

2. ラミブジン投与による HBs 抗原消失

B型慢性肝疾患に対する最終目標であるHBs抗原の陰性化は、なかなか人為的に成し得るものではありません。自然経過でもHBs抗原の消失は起こり得ますが、実際の頻度については明確な答えがなく、また治療の介入による成績も一定した見解は得られていないのが現状であります。

LAMのHBs抗原消失に対する効果は当院の保坂らが解析しておりますので、一部を紹介いたします。LAMで治療を開始した850例以上の症例を投与期間中央値6.3年で検討しますと、33例(3.3%)にHBs抗原の消失が得られました。累積の結果を示したのが図4になりますが、10年間で7.3%に消失が認められました。この数字が高いのかどうかについては、今後さらなる検討を行わなければならないと考えますが、症例の背景をじっくりと見据えた上でうまく核酸アナログを使用することで、HBs抗原の消失を得ることも可能なことであると思われまます。

III. ラミブジン耐性ウイルスへの対策

当院では、これまでにのべにしますとB型慢性肝疾患症例1,000例以上に対してLAMの投与を行ってきましたが、5年で62%の症例に耐性ウイルスの出現が認められ、35%にBTHを認めております。これらの症例に対して当初はIFNの併用投与が行われ、2004年以降はADVの併用投与が行われ効果を上げてきました。詳しい成績はそれぞれの稿に譲りますが、現在も投与を行っており、今後、耐性ウイルスの出現が危惧される症例を、どのように診察していくかも大きな問題と考えます。

ひとたび耐性ウイルスが出現した場合は、肝炎の再燃が起こればADVを併用せざるを得ず、またETVに切り替えれば、さらなる耐性の出現を高頻度に起こすことは明らかであります。このような問題点に対しては、厚生労働省の研究班である熊田班より切り替えを含めた指針が提示されています。そして平成22年のガイドラインでは、大きな変更がなされ、LAM投与例においてはウイルス量が低値で安定しているうちにETVに切り替えるということが提唱されています。詳しくはガイドラインの稿(⊙ B型肝疾患の最新のガイドライン(89頁))を参照していただければと思いますが、この根拠となるものは平成22年に当院から報告しており、HBV DNAを三段階に分けてその耐性ウイルス出現率を示しております³⁾。

図5に示しますように、234例の大規模検討ですが、LAMによる抗ウイルス効果が

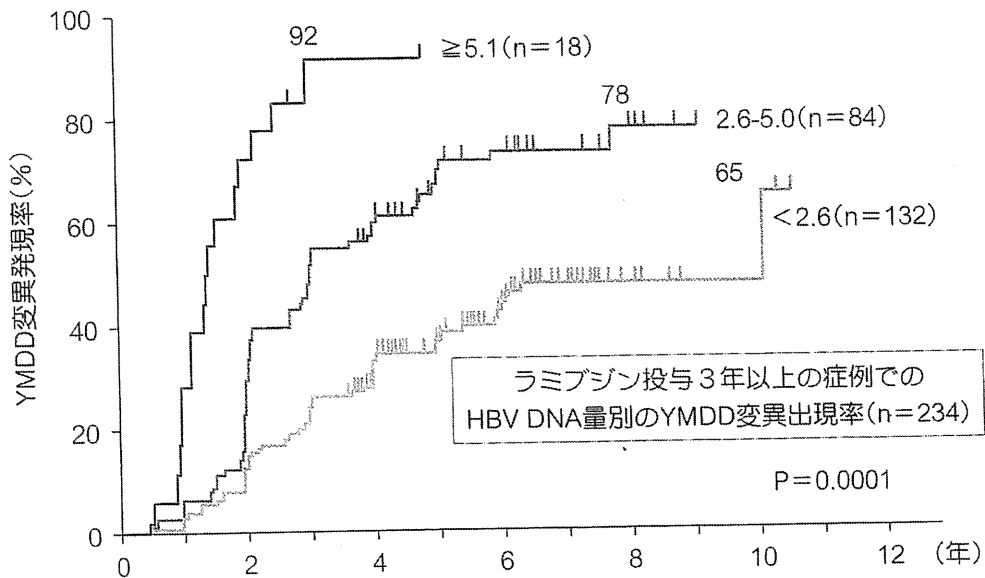


図5 ラミブジン長期投与症例のYMDD変異出現率

LAM投与により、HBV DNAが2.6 log copies/mL >に抑えられていても、YMDD変異は投与期間が長くなればなるほど出現してくることがわかります。
(文献3より改変引用)

持続し、HBV DNAが2.6 log copies/mL未満の状態を保っている間は、LAM耐性ウイルスの出現は少なくなっています。しかしながら、図5では、2.6 log copies/mL未満の症例でも5年を過ぎた後もさらに耐性ウイルスは出現し、YMDD変異は年を経るごとに増加し、肝炎の再燃が起こりうることを示しています。このような理由から、耐性が出る前にLAMをETVに切り替えるということが推奨されています。ありますが、個々の症例による事情がありますので、切り替えに当たっては十分な検討を行い、判断していくことが肝要であると思われます。

おわりに

B型慢性肝炎治療はLAMの登場により大きく様変わりしたといえますが、一方で耐性株の出現という問題も起こり、この10年の間にさらに変化を遂げました。本稿では、LAMの歴史を語るような形となってしまいましたが、HBs抗原消失の問題、発癌抑制の問題等、今後さらなる症例と時間の積み重ねがなければ解決し得ない問題も山積しており、やはり核酸アナログには、まだまだ筆者らの知らない問題点が

隠されているように感じざるを得ません。一例一例しっかりと経過をみながら、慎重に使用していくことが望ましいと考えております。

(鈴木 義之)

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Long-term effect of lamivudine treatment on the incidence of hepatocellular carcinoma in patients with hepatitis B virus infection

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Abstract

Background Nucleotide analogues have recently been approved for the treatment of patients with hepatitis B virus (HBV) infection. However, it is still controversial whether the decrease of HBV-DNA amount induced by treatment with nucleotide analogues can reduce the risk of hepatocellular carcinoma (HCC) development in HBV patients.

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Methods A total of 293 HBV patients without HCC who were treated with lamivudine (LAM) were enrolled in a multicenter trial. The incidence of HCC was examined after the start of LAM therapy, and the risk factors for liver carcinogenesis were analyzed. The mean follow-up period was 67.6 ± 27.4 months.

Results On multivariate analysis for HCC development in all patients, age ≥ 50 years, platelet count $< 14.0 \times 10^4/\text{mm}^3$, cirrhosis, and median HBV-DNA levels of ≥ 4.0 log copies/ml during LAM treatment were significant risk factors. The cumulative carcinogenesis rate at 5 years was

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3% in patients with chronic hepatitis and 30% in those with cirrhosis. For the chronic hepatitis patients, the log-rank test showed the significant risk factors related to HCC development to be age ≥ 50 years, platelet count $< 14.0 \times 10^4/\text{mm}^3$, and hepatitis B e antigen negativity, but median HBV-DNA levels of < 4.0 log copies/ml (maintained viral response, MVR) did not significantly suppress the development of HCC. In cirrhosis patients, however, the attainment of MVR during LAM treatment was revealed to reduce the risk of HCC development.

Conclusions These results suggest that the incidence of HCC in HBV patients with cirrhosis can be reduced in those with an MVR induced by consecutive LAM treatment.

Keywords Lamivudine · Chronic hepatitis B · Cirrhosis · Hepatocellular carcinoma · HBV-DNA level

Abbreviations

HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
LAM	Lamivudine
ADV	Adefovir
ETV	Entecavir
Hbs Ag	Hepatitis B surface antigen
PCR	Polymerase chain reaction
TMA	Transcription-mediated amplification
IVR	Initial viral response
MVR	Maintained viral response
HBe Ag	Hepatitis B e antigen
CT	Computed tomography
MRI	Magnetic resonance imaging
ALT	Alanine aminotransferase

Introduction

More than 350 million people worldwide suffer from chronic infection with hepatitis B virus (HBV) [1–3]. Chronic HBV infection eventually leads to the development of cirrhosis and hepatocellular carcinoma (HCC), and raises the risk of hepatic disease-related death [4–6]. In Japan, up to 15% of HCC patients are diagnosed with HBV-related liver disease [7].

HCC is one of the most common malignancies in Japan and its incidence has been increasing over the past 30 years. Recently, various treatments such as transcatheter arterial embolization/chemoembolization, radio-frequency ablation, and hepatic resection have been reported to yield significant improvements in overall patient survival [8–11]. However, HCC relapse has thus far been observed in a majority of treated patients due to its highly malignant potential. In this regard, successful treatment of chronic

HBV infection should prevent the patient's liver from progressing to cirrhosis and reduce the risk of HCC development. In recent years, the treatment of chronic hepatitis has changed greatly with the development of various antiviral therapies with nucleoside/nucleotide analogues such as lamivudine (LAM), adefovir (ADV), and entecavir (ETV) [12–15]. LAM has long been used against chronic hepatitis, and many reports have demonstrated that LAM is effective in stabilizing inflammatory activity, suppressing HBV-DNA replication, and improving liver histological findings in chronic hepatitis patients [16, 17] and in HBV-related cirrhosis patients [18]. Furthermore, LAM has been reported to reduce the incidence of HCC in patients with chronic hepatitis B [19]. However, it is still controversial whether or not treatment using nucleotide analogues can reduce the risk of HCC development in HBV-infected patients [20, 21], and the relationship between the effect of HBV suppression and HCC development during LAM treatment has not yet been discussed in detail. Also, the risk factors for HCC development in HBV-infected patients who have been treated with LAM have not been sufficiently evaluated. In this study, we aimed to clarify whether the decrease of HBV-DNA amount induced by LAM therapy could reduce the incidence of HCC in HBV-infected patients.

