Publication: Antiviral Therapy; Type: Original article
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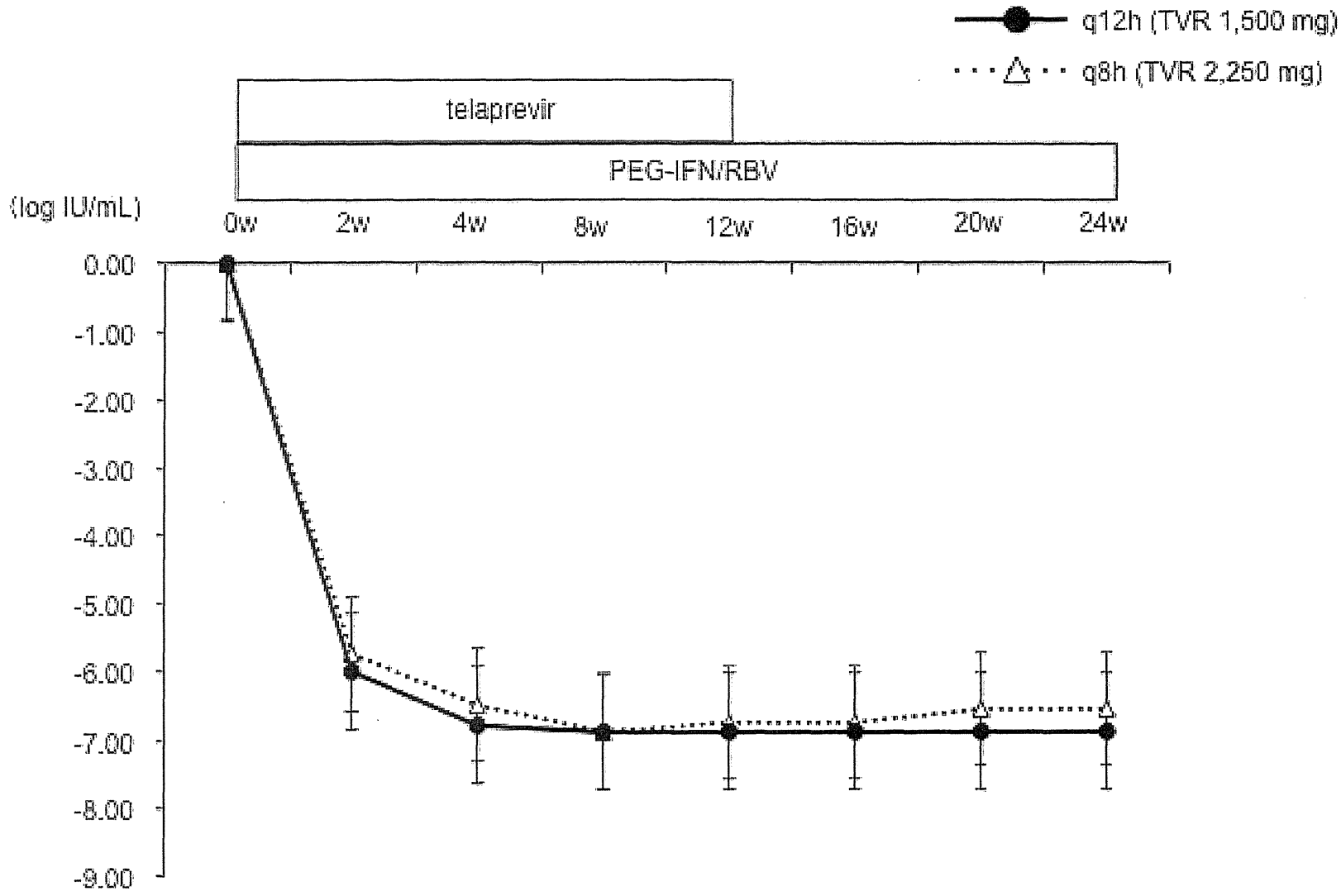
Table 3. Adverse events occurring in more than 5% of participants

	750mg q8h group (n=26)	750mg q12h group (n=26)	P value	All (n=52)
White blood cell count decreased	26(100)	26(100)	1.00	52(100)
Platelet count decreased	26(100)	26(100)	1.00	52(100)
Anemia	26(100)	26(100)	1.00	52(100)
Blood creatinine increased (eGFR decreased)	21(80.8)	12(46.2)	0.02	33(63.5)
Skin rash	11(42.3)	13(50)	0.59	24(46.2)
Blood uric acid increased	10(38.5)	6(23.1)	0.37	16(30.1)
Anorexia	4(15.4)	2(7.7)	0.67	6(11.5)
General fatigue	3(11.5)	1(3.8)	0.61	4(7.7)

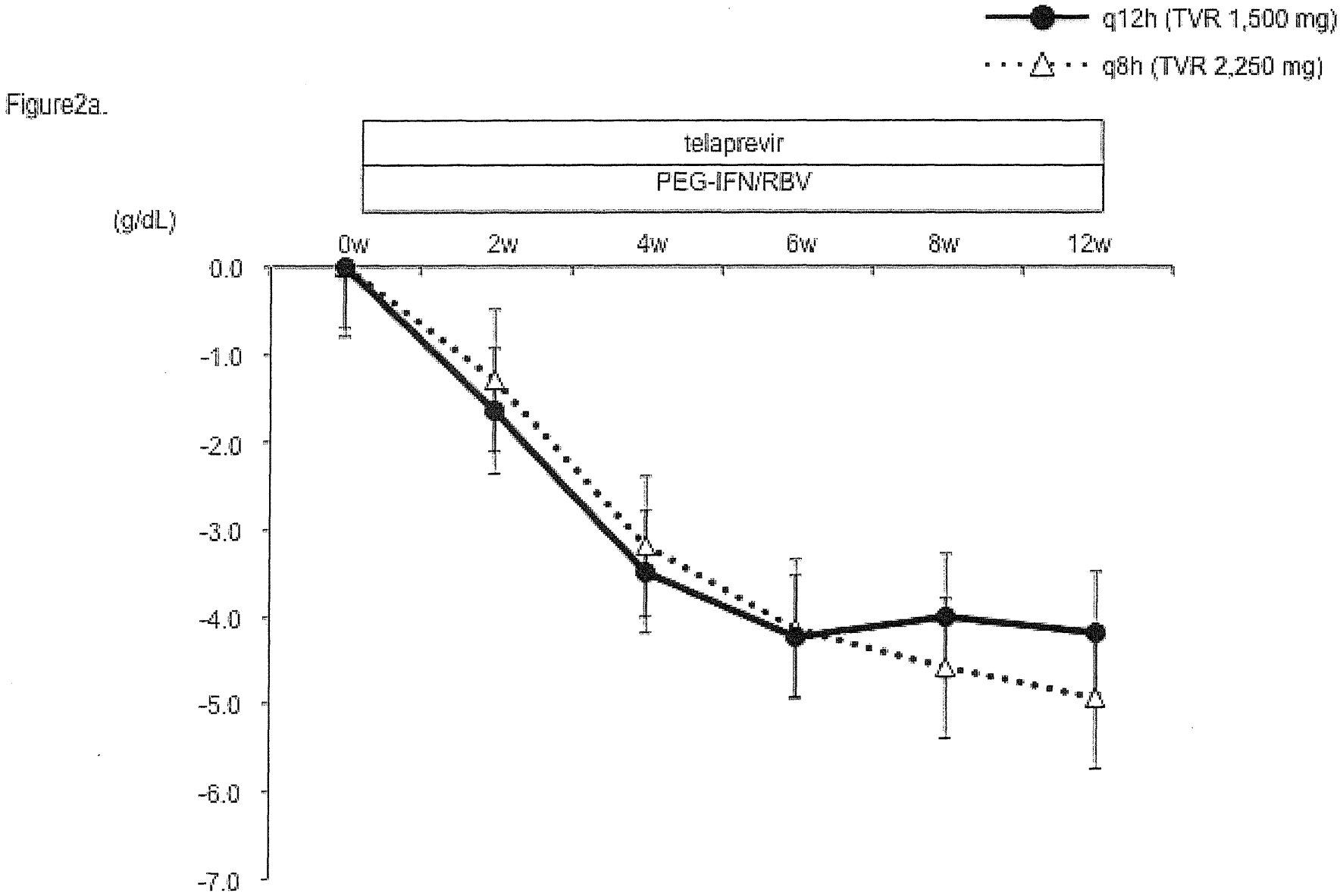
Figure1.



