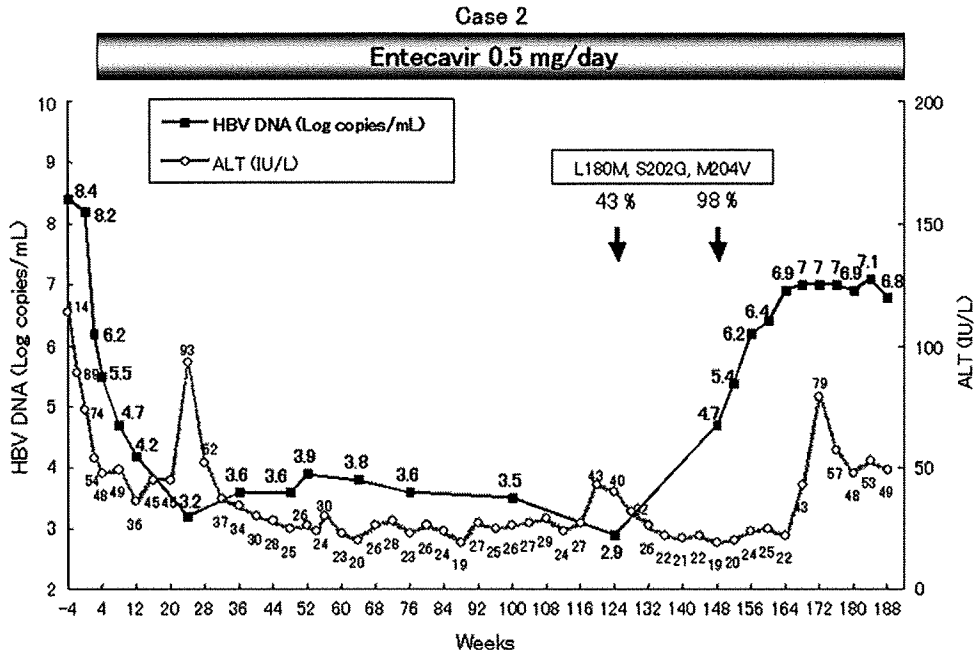

Fig. 2 Clinical course of case 2, a 47-year-old man with nucleoside-naive CHB. ETV treatment reduced ALT level to below the upper normal limit at week 30 and reduced serum HBV DNA level to a nadir of 2.9 $\log_{10}$ copies/ml at week 124. However, HBV DNA level re-elevated to 4.7 $\log_{10}$ copies/ml (virologic breakthrough) at week 148 and 7.0 $\log_{10}$ copies/ml at week 168, as well as ALT level re-elevated to 79 IU/l at week 172. Sequence analysis of the HBV DNA polymerase gene using serum sample obtained at weeks 124 and 148 revealed the emergence of L180M, M204V (related to LVD resistance), and S202G (related to ETVr) substitutions. SNP-PCR assay revealed that the resistant isolate was enriched to 43 (M204V) and 98% (M204V), respectively

Table 4 Population sequence analysis of isolates from case 2 on ETV therapy

Week	RT position								
	55	76	180	191	195	202	204	221	269
0	H/R	S	L	V	F	S	M	Y/F	I/L
24	H/R	S	L	V	F	S	M	Y/F	I/L
52	H/R	S	L	V	F	S	M	Y/F	I/L
100	H	S	L	V	F	S	M	Y/F	I/L
124	H	S/T	L/M	V/I	F/S	S/G	M/V	Y	I/L
148	H	S	M	V	F	G	V	Y	I

Table 5 SNP-PCR analysis of case 2 isolates

Week	S202G[a]	M204V (GTG, %)	M204I (ATA, %)	M204I (ATT, %)
0	Negative	0.016	0.020	0.0065
24	Positive	0.65	0.029	0.018
52	Negative	0.021	0.020	0.018
100	Negative	0.020	0.021	0.010
124	Positive	43	0.33	0.010
148	Positive	98	2.9	0.016

[a] S202G PCR was non-quantitative. A positive indicates 4-fold, 5085-fold, and 10475-fold the wild-type background for weeks 24, 124, and 148, respectively. The baseline isolate gave 1.1-fold the wild-type background

Discussion

The most important limitation of long-term nucleoside analogue treatment for CHB is the emergence of drug-resistant mutant HBV followed by viral breakthrough and hepatitis flare [12]. The most common mutation associated with LVDr involves substitution of methionine in the tyrosine-methionine-aspartate-aspartate (YMDD) motif of the HBV DNA polymerase gene RT domain with valine or isoleucine (M204V/I), with or without a leucine-to-methionine substitution in an upstream region (rtL180M) [20]. It was reported that LVDr was detected at a rate of 14 to 32% after 1 year and 60 to 70% after 5 years of LVD treatment [12]. The substitutions conferring resistance to ADV are asparagine to threonine (N236T) and alanine to valine or threonine (A181V/T) [21], and the cumulative probability of ADV resistance with elevation of HBV DNA level has been reported to be 20% at 5 years in HBeAg-negative patients [22] and as high as 42% in HBeAg-positive patients [23].

In the case of ETV, it has been reported that resistance to the drug requires at least one of three substitutions in HBV RT, that is, rtT184, rtS202, and rtM250, as well as LVDr-related substitutions rtL180M and M204V [24]. Phenotypic analyses of samples associated with virologic breakthrough confirmed that ETV susceptibility correlates

with the spectrum of these additional substitutions conferring genotypic resistance and the increased level of circulating HBV DNA [25].

There is a high genetic barrier to resistance to ETV in nucleoside-naive patients and <1% experience virologic breakthrough with ETVr through 4 years of therapy [15]. However, in LVD-refractory patients, the barrier to resistance is lower because the suppression of HBV replication is not as great and these patients mostly harbor virus with two of the three substitutions required for high-level ETVr [26]. This results in virologic breakthrough with ETVr in LVD-refractory patients at 1% in the first year but increasing to 39.5% after 4 years of therapy [15].

In this article, we report two cases with confirmed genotypic resistance to ETV, virologic rebound, and biochemical breakthrough during long-term ETV treatment for nucleoside-naive CHB patients. In the first case, the patient received a lower dose of ETV (0.1 mg daily for 52 weeks) than is currently recommended in product labeling. It was shown that LVD–ADV combination therapy was apparently effective for the ETV-resistant strain, presumably because there is no cross-resistance between ETV and ADV [26, 27].

SNP-PCR analysis for resistance substitutions revealed that the LVDr M204V(GTG) and the ETVr S202G(GGT) substitutions were negative at baseline and emerged simultaneously at week 124 in both patients. The three resistance substitutions L180M, M204V, and S202G appeared to be genetically linked and did not arise in a stepwise manner in nucleoside-naive patients, as has been described previously.

ETV displays several properties for consideration as the first-line nucleoside analogue because of its potent antiviral activity and a lower frequency of drug resistance than LVD, ADV, or telbivudine [13]. Although ETV is effective in LVD-refractory patients, the potency is reduced somewhat and the barrier to resistance is diminished by the presence of rtM204I/V and rtL180M substitutions. The fact that ETVr may develop in nucleoside-naive patients, even if the chance is small, is noteworthy. In case 1, the patient received a lower dose of ETV (0.1 mg daily), which may be a possible contributing factor to resistance. The common features of our two cases were: HBeAg-positivity, male, high viral load, slow decrease of HBV DNA, and persistently detectable HBV DNA by PCR (>2.6 $\log_{10}$ copies/ml) during the treatment course; however, these characteristics were also present in some other patients who did not develop ETVr. Patient compliance with prescribed therapy also should be assessed in such situations. It is believed that some subpopulations of HBV that proliferate very actively and are not completely suppressed by ETV may have a chance of being selected for the resistance substitutions required for ETV virologic failure. Accordingly, such cases with persistent HBV DNA after extended ETV treatment should be evaluated for emergence of drug-resistance substitutions with close monitoring of HBV DNA level, even in nucleoside-naive patients.

The rate at which resistant mutants are selected is related to pretreatment serum HBV DNA level, rapidity of viral suppression, duration of treatment, and prior exposure to nucleoside analogue therapies [12]. For the management of the emergence of drug resistance in nucleoside analogue treatment of CHB and cirrhosis, prediction and early detection of drug-resistant HBV by close monitoring of serum viral load and genotypic resistance are necessary. Keeffe et al. [28] reported the "road-map concept," that is, on-treatment monitoring strategy, for selection of nucleoside analogues by early prediction of efficacy and resistance using assessment of viral responses at weeks 12 and 24. Although this "concept" seems imperfect because the probability of emergence of resistance to particular nucleoside analogue is not taken into account, a similar strategy for management of drug resistance by close monitoring of viral load and confirming genotypic resistance with consideration of the property of each nucleoside analogue should be established for antiviral treatment of CHB using nucleoside analogues.

Conclusions

We reported two cases of emergence of genotypic resistance to ETV accompanied by virologic breakthrough in nucleoside-naive CHB patients. One patient was treated with a lower than recommended dose of ETV. Although development of ETVr-related gene mutations is rare in nucleoside-naive patients, the patients with a slow decline of HBV DNA or persistent HBV DNA (>2.6 $\log_{10}$ copies/ml) after ETV administration should be evaluated carefully for the potential emergence of ETVr.

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ORIGINAL ARTICLE

A randomized trial of 24 versus 48 weeks of peginterferon α-2a in patients infected with chronic hepatitis C virus genotype 2 or low viral load genotype 1: a multicenter national study in Japan

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Abstract In a country such as Japan with the average age of patients with chronic hepatitis C treated with antivirals sometimes well above 60 years, the standard combination therapy is not well tolerated. In this randomized, prospective, controlled trial, we investigated the efficacy of 24-week peginterferon α monotherapy for easy-to-treat patients. A total of 132 patients chronically infected with hepatitis C virus (HCV) genotype 2 ($n = 115$) or low viral load HCV genotype 1 (<100 kIU/ml, $n = 17$) were treated with peginterferon α-2a (180 μg/week). Patients with a rapid virological response (RVR, HCV RNA negative or <500 IU/ml at week 4) were randomized for a total treatment duration of 24 (group A) or 48 (group B) weeks. Patients who did not show RVR (group C) were treated for 48 weeks. Sustained virological response (SVR) was assessed by qualitative reverse-transcription polymerase chain reaction. One hundred eight of 132 (82%) patients with RVR were randomized. SVR rates were 60% (group A), 79% (group B), and 27% (group C), respectively. Similar SVR rates were achieved in patients infected with HCV genotype 2 with low pretreatment viral load (<1000 kIU/ml) in group A (81%) and group B (79%) ($P = 0.801$), whereas in those with higher viral load (≥1000 kIU/ml), a lower SVR rate was identified in group A (26%) than in group B (67%) ($P = 0.041$). In

This study is conducted on behalf of the Japanese Consortium for the Study of Liver Diseases.