Patients and methods

Patient selection and study design

This study was conducted at Osaka University Hospital and other institutions participating in the Osaka Liver Forum in Japan. The subjects were 293 consecutive patients with HBV infection who underwent continuous LAM therapy for more than 24 weeks from September 2000 to September 2006. All patients tested positive for hepatitis B surface antigen (HBs Ag) or had detectable levels of HBV DNA in their sera according to findings from a polymerase chain reaction (PCR)-based method or a transcription-mediated amplification (TMA) method. Exclusion criteria were patients with anti-hepatitis C antibody, anti-human immunodeficiency virus antibody, and other liver diseases (alcoholic liver disease, drug-induced liver disease, and autoimmune hepatitis). Also excluded were patients with a history of HCC and those who developed HCC within the first 24 weeks of the follow-up period after the initiation of LAM therapy (because of the possibility that microscopic HCC had been present before the initiation of treatment).

All patients were treated with 100 mg of LAM daily. Of the 293 patients, 129 underwent ADV (10 mg/day) therapy in addition to receiving ongoing LAM treatment. For 43 patients who started ETV administration in lieu of LAM, the observation period was terminated when they started

ETV. LAM resistance was confirmed by virological breakthrough and was defined as an increase in serum HBV-DNA by $>1 \log_{10}$ greater than the nadir [22]. If virological breakthrough developed and alanine aminotransferase (ALT) was elevated over the upper normal limit, the patients received add-on ADV at 10 mg/day.

In this study, all patients were examined for serum HBV-DNA level just before therapy initiation and every 6 months during treatment. The initial viral response (IVR) was defined as HBV-DNA $<4.0 \log$ copies/ml in the first 24 weeks of the follow-up period after the initiation of LAM therapy, and the maintained viral response (MVR) was defined as median HBV-DNA levels of less than 4.0 log copies/ml measured every 6 months during therapy.

This study protocol followed the ethical guidelines of the Declaration of Helsinki amended in 2008, and informed consent was obtained from each patient.

HBV testing

HBs Ag, hepatitis B e antigen (HBe Ag) and anti-hepatitis B e antibody (anti-HBe) levels were examined by chemiluminescence immunoassay or enzyme immunoassay. HBV DNA was measured by a PCR-based method (Amplicor HBV monitor; Roche Diagnostics, Tokyo, Japan) or a TMA method (TMA-HPA; Fujirebio, Tokyo, Japan), which have lower detection limits of 2.6 and 3.7 log copies/ml, respectively. The LAM-resistant YMDD mutant virus was examined by a PCR-ELMA method. Serum samples were stored frozen at -80°C .

Diagnosis of HCC and cirrhosis

Ultrasonography was carried out before LAM therapy and every 3–6 months during the follow-up period. New space-occupying lesions detected or suspected at the time of ultrasonography were further examined by computed tomography (CT), magnetic resonance imaging (MRI), or hepatic angiography. HCC was diagnosed by the presence of typical hypervascular characteristics on angiography, in addition to the findings from CT or MRI. If no typical image of HCC was observed, fine-needle aspiration biopsy was carried out with the patient's consent or the patient was carefully followed until a diagnosis was possible with definite observation by CT, MRI, or hepatic angiography. Cirrhosis was diagnosed by liver biopsy or laparoscopy, and for patients without this information, by clinical data, imaging modalities, and portal hypertension.

Statistical analysis

Quantitative variables were expressed as means \pm SD. Quantitative variables at the baseline were compared

among two groups, the chronic hepatitis and cirrhosis groups, using the Mann–Whitney *U*-test. Categorical data, such as gender and status of HBe Ag, were compared using Fisher's exact test. The cumulative incidence of HCC was evaluated with a Kaplan–Meier curve and the differences between groups were analyzed by the log-rank test. For multivariate analysis to investigate factors affecting the cumulative incidence of HCC, Cox's regression analysis was carried out. A value of $p < 0.05$ (two-tailed) was considered to be statistically significant. All calculations were performed with SPSS version 15.0J (SPSS, Chicago, IL, USA).

Results

Baseline characteristics of patients

The baseline clinical features of the enrolled patients before LAM administration are shown in Table 1. The mean age of the patients was 48.0 ± 10.7 years, 214 (73%) of the entire group were male, and 163 (56%) tested positive for HBe Ag. Of the 293 patients, 205 (70%) were diagnosed as having chronic hepatitis and 88 (30%) as having cirrhosis. The median HBV-DNA level was 7.0 (range 3.0 to 8.5) log copies/ml. At baseline, the aspartate aminotransferase (AST) level was 131 ± 151 IU/l, the ALT level was 203 ± 252 IU/l, the total bilirubin level was 1.2 ± 1.6 mg/dl, the albumin (Alb) level was 3.8 ± 0.5 g/dl, and the platelet count was $13.7 \pm 5.4 \times 10^4/\text{mm}^3$. The mean follow-up period for all patients was 67.6 ± 27.4 months, with a range of 12–110 months from the start of LAM treatment. There were significant differences between patients with chronic hepatitis and those with liver cirrhosis in age, AST, ALT, total bilirubin, Alb, and platelet counts.

Cumulative incidence of development of HCC

Figure 1a shows the Kaplan–Meier curve of the cumulative HCC incidence for all HBV patients treated with LAM or LAM plus ADV. Of the 293 patients with HBV infection, 32 (10.9%) developed HCC and the cumulative carcinogenesis rate was 6% at 3 years, 12% at 5 years, and 15% at 7 years.

Figure 1b shows the Kaplan–Meier curve of the cumulative HCC incidence according to initial diagnosis (chronic hepatitis vs. cirrhosis). Eight (4%) of the 205 enrolled chronic hepatitis patients developed HCC and the cumulative carcinogenesis rate was 2% at 3 years, 3% at 5 years, and 5% at 7 years. On the other hand, 24 (27%) of the 88 enrolled cirrhosis patients developed HCC and the cumulative carcinogenesis rate was 15% at 3 years, 30% at 5 years, and 35% at 7 years.

Table 1 Patient characteristics

Factor	All	Chronic hepatitis	Cirrhosis	<i>p</i> value
<i>HBe</i> Ag Hepatitis B e antigen, <i>HBV</i> hepatitis B virus, <i>AST</i> aspartate aminotransferase, <i>ALT</i> alanine aminotransferase, <i>Alb</i> albumin				
Number of patients	293	205	88	
Age (years)	48.0 ± 10.7	46.3 ± 10.7	51.9 ± 9.8	<0.001**
Sex (male/female)	214/79	147/58	67/21	0.475
<i>HBe</i> Ag (positive)	163 (56%)	121 (59%)	42 (48%)	0.068
^a Values are expressed as medians				
<i>HBV</i> DNA (log copies/ml) ^a	7.0 (3.0 to 8.5<)	6.8±1.1	6.6 ± 1.1	0.162
<i>AST</i> (IU/l)	131 ± 151	143 ± 162	104 ± 120	0.045*
<i>ALT</i> (IU/l)	203 ± 252	235 ± 269	129 ± 189	<0.001**
Total bilirubin (mg/dl)	1.2 ± 1.6	0.9 ± 0.6	1.8 ± 2.7	<0.001**
<i>Alb</i> (g/dl)	3.8 ± 0.5	3.9 ± 0.4	3.5 ± 0.6	<0.001**
Platelets (×10 ⁴ /mm ³)	13.7 ± 5.4	15.6 ± 9.3	9.3 ± 3.8	<0.001**
Follow-up period (months)	67.6 ± 27.4	68.5 ± 26.5	65.5 ± 29.5	0.393

* $p < 0.05$, ** $p < 0.001$, comparing patients with chronic hepatitis and those with liver cirrhosis using the Mann–Whitney *U*-test for quantitative variables and Fisher's exact test for categorical variables

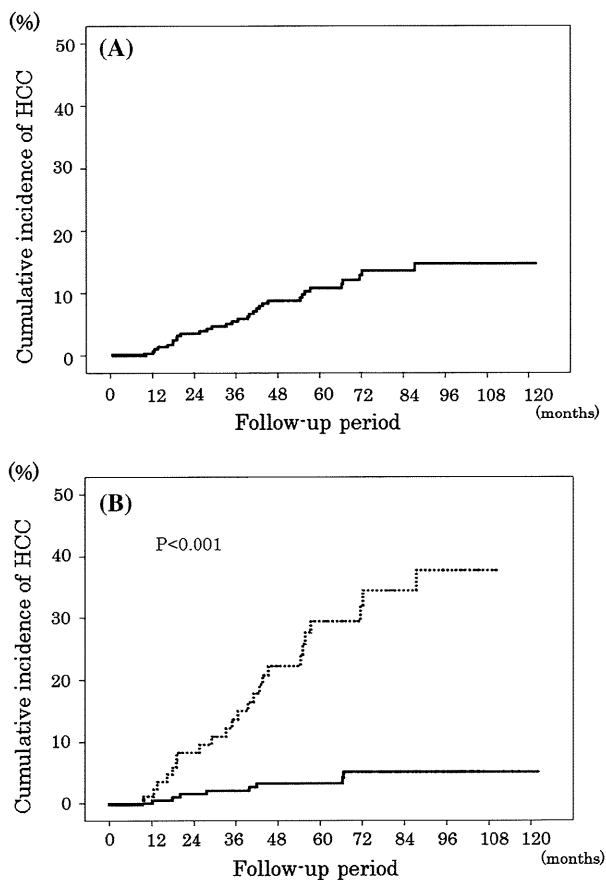


Fig. 1 Cumulative incidence of development of hepatocellular carcinoma (HCC) in patients with hepatitis B virus infection treated with lamivudine (LAM). **a** All cases; **b** chronic hepatitis or cirrhosis. Solid line Chronic hepatitis, dotted line cirrhosis

Risk factors for cumulative incidence of HCC development in all HBV-infected patients

Univariate analysis with the log-rank test was performed for all HBV-infected patients treated with LAM, with the

results shown in Table 2. Univariate analysis with the log-rank test showed that the following were significant risk factors for the development of HCC: older age (≥ 50 years) ($p < 0.001$), cirrhosis ($p < 0.001$), high total bilirubin level (> 1.2 g/dl) ($p = 0.004$), low *Alb* level (< 3.8 g/dl) ($p = 0.019$), low platelet count ($< 14 \times 10^4/\text{mm}^3$) ($p < 0.001$), and non-MVR ($p = 0.035$).