Patients remaining on study

q12h (n)	26	24	24	22	22	22	22	22
q8h (n)	26	25	24	23	23	23	23	23

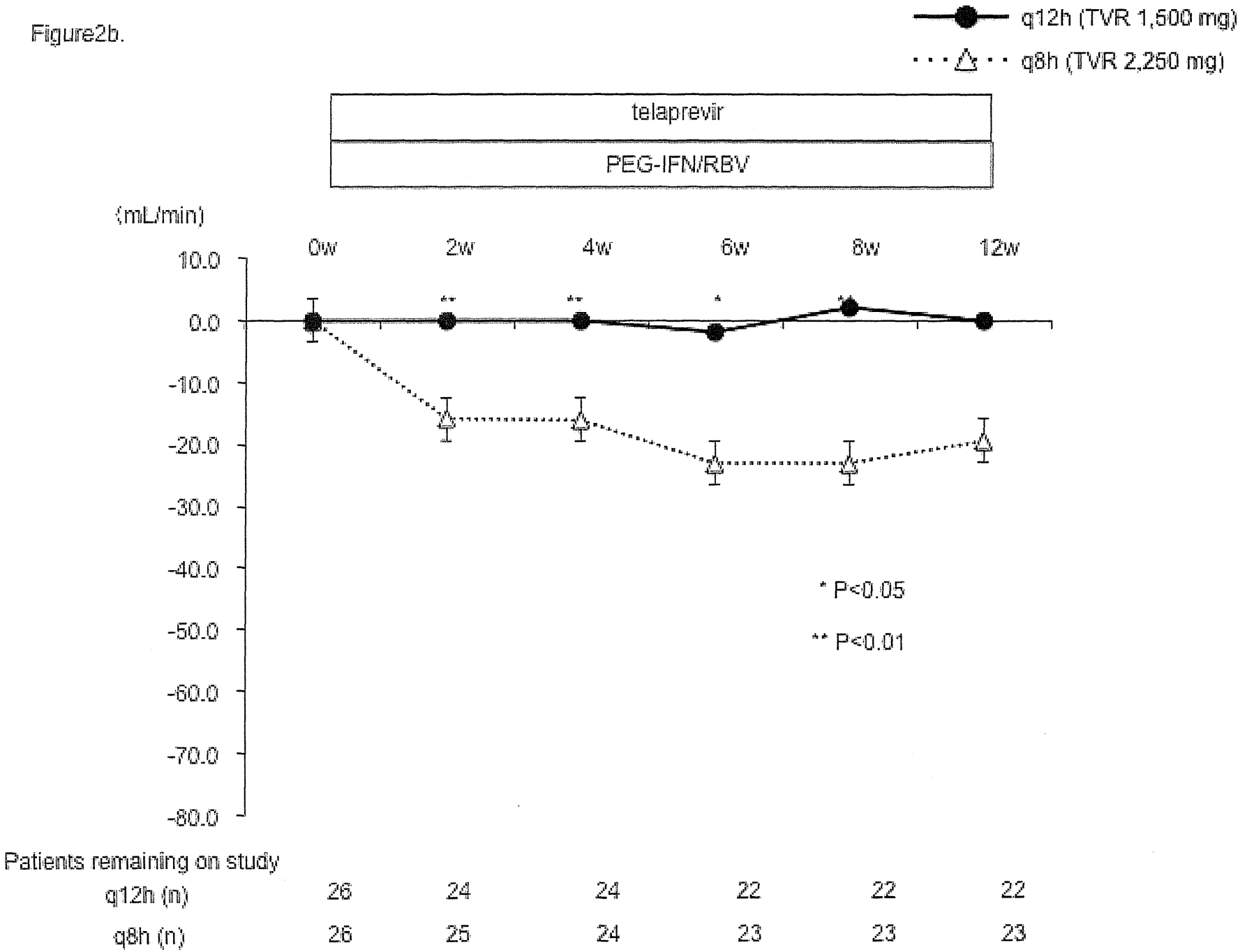
Figure2a.



Patients remaining on study

q12h (n)	26	24	24	22	22	22
q8h (n)	26	25	24	23	23	23

Figure2b.


q12h (TVR 1,500 mg)
q8h (TVR 2,250 mg)
telaprevir
PEG-IFN/RBV
(mL/min)
0w
2w
4w
6w
8w
12w
10.0
0.0
-10.0
-20.0
-30.0
-40.0
-50.0
-60.0
-70.0
-80.0
* P<0.05
** P<0.01
Patients remaining on study
q12h (n) 26 24 24 22 22 22
q8h (n) 26 25 24 23 23 23

Supplementary Figures

Supplementary Figure 1. Study design. Administration of drugs, randomization, and time points when PK study was performed are noted.

Supplementary Figure 2. Enrolment and outcomes. Patients who completed the 24 weeks therapy were classified as having completed treatment.

Supplementary Figure 3. Cumulative rate of undetectable HCV RNA in serum during treatment. Statistical analysis was performed on differences between 1,500mg and 2,250mg patients at 2 and 4 weeks from the start of the therapy and at the end of the observation period (SVR_{12}).

Supplementary Table 1. Comparison between IFNL3 (rs8099917) and IFNL4 (ss469415590) genotypes

	rs8099917				
ss469415590		TT	TG	GG	total
	TT/TT	50	0	0	50
	TT/ΔG	1	1	0	2
	ΔG/ΔG	0	0	0	0
	total	51	1	0	52

discrepancy

	rs12979860				
ss469415590		CC	CT	TT	total
	TT/TT	50	0	0	50
	TT/ΔG	0	2	0	2
	ΔG/ΔG	0	0	0	0
	total	50	2	0	52

Supplementary Table 2. SVR rates stratified by response to previous therapy, age , gender and platelet count

	750mg q8h group (n=26)	750mg q12h group (n=26)	P-value
Previous IFN therapy −n/N (%)			
naïve	13/14 (92.9%)	9/11 (81.8%)	0.56
relapse	8/9(88.9%)	11/11 (100%)	0.45
non-response	3/3 (100%)	4/4 (100%)	1.00
Age −n/N (%)			
≤59	11/11 (100%)	11/11 (100%)	1.00
≥60	13/15 (86.7%)	13/15 (86.7%)	1.00
Gender −n/N (%)			
Male	16/17 (94.1%)	18/18 (100%)	0.49
Female	8/9(88.9%)	6/8(75%)	0.58
Platelet −n/N (%)			
10≤	21/23 (91.3%)	23/25 (92%)	1.00
>10	3/3 (100%)	1/1 (100%)	1.00

Supplementary Table 3. Adverse events leading to discontinuation of all treatment or TVR only.

