity based on a combination of location and quantity of scarring as well as whether the fibrous tissue forms septa, bridges, or nodules. Fibrosis area represents only the quantity of fibrosis in liver tissues. Our results indicate that not only the quantity of fibrosis but also other histological factors such as patterns of fibrosis also affect ARFI.

The present study demonstrated that ARFI correlated with fibrosis stage but was not associated with inflammation. BMI negatively correlated with ARFI in the patients with stage *F*0 or *F*1. γ-GTP and hyaluronic acid levels were positively correlated in those with stage *F*2 and in those with *F*3 or *F*4, respectively. Thus, careful attention should be paid to BMI, γ-GTP levels, and hyaluronic acid levels when estimating fibrosis stage by ARFI. Fibrosis stage showed a better correlation with ARFI than fibrosis area, indicating that not only the quantity of fibrosis but also other factors such as patterns of fibrosis also affect ARFI. Since the number of the patients studied is small, further studies are needed to confirm the conclusion of the present study.

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COMMENTS

Background

Most studies reported that liver stiffness measurement by Fibroscan was affected by inflammation. There have been both of the reports which demonstrated the correlation of inflammation and acoustic radiation force impulse (ARFI) and those which denied their correlation. The present study confirmed findings reported previously that ARFI correlates with fibrosis stage, and demonstrated that aspartate aminotransferase, alanine aminotransferase and inflammatory grade did not independently correlate with ARFI in the multiple regression analysis. The present study also demonstrated the correlation of body mass index (BMI) and ARFI for the first time.

Innovations and breakthroughs

The new findings of this study are the correlation of BMI and ARFI, and the denial of the correlation between ARFI and inflammation.

Applications

The results showed that ARFI correlated significantly with liver fibrosis stage and hyaluronic acid in all patients. ARFI correlated significantly with BMI in fibrosis stage F0-1, with γ-glutamyltranspeptidase (GTP) in F2, and with fibrosis stage and hyaluronic acid in F3-4. In conclusion, ARFI correlated with fibrosis stage and hyaluronic acid but not with inflammation. ARFI was affected by BMI, γ-GTP, and hyaluronic acid in each fibrosis stage.

Peer review

The authors reported the utilities of ARFI elastography for evaluation of hepatic fibrosis in patients with chronic hepatitis C. This paper looks very important and has a novelty in this study field.

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