Stepwise multivariate analyses of four of these variables were performed by Cox's regression analysis for all patients treated with LAM with the results shown in Table 3. The analysis indicated the following factors as independent significant risk factors related to the development of HCC: age ≥ 50 years [hazard ratio (HR) 3.20, 95% confidence interval [CI] 1.08–9.53, $p = 0.036$], platelet count $< 14.0 \times 10^4/\text{mm}^3$ (HR 4.76, 95% CI 0.05–0.96, $p = 0.045$), cirrhosis (HR 4.64, 95% CI 1.75–12.4, $p = 0.002$), and non-MVR (HR 2.70, 95% CI 1.09–6.56, $p = 0.032$).

Cumulative incidence of and risk factors for HCC development in patients with chronic hepatitis and cirrhosis

The results of univariate analysis with the log-rank test for the development of HCC in chronic hepatitis patients treated with LAM are shown in Table 4, and the following were significant risk factors: older age (≥ 50 years) ($p = 0.002$), *HBe* Ag negativity ($p = 0.005$), and low platelet count ($< 14 \times 10^4/\text{mm}^3$) ($p = 0.004$). Suppression of median *HBV*-DNA levels to < 4.0 log copies/ml by LAM treatment was not associated with the development of HCC in the chronic hepatitis patients. Only non-MVR (median *HBV*-DNA amount ≥ 4.0 log copies/ml) was shown to be a significant risk factor for the development of HCC in the cirrhosis patients ($p = 0.029$), while the factors of age, *HBe* Ag status, and platelet count were not significant in these patients (Table 4).

Table 2 Risk factors for HCC development in all HBV-infected patients by univariate analysis

Factor	95% CI	p value
Age (years) (<50/≥50)	2.15–14.5	<0.001
Sex (male/female)	0.33–1.76	0.520
Initial diagnosis (chronic hepatitis/cirrhosis)	3.75–1.176	<0.001
HBe Ag (positive/negative)	0.31–1.29	0.209
HBV DNA (log copies/ml) (<7.0/>7.0)	0.33–1.35	0.262
AST (IU/l) (<40/≥40)	0.33–2.22	0.742
ALT (IU/l) (<40/≥40)	0.17–1.16	0.188
Total bilirubin (mg/dl) (<1.2/≥1.2)	1.43–6.72	0.004
Alb (g/dl) (<3.8/≥3.8)	0.19–0.86	0.019
Platelets (×10 ⁴ /mm ³) (<14/≥14)	0.02–0.31	<0.001
Emergence of LAM-resistant viruses (positive/negative)	0.51–2.03	0.968
IVR (positive/negative)	0.52–3.25	0.575
MVR (positive/negative)	1.04–5.95	0.035

HCC Hepatocellular carcinoma, HBV hepatitis B virus, CI confidence interval, HBe Ag hepatitis B e antigen, HBV hepatitis B virus, AST aspartate aminotransferase, ALT alanine aminotransferase, Alb albumin, IVR initial viral response, MVR maintained viral response, LAM lamivudine

Table 3 Risk factors for HCC development in all HBV-infected patients by multivariate analysis

Factor	Category	Risk ratio	95% CI	p value
Age (years)	<50	1	1.08–9.53	0.036
	≥50	3.20		
Initial diagnosis	Chronic hepatitis	1	1.75–12.4	0.002
	Cirrhosis	4.64		
Platelets (×10 ⁴ /mm ³) (<14/≥14)	≥14	1	0.05–0.96	0.045
	<14	4.76		
MVR	Negative	1	1.09–6.56	0.032
	Positive	0.37		

HCC Hepatocellular carcinoma, HBV hepatitis B virus, CI confidence interval, MVR maintained viral response

Cumulative incidence of HCC development according to effectiveness of treatment (MVR vs. non-MVR)

Figure 2a shows the Kaplan–Meier curve of cumulative HCC incidence in all HBV-infected patients treated with LAM according to the effectiveness of treatment (MVR vs. non-MVR). The cumulative carcinogenesis rate for MVR-positive patients was 2% at 3 years, 4% at 5 years, and 6% at 7 years. On the other hand, the cumulative carcinogenesis rate for MVR-negative patients was 5% at 3 years, 13% at 5 years, and 16% at 7 years. MVR during LAM significantly suppressed the cumulative HCC incidence

Table 4 Risk factors for HCC development by univariate analysis (chronic hepatitis/cirrhosis)

	95% CI	p value
Chronic hepatitis		
Age (years) (<50/≥50)	0.26–8.38	0.002
Sex (male/female)	0.37–6.42	0.556
HBe Ag (positive/negative)	0.01–0.74	0.005
HBV DNA (log copies/ml) (<7.0/≥7.0)	0.11–1.99	0.296
AST (IU/l) (<40/≥40)	0.11–2.64	0.482
ALT (IU/l) (<40/≥40)	0.06–1.41	0.101
Total bilirubin (mg/dl) (<1.2/≥1.2)	0.67–6.67	0.574
Alb (g/dl) (<3.8/≥3.8)	0.13–8.58	0.960
Platelets (×10 ⁴ /mm ³) (<14/≥14)	0.01–0.72	0.004
Emergence of LAM-resistant viruses (positive/negative)	0.27–4.28	0.927
IVR (positive/negative)	0.29–8.67	0.590
MVR (positive/negative)	0.51–37.10	0.144
Cirrhosis		
Age (years) (<50/≥50)	0.86–6.17	0.089
Sex (male/female)	0.21–1.82	0.380
HBe Ag (positive/negative)	0.80–4.17	0.149
HBV DNA (log copies/ml) (<7.0/≥7.0)	0.40–2.01	0.795
AST (IU/l) (<40/≥40)	0.27–3.07	0.873
ALT (IU/l) (<40/≥40)	0.13–1.47	0.167
Total bilirubin (mg/dl) (<1.2/≥1.2)	0.82–4.80	0.126
Alb (g/dl) (<3.8/≥3.8)	0.28–1.58	0.354
Platelets (×10 ⁴ /mm ³) (<14/≥14)	0.03–1.51	0.084
Emergence of LAM-resistant viruses (positive/negative)	0.44–2.18	0.948
IVR (positive/negative)	0.90–8.32	0.063
MVR (positive/negative)	1.07–0.029	

HCC Hepatocellular carcinoma, HBV hepatitis B virus, CI confidence interval, HBe Ag hepatitis B e antigen, HBV hepatitis B virus, AST aspartate aminotransferase, ALT alanine aminotransferase, Alb albumin, IVR initial viral response, MVR maintained viral response

compared with non-MVR in all HBV-infected patients ($p = 0.035$).

Figure 2b shows the Kaplan–Meier curve of the cumulative HCC incidence in chronic hepatitis patients according to the effectiveness of treatment (MVR vs. non-MVR). The cumulative carcinogenesis rate for MVR-positive patients was 0% at 3 years, 0% at 5 years, and 2% at 7 years. On the other hand, the cumulative carcinogenesis rate for MVR-negative patients was 2% at 3 years, 4% at 5 years, and 6% at 7 years. MVR during LAM did not significantly suppress the cumulative HCC incidence compared with non-MVR in the chronic hepatitis patients ($p = 0.144$).

Figure 2c shows the Kaplan–Meier curve of the cumulative HCC incidence in cirrhosis patients according to the effectiveness of treatment (MVR vs. non-MVR).

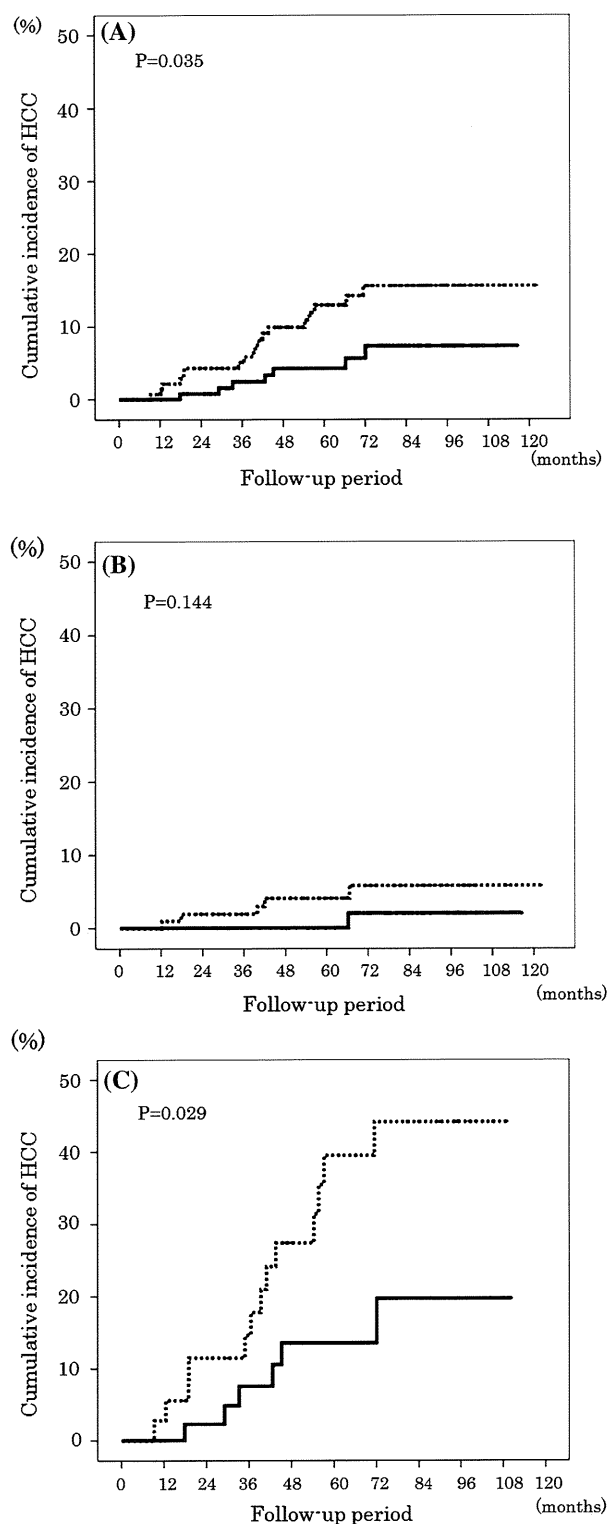


Fig. 2 Cumulative incidence of development of HCC according to the effectiveness of treatment (MVR vs. non-MVR). **a** All cases; **b** chronic hepatitis; **c** cirrhosis. Solid lines MVR, dotted lines non-MVR. MVR Maintained viral response

