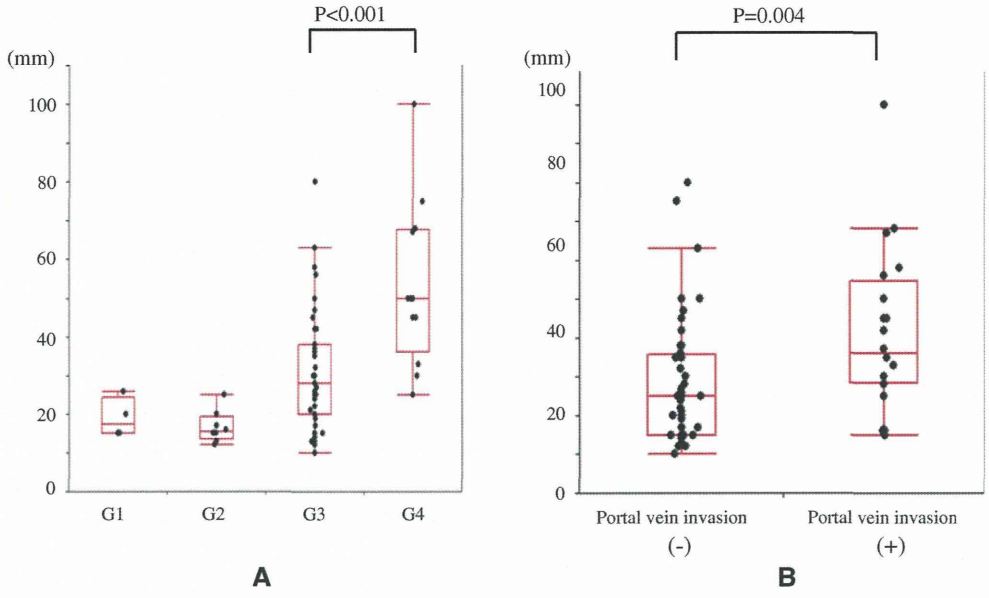

Fig. 4 Correlations between tumor size and malignant grading system and portal vein invasion. **a** Correlation with malignant grading system. Tumor sizes were similar between Grades 1 and 2, but there was a greater increase in size between Grades 3 and 4. **b** Correlation with portal vein invasion. Although HCCs positive for portal vein invasion were larger than negative HCCs, discriminating the positive cases only using tumor size was difficult

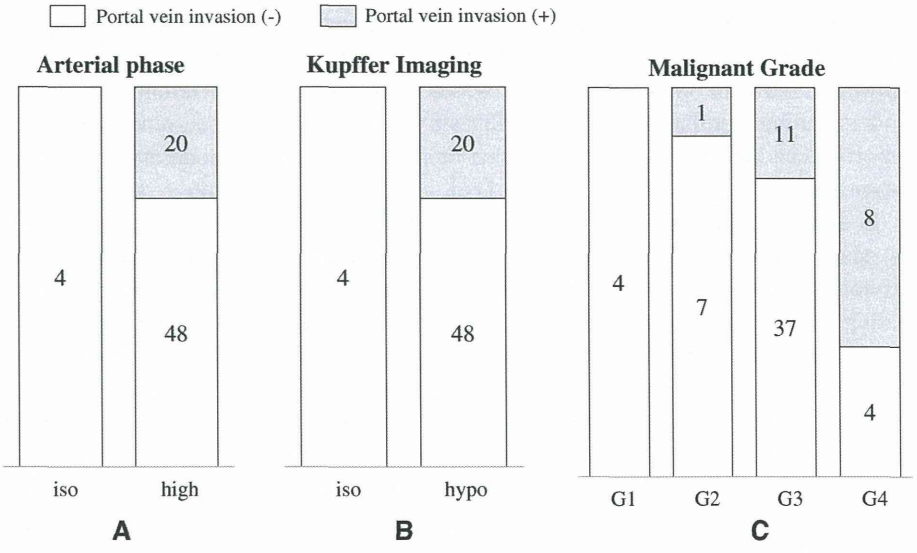

Fig. 5 Correlations between portal vein invasion and CEUS parameters. Arterial phase (**a**) and Kupffer imaging (**b**) showed the same patterns when these parameters were analyzed with portal vein invasion. Most cases, including all portal vein-positive HCCs, were hyper-echoic in the arterial phase and hypo-echoic in Kupffer imaging. Portal vein invasion-positive rates increased with increasing malignant grade (**c**)

invasion increased with the CEUS malignant grade: Grade 1, 0 % (0/4); Grade 2, 13 % (1/8); Grade 3, 23 % (11/47); and Grade 4, 67 % (8/12) (Fig. 5c). This correlation was closer ($r = 0.385$, $P < 0.001$) than the correlation between portal vein invasion and tumor size ($r = 0.359$, $P = 0.002$).

The significance of the tumor markers, such as AFP and DCP, were also evaluated for their ability to detect portal vein invasion (Fig. 6). However, no correlation was demonstrated between tumor markers and portal vein invasion.

Discussion

This study proposes a new malignant grading system that incorporates two key features of CEUS and demonstrates a strong correlation between this new grading system and histological differentiation of HCC. Both Kupffer imaging and MIP are unique imaging techniques that utilize characteristics of Sonazoid CEUS. These images are difficult to obtain by other imaging modalities, such as CT or MRI. Detection of small HCCs by CT or MRI without contrast medium can be difficult and use of contrast media for both

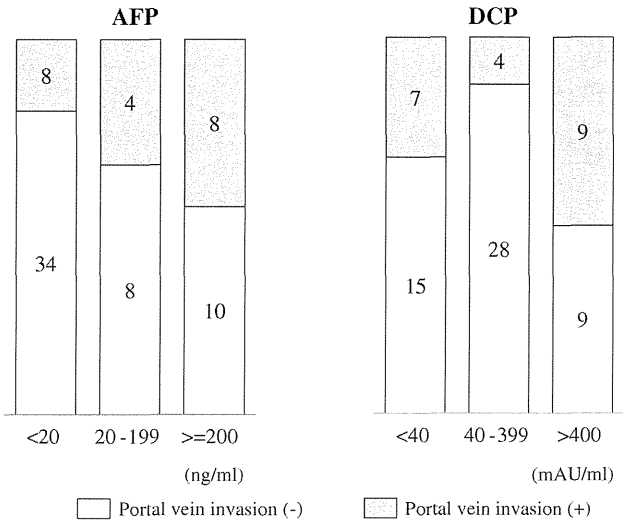

Fig. 6 Correlations between portal vein invasion and tumor markers. **a** Alpha-fetoprotein (AFP). **b** Des-gamma carboxyprothrombin (DCP). No correlation was demonstrated between tumor markers and portal vein invasion

CT and MRI is sometimes restricted by renal function and allergy. Hence, our new malignant grading system using CEUS, which is associated with little or no side effects, is of particular value. Notably, it is difficult to estimate the degree of malignancy using only an imaging technique. However, the term "malignant grade" was used here to aid understanding, similar to the use by Hayashi et al., in their report indicating the correlation between blood supply and progression of hepatocellular nodules [16].

In addition, ultrasonography yielded several details such as size, shape, and internal echo pattern of tumors. Although tumor size is also an important factor for evaluating the differentiation of HCC, it is difficult to distinguish the histological differentiation especially in small nodules. This tendency was confirmed in our results. Although tumor sizes in Grades 1 and 2 were similar (Grade 1, 18.2 ± 4.7 mm; Grade 2, 16.6 ± 4.2 mm), histological differences were identified between malignant Grades 1 and 2 ($P = 0.001$) (Fig. 3a). As just described, it is difficult to distinguish histological differentiation only by tumor size. US also demonstrated characteristic findings in internal echo patterns. Nevertheless, internal echo patterns were not easily distinguishable and, in this way, the objectivity of these results was limited.

Using the Sonazoid contrast medium, additional arterial phase, Kupffer imaging, and MIP patterns imaging were also obtained. This study showed a definite correlation between each of these findings and histological differentiation, although no significant difference was achieved only in arterial phase due to the small population size. Therefore, all these results are of value. Kupffer imaging is a unique imaging technique that, since Levovist was discontinued, currently can only be accomplished using Sonazoid CEUS or super paramagnetic iron oxide magnetic resonance images (SPIO-MRI). HCC intensity changes on Kupffer imaging according to the progression of histological differentiation [10, 11]. For example, the tumor intensity, on Kupffer imaging by Sonazoid CEUS, changes from an iso- to a hypo-echoic pattern as the histological differentiation changes from well- to moderately differentiated HCC. Kupffer imaging can also be obtained by SPIO-MRI. However, SPIO-MRI cannot obtain vascular imaging and the resolution is inadequate.

MIP is a CEUS modality in which the imaging is based on an accumulation of images. MIP combines the flash-replenishment sequence with maximum-holding image processing to distinctly delineate the blood vessels in tissue [14]. HCC is known to undergo changes in vascular structure as it progresses. MIP can help visualize this fine vascular structure without angiography of the liver. In this study, half of the well-differentiated HCCs demonstrated fine patterns. The proportion of fine patterns of well-differentiated HCC was higher (73 %), when analyzed with all 121 HCCs. In contrast, all 12 patients with irregular patterns had either moderately or poorly differentiated HCC and all poorly differentiated HCCs demonstrated irregular patterns (Fig. 2c). The prognosis associated with these tumors was poor. All 6 patients with moderately differentiated HCC with an irregular pattern also had a recurrence within 2 years (data not shown). Moreover, the moderately differentiated HCCs could be further divided into a less malignant group (fine pattern) and a more malignant group (irregular pattern) using our grading system. Some of the CEUS results showed similar tendencies in the correlation with histological differentiation. For example, both arterial phase and Kupffer imaging showed similar patterns with respect to the degree of histological differentiation (Table 1). These results indicate that combining these 2 imaging modalities would yield little additional information. In this study, we did not combine arterial phase findings and MIP patterns, although this combination would allow for shortening the time of examination. These techniques were not combined because both reflect the vascularity of a HCC and contain similar information and because the arterial phase evaluation would have to be limited to 20 s, causing the optimal moment for appropriate assessment to be missed in some cases. In contrast, Kupffer phase imaging could be assessed repeatedly from various angles and serves as an indispensable phase of the CEUS examination. Thus, the combination of Kupffer imaging and MIP patterns would be the most sensible and complementary method for evaluating the histological differentiation of HCC. For example, 7 moderately differentiated HCCs with Fine patterns were indistinguishable from well-differentiated HCCs only using

MIP patterns (Fig. 3a). All of these tumors were of malignant Grade 2 and could be distinguished by adding Kupffer imaging.

