mutations (including resistance unproven mutations), and clinical characteristics including HCV RNA levels and responses to PEG-IFN/RBV therapy were compared. To assess the influence of PEG-IFN/RBV therapy on NS3 mutational status, posttreatment HCV-NS3 sequences in 39 of 58 non-SVR patients were also examined.

Statistical analysis

Statistical differences in the data, including all available patients' demographic, biochemic, hematologic, and virologic data such as sequence variation factors, were determined among the various groups by Student's t test or Mann–Whitney U test for numerical variables and Fisher's exact probability test for categorical variables.

Results

Prevalence of dominant PI-resistance-associated nonstructural 3 mutations in untreated patients

Figure 1 shows the frequency of substitutions in 261 patients for each of 181 NS3 protease amino acid residues compared to the consensus sequence. A total of 41 resistance proven mutations were detected in 35 (13.4%) patients: T54S (14 patients, 5.4%), Q80K (1 patient, 0.4%), I153V (22 patients, 8.4%), D168E (4 patients, 1.5%), T54S plus I153V double mutation (4 patients, 1.5%), and I153V plus D168E double mutation (2 patients, 0.8%). The mutation number increased to 54 in 47 (18.0%) patients when resistance unproven mutations were included: V36I (2 patients, 0.8%), I153L (11 patients, 4.2%), and I153V plus V36I double mutation (2 patients, 1.5%). Double mutations were found in 7 patients (2.7%) (Table 1). Q80L was observed in 47 (18%) patients but these were excluded from consideration because a previous study demonstrated that this mutation does not confer resistance [15]. All mutations observed in this study would confer low- to moderate-level PI resistance according to previous studies [6, 15–19]. No mutations conferring high-level resistance such as R155 or A156 [11, 17, 19–22] were observed.

Clinical characteristics of patients with PI-resistance mutations

Table 2 presents the characteristics of patients classified according to the presence of PI-resistance mutations

Table 1 Prevalence of PI-resistance-associated NS3 mutations

Drug-resistance mutations described in the literature				Detected resistance mutations
NS3 residue	Resistance mutations	Drugs	References	Genotype 1b ($N = 261$), (%)
V36	A, M, L, G, C	Telaprevir, Boceprevir	[1, 3, 4, 10, 11, 19, 31, 37]	I × 2 (0.8)
Q41	R	ITMN-191, Boceprevir	[19]	
F43	S, C	ITMN-191, Boceprevir, Telaprevir, TMC435	[15, 19]	
T54	A, S	Telaprevir, Boceprevir, SCH900518	[1, 3, 10, 11, 19, 20, 31, 38]	**S × 14 (5.4)**
V55	A	Boceprevir	[1]	
Q80	R, K	TMC435	[6, 15]	**K × 1 (0.4)**
R109	K	SCH446211	[17]	
I153	V	SCH446211	[17]	**V × 22 (8.4)**, L ×11 (4.2)
R155	K, T, I, M, G, L, S, Q	Telaprevir, Boceprevir, ITMN-191, BILN2061, TMC435	[1, 3, 4, 6, 10, 11, 15, 19, 20]	
A156	S, T, V, I, G	Telaprevir, Boceprevir, ITMN-191, BILN2061, SCH446211, TMC435, SCH900518	[1, 3, 4, 10, 11, 15, 17, 19, 20, 38]	
D168	A, V, E, N, T, H	BILN2061, ITMN-191, TMC435	[6, 15, 20]	**E × 4 (1.5)**
V170	A	Telaprevir, Boceprevir	[1, 19, 20]	
M175	L	Boceprevir	[39]	
Total number (%) of patients with resistance proven mutations				35 (13.4)
Total number (%) of patients with resistance proven and unproven mutations				47 (18.0)

Amino acid mutations conferring PI resistance in the literatures and those observed in PI-treatment-naive patients in this study are indicated. Bold indicates resistance proven mutations, and the others indicate resistance unproven mutations

Double mutations found were as follows: V36I and I153V × 1, T54S and I153V × 4, I153V and D168E × 2

(including resistance unproven mutations). Age, sex ratio, body mass index, alanine aminotransferase (ALT) levels, serum albumin, platelet count, and fibrosis stage did not differ between the NS3 mutation and wild-type groups. No significant difference was observed between the two groups in the parameters of PEG-IFN/RBV treatment response, HCV sequence variations in interferon sensitivity determining region (ISDR), Core 70, interferon plus ribavirin resistance-determining region (IRRDR), or interleukin 28B (IL28B) single nucleotide polymorphism (SNP) (rs8099917; T/G and G/G vs. T/T) [23–30]. These clinical variables were also compared between the mutation group defined as resistance proven mutations and the wild-type group, but no notable differences were observed.