Clinical Trial Registry: www.umin.ac.jp/ctr/index.htm; identifier: UMIN000001067.

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conclusion, in patients infected with HCV genotype 2 and pretreatment viral load below 1000 kIU/ml who achieve RVR, 24-week treatment with peginterferon α-2a alone is clinically sufficient. Those who show no RVR or have higher baseline viral load, require alternative therapies.

Keywords Randomized trial · Chronic hepatitis C · Peginterferon-α monotherapy · Rapid virological response · Genotype 2 · Pretreatment viral load

Introduction

Hepatitis C virus (HCV) infection may progress to chronic hepatitis, cirrhosis, and hepatocellular carcinoma [1–3]. Interferon (IFN)-based treatment of HCV-infected patients can achieve viral clearance and thereby improve histology and prognosis [4, 5]. Thus, the primary aim of antiviral therapy in patients with chronic hepatitis C is a sustained virological response (SVR), defined as undetectable serum HCV RNA by a sensitive molecular assay 24 weeks after the end of treatment.

A combination therapy of peginterferon and ribavirin is currently recognized as the standard treatment of chronic hepatitis C, resulting in 40–50% of SVR rate in patients infected with HCV genotype 1 and around 80% in those infected with HCV genotype 2 or 3 [6–8]. The combination therapy, however, tends to be associated with adverse events more frequently than those that occur with IFN monotherapy [9–14], resulting in dose reduction or discontinuation of therapy and thus impaired response rate particularly in elderly patients [15]. Furthermore, patients with renal failure, ischemic vascular disease, and congenital hemoglobin abnormalities never tolerate ribavirin treatment of their chronic hepatitis C.

In Japan, the Bureau of National Health Insurance provides reimbursement for 24-week interferon α-2b plus ribavirin combination therapy for patients with chronic hepatitis C with high viral load or re-treatment, irrespective of viral load, since December 2001 and for 48 weeks of peginterferon α-2a monotherapy for all patients with chronic hepatitis C since December 2003. The bureau started to provide reimbursement for 24-week peginterferon α-2b and ribavirin therapy for those infected with HCV genotype 2 and high viral load or re-treatment, irrespective of viral load, since December 2005. Thus, Japanese patients infected with HCV genotype 2 and high viral load or re-treatment irrespective of viral load have been able to receive either peginterferon α monotherapy or combination therapy with ribavirin since December 2003.

There are three major phase II/III or phase III clinical trials of peginterferon α monotherapy in patients with chronic hepatitis C [16–18]. All three studies indicate that the long-acting pegylated forms of IFN-α are more potent than standard IFN-α monotherapies. Factors independently associated with SVR to peginterferon α include viral genotype, low pretreatment viral load, age, no cirrhosis, and body surface area [18]. The reported SVR rate in patients with HCV genotype 2 infection and a baseline viral load of less than 2 million copies/ml is around 60% or more [16, 17]. A phase II study of 48-week peginterferon α-2a therapy conducted in Japan demonstrated an SVR rate as high as 71% in patients with HCV genotype 2 infection [19]. Furthermore, 85% of the patients, who had undetectable levels of HCV RNA after 4 weeks of therapy, had an SVR [19]. Thus, data on viral kinetics have led to the hypothesis that in these patients, 24 weeks of treatment may be as effective as the recommended course of 48 weeks. Therefore, 48-week therapy may lead to overtreatment in some patients who have a rapid virological response (RVR). Shorter treatment duration should also be associated with better tolerability and lower rate of premature discontinuation of therapy. This is particularly relevant to elderly patients with HCV genotype 2 infection who can less tolerate the combination therapy with ribavirin and/or a longer treatment period. However, whether the duration of treatment with peginterferon α alone can be reduced from 48 to 24 weeks in patients chronically infected with HCV genotype 2 or low viral load HCV genotype 1 without compromising antiviral efficacy is not clear at present.

Therefore, the aim of this study was to compare the efficacy and safety of peginterferon α-2a administered alone for 24 or 48 weeks in patients with chronic HCV genotype 2 infection or low viral load HCV genotype 1 and had a virological response at week 4.

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Materials and methods

Patients

Adult patients with chronic HCV infection who had the following characteristics were eligible for the study: (1) a positive test for anti-HCV antibody, (2) HCV genotype 1 and an HCV RNA level less than 100,000 IU/ml or HCV genotype 2 irrespective of viral load, (3) entry neutrophil and platelet counts and hemoglobin level of at least 1500/μl, 90,000/μl, and 10 g/dL, respectively. Patients with the following criteria were excluded: other viral infections such as infection with hepatitis B virus or human immunodeficiency virus; any other cause of liver disease such as autoimmune hepatitis, primary biliary cirrhosis, drug-induced liver disease, and excessive daily intake of alcohol; relevant disorders including decompensated liver disease, hepatocellular carcinoma, and other malignant neoplastic disease; concomitant use of immunosuppressive or herbal medications such as Sho-saiko-to; current illicit drug use; neurological or psychiatric diseases; and allergic to peginterferon α-2a or other interferons and biological preparations including vaccines.

Study design

The current study was an investigator-initiated study. This multicenter, open-label, randomized, controlled trial was originally discussed and designed on 12 July 2003, by a committee composed of 36 staff members from 33 participating hospitals and universities (the Japanese Consortium for the Study of Liver Diseases). The diagnostic criteria for chronic hepatitis C, treatment regimens, and follow-up protocols were finalized by the committee on 9 November 2003. This study compared the efficacy and safety of 24 vs. 48 weeks of treatment with peginterferon α-2a in patients with chronic HCV genotype 2 infection or low viral load HCV genotype 1 and showed an RVR (serum HCV RNA negative [<50 IU/ml] or <500 IU/ml by HCV RNA test at week 4 of therapy).

Eligible patients were treated with peginterferon α-2a (PEGASYS; Chugai Pharmaceuticals Inc., Tokyo, Japan) at a dose of 180 μg once per week subcutaneously. Patients who showed RVR at 4 weeks of treatment were randomized into either total treatment duration of 24 (group A) or 48 (group B) weeks. Randomization was performed at Okayama University Graduate School, centrally accessed through fax. Patients were assigned upon a report of RVR to group A or B with a computer-based random allocation system by a researcher who was independent of the study, and the allocation system was not accessible by any of the investigators who enrolled patients for the study. Randomization was stratified according to genotype (genotype 1 or 2) and previous IFN treatment (naive or re-treated) and was not blocked. Patients who were still positive for HCV RNA (by qualitative or quantitative HCV RNA tests) at week 4 were treated for 48 weeks (group C, Fig. 1). After the end of treatment, all patients were followed for an additional 24-week period.

The study was approved by ethics committee of each center and carried out according to the Declaration of Helsinki and the guidelines of the International Conference

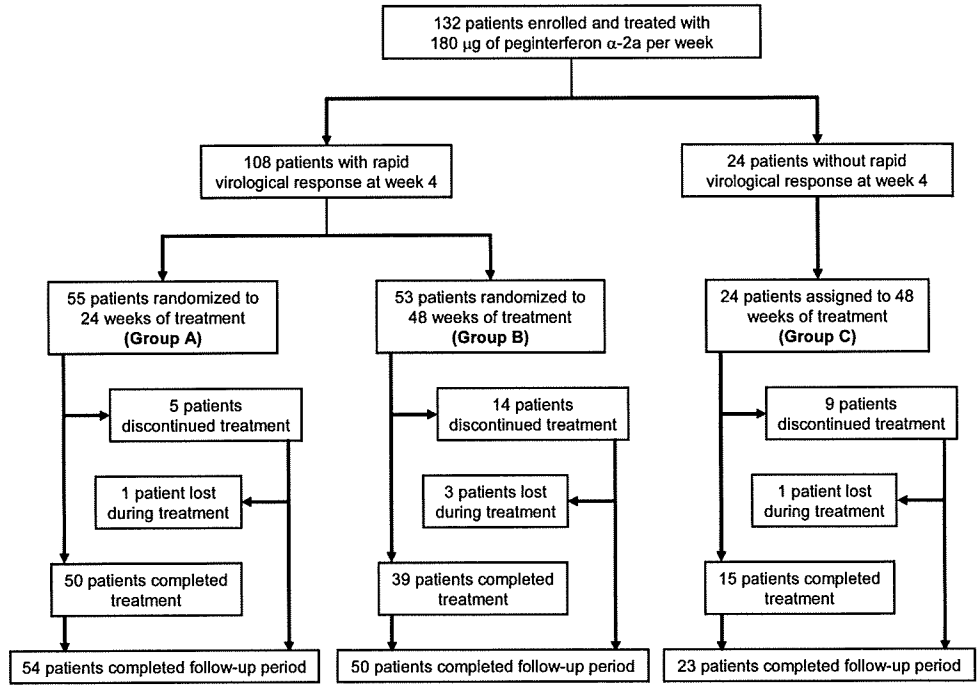

Fig. 1 Trial profile. Patients were randomized at week 8 for a total treatment duration of 24 (group A) or 48 (group B) weeks on the basis of the virological response at week 4. Patients who withdrew prematurely from treatment were encouraged to return for follow-up. For this reason, the number of patients who completed follow-up is higher than the number of patients who completed treatment

on Harmonization for Good Clinical Practice. All patients provided written informed consent before enrollment. Enrollment started in March 2004 and ended in December 2005.

Virological and histological evaluation

Serum HCV RNA was detected by qualitative reverse-transcription polymerase chain reaction (RT-PCR, Amplicor HCV, Roche Diagnostics Japan, Tokyo, Japan; low limit of detection 50 IU/ml). The serum HCV load was determined by quantitative RT-PCR (Amplicor HCV Monitor Test, Version 2.0, Roche Diagnostics Japan; low limit of detection 500 IU/ml). HCV RNA genotype was determined by RT-PCR with genotype-specific primers [20] or by serological grouping of serum antibodies determined by enzyme-linked immunosorbent assay (SRL Inc., Hachi-Oji, Tokyo) according to the method of Tanaka et al. [21] assuming that genotypes 1a and 1b corresponded to serological group 1 (genotype 1) and genotypes 2a and 2b corresponded to serological group 2 (genotype 2) [22].