Our grading system was also correlated with portal vein invasion ($r = 0.385$). Tumor size is also one of the important factors for the prediction of portal vein invasion and was correlated with portal vein invasion in this study ($r = 0.359$). However, AFP and DCP, as the representative tumor markers for HCC, also did not show correlations with portal vein invasion in this study (AFP, $r = 0.050$; DCP, $r = 0.203$). Thus, both our malignant grade and tumor sizes are important for the prediction of the portal vein invasion. Since some cases with small HCC and portal vein invasion exist, our malignant grade could be expected to identify these cases with small portal vein invasion.

Sugimoto et al. [14] reported the classification of HCC using MIP patterns and the MIP pattern concept used in this study was similar to theirs in that the categories, fine, vascular, and irregular, are parallel to the normal or cotton, vascular, and dead wood categories used in the previous study. An advantage of our MIP classification is the simplicity of classification. If some irregular vessels can be detected, the case is classified as "irregular pattern". Similarly, if some tumor vessels thicker than the surrounding fifth or sixth branch can be detected, the case is classified as "vascular pattern". Other cases were classified into "fine pattern," including cases in which the difference between the tumor vessels and surrounding hepatic parenchyma could not be detected. In addition, the "cotton pattern" terminology could be misunderstood to indicate the classical contrast-enhanced pattern of a hemangioma [17, 18]. Furthermore, the term "dead wood" is associated with large HCCs. However, to the extent possible, a system should be able to detect irregular vessels in a small size. Therefore, the current MIP patterns were chosen in this study.

There are some limitations associated with this malignant grading system. First, it is possible that the grading system contains some bias due to tumor size. In this study, only resected cases were analyzed, because a sample taken by fine-needle biopsy may not always represent the majority histological characteristics of HCC, especially in nodule-in-nodule cases. As a result, the proportion of well-differentiated HCC cases was small. To address this limitation, our grading system was validated using 49 HCCs diagnosed by fine needle biopsy and ablated by radiofrequency ablation (Fig. 3b). This validation raised the stratification of our grading system and could indicate following association, an HCC grade of 1 or 3 indicates a well- or moderately differentiated HCC, respectively. An additional limitation of this study is objectivity, which is a problem inseparable from ultrasonography. However, the subjectivity of our MIP classification is minimized by the simplicity of classification, as previously described. A possible limitation of this MIP pattern was the possibility that key images could not be detected because only the two dimensional image of MIP patterns, but not the entire tumor, was detectable. Hence, these cases might be classified into a less malignant grade. However, misclassified cases could be minimized using the CEUS method introduced in "Methods".

This study was an exploratory study. Our dataset includes both early and advanced HCC and is therefore a true representation of daily clinical practice. Since the dataset was small, the focus was on the analysis of the value of the CEUS malignant grading system. Future studies are necessary to support an association between our malignant grading system and overall survival.

Conclusions

This malignant grading system for HCC, using a combination of Kupffer imaging and MIP pattern, could evaluate not only the histological differentiation of HCC but also portal vein invasion.

Conflict of interest Shuhei Nishiguchi received financial support from Chugai Pharmaceutical, MSD, Dainippon Sumitomo Pharma, Ajinomoto Pharma, and Otsuka Pharmaceutical. Hiroko Iijima received financial support from Chugai Pharmaceutical. Hiroyasu Imanishi received financial support from Chugai Pharmaceutical. The remaining authors have no conflict of interest.

References

1. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology. 2007;132:2557–76.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005;55:74–108.
3. Tamura S, Kato T, Berho M, Misiakos EP, O'Brien C, Reddy KR, et al. Impact of histological grade of hepatocellular carcinoma on the outcome of liver transplantation. Arch Surg. 2001;136:25–30 Discussion 31.
4. Kumada T, Nakano S, Takeda I, Sugiyama K, Osada T, Kiriyama S, et al. Patterns of recurrence after initial treatment in patients with small hepatocellular carcinoma. Hepatology. 1997;25:87–92.
5. Sakurai M, Okamura J, Seki K, Kuroda C. Needle tract implantation of hepatocellular carcinoma after percutaneous liver biopsy. Am J Surg Pathol. 1983;7:191–5.
6. Smith EH. Complications of percutaneous abdominal fine-needle biopsy. Review. Radiology. 1991;178:253–8.
7. Watanabe R, Matsumura M, Chen CJ, Kaneda Y, Ishihara M, Fujimaki M. Gray-scale liver enhancement with Sonazoid (NC100100), a novel ultrasound contrast agent; detection of hepatic tumors in a rabbit model. Biol Pharm Bull. 2003;26:1272–7.
8. Hagen EK, Forsberg F, Aksnes AK, Merton DA, Liu JB, Tornes A, et al. Enhanced detection of blood flow in the normal canine prostate using an ultrasound contrast agent. Invest Radiol. 2000;35:118–24.

9. Yao J, Teupe C, Takeuchi M, Avelar E, Sheahan M, Connolly R, et al. Quantitative 3-dimensional contrast echocardiographic determination of myocardial mass at risk and residual infarct mass after reperfusion: experimental canine studies with intravenous contrast agent NC100100. J Am Soc Echocardiogr. 2000;13:570–81.
10. Korenaga K, Korenaga M, Furukawa M, Yamasaki T, Sakaida I. Usefulness of Sonazoid contrast-enhanced ultrasonography for hepatocellular carcinoma: comparison with pathological diagnosis and superparamagnetic iron oxide magnetic resonance images. J Gastroenterol. 2009;44:733–41.
11. Imai Y, Murakami T, Yoshida S, Nishikawa M, Ohsawa M, Tokunaga K, et al. Superparamagnetic iron oxide-enhanced magnetic resonance images of hepatocellular carcinoma: correlation with histological grading. Hepatology. 2000;32:205–12.
12. Inoue T, Kudo M, Watai R, Pei Z, Kawasaki T, Minami Y, et al. Differential diagnosis of nodular lesions in cirrhotic liver by post-vascular phase contrast-enhanced US with Levovist: comparison with superparamagnetic iron oxide magnetic resonance images. J Gastroenterol. 2005;40:1139–47.
13. Wilson SR, Jang HJ, Kim TK, Iijima H, Kamiyama N, Burns PN. Real-time temporal maximum-intensity-projection imaging of hepatic lesions with contrast-enhanced sonography. Am J Roentgenol. 2008;190:691–5.
14. Sugimoto K, Moriyasu F, Kamiyama N, Metoki R, Yamada M, Imai Y, et al. Analysis of morphological vascular changes of hepatocellular carcinoma by microflow imaging using contrast-enhanced sonography. Hepatol Res. 2008;38:790–9.
15. Terminology of nodular hepatocellular lesions. International Working Party. Hepatology. 1995;22:983–93.
16. Hayashi M, Matsui O, Ueda K, Kawamori Y, Kadoya M, Yoshikawa J, et al. Correlation between the blood supply and grade of malignancy of hepatocellular nodules associated with liver cirrhosis: evaluation by CT during intraarterial injection of contrast medium. Am J Roentgenol. 1999;172:969–76.
17. Johnson CM, Sheedy PF, Stanson AW, Stephens DH, Hattery RR, Adson MA. Computed tomography and angiography of cavernous hemangiomas of the liver. Radiology. 1981;138:115–21.
18. Wen YL, Kudo M, Zheng RQ, Ding H, Zhou P, Minami Y, et al. Characterization of hepatic tumors: value of contrast-enhanced coded phase-inversion harmonic angio. Am J Roentgenol. 2004;182:1019–26.

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Original Article

Hepatitis C virus relapse was suppressed by long-term self-injection of low-dose interferon in patients with chronic hepatitis C after pegylated interferon plus ribavirin treatment

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Aim: The recommended treatment for chronic hepatitis C is a combination of pegylated interferon (PEG IFN) plus ribavirin (RBV). However, the sustained virological response (SVR) rate of PEG IFN-RBV therapy was approximately 50% in patients with genotype 1b and a high viral load. Thus, we compared the efficiencies and side-effects of PEG IFN-RBV and self-injected low-dose natural (n) IFN-α in patients with hepatitis C virus (HCV).

Methods: A prospective, multicenter, open-label study was conducted in 12 Japanese institutions. A total of 129 patients with chronic hepatitis C and no detectable HCV after 24–72 weeks of PEG IFN-RBV treatment were assigned to the control ($n = 82$) or treated ($n = 47$) group. Treated patients received 3 million units of nIFN-α 2–3 times/week over 96 weeks. The groups were compared regarding treatment efficiency and side-effects.

Results: Significant treatment success regarding virus negativation rates was found, with 89% and 73% for the treated and control groups, respectively ($P = 0.039$). In contrast, there was no difference in relapse rate between the groups 24 weeks after the 96-week nIFN-α treatment ($P = 0.349$). However, when early viral responders and late viral responders (LVR) were separated, LVR patients responded significantly to the treatment with 90% sustained virological response, compared to 53% for the control group ($P = 0.044$). The side-effects of nIFN-α were less than that of PEG IFN-RBV treatment.

Conclusion: Self-injected nIFN-α has larger benefits than prolonged PEG IFN-RBV for chronic hepatitis C patients with high viral loads of genotype 1b who fail to achieve early viral response during initial combination treatment.