Unimpaired in vivo fitness of viral strains with resistance mutations

Because most PI-resistance mutations described till date have been associated with reduced replicative capacity of varying degrees [1, 10, 11, 13, 17, 20–22, 31, 32], we examined viral replication levels in patients with drug-resistance mutations (Fig. 2). The estimated P value indicated no significant difference between the mutation (median 1,500 KIU/ml) and wild-type (median 1,800 KIU/ml) groups ($P = 0.69$). The results indicate that drug-resistant HCVs were not necessarily impaired in their ability to replicate in vivo. However, patients with double mutations ($N = 7$) tended to have low viral loads (median 1,200 KIU/ml) ($P = 0.09$).

Resistance mutations and virologic response to PEG-IFN/RBV therapy

To determine the difference in virologic response to PEG-IFN/RBV therapy according to the PI mutation, frequency of HCV RNA levels below detection at 4 weeks (rapid viral response, RVR) and 12 weeks (complete early viral response, cEVR), and SVR rate (%) were investigated in each group. The frequency of HCV RNA levels below detection at 4 and 12 weeks was 14 and 50%, respectively, in the mutation group, and was 11 and 46%, respectively, in the wild-type group. The SVR rate was 48 and 40% in the mutation and wild-type groups, respectively ($P = 0.38$). No significant difference was observed between the two groups in any of the indexes investigated (Table 2). The time-dependent viral clearance rate during PEG-IFN/RBV therapy was estimated in 133 patients including 25 patients (19%) with PI-resistance mutations available for the analysis. Kaplan–Meier analysis demonstrated that HCV clearance did not differ between the two groups with and without resistance mutations (log-rank test, $P = 0.30$) (Fig. 3).

Changes in nonstructural 3 amino acid sequence diversity during PEG-IFN/RBV therapy

Full-length NS3 protease sequences were determined in 39 non-SVR patients after PEG-IFN/RBV therapy. A single amino acid change at resistance-associated sites in two patients was observed. In one patient, isoleucine (Ile) at position 153 changed to valine (Val), and glutamic acid (Glu) changed to aspartic acid (Asp) at position 168 in the second (Fig. 4). At the nucleotide level, ATC (Ile) changed to GTC (Val) in I153V, and GAA (Glu) changed to GAC (Asp) in E168D. Both mutations were caused by one nucleotide exchange. No other changes were observed in the other 37 patients.

Discussion

Here we report that in 18% (47/261) HCV genotype 1b-infected patients who had not been previously treated with NS3 PIs, the viral genome contained dominant amino acid mutations within the NS3 PI-resistance sites. Even after confining the data to established PI-resistance mutations, the mutation rate was still significant in 13.4% (35/261). No clinical differences were observed between patients

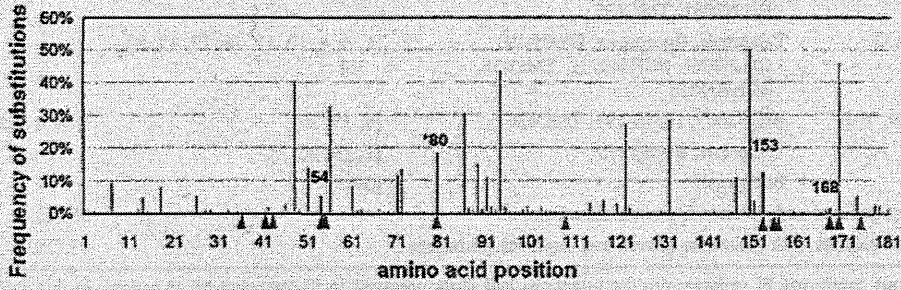


Fig. 1 Frequency of polymorphic mutations for each of the 181 NS3 protease amino acid residues in 261 patients. *Arrowheads* indicate the sites reported to confer PI resistance. *Dark bars* denote the amino acid variations at the resistant sites in this study. **80*, we detected one resistant mutation (Q80K) and 47 (18%) non-resistant variations (Q80L) at the 80th residue

Table 2 Characteristics of patients with or without HCV genomes harboring drug-resistance mutations