The cumulative carcinogenesis rate for MVR-positive patients was 8% at 3 years, 14% at 5 years, and 14% at 7 years. On the other hand, the cumulative carcinogenesis rate for MVR-negative patients was 18% at 3 years, 40% at 5 years, and 44% at 7 years. MVR during LAM significantly suppressed the cumulative HCC incidence compared with non-MVR in the cirrhosis patients ($p = 0.029$).

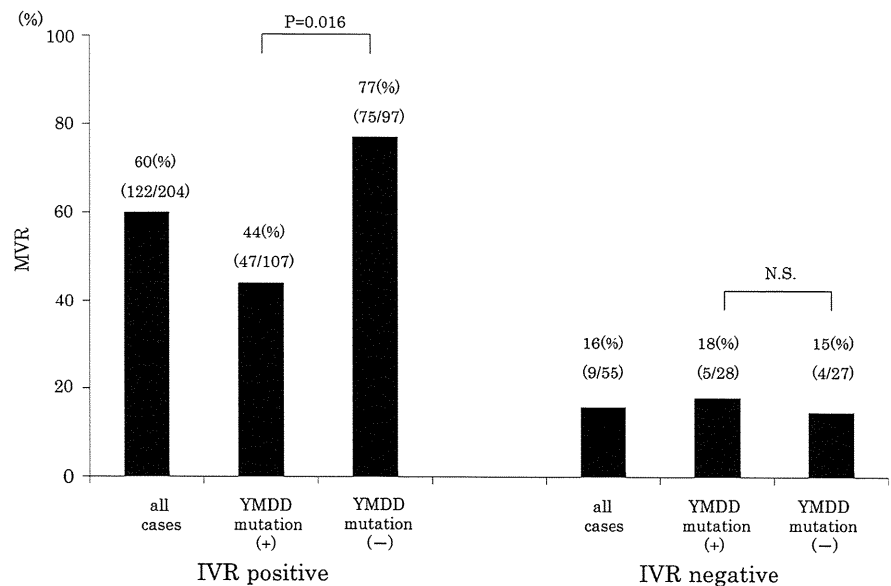
Relationship between IVR and MVR

Maintained viral response (MVR) was achieved by 142 (48%) of the 293 patients enrolled in this study. IVR was achieved by 204 (79%) of the 259 patients who were examined for IVR. The relationship between IVR and MVR is shown in Fig. 3; 60% (122/204) of the IVR-positive patients achieved an MVR, while only 16% (9/55) of the IVR-negative patients achieved an MVR ($p < 0.001$). The LAM-resistant YMDD mutant virus was found in 149 (51%) of all patients during follow-up, and in 52% (107/204) of the IVR-positive patients, a finding which was nearly equal to that for the IVR-negative patients (51%, 28/55). Among the IVR-positive patients, the MVR rate was lower in patients with the YMDD mutation, compared with that in those without the YMDD mutation (44%, 47/107 vs. 77%, 75/97, $p = 0.016$), while the MVR rates were low in the IVR-negative patients, irrespective of their YMDD mutation status (with and without the mutation, 15 vs. 18%, respectively). ADV was added to LAM treatment for 73 (68%) of the 107 IVR-positive patients with the YMDD mutation and 20 (36%) of the 55 IVR-negative patients with the YMDD mutation. However, MVR was only achieved at the low rates of 33% (24/73) for the former patients and 20% (4/20) for the latter.

Discussion

Lamivudine treatment has been shown to improve the liver histological findings in patients with HBV-infected liver disease by reducing the HBV load and stabilizing inflammatory activity [16–18]. One report has shown that LAM effectively reduced the incidence of HCC in patients with chronic hepatitis B, but the study only compared LAM-treated patients with non-treated patients in a matched case-controlled study [19]. However, there have been few detailed reports about the relationship between virological response and HCC development in HBV-infected patients during LAM treatment. In the present study, we retrospectively examined the incidence of HCC to clarify the indicators of LAM therapy, including median HBV-DNA levels, for reducing the risk of HCC in HBV-infected patients.

Fig. 3 Relationship between IVR and MVR. *IVR* Initial viral response, *MVR* maintained viral response, *N.S.* not significant



Many investigators have reported that serum HBV DNA levels higher than 4.0–4.5 log copies/ml before HBV treatment serve as a strong risk predictor of HCC [23–25]. Di Marco et al. [26] have reported that the incidence of HCC was higher in patients with serum HBV levels of more than 5.0 log copies/ml, at least once, during LAM therapy than in those in whom serum HBV levels were maintained at 5.0 log copies/ml or less. However, the add-on ADV therapy had not been adopted when the study of Di Marco et al. was reported. When the use of ADV is possible, an evaluation method is needed to measure the antiviral effects of nucleoside/nucleotide analogues against HBV-related liver disease. In the present study, we set the cut-off value for HBV-DNA at 4.0 log copies/ml. The basis of this cut-off value is that a serum HBV DNA level higher than 4.0 log copies/ml before HBV treatment was reported to serve as a strong risk predictor of HCC [23]. MVR, defined as a median HBV-DNA level of less than 4.0 log copies/ml measured every 6 months during therapy, was adopted as an indicator of viral replication, and non-MVR (median HBV-DNA >4.0 log copies/ml) during LAM therapy was shown to be significantly associated with the development of HCC in HBV-infected patients. We also found that a median HBV-DNA level of >4.0 log copies/ml during LAM therapy was a risk factor for HCC development. On the other hand, IVR, defined as HBV-DNA of <4.0 log copies/ml in the first 6 months of the follow-up period after the initiation of therapy, was not associated with the development of HCC in HBV patients in this study. As shown in Fig. 3, 84% of the IVR-negative patients could not achieve an MVR, suggesting that it is crucial to achieve an IVR in order to achieve an MVR. The reason why IVR was not a significant factor for MVR seemed to be the appearance of the YMDD mutation, which reduced the antiviral effect of

LAM for HBV in IVR-positive patients. The LAM-resistant YMDD mutant virus was found in 52% of the IVR-positive patients. Although ADV was added to LAM treatment for 73 patients, only 33% of these patients could achieve an MVR. We speculate that the antiviral effect of ADV is not very strong [27] and it takes time to reduce the YMDD mutant virus, which may explain the low MVR rate (33%) in patients with the add-on ADV therapy. The immediate administration of ADV when the LAM-resistant YMDD mutant virus appears can be important [28]. A switch to ETV, which induces resistant virus less frequently, could also raise MVR rates among IVR-positive patients without the YMDD mutant virus.

As the duration of the add-on ADV therapy was included in this study, we compared the cumulative incidence of HCC in patients receiving LAM monotherapy with that in patients who also received the add-on ADV therapy. Sixteen (10%) of the 164 patients who received the LAM monotherapy developed HCC and the cumulative carcinogenesis rate was 6% at 3 years, 10% at 5 years, and 15% at 7 years. On the other hand, 16 (12%) of the 129 patients who received LAM plus ADV developed HCC and the cumulative carcinogenesis rate was 6% at 3 years, 12% at 5 years, and 14% at 7 years. No significant difference was found between these two groups ($p = 0.986$). In addition, we examined the cumulative incidence of HCC development according to the effectiveness of treatment (MVR vs. non-MVR) in patients for whom the observation period was terminated when ADV was added, and the same results were obtained (data not shown).

Older age (≥ 50 years), cirrhosis, and low platelet count ($<14 \times 10^4/\text{mm}^3$) were shown to be significantly associated with the development of HCC in patients with HBV infection. These results were consistent with those of

previous reports [29–31], suggesting that patients of older age with advanced fibrosis should be followed up carefully for longer periods in order to detect early stages of HCC even if LAM therapy does effectively suppress HBV. Of note, in the present study we estimated the cumulative HCC incidence according to the initial diagnosis of chronic hepatitis or cirrhosis. In the chronic hepatitis patients, older age (≥ 50 years), HBe Ag negativity, and low platelet count ($< 14 \times 10^4/\text{mm}^3$) were significant risk factors for the development of HCC, but this was not the case in the cirrhosis patients. Because liver biopsies had not been performed, the liver fibrosis stage could not be evaluated with respect to the risk factors for HCC in this study. Instead, the factors of age, HBe Ag status, and platelet count may reflect the degree of liver fibrosis in chronic hepatitis patients. In fact, cirrhotic patients, in comparison with chronic hepatitis patients, were of older age (chronic hepatitis vs. cirrhosis: 46.3 ± 10.7 vs. 51.9 ± 9.8 years, $p < 0.001$), had higher rates of HBe Ag negativity (chronic hepatitis vs. cirrhosis: 39 vs. 51%, $p = 0.065$), and had lower platelet counts (chronic hepatitis vs. cirrhosis: 15.6 ± 4.9 vs. $9.3 \pm 3.8 \times 10^4/\text{mm}^3$, $p < 0.001$). This seems to explain why none of these factors were significant risk factors for HCC in cirrhotic patients. On the other hand, in the chronic hepatitis patients, MVR was not a significant factor for HCC development, while MVR was a significant factor for HCC development in the cirrhotic patients. We speculate that HBV suppression induced by LAM therapy could reduce the incidence of HCC in patients infected with HBV, especially those with cirrhosis, who displayed higher malignant potential. Investigation over a longer period is needed to clarify the effect of HBV suppression on the development of HCC in chronic hepatitis patients.