Most patients underwent liver biopsy before therapy. In 40 patients, a liver biopsy was not available because the patients declined to have a biopsy specimen taken. Histopathological results were classified by local pathologists according to the METAVIR criteria reported previously [23, 24]. Treatment commenced within 12 months of liver biopsy.

Follow-up of patients

Patients were evaluated as outpatients for treatment safety, tolerance, and efficacy by each attending physician every week during treatment and every 4 weeks after the end of treatment for the rest of the study period.

Assessment of efficacy

During treatment, HCV RNA was quantified by PCR assay and was tested by qualitative test if HCV RNA became undetectable by the quantitative test. The end-of-treatment response (ETR) and SVR were assessed by qualitative PCR assay. ETR was defined as an undetectable serum HCV RNA level at the end of treatment. SVR was defined as an undetectable serum HCV RNA level by the end of treatment and throughout the follow-up period.

Safety analysis

Patients were assessed for safety and tolerance by the attending physician by monitoring adverse events and laboratory abnormalities. The study protocol permitted dose modification for patients who had clinically significant adverse events or important abnormalities in laboratory values. Adverse events were handled according to the instructions provided by the manufacturer for peginterferon α-2a, and therapy adjustments were applied. In general, dose reductions and discontinuation of therapy, if any, were made following the recommendations of the manufacturer. The dose was also reduced or the drug was discontinued at the discretion of the investigator at each of the participating clinical centers on the basis of the results of hematological, neuropsychiatric, and cutaneous or other adverse effects that were considered related to the medication. The dose of peginterferon could be restored to their original levels upon resolution of the event or abnormality.

Adherence to therapy was assessed as described previously [15], namely, by calculating the actual doses of IFN received as a percentage of the expected dose. Thus, patients who received 80% or more of their total IFN doses for 80% or more of the expected duration of therapy were considered to be 80% adherent. The dose of peginterferon received during the first 4 weeks was also assessed.

Sample size

The noninferiority margin was set at 10% between groups A and B. To obtain 80% statistical power with one-sided 5% significance level, a sample size of 81 patients per treatment group was necessary. With a dropout rate of 10% allowed, 90 patients per group were to be recruited. It was assumed that 70% of the patients would have undetectable HCV RNA at week 4. On the basis of this, the original plan specified enrollment of 270 patients to ensure randomization of an adequate number of patients at week 8. However, the Japanese Bureau of National Health Insurance started to provide reimbursement for peginterferon α-2b and ribavirin therapy for patients with HCV genotype 2 infection and high viral load or re-treatment irrespective of viral load since December 2005 and thus difficulty in new enrollment was anticipated; the enrollment was terminated by the end of the year.

Statistical analysis

Intention-to-treat analysis was used for all measures of efficacy. Patients who missed the examination at the end of the follow-up period were considered not to have had a response at that point. Patients who received at least one dose of study medication were included in the analysis of safety. The primary objective of the study was to establish the difference in SVR rates between treatment groups A and B.

Differences in baseline clinical characteristics, efficacy, and safety between the treatment groups were compared statistically by analysis of variance, χ^2 test, Fisher's exact test, Mann–Whitney *U* test, and Kruskal–Wallis test, where appropriate, using SAS, Version 9.1.3, software (SAS Institute, Inc., Cary, NC). Univariate and multivariate logistic regression analyses were used to establish those factors that contributed to the efficacy of peginterferon α-2a monotherapy. Variables with more than marginal statistical significance ($P < 0.10$) in univariate analysis were entered into multivariate analysis. A risk ratio with a 95% confidence interval was denoted for each analysis. Unless otherwise stated, *P* values below 0.05 were considered significant.

Results

Characteristics of patients

This study was performed between March 2004 and June 2007 at 33 centers in Japan. On the basis of the inclusion and exclusion criteria, 132 patients were enrolled (Fig. 1): 17 (13%) and 115 (87%) patients were infected with HCV genotypes 1 and 2, respectively. The baseline characteristics of the patients are summarized in Table 1.

Virological response

After 4 weeks of treatment with peginterferon α-2a, HCV RNA was below 500 IU/ml in 108 of 132 (82%) patients (Fig. 1). Among these 108 patients with RVR, HCV RNA was undetectable by qualitative test in 97 of 108 (90%) patients, whereas it was not tested by qualitative test in the rest of the patients. The RVR was achieved by 15 of 17 (88%) patients infected with HCV genotype 1 and low viral load and by 93 of 115 (81%) patients infected with HCV genotype 2 ($P = 0.737$). These patients were randomly assigned to group A ($n = 55$) and group B ($n = 53$). Patients with HCV RNA of 500 IU/ml or higher at week 4 were assigned to group C ($n = 24$) (Fig. 1). There were no significant differences in baseline parameters between groups A and B (Table 2).

An overall intention-to-treat virological response at the ETR was achieved in 122 of 132 (92%) patients and SVR in 81 of 132 (61%) patients. In groups A and B, 53 of 55 (96%) patients and 51 of 53 (96%) patients achieved ETR and 33 of 55 (60%) patients and 42 of 53 (79%) patients achieved SVR, respectively (Fig. 2). The SVR rate was

Table 1 Demographic, biochemical, molecular, and histological profiles of patients at baseline

	All patients	HCV RNA (kIU/ml) at week 4		*P*-value†
		<500	≥500	
Patients, *n*	132	108	24	
Gender, male/female (% male)	81/51 (61)	68/40 (63)	13/11 (54)	0.423‡
Age (years)[a]	56.4 ± 12.2	56.0 ± 12.2	57.8 ± 12.2	0.536§
Weight (kg)[a]	61.5 ± 12.6	62.0 ± 13.1	58.6 ± 9.5	0.257§
Naive/re-treatment	119/13	98/10	21/3	0.704#
Fibrosis staging, *n* (F1/F2/F3/F4)	57/26/8/1	48/22/6/1	9/4/2/0	0.881‡
Grading, *n* (A0-1/A2/A3)	52/38/2	44/31/2	8/7/0	0.868‡
Genotype, 1/2 (% genotype 1)	17/115 (13)	15/93 (14)	2/22 (8)	0.737#
HCV RNA (kIU/ml)[b]	285 (46–1620)	130 (37–1006)	1350 (360–3060)	<0.001¶
ALT (IU/l)[b] [7–42][c]	66 (35–117)	64 (36–119)	69 (31–109)	0.571¶
γ-GTP (IU/l)[b] [5–50][c]	45 (24–92)	54 (26–97)	33 (21–49)	0.014¶
Neutrophil count (/μl)[a] [1000–7500][c]	2844 ± 1036	2898 ± 1042	2615 ± 996	0.230§
Hemoglobin (g/dl)[a] [13.5–17.5][c]	14.0 ± 1.2	14.1 ± 1.2	14.0 ± 1.4	0.751§
Platelet count (10^3/μl)[a] [150–400][c]	175 ± 63	179 ± 66	155 ± 40	0.089§

ALT alanine aminotransferase, *γ-GTP* gamma glutamyl transpeptidase

† Comparison between groups according to HCV RNA at week 4

‡ Chi-square test

Fisher's exact test

§ Unpaired-t test

¶ Mann–Whitney *U*-test

Data are [a] mean ± SD or [b] median (interquartile range), [c] normal range

Table 2 Comparison of baseline profiles between groups A (24 weeks) and B (48 weeks)

	Group A	Group B	*P*-value[†]
Patients, *n*	55	53	
Gender, male/female (% male)	35/20 (64)	33/20 (62)	0.883[‡]
Age (years)[a]	56.9 ± 11.3	55.2 ± 13.1	0.473[§]
Weight (kg)[a]	59.9 ± 11.6	64.3 ± 14.3	0.087[§]
Naive/re-treatment	48/7	49/4	0.374[#]
Fibrosis staging, *n* (F1/F2/F3/F4)	26/12/2/0	22/10/4/1	0.558[‡]
Grading, *n* (A0-1/A2/A3)	28/11/1	16/20/1	0.097[‡]
Genotype, 1/2 (% genotype 1)	9/46 (16)	6/47 (11)	0.580[#]
HCV RNA (kIU/ml)[b]	190 (35–1660)	120 (38–580)	0.282[¶]
ALT (IU/l)[b] [7–42][c]	70 (37–117)	59 (36–120)	0.813[¶]
γ-GTP (IU/l)[b] [5–50][c]	43 (26–78)	63 (28–116)	0.242[¶]
Neutrophil count (/μl)[a] [1000–7500][c]	2936 ± 1047	2859 ± 1088	0.711[§]
Hemoglobin (g/dl)[a] [13.5–17.5][c]	14.1 ± 1.0	14.1 ± 1.3	0.943[§]
Platelet count (10^3/μL)[a] [150–400][c]	177 ± 47	181 ± 81	0.793[§]

ALT alanine aminotransferase, *γ-GTP* gamma glutamyl transpeptidase

[†] Comparison between groups A and B

[‡] Chi-square test

[#] Fisher's exact test

[§] Unpaired *t*-test

[¶] Mann–Whitney *U* test

Data are [a] mean ± SD or [b] median (interquartile range), [c] normal range

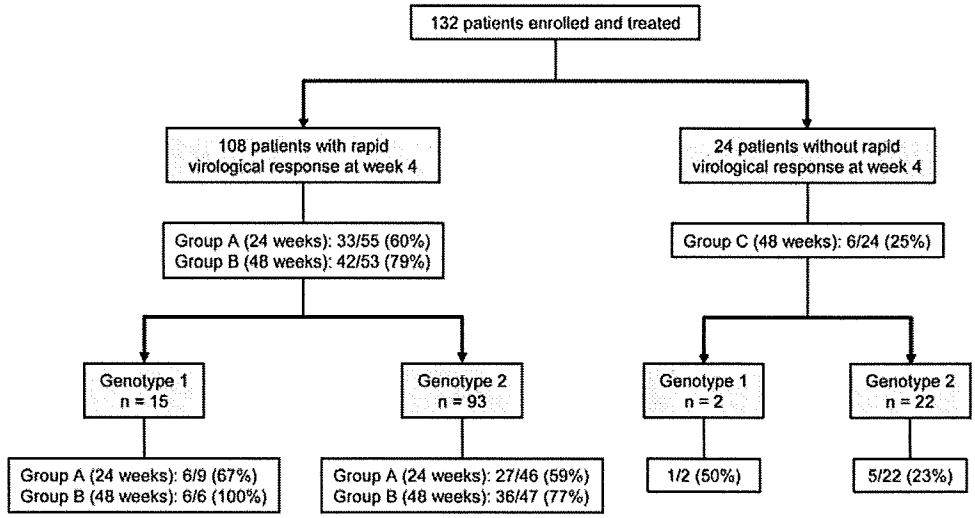

Fig. 2 Sustained virological response rate according to genotype in each treatment group

significantly higher in patients randomized to 48 weeks of therapy (group B) than in those randomized to 24 weeks of therapy (group A) ($P = 0.030$), namely, the relapse rate in group A was 40% (22/55), which was significantly higher than in group B (21%, 11/53, $P = 0.030$). Among patients with RVR confirmed by qualitative test (HCV RNA < 50 IU/ml), 29 of 48 (60%) patients achieved SVR in group A vs. 39 of 46 (85%) in group B ($P = 0.008$). The ETR and SVR rates in patients who did not show RVR and who were treated for 48 weeks (group C) were lower than in those who showed RVR (groups A and B) (75% vs. 96%, $P = 0.003$ for ETR and 25% vs. 69%, $P < 0.001$ for SVR, respectively) (Fig. 2).