Characteristics	Mutation type ($N = 47$)	Wild-type ($N = 214$)	P value
Patients' characteristics			
Age, median (range)	59 (46–72)	57 (19–77)	0.17
Male, no. (%)	26 (55)	112 (52)	0.70
BMI, median (range)	23.2 (15.5–31.9)	22.8 (16.1–31.9)	0.41
ALT IU/ml	81.3 ± 72.6[a]	74.8 ± 51.9	0.93
Serum albumin g/dl	4.00 ± 0.37	4.01 ± 0.36	0.81
Platelet count × 10^4/μl	15.8 ± 4.3	14.5 ± 4.8	0.18
HCV RNA KIU/ml, median (range)	1,500 (58–6,310)	1800 (28–15,849)	0.69
Fibrosis, no. (%)			0.97
F0	0 (0)	7 (3)	
F1	23 (50)	89 (42)	
F2	9 (20)	52 (24)	
F3	9 (20)	40 (19)	
F4	5 (11)	26 (12)	
IFN pre-treatment no. (%)	15/40 (38)[b]	66/172 (38)	1.00
IL28B (rs8099917) T/G or G/G no. (%)	6/20 (30)	19/67 (28)	1.00
Response to PEG-IFN/RBV therapy			
SVR total cases no. (%)	22/46 (48)	83/210 (40)	0.38
RVR in total cases no. (%)	6/44 (14)	22/195 (11)	0.83
cEVR in total cases no.(%)	22/44 (50)	92/200 (46)	0.75
SVR 48w treatment no. (%)	16/29 (55)	55/130 (42)	0.29
End of treatment response no. (%)	26/41 (63)	123/202 (61)	0.91
HCV genome sequence variation			
ISDR mutation ≤1 no. (%)	32/46 (70)	167/210 (80)	0.21
Core70 R no. (%)	26/44 (59)	136/210 (65)	0.56
IRRDR mutation >3 no. (%)	25/38 (66)	107/190 (56)	0.34

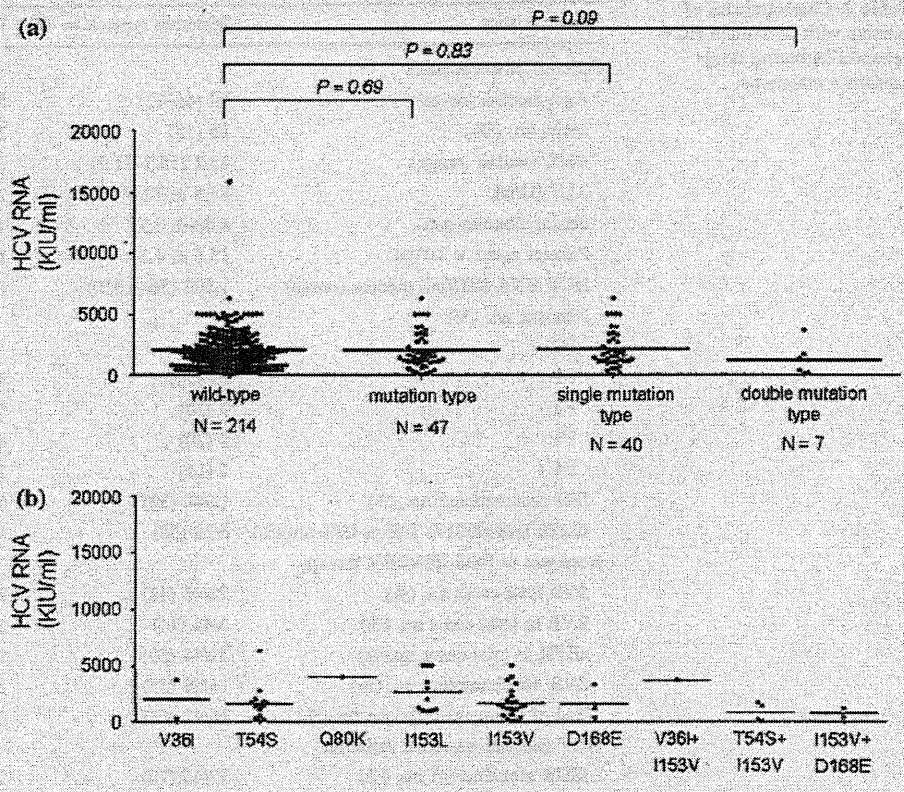
[a] Mean ± SD

[b] Number/total number (%)

harboring viruses with and without these mutations. Moreover, no differences were observed in the responses of either group to PEG-IFN/RBV therapy.

Recent studies reported that significant number of patients who were never treated with PI possess viral sequences with PI-resistance-associated NS3 mutations. In these studies, the prevalence of PI-resistance mutations was determined to be 8.6–16.2% [13, 14], in HCV genotype 1- and 3-infected patients in European–American populations. These patients were often coinfected with HIV. Analysis of the public HCV databases (EuHCVdb and Los Alamos) also reported the presence of naturally occurring PI-resistance-associated NS3 mutations in worldwide isolates [33]. However, in vivo and in vitro studies demonstrated that most of the mutations observed conferred only low- to moderate-level PI resistance [7, 13, 14, 34, 35]. Regarding viral fitness, PI-resistant HCVs show lower fitness at varying degrees as revealed by in vitro studies [1, 10, 11, 17, 20–22, 31, 32], but HCV RNA levels in a clinical study did not differ significantly. The response to PEG-IFN/RBV therapy was almost comparable to that in HCV-infected patients without PI-resistance mutations either in HCV replicon experiments or in a clinical study of small number of treated patients [34].