In conclusion, the present study shows that the attainment of an MVR induced by LAM therapy has a significant beneficial effect on the clinical course of HBV-infected patients by decreasing the incidence of HCC. The newer nucleotide analogues, such as ETV and tenofovir, should be able to further reduce the incidence of HCC, given their greater potency.

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Conflict of interest The authors declare that they have no conflict of interest.

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Reducing Peg-IFN doses causes later virologic response or no response in HCV genotype 1 patients treated with Peg-IFN alfa-2b plus ribavirin

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Abstract

Background The timing to the first undetectable hepatitis C virus (HCV) RNA level is strongly associated with sustained virologic response in pegylated interferon (Peg-IFN) plus ribavirin combination therapy for patients with chronic hepatitis C (CH-C) with genotype 1. This study was conducted to clarify the impact of drug exposure to Peg-IFN on the timing of HCV RNA negativity in Peg-IFN plus ribavirin combination therapy for CH-C patients with genotype 1.

Methods A total of 1409 patients treated with Peg-IFN alfa-2b plus ribavirin were enrolled and classified into four categories according to the Peg-IFN dosage. Furthermore, 100 patients were extracted from each Peg-IFN dosage category to adjust for characteristic factors, using the propensity score method.

Results Peg-IFN exposure was dose-dependently associated with the timing of HCV RNA negativity ($p \leq 0.001$). The HCV RNA negative rate at week 4 decreased from 12% with a Peg-IFN dose of $>1.5 \mu\text{g}/\text{kg}/\text{week}$ to 1–3% with a dose of $<1.5 \mu\text{g}/\text{kg}/\text{week}$ ($p \leq 0.001$), and at week 12 the rate had decreased from 44% with a dose of

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≥ 1.2 $\mu\text{g}/\text{kg}/\text{week}$ to 18% with a dose of < 1.2 $\mu\text{g}/\text{kg}/\text{week}$ ($p = 0.001$). Treatment failure (patients without a 1-log decrease of HCV RNA at week 4 or a 2-log decrease of HCV RNA at week 12, or positive at week 24) was found in 54–66% of patients given < 1.2 $\mu\text{g}/\text{kg}/\text{week}$ ($p \leq 0.001$), and these patients accounted for 64% of the non-responders.

Conclusions The timing of HCV RNA negativity depends significantly on the Peg-IFN dose. Reducing the Peg-IFN dose can induce a later virologic response or non-response in HCV genotype 1 patients treated with Peg-IFN plus ribavirin.

Keywords Chronic hepatitis C · Pegylated interferon plus ribavirin · Drug adherence · HCV RNA negativity · Propensity score matched study

Introduction

The timing to the first undetectable hepatitis C virus (HCV) RNA level during pegylated interferon (Peg-IFN) plus ribavirin combination therapy for patients with chronic hepatitis C (CH-C) genotype 1 is strongly associated with a sustained virologic response (SVR), defined as undetectable HCV RNA at 24 weeks after the finishing of the treatment. The SVR rate was 87–100% in patients with undetectable HCV RNA at week 4, 73–81% at week 12, and 14–44% between weeks 12 and 24 in patients receiving the standard 48-week treatment [1–8]. These results suggest that HCV RNA negativity should be achieved as soon as possible during treatment in order to attain a higher SVR rate.

Previous studies have revealed that many factors affect the complete virologic response (c-EVR) and SVR, such as age, gender, degree of liver fibrosis, HCV genotype, HCV viral load, and the amount of drug exposure [1–3, 9–16]. Of these factors, only the amount of drug exposure can be controlled in order to try to improve the antiviral effect, as the other factors are fixed for individual patients. Also, recently, the single-nucleotide polymorphisms (SNPs) of the *IL28B* gene have been revealed to be associated with the antiviral effects of pegylated interferon-alpha and ribavirin therapy [17–19].

Peg-IFN has been reported to be dose-dependently correlated with c-EVR [13]. Patients' characteristic factors can be related to drug adherence, as aged patients, female patients, and patients with progression of liver fibrosis have a tendency to show low drug adherence. This suggests that patients with low drug adherence could be those who are difficult to treat. Therefore, patients with similar characteristic factors should be compared in order to precisely assess the actual impact of drug exposure on the timing to the first undetectable HCV RNA level.

Only a few randomized controlled trials (RCTs) have examined the relationship between drug dose reduction and antiviral effect with Peg-IFN plus ribavirin combination therapy [1–3, 20–23], and the findings are controversial. Manns et al. [1] reported that the SVR rate was significantly lower in patients given 0.5 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN than in those given 1.5 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN (34 vs. 42%, $p < 0.05$). McHutchison et al. [3] reported that the SVR rate did not differ between groups given 1.0 and 1.5 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN (38 vs. 40%, $p = 0.20$). No detailed study of the relationship between dose reduction and delay of HCV RNA negativity or the relationship between dose reduction and an increase of non-responders to the treatment has been reported, and the real impact of drug exposure on the anti-viral effect remains unclear.

In this present work, we conducted a matched study in which characteristic factors other than drug exposure were adjusted using propensity scores. We investigated the impact of drug exposure to Peg-IFN on the timing to the first undetectable HCV RNA level.

Patients and methods

Patients

The present study was a retrospective, multicenter trial conducted by Osaka University Hospital and other institutions participating in the Osaka Liver Forum. A total of 1409 Japanese patients with CH-C treated with a combination of Peg-IFN alfa-2b plus ribavirin were enrolled in this study between December 2004 and July 2008.

Patients eligible for this study were those who were infected with HCV genotype 1 and had a viral load of $\geq 10^5$ IU/ml, but were negative for hepatitis B surface antigen and anti-human immunodeficiency virus. Patients were excluded from this study if they had decompensated cirrhosis or other forms of liver disease (alcoholic liver disease, autoimmune hepatitis). Informed consent was obtained from each patient included in this study, which was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki.

Treatment

All patients received Peg-IFN alfa-2b (PEGINTRON; Schering-Plough, Kenilworth, NJ, USA) plus ribavirin (REBETOL; Schering-Plough). Peg-IFN alfa-2b was given subcutaneously once weekly at a dosage of 60–150 μg based on body weight (body weight 35–45 kg, 60 μg ; 46–60 kg, 80 μg ; 61–75 kg, 100 μg ; 76–90 kg, 120 μg ; 91–120 kg, 150 μg) and ribavirin was given orally twice a day at a total dose of 600–1000 mg/day based on body weight

(body weight <60 kg, 600 mg; 60–80 kg, 800 mg; >80 kg, 1000 mg), according to the standard treatment protocol for Japanese patients.

Dose reduction

As a rule, dose modification, which was performed according to the intensity of the adverse hematologic effects, was done by following the manufacturer's drug information. The dose of Peg-IFN alfa-2b was reduced to 50% of the assigned dose if the white blood cell (WBC) count declined to $<1500/\text{mm}^3$, the neutrophil count declined to $<750/\text{mm}^3$, or the platelet (Plt) count declined to $<8 \times 10^4/\text{mm}^3$, and was discontinued if the WBC count declined to $<1000/\text{mm}^3$, the neutrophil count declined to $<500/\text{mm}^3$, or the Plt count declined to $<5 \times 10^4/\text{mm}^3$. Ribavirin was also reduced from 1000 to 600 mg, or from 800 to 600 mg, or from 600 to 400 mg if the hemoglobin (Hb) level decreased to $<10 \text{ g/dl}$, and was discontinued if the Hb level decreased to $<8.5 \text{ g/dl}$.

Virologic assessment and definition of virologic response

Serum HCV RNA level was quantified using the COBAS AMPLICOR HCV MONITOR test, version 2.0 (detection range 6–5000 KIU/ml; Roche Diagnostics, Branchburg, NJ, USA) and qualitatively analyzed using the COBAS AMPLICOR HCV test, version 2.0 (lower limit of detection 50 IU/mL). The HCV RNA level was evaluated every 4 weeks during treatment. A rapid virologic response (RVR) was defined as undetectable serum HCV RNA at week 4, a c-EVR as undetectable serum HCV RNA at week 12, and a late virologic response (LVR) as detectable HCV RNA at week 12 but undetectable at week 24. Patients with $<a$ 1-log decrease in the HCV RNA level at week 4 or $<a$ 2-log decrease at week 12 compared with the baseline or detectable HCV RNA at week 24 were considered to have experienced treatment failure (non-response, NR) and had to stop treatment. If patients discontinued the treatment due to adverse events, without HCV RNA negativity being attained, they were also regarded as having had treatment failure.

Assessment of drug exposure

The amounts of Peg-IFN alfa-2b and ribavirin actually taken were evaluated by reviewing the medical records and calculating the amount taken from the start until the timing of the first undetectable HCV RNA level for the patients achieving HCV RNA negativity, and calculating the amount taken throughout the treatment for the patients not attaining HCV RNA negativity. For patients who

discontinued the treatment, if their HCV RNA had become negative before discontinuation, the drug amount data were calculated from the start of treatment until the timing of the first undetectable HCV RNA level, and if HCV RNA had not become negative before discontinuation, the data throughout the treatment before discontinuation were used. The amounts of both drugs were divided individually on the basis of body weight at baseline as the average: Peg-IFN alfa-2b was expressed as $\mu\text{g/kg/week}$ and ribavirin as mg/kg/day .