(a)

All treatment discontinuation	750mg q8h group(n=26)	750mg q12h group (n=26)	P-value
anemia	2(7.7)	0(0)	0.49
renal damage (creatinine increase)	1(3.8)	0(0)	1.00
anemia or renal damage	3(11.5)	0(0)	0.10
skin rash	0(0)	1(3.8)	1.00
syncope	0(0)	1(3.8)	1.00
anorexia	0(0)	2(7.7)	0.49
total	3(11.5)	4(15.4)	0.17

(b)

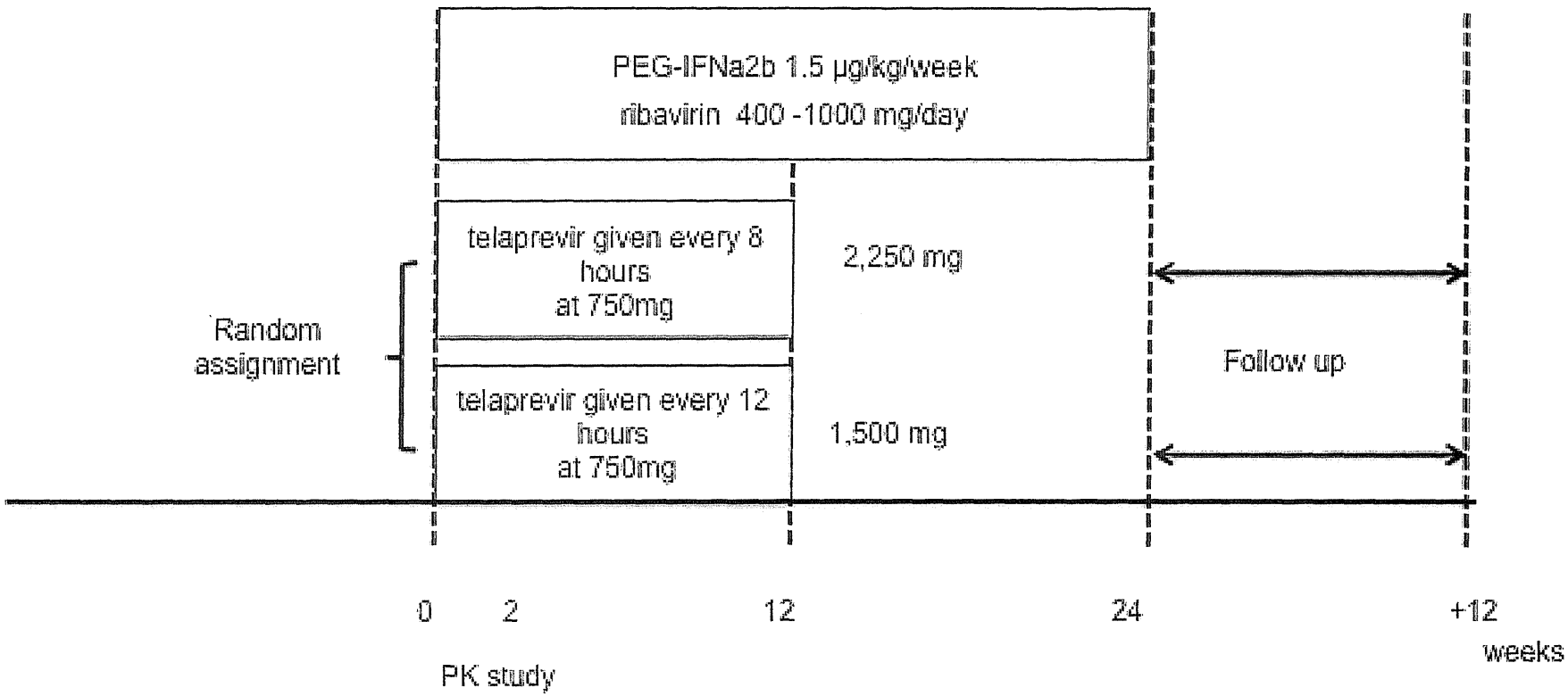
TVR discontinuation	750mg q8h group (n=26)	750mg q12h group (n=26)	P-value

anemia	2(7.7)	0(0)	0.49
renal damage (creatinine increase)	4(15.4)	0(0)	0.11
anemia or renal damage	6(23.0)	0(0)	0.02
skin rash	0(0)	1(3.8)	1.00
syncope	0(0)	1(3.8)	1.00
pneumonia	1(3.8)	0(0)	1.00
anorexia	4(15.4)	4(15.4)	1.00
total	11(42.3)	6(23.1)	0.14

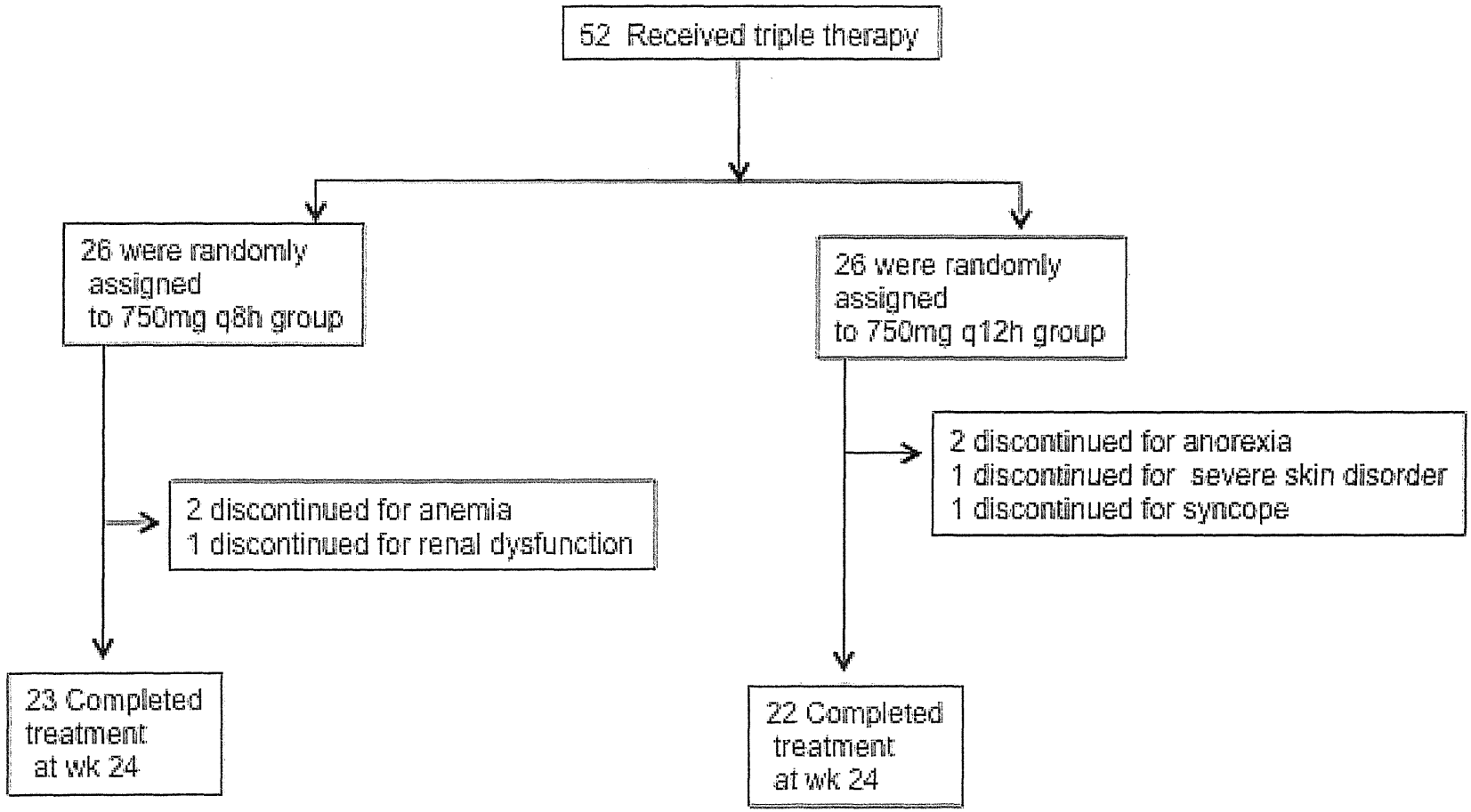
Supplementary Table 4. Rate of treatment completion without reduction or discontinuation.

