The mechanism for the loss of HBeAg in patients with PC mutant has been thought to be that HBV with PC mutant cannot produce HBeAg. In the present study, 85.9% of HBeAg-negative patients had PC mixed type or PC mutant, and only 11.1% had PC wild type. This frequency of PC wild type in HBeAg-negative patients roughly coincides with the result of Yoo et al. (6.3%) [25]. The mechanism by which patients with PC wild type lose HBeAg has not been elucidated. Host defense system or mutations in region other than PC region may be responsible. The reason why HBeAg-negative patients with PC wild type had lower HBV DNA levels and ALT levels than those with PC mutant should be elucidated. Since HBeAg-positive patients with PC wild type had significantly higher HBV DNA levels than did HBeAg-negative patients with PC mutant, HBV with PC mutant probably propagates less efficiently than HBV with PC wild type. Therefore, host defense system or mutations in region other than PC region may be responsible for more strongly decreased HBV DNA levels in HBeAg-negative patients with PC wild type than in HBeAg-negative patients with PC mutant. Previous report showed that high serum viral load was the most important predictor of abnormal ALT levels among HBeAg-negative patients [26]. Another study reported an association between serum viral loads and histologic inflammation and fibrosis scores and ALT levels in HBeAg-negative patients [27]. In the present study, HBeAg-negative genotype C patients with PC wild type had lower HBV DNA levels and also lower ALT levels, which probably suggest better prognosis. Thus the follow-up of these patients may be reduced in frequency.

Previous studies showed that HBV genotype C patients had significantly higher serum viral loads, and were more likely to have PC and BCP mutants compared with HBV genotype B patients [5, 15, 17]. Other report also showed that HBV genotype C patients were more likely to have BCP mutants compared with genotype B patients among HBeAg-negative patients [18]. However, these studies have not shown the difference of serum viral loads between HBV genotype C patients and genotype B patients among HBeAg-negative patients. The present study demonstrated that, among HBeAg-negative patients, the patients with genotype B had significantly lower HBV DNA levels than those with genotype C, and that the prevalence of BCP wild type was significantly higher in patients with genotype B than those with genotype C. There was no difference in the prevalence of PC mutation between patients with genotype B and those with genotype C. Either among genotype C patients or among genotype B patients, there was no difference in HBV DNA levels between patients with BCP wild type and those with BCP mutant, although the number of genotype B patients with BCP mutant was too small. Thus the lower HBV DNA levels in genotype B may not correlate with the low prevalence of BCP mutation in genotype B. Further sequence studies on other regions of HBV are necessary to elucidate the mechanism of lower HBV DNA levels in HBeAg-negative genotype B patients.

In contrast to genotype C patients, the patients with PC mutant had similarly low HBV DNA levels and ALT levels compared with those with PC wild type in genotype B patients, although the number of genotype B patients with PC wild type was too small. Actually those with PC mutant tended to have lower HBV DNA levels and ALT levels in genotype B patients than genotype C patients. These findings may explain the lower HBV DNA levels in genotype B patients than in genotype C patients among HBeAg-negative patients. The cause of the difference between the two genotypes is not clear. The differences of specific sequence of HBV or host immune response between the two genotypes may be responsible for the difference. Further studies are needed to elucidate the cause of the difference between the two genotypes.

Recent study reported that HBV genotype B was classified into two subgenotypes called Ba (found in Asia) and Bj (found exclusively in Japan) [28, 29]. We did not determine subgenotypes of HBV genotype B. It was also reported that HBV subgenotype Bj accounted for 93% of carriers of HBV genotype B in Japan, and that higher prevalence rate of HBeAg and BCP mutations were observed in carriers of HBV subgenotype Ba than Bj [29]. For these reasons, most of the patients with HBV genotype B in the present study probably had subgenotype Bj.

The prevalence of genotype C patients and genotype B patients in our study was consistent with that in Japanese population [15], but we acknowledge that the number of genotype B patients was far smaller than that of genotype C patients in the present study. Thus, we may need further investigation such as a case–control study to confirm our results in the future. Furthermore, studies on the exact mechanism of active replication by the presence of PC and BCP mutations are also needed.

In conclusion, we showed that, among HBeAg-negative patients with genotype C, the patients with PC wild type had significantly lower viral loads and ALT levels than those with PC mutant, which probably suggests better prognosis for the patients with PC wild type. It is also suggested that host defense system or mutations in region other than PC region may be responsible for more strongly decreased HBV DNA levels in HBeAg-negative patients with PC wild type than in HBeAg-negative patients with PC mutant. We also showed that, among HBeAg-negative patients, the patients with genotype B had significantly lower viral loads than those with genotype C, and that the prevalence of BCP wild type was significantly higher in patients with genotype B than in those with genotype C. Further studies are needed to confirm our observations and

to determine if HBV genotyping and the mutations in PC and BCP region should be included in the clinical evaluation of chronic HBV infection and in the decision of antiviral therapy.

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Original Article

Liver stiffness measured by transient elastography correlates with fibrosis area in liver biopsy in patients with chronic hepatitis C

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Aim: Liver stiffness (LS) measured by transient elastography (TE) has been reported to correlate with liver fibrosis, which is usually semiquantitatively assessed. In the present study, the fibrosis area was measured by image analysis software in liver biopsy specimens and its correlation with LS was assessed.

Methods: LS was measured by TE in all 165 patients with chronic hepatitis C virus (HCV) infection who underwent liver biopsy consecutively in Fujita Health University Hospital from July 2004 to September 2007.

Results: Fibrosis area was significantly correlated with fibrosis stage as assessed by the Metavir score ($\rho = 0.733$, $P < 0.0001$). The optimal cut-off value of fibrosis area was 1.6% for F > or = 2, 3.1% for F > or = 3, and 3.8–6.4% for F4. LS was significantly correlated with fibrosis stage ($\rho = 0.734$, $P < 0.0001$). The optimal cut-off value of LS was 7.1 kPa for F > or = 2, 9.6 kPa for F > or = 3 and 11.6–16.9 kPa for F4. Multiple linear regression analysis selected fibrosis area ($P = 0.0002$), alanine aminotransferase (ALT) ($P = 0.0237$), γ-glutamyltransferase (γ-GTP) ($P = 0.0114$), prothrombin time ($P = 0.0114$) and hyaluronic acid ($P < 0.0001$) as factors correlating with LS.

Conclusion: The correlation between LS and liver fibrosis was confirmed by the objective measurement of fibrosis area. ALT was significantly correlated with LS, suggesting that inflammatory activity also affects LS values. Despite some limitation, LS measurement is a useful method for the diagnosis of liver fibrosis.

INTRODUCTION

NON-INVASIVE ASSESSMENT OF liver fibrosis is a major objective that has encouraged many new approaches. The prognosis and treatment of chronic liver diseases depend on the stage of liver fibrosis.[1] In chronic viral hepatitis, the presence of significant fibrosis (F > or = 2) indicates the necessity of antiviral therapies, and the outcome of therapy should be assessed by the alleviation of fibrosis stage. Diagnosis of advanced fibrosis is also important, since the risk of hepatocellular carcinoma or bleeding from esophageal varices is high in patients with advanced fibrosis.[2,3] Liver biopsy, the gold standard of assessment of liver fibrosis, is an invasive and expensive procedure, and its accuracy is sometimes questionable because of sampling errors, inadequate specimens and the subjective diagnosis of observers.[4,5]

Recently, transient elastography (TE) with the use of a new apparatus, FibroScan, for the non-invasive measurement of liver stiffness (LS) was developed.[6] LS measured by a FibroScan has been reported to correlate with stage of liver fibrosis in various liver diseases.[6–17]

Liver fibrosis is usually semiquantitatively assessed by the numerical systems of Scheuer,[18] the Metavir group,[19]

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or Ishak *et al.*[20] Recently, direct measurement of the amount of fibrosis in the biopsy specimen by computer-assisted morphometric image analysis has been reported, where the morphometric collagen content was measured quantitatively and correlated well with the score of numerical systems of liver biopsy assessment.[21–23]

In the present study, the proportion of fibrosis area was quantitatively measured by image analysis software in the liver biopsy specimen and the correlation with LS was assessed.