Evaluation of impact of drug exposure on HCV RNA negativity

We evaluated the relationship between the exposure to both drugs and HCV RNA negativity at week 24 by univariate and multivariate analyses for the patients who completed 24 weeks of treatment, using the mean administration doses of both drugs during the first 24 weeks and the characteristic factors other than drug exposure at baseline.

The patients were divided into four categories according to the Peg-IFN dose: up to $0.9 \mu\text{g/kg/week}$ of Peg-IFN; from 0.9 to less than $1.2 \mu\text{g/kg/week}$; from 1.2 to less than $1.5 \mu\text{g/kg/week}$; and from $1.5 \mu\text{g/kg/week}$. The propensity score matching method was used to adjust the patients' characteristic factors among these categories. This score was calculated for each patient by logistic regression analysis, with four patient characteristic factors as independent variables; age, gender, Plt values, and history of IFN treatment. We then performed 1:1 nearest neighbor matching within a caliper of 0.15 standard deviation of the propensity score: one patient in each group with $0.9\text{--}1.2 \mu\text{g/kg/week}$, $1.2\text{--}1.5 \mu\text{g/kg/week}$, and $\geq 1.5 \mu\text{g/kg/week}$ to one patient with $<0.9 \mu\text{g/kg/week}$, and extracted 100 patients from each category.

Statistical analysis

Baseline data for various demographic, biochemical, and virologic characteristics of the patients were expressed as means \pm SD or median values. Factors associated with HCV RNA negativity at week 24 were assessed by univariate analysis using the Mann–Whitney U -test or the χ^2 test, and by multivariate analysis using logistic regression analysis. To analyze the difference between baseline data among the four Peg-IFN groups, analysis of variance (ANOVA) or the χ^2 test was performed. The significance of trends in values for the timing to the first undetectable HCV RNA level was determined with the Mantel–Haenszel χ^2 test. A two-tailed p value of <0.05 was considered significant. Statistical analysis was conducted with SPSS version 15.0J (SPSS, Chicago, IL, USA).

Table 1 Baseline characteristics of patients before matching

Factor	All patients	<0.9 µg/kg/week of Peg-IFN	0.9–1.2 µg/kg/week of Peg-IFN	1.2–1.5 µg/kg/week of Peg-IFN	≥1.5 µg/kg/week of Peg-IFN	<i>p</i> value
Number	1409	153	159	670	427	
Age (years)	56.3 ± 10.4	58.0 ± 9.9	57.3 ± 10.2	55.9 ± 10.6	56.3 ± 10.4	0.069
Sex: male/female	722/687	70/83	69/90	376/294	207/220	0.004
History of IFN treatment: naïve/experienced	862/547	98/55	96/63	408/262	260/167	0.894
White blood cells (/mm ³)	5060 ± 1532	4325 ± 1419	4566 ± 1394	5246 ± 1562	5215 ± 1456	<0.001
Neutrophils (/mm ³)	2578 ± 1073	2129 ± 1049	2258 ± 949	2699 ± 1080	2667 ± 1052	<0.001
Red blood cells (×10 ⁴ /mm ³)	440 ± 46	424 ± 42	429 ± 45	445 ± 46	441 ± 45	<0.001
Hemoglobin (g/dl)	14.0 ± 1.4	13.6 ± 1.2	13.7 ± 1.5	14.1 ± 1.4	14.1 ± 1.4	<0.001
Platelets (×10 ⁴ /mm ³)	16.3 ± 5.6	11.9 ± 3.9	13.2 ± 4.8	17.4 ± 5.7	17.4 ± 5.2	<0.001
ALT (IU/l)	78 ± 61	93 ± 64	87 ± 68	75 ± 58	73 ± 60	0.001
Serum HCV RNA (KIU/ml) ^a	1450	1300	1900	1700	1900	0.176
Histology (METAVIR) ^b						
Fibrosis, 0–2/3–4 (%) ^c	810/186 (17%)	78/34 (30%)	70/30 (30%)	392/77 (16%)	270/45 (14%)	<0.001
Activity, 0–1/2–3	527/468	43/67	38/62	259/210	187/129	<0.001

ALT alanine aminotransferase, HCV hepatitis C virus, IFN interferon, Peg-IFN pegylated interferon

^a Data shown are median values

^b Data missing for 413 patients

^c Percent of patients with 3–4

Results

Clinical characteristics of all patients according to Peg-IFN dosage before matching

A total of 1409 patients were enrolled in this study, and the baseline characteristics of the patients are shown in Table 1. Based on the Peg-IFN dosage, these patients were classified into four categories. With the decrease of Peg-IFN dosage, the ratio of female-to-male patients increased, the peripheral blood cell count decreased, and the number of patients with progression of liver fibrosis (METAVIR fibrosis score 3 or 4) increased significantly ($p < 0.001$). Patients with a lower Peg-IFN dosage tended to be older ($p = 0.07$).

Next, we analyzed the factors associated with HCV RNA negativity at week 24 for the 1226 patients who completed 24 weeks of treatment, using the baseline characteristic variables, excluding liver histology, shown in Table 1 and the mean doses of both drugs during the first 24 weeks. The HCV RNA negative rate at week 24 was 68% (829/1226). The results of univariate analysis are shown in Table 2. The factors evaluated by multivariate analysis were those for which the p value was <0.10 by univariate analysis for HCV RNA negativity at week 24: age, gender, history of IFN treatment, WBC, neutrophils, red blood cells (RBC), Hb, Plt, alanine aminotransferase, and the mean doses of Peg-IFN and ribavirin during the first 24 weeks. By the multivariate analysis, in addition to the RBC value ($p = 0.02$), Plt value ($p < 0.001$), and

Table 2 Univariate analysis of factors associated with HCV RNA negativity at week 24

Factor	Negative	Positive	<i>p</i> value
Number	829	397	
Age (years)	55.1 ± 10.5	57.6 ± 10.1	<0.001
Sex: male/female	437/392	189/208	0.094
History of IFN treatment: naïve/experienced	523/306	229/168	0.069
White blood cells (/mm ³)	5175 ± 1498	4566 ± 1394	<0.001
Neutrophils (/mm ³)	2665 ± 1087	2429 ± 1059	<0.001
Red blood cells (×10 ⁴ /mm ³)	445 ± 44	434 ± 47	<0.001
Hemoglobin (g/dl)	14.1 ± 1.4	13.9 ± 1.4	0.004
Platelets (×10 ⁴ /mm ³)	17.2 ± 5.6	14.8 ± 5.3	<0.001
ALT (IU/l)	73 ± 55	83 ± 63	0.001
Serum HCV RNA (KIU/ml)	1750	1800	0.673
Mean Peg-IFN dose (µg/kg/week)	1.39 ± 0.24	1.25 ± 0.32	<0.001
Mean ribavirin dose (mg/kg/day)	10.6 ± 1.8	9.7 ± 2.2	<0.001

ALT alanine aminotransferase, HCV hepatitis C virus, IFN interferon, Peg-IFN pegylated interferon

history of IFN treatment ($p = 0.04$), the factor of Peg-IFN exposure was an independent factor for HCV RNA negativity at week 24 ($p < 0.001$) (Table 3). The mean dose of ribavirin did not show a significant correlation with HCV RNA negativity at week 24 ($p = 0.07$).

Clinical characteristics of patients extracted from each Peg-IFN dosage category after matching

Patients in the four Peg-IFN categories were matched by the propensity score method and 100 patients were extracted from each category. The c-statistics for the propensity score model between the patients with <0.9 µg/kg/week of Peg-IFN and those given different levels of Peg-IFN were 0.62 for 0.9–1.2 µg/kg/week of Peg-IFN, 0.82 for 1.2–1.5 µg/kg/week of Peg-IFN, and 0.82 for ≥1.5 µg/kg/week of Peg-IFN.

The baseline characteristics of the patients extracted according to the Peg-IFN dosage category are shown in Table 4. There was no significant difference among the four Peg-IFN categories in any of the factors, indicating that the extracted cohort of 400 patients was well matched according to propensity score methods.

Table 3 Multivariate analysis of factors associated with HCV RNA negativity at week 24

Factor	Category	Odds ratio	95% CI	p value
Age	1 year	–	–	NS
Sex	Male/female	–	–	NS
History of IFN treatment	Naïve/experienced	0.756	0.581–0.984	0.037
White blood cells	1 × 10 ³ /mm ³	–	–	NS
Neutrophils	1 × 10 ³ /mm ³	–	–	NS
Red blood cells	1 × 10 ⁴ /mm ³	1.004	1.001–1.007	0.02
Hemoglobin	1 g/dl	–	–	NS
Platelets	1 × 10 ⁴ /mm ³	1.054	1.026–1.083	<0.001
ALT	1 IU/l	–	–	NS
Mean Peg-IFN dose	0.1 µg/kg/week	1.096	1.045–1.149	<0.001
Mean ribavirin dose	1 mg/kg/day	1.060	0.994–1.130	0.074

ALT alanine aminotransferase, CI confidence interval, IFN interferon, NS not significant, Peg-IFN pegylated interferon

Timing to the first undetectable HCV RNA level according to Peg-IFN dosage

We evaluated the relationship between the virologic response during the treatment and the drug exposure to Peg-IFN using our matched cohort of 400 patients (Fig. 1). Of the 400 patients, 23 had discontinued treatment due to adverse events by week 24 (<0.9 µg/kg/week, n = 5; 0.9–1.2 µg/kg/week, n = 4; 1.2–1.5 µg/kg/week, n = 5; ≥1.5 µg/kg/week, n = 9). The proportion of patients with treatment failure increased according to the decrease in the dose of Peg-IFN: 66% among patients with <0.9 µg/kg/week of Peg-IFN, 54% among those with 0.9–1.2 µg/kg/week of Peg-IFN, 35% among those with 1.2–1.5 µg/kg/week of Peg-IFN, and 32% among those with ≥1.5 µg/kg/week of Peg-IFN (p < 0.001). Additionally, the timing to the first undetectable HCV RNA level tended to shift to an earlier time