Drug name	750mg q8h group (n=26)	750mg q12h group (n=26)	P-value
telaprevir	14 (53.8%)	20(76.9%)	0.09
ribavirin	3 (11.5%)	8(30.8%)	0.17
pegylated IFN	19 (73.1%)	20 (76.9%)	1.0

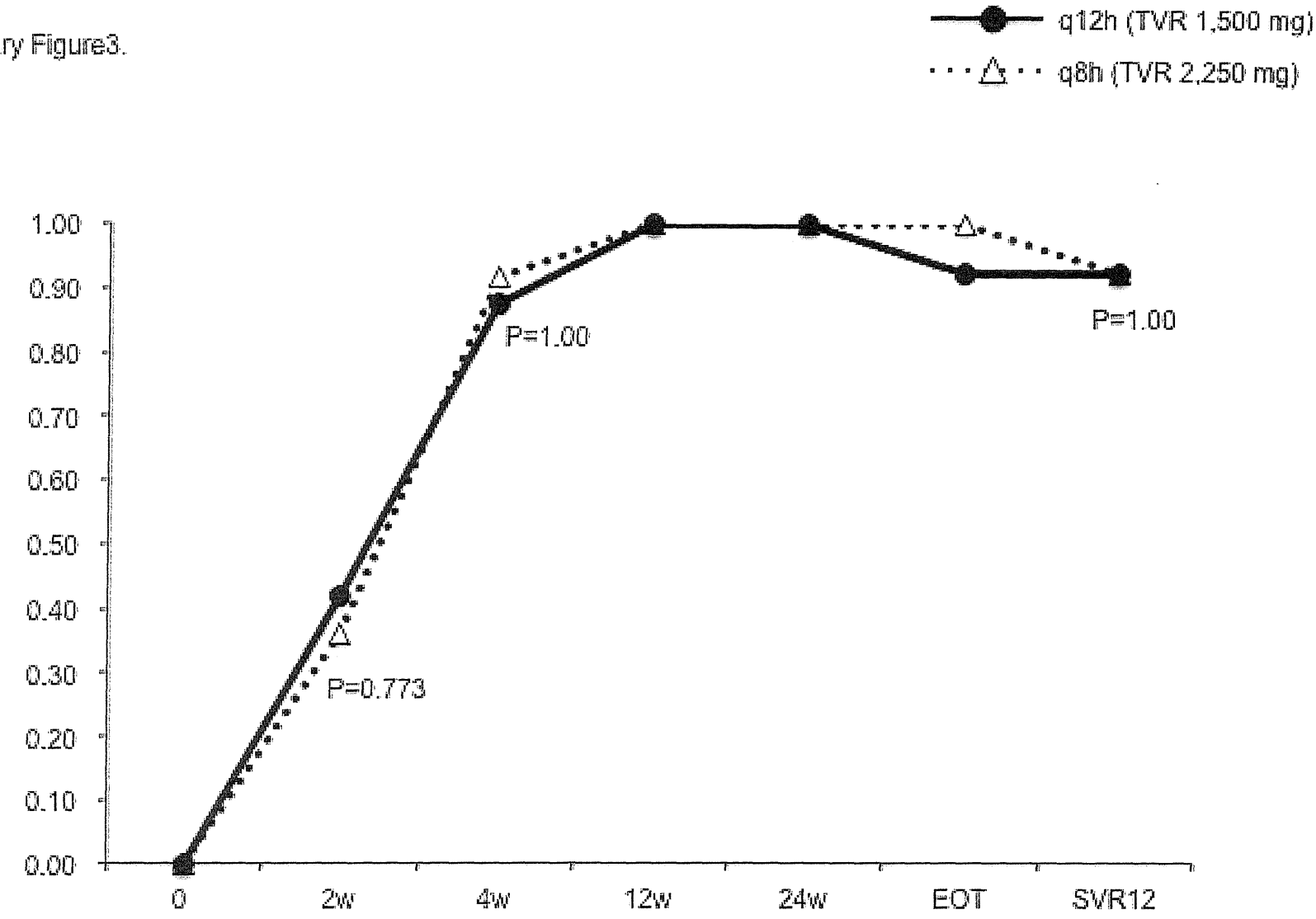
Supplementary Figure1.



Supplementary Figure2.



Supplementary Figure3.



Patients remaining on study

q12h (n)	26	24	24	22	22	26	26
q8h (n)	26	25	24	23	23	26	26

Journal of Medical Virology 86:169–175 (2014)

Impact of Virus Clearance for the Development of Hemorrhagic Stroke in Chronic Hepatitis C

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The aim of this retrospective cohort study was to assess the cumulative incidence and predictive factors for intracerebral hemorrhagic stroke after the termination of interferon (IFN) therapy in Japanese patients with hepatitis C virus (HCV). A total of 4,649 HCV-positive patients treated with IFN were enrolled. The primary goal is the first onset of intracerebral hemorrhagic stroke. The mean observation period was 8.0 years. Evaluation was performed using the Kaplan–Meier method and the Cox proportional hazard model. A *P*-value of less than 0.05 was considered statistically significant. A total of 28 developed intracerebral hemorrhagic stroke. The cumulative incidence of intracerebral hemorrhagic stroke was 0.3% at 5 years, 0.8% at 10 years, and 1.7% at 15 years. Intracerebral hemorrhagic stroke occurred when patients had age increments of 10 years (hazard ratio: 2.77; 95% confidence interval (CI) 1.48–5.18; $P=0.001$), hypertension (hazard ratio: 2.30; 95% CI 1.09–4.83; $P=0.021$), liver cirrhosis (hazard ratio: 4.50; 95% CI 2.07–9.78; $P<0.001$), and HCV non-clearance (hazard ratio: 3.22; 95% CI 1.22–8.53; $P=0.018$). On the intracerebral hemorrhagic stroke based on the difference of liver fibrosis and efficacy of IFN therapy, HCV clearance reduced to 24.3% (1/4.11) compared to HCV non-clearance in cirrhotic patients ($P=0.040$). In conclusion, HCV clearance reduced the development of intracerebral hemorrhagic stroke. In particular, HCV clearance reduced intracerebral hemorrhagic stroke to about one-fourth in cirrhotic patients. ***J. Med. Virol. 86:169–175, 2014.***

KEY WORDS: hepatitis C virus; interferon therapy; hemorrhagic stroke

INTRODUCTION

There are 170 million people affected with chronic hepatitis C virus (HCV) infection worldwide, which may cause an insidiously progressive form of liver disease that relentlessly but silently progresses to cirrhosis in 20–50% of cases over a period of 10–30 years [Kiyosawa and Furuta, 1991; Alter et al., 1992]. In addition, HCV is a major risk for hepatocellular carcinoma (HCC) [Hasan et al., 1990; Kew et al., 1990; Ikeda et al., 1993; Tsukuma et al., 1993; Arase et al., 2012]. In addition, several authors have reported that HCV clearance decreases the rate of fibrosis progression and the development of HCC in patients with chronic HCV infection [Kasahara et al., 1998; Yoshida et al., 2002; Arase et al., 2013].