METHODS

Patients

ALL 165 PATIENTS with chronic hepatitis C virus (HCV) infection where liver biopsy was consecutively performed at Fujita Health University Hospital from July 2004 to September 2007 were studied (Table 1). Sections were stained with hematoxylin–eosin (HE) stain and azan stain. Liver biopsy specimens were assessed by a hepatologist (K.Y.) blinded of clinical data according to Metavir score.[24] Fibrosis was staged as: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Activity was graded as A0, none; A1, mild; A2, moderate; and A3, severe activity.

LS measurement

LS measurement by TE was performed with a FibroScan (EchoSens, Paris, France) within a week of liver biopsy. Ten validated measurements were made on each patient. The results were expressed in kilopascals (kPa). Only procedures with 10 validated measurements and a success rate of at least 60% (ratio of the number of successful acquisitions over the total number of acquisitions) were considered reliable. The median value was considered representative of the liver elastic modulus.

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 (Fig. 1). Liver biopsy specimens were stained with azan stain. The fibrosis area that was stained blue with azan was marked and measured with the image analysis software, Image Pro Plus 4.0 (Nippon Roper Co, Tokyo, Japan). Each specimen was examined at three different fields or more with the use of a 5X objective.

Statistical analysis

Patients were divided according to fibrosis stage. The groups were compared by χ^2-test and the Mann–Whitney *U*-test with Bonferroni's adjustment. Factors correlating with LS and fibrosis stages were estimated by the Spearmen's ρ coefficient. Factors independently correlating with LS were assessed by multiple regression analysis. The diagnostic performance of LS and fibrosis area was determined in terms of sensitivity, specificity, positive and negative predictive values, positive likelihood ratio, diagnostic accuracy and area under receiver operating characteristics (ROC) curves. Optimal cut-off values between fibrosis categories were determined at the maximum total of sensitivity and specificity with sensitivity of more than 80%. When the positive predictive value was less than 50%, other cut-off values were assessed by lowering the sensitivity less than 80%.

RESULTS

Semiquantitative histological assessment by the Metavir system

THE LIVER BIOPSIES of 165 patients were assessed by the Metavir system. Fibrosis stage was F0 in 14 patients, F1 in 52, F2 in 42, F3 in 33 and F4 in 24 (Table 1). All the patients with F4 were assessed as grade A by the Child–Pugh score. Age ($P = 0.0058$), inflammatory grade ($P < 0.0001$), platelet count ($P = 0.0021$), prothrombin time ($P = 0.0017$) and hyaluronic acid ($P = 0.0224$) differed significantly between F0–1 and F2. Inflammatory grade ($P < 0.0001$), aspartate aminotransferase (AST) ($P = 0.0024$), alanine aminotransferase (ALT) ($P = 0.0371$) and γ-glutamyltranspeptidase (γ-GTP) ($P = 0.0290$) differed significantly between F2 and F3. Platelet count ($P = 0.0351$) differed significantly between F3 and F4.

Fibrosis area in the liver biopsy specimen

The proportion of fibrosis area was significantly correlated with fibrosis stage as assessed by the Metavir system ($\rho = 0.733$, $P < 0.0001$) (Fig. 2). The values of fibrosis area significantly differed between F0–1 and F2 ($P < 0.0001$); between F2 and F3 ($P < 0.0001$); and between F3 and F4 ($P < 0.0001$) (Table 1).

ROC analysis was done to assess the diagnostic value of fibrosis area for different fibrosis stages. Area under

Table 1 Characteristics of 165 patients with chronic hepatitis C virus infection

	All patients ($n = 165$)	F0–1 ($n = 66$)	*P* values of Mann–Whitney *U* test between F0–1 and F2	F2 ($n = 42$)	*P* values of Mann–Whitney *U* test between F2 and F3	F3 ($n = 33$)	*P* values of Mann–Whitney *U* test between F3 and F4	F4 ($n = 24$)
Age (year)†	57 (18–71)	49 (21–69)	$P = 0.0058$	59 (18–71)	NS	61 (22–71)	NS	61 (43–71)
Gender (female/male)‡	73/92	27/39	NS	17/25	NS	19/14	NS	10/14
Fibrosis stage (F0/F1/F2/F3/F4)	14/52/42/33/24	–	–	–	–	–	–	–
Inflammatory grade (A0/A1/A2/A3)	13/73/49/30	13/45/8/0	$P < 0.0001$	0/22/17/3	$P < 0.0001$	0/2/20/11	NS	0/4/4/16
AST (IU/L)†	42 (15–216)	32 (15–148)	NS	33 (16–187)	$P = 0.0024$	66 (23–216)	NS	75 (19–155)
ALT (IU/L)†	56 (9–330)	42 (12–226)	NS	53 (9–178)	$P = 0.0371$	82 (21–330)	NS	91 (16–221)
γ-GTP (IU/L)†	44 (11–556)	30 (11–556)	NS	42 (14–164)	$P = 0.0290$	67 (21–216)	NS	50 (18–195)
Platelet count ($\times 10^4/\mu L$)†	16.3 (6.3–37.1)	18.3 (6.3–37.1)	$P = 0.0021$	15.6 (6.7–26.0)	NS	14.9 (7.7–29.2)	$P = 0.0351$	11.6 (6.5–17.2)
Prothrombin time (%)†	93 (62–120)	99 (72–120)	$P = 0.0017$	93 (71–120)	NS	88 (77–112)	$P = 0.0790$	82 (62–99)
Albumin (g/dL)†	4.3 (3.4–5.0)	4.4 (4.0–5.0)	NS	4.4 (3.5–4.8)	NS	4.2 (3.6–4.6)	NS	4.1 (3.4–4.9)
γ-globulin (g/dL)†	1.5 (0.9–2.7)	1.3 (0.9–2.1)	NS	1.5 (0.9–2.1)	NS	1.6 (1.2–2.4)	NS	1.8 (1.2–2.7)
Hyaluronic acid (ng/mL)†	63 (9–765)	34 (9–328)	$P = 0.0224$	72 (9–427)	NS	96 (9–675)	$P = 0.0683$	145 (59–765)
Fibrosis area (%)†	2.2 (0.2–13.4)	1.0 (0.2–5.4)	$P < 0.0001$	2.2 (0.6–5.2)	$P < 0.0001$	4.3 (1.0–7.6)	$P < 0.0001$	8.0 (0.6–13.4)
Liver stiffness (kPa)†	8.0 (2.8–48.8)	5.4 (2.8–12.5)	$P < 0.0001$	8 (3.7–19.1)	$P = 0.0012$	12.0 (4.3–26.3)	$P = 0.0207$	17.7 (7.4–48.8)

†Data are shown as median (range).
‡Difference of frequency of gender was assessed by χ^2-test.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyltranspeptidase.

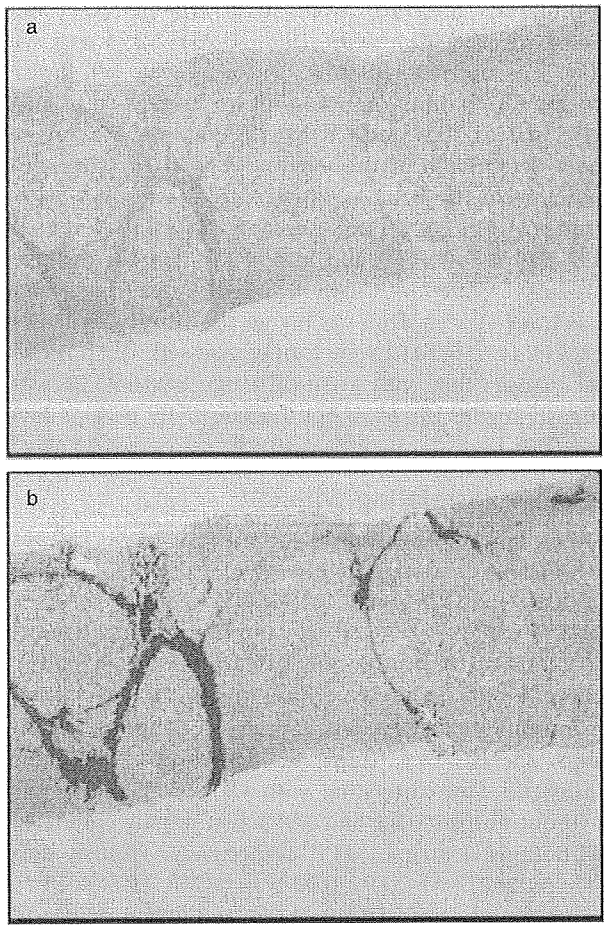

Figure 1 The proportion of fibrosis area in the biopsy specimens was measured by computer-assisted morphometric image analysis. (a) Liver biopsy specimens were stained with azan stain. (b) The fibrosis area that was stained blue with azan was marked and measured with the image analysis software, Image Pro Plus 4.0 imaging software (Nippon Roper Co, Tokyo, Japan). Each specimen was examined at three different fields or more with the use of a 5X objective.