Table 4 Baseline characteristics of patients after matching

Factor	All patients	<0.9 µg/kg/week of Peg-IFN	0.9–1.2 µg/kg/week of Peg-IFN	1.2–1.5 µg/kg/week of Peg-IFN	>1.5 µg/kg/week of Peg-IFN	p value
Number	400	100	100	100	100	
Age (years)	56.9 ± 9.6	57.4 ± 10.0	56.6 ± 9.9	56.8 ± 9.5	56.7 ± 9.1	0.941
Sex: male/female	190/210	47/53	47/53	46/54	50/50	0.948
History of IFN treatment: naïve/experienced	286/114	70/30	71/29	73/27	72/28	0.970
White blood cells (/mm ³)	4557 ± 1344	4331 ± 1310	4532 ± 1372	4642 ± 1399	4725 ± 1280	0.186
Neutrophils (/mm ³)	2261 ± 955	2070 ± 855	2200 ± 883	2416 ± 1068	2357 ± 972	0.054
Red blood cells (×10 ⁴ /mm ³)	429 ± 43	423 ± 40	427 ± 42	432 ± 44	434 ± 46	0.300
Hemoglobin (g/dl)	13.8 ± 1.5	13.7 ± 1.3	13.7 ± 1.6	13.9 ± 1.4	14.0 ± 1.5	0.300
Platelets (×10 ⁴ /mm ³)	12.1 ± 3.7	11.8 ± 3.9	12.0 ± 3.8	12.1 ± 3.5	12.4 ± 3.6	0.625
ALT (IU/l)	89 ± 67	98 ± 65	92 ± 70	86 ± 61	80 ± 69	0.254
Serum HCV RNA (KIU/ml) ^a	1700	1400	1800	1700	1750	0.742
Histology (METAVIR) ^b						
Fibrosis, 0–2/3–4 (%) ^c	186/89 (32%)	47/22 (32%)	46/22 (32%)	48/21 (30%)	45/24 (35%)	0.958
Activity, 0–1/2–3	115/159	25/42	26/42	32/37	32/38	0.585

ALT alanine aminotransferase, HCV hepatitis C virus, IFN interferon, Peg-IFN pegylated interferon

^a Data shown are median values

^b Data missing for 125 patients

^c Percent of patients with 3–4

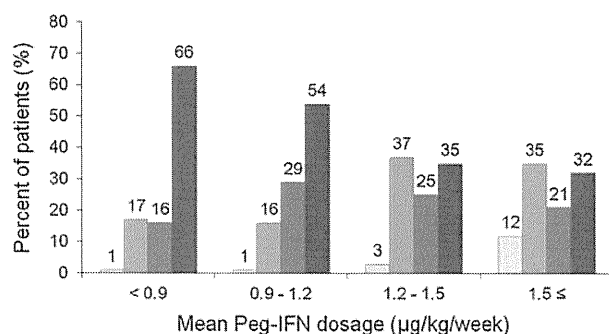


Fig. 1 Timing to the first undetectable hepatitis C virus (HCV) RNA level according to pegylated interferon (*Peg-IFN*) dosage. *Light gray bars* patients with undetectable HCV RNA at week 4. *Medium gray bars* patients with undetectable HCV RNA during 5 to 12 weeks. *Dark gray bars* patients with undetectable HCV RNA during 13–24 weeks. *Black bars* patients with treatment failure (patients with less than a 1-log decrease in HCV RNA level at week 4 or less than a 2-log decrease at week 12 compared with the baseline or detectable HCV RNA at week 24 and those with treatment discontinuance without HCV RNA negativity). Peg-IFN exposure was dose-dependently associated with the timing of HCV RNA negativity ($p \leq 0.001$)

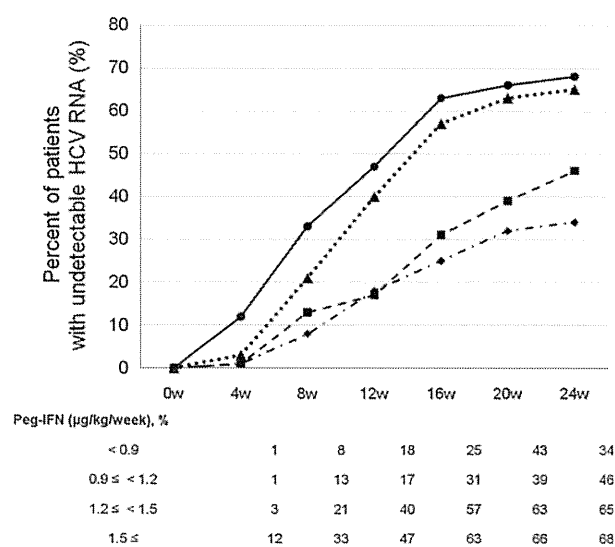


Fig. 2 Longitudinal negative HCV RNA rates from the start to 24 weeks of the treatment. *Filled circles* Peg-IFN ≥ 1.5 μg/kg/week, *filled triangles* Peg-IFN 1.2–1.5 μg/kg/week, *filled squares* Peg-IFN 0.9–1.2 μg/kg/week, *filled diamonds* Peg-IFN < 0.9 μg/kg/week. The HCV RNA negative rate at week 4 was significantly higher among the patients with Peg-IFN ≥ 1.5 μg/kg/week than among those with Peg-IFN < 1.5 μg/kg/week ($p \leq 0.001$). The HCV RNA negative rates at weeks 12 and 24 were significantly higher among the patients with Peg-IFN ≥ 1.2 μg/kg/week than among those with Peg-IFN < 1.2 μg/kg/week ($p = 0.001$, $p = 0.002$, respectively). *w* week

during the treatment according to the increase in the Peg-IFN dose ($p \leq 0.001$).

Figure 2 shows the longitudinal data of the HCV RNA negative rate. The data for patients with treatment failure were included until the end of each patient's treatment. The

percentage of patients with undetectable HCV RNA at week 4 decreased from 12 to 1–3% if they were given < 1.5 μg/kg/week of Peg-IFN ($p \leq 0.001$). As for the HCV RNA negative rates at week 12 and week 24, there was no significant difference between patients with 1.2–1.5 μg/kg/week and those with > 1.5 μg/kg/week of Peg-IFN. The two groups with < 1.2 μg/kg/week of Peg-IFN showed significantly lower HCV RNA negative rates than the other two groups given ≥ 1.2 μg/kg/week of Peg-IFN (week 12, 18 vs. 44%, $p = 0.0001$, week 24, 40 vs. 67%, $p = 0.0002$). The patients with < 0.9 μg/kg/week tended to show a decreased HCV RNA negative rate at week 24 compared to the patients given 0.9–1.2 μg/kg/week (34 vs. 46%, $p = 0.08$).

Figure 3 shows the proportion of patients, according to Peg-IFN exposure, among those with undetectable HCV RNA at week 4 ($n = 17$) and at week 12 ($n = 122$), as well as the proportion of patients with detectable HCV RNA at week 24 ($n = 213$). The patients given ≥ 1.5 μg/kg/week of Peg-IFN accounted for 70% of the patients with undetectable HCV RNA at week 4, and those given ≥ 1.2 μg/kg/week of Peg-IFN accounted for 71% of the patients with undetectable HCV RNA at week 12. On the other hand, the patients given < 1.2 μg/kg/week of Peg-IFN accounted for 64% of the patients with detectable HCV RNA at week 24.

Discussion

The association between drug exposure and HCV RNA negativity has been reported [9–13]. However, most studies have shown only the fixed-point relationship at week 12, and it remains unclear whether dose modification accelerates or delays the timing to the first undetectable HCV RNA level. The present study is the first to clarify this. Induction regimens in which a high dose (360 μg/week) of Peg-IFN alfa-2a was administered for the first 12 weeks failed to improve SVR rates compared to treatment with a standard dose of Peg-IFN in the CHARIOT [22] and PROGRESS [23] RCTs. In contrast, the adherence study of McHutchison et al. revealed that patients who received $\geq 80\%$ of the planned dose of Peg-IFN and ribavirin for $\geq 80\%$ of the full 48 weeks of treatment had a significantly higher SVR rate (51%) than those who received $< 80\%$ of the planned dose of one or both drugs for $\geq 80\%$ of the full 48 weeks of treatment (34%) ($p = 0.011$) [10]. These apparently paradoxical results for the relationship between drug dosage and antiviral effect imply that the dose-dependent increase of the antiviral effect was observed up to a certain dose and the antiviral effect then reached a plateau above the regular dose. This paradoxical effect could explain the impact of Peg-IFN reduction from a regular dose on the timing to the first undetectable HCV

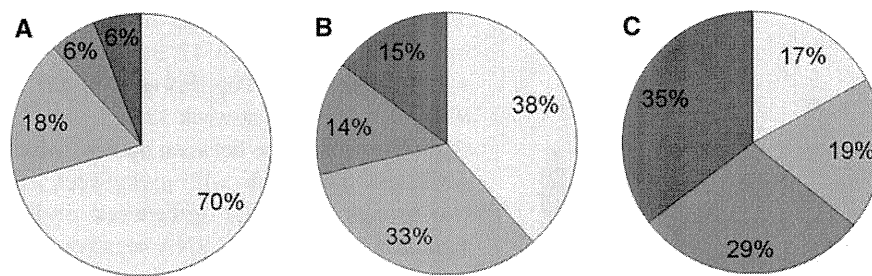


Fig. 3 Proportions of patients according to Peg-IFN exposure among patients with undetectable HCV RNA at weeks 4 and 12 and those with detectable HCV RNA at week 24. **a** Week 4 ($n = 17$), **b** week 12 ($n = 122$), **c** week 24 ($n = 213$). *Light gray segments* Peg-IFN

*<math><0.9 \mu\text{g/kg/week}</math>. *Medium gray segments* Peg-IFN $0.9\text{--}1.2 \mu\text{g/kg/week}$. *Dark gray segments* Peg-IFN $1.2\text{--}1.5 \mu\text{g/kg/week}$. *Black segments* Peg-IFN $\geq 1.5 \mu\text{g/kg/week}$*

RNA level in the present study differing from that of the induction therapy with a high Peg-IFN dose in the above two studies.