Virological response according to HCV genotype and pretreatment viral load

The SVR rate in HCV genotype 1 and low viral load were not significantly different between treatment groups A and B (67% vs. 100%, respectively; $P = 0.229$) (Fig. 2),

although the number of patients of this subgroup was small for meaningful comparison.

The SVR rate in patients infected with HCV genotype 2 was higher in group B (77%) than in group A (59%, $P = 0.065$). There was an inverse correlation between SVR rate and baseline viral load (Fig. 3). This observation was significant in group A ($P < 0.001$) but not in group B ($P = 0.096$). On the basis of receiver operating characteristics analysis, 1,000,000 IU/ml was optimal for use as the cutoff point of baseline viral load to best discriminate patients who might achieve SVR in group A. The SVR rate of patients with HCV genotype 2 infection and a baseline viral load below 1,000,000 IU/ml was not compromised by 24-week treatment (group A) compared with 48-week treatment (group B) (81% and 79%, respectively), without significant difference between the two groups ($P = 0.801$). On the other hand, the SVR rate in those with a baseline viral load of 1,000,000 IU/ml or higher was significantly lower in group A than in group B (26% and 67%, respectively, $P = 0.041$) (Fig. 3).

Factors associated with RVR

Next, we analyzed the factors associated with RVR using data of all patients. The variables included were demographic features, baseline viral load, liver enzymes, and administered dose of peginterferon during the first 4 weeks (Table 3). Pretreatment HCV RNA level was lower and

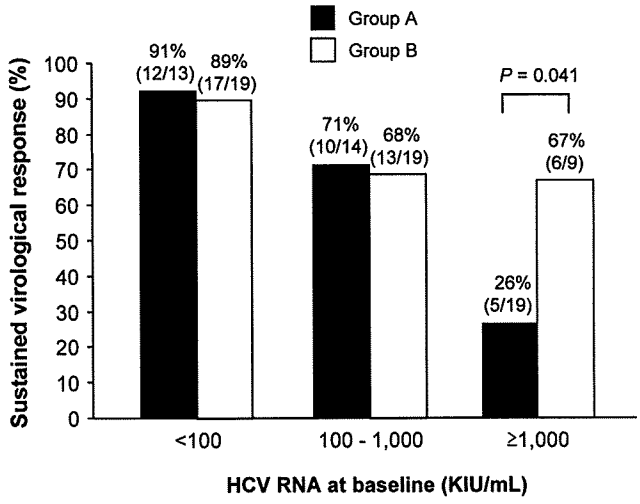

Fig. 3 Sustained virological response (SVR) rates stratified according to pretreatment HCV RNA level for patients of groups A (24 weeks) and B (48 weeks) infected with HCV genotype 2. The SVR rate was significantly lower in patients with higher baseline viral load in group A ($P < 0.001$) but not in group B ($P = 0.096$). The SVR rate in patients with a baseline viral load of less than 1,000,000 IU/ml was similar between group A (81%, 22/27) and group B (79%, 30/38) ($P = 0.801$). However, the SVR rate in those with a baseline viral load of 1,000,000 IU/ml or higher was lower in group A (26%, 5/19) than in group B (67%, 6/9) ($P = 0.041$)

γ-glutamyl transpeptidase (γ-GTP) level and platelet count were higher in patients with RVR (groups A and B) than in those without RVR (group C) (Table 1). On the basis of receiver operating characteristic analyses, 41 IU/l and 191 × 10^3/μl were optimal for use as the cutoff points of baseline γ-GTP level and platelet count, respectively. Multivariate analysis identified low baseline viral load (<1,000,000 IU/ml), high γ-GTP level (≥41 IU/l), and high platelet count (≥191 × 10^3/μl) were significant determinants of RVR (Table 3).

Factors associated with SVR

Next, the factors associated with SVR were analyzed using data of all patients. Univariate analysis indicated that grading score, pretreatment viral load, alanine aminotransferase (ALT) and γ-GTP levels, neutrophil count, RVR, and adherence to treatment were associated with SVR. On the basis of receiver operating characteristic analyses, 41 IU/l, 28 IU/l, and 3,155/μl were optimal for use as the cutoff points of baseline ALT level, γ-GTP level, and neutrophil count, respectively. Multivariate analysis was performed with the following variables: pretreatment viral load, ALT and γ-GTP levels, neutrophil count, RVR, and adherence to treatment but excluding grading score due to a significant association with ALT level and a substantial number of cases (40 cases) missing histological data. The analysis identified low viral load (<100,000 IU/ml), RVR, high ALT level (≥41 IU/l), and high γ-GTP level (≥28 IU/l) as independent determinants of SVR (Table 4).

Multivariate analysis for factors associated with SVR in patients with RVR identified low viral load (<100,000 IU/ml) and high ALT level (≥41 IU/l) as independent determinants of SVR. The SVR rates in patients with RVR and with high ALT levels (≥41 IU/l) were generally high except for group A patients with high viral load (≥1,000,000 IU/ml). On the other hand, the SVR rates for both groups A (black bars) and B (open bars) were entirely low in patients with low ALT levels (<41 IU/l) except those with low viral load (<100,000 IU/ml) (Fig. 4). In patients with high viral load (≥100,000 IU/ml) and low ALT levels (<41 IU/l), SVR was achieved only in group B patients, though at low rate.

Safety

Twenty-eight (21%) patients discontinued therapy and 14 of them discontinued because of adverse events, 4 because of laboratory abnormalities, 3 because of refusal of treatment, 2 because of insufficient response, and 5 because of failure to return (Table 5). Fatigue was the most common adverse event leading to discontinuation of therapy. The frequencies of discontinuation and discontinuation due to

Table 3 Logistic regression analysis of the factors associated with rapid virological response

Variable	RR (95% CI)	*P* value
Univariate analysis		
Pretreatment variables		
Gender (male vs. female)	1.438 (0.589–3.513)	0.425
Age (<55 vs. ≥55 years)	1.536 (0.583–4.049)	0.512
Weight (≥60 vs. <60 kg)	2.511 (0.938–6.723)	0.067
Treatment (naive vs. re-treatment)	1.400 (0.354–5.530)	0.631
Genotype (1 vs. 2)	1.773 (0.377–8.333	0.468
Fibrosis staging (F2-4 vs. F0-1)†	1.104 (0.356–3.425)	0.865
Grading (A2-3 vs. A0-1)†	1.167 (0.384–3.546)	0.786
HCV RNA (kIU/mL)		
<100	1	
100–1000	0.562 (0.140–2.251)	0.415
≥1000	0.159 (0.048–0.526)	0.003
ALT (≥60 vs. <60 IU/l)	1.437 (0.579–3.571)	0.435
γ-GTP (IU/l) (≥41 vs. <41 IU/l)	3.946 (1.504–10.352)	0.005
Neutrophil count (≥2500 vs. <2500/μL)	1.135 (0.465–2.771)	0.782
Hemoglobin (<14 vs. ≥14 g/dl)	1.427 (0.582–3.497)	0.437
Platelet count (≥191 vs. <191 × 10^3/μL)	6.567 (1.466–29.424)	0.014
Treatment-associated variables		
Adherence during 4 weeks of treatment (≥80% vs. <80%)	1.714 (0.419–7.011)	0.453
Stepwise multivariate analysis		
HCV RNA (kIU/ml)		
<100	1	
100–1000	0.399 (0.091–1.759)	0.225
≥1000	0.126 (0.034–0.464)	0.002
Platelet count (≥191 vs. <191 × 10^3/μl)	10.230 (2.056–50.902)	0.005
γ-GTP (IU/l) (≥41 vs. <41 IU/l)	3.989 (1.355–11.744)	0.012

ALT alanine aminotransferase, *γ-GTP* gamma glutamyl transpeptidase

† A biopsy was not available from 40 patients

adverse events were significantly lower in the 24-week treatment group (group A) than in the 48-week treatment group (groups B and C) (9% vs. 30%, $P = 0.005$ and 4% vs. 16%, $P = 0.042$, respectively).

Adherence to scheduled therapy (median and interquartile range) was 100% (63–100%), 77% (54–100%), and 85% (55–100%), respectively, for groups A, B, and C ($P = 0.012$ by Kruskal–Wallis test). The rate of adherence in group A was higher than in groups B and C ($P = 0.003$ and $P = 0.082$, respectively, by Mann–Whitney U test). There was no difference in adherence to therapy between groups B and C ($P = 0.597$). Thus, adherence to therapy in the longer treatment course (48 weeks) was lower than in the shorter treatment course (24 weeks).

Costs

Based on the current prices in the United States, spending on medication for 48 weeks of peginterferon α-2a monotherapy is $26,305 and that for 24 weeks treatment is $13,152. If we consider re-treatment for 48 weeks of the 40% of patients with RVR who relapse after 24 weeks of treatment, the mean cost of treating HCV genotype 2 infection or low viral load HCV genotype 1 patients with RVR would be $23,674 (Fig. 5). Thus, if all relapsers after 24 weeks of treatment receive re-treatment for 48 weeks, the mean saving per patient with this concept vs. 48 weeks to all would be $2,630 (10%).