On the other hand, hemorrhagic stroke is a medical emergency and can cause permanent neurological damage and death [Truelsen et al., 2003; Iso et al., 2007; Donnan et al., 2008]. It is becoming a great health burden in most countries. However, there is a little information on the incidence and risk factors on the incidence of hemorrhagic stroke in HCV patients treated with interferon (IFN). Furthermore, it is not clear whether the HCV clearance is useful for

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; CT, computed tomography; GGT, gamma-glutamyltransferase; HbA_{1C}, hemoglobin A_{1C}; HCV, hepatitis C virus; HDL, high density lipoprotein; IFN, interferon; LDL, low density lipoprotein

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reducing the development of hemorrhagic stroke in HCV patients.

With this background in mind, the present retrospective cohort study was initiated to investigate the cumulative incidence and risk factors of cerebral stroke after prolonged follow-up in HCV patients treated with IFN. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

PATIENTS AND METHODS

Patients

The number of patients who were diagnosed with chronic HCV infection and treated for the first time with IFN monotherapy or combination therapy between September 1990 and May 2010 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan was 7,635. Of these, 4,649 patients satisfied with the following enrolled criteria: (1) features of chronic hepatitis or cirrhosis diagnosed via laparoscopy and/or liver biopsy within 1 year before the initiation of IFN therapy; (2) positivity for serum HCV-RNA before the initiation of IFN therapy; (3) period of $\geq$1 month to $\leq$1 year of IFN therapy; (4) negativity for hepatitis B surface antigens (HBsAg), antibody to hepatitis B core, or antimitochondrial antibodies in serum, as determined by radioimmunoassay, enzyme-linked immunosorbent assay or indirect immunofluorescence assay; (5) age of $\geq$30 to $\leq$80 years; and (6) no autoimmune systemic disease, such as systemic lupus erythematosus or rheumatic arthritis. Patients with either of the following criteria were excluded from the study: (1) they had illnesses that could seriously reduce their life expectancy; (2) they had a history of coronary and/or cerebrovascular disease; (3) they had a history of carcinogenesis; and (4) they had been given anticoagulant and antiplatelet drugs.

The primary outcome is the first development of hemorrhagic stroke. Hemorrhagic stroke was regarded as intracerebral hemorrhagic stroke in the present study. Thus, patients with subarachnoid hemorrhagic stroke or subdural hematoma were excluded from analyses. The development of hemorrhagic stroke was diagnosed by clinical symptoms and imaging (computed tomography and/or magnetic resonance imaging) based on the World Health Organization definition [Truelsen et al., 2003; Iso et al., 2007; Donnan et al., 2008]. All of the studies were performed retrospectively by collecting and analyzing data from the patient records. The physicians in charge explained the purpose, method, and side effect of IFN therapy to each patient and/or patients' family. In addition, the physicians in charge got permission of serum stores and future uses of stored serum. Informed consent for IFN therapy and future uses of stored serum was obtained from all patients. This study had been approved by Institutional Review Board of our hospital.

Medical Evaluation

Body weight was measured in light clothing and without shoes to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm. Height and weight were recorded at baseline, and the body mass index (BMI) was calculated as kg/m^2. All patients were interviewed by physicians or nurse staff in the Toranomon Hospital using a questionnaire that gathered information on demographic characteristics, medical history, and health-related habits including questions on alcohol intake and smoking history.

Hemoglobin A_{1C} (HbA_{1C}) was estimated as National Glycohemoglobin Standardization Program equivalent value (%) and fasting plasma glucose [American Diabetes Association, 2010]. Patients were defined as having type 2 diabetes mellitus when HbA_{1C} level was $\geq$6.5% and/or fasting plasma glucose level was $\geq$126 mg/dl. Patients were defined as hypertensive when blood pressure was $\geq$140/90 mmHg or pharmacological treatment for high blood pressure was given. Smoking index (package per day $\times$ year) and total alcohol intake were evaluated by the sum of before, during, and after the IFN therapy.

Laboratory Investigation

Diagnosis of HCV infection was based on detection of serum HCV antibody and positive RNA. Anti-HCV was detected using an enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL). HCV-genotype was examined via polymerase chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported [Dusheiko et al., 1994]. HCV-RNA was determined by the COBAS TaqMan HCV test (Roche Diagnostics, Basel, Switzerland). The serum samples stored at -80°C before IFN therapy were used. The linear dynamic range of the assay was 1.2–7.8 log IU/ml, and the undetectable samples were defined as negative. A HCV clearance was defined as clearance of HCV RNA using the COBAS TaqMan HCV test 6 months after the cessation of IFN therapy.

Evaluation of Liver Cirrhosis

Status of liver was mainly determined on the basis of peritoneoscopy and/or liver biopsy. Liver biopsy specimens were obtained using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas [Desmet et al., 1994].

Follow-Up

The observation starting point was 6 months after the termination of IFN therapy. After that, patients were followed up at least twice a year in our hospital.

Biochemical tests were conducted at each examination together with regular check-up. Four hundred fifty patients were lost to follow-up. The final date of follow-up in 452 patients with loss of follow-up was regarded as last consulting day.

Patients with either of the following criteria during follow-up were regarded as censored data in statistical analysis [Fleming et al., 1984]: (1) they were retreated with IFN (N = 949); (2) they had new onset of carcinogenesis (N = 645); and (3) they had been given anticoagulant and antiplatelet drugs (N = 28). The final date of follow-up in these patients with censored data was regarded as the time of the initiation of criteria described above. The mean follow-up period was 6.7 [standard deviation (SD) 4.3] years in 452 patients with loss of follow-up and 7.4 (SD 4.7) years in 1,722 patients who had censored data. Patients with loss of follow-up and censored data were counted in the analysis.

Statistical Analysis

Clinical differences between patients with hemorrhagic stroke and those without events were evaluated using Mann–Whitney test. The cumulative incidence of hemorrhagic stroke were calculated by using the Kaplan–Meier technique, and differences in the curves were tested using the log-rank test [Kaplan and Meier, 1958; Harrington and Fleming, 1983]. Independent risk factors associated with hemorrhagic stroke were studied using the stepwise Cox regression analysis [Cox, 1972]. The following

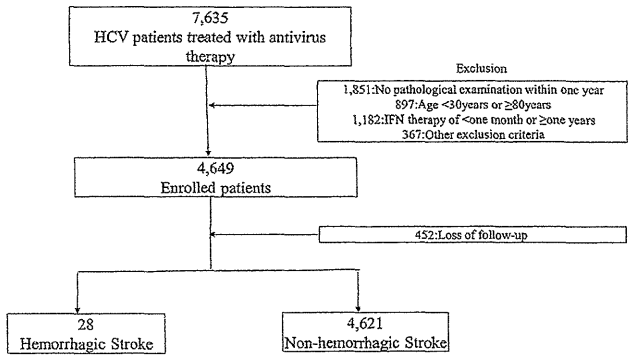


Fig. 1. An algorithm of the study population.