the ROC (AUROC) values (95% confidence interval) were 0.87 (0.81–0.92) for F > or = 2, 0.91 (0.87–0.96) for F > or = 3, and 0.93 (0.85–1.00) for F4. Based on the fibrosis area distribution according to fibrosis stage and ROC curves, the optimal discriminate cut-off values were determined at the maximum total of sensitivity and specificity with sensitivity of more than 80% (Table 2). The cut-off values were 1.6% for F > or = 2, 3.1% for F > or = 3, and 3.8% for F4. The positive predictive value for F4 with the cut-off value of 3.8% was low (45.1%). Thus the higher cut-off values such as 4.9

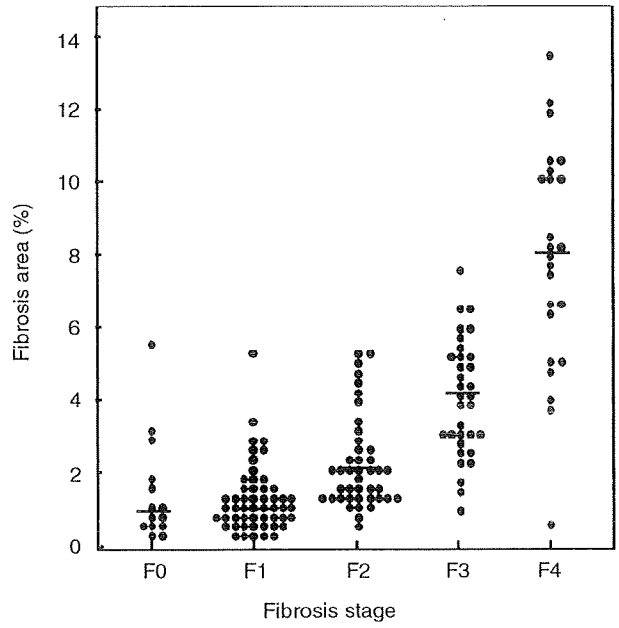

Figure 2 The proportion of fibrosis area was significantly correlated with fibrosis stage, as assessed by the Metavir system ($\rho = 0.733$, $P < 0.0001$).

or 6.4% were further assessed. The positive predictive value with a cut-off value of 6.4% was reasonably high (85.7%), and the sensitivity became slightly lower (75.0%).

Liver stiffness

LS was significantly correlated with fibrosis stage ($\rho = 0.734$, $P < 0.0001$) (Fig. 3). Values of LS signifi-

Table 2 Optimal cut-off value of fibrosis area for each fibrosis stage was determined at the maximum total of sensitivity and specificity with sensitivity of more than 80%. For F4, the higher cut-off values such as 4.9 and 6.4% were further assessed

	F > or = 2	F > or = 3	F4		
Cut-off value (%)	1.6	3.1	3.8	4.9	6.4
Positive predictive value (%)	83.3	78.7	45.1	55.6	85.7
Negative predictive value (%)	72.5	91.3	99.1	96.9	95.8
Sensitivity (%)	80.8	84.2	95.8	83.3	75.0
Specificity (%)	75.8	88.0	80.1	88.7	97.9
Positive likelihood ratio	3.3	7.0	4.8	7.3	35.3
Diagnostic accuracy (%)	78.8	86.7	82.4	87.9	94.5

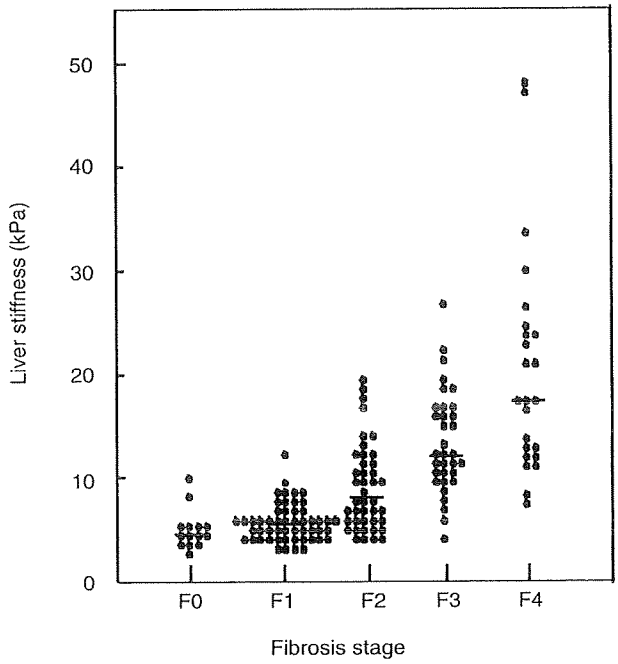

Figure 3 Liver stiffness was significantly correlated with fibrosis stage ($\rho = 0.734$, $P < 0.0001$).

cantly differed between F0–1 and F2 ($P < 0.0001$); between F2 and F3 ($P = 0.0012$); and between F3 and F4 ($P = 0.0207$) (Table 1). LS was significantly correlated with fibrosis area ($\rho = 0.590$, $P < 0.0001$) (Fig. 4).

ROC analysis was done to assess the diagnostic value of LS for different fibrosis stages. AUROC values (95% confidence interval) were 0.88 (0.83–0.93) for F > or = 2 (Fig. 5a), 0.90 (0.86–0.95) for F > or = 3 (Fig. 5b), and 0.90 (0.84–0.96) for F4 (Fig. 5c). Based on the LS distribution according to fibrosis stage and ROC curves, the optimal discriminate cut-off values were determined at the maximum total of sensitivity and specificity with sensitivity of more than 80% (Table 3). The cut-off values were 7.1 kPa for F > or = 2, 9.6 kPa for F > or = 3, and 11.6 kPa for F4. The positive predictive value for F4 with the cut-off value of 11.6 kPa was low (41.5%). Thus the higher cut-off values such as 13.6 kPa or 16.9 kPa were further assessed. The positive predictive value with a cut-off value of 16.9 kPa was slightly higher (55.6%), but the sensitivity became low (62.5%).

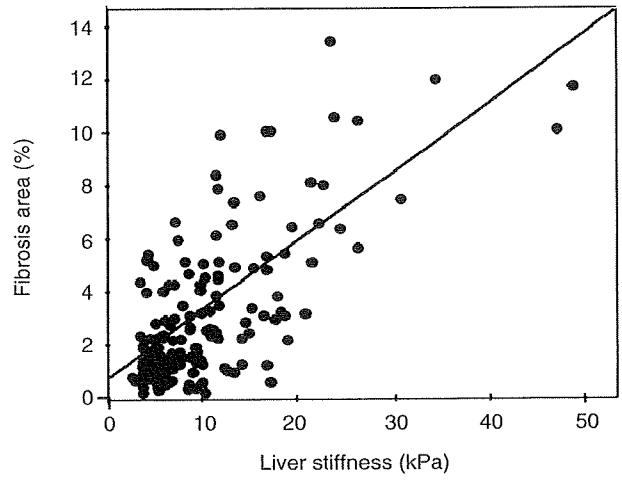

Figure 4 Liver stiffness was significantly correlated with proportion of fibrosis area ($\rho = 0.590$, $P < 0.0001$).

Factors correlating with LS

Other than fibrosis stage and fibrosis area, LS was significantly correlated with age ($P < 0.0001$), inflammatory grade ($P < 0.0001$) (Fig. 6), AST ($P < 0.0001$), ALT ($P < 0.0001$), γ-GTP ($P < 0.0001$), platelet counts ($P < 0.0001$), prothrombin time (%) ($P < 0.0001$), albumin ($P < 0.0001$), γ-globulin ($P < 0.0001$), and hyaluronic acid ($P < 0.0001$) (Table 4). Multiple regression analysis was done to determine the factors independently correlating with LS among the factors without fibrosis stage and AST which highly correlated with fibrosis area or ALT, respectively. Fibrosis area ($P = 0.0002$), ALT ($P = 0.0237$), γ-GTP ($P = 0.0114$), prothrombin time ($P = 0.0114$) and hyaluronic acid ($P < 0.0001$) were selected as factors independently correlating with LS (adjusted R square = 0.547) (Table 4).