The prevalence of 13.4% for PI-resistance-proven patients observed in the present study was almost comparable to the results of previous studies. Although HIV is known to increase HCV replication in coinfection with HCV [36], and HIV patients are often treated with the HIV-specific PIs, the HIV infection might not affect the natural occurrence of HCV-specific PI-resistance mutations since our studied patients were all proven to be free from coinfection with HIV infection. As shown in Table 1 and Fig. 1, I153 V (22/261, 8.4%), T54S (14/261, 5.4%), and D168E (4/261, 1.5%) were among the most prevalent PI-resistance-proven mutations in the present study. The most frequent mutation detected in our study I153V was reported to appear secondarily to the occurrence of R109K mutations in a HCV replicon system [17]. Although the role of this mutation is not understood, the I153V mutation on its own conferred SCH446211 resistance to the HCV replicon to a lesser degree [17]. Interestingly, I153V was often found in double mutations in our study, as shown in Fig. 2. This suggests analogy between in vitro and in vivo data. T54S and D168E, the other frequent mutations, have been also reported to occur as single dominant mutations in previous in vitro or in vivo studies in HCV genotype 1



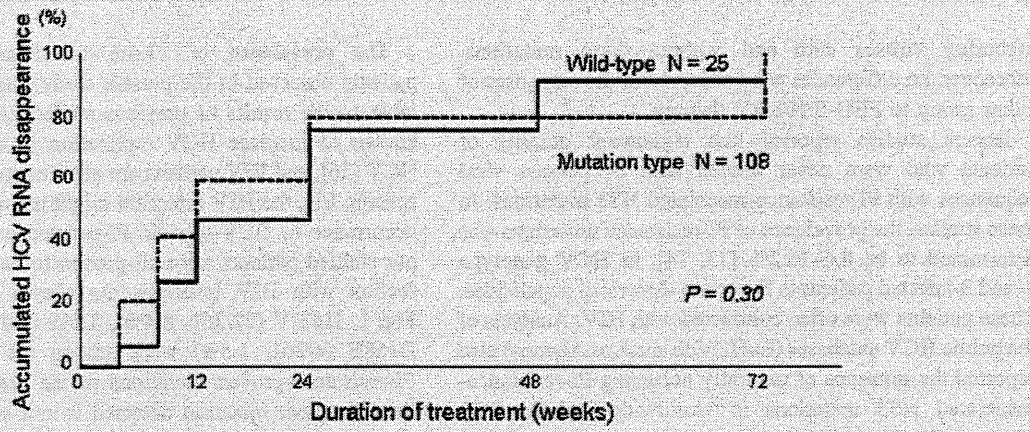


Fig. 2 In vivo fitness of HCV with PI-resistance-associated NS3 mutations. HCV RNA levels were compared between patients with and without NS3 PI-resistance-associated mutations (a) and between patients with each resistance mutation (b). The estimated *P* value (Mann–Whitney *U* test) indicates no significant difference between the wild-type and other groups (wild-type vs. mutation type, wild-type vs. single mutation type, and wild-type vs. double mutation type). (Wild-type, $N = 214$; mutation type, $N = 47$; single mutation type, $N = 40$; double mutation type, $N = 7$; V36I, $N = 2$; T54S, $N = 14$; Q80K, $N = 1$; I153L, $N = 11$; I153V, $N = 22$; D168E, $N = 4$; E176A, $N = 1$; V36I + I153V, $N = 1$; T54S + I153V, $N = 4$, and I153V + D168E, $N = 2$)

Fig. 3 Comparison of virologic response to PEG-IFN/RBV therapy between HCV-infected patients with and without PI-resistance-associated NS3 mutations. Time-dependent HCV clearance rate analysis was based on serum HCV RNA positivity during PEG-IFN/RBV therapy for HCV isolates with resistance mutations or wild-type sequences. A total of 133 patients for whom the limit of viral genome detection could be determined were analyzed. Among this group, NS3 mutations were detected in 25 patients (19%). The estimated *P* value (log-rank test) shows no significant difference between the two groups ($P = 0.30$)

infections showing moderate degrees of resistance [16, 18, 19].