Discussion

The key finding of this study is that in patients infected with HCV genotype 2 and low viral load (<1,000,000 IU/ml) who achieve RVR, 24-week treatment with peginterferon α-2a alone may be sufficient in terms of efficacy. Patients treated for 24 weeks also discontinued treatment less frequently and showed higher adherence than those

Table 4 Logistic regression analysis of the factors associated with sustained virological response

Variable	RR (95% CI)	*P*-value
Univariate analysis		
Pretreatment variables		
Gender (male vs. female)	1.190 (0.581–2.438)	0.635
Age (≥55 vs. <55 years)	1.273 (0.618–2.625)	0.512
Weight (<60 vs. ≥60 kg)	1.064 (0.520–2.175)	0.865
Treatment (re-treatment vs. naive)	1.468 (0.428–5.051)	0.541
Genotype (1 vs. 2)	2.247 (0.690–7.299)	0.179
Fibrosis staging (F2-4 vs. F0-1)†	1.964 (0.812–4.749)	0.134
Grading (A2-3 vs. A0-1)†	4.343 (1.727–10.922)	0.002
HCV RNA (kIU/mL) <100	1	
100–1000	0.367 (0.138–0.975)	0.044
≥1000	0.115 (0.044–0.298)	<0.001
ALT (≥41 vs. <41 IU/l)	4.570 (2.104–9.927)	<0.001
γ-GTP (IU/l) (≥28 vs. <28 IU/l)	6.182 (2.657–14.384)	<0.001
Neutrophil count (<3155 vs. ≥3155/μl)	3.135 (1.479–6.623)	0.003
Hemoglobin (≥14 vs. <14 g/dl)	1.125 (0.555–2.283)	0.744
Platelet count (<150 vs. ≥150 × 10^3/μl)	1.091 (0.507–2.347)	0.824
Treatment-associated variables		
RVR (yes vs. no)	6.818 (2.482–18.733)	<0.001
Adherence (≥80% vs. <80%)	1.940 (0.949–3.966)	0.070
Stepwise Multivariate Analysis‡		
HCV RNA (kIU/ml)		
<100	1	
100–1000	0.165 (0.046–0.589)	0.006
≥1000	0.102 (0.029–0.352)	<0.001
RVR (yes vs. no)	6.223 (1.821–21.305)	0.003
ALT (≥41 vs. <41 IU/l)	4.775 (1.373–16.601)	0.014
γ-GTP (IU/l) (≥28 vs. <28 IU/l)	3.466 (1.092–11.000)	0.035

ALT alanine aminotransferase, *γ-GTP* gamma glutamyl transpeptidase, *RVR* rapid virological response

† A biopsy was not available from 40 patients

‡ Grading was not included

treated for 48 weeks. Furthermore, the drug cost can be reduced by truncating treatment duration. Thus, by reducing the treatment period, these patients can avoid unnecessary treatment without compromising the chance for an SVR. In particular, the SVR rate in patients with HCV genotype 2 infection and low viral load (<1,000,000 IU/ml) who achieved RVR was as high as 81% by 24-week monotherapy. The SVR rate was comparable with that (84%, 81/96) reported previously in patients with HCV genotype 2 and 3 infection who received 24-week combination therapy of peginterferon α-2a plus ribavirin [7], although the latter included patients with HCV genotype 3 infection. On the other hand, the results of this study were not conclusive regarding patients with HCV genotype 1 infection and low viral load (<100,000 IU/ml). Further prospective controlled trial is warranted to confirm our findings in patients with HCV genotype 1 infection and low viral load or HCV genotype 2 infection and baseline viral loads of less than 1,000,000 IU/ml who achieve RVR.

In patients with HCV genotype 2 infection and high viral load (≥1,000,000 IU/ml), the SVR rate was lower for the 24-week treatment group than for the 48-week treatment group, even if the patients achieved RVR. Thus, a longer treatment (>24 weeks) with peginterferon is recommended for this group of patients. Furthermore, since the SVR rate was not more than 67% in this subgroup of patients, even if they were treated for 48 weeks, a combination with ribavirin or further extended treatment duration may be necessary as long as patients can tolerate the treatment.

The combination therapy of peginterferon and ribavirin is currently the therapeutic standard for chronic hepatitis C. However, the combination therapy tends to be associated with adverse events more frequently than those that occur with IFN monotherapy, resulting in dose reduction or discontinuation of therapy and thus impaired response rate [9–14]. This is true particularly in elderly patients. In a country such as Japan where the average age of patients with chronic hepatitis C to be treated by antivirals sometimes is well above 60, standard combination therapy is not well tolerated [15]. For example, in a phase III study of 48-week peginterferon α-2a plus ribavirin combination therapy

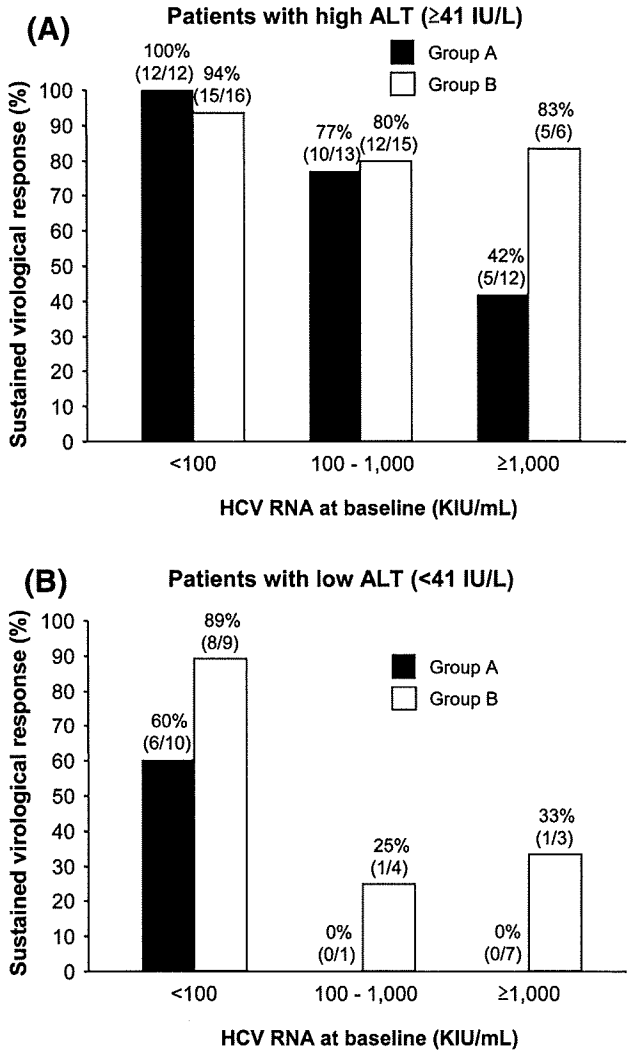

Fig. 4 Sustained virological response (SVR) rates stratified according to pretreatment HCV RNA and ALT levels in group A and B patients. The SVR rates in patients with high (≥41 IU/l) and low (<41 IU/l) ALT levels are shown in panels **a** and **b**, respectively. **a** The SVR rates in patients with high ALT levels (≥41 IU/l) were generally high except for group A patients with high viral load (≥1,000,000 IU/ml). **b** The SVR rates in both groups A and B were low in patients with low ALT levels (<41 IU/l) except those with low viral load (<100,000 IU/ml)

conducted in Japan [25], the SVR rate in the combination arm (78%, 18/23) was rather inferior to that of peginterferon α-2a monotherapy (placebo) arm (100%, 14/14) among patients with RVR ($P = 0.061$), although the difference did not reach statistical significance. In the same study, all of the patients who failed to achieve SVR in the combination arm discontinued treatment [25]. Thus, the combination therapy with ribavirin does not always lead to a better response than with monotherapy, at least in a subgroup of patients. It is noteworthy that most of the patients in the present trial were those who preferred peginterferon α monotherapy to combination therapy in spite

Table 5 Incidence and reason of discontinuation according to treatment group

Variable	All patients	Age (years)		
		A	B	C
n	132	55	53	24
Discontinuation	28 (21)	5 (9)	14 (26)	9 (38)
Adverse events	14 (11)	2 (4)	8 (15)	4 (17)
Fatigue	4	0	1	3
Depression	2	1	1	0
Arthralgia	2	0	2	0
Arrhythmia	2	0	1	1
Pyrexia	1	1	0	0
Headache	1	0	1	0
Hyperthyroidism	1	0	1	0
Colon cancer	1	0	1	0
Laboratory abnormality	4 (3)	1 (2)	1 (2)	2 (8)
High aminotransferase	2	1	0	1
Anemia	1	0	1	0
Neutropenia	1	0	0	1
Refusal of treatment	3 (2)	1 (2)	1 (2)	1 (4)
Insufficient response	2 (2)	0	1 (2)	1 (4)
Failure to return	5 (4)	1 (2)	3 (6)	1 (4)

Data are number of patients (percentage in each patient group)

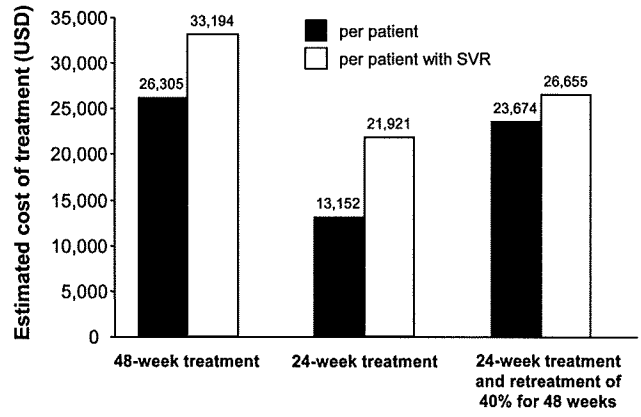

Fig. 5 The cost of treating patients infected with HCV genotype 2 or low viral load genotype 1 and RVR for 48, 28, or 24 weeks followed by 48 weeks of re-treatment of 40% of patients who relapse after the initial treatment

of the coverage for the latter therapy by the Bureau of National Health Insurance, as described previously.

In addition to elderly patients, those with renal failure, ischemic vascular diseases, and congenital hemoglobin abnormalities never tolerate ribavirin for chronic hepatitis C treatment [26]. Therefore, data on peginterferon α monotherapy are particularly relevant to these patients. The possibility of shorter combination therapy with peginterferon α and ribavirin in easy-to-treat patients such as those

chronically infected with HCV genotype 2 or 3 has been investigated in several trials [27–31]. However, to the best of our knowledge, there has been no randomized, controlled trial to identify optimal treatment duration of peginterferon α monotherapy. A further prospective randomized, controlled trial aiming at patients who cannot receive combination therapy with ribavirin is warranted.