To determine the factors correlating with LS in each fibrosis stage, multiple regression analysis was done with the patients with same fibrosis stage. In the patients with F0–1, γ-GTP ($P = 0.0227$) and prothrombin time ($P = 0.0286$) were selected, although the R value of multiple regression analysis was rather low (adjusted R square = 0.112). In those with F2, ALT ($P = 0.0047$) and albumin ($P = 0.0029$) were selected (adjusted R square = 0.362). In those with F3, albumin ($P = 0.0166$) and γ-globulin ($P = 0.0002$) were selected (adjusted R square = 0.512). In those with F4, fibrosis area ($P = 0.0177$) and hyaluronic acid ($P = 0.0349$) were selected (adjusted R square = 0.371).

DISCUSSION

THE PRESENT STUDY demonstrated that LS correlates with the proportion of fibrosis area quantitatively measured in the liver specimens. This finding

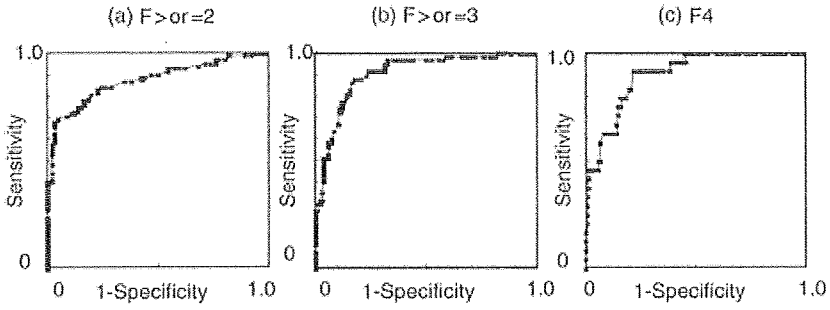

Figure 5 The receiver operating characteristics curve analysis was done to assess the diagnostic value of liver stiffness for different fibrosis stages. The values of area under the receiver operating characteristics curve (95% confidence interval) were 0.88 (0.83–0.93) for F > or = 2 (a); 0.90 (0.86–0.95) for F > or = 3 (b); and 0.90 (0.84–0.96) for F4 (c).

confirmed the correlation between LS and liver fibrosis stage which has been semi-quantitatively measured by numerical systems. In most studies dealing with the correlation between LS and hepatic fibrosis, fibrosis has been measured numerically with the Metavir system,[19] using stages that range from 0 to 4. The numbers actually represent categories of increasing severity of fibrosis based on a combination of location and quantity of scarring, and whether the fibrous tissue forms septa, bridges, or nodules. Thus the numerical systems are not essentially a quantitative method for assessing the amount of fibrosis in the liver, although they are intended to be semi-quantitative. The present study demonstrated that fibrosis area quantitatively measured correlates well with the numerical fibrosis staging and also with LS.

In the present study, the fibrosis areas in the biopsy specimens were measured by computer-assisted morphometric image analysis. Although the methodology is not standardized, a number of publications have described methods for quantifying hepatic fibrosis by image analysis, and all appear to yield similar results.[21–23] Goodman *et al.* reported that the mean morphometric collagen content was 0.0856 in stage 5 (incomplete cirrhosis), and 0.1163 in stage 6 (cirrhosis, probable or definite) of the Ishak score,[23] which correspond with the values of fibrosis area of F4 in the present study. Since the methods were time consuming, and needed the equipment and expertise, they had not been made widely available. However, recently these problems have been gradually fixed. The apparatus for microscopic image acquisition and image analysis software are available in many institutions now, and their functions are being improved. Thus in a study to evalu-

Table 3 Optimal cut-off value of liver stiffness for each fibrosis stage was determined at the maximum total of sensitivity and specificity with sensitivity of more than 80%. For F4, the higher cut-off values such as 13.6 kPa and 16.9 kPa were further assessed

	F > or = 2	F > or = 3	F4		
Cut-off value (kPa)	7.1	9.6	11.6	13.6	16.9
Positive predictive value (%)	86.0	72.5	41.5	44.4	55.6
Negative predictive value (%)	73.6	92.7	98.2	93.8	93.5
Sensitivity (%)	80.8	87.7	91.7	66.7	62.5
Specificity (%)	80.3	82.4	78.0	85.8	91.5
Positive likelihood ratio	4.1	5.0	4.2	4.7	7.3
Diagnostic accuracy (%)	80.6	84.2	80.0	83.0	87.3

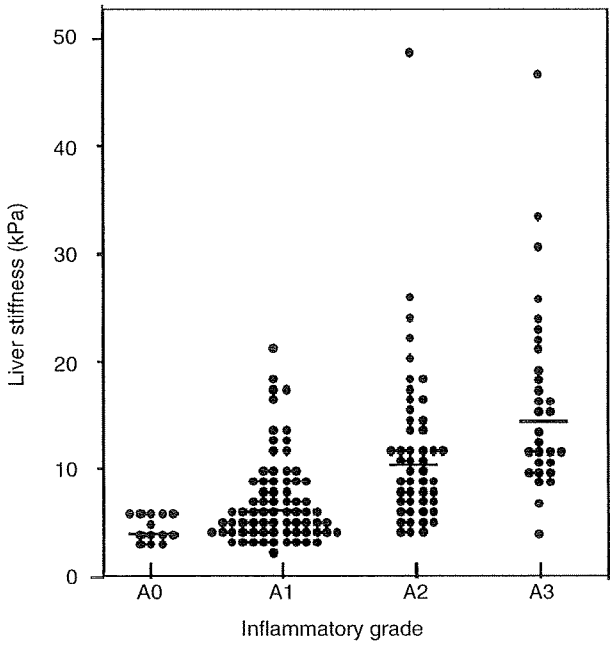

Figure 6 Liver stiffness was significantly correlated with inflammatory grade ($\rho = 0.598$, $P < 0.0001$).

Table 4 Factors correlating with liver stiffness in all patients or patients with each fibrosis stage, as assessed by Spearman's rank correlation test and multiple regression analysis

	All patients				F0–F1			
	Spearman's rank correlation test		Multiple regression analysis		Spearman's rank correlation test		Multiple regression analysis	
	ρ	*P*	β	*P*	ρ	*P*	β	*P*
Age	0.483	$P < 0.0001$		NS		NS	–	–
Gender		NS	–	–		NS	–	–
Fibrosis stage	0.734	$P < 0.0001$	–	–	–	–	–	–
Inflammatory grade	0.598	$P < 0.0001$		NS	0.226	$P = 0.0679$		NS
Fibrosis area	0.590	$P < 0.0001$	0.204	$P = 0.0002$		NS	–	–
AST	0.583	$P < 0.0001$	–	–	0.269	$P = 0.0290$		NS
ALT	0.486	$P < 0.0001$	0.064	$P = 0.0237$		NS	–	–
γ-GTP	0.413	$P < 0.0001$	0.061	$P = 0.0114$	0.275	$P = 0.0255$	0.103	$P = 0.0227$
Platelet counts	–0.449	$P < 0.0001$		NS		NS	–	–
Prothrombin time (%)	–0.447	$P < 0.0001$	–0.013	$P = 0.0114$	–0.208	$P = 0.0931$	–0.015	$P = 0.0286$
Albumin	–0.471	$P < 0.0001$		NS		NS	–	–
γ-globulin	0.351	$P < 0.0001$		NS		NS	–	–
Hyaluronic acid	0.599	$P < 0.0001$	0.207	$P < 0.0001$	0.226	$P = 0.0728$		NS
R			0.739				0.373	
Adjusted R square			0.547				0.112	
F			37.4				5.1	
p			$P < 0.0001$				$P = 0.0088$	