Most PI-resistance mutations described to date have been associated with varying degrees of reduced replicative capacity [10, 11, 17, 20–22, 31, 32]. In the present study, HCV RNA levels of those patients with low- to moderate-level resistance mutations were similar to those in patients in the wild-type groups, suggesting that in vitro viral fitness

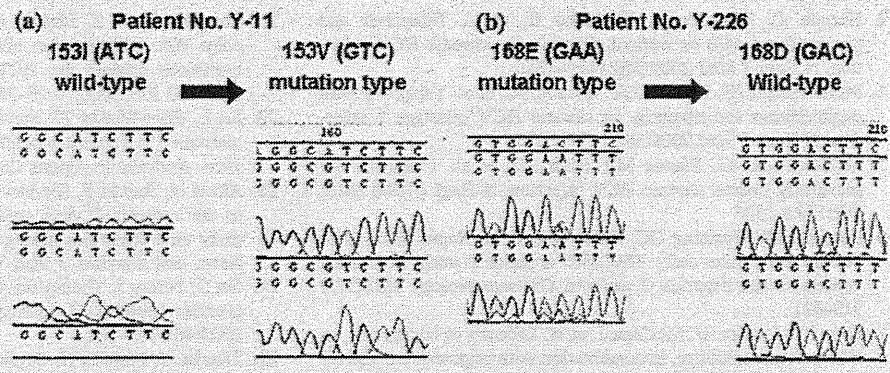


Fig. 4 Appearance of PI-resistance-associated NS3 mutations during the PEG-IFN/RBV therapy. Chromatograms show part of the HCV NS3 sequence demonstrating PI-resistance mutations in two patients receiving therapy. a Site 153 isoleucine (Ile) (ATC) changed to valine (Val) (GTC), b Site 168 glutamic acid (Glu) (GAA) changed to aspartic acid (Asp) (GAC)

does not necessarily reflect in vivo viral fitness. This, however, does not rule out the possibility that some unknown compensatory viral mutations might have resulted in upregulation of reduced viral fitness. Interestingly, although the replicative capacity conferred by a single mutation seemed to be the same, the HCV RNA levels of double mutations were frequently low, suggesting that double mutations might weaken viral fitness.

In previous studies, clinical characteristics representing the state of liver disease other than HCV RNA levels were not studied in patients with PI-resistance mutations. In this study, we show that those clinical characteristics did not differ according to the presence of viral NS3 mutations. As shown in Table 2, age, sex ratio, fibrosis stage, ALT levels, serum albumin, platelet count, and past history of IFN pretreatment did not differ according to the presence of NS3 mutations. These results suggest that NS3 mutations occur independently of disease progression. Moreover, no evident differences were observed between viral and host factors known to affect IFN-based treatment responses. However, viral amino acid variations in the core and NS5A or the allelic frequency of IL28B SNPs, which were recently reported for the close relationship of responses to PEG-IFN/RBV therapy, did not differ between the two groups.

A significant outcome of the present study is the demonstration that PI-resistance mutations might not affect responses to PEG-IFN/RBV therapy. Previous in vitro studies demonstrated that HCV replicons harboring PI-resistance mutations were also sensitive to IFN treatment [31]. In addition, recent clinical studies also indicated that PI-resistance mutations were sensitive to the PEG-IFN/RBV [10, 34]. However, our analysis was more comprehensive because viral and host factors that contribute to treatment responses were simultaneously analyzed. A unique aspect of the present study is that we investigated the influence of the PEG-IFN/RBV treatment on the occurrence of new PI mutations by direct nucleotide sequencing, and were able to show that the PEG-IFN/RBV might not induce amino acid mutations.

Will the pre-existence of naturally occurring PI-resistance mutations have an influence on future treatment of HCV infections? Since new PIs are on the verge of clinical use, all clinicians should bear in mind the substantial numbers of HCV-infected patients with PI-resistance mutations. Although the degree of resistance is considered to be low or moderate in untreated patients, weak resistance might progress to more potent resistance with additional mutations, when PIs become widely used. Therefore, all clinicians need to be sufficiently prepared for the possibility of later onset of PI-resistance mutations that confer greater drug resistance and concomitant poorer responses to therapy. In SPRINT-1 study, the lead-in therapy was associated with a modestly lower rate of breakthrough than with no lead in [7]. Considering that PEG-IFN/RBV was equally effective for PI-resistant viruses, sufficient "lead-in" therapy before the administration of PIs could be an option in the forthcoming triple therapy modality.

In conclusion, we demonstrate here that PI-resistance-associated NS3 mutations exist in a substantial proportion of untreated HCV-1b-infected patients. Although the degree of resistance might not be strong, clinicians will need to consider this upon the introduction of triple therapy.

Acknowledgements This work was supported in part by a grant-in-aid scientific research fund from the Ministry of Education, Science, Sports, and Culture [grant number 20390206] and in part by a grant-in-aid from the Ministry of Health, Labor, and Welfare of Japan [grant number H19-kanen-002].