Key words: chronic hepatitis C, hepatitis C virus, interferon-α, pegylated interferon

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Conflict of interest: Shuhei Nishiguchi received financial support from Chugai Pharmaceutical, MSD, Dainippon Sumitomo Pharma, Ajinomoto Pharmaceuticals, and Otsuka Pharmaceutical. Hiroko Iijima and Hiroyasu Imanishi received financial support from Chugai Pharmaceutical. The remaining authors have no conflicts of interest to declare.

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INTRODUCTION

CHRONIC HEPATITIS C virus (HCV) infection currently affects nearly 200 million people worldwide, and is the leading cause of liver cirrhosis and liver cancer.[1–4] Hepatocellular carcinoma (HCC) is induced by viral proteins activating signaling pathways, and indirectly by chronic inflammation. Accordingly, standard treatments currently combine antiviral ribavirin (RBV) and a pegylated form of interferon-α (PEG IFN-α). Unfortunately, the success rate of this treatment depends upon the genotype of the virus. Clinical studies demonstrate that PEG IFN plus RBV is effective in 90% of patients with genotype 2 HCV after 24 weeks,[5,6] whereas only 50% of patients with intractable hepatitis (genotype 1 HCV and high viral load) respond even after 48 weeks of treatment.[7–11] Therefore, further research is necessary to address patients whom are refractory to standard PEG IFN plus RBV treatments.

A combination of PEG IFN plus RBV is used to treat high HCV genotype 1b viral loads, and a sustained virological response (SVR) rate of 70–80% can be achieved in patients who show a complete disappearance of the virus at week 12, considered a complete early virological response (cEVR). However, SVR rates are low in late virological response (LVR) patients who show a complete disappearance of the virus after 13 weeks.[12–17] Prolonged administration of PEG IFN plus RBV over a 72-week period increases the SVR rates[18–24] and has guaranteed treatment efficacy; but, the longer the treatment duration, the more frequently the patient must attend the hospital, which will be a burden on the patient, and the incidence rate of side-effects also increases.

Accordingly, the current guidelines for the treatment of hepatitis C state that, for the prevention of carcinogenesis, long-term administration of low-dose IFN is preferable in patients for whom RBV combination therapy is contraindicated, or in patients who are unresponsive to RBV combination therapy.[25–35] Since April 2005, self-injection of IFN-α at home has been covered by health insurance in Japan, and administration of the medication at home has been made possible. Particularly when administrated at night, which is more possible to do at one's home, IFN-α is less likely to affect the circadian rhythm of endogenous cortisol than when administrated in the morning,[36] which is promising in reducing its side-effects.

However, the treatment efficiency of self-injected natural IFN-α (nIFN-α) has not been evaluated. In addition, the therapeutic efficacy of self-injected IFN-α after combination therapy including RBV for over 48 weeks is unclear and the therapy's use could result in concerns of poor adherence due to side-effects such as anemia. Therefore, a reduction of the dose of IFN and a transition to self-injection would be preferable.

To this end, we conducted a multicenter study involving 12 medical institutions located in Kinki district, Japan. The aim was to test the efficiency of low-dose self-injected IFN-α in patients diagnosed with high viral loads of HCV genotype 1b. Patients who achieved an EVR or LVR at the end of treatment proceeded with self-injections of low-dose nIFN-α for 96 weeks and were periodically monitored for clinical symptoms and relapse of HCV infection.

METHODS

Patients

THE PROSPECTIVE MULTICENTER study was conducted between 1 April 2006 and 1 March 2012 at 12 medical institutions located in Kinki district, Japan. The patients enrolled were diagnosed with high viral loads (HCV TaqMan ≥5.0 logIU/mL) of HCV genotype 1b, had completed a 24–72-week combination therapy of PEG IFN-α plus RBV, and were aged 20 years or older on the day they provided consent.

The following subjects were excluded from the study: (i) patients with a history of hypersensitivity to IFN formulations; (ii) patients with chronic liver diseases other than hepatitis C, such as chronic hepatitis B, autoimmune hepatitis and alcoholic hepatitis; (iii) patients with severe depression or neuropsychiatric disorders; (iv) patients with severe complications (renal disorders, heart disease, hypertension, diabetes, autoimmune diseases and asthma); (c) patients with a history of interstitial pneumonia; and (vi) pregnant women, lactating women or women who were likely to be pregnant.

Study design

The main purpose of this study was to investigate the efficiency of low-dose self-injected IFN-α in patients diagnosed with high viral loads of HCV genotype 1b. We investigated several concerns, including side-effects and relapse rates, as well as prevention of disease progression, including HCC development. This study was an open-label prospective multicenter study in which a combination therapy of RBV with PEG IFN-α-2a (Pegasys; Chugai Co. Ltd., Tokyo, Japan) or -α-2b (Pegintron; MSD Co. Ltd., Tokyo, Japan) was conducted for 24–72 weeks on patients with high viral loads of HCV genotype 1b. Among the patients whose serum HCV RNA was negative at the completion of treatment,

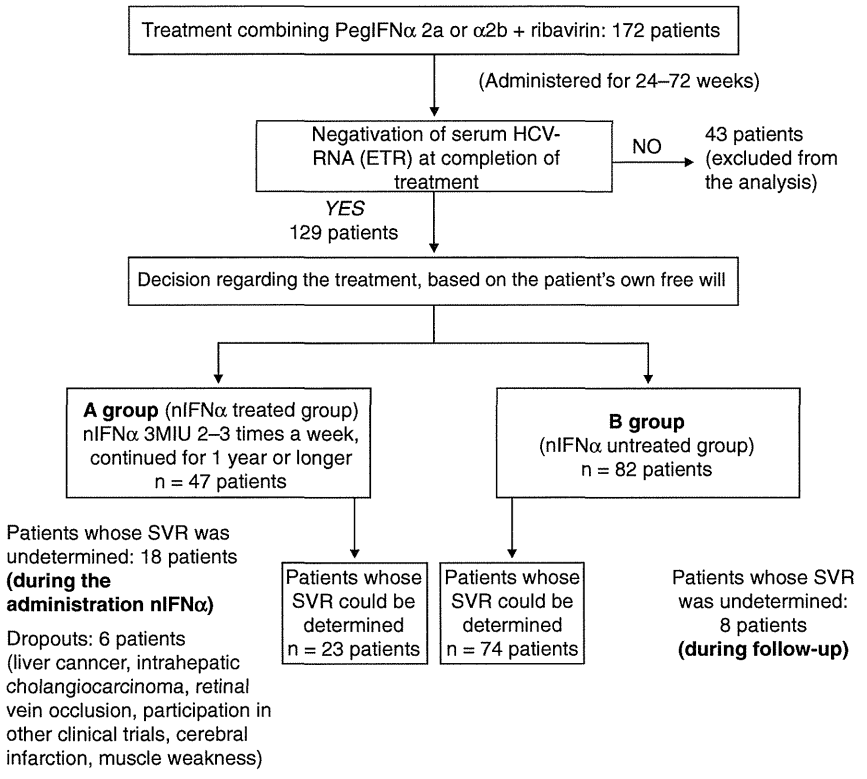


Figure 1 Algorithm used for patient selection. ETR, end of treatment response; HCV, hepatitis C virus; MU, million units; PEG IFN, pegylated interferon; SVR, sustained virological response.

47 were treated with a prolonged administration of nIFN-α (Sumiferon; Dainippon Sumitomo Co. Ltd., Osaka, Japan) by self-injection at a dose of 3 million units (MU) 2–3 times a week for year or longer (group A) and the therapeutic efficacy was prospectively compared with that of 82 non-treated patients (group B). In the present study, treatment was selected based on the patient's own free will (Fig. 1). The combination therapy of PEG IFN-α plus RBV was conducted in accordance with the recommended initial dose described in the attached document, and in consideration of each patient's bodyweight.

This study design was reviewed and approved by our university hospital's ethics committee (approval no. 352). The determination of genetic polymorphism in interleukin (IL)-28B rs8099917 was also reviewed and approved by our ethics committee on genetics (approval no. rin-hi-92). This study was conducted in compliance with the Declaration of Helsinki. Each patient signed an informed consent form for the research and for the disclosure of their genetic information.

Measurements pertaining to IL-28B were conducted using the TaqMan probe method,[37] and those pertaining to HCV amino acid 70 in the core region (core70) and the interferon sensitivity-determining region (ISDR) were conducted using a direct sequencing method.[38,39]

Questionnaire survey

For the 41 patients who performed self-injections of nIFN-α (group A) (recovery rate, 87.2%), a patient survey was conducted after the transition from PEG IFN plus RBV treatment to the nIFN-α self-injection protocol.

The contents of the questionnaire survey included technical issues pertaining to the self-injection of nIFN-α, the severity of side-effects, and changes in daily life due to the switch from PEG IFN plus RBV to nIFN-α, as well as any inconveniences encountered during the administration of each medication.

Treatment success assessment through HCV RNA levels and viral load

In group A, therapeutic efficacy was evaluated at two time points, 24 weeks after completion of PEG IFN plus RBV and 24 weeks after completion of nIFN-α. In group B, the therapeutic efficacy was evaluated 24 weeks after completion of PEG IFN plus RBV. In both groups, the evaluations were based on the amount of HCV RNA (TaqMan PCR method) present in the serum.

Table 1 Comparison of patient backgrounds (all patients)

No. of patients	Group A 47	Group B 82	*P*-value
Age (mean value ± SD)	58.8 ± 9.8	56.3 ± 10.5	0.1710
Sex (male/female)	30/17	47/35	0.4680
Median ALT (IU/L) (range)	43 (18–157)	54 (3–228)	0.1151
Median HCV RNA (log IU/mL) (range)	6.5 (5.1–7.5)	6.2 (5.1–7.4)	0.1026
PEG IFN-α-2a/PEG IFN-α-2b	5/42	12/70	0.5184
Mean duration of PEG IFN treatment (weeks)	53.4 ± 11.6	49.2 ± 13.2	0.0301
†F stage (F0/F1/F2/F3/F4)	(2/12/13/5/3)	(4/26/13/7/1)	0.4009
†A grade (A0/A1/A2/A3)	(1/24/8/2)	(1/19/27/4)	0.0449
History of liver cancer	2	1	0.2657
‡IL-28B SNP rs8099917 (TT/non-TT)	23/2	41/7	0.4168
‡Core70 (wild/mutant)	9/9	23/7	0.0577

†Only patients who signed an informed consent to undergo liver biopsy were analyzed. ‡Only patients who signed an informed consent to check each marker were analyzed.
ALT, alanine aminotransferase; HCV, hepatitis C virus; IL, interleukin; PEG IFN, pegylated interferon; SD, standard deviation; SNP, single nucleotide polymorphism.