TABLE I. Clinical Backgrounds at the Initiation of Follow-Up in Enrolled Patients

	Total	Hemorrhagic stroke group	Without events group	*P*-value
N	4,649	28	4,621	
Age (years)	51.9 ± 11.8	60.4 ± 6.7	51.8 ± 11.9	<0.001
Gender (M/F)	2,966/1,883	16/12	2,950/1,871	0.781
Height (cm)	163.1 ± 9.2	159.5 ± 9.4	163.2 ± 9.2	0.171
Weight (kg)	61.4 ± 12.8	57.9 ± 8.0	61.4 ± 12.7	0.113
BMI	22.7 ± 3.1	23.4 ± 2.8	22.7 ± 3.1	0.582
BP (systolic, mmHg)	128 ± 18	140 ± 20	127 ± 18	0.007
BP (diastolic, mmHg)	77 ± 13	86 ± 15	77 ± 13	0.001
Total alcohol intake (kg)[a]	95 ± 92	148 ± 105	94 ± 92	0.002
Smoking index[a]	6.5 ± 9.5	11.8 ± 12.4	6.4 ± 9.4	<0.001
AST (IU/L)	41 ± 43	48 ± 28	41 ± 43	<0.001
ALT (IU/L)	44 ± 53	53 ± 38	43 ± 52	0.004
GGT (IU/L)	53 ± 60	59 ± 47	52 ± 61	0.078
Albumin (g/dl)	4.0 ± 0.3	3.5 ± 0.4	4.0 ± 0.3	0.110
Triglyceride (mg/dl)	101 ± 52	108 ± 46	100 ± 52	0.097
Cholesterol (mg/dl)	170 ± 31	171 ± 27	170 ± 31	0.893
HDL-C (mg/dl)	48 ± 14	45 ± 12	48 ± 14	0.002
LDL-C (mg/dl)	104 ± 29	108 ± 37	103 ± 29	0.049
Fasting plasma glucose (mg/dl)	99 ± 22	103 ± 23	100 ± 22	0.093
HbA_{1C} (%)	5.7 ± 1.1	5.9 ± 1.2	5.7 ± 1.1	0.024
Platelet ($\times 10^4/mm^3$)	17.2 ± 5.2	14.1 ± 6.2	17.3 ± 5.4	0.001
Staging (cirrhosis/non-cirrhosis)[b]	485/4,164	12/16	473/4,148	<0.001
HCV genotype (1b/2a/2b/other)[b]	2,859/1,109/497/184	22/5/1/0	2,837/1,104/496/184	0.104
HCV RNA (log IU/ml)[b]	6.07 ± 1.05	6.03 ± 1.03	6.08 ± 1.05	0.387
IFN monotherapy/combination therapy[c]	3,000/1,649	24/4	2,976/1,645	<0.001
Efficacy (HCV; clearance/non-clearance)	2,103/2,546	5/23	2,098/2,523	0.006

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; GGT, gamma-glutamyltransferase; HbA_{1C}; hemoglobin A_{1C}; HCV, hepatitis C virus; HDL, high density lipoprotein; IFN, interferon.
Data are number of patients or mean ± standard deviation.
[a]Smoking index is defined as package per day × year; total alcohol intake and smoking index indicate the sum before and after first consultation.
[b]Value before IFN treatment.
[c]Outbreak of IFN monotherapy: recombinant IFN alpha 2a, 238 cases; recombinant IFN alpha 2b, 183 cases; natural IFN alpha, 1,750 cases; natural IFN beta, 750 cases; total dose of IFN = 554 ± 164 MU. Outbreak of peg IFN monotherapy: peg IFN alpha 2a, 93 cases, total dose of peg IFN = 7.54 ± 2.20 mg.
Outbreak of combination therapy: recombinant IFN alpha 2b + ribavirin, 335 cases, total dose of IFN = 508 ± 184 MU, total dose of ribavirin = 160 ± 68 g; natural IFN beta + ribavirin, 127 cases, total dose of IFN = 502 ± 177 MU, total dose of ribavirin = 155 ± 67 g; peg IFN alpha 2b + ribavirin, 1,173 cases, total dose of peg IFN = 4.12 ± 1.10 mg, total dose of ribavirin = 205 ± 58 g.

variables were analyzed for potential covariates for incidence of primary outcome: (1) age, gender, type 2 diabetes mellitus, hypertension, BMI at the initiation time of follow-up, (2) HCV genotype, HCV load, and hepatic fibrosis before IFN therapy, (3) average value of aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and platelet during follow-up, (4) sum value of smoking and alcohol before, during, and after the IFN therapy, (5) efficacy of IFN therapy, combination of ribavirin, type of IFN, and total dose of IFN. A P-value of less than 0.05 was considered statistically significant. Data analysis was performed using SPSS 11.5 for Windows (SPSS, Chicago, IL).