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Clinical and Applied
Thrombosis/Hemostasis
18(3) 272-280

DOI: 10.1177/1076029611429124
http://cath.sagepub.com
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Causes of Thrombocytopenia in Chronic Hepatitis C Viral Infection

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Abstract

We retrospectively studied 89 patients with chronic hepatitis C virus (HCV) infection, including 50 chronic hepatitis (CH) cases, 18 liver cirrhosis (LC) cases, and 21 LC with hepatocellular carcinoma (LC + HCC) cases, with regard to various factors related with thrombocytopenia. The platelet count decreased with the stage advancement of liver diseases. Multiple regression analysis revealed that splenomegaly and von Willebrand factor (vWF) were explanatory variables that correlated with thrombocytopenia. Splenomegaly appears to be the most responsible factor, although there are a considerable number of thrombocytopenic cases without splenomegaly, suggesting other factors may also be responsible. The vWF level is inversely correlated with the platelet count. Soluble thrombomodulin, a marker of endothelial dysfunction, increases with the advancement of liver fibrosis. It is positively correlated with vWF and inversely with the platelet count. Our present results imply that vascular endothelial dysfunction is also involved in thrombocytopenia during chronic HCV infection.

Keywords

thrombocytopenia, hepatitis C, splenomegaly, von Willebrand factor, thrombomodulin

Introduction

The platelet count is known to decrease in proportion to the advancement of the stage of liver disease in chronic hepatitis C virus (HCV) infection. Liver biopsy is the golden standard for evaluating the stage of fibrosis in HCV patients. It is, however, a considerably invasive procedure and more simple, noninvasive laboratory methods capable of predicting the stage of fibrosis would be of great help in clinical settings. A strong correlation between liver fibrosis and thrombocytopenia has been noted in a number of papers, and the platelet count is presently used as an index for fibrosis staging.[1,2]

Thrombocytopenia in liver fibrosis can be attributed to (1) platelet destruction/sequestration by the spleen, (2) the decreased production of platelets, and (3) platelet consumption. Based on several papers that have reported a strong correlation between spleen size and thrombocytopenia,[3-5] platelet destruction/sequestration as a result of splenomegaly caused by portal hypertension has been considered to be the most important determinant. On the other hand, a splenectomy or portal vein shunting does not necessarily normalize the platelet count,[6,7] suggesting that factors other than splenomegaly are also operative in reducing the platelet count during liver fibrosis.

Since thrombopoietin (TPO), which facilitates the proliferation and differentiation of the megakaryocytic lineage (resulting in the production of platelets), is released from the liver, some reports have suggested that inadequate TPO production is at least partly responsible for thrombocytopenia in liver fibrosis.[4,8-11] The expression level of c-mpl, the TPO receptor, is also reportedly low in patients with liver cirrhosis.[12] On the other hand, some reports have argued against a correlation between thrombocytopenia and hepatic TPO production,[13] and whether decreased thrombopoiesis contributes to thrombocytopenia during liver fibrosis awaits further elucidation.

As for platelet consumption, several hypotheses related to von Willebrand factor (vWF) have been proposed.

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Inflammatory changes that accompany chronic HCV hepatitis and liver cirrhosis may lead to an increase in the vWF level, resulting in platelet consumption.[14-16] The impaired hepatic production of ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13) and its activity, which cleaves vWF, may also lead to an increase in ultra-large multimers of vWF, resulting in platelet microthrombi formation[17] and consumptive thrombocytopenia, similar to the conditions observed with thrombotic thrombocytopenic purpura (TTP).

In the present study, we evaluated various factors, including spleen size, TPO, vWF, and ADAMTS13, as well as general hematological, biochemical, and coagulation parameters in patients with chronic HCV infection, with fibrosis stages ranging from a relative early phase to an advanced stage of liver cirrhosis and attempted to determine the contributing power of each factor to thrombocytopenia using a multiple regression analysis and other analytical methods.

Materials and Methods

Patients

Our study was made up of 89 patients with chronic HCV infection who had been referred to the outpatient clinic of the University of Yamanashi Hospital. Based on the chronic HCV staging, 50 patients had chronic hepatitis (CH), 18 patients had liver cirrhosis (LC), and 21 patients had liver cirrhosis plus hepatocellular carcinoma (LC + HCC). The diagnosis of chronic hepatitis and LC was made by an expert in hepatology (M.S.), fundamentally using the score of Fib-4.[18] Fib-4 was calculated according to the formula of (age × AST/(platelets × $ALT^{0.5}$)), and the cases exceeding the score of 3.25 were diagnosed as LC. The study was endorsed by the Institutional Review Board of the University of Yamanashi (No. 224), and all the patients gave their written informed consent prior to participation in the study. Blood was withdrawn from the ante-cubital vein; after laboratory measurements for diagnostic purposes, the residual samples were used to assess various factors that may be involved in thrombocytopenia.