In addition, evaluations according to the timing of the negativation of viral load were also conducted to determine whether the recurrence of HCV could be inhibited in patients whose HCV RNA was successfully maintained below detectable levels 24 weeks after completion of PEG IFN plus RBV. The rates of SVR in group A and B were evaluated and were also assessed according to the negativation of viral load.

Statistical analysis

Per protocol-based (PPB) analysis was performed and JUMP9 analysis software was used for analysis. The patients' backgrounds (normally distributed data) were analyzed using Student's *t*-test and the χ^2-test, the recurrence rate and SVR rate were analyzed using the χ^2-test and the questionnaire count (non-normally distributed data) was analyzed by the Mann–Whitney *U*-test. $P < 0.05$ was considered statistically significant.

RESULTS

Patient selection and characteristics

DURING THIS MULTICENTER prospective study, a total of 172 patients diagnosed with high viral loads of HCV genotype 1b received PEG IFN plus RBV treatment. Of these 172 patients, 129 had HCV RNA levels that fell below the level of detection after 24–72 weeks of PEG IFN plus RBV treatment (Table 1). These 129 patients elected to initiate the secondary treatment of self-injected nIFN-α. They were assigned to the control group (untreated; $n = 82$) or those receiving nIFN-α (treated; $n = 47$).

The algorithm of patient selection is shown in Figure 1. The study was multicenter, prospective and based on each patient's choice; the entries included 47 patients in group A and 82 patients in group B.

In group A, six patients discontinued treatment during administration of nIFN-α for the following reasons: development of liver cancer (one patient), intrahepatic cholangiocarcinoma (one patient), retinal vein occlusion (one patient), participation in other clinical trials (one patient), cerebral infarction (one patient), and muscle weakness and gait disturbance (one patient). The characteristics of the two groups did not differ significantly in terms of age, sex, viral load, history of liver cancer and stage of liver disease (Table 1). The two groups also received similar primary PEG IFN plus RBV treatment, except for slightly shorter durations in group B.

The study was conducted on consenting patients whose core70, ISDR mutations and IL-28B could be measured. Core70 mutants were found in 50.0% (9/18) of group A and 23.3% (7/30) of group B ($P = 0.057$). ISDR 0–1 amino acid substitutions were present in 73.3% (11/15) patients in group A and 42.8% (9/21) in group B ($P = 0.069$), with no significant difference between the two groups. However, refractory virological cases tended to be higher in group A. The non-TT type IL-28B were found in 8.0% (2/25) of patients in group A and 14.5% (7/48) in group B ($P = 0.416$); there was no

Table 2 Comparison of patient backgrounds (patients whose SVR could be determined)

No. of patients	Group A 23	Group B 74	*P*-value
Age (years) (mean value ± SD)	55.4 ± 9.30	56.9 ± 10.23	0.5332
Sex (male/female)	13/10	41/33	0.9250
Median ALT (IU/L) (range)	43 (18–157)	54 (3–228)	0.5088
Median HCV RNA (log IU/mL) (range)	6.4 (5.2–7.0)	6.2 (5.1–7.4)	0.8229
PEG IFN-α-2α/PEG IFN-α-2b	1/22	9/65	0.2817
Mean duration of PEG IFN treatment (weeks)	48.7 ± 6.0	48.7 ± 13.0	0.9897
Mean duration of nIFN-α treatment (weeks)	84.0 ± 36.7	NA	NA
†F stage (F0/F1/F2/F3/F4)	(1/6/8/2/0)	(2/26/13/7/1)	0.5805
†A grade (A0/A1/A2/A3)	(0/12/4/1)	(1/17/27/4)	0.1001
History of liver cancer	1	1	0.3770
‡IL-28B SNP rs8099917 (TT/non-TT)	17/2	30/4	0.8914
EVR	10/0	24/3	0.2715
LVR	7/2	6/1	0.6865
‡Core70 (wild/mutant)	9/7	12/6	0.5327
EVR	5/4	10/5	0.5862
LVR	4/3	2/1	0.7781

†Only patients who signed an informed consent to undergo liver biopsy were analyzed. ‡Only patients who signed an informed consent to check each marker were analyzed.
ALT, alanine aminotransferase; EVR, early virological response; HCV, hepatitis C virus; IL, interleukin; LVR, late virological response; NA, not applicable; nIFN-α, natural interferon-α; PEG IFN, pegylated interferon; SD, standard deviation; SNP, single nucleotide polymorphisms; SVR, sustained virological response.

significant difference in genetic background between the two groups in patients whose SVR could be determined (Table 2).

In group A, five patients were treated with PEG IFN-α-2a plus RBV and 42 patients were treated with PEG IFN-α-2b plus RBV, whereas in group B, 12 patients were treated with PEG IFN-α-2a plus RBV and 70 patients were treated with PEG IFN-α-2b plus RBV. The mean duration of treatment was 54.3 ± 11.6 weeks of patients in group A and 49.2 ± 13.2 weeks in group B.

The backgrounds of patients in group A (23 patients) and group B (74 patients) were examined 24 weeks after completion of nIFN-α or PEG IFN plus RBV treatment, respectively (Table 2). In group A, one patient received PEG IFN-α-2a plus RBV and 22 patients received PEG IFN-α-2b plus RBV, whereas in group B, nine patients received PEG IFN-α-2a plus RBV and 65 patients received PEG IFN-α-2b plus RBV. The mean duration of treatment was 48.7 ± 6.0 weeks for group A and 48.7 ± 13.0 weeks for group B. In addition, in group A, nIFN-α treatment was initiated after completion of PEG IFN-α-2a plus RBV or PEG IFN-α-2b plus RBV, and the mean duration of nIFN-α treatment was 84.0 ± 36.7 weeks.

Virological relapse rates in early and late viral responders

The capacity of nIFN-α self-injection to control HCV infection was tested by comparing the virus negativation rates of the two groups after 24 weeks of treatment (Fig. 2). A total of 10 patients (two in group A and eight in group B) who had not passed 24 weeks after the completion of the PEG IFN plus RBV treatment were excluded from this analysis. Therefore, Figure 2 includes the results obtained from the 45 (group A) and 74 cases (group B), respectively.

The treated group showed a significant treatment success rate compared to the control group, with virus negativation rates of 89.0% and 73.0%, respectively ($P = 0.039$). This comparison was performed under the condition that the patients in group A continue the low-dose nIFN-α treatment, and did not directly indicate the increased SVR rate. However, our result suggests that the self-injection IFN treatment can suppress HCV relapse.

Analysis of the time of negativation after completion of PEG IFN plus RBV treatment revealed that the relapse rate among EVR patients was 4.5% (1/22) in group A

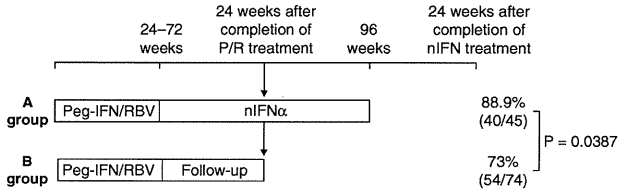


Figure 2 Virus negativation rate 6 months after completion of PEG IFN plus RBV treatment. The negativation rates of HCV RNA were compared 6 months after completion of PEG IFN plus RBV treatment. In group A, during the administration of nIFN-α, the recurrence rate was 11% and the negativation rate was 88.9%. In group B, determination of the SVR was possible, and the SVR rate was 73%. The rates were significantly higher in group A than in group B ($P = 0.0387$). nIFN-α, natural interferon; PEG IFN, pegylated interferon; RBV, ribavirin; SVR, sustained virological response.

and 20.0% (11/55) in group B, and that the relapse rate was lower in group A than in group B ($P = 0.0912$, χ^2-test). Among LVR patients, the relapse rate was 17.4% (4/23) in group A and 47.4% (9/19) in group B, with a significant difference ($P = 0.0364$, χ^2-test) between groups.

SVR rates

In group A, it was possible to follow up with 23 patients up to 24 weeks after completion of nIFN-α treatment, and SVR was found in 19 patients (82.6%). This percentage was slightly higher than the SVR rate in group B (73.0%), but no significant difference was found ($P = 0.3495$, χ^2-test) between the groups (Fig. 3).

Treatment success was further assessed by separating the early and late virological responders in each group. In EVR patients, the SVR was not affected by nIFN-α self-injection. In contrast, LVR patients responded

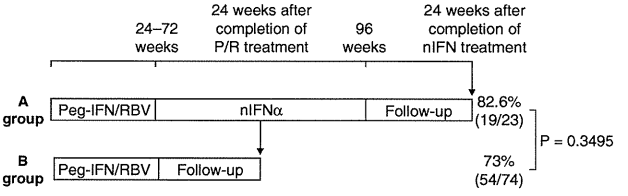


Figure 3 SVR rate after completion of IFN treatment. In group A, 23 patients were evaluated 6 months after completion of nIFN-α treatment, and 19 of them (82.6%) showed an SVR. Although the SVR rate was higher than that found in group B, there was no significant difference between the two groups. nIFN-α, natural interferon; PEG IFN, pegylated interferon; RBV, ribavirin; SVR, sustained virological response.