A high ALT level has been identified as a significant factor for SVR [18]. The reason why patients with low or normal ALT levels do not respond well to peginterferon α monotherapy is currently unknown. The SVR rates were low in our patients with low ALT levels and HCV RNA levels of 100,000 IU/ml or higher in the two randomized groups (Fig. 4). Thus, these patients may not benefit by simply extending therapy from 24 to 48 weeks. Since a similar efficacy has been demonstrated in patients with persistently normal ALT levels compared with those with elevated ALT levels by combination therapy of peginterferon α-2a plus ribavirin [32], combination therapy should be considered for these patients.

A high γ-GTP level was unexpectedly identified as a factor for both RVR and SVR, independent of ALT levels. Again, the reason for this finding is unknown at present. It is well known that a low γ-GTP level is associated with SVR to combination therapy comprising peginterferon and ribavirin; the reason also being unexplained so far [33]. Thus, the present finding at least suggests that entirely different mechanisms may underlie these observations.

In this trial, RVR was defined as serum HCV RNA level below 500 IU/ml at week 4, although most of the patients who achieved RVR had HCV RNA levels below 50 IU/ml. The criterion of RVR used in this study was less strict than those reported recently, in which serum HCV RNA level below 50 IU/ml at week 4 has been utilized [30, 31]. This may result in a higher rate of achieving RVR and lower SVR rates, resulting in more relapsers, particularly in patients treated for a shorter duration of 24 weeks than a standard duration of 48 weeks. By using more strict criteria of serum HCV RNA level below the detection limit of qualitative PCR (≤50 IU/ml) at week 4 or negativity of HCV RNA at earlier time points during therapy, such as at week 2 [34], a subgroup of patients, who can be sufficiently treated with a shorter duration of therapy (such as 24 weeks) without compromising the chance for SVR, could be more specifically identified.

In conclusion, patients infected with HCV genotype 2 and have low baseline viral load (<1,000,000 kIU/ml), who can achieve RVR, can satisfactorily be treated for 24 weeks with peginterferon α-2a alone without compromising the SVR. We propose that these patients should first be treated with peginterferon α monotherapy for 24 weeks, as long as RVR is achieved, otherwise they should be switched to combination therapy with ribavirin at the time for another 24–48 weeks, depending on the response thereafter. However, the data of this study are less conclusive for patients with low viral load genotype 1 or 2 and viral load of more than 1,000,000 IU/ml. Additional trials are required to optimize treatment schedule in these patients.

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Liver, Pancreas and Biliary Tract

Clinical features of autoimmune hepatitis diagnosed based on simplified criteria of the International Autoimmune Hepatitis Group

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ABSTRACT

Background: Recently, simplified diagnostic criteria for autoimmune hepatitis have been proposed.
Aim: We aimed to evaluate usefulness of the simplified criteria.
Methods: We applied the simplified criteria to 176 autoimmune hepatitis patients diagnosed according to the revised scoring system proposed in 1999 (original criteria). Furthermore, in order to compare predictabilities between these two diagnostic criteria, we included 168 patients with other liver disease than autoimmune hepatitis.
Results: Of 176 autoimmune hepatitis patients, 85% were diagnosed with autoimmune hepatitis according to the simplified criteria, and patients diagnosed according to the simplified criteria showed a higher frequency of antinuclear antibodies and/or smooth muscle antibodies of 1:80 or greater and slightly higher serum levels of immunoglobulin G than those diagnosed according to the original criteria. However, 30% of male patients, 23% of patients with acute presentation, 50% of patients showing histological acute hepatitis and 46% of patients negative for antinuclear antibodies at presentation were not diagnosed with autoimmune hepatitis according to the simplified criteria. The simplified criteria showed lower sensitivity (85% vs. 100%) and higher specificity (99% vs. 93%) for autoimmune hepatitis than the original criteria.
Conclusions: The simplified criteria may be useless for the diagnosis of patients with atypical features, especially patients with histological acute hepatitis.

1. Introduction

The clinical characteristics of autoimmune hepatitis (AIH) are circulating autoantibodies, hypergammaglobulinemia, and histological interface hepatitis with lymphocytic and plasma cell infiltration into portal tracts [1,2]. The disease predominantly affects women and has generally good prognosis with immunosuppressive treatment.

In 1993, the International Autoimmune Hepatitis Group (IAIHG) proposed a scoring system to establish diagnostic criteria for AIH [3]. The specificity of this scoring system was insufficient, although the sensitivity was more than 90% [4]. Thus, in 1999, a revised scoring system (hereafter referred to as the original criteria) with sufficient specificity was proposed [4,5]. Even though the criteria were improved in this revision, the original criteria are complex and include a variety of parameters of questionable value. Several drugs (infliximab, minocycline, atorvastatin, hepatitis A vaccine) have been reported as possible triggers for AIH; however, "history of recent or current use of known or suspected hepatotoxic drugs" has been scored as −4 points [4,6–9]. Furthermore, 7.5% of AIH patients have circulating antimitochondrial antibodies (AMA); however, "positivity of AMA" has been scored as −4 points [10].

In 2008, the IAIHG proposed a simplified set of diagnostic criteria (hereafter referred to as the simplified criteria) that included autoantibodies such as antinuclear antibodies (ANA), smooth muscle antibodies (SMA), liver–kidney microsomal antibodies and antibodies to soluble liver antigen, immunoglobulin G (IgG), histology, and exclusion of viral hepatitis [11]. These criteria have 88% sensitivity and 99% specificity. The variables included in the simplified criteria are typical characteristics of AIH, so it is not clear whether AIH patients with atypical features (male, acute presentation, histological acute hepatitis, negativity for ANA) can be appropriately diagnosed. To determine the usefulness of the simplified criteria, we applied them to 176 AIH patients diagnosed according to the original criteria and 168 patients with other liver disease than AIH.

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2. Methods

We included 176 patients who were admitted to the Okayama University Hospital or 6 affiliated hospitals between March 1989 and April 2008 and were diagnosed with definite or probable AIH based on the original criteria [4]. A definite diagnosis required a pretreatment score greater than 15, while a probable diagnosis required a score between 10 and 15. All patients were seronegative for hepatitis B surface antigen, anti-hepatitis C virus antibody, hepatitis C virus-RNA (as determined via polymerase chain reaction after reverse transcription), and anti-mitochondrial antibody, and all underwent liver biopsy. Patients with an overlapping syndrome or a coexistent liver disease (for example, primary biliary cirrhosis, primary sclerosing cholangitis, nonalcoholic fatty liver disease, or alcohol-induced liver injury) were excluded from AIH patients.

Forty-two patients (26%) had concurrent autoimmune diseases: 18 had autoimmune thyroiditis, 4 had Sjögren's syndrome, 3 each had systemic lupus erythematosus, Graves' disease, or ulcerative colitis, 2 each had autoimmune haemolytic anaemia, idiopathic thrombocytopenic purpura, progressive systemic sclerosis, or rheumatoid arthritis, one each had both autoimmune thyroiditis and autoimmune haemolytic anaemia, both systemic lupus erythematosus and Sjögren's syndrome, and both autoimmune thyroiditis and Sjögren's syndrome.

The titres of ANA were measured using an indirect immunofluorescence (IIF) technique with HEp-2 cells. SMA was assayed by the IIF technique using rat kidney and stomach cells. A serum titre of 1:40 or greater was positive for ANA or SMA. Antibodies to liver/kidney microsome type 1 (anti-LKM-1) were measured using an enzyme-linked immunosorbent assay using recombinant cytochrome P4502D6 as the antigen, and a serum value of 50.0 index or greater was positive. Twenty-four patients (13%) were negative for ANA (<1:40). Fifteen patients (9%) were positive for ANA titres of 1:40. Forty-five of 122 patients (37%) who were screened for SMA were negative for SMA (<1:40), and 24 were positive for SMA titres of 1:40. One hundred and fifty patients (85%) were positive for ANA and/or SMA titres of ≥1:80. None had anti-LKM-1. Sixty of 87 patients (69%) screened for human leukocyte antigen (HLA) DR status by the polymerase chain reaction sequence specific oligonucleotide hybridization method had DR4. None had DR3.

An acute presentation was defined by the presence of acute onset of symptoms (for example, jaundice and/or fatigue and/or anorexia) in conjunction with serum bilirubin levels ≥5 mg/dL and/or serum alanine aminotransferase (ALT) levels higher than 10-fold the upper normal limit.

Liver biopsy was performed with a Vim-Silverman needle (14-G) under laparoscopy, or with a 17-G needle under ultrasonography guidance, before or just after the introduction of initial treatment. Liver biopsy specimens were evaluated by two pathologists and diagnosed as acute or chronic hepatitis. A diagnosis of acute hepatitis was made on the basis of the presence of histologically predominant zone 3 necrosis with minimal lymphocytic and plasma cell infiltration into portal tracts, in the absence of interface hepatitis or portal fibrosis. Liver biopsy specimens diagnosed as showing chronic hepatitis underwent histological staging based on the classification of Desmet et al. [12].

All patients were re-scored according to the simplified criteria [11]. A definite diagnosis of AIH based on these simplified criteria required a pretreatment score greater than 6, while a probable diagnosis required a score of 6. Histologically, the required typical features were interface hepatitis, lymphocytic/lymphoplasmocytic infiltration into portal tracts, and rosetting of liver cells, while the compatible feature was chronic hepatitis with lymphocytic infiltration without all the other features considered typical.

To compare the clinical features of patients diagnosed with definite or probable AIH based on the original criteria to those of patients diagnosed with definite or probable AIH based on the simplified criteria, we analysed gender, age, frequency of acute presentation, concurrent autoimmune disease, laboratory data [albumin, bilirubin, aspartate aminotransferase (AST), ALT, IgG, ANA and/or SMA titre, HLA DR4], and histological features (staging of fibrosis, rosetting of liver cells, zone 3 necrosis).