Methods

A complete blood count (CBC) analysis was performed using an SE-3000 (Sysmex Co, Kobe, Japan). Serum and plasma anticoagulated with citrate were obtained by centrifugation of the whole blood within 2 hours of blood collection and were stored at −80°C until measurement.

Repeated freezing was avoided as much as possible; when necessary, the thawing of the frozen serum and plasma was performed at 37°C. Biochemical parameters, including albumin, alanine aminotransferase (ALT), and total bilirubin, were measured using a BM-2000 (JEOL Ltd, Tokyo, Japan). The vWF antigen level was determined using STA-LIA kits (Roche Diagnostics K.K., Tokyo Japan), and the ADAMTS13 antigen level was determined using ADAMTS13 ELISA kits (Mitsubishi Chemical Medience Co, Tokyo, Japan).[19] ADAMTS13 activity was measured using the ADAMTS13 Activity Kit (Kainos Laboratories, Tokyo, Japan),[20] thrombomodulin (TM) was measured using TM (MKI) EIA kits (Mitsubishi Kagaku Iatron Co Ltd), and TPO was measured using Human TPO Immunoassay kits (R&D Systems, Minneapolis). For coagulation and fibrinolysis testing, an LPIA A-700 (Mitsubishi Chemical Medience Co) was used to measure the prothrombin time, with results expressed as international normalized ratio (PT-INR), D-dimers, and tissue plasminogen activator inhibitor (t-PAI). The spleen size was determined using computed tomography (CT), magnetic resonance imaging (MRI), or ultrasonographic measurements. The splenic index (SI $^{1/4}$(long axis/2) × short axis) was calculated, and spleens with an SI value higher than 20 were considered to exhibit splenomegaly.

Statistical Analysis

The analysis of the biochemical parameters, coagulation/fibrinolysis factors, SI index, and so on, and the multiple regression analysis were preformed using the data analysis software STAT FLEX, Ver. 4.1 (Artec, Ltd, Osaka, Japan).

Results

Patient Background

The numbers and sex (male/female) of patients at each stage was 50 patients (37/13) with CH, 18 patients (7/11) with LC, and 21 patients (9/12) with LC + HCC. The patient age tended to increase with the progression of the fibrosis stages: 57.4 ± 10.6 years among patients with CH, 65.3 ± 9.5 years among patients with LC, and 70.0 ± 9.1 years among patients with LC + HCC. The CBC profile, biochemical parameters, coagulation/fibrinolysis factors, and inflammation markers are summarized according to each stage in Table 1.

Platelet Count

The platelet count decreased with the progression of fibrosis staging, and significant differences in the platelet count were observed between the stages: 182.8 ± 47.5 × 10^3/μL among patients with CH, 85.9 ± 33.6 × 10^3/μL among patients with LC, and 66.7 ± 25.2 × 10^3/μL among patients with LC + HCC (Table 1 and Figure 1).

Liver Function

Prothrombin time–international normalized ratio (PT-INR), which represents the overall coagulation capacity of the extrinsic pathway, is a good marker for hepatic protein synthesis. Prothrombin time–international normalized ratio was positively correlated with the progression of the fibrosis stages; 1.03 ± 0.05 among patients with CH, 1.18 ± 0.16 among patients with LC, and 1.22 ± 1.13 among patients with LC + HCC; significant differences were observed among the stages ($P < .01$; Table 1). A negative correlation was seen between PT-INR and the platelet count ($r = -.71$,

Table 1. Clinical Characteristics of Patients With Chronic HCV Infection

Variable	CH	LC	LC + HCC
Platelet count ($\times 10^3/\mu L$)	182.4 ± 48.7[a,b]	91.3 ± 33.0	65.7 ± 23.1[c]
Splenic index (SI)	14.7 ± 4.2[a,b]	23.4 ± 9.5	24.2 ± 7.3
PT-INR	1.03 ± 0.05[a,b]	1.18 ± 0.16	1.22 ± 0.13
Albumin, g/dL	4.3 ± 0.4[a,b]	3.6 ± 0.7	3.5 ± 0.4
Total bilirubin, mg/dL	0.5 ± 0.2[a,b]	0.7 ± 0.4	0.88 ± 0.33
Thrombopoietin, pg/mL	42.5 ± 33.1[d]	39.9 ± 37.8	70.3 ± 68.7
von Willebrand factor, %	153.4 ± 52.6[a,b]	208.7 ± 83.1	243.6 ± 65.3
Thrombomodulin, U/mL	16.0 ± 5.7[a,b]	22.9 ± 8.8	25.8 ± 7.5
ADAMTS13 antigen, %	103.2 ± 32.9[a]	138.6 ± 58.1	107.6 ± 31.2
ADAMTS13 activity, %	97.2 ± 28.8[a]	123.0 ± 43.2	102.1 ± 27.5
vWF/ADAMTS13 activity	1.7 ± 0.7[b]	2.0 ± 1.3	2.7 ± 1.5
D-Dimer, μg/mL	0.44 ± 0.28[b,c]	0.72 ± 0.60	0.87 ± 0.82
PAI-1, ng/mL	18.9 ± 6.5	19.8 ± 7.1	19.1 ± 10.5
CRP, mg/dL	0.11 ± 0.02	0.11 ± 0.02	0.13 ± 0.05