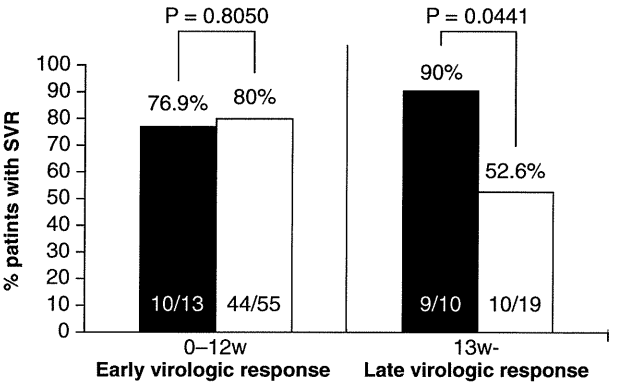


Figure 4 Timing of the HCV viral load negativation during administration of PEG IFN plus RBV treatment and the subsequent SVR rate. In EVR patients, no difference in SVR rates was found between group A and group B. In LVR patients, group A had a significantly higher SVR rate than group B. EVR, early virological response; HCV, hepatitis C virus; LVR, late viral responders; PEG IFN, pegylated interferon; RBV, ribavirin; SVR, sustained virological response. ■, group A; □, group B.

significantly to treatment, with 90% SVR, compared to 53% in the control group ($P = 0.044$) (Fig. 4). These data suggest that treatment may be particularly efficient for a subset of patients.

We also analyzed the SVR rate for each type of IL-28B, core70 and ISDR. Patients with the IL-28B major allele (type TT) had an SVR rate of 69.6% (16/23) in group A and 78.0% (32/41) in group B, and patients with the minor allele (type TG/GG) had an SVR rate of 100% (2/2) in group A and 57.1% (4/7) in group B. Patients with wild-type core70 had an SVR rate of 88.9% (8/9) in group A and 91.3% (21/23) in group B, and patients with mutant core70 had an SVR rate of 77.8% (7/9) in group A and 71.4% (5/7) in group B. Patients with two or more ISDR mutations had an SVR rate of 100% (4/4) in group A and 75.0% (9/12) in group B, and patients with a 0–1 ISDR mutation, had an SVR rate of 72.7% (8/11) in group A and 77.8% (7/9) in group B. Although a significant difference of SVR rate was found in the late responders, the frequency of IL-28B single nucleotide polymorphisms were not different between groups A and B (Table 2).

Quality of life

A questionnaire survey regarding the subjective symptoms accompanying PEG IFN plus RBV and self-injected nIFN-α, as well as the patients' impression of the treatment, was conducted on 41 patients (recovery rate, 87.2%) using by asking the following questions:

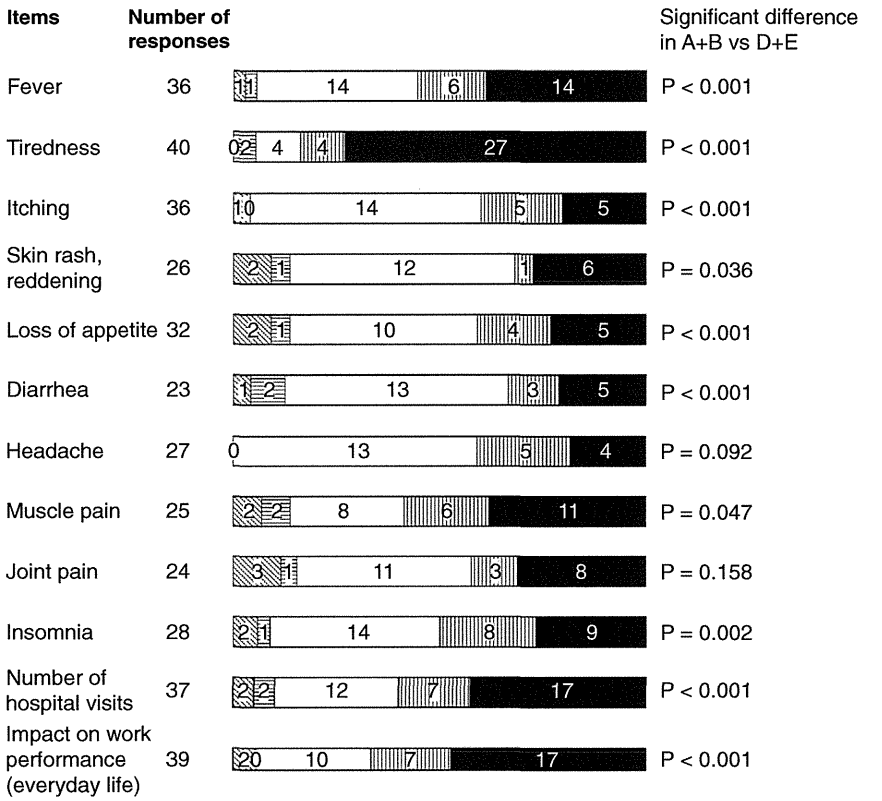
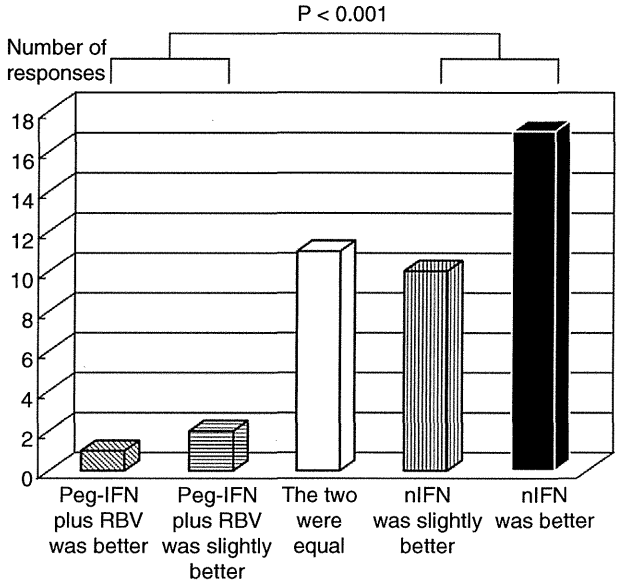


Figure 5 Comparison between PEG IFN plus RBV and self-injected nIFN-α regarding criteria for the evaluation of side-effects and treatment. The following question was asked of 41 patients in group A during administration of nIFN-α: "When the treatment was switched from PEG IFN plus RBV to nIFN-α, which treatment was better in terms of the symptoms and impact on everyday life? PEG IFN plus RBV or self-injected nIFN-α?" The results obtained from this question revealed that the self-injection of nIFN-α was better, with a significant difference ($P < 0.05$, Mann–Whitney *U*-test) in terms of fever, general fatigue, itching, skin rash or redness, loss of appetite, diarrhea, muscle pain, insomnia, number of hospital visits and impact on performance at work. In contrast, there was no difference between the two treatments with respect to headache and joint pain. nIFN-α, natural interferon; PEG IFN, pegylated interferon; RBV, ribavirin. ▨, (A) PEG IFN plus RBV was better; ▤, (B) PEG IFN plus RBV was slightly better; □, (C) the two were equal; ▥, nIFN-α was slightly better; ■, (E) nIFN-α was better. nIFN-α, natural interferon; PEG IFN, pegylated interferon; RBV, ribavirin.

1 "When the treatment was switched from PEG IFN plus RBV to nIFN-α, which treatment was better in terms of the items pertaining to symptoms and everyday life? PEG IFN plus RBV or the self-injection of nIFN-α?" The results obtained from this question showed that self-injection of nIFN-α was significantly better ($P < 0.05$, Mann–Whitney *U*-test) in terms of fever, general fatigue, itching, skin rash or redness, loss of appetite, diarrhea, muscle pain, insomnia, number of hospital visits and impact on performance at work. In contrast, there was no difference between the two treatments with respect to headache and joint pain (Fig. 5).

2 "Based on your overall impression, which treatment was better when conducted after the 1-year administration of PEG IFN plus RBV? The treatment consisting of continuing PEG IFN plus RBV or the treatment when switched to self-injection of nIFN-α?" Figure 6 shows that nearly 42% of the patients stated that nIFN-α self-injection was better than prolonging the combination treatment. When the answers quoting nIFN-α as "better" or "slightly better" were included, the results showed that the overall side-effects of self-injection of nIFN-α as well as the severity of its interference with everyday life were significantly lower than those associated with PEG IFN plus RBV



The number in parentheses are actual numbers
Total: 41 patients

%	2.4%	4.9%	26.8%	24.4%	41.5%
Patients	1	2	11	10	17

Figure 6 Overall impressions regarding the relative merits of PEG IFN plus RBV and the self-injection of nIFN-α as a treatment. The following question was asked of the 41 patients in group A: "Your treatment has been changed to a self-injection of sumiferon after the 1-year administration of PEG IFN plus RBV. Do you think that a continuation of PEG IFN plus RBV would have been better? Or, do you think that changing the treatment to a self-injection of nIFN-α was better?" Statements of "self-injection of nIFN-α was better" accounted for 65.9% of responses, and were significantly larger in number. nIFN-α, natural interferon; PEG IFN, pegylated interferon; RBV, ribavirin.

treatment ($P = 0.00000011$, Mann–Whitney *U*-test) (Fig. 6).

3 "Are there specific side-effects or problematic issues associated with the treatment during the administration of PEG IFN plus RBV or after the transition to self-injection of nIFN-α? Multiple answers are allowed." The respondents cited a total of 65 problems and side-effects associated with PEG IFN plus RBV, including seven answers mentioning "feeling sluggish", five that mentioned "attending outpatient treatment was tiresome", five mentioned a loss of appetite, seven mentioned itchy skin, two mentioned a skin rash and two mentioned fever. The respondents mentioned 42 troubles associated with the transition to self-injection of nIFN-α, including seven that were reluctant to self-inject, and two that reported the storage (management) of the medication was tiresome. Although there was dissatisfaction with the self-injection itself, the side-effects of nIFN-α were of minor severity, and therapeutic problems were fewer, compared to those found with PEG IFN plus RBV treatment.