Furthermore, in order to compare the predictability between the simplified criteria and the original criteria, we included 168 patients with other liver disease than AIH, who were admitted to the Okayama University Hospital between April 2005 and March 2008 and underwent liver biopsy (23 patients with chronic hepatitis B, 87 patients with chronic hepatitis C, 10 patients with drug-induced liver injury, 18 patients with primary biliary cirrhosis, 4 patients with primary sclerosing cholangitis, 17 patients with nonalcoholic steatohepatitis, 6 patients with simple steatosis, 3 patients with alcoholic liver disease).

Chronic hepatitis B and C were diagnosed by positive serology tests for serum hepatitis B surface antigen and anti-hepatitis C virus antibodies, respectively. Primary biliary cirrhosis was diagnosed with the presence of detectable antimitochondrial antibodies in serum and histologic findings [13]. Primary sclerosing cholangitis was diagnosed with cholangiographic findings and the presence of histological onion skin lesion [14]. The diagnosis of drug-induced liver injury was made based on the temporal relationship between drug ingestion and adverse reaction, exclusion of other diseases, some findings on liver biopsy [15]. Nonalcoholic steatohepatitis and simple steatosis were diagnosed by ultrasonography and histology after exclusion of other possible etiologies of fatty liver [16].

2.1. Statistics

Statistical analysis was performed using the SPSS statistical program (release 11.0.1J, SPSS Inc., Chicago, Illinois).

Continuous variables were expressed as medians and ranges. The Mann–Whitney *U*-test was used to evaluate differences in the continuous variables between two groups, and the Kruskal–Wallis *U*-test was performed among three groups. Dichotomous variables were compared by the χ^2-test. *P* values of less than 0.05 were considered significant.

3. Results

3.1. Diagnosis according to the simplified criteria in 176 AIH patients

Of 176 patients diagnosed with AIH according to the original criteria, 150 (85%) were also diagnosed with AIH according to the simplified criteria. Of 136 patients with definite AIH according to the original criteria, 107 (79%) scored ≥7 points (definite AIH), 20 (15%) scored 6 points (probable AIH), and 9 (6%) scored ≤5 points based on the simplified criteria. On the other hand, of 40 patients with probable AIH according to the original criteria, 12 (30%) scored ≥7 points, 11 (28%) scored 6 points, and 17 (42%) scored ≤5 points based on the simplified criteria. Thus, 26 patients (15%) who consisted of 9 patients with definite AIH and 17 with probable AIH according to the original criteria were not diagnosed as having AIH based on the simplified criteria (Fig. 1).

3.2. Comparison of clinical features between patients diagnosed according to the original criteria and those diagnosed according to the simplified criteria

Patients diagnosed according to the original criteria and those diagnosed according to the simplified criteria were indistinguishable by clinical and histological features. However, patients

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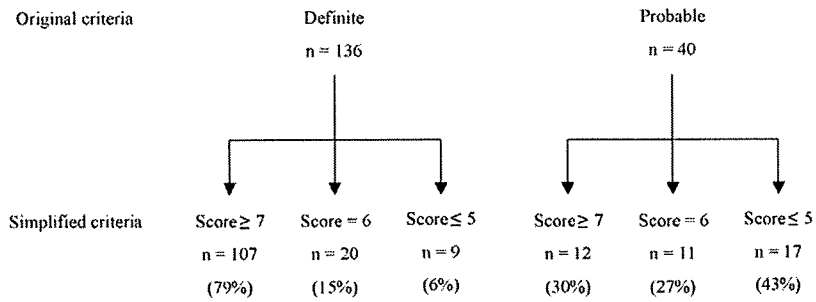

Fig. 1. Relation between the original criteria and the simplified criteria.

diagnosed according to the simplified criteria showed a higher frequency of ANA and/or SMA titres of 1:80 or greater and a slightly higher serum IgG levels ($P = 0.07$) than those diagnosed according to the original criteria (Table 1).

In AIH patients diagnosed according to the simplified criteria, patients with definite AIH had lower frequencies of acute presentation and histological acute hepatitis, a higher frequency of ANA and/or SMA titres of 1:80 or greater, and higher serum IgG levels than those with probable AIH. The same findings were shown in AIH patients diagnosed according to the original criteria, too. In particular, no patient with definite AIH according to the simplified criteria showed histological acute hepatitis. In patients diagnosed according to the original criteria, patients with definite AIH consisted of a higher proportion of females than those with probable AIH, while, in patients diagnosed according to the simplified criteria, no difference in the proportion of females was found between patients with definite AIH and those with probable AIH. Patients with probable AIH according to the simplified criteria showed a higher frequency of ANA and/or SMA titres of 1:80 or greater than those with probable AIH according to the original criteria (Table 2).

3.3. *Simplified criteria in 136 patients with definite AIH based on the original criteria*

According to the simplified criteria, patients with definite AIH showed higher serum IgG levels, a higher frequency of ANA and/or

Table 1
Clinical features of AIH patients diagnosed according to the original criteria and those diagnosed according to the simplified criteria.

	Original criteria	Simplified criteria
Patients, *n*	176	150
Gender, female (%)	153 (87)	134 (89)
Age (yr)	55 (16–79)	55 (16–79)
Form of clinical onset, acute presentation (%)	53 (30)	41 (27)
Concurrent autoimmune disease, *n* (%)	42 (24)	37 (25)
Laboratory data		
Albumin (g/dL)	3.8 (2.1–5.1)	3.8 (2.1–5.1)
Bilirubin (mg/Dl)	1.1 (0.3–29.2)	1.0 (0.3–22.6)
AST (IU/L)	162 (28–2330)	153 (33–2330)
ALT (IU/L)	203 (23–2161)	179 (25–2132)
IgG (mg/dL)	2541 (724–6562)	2625 (1085–6562)
ANA or ASMA ≥ 1:80, *n* (%)	150 (85)[a]	141 (94)[a]
HLA DR4, *n* (%)	60/87 (69)	50/70 (71)
Fibrosis staging, *n* (%)		
Acute hepatitis	10 (6)	5 (3)
Chronic hepatitis		
F1	51 (29)	45 (30)
F2	53 (30)	47 (31)
F3	44 (25)	37 (25)
F4	18 (10)	16 (11)
Rosetting of liver cells, *n* (%)	49 (28)	44 (29)
Zone 3 necrosis, *n* (%)	52 (30)	42 (28)

Significant difference from each other at level of [a] $P < 0.05$.

Table 2
Comparison of clinical features between AIH patients diagnosed according to the original criteria and those according to the simplified criteria.

	Original criteria		Simplified criteria	
	Definite	Probable	Definite	Probable
Patients, *n*	136	40	119	31
Gender, female (%)	127 (93)[a]	26 (65)[a]	108 (91)	26 (84)
Age (yr)	55 (16–79)	57 (16–77)	56 (18–79)	54 (16–74)
Form of clinical onset, acute presentation (%)	34 (25)[b]	19 (48)[b]	28 (24)[c]	13 (42)[c]
Concurrent autoimmune disease, *n* (%)	36 (26)	6 (15)	31 (26)	6 (19)
Laboratory data				
Albumin (g/dL)	3.8 (2.1–5.1)	3.8 (2.3–4.8)	3.8 (2.1–5.1)	4.0 (3.2–4.8)
Bilirubin (mg/Dl)	1.0 (0.3–29.2)	1.4 (0.3–25.8)	1.0 (0.3–22.6)	1.1 (0.3–17.0)
AST (IU/L)	155 (33–1716)	251 (28–2330)	153 (33–1716)	142 (40–2330)
ALT (IU/L)	184 (33–2132)	336 (23–2161)	171 (25–2132)	337 (28–1820)
IgG (mg/dL)	2610 (1085–6562)[d]	2003 (724–3990)[d]	2684 (1779–6562)[e]	1885 (1086–5894)[e]
ANA or ASMA ≥ 1:80, *n* (%)	129 (95)[f]	21 (53)[f,g]	117 (98)[h]	24 (77)[g,h]
HLA DR4, *n* (%)	48/66 (73)	12/21 (57)	40/55 (73)	10/15 (67)
Fibrosis staging, *n* (%)				
Acute hepatitis	5 (4)[i,j]	5 (13)[i]	0 (0)[j,k]	5 (16)[k]
Chronic hepatitis				
F1	38 (28)	13 (33)	35 (29)	10 (33)
F2	39 (29)	14 (34)	36 (30)	11 (35)
F3	38 (28)	6 (15)	33 (28)	4 (13)
F4	16 (11)	2 (5)	15 (13)	1 (3)
Rosetting of liver cells, *n* (%)	41 (30)	8 (20)	32 (27)	12 (39)
Zone 3 necrosis, *n* (%)	39 (29)	13 (33)	31 (26)	11 (35)

Significant difference from each other at level of [a,b,c,d,e,f,g,h,i,j,k] $P < 0.05$.

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Table 3
The simplified criteria in 136 patients diagnosed with definite AIH based on the original criteria.

	Score ≥ 7	Score = 6	Score ≤ 5
Patients, *n*	107	20	9
Gender, female (%)	100 (93)	18 (90)	9 (100)
Age (yr)	55 (18–79)	55 (16–74)	58 (34–78)
Form of clinical onset, acute presentation (%)	25 (23)	7 (35)	2 (22)
Concurrent autoimmune disease, *n* (%)	29 (27)	4 (20)	3 (33)
Laboratory data			
Albumin (g/dL)	3.8 (2.1–5.1)	4.0 (3.3–4.5)	4.0 (3.0–4.2)
Bilirubin (mg/dL)	1.0 (0.3–22.6)	1.1 (0.4–17.0)	1.1 (0.6–29.2)
AST (IU/L)	167 (33–1716)	133 (53–1704)	97 (37–861)
ALT (IU/L)	183 (33–2132)	211 (56–1820)	132 (47–720)
IgG (mg/dL)	2747 (1779–6562)[a,b]	1821 (1085–5894)[a]	1554 (1370–1960)[b]
ANA or ASMA ≥ 1:80, *n* (%)	106 (99)[c,d]	16 (80)[c]	7 (78)[d]
HLA DR4, *n* (%)	38/52 (73)	7/10 (70)	3/4 (75)
Fibrosis staging, *n* (%)			
Acute hepatitis	0 (0)[e,f]	4 (20)[e]	1 (11)[f]
Chronic hepatitis			
F1	29 (27)	6 (30)	3 (33)
F2	33 (31)	5 (25)	1 (11)
F3	30 (28)	4 (20)	4 (45)
F4	15 (14)	1 (5)	0 (0)
Rosetting of liver cells, *n* (%)	30 (28)	10 (50)[g]	1 (11)[g]
Zone 3 necrosis, *n* (%)	28 (26)	8 (40)	3 (33)

Significant difference from each other at level of [a,b,c,d,e,f,g] $P < 0.05$.