Abbreviations: CH, chronic hepatitis; LC, liver cirrhosis; LC + HCC, LC complicated with hepatocellular carcinoma; PT-INR, prothrombin time–international normalized ratio; vWF, von Willebrand factor; CRP, C-reactive protein; PAI-1, plasminogen activiator inhibitor 1; ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13.
[a] $P < .01$ versus LC.
[b] $P < .01$ versus LC + HCC.
[c] $P < .05$ versus LC.
[d] $P < .05$ versus LC + HCC.

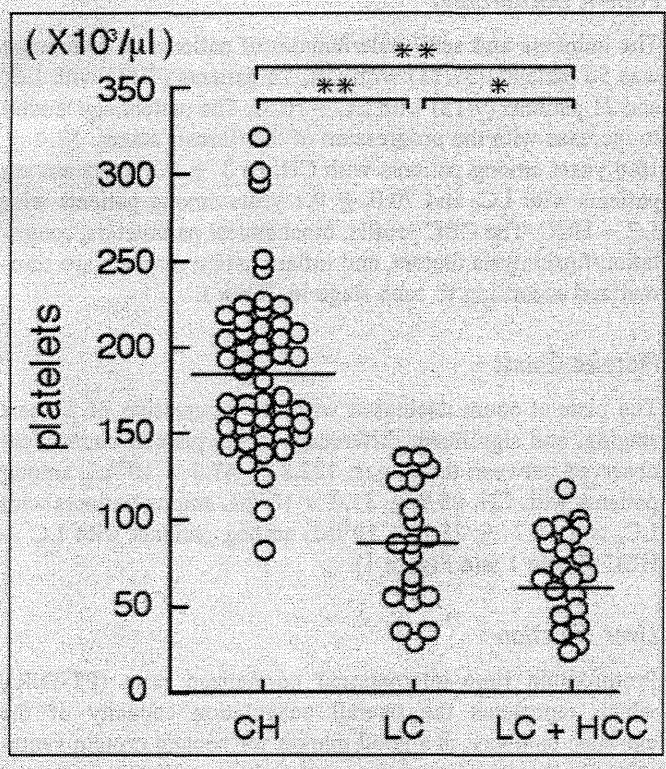

Figure 1. Platelet counts in chronic hepatitis C virus (HCV) infection patients with chronic hepatitis (CH), liver cirrhosis (LC), and liver cirrhosis complicated by hepatocellular carcinoma (LC + HCC). The open circles represent each individual patient, and the mean of each patient group is indicated by the horizontal line. Statistical differences are indicated with *P < .05 and **P < .01.

$P < .0001$), suggesting that the platelet count decreases with impaired liver function in patients with chronic HCV infection (Figure 2). A positive correlation ($r = .59$, $P < .0001$) was observed between the platelet count and the albumin level, which represents hepatic protein synthesis, and an inverse correlation was observed between the platelet count and the total bilirubin level, the elevation of which represents a fibrosis-related impairment in bile secretion ($r = -.49$, $P < .0001$). Of these markers of liver function, the coefficient value of PT-INR exceeded those of the others. A negative correlation was also observed between PT-INR and the albumin level ($r = -.73$, $P < 0.0001$), and a positive correlation was observed between PT-INR and the total bilirubin level ($r = .76$, $P < .0001$).

Splenomegaly

Splenomegaly is considered to be one of the major causes of thrombocytopenia during chronic HCV infection. The SI was 14.7 ± 4.2 among the patients with CH, 23.7 ± 9.2 among the patient with LC, and 25.3 ± 8.2 among the patients with LC + HCC. These values were significantly different ($P < .01$; Table 1), suggesting that splenomegaly increases in size with the progression of the fibrosis stage. A negative correlation was observed between the platelet count and the splenomegaly ($r = -.65$, $P < .0001$; Figure 3), confirming the previous notion that the thrombocytopenia was attributable to splenomegaly in proportion to the stage of progression. On the other hand, of the 33 patients with a platelet count of less than $100 \times 10^3/\mu L$, 8 patients had no significant splenomegaly, while 25 patients had splenomegaly with SI values higher than 20. These findings imply that there are some cases of thrombocytopenia that are unexplainable by splenomegaly.