DISCUSSION

SEVERAL PAPERS HAVE examined the utility of prolonging PEG IFN plus RBV treatment by 72 weeks in cases of slow virological response for high viral loads of HCV genotype 1b. Previous reports have stated that the SVR rate was 38–48% in Europe and the USA[18–23] and 36–60% in Japan.[24] In this study, only patients who achieved an end of treatment response were examined, but when only LVR patients were considered, the administration of nIFN-α resulted in an SVR rate of 90.0% (9/10), which indicated a favorable outcome. As nIFN-α self-injection is a properly validated drug for treatment of chronic hepatitis C, it was ethically difficult to design a completely randomized clinical trial, and the patients in this study were therefore not randomly assigned to the two groups. Furthermore, the duration of PEG IFN plus RBV treatment in the present study varied between 24 and 72 weeks, and the differing durations would influence SVR rate, as well as the prevalence of adverse events. Therefore, results obtained from this study may have some limitations. However, our results suggest that nIFN-α self-injection improves the SVR rate in patients treated with PEG IFN plus RBV, particularly in patients with LVR.

Administration of nIFN-α, even at low doses, resulted in a decreased recurrence rate in LVR patients whose viral loads had once decreased below detectable levels due to treatment with PEG IFN plus RBV. Thus, when prolonging treatment beyond 48 weeks, sufficient therapeutic efficacy with few side-effects can be achieved with self-injected nIFN-α monotherapy, even at low doses. In addition, low doses of nIFN-α monotherapy had fewer side-effects than continuation of PEG IFN plus RBV; the switch to self-injection also proved to be effective, and was better in terms of acceptability.

The results of the patient questionnaire in this study demonstrated that, taking into consideration symptoms due to IFN administration and the changes affecting everyday life, the self-injection of nIFN-α was significantly more acceptable than administration of PEG IFN plus RBV. The differences were particularly large regard-

ing items such as fever, general fatigue and the number of hospital visits; switching the treatment to self-injected nIFN-α resulted in an improvement of the quality of life. However, we did not directly compare the prolonged PEG IFN plus RBV for 24 weeks or self-injected nIFN-α for 1 year or longer; therefore we cannot conclusively state the difference in SVR rate or quality of life between the two therapies (prolonged PEG IFN plus RBV for 24 weeks and self-injected nIFN-α). Furthermore, despite the fact that severe IFN therapy-related adverse events often occur in elderly patients, we did not assure the low adverse events of the long-term nIFN-α treatment in the elderly patients because we conducted an anonymous survey. The prolonged PEG IFN plus RBV for 24 weeks may have some advantages in elderly patients because of its shorter duration of treatment when compared with self-injected nIFN-α for 1 year or longer.

Due to the small number of cases that could be analyzed, no significant difference was found; however, in refractory cases with mutant-type core70 and IL-28B minor allele, the SVR rate was found to be more elevated in group A than in group B, suggesting that continuous administration of nIFN-α may also affect the SVR in more refractory cases.

A new triple therapy (PEG IFN-RBV plus a protease inhibitor) with an increased SVR rate was recently approved for use in Japan. PEG IFN plus RBV is not a standard therapy for patients with high viral loads of genotype 1b, and our conclusion that self-injected nIFN-α has larger benefits may have less impact on the triple therapy. However, the highly efficient therapy may cause certain severe adverse events,[40] and many patients in the present study selected the self-injection therapy as the less stressful therapy (Fig. 6), suggesting that self-injection therapy may be an acceptable method for raising the SVR rate with comparatively mild side-effects.

The combination therapy with the direct anti-viral agent (DAA) allows for the shortened duration of treatment. Although we did not investigate the inhibitory effect of hepatocarcinogenesis in the observation period of this study, IFN treatment is known to suppress the development of HCC even in patients without SVR,[29–31] suggesting that IFN treatment inhibits hepatocarcinogenesis through several mechanisms, in addition to HCV eradication. Therefore, it remains unknown whether the rate of carcinogenesis in patients who achieve SVR with a shortened-period IFN treatment or IFN-free treatment with DAA is equal to that in the patients who achieve SVR with a long-term IFN treatment. Further work is needed to investigate the rate of HCC development in patients who achieved SVR by means of the shortened treatment. No significant difference was found regarding the inhibitory effect on carcinogenesis, but the administration of 3 MU of nIFN-α tended to increase the number of patients with stable alanine aminotransferase levels of less than 20 IU and α-fetoprotein levels of less than 5 ng/mL (data not shown). Therefore, non-responders for the triple therapy as well as DAA combination therapy may require low-dose IFN therapy to prevent HCC development. An inhibitory effect could be promising if the number of such cases increases and a long-term follow up is performed.

In conclusion, this study suggests that there are more benefits of low-dose nIFN-α self-injection than of prolonged PEG IFN plus RBV treatment for chronic hepatitis C patients with high viral loads of genotype 1b who fail to achieve early viral response during the initial combination treatment.

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REFERENCES

1 Poynarl T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997; **349**: 825–32.
2 Kiyosawa K, Sodeyama T, Tanaka E *et al.* Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990; **12**: 671–5.
3 Shiffman RN, Shekelle P, Overhage JM, Slutsky J, Grimshaw J, Deshpande AM. Standardized reporting of clinical practice guidelines: a proposal from the Conference on Guideline Standardization. *Ann Intern Med* 2003; **139**: 493–8.
4 Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006; **144**: 705–14.
5 Zeuzem S, Hultcrantz R, Bourliere M *et al.* Peginterferon alfa-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients infected with HCV genotypes 2 or 3. *J Hepatol* 2004; **40**: 993–9.

6 McHutchison JG, Lawitz EJ, Shiffman ML *et al.* Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. *N Engl J Med* 2009; **361:** 580–93.
7 Hayashi N, Takehara T. Antiviral therapy for chronic hepatitis C: past, present, and future. *J Gastroenterol* 2006; **41:** 17–27.
8 Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49:** 1335–74.
9 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358:** 958–65.
10 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347:** 975–82.
11 Hadziyannis SJ, Sette H Jr, Morgan TR *et al.* Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140:** 346–55.
12 Yu JW, Wang GQ, Sun LJ, Li XG, Li SC. Predictive value of rapid virological response and early virological response on sustained virological response in HCV patients treated with pegylated interferon alfa 2a and ribavirin. *J Gastroenterol Hepatol* 2007; **22:** 832–6.
13 Jensen DM, Morgan TR, Mercellin P *et al.* Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha 2a (40 kd)_ribavirin therapy. *Hepatology* 2006; **43:** 954–60.
14 Davis GL, Wong JB, McHuchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa 2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 2003; **38:** 645–52.
15 Carlsson T, Reichard O, Norkrans G *et al.* Hepatitis C virus RNA kinetics during the initial 12 weeks treatment with pegylated interferon alfa 2a and ribavirin according to virological response. *J Viral Hepat* 2005; **12:** 473–80.
16 Ferrenci P, Fried MW, Shiffman ML *et al.* Predicting sustained virological response in chronic hepatitis C patients treated with peginterferon alfa 2a (40 kd)_ribavirin. *J Hepatol* 2005; **43:** 425–33.
17 Berg T, Sarrazin C, Herrmann E, Hinrichsen H, Gerlach T, Zachoval R. Prediction of treatment outcome in patients with chronic hepatitis C: significance of baseline parameters and viral dynamics during therapy. *Hepatology* 2003; **37:** 600–9.
18 Berg T, von Wagner M, Nasser S *et al.* Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin. *Gastroenterology* 2006; **130:** 1086–97.
19 Pearlman BL, Ehleben C, Saifee S. Treatment extension to 72 weeks of peginterferon and ribavirin in hepatitis C genotype 1-infected slow responders. *Hepatology* 2007; **46:** 1688–94.
20 Mangia A, Minerva N, Bacca D *et al.* Individualized treatment duration for hepatitis C genotype 1 patients: a randomized controlled trial. *Hepatology* 2008; **47:** 43–50.
21 Ferenci P, Laferl H, Scherzer TM *et al.* Peginterferon alfa-2a/ribavirin for 48 or 72 weeks in hepatitis C genotypes 1 and 4 patients with slow virologic response. *Gastroenterology* 2010; **138:** 503–12.
22 Sanchez-Tapias JM, Diago M, Escartin P *et al.* Peginterferon-alfa2a plus ribavirin for 48 versus 72 weeks in patients with detectable hepatitis C virus RNA at week 4 of treatment. *Gastroenterology* 2006; **131:** 451–60.
23 Buti M, Lurie Y, Zakharova NG *et al.* Randomized trial of peginterferon alfa-2b and ribavirin for 48 or 72 weeks in patients with hepatitis C virus genotype 1 and slow virologic response. *Hepatology* 2010; **52:** 1201–7.
24 Oze T, Hiramatsu N, Yakushijin T *et al.* Indications and limitations for aged patients with chronic hepatitis C in pegylated interferon alfa-2b plus ribavirin combination therapy. *J Hepatol* 2011; **54:** 604–11.
25 Nishiguchi S, Kuroki T, Nakatani S *et al.* Randomized trial of effects of interferon alfa on incidence of hepatocellular carcinoma in chronic hepatitis C with cirrhosis. *Lancet* 1995; **346:** 1051–5.
26 Shiratori Y, Imazeki F, Moriyama M *et al.* Histologic improvement of fibrosis in patients with chronic hepatitis C who have sustained response to interferon therapy. *Ann Intern Med* 2000; **132:** 517–24.
27 Hung CH, Lee CM, Lu SN *et al.* Long-term effect of interferon alfa-2b plus ribavirin therapy on incidence of hepatocellular carcinoma in patients with hepatitis C-related cirrhosis. *J Viral Hepat* 2006; **13:** 409–14.
28 Hiramatsu N, Hayashi N, Kasahara A *et al.* Improvement of liver fibrosis in chronic hepatitis C patients treated with natural interferon alpha. *J Hepatol* 1995; **22:** 135–42.
29 Kasahara A, Hayashi N, Mochizuki K *et al.* Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. *Hepatology* 1998; **27:** 1394–402.
30 Yoshida H, Shiratori Y, Moriyama M *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. *Ann Intern Med* 1999; **131:** 174–81.
31 Ikeda K, Saitoh S, Arase Y *et al.* Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1, 643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* 1999; **29:** 1124–30.
32 Kurokawa M, Hiramatsu N, Oze T *et al.* Effect of interferon alpha-2b plus ribavirin therapy on incidence of hepatocellular carcinoma with chronic hepatitis. *Hepatol Res* 2009; **39:** 432–8.
33 Kasahara A, Tanaka H, Okanoue T *et al.* Interferon treatment improves survival in chronic hepatitis C patients showing biochemical as well as virological responses