Table 4 *Continued*

	F2				F3				F4			
	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*	β	*P*	ρ	*P*	β	*P*	ρ	P	β	*P*
Age	0.299	$P = 0.0546$		NS	0.421	$P = 0.0146$		NS		NS	–	–
Gender		NS	–	–		$P = 0.0275$		NS		NS	–	–
Fibrosis stage	–	–	–	–	–	–	–	–	–	–	–	–
Inflammatory grade		NS	–	–	0.324	$P = 0.0655$		NS		NS	–	–
Fibrosis area		NS	–	–	0.357	$P = 0.0413$		NS	0.518	$P = 0.0095$	1.575	$P = 0.0177$
AST	0.492	$P = 0.0009$	–	–		NS	–	–		NS	–	–
ALT	0.520	$P = 0.0004$	0.037	$P = 0.0047$	0.319	$P = 0.0700$		NS		NS	–	–
γ-GTP	0.413	$P = 0.0066$	–	–		NS	–	–	0.360	$P = 0.0838$		NS
Platelet counts		NS	–	–	−0.376	$P = 0.0310$		NS		NS	–	–
Prothrombin time (%)		NS	–	–		NS	–	–		NS	–	–
Albumin	−0.432	$P = 0.0043$	−5.201	$P = 0.0029$	−0.442	$P = 0.0100$	−6.737	$P = 0.0166$	−0.316	$P = 0.0629$		NS
γ-globulin		NS	–	–	0.635	$P = 0.0002$	8.027	$P = 0.0002$		NS	–	–
Hyaluronic acid	0.469	$P = 0.0017$		NS	0.503	$P = 0.0033$		NS	0.394	$P = 0.0121$	0.020	$P = 0.0349$
R			0.602				0.739				0.655	
Adjusted R square			0.362				0.512				0.371	
F			11.1				16.2				7.5	
p			$P = 0.0002$				$P < 0.0001$				$P = 0.0037$	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyl transpeptidase; R, multiple correlation coefficient; F, ratio of mean square for the model divided by mean square for error, where the mean squares are the respective sums of squares divided by the degrees of freedom; p, probability.

ate fibrosis, quantitative measurement of fibrosis area by computer-assisted morphometric image analysis is becoming readily available.

Recently several reports questioned the generally accepted supposition that LS is determined entirely by hepatic fibrosis. In these studies, the association between LS and necroinflammatory activity was demonstrated. Coco *et al.* reported that, in patients with biochemical remission, either spontaneous or after antiviral therapy, LS was lower than in those with an identical fibrosis stage but elevated ALT.[25] In the studies of Sagir *et al.* and Arena *et al.*, patients with acute liver damage showed high values of LS suggestive of cirrhosis, while none of them had any other signs of cirrhosis, and they showed a decrease in LS values below the cut-off value of cirrhosis in the convalescent period.[26,27] The significant correlation between aminotransferases and LS at the onset of acute viral hepatitis was also described. In the present study, ALT was shown to correlate with LS in multiple regression analysis with all patients, and in the analysis with those with F2. Inflammatory grade also correlated with LS in univariate analysis, but not in multiple regression analysis. These results confirmed that inflammatory activity affects LS values in chronic hepatitis, especially in those with F2. Thus the interpretation of LS in patients with high levels of ALT should be made cautiously. Inflammatory activity is associated with inflammatory infiltrate, tissue edema and hepatocyte swelling, all of which are likely to affect LS,[25–27] although the exact mechanism by which inflammatory activity or elevation of ALT affects LS has not been elucidated.

In the present study, the positive predictive value for F4 with a cut-off value of 11.6 kPa was low (41.5%). When the higher cut-off values such as 16.9 kPa was adopted, the positive predictive value became higher (55.6%), while the sensitivity became lower (62.5%). Thus it should be noted that the false positive rate for the diagnosis of F4 is high, even if the higher cut-off value is adopted.

Ganne-Carrie *et al.* reported that most false negative diagnoses of cirrhosis by FibroScan are attributable to inactive or macronodular cirrhosis.[11] 29% of patients with cirrhosis with LS less than the cut-off value for cirrhosis were reported to have macronodular cirrhosis with limited amount of fibrosis tissue, characterized by large nodules with very thin septa. The present study demonstrated that the fibrosis area significantly correlated with LS in F4; LS was low in those with a small fibrosis area and was high in those with a large fibrosis area. When the cut-off value of 16.9 kPa is adopted for the diagnosis of F4, only 62.5% of patients with cirrhosis are correctly diagnosed as cirrhosis. 33% of the false negative patients with cirrhosis had a small fibrosis area of less than 6.4% (data not shown). Thus it should be noted that the patients with cirrhosis and a small fibrosis area may be misdiagnosed as not being cirrhosis when the higher cut-off value is adopted.

γ-GTP, prothrombin time and hyaluronic acid were also shown to correlate with LS in multiple regression analysis in all patients. Albumin in F2 and in F3, γ-globulin in F3, and hyaluronic acid in F4 were also shown to correlate with LS. These factors have been shown to bear direct or indirect correlation with fibrosis,[28–32] which is probably the reason why they correlated with LS.

The correlation between LS and liver fibrosis was confirmed by the objective measurement of fibrosis area. ALT was significantly correlated with LS, suggesting that inflammatory activity also affects LS values. The positive predictive value for F4 was low, even if the higher cut-off value is adopted. Despite some limitation, LS measurement is a useful method for the diagnosis of liver fibrosis.

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Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: a Japanese multi-center study

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Abstract
Background Chronic hepatitis C (CHC) genotype 1b patients with high viral load are resistant to peginterferon (PEG-IFN) and ribavirin (RBV) combination therapy, especially older and female patients.
Methods To elucidate the factors affecting early and sustained viral responses (EVR and SVR), 409 genotype 1b patients CHC with high viral loads who had received 48 weeks of PEG-IFN/RBV therapy were enrolled. The amino acid (aa) sequences of the HCV core at positions 70 and 91 and of the interferon sensitivity determining region (ISDR) were analyzed. Host factors, viral factors, and treatment-related factors were subjected to multivariate analysis.
Results Male gender, low HCV RNA load, high platelet count, two or more aa mutations of ISDR, and wild type of core aa 70 were independent predictive factors for SVR. In patients with over 80% adherences to both PEG-IFN and RBV, male gender, mild fibrosis stage, and wild type of core aa 70 were independent predictors for SVR.
Conclusions Independent predictive factors for SVR were: no aa substitution at core aa 70, two or more aa mutations in the ISDR, low viral load, high values of platelet count, mild liver fibrosis and male gender.

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Keywords Chronic hepatitis C · Peginterferon and ribavirin · Core amino acid · Interferon sensitivity determining region

Abbreviations

CHC	Chronic hepatitis C
PEG-IFN	Peginterferon
RBV	Ribavirin
RVR	Rapid viral response
cEVR	Complete early viral response
LVR	Late viral response
ETR	End of treatment response
NR	Non response
SVR	Sustained viral response
ISDR	Interferon sensitivity determining region
Aa	Amino acid
ALT	Alanine aminotransferase
PLT	Platelet
HCC	Hepatocellular carcinoma

Introduction

A combination of pegylated interferon (PEG-IFN) and ribavirin (RBV) therapy for 48 weeks achieves a sustained viral response (SVR) rate of 40–50% in chronic hepatitis C (CHC) patients with a high viral load of genotype 1 [1–4]. The dose-reduction rate and the frequency of discontinuation of this treatment are high in aged patients [5]. The SVR rate of the therapy is lower in females than males, especially in older patients in Japan [6].

Around 30% of HCV carriers have serum alanine aminotransferase (ALT) levels within the upper limit of normal ranges [7, 8] and HCV carriers with persistently normal serum ALT (PNALT) and serum platelet (PLT) counts of over $15 \times 10^4/mm^3$ show low grade hepatic fibrosis and good prognosis [9]. Before treating HCV carriers, it is very important to predict non-response to PEG-IFN plus RBV therapy because of its medical cost, adverse effects, and its impact on the long term quality of life.

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There are many factors affecting response to IFN monotherapy and PEG-IFN/RBV therapy, including body mass index (BMI) [10, 11], steatosis [12, 13], insulin resistance [14], stage of liver fibrosis [15, 16], total cholesterol (T. Chol), triglyceride (TG), adherence to both PEG-IFN and RBV [17], race [18, 19], age [1, 2, 20], and viral factors including serum quantity of HCV RNA, HCV genotype and substitution of amino acids (aa) in the interferon sensitivity determining region (ISDR, 2209–2248) of the nonstructural protein 5A (NS5A) [21] and in the core protein [22, 23]. Early viral response is an important predictive factor in PEG-IFN/RBV therapy for CHC patients with genotype 1 and high viral loads [24–27].