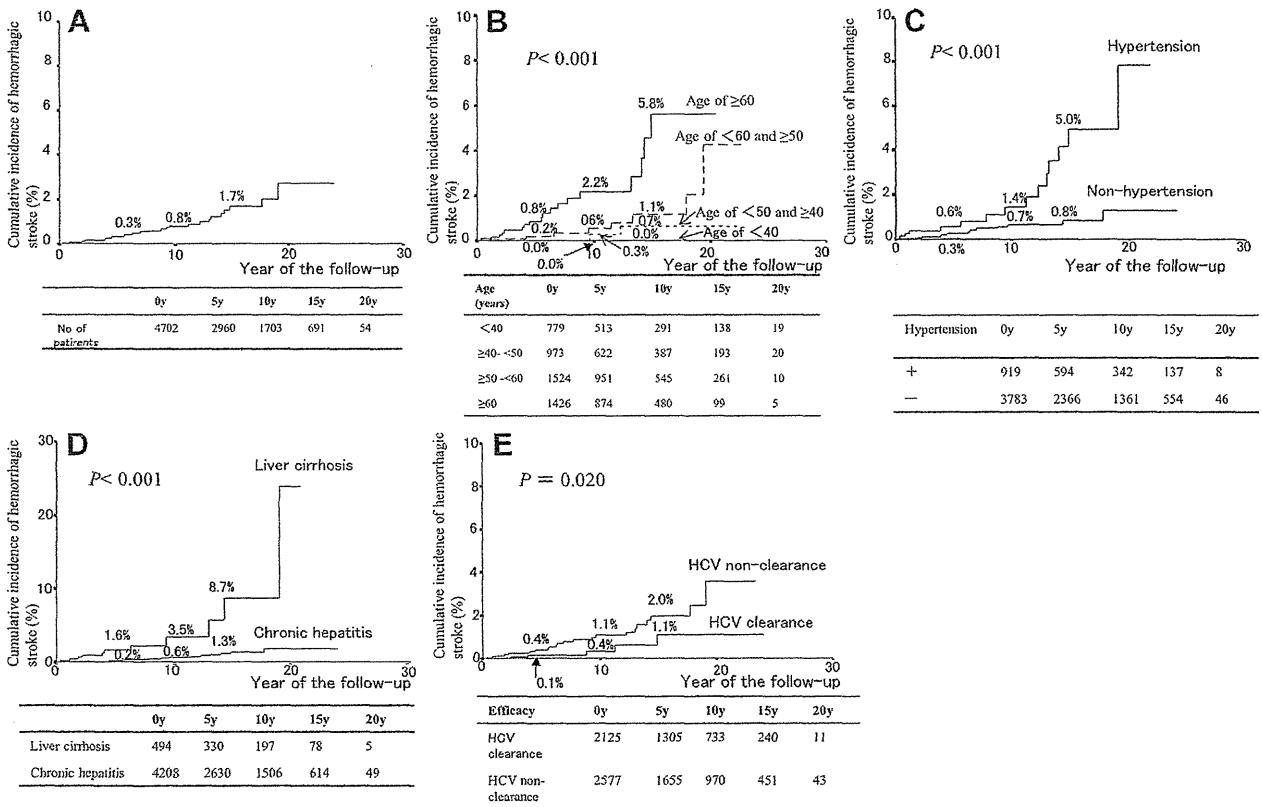
RESULTS

Patients Characteristics

Figure 1 shows the algorithm of the study population. For the mean observation period of 8.0 years, 28 of 4,649 patients developed hemorrhagic stroke. Table I shows the baseline characteristics of the enrolled 4,649 patients at the initiation of follow-up. The patients are divided into two groups of patients with hemorrhagic stroke and without event. There are significant differences in several baseline characteristics between the two groups. The HCV clearance rate was 34.7% (1,042/3,000) in IFN monotherapy and 64.3% (1,061/1,649) in combination therapy of IFN and ribavirin. Thus, the number of patients with HCV clearance was 2,103. The mean follow-up was 8.0 (SD 5.0) years. The 28-day vascular disease-related mortality rate was 33% (10/28) in hemorrhagic stroke.

Predictive Factors for the Development of Intracerebral Hemorrhagic Stroke

The cumulative incidence of intracerebral hemorrhagic stroke was 0.3% at 5 years, 0.8% at 10 years, and 1.7% at 15 years (Fig. 2A). The factors associated with the development of intracerebral hemorrhagic stroke are shown in Table II. Intracerebral hemorrhagic stroke occurred when patients had age increments of 10 years [hazard ratio: 2.77; 95% confidence interval (CI) 1.48–5.18; $P = 0.001$], hypertension



	0y	5y	10y	15y	20y
No of patients	4702	2960	1703	691	54

Age (years)	0y	5y	10y	15y	20y
<40	779	513	291	138	19
≥40-<50	973	622	387	193	20
≥50-<60	1524	951	545	261	10
≥60	1426	874	480	99	5

Hypertension	0y	5y	10y	15y	20y
+	919	594	342	137	8
−	3783	2366	1361	554	46

	0y	5y	10y	15y	20y
Liver cirrhosis	494	330	197	78	5
Chronic hepatitis	4208	2630	1506	614	49

Efficacy	0y	5y	10y	15y	20y
HCV clearance	2125	1305	733	240	11
HCV non-clearance	2577	1655	970	451	43

Fig. 2. **Panel A**: Cumulative development rate of intracerebral hemorrhagic stroke in total HCV patients treated with IFN therapy. **Panel B**: Cumulative development rate of intracerebral hemorrhagic stroke based on difference of age. **Panel C**: Cumulative development rate of ischemic stroke based on the difference of blood pressure. **Panel D**: Cumulative development rate of intracerebral hemorrhagic stroke based on difference of liver fibrosis. **Panel E**: Cumulative development rate of intracerebral hemorrhagic stroke based on difference of interferon efficacy.

TABLE II. Predictive Factors for the Development of Intracerebral Hemorrhagic Stroke

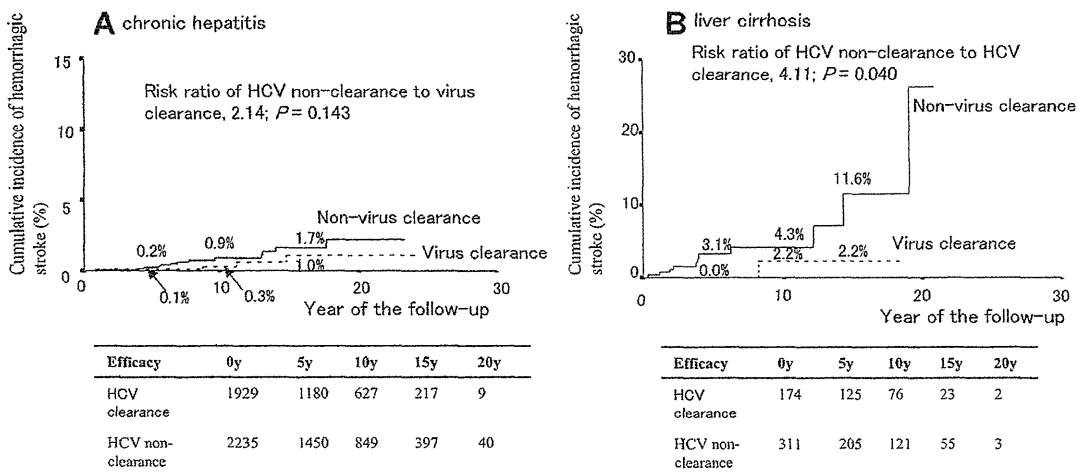
	Univariate analysis		Cox regression	
Variables	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (years, per 10)	3.55 (1.96–6.43)	<0.001	2.77 (1.48–5.18)	0.001
Gender (M/F)	1.26 (0.65–2.44)	0.334		
BMI (≥22/<22)	0.97 (0.75–1.24)	0.767		
Diabetes (+/−)	3.40 (1.26–9.15)	0.015		
Hypertension (+/−)	4.07 (1.94–8.54)	<0.001	2.30 (1.09–4.83)	0.021
Smoking index (≥20/<20)[a]	2.12 (0.95–4.76)	0.068		
Total alcohol intake (kg, ≥200/<200)[a]	1.10 (0.53–4.37)	0.138		
AST (IU/L, ≥34/<34)	2.79 (1.17–6.66)	0.020		
ALT (IU/L, ≥36/<36)	2.68 (1.14–6.29)	0.023		
GGT (IU/L, ≥109/<109)	1.28 (0.610–1.89)	0.655		
Albumin (g/dl, <3.9/≥3.9)	2.96 (1.24–7.09)	0.015		
Triglyceride (mg/dl, ≥100/<100)	1.19 (0.83–1.49)	0.283		
Total cholesterol (mg/dl, <150/≥150)	1.06 (0.48–1.91)	0.936		
HDL-C (mg/dl, ≥40/<40)	0.96 (0.38–2.50)	0.960		
LDL-C (mg/dl, ≥120/<120)	0.81 (0.50–2.51)	0.572		
Platelet ($\times 10^4$/mm^3, <15/≥15)	3.22 (1.41–7.35)	0.005		
Histological diagnosis (cirrhosis/non-cirrhosis)	7.40 (3.30–16.77)	<0.001	4.50 (2.07–9.78)	<0.001
Combination of ribavirin (+/−)	0.80 (0.25–2.54)	0.701		
Type of IFN (α/β)	1.29 (0.65–2.33)	0.116		
Total dose of IFN (MU, ≥500/<500)	0.87 (0.39–1.99)	0.744		
HCV genotype (1/2)	1.53 (0.62–3.80)	0.360		
HCV RNA (log IU/ml, ≥5/<5)	1.35 (1.02–1.79)	0.035		
Efficacy (HCV: non-clearance/clearance)	2.98 (1.13–6.59)	0.020	3.22 (1.22–8.53)	0.018

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; GGT, gamma-glutamyltransferase; HCV, hepatitis C virus; IFN, interferon.
[a]Smoking index is defined as package per day × year; total alcohol intake and smoking index indicate the sum before and after first consultation.