by preventing liver-related death. *J Viral Hepat* 2004; **11:** 148–56.
34 Imai Y, Kasahara A, Tanaka H *et al.* Interferon therapy for aged patients with chronic hepatitis C: improved survival in patients exhibiting a biochemical response. *J Gastroenterol* 2004; **39:** 1069–77.
35 Simomura S, Nishiguchi S. Anticarcinogetic impact of infection therapy on the progression of hepatocellular carcinoma in patients with chronic viral infection. *Hepatol Res* 2012; **42:** 22–32.
36 Morgano A, Puppo F, Criscuolo D *et al.* Evening administration of alpha Interferon: relationship with the circadian rhythm of Cortisol. *Med Sci Res* 1987; **15:** 615–16.
37 Tanaka Y, Nishida N, Sugiyama M *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41:** 1105–9.
38 Enomoto N, Sakuma I, Asahina Y *et al.* Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 1996; **334:** 77–81.
39 Akuta N, Suzuki F, Arase Y *et al.* Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. *Intervirology* 2005; **48:** 372–80.
40 Kumada H, Toyota J, Okanoue T, Chayama K, Tsubouchi H, Hayashi N. Telaprevir with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan. *J Hepatol* 2012; **56:** 78–84.

Hepatology Research

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Original Article

Nationwide survey in Japan regarding splenectomy/partial splenic embolization for interferon treatment targeting hepatitis C virus-related chronic liver disease in patients with low platelet count

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Aim: In chronic liver disease associated with hepatitis C virus (HCV), a low platelet count is a major obstacle in carrying out interferon (IFN) treatment. We used a questionnaire to clarify the extent to which splenectomy/partial splenic embolization (PSE) is performed before IFN treatment, as well as the efficacy and complications thereof.

Methods: Two questionnaires were distributed to 413 medical institutes in Japan specializing in the treatment of liver diseases, and responses were obtained from 204 institutes. Furthermore, a more detailed questionnaire was completed by 10 institutes that experienced cases of death.

Results: In patients with HCV genotype 1b and a high viral load (HCV1b/High), the sustained viral response (SVR) rate was 28% for the splenectomy group and 22% for the PSE group, with no significant difference between these groups. In patients that were not HCV1b/High, the SVR rate was higher in those that underwent splenectomy (71%) compared to the PSE group (56%; $P = 0.025$). There were cases of death in seven of 799 splenectomy cases (0.89%) and four of 474 PSE cases (0.84%). Infectious diseases were involved in nine of 11 cases of death, with a peculiar patient background of Child–Pugh B (6/10) and an age of 60 years or greater (7/11).

Conclusion: The application of splenectomy/PSE before IFN treatment should be avoided in patients with poor residual hepatic function and/or elderly patients. In HCV1b/High patients, splenectomy/PSE should be performed only after selecting those in which IFN treatment should be highly effective.

Key words: chronic hepatitis C, interferon, low platelet count, partial splenic embolization, questionnaires, splenectomy

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INTRODUCTION

IN JAPAN, MANY patients with hepatitis C virus (HCV)-related chronic liver disease (CLD) exhibit advanced aging and a long disease duration. These patients often have pancytopenia due to hypersplenism caused by advanced liver fibrosis, thus leading to difficulty in receiving a sufficient dose of interferon (IFN). Because low adherence to IFN often results in treatment failure, pancytopenia, including low platelet count, is a major challenge for IFN therapy in Japanese HCV-infected patients.

The rate of hepatocellular carcinoma (HCC) development increases with the progression of liver fibrosis, and the annual occurrence rate in HCV positive Japanese cirrhotic patients is high (~7–8%).[1] The incidence of HCC significantly decreases after viral eradication by IFN treatment (sustained viral response [SVR]) in patients with HCV-related CLD, even in patients with liver cirrhosis.[2–5] However, the SVR rate in cirrhotic patients with HCV genotype 1b is approximately 25%, that is significantly lower than that of chronic hepatitis patients (~50%). Hypersplenism due to portal hypertension is believed to be one of the causes of the low SVR rate of IFN treatment observed in cirrhotic patients.[6–9] In patients with hypersplenism, reducing the dose or discontinuing IFN is often required because of their thrombocytopenia and/or granulocytopenia. Furthermore, with the combined use of ribavirin (RBV), the adherence to treatment declines in association with the degree of anemia. Discontinuing or reducing the dose of antiviral agents (IFN and/or RBV) decreases the SVR rate,[10,11] and the presence of hypersplenism-related pancytopenia can be a major cause of this decrease. In particular, a low platelet count is the main factor that is linked to the discontinuation of IFN treatment. Therefore, in order to increase the platelet count and improve adherence to antiviral agents, splenectomy/partial splenic embolization (PSE) before the initiation of IFN therapy is considered a useful option for patients with hypersplenism-linked pancytopenia.[12–23]

In Japan, it is recommended that splenectomy/PSE be performed on patients with a low platelet count before IFN treatment, as specified in the Guidelines for Chronic Hepatitis C of 2008 and onwards.[24] However, neither splenectomy nor PSE is recommended in guidelines outside of Japan. Therefore, it is important and necessary to investigate whether these surgical or interventional treatments for anti-hypersplenism should be a standard precursor to IFN treatment for patients with a low platelet count. In addition, if splenectomy/PSE is indeed a valid therapeutic option, the patients that would most benefit from these treatments should be identified.[9,10,25–27]

In the present study, we investigated the current state of the treatment of splenectomy/PSE in HCV positive patients with low platelet count. We conducted a survey in the form of a questionnaire that probed the following topics with regard to splenectomy/PSE: the current status of implementation, associated complications, degree of increased platelets and its effect on IFN treatment.[15]

METHODS

Subjects

A SURVEY WAS conducted as a part of research by the "Standards and Clinical Research Aimed at Establishment of IFN Treatment Towards Cases with Low Platelet Counts" group from the scientific research grant from the Ministry of Health, Labor and Welfare in Japan. The sample included the 413 medical institutes to which the liver disease specialists (Japan Society of Hepatology, Board of Councilors of the Western and Eastern Association, Director of the Liver Cancer Study Group of Japan, and Councilor of The Japanese Society of Interventional Radiology) belong. Approval was obtained from the three associations (the Japan Society of Hepatology, the Liver Cancer Study Group of Japan and the Japanese Society of Interventional Radiology) prior to distributing this questionnaire. This study is a summary of the questionnaire responses, and ethical considerations toward patients were ensured by anonymizing personal information.

Questionnaires

Three types of questionnaires were prepared with internists, surgeons and radiologists as the subjects; each type of questionnaire was sent to 336, 46 and 31 institutes, respectively. As a general rule, one questionnaire was sent to one medical institute.

In this study, thrombocytopenia was defined as a platelet count of less than 100×10^9 platelets/L. A survey was conducted to determine whether splenectomy or PSE was performed to improve adherence to IFN treatment in patients with thrombocytopenia, and the selection criteria for splenectomy/PSE (including a platelet count and the liver function tests) were also queried in the first questionnaires (sent in September 2009 and collected on 22 December 2009). The state of the implementation of splenectomy/PSE was questioned again in the second questionnaire. The second questionnaire also focused on the appropriateness of performing splenectomy or PSE for IFN treatment in the patients, and investigated the aforementioned topics, including the efficacy of splenectomy/PSE, complications, and the prevalence of prophylactic administration of pneumococcal vaccine (sent in September 2010 and collected on 14 January 2011).[28] The third questionnaire (sent in November 2011 and collected on 6 December 2011) was performed as a detailed investigation of the 11 cases in which death was reported in the second questionnaire.

Statistical analysis

Data were expressed as mean ± standard deviation. A χ^2-test was used to compare splenectomy/PSE implementation cases, and Student's *t*-test and Mann–Whitney *U*-test were used for other comparisons. $P < 0.05$ indicated statistically significant difference.

RESULTS

FOR THE FIRST and second questionnaire, responses were obtained from internists, surgeons and radiologists (Table 1). For the third questionnaire, responses were obtained from all 10 institutes (11 patients died in 10 institutes: 100% recovery of the questionnaire sheets).

Standard platelet count required to initiate IFN treatment and the initial dose of IFN for patients with low platelet count

Eighty-nine percent (95/107) of institutes began IFN treatment even when the platelet count was less than 100×10^9 platelets/L. The adherence to IFN treatment of the patients was also answered. In the patients with a platelet count of 80×10^9 platelets/L or more before IFN treatment, 90% (72/80) of the institutions initiated therapy with a sufficient (≥80% of the normal dose) initial dose of IFN. However, among patients with a platelet count of less than 80×10^9 platelets/L, only 27% (25/93) started treatment with an insufficient (<80% of the normal dose) initial dose of IFN. Thus, many patients with a platelet count of less than 80×10^9 platelets/L prior to IFN introduction received a dose of IFN that was reduced to a level at which the IFN SVR rate was predicted to be low.

Implementation status of splenectomy/PSE prior to IFN treatment in patients with low platelet counts

The questionnaire results clarified that splenectomy and/or PSE were performed in 61% of the specialized institutes providing IFN therapy.

The platelet count that each institute considered when performing splenectomy/PSE before IFN treatment was $64 \times 10^9 \pm 18 \times 10^9$ platelets/L ($n = 25$) for splenectomy and $79 \times 10^9 \pm 14 \times 10^9$ platelets/L ($n = 24$) for PSE, with splenectomy having a significantly low value compared to PSE ($P = 0.002$).

Reasons for not performing splenectomy

In the questionnaire given to the internists (114 institutes), 60 institutes responded "splenectomy is not performed for IFN treatment".

In these 60 institutes, 28 described the possible severe complications as the reason for not carrying out splenectomy. Of these 28 institutes, four institutes that performed splenectomy for IFN treatment in the past experienced cases of portal thrombosis.