The aim of this study was to elucidate the valuable predictive factors of SVR in Japanese patients with HCV genotype 1b high viral loads following 48 weeks of PEG-IFN/RBV therapy, focusing on the relationship between aa substitutions in the ISDR and at core aa 70 and 91 and early viral kinetics.

Patients and methods

Selection of patients

This retrospective study was conducted at 15 clinical sites in Japan which are part of the Study Group of Optimal Treatment of Viral Hepatitis supported by the Ministry of Health, Labor and Welfare, Japan. Eligible subjects were CHC patients, who (1) had received liver biopsy; (2) were genotype 1b with high viral load ($\geq$100 KIU/ml by Cobas Amplicor Hepatitis C Virus Test, version 2.0) at the start of PEG-IFN/RBV therapy; (3) received weekly injections of PEG-IFN-α-2b (PEG-INTRON; Shering-Plough, Kenilworth, NJ) of 1.5 μg/kg bw and oral administration of RBV (Rebetol; Shering-Plough) for 48 weeks. The amount of RBV was adjusted based on the subject's body weight; (600 mg for $\leq$60 kg bw, 800 mg for 60–80 kg bw, 1,000 mg for > 80 kg bw); (4) were examined serially for quantitative and qualitative HCV RNA; and (5) the aa sequences at positions 70 and 91 in the core region and of the ISDR in the NS5A had been determined in pretreatment sera.

Hepatitis B virus (HBV) infection, human immunodeficiency virus (HIV) infection, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, and Wilson's disease were excluded. Histopathological diagnosis was based on the scoring system of Desmet et al. [28]. The definition of alcohol abuse included patients having a history of more than 100 kg of total ethanol intake. Complete blood counts, liver function tests, serum lipids, serum ferritin, serum fibrosis markers, fasting plasma glucose (FPG), and immune reactive insulin (IRI) were examined in most cases. Written informed consent was obtained from all

patients before treatment, and the protocol was approved by the ethics committees in each site.

Study design

Four hundred and nine patients who completed 48 weeks of treatment and were followed for more than 24 weeks after treatment were enrolled in the first study (*Study design 1*).

To elucidate the effect of aa substitution of HCV core and in the ISDR on HCV dynamics, including a rapid viral response (RVR), complete early viral response (cEVR), a late viral response (LVR) and SVR, according to gender and age (<60 years ≥ 60 years), 201 of the 409 patients maintaining over 80% adherences to both PEG-IFN and RBV were enrolled in the second study (*Study design 2*).

Nucleotide sequencing of the core and NA5A gene

The nucleotide sequences encoding aa 1–191 (HCV core) and aa 2209–2248 (ISDR) were analyzed by direct sequencing as described by Akuta et al. [22, 27] and Enomoto et al. [21]. In brief, RNA was extracted from the sera and converted to cDNA and two nested rounds of polymerase chain reaction (PCR) were performed. Primers used in the PCR were as follows; (a) Nucleotide sequences of the core region: the first-round PCR was performed with CC11 (sense) and e14 (antisense) primers [22, 27], and the second-round PCR with CC9 (sense) and e14 (antisense) primers [22, 27]. (b) Nucleotide sequences of the ISDR in NS5A: the first-round PCR was performed with ISDR1 (sense) and ISDR2 (antisense) primers [21], and the second-round PCR with ISDR3 (sense) and ISDR4 (antisense) primers [21]. These sequences were compared with the consensus sequence of genotype 1b (HCV-J) [29]. Wild types virus encoded arginine and leucine at aa 70 and 91, respectively, and the aa substitutions were glutamine or histidine at aa 70 and methionine at aa 91.

Viral kinetic study

Serum HCV RNA levels were measured by PCR (Amplicor HCV RNA kit, version 2.0, Roche Diagnostics) using samples taken before treatment and at 4, 12, 24, and 48 weeks after the therapy. SVR was defined as HCV RNA negativity by qualitative analysis by PCR at 24 weeks after the treatment. RVR was defined as HCV RNA negativity at 4 weeks, cEVR as HCV RNA negativity at 12 weeks, LVR as HCV RNA negativity during 13–24 weeks and an end of treatment response (ETR) as HCV RNA negativity at the end of treatment. Patients who remained positive for HCV RNA at the end of the treatment and at 24 weeks after the therapy were defined as non-responders (NR).

Adherences to PEG-IFN and RBV

Adherences to PEG-IFN and RBV were assessed by separately calculating the actual doses of PEG-IFN and RBV received as percentages of the intended dosages. Adherences to PEG-IFN and RBV were divided into two groups; 80%≤ and <80%.

Statistical analysis

All data analyses were conducted using the SAS version 9.1.3 statistical analysis packages (SAS Institute, Cary, NC, USA). Individual characteristics between groups were evaluated by Mann–Whitney U test for numerical variables or Fisher's exact test for categorical variables. Variables exhibiting values of $p < 0.1$ in the univariate analysis were subjected to stepwise multivariate logistic regression analysis. The grade of steatosis and iron deposition in liver tissue, BMI, albumin (Alb), low density lipoprotein-cholesterol (LDL-C), homeostasis model assesment-insulin resistance (HOMA-IR), ferritin, and hyaluronic acid were excluded from multivariate logistic regression analysis because of the absence of those data in more than 10% of the patients. All p values of $p < 0.05$ by the two-tailed test were considered statistically significant.

Results

Study design 1

Baseline backgrounds, characteristics and adherences of peginterferon and ribavirin in males and females

The treatment outcome of PEG-IFN and RBV combination therapy depends on gender in Japanese patients, so in addition to aa substitutions in the ISDR in NS5A [21] or at HCV core 70 and 91 [22, 27], we compared the baseline characteristics according to gender (Table 1). Males were younger and the grade of hepatic inflammation was milder in males. The serum levels of LDL-C, PLT count, and aa substitutions of ISDR and at core 70 and 91 did not differ significantly different between males and females. The frequency of no alcohol abuse was significantly ($p < 0.0001$) higher in females than males (Some of them are not described in Table 1).

The rates of over 80% adherences to PEG-IFN and RBV were significantly lower ($p = 0.0066$, $p < 0.00001$, respectively) in females than males. Only in those above 60 years did the rate of over 80% adherence to PEG-IFN not differ significantly between males and females, but the rate of over 80% adherence to RBV was significantly lower ($p = 0.035$) in females than males (Table 1).

Table 1 Backgrounds and characteristics of male and female patients

Factors	Gender		*p* value
	Male	Female	
No. of patients	256 (62.6%)	153 (37.4%)	
Age			
Median (range)	53 (18–73)	59 (23–75)	0.00001
F stage			
F0–2	206 (80.5%)	119 (77.8%)	0.592
F3–4	50 (19.5%)	34 (22.2%)	
Grade (A factor)			
A0–1	163 (63. 7%)	79 (51.6%)	0.026
A2–3	93 (36.3%)	74 (48.4%)	
HCV RNA load 0 week (KIU/mL)			
Median (range)	1500 (100–5000 <)	1280 (100–5000<)	0.384
ALT 0 week (IU/L)			
Median (range)	74.5 (16–504)	59 (19–391)	0.001
BMI			
Median (range)	23.6 (17.5–31.2)	22.1 (16.1–33.9)	0.00033
Alb (g/dL)			
Median (range)	4.0 (3.0–5.2)	3.8 (3.0–4.8)	0.011
LDL-C (mg/dL)			
Median (range)	97 (30–185)	90 (34–174)	0.612
T-Chol (mg/dL)			
Median (range)	167 (85–273)	176 (114–261)	0.0016
PLT count ($\times 10^4/mm^3$)			
Median (range)	17.0 (8.0–31.9)	16.4 (8.1–39.9)	0.350
Amino acid mutation of ISDR			
0–1	200 (78.1%)	121 (79.1%)	0.608
2≤	56 (21.9%)	32 (20.9%)	
Amino acid substitution of core 70			
Wild	177 (69.1%)	114 (74.5%)	0.261
Mutant	79 (30.9%)	39 (25.5%)	
Amino acid substitution of core 91			
Wild	153 (59.8%)	98 (64.1%)	0.403
Mutant	103 (40.2%)	55 (35.9%)	
PEG-IFN adherence			
<80%	41 (17.7%)	42 (30.4%)	0.0066
80%≤	190 (82.3%)	96 (69.6%)	
Ribavirin adherence			
<80%	54 (23.6%)	73 (52.1%)	<0.00001
80%≤	175 (76.4%)	67 (47.9%)	
Age: <60 years			
PEG adherence			
<80%	30 (17.8%)	23 (31.5%)	0.027
80%≤	139 (82.2%)	50 (68.5%)	
Ribavirin adherence			
<80%	27 (16.2%)	31 (42.5%)	0.000029
80%≤	140 (83.8%)	42 (57.5%)	
Age: 60 years≤			
PEG adherence			
<80%	11 (17.7%)	19 (29.2%)	0.147
80%≤	51 (82.3%)	46 (70.8%)	
Ribavirin adherence			
<80%	27 (43.5%)	42 (62.7%)	0.035
80%≤	35 (56.5%)	25 (37.3%)	