In the present study, characteristic matched patients were extracted from a large retrospective cohort to examine the impact of Peg-IFN dosage on viral dynamics. The reason for using a matched cohort was that performing an RCT according to Peg-IFN doses poses an ethical problem, because a low dose of Peg-IFN is known to show little efficacy. The reason for our focusing on Peg-IFN dosage was based on the finding that ribavirin was indeed a significant factor for HCV RNA negativity at week 24 on univariate analysis, but not on multivariate analysis, and Peg-IFN was significantly correlated with HCV RNA negativity at week 24 in an independent manner in this study cohort. Our previous report that Peg-IFN, but not ribavirin, was correlated with c-EVR supports this [13].

To calculate the propensity score, we chose four covariates as candidates for adjustment: age, gender, Plt values, and history of IFN treatment, because there was a need to match universal features such as age, gender, and factors associated with HCV RNA negativity at week 24, such as Plt values and the history of IFN treatment. As shown in Table 4, the baseline characteristic factors in the different Peg-IFN patient categories were well matched after propensity score adjustment. That is, c-statics, the hallmark of application to logistic regression analysis, was regarded as adequate for random assignment. Only the c-statics for the patients given $<0.9 \mu\text{g/kg/week}$ of Peg-IFN and the patients given $0.9\text{--}1.2 \mu\text{g/kg/week}$ of Peg-IFN showed a low value (0.62), because the number of patients in the Peg-IFN category of $0.9\text{--}1.2 \mu\text{g/kg/week}$ ($n = 153$) was not very large. However, the patient characteristic factors in two categories after extraction were well matched and were considered to be adequate for further analysis. In this study, the populations extracted after matching were composed of patients with relatively advanced liver fibrosis compared to the original population; the mean Plt value was lower and the proportion of patients with

progression of liver fibrosis (METAVIR fibrosis score 3 or 4) was higher in the extracted population than in the original one (mean Plt value $12.1 \times 10^4/\text{mm}^3$ vs. $16.3 \times 10^4/\text{mm}^3$, proportion of patients with progression of liver fibrosis, 32 vs. 19%, respectively). The patients with $<0.9 \mu\text{g/kg/week}$ of Peg-IFN, which was the smallest population among the four Peg-IFN categories and included more patients with advanced liver fibrosis, were used as the control for the propensity score matching.

Recently, the usefulness of extended therapy has been revealed for patients with LVR, defined as HCV RNA negativity between week 12 and week 24 (or week 36). In addition, we have reported that, even with extended treatment of 72 weeks, the timing of HCV RNA disappearance showed a strong correlation with relapse after treatment [24]. Accordingly, at present, it is necessary to verify how reducing drug doses affects the delay of the timing to the first undetectable HCV RNA level or treatment failure, and in the present study we demonstrated the appropriate dose of Peg-IFN required to attain HCV RNA negativity by 24 weeks. As shown in Fig. 1, Peg-IFN dose-dependently affected the timing to the first undetectable HCV RNA level during the treatment. These results indicate that dose reduction of Peg-IFN can cause a shift from c-EVR to LVR and a shift from LVR to HCV RNA-positivity at week 24. The proportion of patients with treatment failure among those given $<0.9 \mu\text{g/kg/week}$ of Peg-IFN (66%) was decreased by half among the patients given $\geq 1.2 \mu\text{g/kg/week}$ of Peg-IFN (32–35%). Considering that the effectiveness of extended treatment for patients with LVR is obvious, if patients without a c-EVR were to attain HCV RNA negativity by 24 weeks, those patients would have the potential to attain an SVR with extended treatment. However, if patients do not attain HCV RNA negativity, those patients must discontinue the treatment. Therefore, causing patients to shift from HCV RNA negativity by week 24 to being HCV RNA-positive at week 24 would be missing the chance to obtain SVR even with extended treatment. As shown in Fig. 2, the longitudinal negative

rate of HCV RNA was dose-dependently affected by Peg-IFN at all points during the treatment. Therefore, a marked dose reduction of Peg-IFN should not be done at the start of treatment even for patients with lower Plt values (which are indicative of advanced fibrosis), because dose reduction of Peg-IFN before HCV RNA negativity is attained can lead to an increased possibility of treatment failure.

Next, as shown in Fig. 3, 70% of the patients with undetectable HCV RNA at week 4 were given ≥ 1.5 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN, 71% of those with undetectable HCV RNA at week 12 were given ≥ 1.2 $\mu\text{g}/\text{kg}/\text{week}$, and 64% of those with detectable HCV RNA at week 24 were given ≤ 1.2 $\mu\text{g}/\text{kg}/\text{week}$. Therefore, in HCV genotype 1 patients treated with Peg-IFN plus ribavirin, the treatment goal for c-EVR or non-NR should be to maintain a Peg-IFN dose of ≥ 1.2 $\mu\text{g}/\text{kg}/\text{week}$, and that for RVR should be to maintain a Peg-IFN dose of ≥ 1.5 $\mu\text{g}/\text{kg}/\text{week}$. Using granulocyte-macrophage colony-stimulating factor for patients who develop a severe decrease of blood cells and are forced to decrease Peg-IFN can be beneficial, as long as HCV RNA is positive.

A limitation of the present study is that the actual SVR rate could not be compared among the four Peg-IFN categories because some patients with LVR were treated for 72 weeks and some were treated for 48 weeks; actual SVR rates were 20% in patients with Peg-IFN < 0.9 $\mu\text{g}/\text{kg}/\text{week}$, 18% in those with 0.9 – 1.2 $\mu\text{g}/\text{kg}/\text{week}$, 36% in those with 1.2 – 1.5 $\mu\text{g}/\text{kg}/\text{week}$, and 48% in those ≥ 1.5 $\mu\text{g}/\text{kg}/\text{week}$. On the assumption that the SVR rate for patients with RVR is 90%, the SVR rate for those with c-EVR without RVR is 75% for 48-week treatment, and the SVR rate for those with LVR is 60% for 72-week treatment, the SVR rate of response-guided therapy was calculated to be 23% for patients given < 0.9 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN, 30% for those given 0.9 – 1.2 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN, 45% for those given 1.2 – 1.5 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN, and 50% for those given ≥ 1.5 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN in the matched cohort in the present study. Thus, dose reduction of Peg-IFN can reduce the SVR rate even if response-guided therapy is done. Another limitation of this study is that the *IL28B* SNP, which is known to be a host factor affecting the antiviral effect, could not be examined in all cases, because the characteristic matched patients were extracted from a large retrospective cohort. However, we had the result of the *IL28B* SNP (rs8099917) for 290 patients; 214 patients had TT and 76 had TG or GG. The proportions of patients with the *IL28B* SNP TT were similar among the four Peg-IFN categories (≤ 0.9 $\mu\text{g}/\text{kg}/\text{week}$, 76%, 31/41; 0.9 – 1.2 $\mu\text{g}/\text{kg}/\text{week}$, 71%, 27/38; 1.2 – 1.5 $\mu\text{g}/\text{kg}/\text{week}$, 67%, 99/147; ≥ 1.5 $\mu\text{g}/\text{kg}/\text{week}$, 77%, 57/74, $p = 0.853$). Therefore, it would appear that there was no bias for any cases. Among the patients with the *IL28B* SNP TT, the HCV negative rates at weeks 4, 12, and 24 were 0% (0/58), 33% (19/58),

and 69% (40/58) among the patients with < 1.2 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN and 4% (7/156), 62% (97/156), and 82% (128/156) among those with ≥ 1.2 $\mu\text{g}/\text{kg}/\text{week}$. There were significant differences between these two Peg-IFN groups in the HCV RNA negative rates at weeks 12 and 24 ($p = 0.002$, $p = 0.04$, respectively). Similarly, among the patients with *IL28B* SNP TG or GG, the HCV negative rates at weeks 4, 12, and 24 were 0% (0/21), 0% (0/21), and 10% (2/21) among the patients with < 1.2 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN and 2% (1/55), 9% (5/55), and 27% (15/55) among those with ≥ 1.2 $\mu\text{g}/\text{kg}/\text{week}$. The HCV RNA negative rates at weeks 12 and 24 tended to be higher in the patients with ≥ 1.2 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN ($p = 0.06$, $p = 0.13$, respectively). From the above-mentioned results, it appears that the dose-dependent effect of Peg-IFN on the timing of HCV RNA negativity could be considered regardless of the *IL28B* SNP.

In conclusion, this matched study has demonstrated that, in patients with CH-C with genotype 1 receiving Peg-IFN plus ribavirin combination therapy, Peg-IFN dose-dependently affects the timing to the first undetectable HCV RNA level and the failure to attain HCV RNA negativity. Dose reduction of Peg-IFN to < 1.2 $\mu\text{g}/\text{kg}/\text{week}$ before HCV RNA negativity is attained delays HCV RNA clearance dose-dependently and increases the rate of treatment failure. Maintaining the Peg-IFN dose at ≥ 1.2 $\mu\text{g}/\text{kg}/\text{week}$, and preferably at ≥ 1.5 $\mu\text{g}/\text{kg}/\text{week}$, can accelerate the timing to the first undetectable HCV RNA level for CH-C genotype 1 patients treated with Peg-IFN plus ribavirin.

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