In the 26 institutes in which the surgeon-specific questionnaire was completed, 18 (69%) were performing splenectomy before IFN treatment; of these institutes, 59% experienced cases of portal thrombosis. In the questionnaire with internists and surgeons as subjects, the respondents strongly indicated complications as a reason for not performing splenectomy.

Reasons for not performing PSE

From the questionnaire that targeted internists (114 institutes), 70 institutes responded "PSE is not performed for IFN treatment".

In these 70 institutes, 36 described the possible severe complications as the reason for not performing splenectomy.

Of the 10 institutes in which questionnaires were completed by radiologists, one institute responded that PSE should not be performed for IFN treatment because of complication issues.

Platelet count transition and period before IFN treatment initiation following splenectomy/PSE

The changes in platelet count following splenectomy/PSE were investigated at each institute (Fig. 1), and a significantly increased platelet count was observed after carrying out splenectomy or PSE. However, this platelet count increase appeared to be higher and more sustained in patients who underwent splenectomy relative to those that underwent PSE. IFN administration was initiated within 1 – 3 months following PSE and within

Table 1 Questionnaire collection rates

	Internists	Surgeons	Radiologists
1st response	32% (107/336)	52% (24/46)	23% (7/31)
2nd response	34% (114/336)	57% (26/46)	32% (10/31)

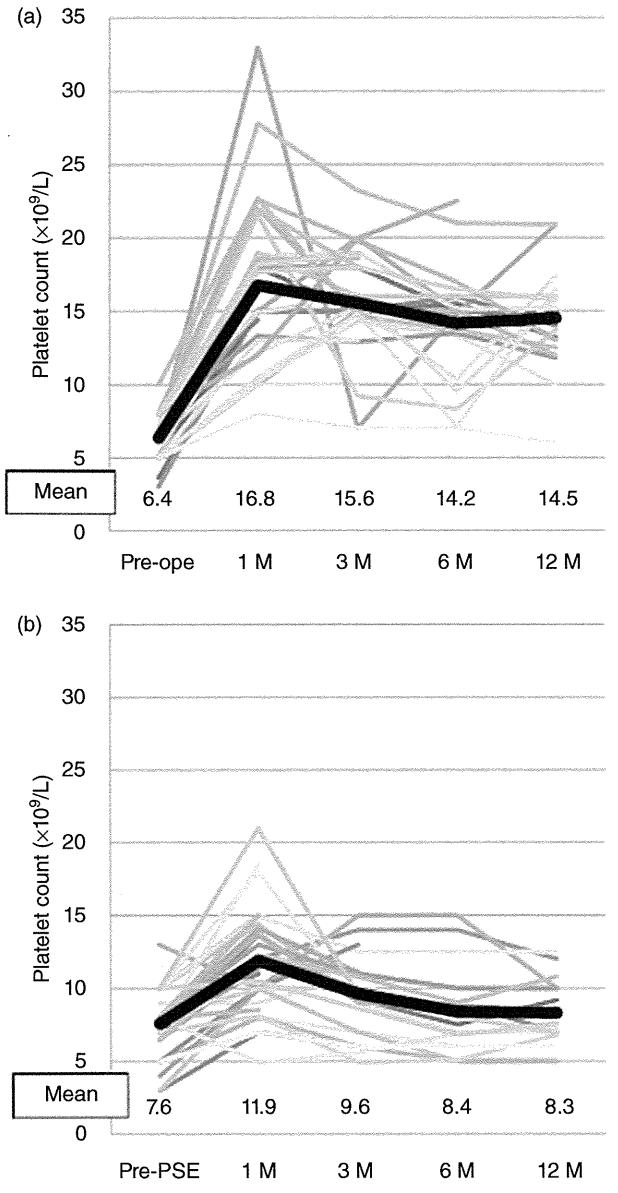

Figure 1 Platelet count transition of each institute following splenectomy/partial splenic embolization (PSE); average platelet count number of institutes. The bold line represents the transition of the mean platelet counts of each institute. (a) Splenectomy cases. (b) PSE cases.

3 – 6 months following splenectomy in the majority of cases (Table 2).

Complications following splenectomy/PSE

The splenectomy-associated complications experienced in each institute included portal thrombosis (28 of 63 institutes), postoperative infectious diseases (11 of 63 institutes) and ascites (12 of 62 institutes). However, the incidence of these complications varied depending on the institute.

In 64 institutes, fever ($n = 28$), thrombosis ($n = 22$), abscess ($n = 4$) and ascites ($n = 12$) were reported as frequent complications after PSE. However, the incidence of these complications also varied depending on the institute.

From 2005 to 2010, patient deaths were observed in seven of 788 (0.89%) cases of splenectomy, and in four of 474 (0.84%) cases of PSE. In nine of the 11 death cases, there appeared to be causal relationship between death and splenectomy as well as PSE (a causal relationship was indicated to exist by five of seven institutes for splenectomy and four of four institutes for PSE).

The age at the time of death ranged 46–70 years, with many patients older than 60 years. The sex included six male cases and five female cases, and there were two cases of chronic hepatitis and nine cases of liver cirrhosis (Child–Pugh classification grade A, two cases; B, six cases; and unknown, one case). The cirrhotic patients that died tended to have higher Child–Pugh scores and poor residual hepatic function. Pneumococcal vaccine inoculation was only performed in one splenectomy patient, and the other 10 patients were not inoculated. The cause of death was related to infectious diseases in nine cases (there was one patient with an apparent pneumococcal infection who was not inoculated with a pneumococcal vaccine). In most cases, death occurred within 3 months after treatment (splenectomy or PSE), although it also occurred over 3 months after treatment. Three patients died during IFN treatment (two cases after splenectomy, one case after PSE). Two patients died within 3 months after IFN treatment (two cases after splenectomy) (Table 3).

SVR rate of cases in which IFN treatment was performed following splenectomy or PSE

Among patients with low platelet count, IFN treatment was introduced in 92% (236/257) of the cases in which

Table 2 Period from splenectomy/PSE to initiation of IFN treatment

	Splenectomy ($n = 64$)	PSE ($n = 56$)
Within 1 month	6 (9%)	23 (42%)
>1 to 3 months	26 (41%)	23 (42%)
>3 to 6 months	17 (27%)	6 (10%)
>6 to 12 months	8 (12%)	3 (5%)
>12 months	7 (11%)	1 (1%)

IFN, interferon; PSE, partial splenic embolization.

Table 3 Period from splenectomy/PSE to death

	Splenectomy (7 cases of death)	PSE (4 cases of death)
Within 3 months	3	3
	§Postoperative bleeding (hemophilia)	§Thrombocytopenia, cerebral hemorrhage
	§Pancreatic fistula, local infection	§Pneumonia, ARDS, sepsis (MRSA)
	§Intra-abdominal abscess (MRSA)	§Peritonitis
Within 6 months	0	1
		†Spondylodiscitis, sepsis
Within 1 year	2	0
	†SAH, bacteremia (MRSA)	
	‡Sepsis	
Within 2 years	1	0
	‡Liver failure, suspect of SBP	
Over 2 years	1	0
	†Pneumococcal infection	

†Death occurred during IFN treatment.
‡Death occurred within 3 months after IFN treatment.
§Death except † and ‡.
ARDS, acute respiratory distress syndrome; MRSA, methicillin-resistant *Staphylococcus aureus*; PSE, partial splenic embolization; SAH, subarachnoid hemorrhage; SBP, spontaneous bacterial peritonitis.

splenectomy was performed for IFN, 94% (295/314) of the cases in which PSE was performed for IFN, and 84% (241/285) of cases in which such pretreatment (splenectomy or PSE) was not performed before the introduction of IFN. Discontinuation of IFN occurred in 22% of cases of splenectomy, 28% of cases of PSE and 33% of those without pretreatment. Due to the pretreatment, the IFN introduction rates were increased ($P < 0.001$) and discontinuation rate declined ($P = 0.02$).

The pretreatment platelet count was $64 \times 10^9 \pm 17 \times 10^9$ platelets/L in splenectomy cases and $76 \times 10^9 \pm 21 \times 10^9$ platelets/L in PSE cases, while that of cases without pretreatment was $85 \times 10^9 \pm 16 \times 10^9$ platelets/L. In patients with a platelet count of 80×10^9 platelets/L or more, the majority of IFN treatments were without pretreatment to increase the platelet count.

The tabulation of the IFN treatment effects of cases in each institute is shown in Table 4. The SVR rate of cases of HCV genotype 1b and high viral load was 42 of 228 (22%) for the PSE group and 63 of 228 (28%) for the splenectomy group, with an odds ratio of 0.78 ($P = 0.19$). The SVR rate of so-called "others" (patients other than those with genotype 1b and high viral load) was 62 of 110 (56%) for the PSE group and 84 of 119 (71%) for the splenectomy group, with an odds ratio of 0.54 ($P = 0.025$). Additionally, in the "others" group, the SVR rate following IFN treatment was higher in patients who underwent splenectomy compared to that of patients who underwent PSE.

DISCUSSION

THE PRESENT STUDY was conducted to clarify the current conditions of splenectomy/PSE performed for the purpose of IFN treatment. This was the first national questionnaire conducted in Japan, and no similar studies have been reported previously. The results of these questionnaires revealed that the lower limit of the platelet count achieved prior to IFN administration varied widely depending on the institute in

Table 4 SVR rate of IFN treatment following splenectomy/PSE

	Splenectomy	PSE	*P* (odds ratio)
1b-high	28% (63/228)	22% (42/190)	0.19 (0.74)
Others	71% (84/119)	56% (62/110)	0.025 (0.54)

A difference in SVR rate was observed between splenectomy and PSE groups. In patients with hepatitis C virus genotype 1b and a high viral load, there was no significant difference in the low SVR rate. The SVR rate was high in cases other than those of a 1b genotype/high viral load, with splenectomy having a significantly higher SVR rate compared to PSE.
IFN, interferon; PSE, partial splenic embolization; SVR, sustained virological response.