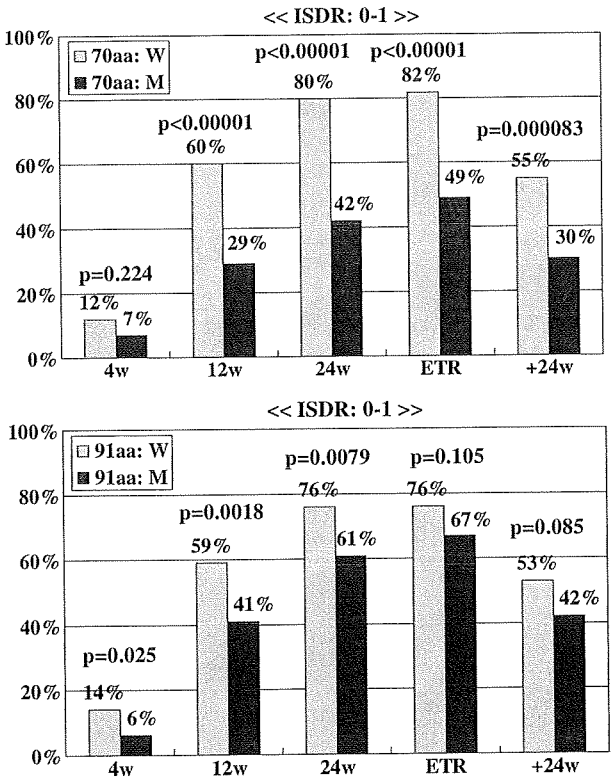

Fig. 1 Relationship between time course of serum HCV RNA negativity and amino acid substitutions in the ISDR and core amino acids 70 and 91. For cases with no or only one amino acid (aa) change in the ISDR, the rates of cEVR, LVR, ETR and SVR were significantly higher in patients with wild type core aa 70 but only the rates of RVR, cEVR, and LVR were significantly higher in patients with wild type core aa 91

Amino acid substitutions

There were no significant differences in the frequency of aa substitutions in the ISDR between males and females. Core aa substitutions at positions 70 and 91 were as follows; 291 (71.1%) were wild type and 118 (28.9%) were mutant at core aa 70, and 251 (61.4%) were wild type and 158 (38.6%) were mutant at core aa 91. There were no significant differences between males and females and between patients below and above 60 years of age.

Virological responses and aa substitutions

The rate of RVR did not differ significantly between males and females. However, more male patients showed HCV RNA negativity at 12 weeks (males vs. females; 60.7 vs. 48.4%, $p = 0.018$), 24 weeks (76.8 vs. 64.2%, $p = 0.0078$) and 48 weeks (78.2 vs. 68.6%, $p = 0.049$), and the proportion of male patients in SVR was significantly higher than that of females (61.3 vs. 37.3%, $p < 0.00001$).

RVR, cEVR and SVR rates were significantly higher in patients with two or more aa mutations in the ISDR compared to patients having no or one aa substitution in that region (20 vs. 11%, $p = 0.044$; 71 vs. 52%, $p = 0.0021$; 66 vs. 49%, $p = 0.0054$, respectively). AA substitution at core position 70 resulted in significantly lower rate of cEVR, LVR, ETR, and SVR (40 vs. 63%, $p = 0.000037$; 51 vs. 81%, $p < 0.00001$; 56 vs. 83%, 41 vs. 57%; $p < 0.00001$, $p = 0.0031$, respectively). Although the patients with the wild type aa at core 91 showed significantly higher rates of RVR and cEVR, the rate of SVR was not significantly higher in those patients ($p = 0.054$).

SVR rates were 30% for patients with no or one aa substitution in the ISDR and the core aa 70 substitution, and were significantly lower compared to those with the wild type aa core 70 (Fig. 1). These findings were not confirmed in patients with no or one aa substitution in the ISDR and the core aa 91 substitution (Fig. 1).

Factors affecting SVR by univariate analysis

Univariate analysis identified nine parameters that influenced non-SVR significantly: female gender, older age, advanced staged liver fibrosis, high viral load, low serum Alb level, low PLT count, no or one aa substitution in the ISDR, aa substitution at core aa 70, and low adherence to RBV (Table 2). The frequency of steatosis and HOMA-IR were significantly ($p = 0.0057$, $p < 0.00001$, respectively) lower in patients with SVR compared with non-SVR (data not shown). However, these factors were not entered in the multivariate analysis because of the absence of the data in many cases.

Factors affecting RVR, cEVR, and SVR by multivariate logistic regression analysis

Multivariate analysis identified four parameters that influenced RVR independently: low HCV RNA load, low serum ALT level, two or more aa mutations in the ISDR and the wild type aa at core position 91 (Table 3).

Concerning cEVR, male gender, mild fibrosis stage, low HCV RNA load, two or more aa mutations in the ISDR, and the wild type aa at core positions 70 and 91 were independent predictors (Table 3).

Concerning SVR, male gender ($p < 0.0001$), low HCV RNA load ($p = 0.013$), high PLT count ($p = 0.0019$), two or more aa mutations in the ISDR ($p = 0.024$), and wild type core aa 70 ($p = 0.0045$) were found to be independent predictors (Table 3).

The predictive values of the combination of gender, PLT count, ISDR and core aa 70 are shown in Fig. 2a. In male patients having PLT of $<15 \times 10^4/mm^3$, and, no or one aa substitution in the ISDR, the SVR rate was 68% when core 70

Table 2 Univariate analysis to identify the factors of SVR

Factors	Negative of HCV RNA after 24 weeks		*p* value
	(−)	(+)	
No. of patients	214 (52.3%)	195	
Gender			
Male	157 (61.3%)	99	<0.00001
Female	57 (37.3%)	96	
Age			
Median (range)	52.5 (18–75)	58 (20–74)	<0.00001
<60 years	155 (58.1%)	112	0.0018
60 years≤	59 (41.5%)	83	
Age: <60 years			
Male	118 (63.4%)	68	0.010
Female	37 (45.7%)	44	
Age: 60 years≤			
Male	39 (55.7%)	31	0.0011
Female	20 (27.8%)	52	
F stage			
F0–2	190 (58.5%)	135	0.000013
F3–4	25 (29.8%)	59	
Grade (A factor)			
A0–1	138 (56.8%)	104	0.130
A2–3	81 (48.5%)	86	
HCV RNA load 0 week (KIU/mL)			
Median (range)	1300 (100–5000<)	1700 (130–5000<)	0.016
ALT 0 week (IU/L)			
Median (range)	66 (16–391)	67 (19–504)	0.892
BMI			
Median (range)	23.0 (17.3–32.4)	23.25 (16.1–33.9)	0.714
Alb (g/dL)			
Median (range)	4.0 (3.2–5.2)	3.8 (3.0–4.8)	0.0088
LDL-C (mg/dL)			
Median (range)	94.5 (31–185)	97.5 (30–182)	0.611
T-Chol (mg/dL)			
Median (range)	169.5 (85–257)	170 (103–273)	0.511
PLT count ($\times 10^4$/mm^3)			
Median (range)	18.2 (8.7–39.9)	15.1 (8.0–31.9)	<0.00001
<15	54 (36.5%)	94	<0.00001
15≤	160 (61.3%)	101	
Amino acid mutation of ISDR			
0–1	156 (48.6%)	165	0.0054
2≤	58 (65.9%)	30	
Amino acid substitution of core 70			
Wild	166 (57.0%)	125	0.0031
Mutant	48 (40.7%)	70	
Amino acid substitution of core 91			
Wild	141 (56.2%)	110	0.054
Mutant	73 (46.2%)	85	
PEG-IFN adherence			
<80%	35 (42.2%)	48	0.063
80%≤	154 (53.8%)	132	
Ribavirin adherence			
<80%	55 (43.3%)	72	0.048
80%≤	132 (54.5%)	110	