SMA titres of 1:80 or greater, and a lower frequency of histological acute hepatitis than did those with probable AIH and those who scored ≤5 points. On the other hand, patients with probable AIH had a higher frequency of histological rosetting of liver cells than those who scored ≤5 points, and a slightly higher frequency of histological rosetting of liver cells compared to those with definite AIH ($P = 0.05$) (Table 3).

3.4. Simplified criteria in 40 patients with probable AIH based on the original criteria

According to the simplified criteria, patients with definite AIH or probable AIH showed higher serum IgG levels and a higher frequency of ANA and/or SMA titres of 1:80 or greater than those who scored ≤5 points. Patients with definite AIH had a slightly lower frequencies of acute presentation and histological acute hepatitis than those who scored ≤5 points (both $P = 0.07$). In particular, 4 of 5 patients with acute hepatitis scored ≤5 points according to the simplified criteria (Table 4).

3.5. Diagnosis according to the simplified criteria in male patients

Of the 23 male patients, 2 (9%) showed histological acute hepatitis, 18 (78%) did not showed histological rosetting of liver cells, 8 (35%) had serum IgG levels under the upper normal limit, and 6 (26%) had both ANA and SMA titres of 1:40 or less. Sixteen patients (70%) were diagnosed with AIH according to the simplified criteria (11 patients with definite diagnosis). The 16 patients were older [64 (31–76) years vs. 38 (19–61) years: $P = 0.004$] and had a lower frequency of histological zone 3 necrosis (13% vs.

Table 4
The simplified criteria in 40 patients diagnosed with probable AIH based on the original criteria.

	Score ≥ 7	Score = 6	Score ≤ 5
Patients, *n*	12	11	17
Gender, female (%)	8 (67)	8 (73)	10 (59)
Age (yr)	61 (20–74)	50 (16–71)	52 (19–77)
Form of clinical onset, acute presentation (%)	3 (25)	6 (55)	10 (59)
Concurrent autoimmune disease, *n* (%)	2 (17)	2 (18)	2 (12)
Laboratory data			
Albumin (g/dL)	3.8 (3.2–4.7)	3.9 (3.2–4.8)	3.7 (2.3–4.7)
Bilirubin (mg/dL)	0.9 (04–14.9)	0.8 (0.3–13.7)	1.8 (0.4–25.8)
AST (IU/L)	91 (35–1502)	430 (40–2330)	393 (28–1690)
ALT (IU/L)	121 (25–1467)	638 (28–1783)	526 (23–2161)
IgG (mg/dL)	2427 (2003–3990)[a]	2759 (1696–3820)[b]	1513 (724–2906)[a,b]
ANA or ASMA ≥ 1:80, *n* (%)	11 (92)[c]	8 (73)[d]	2 (12)[c,d]
HLA DR4, *n* (%)	2/3 (67)	3/5 (60)	7/13 (54)
Fibrosis staging, *n* (%)			
Acute hepatitis	0 (0)	1 (9)	4 (24)
Chronic hepatitis			
F1	6 (50)	4 (36)	3 (18)
F2	3 (25)	6 (55)	5 (28)
F3	3 (25)	0 (0)	3 (18)
F4	0 (0)	0 (0)	2 (12)
Rosetting of liver cells, *n* (%)	2 (17)	2 (18)	4 (24)
Zone 3 necrosis, *n* (%)	3 (25)	3 (27)	7 (41)

Significant difference from each other at level of [a,b,c,d] $P < 0.05$.

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57%: $P=0.02$), higher serum IgG levels [2314 (1085–3987) mg/dL vs.1525 (1170–1932) mg/dL: $P=0.003$], and a higher frequency of ANA and/or SMA titres of 1:80 or greater (94% vs. 29%: $P=0.001$) than the other 7 patients.

3.6. Diagnosis according to the simplified criteria in patients with acute presentation

Of 53 patients with acute presentation, 10 (19%) showed histological acute hepatitis, 34 (64%) did not showed histological rosetting of liver cells, 14 (26%) had serum IgG levels under the upper normal limit, and 14 (26%) had both ANA and SMA titres of 1:40 or less. Forty-one patients (77%) were diagnosed with AIH according to the simplified criteria (28 patients with definite diagnosis). The 41 patients showed a lower frequency of histological acute hepatitis (12% vs. 42%: $P=0.02$), higher serum IgG levels [2630 (1662–4528) mg/dL vs. 1554 (724–2218) mg/dL: $P<0.0001$], and a higher frequency of ANA and/or SMA titres of 1:80 or greater (90% vs. 17%: $P<0.0001$) than the other 12 patients.

3.7. Diagnosis according to the simplified criteria in patients with histological acute hepatitis

Of 10 patients with histological acute hepatitis, 5 (50%) did not showed histological rosetting of liver cells, 4 (40%) had serum IgG levels under the upper normal limit, and 5 (%) had both ANA and SMA titres of 1:40 or less. All five patients (50%) who were diagnosed with AIH according to the simplified criteria were classified into probable diagnosis. The five patients had higher serum IgG levels [2986 (2630–3602) mg/dL vs. 1538 (1370–1724) mg/dL: $P=0.01$] than the other five patients.

3.8. Diagnosis according to the simplified criteria in ANA-negative (<1:40) patients

Of 24 ANA-negative patients, 4 (17%) showed histological acute hepatitis, 18 (75%) did not showed histological rosetting of liver cells, 6 (25%) had serum IgG levels under the upper normal limit. Thirteen patients (54%) were diagnosed with AIH according to the simplified criteria (9 patients with definite diagnosis). The 13 patients had higher serum IgG levels [2722 (2047–3602) mg/dL vs. 1654 (724–2906) mg/dL: $P=0.001$] and a higher frequency of SMA titres of 1:80 or greater (69% vs. 0%: $P=0.002$) than the other 11 patients.

3.9. Predictability of the simplified criteria

Of 168 patients with other liver disease than AIH, according to the original criteria, one with primary biliary cirrhosis had a score of 16 points. Furthermore, 10 patients (1 patient with chronic hepatitis B, 2 patients with chronic hepatitis C, 2 patients with primary biliary cirrhosis, 1 with primary sclerosing cholangitis, and 4 patients with nonalcoholic steatohepatitis) scored between 10 and 15 points. The remaining 157 patients scored <10 points. On the other hand, according to the simplified criteria, one with drug-induced liver injury had a score of 6 points and the other 167 were scored ≤ 5 points.

Sensitivity, specificity, positive predictive value, negative predictive value and accuracy for the diagnosis of AIH were 100%, 93%, 94%, 100% and 97%, respectively, according to the original criteria and 85%, 99%, 99%, 87% and 92%, respectively, according to the simplified criteria.

4. Discussion

The IAIHG proposed new simplified diagnostic criteria for AIH to facilitate the early diagnosis and the initiation of adequate immunosuppressive treatment in routine clinical practice [11]. In this study, 85% of patients diagnosed with AIH according to the original criteria were also diagnosed with AIH according to the simplified criteria. Thus, the simplified criteria are considered useful for the diagnosis of AIH. On the other hand, patients diagnosed according to the simplified criteria showed a higher frequency of ANA and/or SMA titres of 1:80 or greater and a slightly higher serum IgG levels than those diagnosed according to the original criteria. Approximately 20% of patients with atypical features, most of whom had serum IgG levels under the upper normal limit or both ANA and SMA titres of 1:40 or less, were not diagnosed with AIH according to the simplified criteria. Similarly to the report by Czaja [17], the simplified criteria showed greater specificity for a diagnosis of AIH than the original criteria in this study. AIH patients diagnosed according to the simplified criteria may have more typical features of the disease, and roles of autoantibodies and hypergammaglobulinemia for the diagnosis of AIH seem to be more important in the simplified criteria compared with in the original criteria.

Autoantibodies are still essential factors for a diagnosis of AIH; however ANA is negative in 20–30% of patients with type 1 AIH [18,19]. ANA-negative patients are not rare. Czaja [20] reported that 68% of ANA-positive patients lost their ANA during corticosteroid treatment, and that improvements in hypergammablobulinemia and histological necroinflammatory activity affected with the loss of ANA. They also reported that some patients who lost their ANA had recurrent positivity for ANA during relapse. ANA commonly disappear and reappear. On the other hand, in the IAIHG Report [4], the response to immunosuppressive treatment, especially relapse after an initial response, is a characteristic of AIH. Recently, we reported usefulness of the determination of ANA during the follow-up and the response to immunosuppressive treatment in the diagnosis of AIH with negativity for ANA at presentation [21]. The determination of ANA during the follow-up and the response to immunosuppressive treatment may be helpful and essential in order to confirm the diagnosis of AIH in patients negative for ANA at presentation.

In the original criteria, +2 points are assigned to female patients. In the diagnosis of AIH, the original criteria are advantageous to female patients compared with male patients. On the other hand, in the simplified criteria, gender is excluded from parameters associated with the diagnosis of AIH. However, in this study, 30% of the male patients diagnosed according to the original criteria were not diagnosed as AIH according to the simplified AIH. We considered that this was because 35% of male patients showed serum IgG levels under the upper normal limit and 26% had both ANA and SMA titres of 1:40 or less. Of the female patients diagnosed according to the original criteria, 15% showed serum IgG levels under the upper normal limit and 13% had ANA titres of 1:40 or less. Thus, 88% of the female patients were diagnosed as AIH according to the simplified AIH. A diagnosis of AIH for male patients may be distressful.

In this study, 5 of 10 AIH patients with histological acute hepatitis diagnosed according to the original criteria were not diagnosed with AIH according to the simplified criteria. Recently, the number of AIH patients with histological acute hepatitis has been increasing; however the diagnosis in these patients is not easy. They have lower serum IgG levels than those of AIH patients with chronic hepatitis [22,23]. Furthermore, the typical or compatible histological features of the simplified criteria do not include the features of acute hepatitis. In severe and fulminant forms of AIH, corticosteroid therapy is of little benefit, and many patients with these forms of AIH require liver transplantation [24]. In AIH patients with acute hepatitis, an accurate and prompt diagnosis is important. Thus, a new specific marker useful for the diagnosis of AIH with histological acute hepatitis is required.

In the simplified criteria, typical histology requires the presence of rosetting of liver cells. +2 points are assigned to patients

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