(hazard ratio: 2.30; 95% CI 1.09–4.83; $P = 0.021$), liver cirrhosis (hazard ratio: 4.50; 95% 2.07–9.78; $P < 0.001$), and HCV non-clearance (hazard ratio: 3.22; 95% CI 1.22–8.53; $P = 0.018$). Figure 2B–E shows the cumulative incidence of hemorrhagic stroke based on difference of age, blood pressure, liver fibrosis, and efficacy of IFN therapy.

Hemorrhagic Stroke Based on the Difference of Liver Fibrosis and Efficacy

Figure 3A,B shows the cumulative incidence of intracerebral hemorrhagic stroke based on the difference of liver fibrosis and efficacy of IFN therapy. As shown in Figure 3B, HCV clearance reduced



Efficacy	0y	5y	10y	15y	20y
HCV clearance	1929	1180	627	217	9
HCV non-clearance	2235	1450	849	397	40

Efficacy	0y	5y	10y	15y	20y
HCV clearance	174	125	76	23	2
HCV non-clearance	311	205	121	55	3

Fig. 3. **Panel A**: Cumulative development rate of intracerebral hemorrhagic stroke based on difference of efficacy after interferon treatment in HCV patients with chronic hepatitis. **Panel B**: Cumulative development rate of intracerebral hemorrhagic stroke based on the difference of efficacy after interferon treatment in HCV patients with liver cirrhosis.

TABLE III. Comparison in Clinical Backgrounds Between HCV Clearance and HCV Non-Clearance in Patients With Liver Cirrhosis

	HCV clearance group	HCV non-clearance group	*P*-value
N	174	311	
Age (years)	56.7 ± 9.6	57.0 ± 9.9	0.721
Gender (M/F)	108/66	184/127	0.562
BMI	23.8 ± 3.7	23.6 ± 3.5	0.479
BP (systolic, mmHg)	132 ± 18	131 ± 17	0.791
BP (diastolic, mmHg)	80 ± 11	79 ± 12	0.775
Total alcohol intake (kg)[a]	112 ± 97	128 ± 101	0.057
Smoking index[a]	6.2 ± 10.7	5.9 ± 10.2	0.129
AST (IU/L)	33 ± 20	73 ± 47	<0.001
ALT (IU/L)	34 ± 28	79 ± 61	<0.001
GGT (IU/L)	24 ± 26	61 ± 65	<0.001
Albumin (g/dl)	3.7 ± 0.4	3.5 ± 0.4	0.149
Triglyceride (mg/dl)	110 ± 47	104 ± 45	0.243
Cholesterol (mg/dl)	157 ± 29	161 ± 31	0.373
HDL-C (mg/dl)	42 ± 12	45 ± 12	0.257
LDL-C (mg/dl)	96 ± 26	95 ± 30	0.748
Fasting plasma glucose (mg/dl)	104 ± 22	109 ± 26	0.085
HbA_{1C} (%)	5.7 ± 1.2	6.0 ± 1.3	0.024
Platelet ($\times 10^4/mm^3$)	14.1 ± 6.2	17.3 ± 5.4	0.097
HCV genotype (1b/2a/2b/other)[b]	75/72/24/3	209/54/15/33	<0.001
HCV RNA (log IU/ml)[b]	5.32 ± 1.12	6.38 ± 1.00	<0.001
IFN monotherapy/combination therapy[c]	110/64	232/79	0.012

Data are number of patients or mean ± standard deviation, ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; GGT, gamma-glutamyltransferase; HbA_{1C}, hemoglobin A_{1C}; HCV, hepatitis C virus; HDL, high density lipoprotein; IFN, interferon.

[a]Smoking index is defined as package per day × year; total alcohol intake and smoking index indicate the sum before and after first consultation.

[b]Value before IFN treatment.

[c]Outbreak of IFN monotherapy: natural IFN alpha, 252 cases; natural IFN beta, 90 cases; total dose of IFN = 518 ± 156 MU.
Outbreak of combination therapy: natural IFN beta + ribavirin, 41 cases, total dose of IFN = 490 ± 171 MU, total dose of ribavirin = 151 ± 64 g; peg IFN alpha 2b + ribavirin, 102 cases, total dose of peg IFN = 3.96 ± 1.03 mg, total dose of ribavirin = 188 ± 51 g.

hemorrhagic stroke to one-fourth in cirrhotic patients. Table III shows the clinical backgrounds between HCV clearance and HCV non-clearance in patients with liver cirrhosis. There are significant differences in AST, ALT, GGT, HCV genotype, HCV RNA, and HbA_{1C} between HCV clearance group and HCV non-clearance group. However, there are no significant differences in age and hypertension between HCV clearance group and HCV non-clearance group.

DISCUSSION

The incidence of hemorrhagic stroke after the termination of IFN therapy in HCV patients has been described in the present study. The strengths of the present study are a prolonged follow-up in the large numbers of patients included.

The present study shows several findings with regard to the cumulative incidence and predictive factors for hemorrhagic stroke after IFN therapy for HCV patients. First, intracranial hemorrhagic stroke occurred significantly when patients had advanced age of ≥60 years, hypertension, liver cirrhosis, and HCV non-clearance. Several authors have reported that the most common risk factor for hemorrhagic stroke is aging, high levels of blood pressure [Turin et al., 2010; O'Donnell et al., 2010; Naidech, 2011; Cervera et al., 2012]. In addition, antiplatelet and anticoagulant medications also increase the risk of hemorrhagic stroke [Cervera et al., 2012]. Our results evaluated hemorrhagic stroke in HCV patients agreed with these reports concerning aging and hypertension.

Second, HCV clearance reduced hemorrhagic stroke to about one-fourth in cirrhotic patients. In general, patients with advanced liver fibrosis have often the hemorrhagic tendency due to prothrombin deficit and platelets diminution. Thus, our result suggests that the HCV clearance prevent the aggravation of prothrombin deficit and platelets diminution. Our previous reports have indicated that HCV clearance reduces type 2 diabetes mellitus [Arase et al., 2009], bone fracture [Arase et al., 2010], and chronic kidney disease [Arase et al., 2011]. In the present study, HCV clearance reduced the incidence of intracerebral hemorrhagic stroke. In particular, HCV clearance reduced intracerebral hemorrhagic stroke to about one-fourth in cirrhotic patients.

A hemorrhagic stroke is the rapid loss of brain function due to hemorrhage. As a result, a hemorrhagic stroke is a medical emergency and can cause permanent neurological damage and death. Recently, the life span has been long in Japan. Thus, in near the future, a large number of patients with HCV will be >60 years of age. A hemorrhagic stroke might be increasing in HCV positive patients in aging society. Our results show that physicians in charge of HCV

patients with hypertension, liver cirrhosis, and HCV non-clearance should be noted the development of hemorrhagic stroke.

The present study was limited by a retrospective cohort trial. Another limitation of the study was that patients were treated with different types of antivirus therapy for different duration. In addition, these patients were treated with different types of drugs for diabetes, hypertension, and dyslipidemia during follow-up. Finally, our cohort contains Japanese subjects only. On the other hand, the strengths of the present study are a long-term follow-up in the large numbers of patients included.

In conclusion, HCV clearance reduced hemorrhagic stroke to about one-fourth in cirrhotic patients.

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