Table 3 Multivariate logistic regression analysis to identify independent predictive factors of RVR, cEVR, and SVR

	Odds ratio	95% CI	*p* value
RVR factors selected by stepwise method			
F stage			
F0–2/F3–4	2.924	0.988–8.696	0.053
HCV RNA load 0 week (KIU/mL)			
<1000/1000≤	2.151	1.130–4.082	0.020
ALT 0 week (IU/L)			
<60/60≤	2.165	1.127–4.149	0.020
Amino acid mutation of ISDR			
2≤/0–1	2.371	1.187–4.735	0.014
Amino acid substitution of core 91			
W/M	2.137	1.021–4.464	0.044
cEVR factors selected by stepwise method			
Gender			
Male/female	1.912	1.209–3.021	0.0055
F stage			
F0–2/F3–4	2.079	1.133–3.817	0.018
HCV RNA load 0 week (KIU/mL)			
<1000/1000≤	1.608	1.002–2.577	0.049
PLT count ($\times 10^4$/mm^3)			
15≤/<15	1.427	0.882–2.309	0.148
Amino acid mutation of ISDR			
2≤/0–1	2.512	1.407–4.485	0.0018
Amino acid substitution of core 70			
W/M	2.513	1.508–4.184	0.0004
Amino acid substitution of core 91			
W/M	1.965	1.241–3.115	0.004
SVR factors selected by stepwise method			
Gender			
Male/female	3.704	2.132–6.410	<0.0001
F stage			
F0–2/F3–4	1.812	0.888–3.690	0.103
HCV RNA load0 week (KIU/mL)			
<1000/1000≤	2.024	1.163–3.534	0.013
PLT count ($\times 10^4$/mm^3)			
15≤/<15	2.469	1.394–4.372	0.0019
Amino acid mutation of ISDR			
2≤/0–1	2.148	1.107–4.170	0.024
Amino acid substitution of core 70			
W/M	2.415	1.316–4.444	0.0045
Amino acid substitution of core 91			
W/M	1.433	0.828–2.481	0.199
PEG adherence (%)			
80≤/<80	1.562	0.834–2.926	0.164

W Wild, *M* Mutant

was a wild type but only 16% in patients with mutant at core 70. In female patients, no or one aa substitution in ISDR and $<15 \times 10^4$/mm^3 of PLT count, the SVR rates were as low as 10 or 8%, irrespective of aa substitution at core 70. SVR was only 24% in patients with substitution of core aa 70 even when the PLT count was $\geq 15 \times 10^4$/mm^3. In this study, the combination analysis of PLT count, ISDR, and core aa substitution was useful for predicting non-SVR.

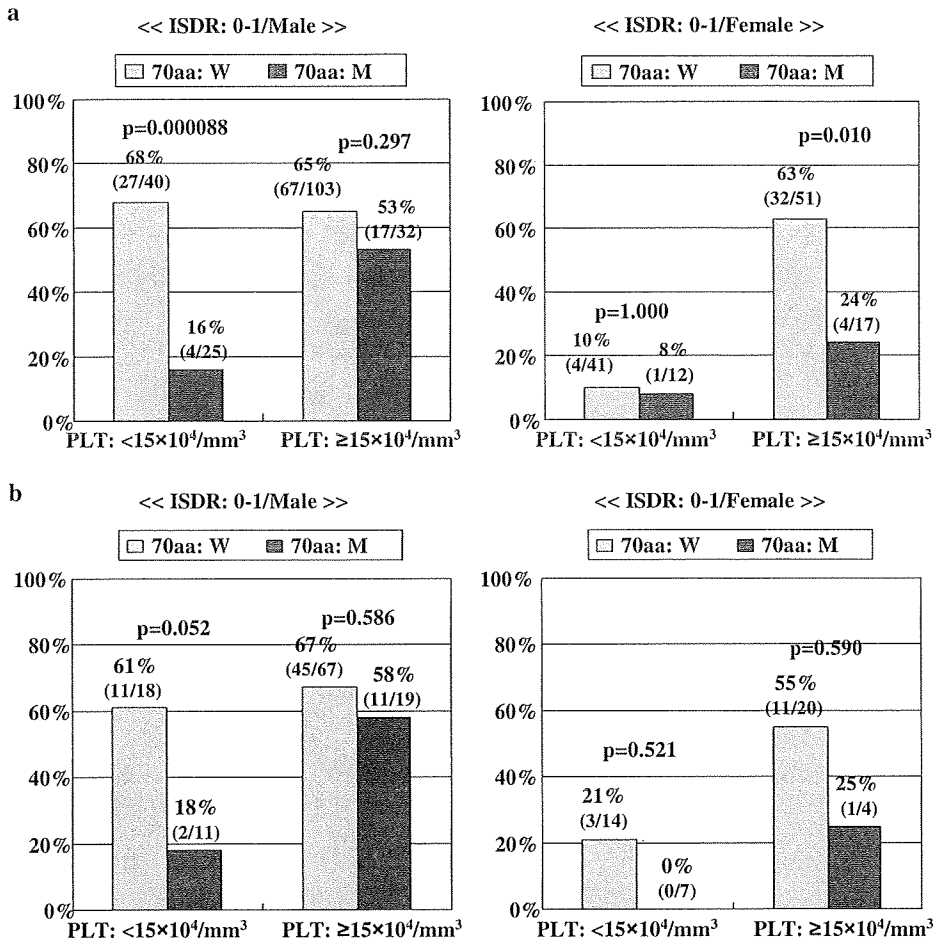

Fig. 2 Relationship between SVR rate and amino acid substitutions in the ISDR and core amino acids 70 and 91, PLT counts and gender difference. The two figures of **a** show the results of *Study 1* and the two figures of **b** show the results of *Study 2*. In male patients with no or only one amino acid (aa) substitution in the ISDR and PLT count of less than $15 \times 10^4/mm^3$, the SVR rate was 68% in those with wild type core aa 70, but only 16% in patients with mutant type of core aa 70, which is significantly different ($p = 0.000088$). There were no significant differences between wild type and mutant type of core aa 70 in the patients with no or one aa substitution in the ISDR and PLT count of over $15 \times 10^4/mm^3$. By contrast, in female patients with no or one aa substitution in the ISDR, there were no significant differences between wild type and mutant type of core aa 70 with PLT count of less than $15 \times 10^4/mm^3$, but there were significant differences between wild type and mutant type of core aa 70 with PLT counts of less than $15 \times 10^4/mm^3$ (**a**). For the patients maintaining over 80% adherences to both PEG-IFN and RBV, in males having no or one aa substitution in the ISDR and PLT counts of less than $15 \times 10^4/mm^3$, a wild type of core aa 70 could predict SVR with a positive predictive value (PPV) of 61% and negative predictive value (NPV) of 82% ($p = 0.052$). However, in male patients with PLT counts of over $15 \times 10^4/mm^3$, core aa 70 was not a useful marker for predicting SVR and non-SVR. The number of female patients with no or one aa substitution in ISDR was too small to reach a definite conclusion (**b**)

Study design 2

The basic features of 201 patients achieving 80% adherences to both PEG-IFN and RBV are as follows: the females were significantly ($p = 0.00006$) older than the males. Iron deposition in liver tissue, alcohol abuse, BMI, serum albumin level, serum ferritin level, and PLT count were significantly higher in males than females. Inflammatory activity was significantly ($p = 0.046$) higher in females than males (data not shown).

AA substitutions in the ISDR were as follows; in males 33 (22.3%) had two or more aa substitutions, in females 8 (15.1%) had two or more aa substitutions. The analysis of core aa position 70 and 91 sequences showed no significant differences in aa substitutions of either core aa 70 or 91 between males and females (data not shown).

In patients less than 60 years of age, SVR rate was significantly higher ($p = 0.0042$) in males than females, but no significant difference was noted between males and females over 60 years old. However, the number of patients over 60 years was small (Table 4).