Table 1 Characteristics of 938 chronic hepatitis C genotype 1 patients treated with a combination of pegylated interferon plus ribavirin according to age (mean ± SD)

	Group A (age < 65 yr) (*n* = 685)	Group B (age ≥ 65 yr) (*n* = 253)	*P*-value
Age (yr)	53.1 ± 8.9	68.6 ± 3.1	< 0.001
Male/female	374/311	122/131	0.090
Body mass index (kg/m^2)	23.7 ± 3.3	22.8 ± 2.7	< 0.001
Prior IFN monotherapy, *n* (%)	163 (23.8)	76 (30.0)	0.052
Prior combined IFN plus RBV treatment, *n* (%)	51 (7.4)	20 (7.9)	< 0.001
Alanine aminotransferase (IU/L)	80.2 ± 62.0	67.9 ± 46.6	0.004
γ-glutamyltranspeptidase (IU/L)	60.2 ± 56.6	57.1 ± 49.2	0.708
Albumin (g/dL)	4.1 ± 0.4	4.0 ± 0.4	< 0.001
White blood cell count (/mm^3)	5200.0 ± 1476.7	4756.3 ± 1458.9	< 0.001
Hemoglobin (g/dL)	14.1 ± 1.4	13. 5 ± 1.4	< 0.001
Platelet count (10^9/L)	16.6 ± 5.3	15.0 ± 5.2	< 0.001
Creatinine (mg/dL)	0.7 ± 0.6	0.8 ± 1.4	0.107
Creatinine clearance (mL/min)	105.5 ± 28.7	75.8 ± 17.5	< 0.001
Serum HCV-RNA level (kIU/mL)	1776.1 ± 1500.0	1986.9 ± 1604.5	0.125
Histological fibrosis			0.008
F0/F1/F2/F3/F4	36/155/121/61/30	9/46/49/31/17	

IFN: Interferon; RBV: Ribavirin; HCV: Hepatitis C virus.

Table 2 Characteristics of 313 chronic hepatitis C genotype 2 patients treated with a combination of pegylated interferon plus ribavirin according to age (mean ± SD)

	Group C (age < 65 yr) (*n* = 252)	Group D (age ≥ 65 yr) (*n* = 61)	*P*-value
Age (yr)	47.7 ± 10.4	69.2 ± 3.4	< 0.001
Male/female	124/128	28/33	0.671
Body mass index (kg/m^2)	23.1 ± 3.5	22.8 ± 2.9	0.577
Prior IFN monotherapy, *n* (%)	47 (18.7)	16 (26.2)	< 0.001
Prior combined IFN plus RBV treatment, *n* (%)	5 (2.0)	4 (6.6)	0.056
Alanine aminotransferase (IU/L)	79.9 ± 78.7	68.9 ± 52.9	0.821
γ-glutamyltranspeptidase (IU/L)	55.8 ± 64.7	44.3 ± 34.7	0.937
Albumin (g/dL)	4.2 ± 0.4	3.9 ± 0.5	< 0.001
White blood cell count (/mm^3)	5276.3 ± 1636.3	4958.0 ± 1495.6	0.005
Hemoglobin (g/dL)	14.1 ± 1.4	13.4 ± 1.3	< 0.001
Platelet count (10^9/L)	18.9 ± 6.3	15.6 ± 4.7	< 0.001
Creatinine (mg/dL)	0.8 ± 1.5	0.7 ± 0.2	0.581
Creatinine clearance (mL/min)	112.1 ± 31.4	74.6 ± 17.2	< 0.001
Serum HCV-RNA level (kIU/mL)	1588.3 ± 1628.7	1195.4 ± 1645.5	0.038
Histological fibrosis			< 0.001
F0/F1/F2/F3/F4	30/77/39/10/10	1/21/9/2/12	

IFN: Interferon; RBV: Ribavirin; HCV: Hepatitis C virus.

Informed consent was obtained from all patients before enrollment in this study. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and the International Conference on Harmonization of guidelines for good clinical practice.

Table 1 (genotype 1) and Table 2 (genotype 2) show the baseline characteristics of the enrolled patients, who were further classified into four groups according to age and genotype status: group A, genotype 1 aged less than 65 years (*n* = 685); group B, genotype 1 aged 65 years or older (*n* = 253); group C, genotype 2 aged less than 65 years (*n* = 252); and group D, genotype 2 aged 65 or older (*n* = 61). In group B, body mass index, prior combined IFN plus RBV treatment, alanine aminotransferase, albumin, white blood cell count, hemoglobin, platelet count, and creatinine clearance calculated using the Modification of Diet in Renal Disease equation[15] were significantly lower than in group A ($P < 0.010$). In group D, albumin, hemoglobin, platelet count, creatinine clearance and serum HCV RNA level were significantly lower than in group C ($P < 0.010$). The percentage of patients with platelet counts below 10 × 10^{10}/L was significantly higher in group B (36 of 253, 14.2%) than in group A (56 of 685, 8.2%) ($P = 0.006$), however, there was no significant difference between group C (16 of 252, 6.3%) and group D (7 of 61, 11.5%).

Liver histology

Liver biopsy was performed in 555 patients (59.2%) with genotype 1 and 209 patients (66.8%) with genotype 2. The other patients refused liver biopsy. Fibrosis was staged on a 0-4 scale as follows: F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis with few septa, F3 = numerous septa without cirrhosis, F4 = cirrhosis. Liver fibrosis was more advanced in group B than in group A

and was more advanced in group D than in group C (P = 0.008, P < 0.001, respectively).

Treatment regimen

All patients were treated with a weight-based, 1.5 μg/kg weekly dose of subcutaneous PEG-IFN α-2b (PegIntron, Schering-Plough, Osaka, Japan), in combination with RBV (Rebetol, Schering-Plough), which was given orally at a daily dose of 600-1000 mg based on body weight (600 mg for patients weighing less than 60 kg, 800 mg for those weighing 60-80 kg, and 1000 mg for those weighing 80 kg or over). The length of treatment was 48 wk for patients with HCV genotype 1 and 24 wk for patients with genotype 2. The above duration and dosage are those approved by the Japanese Ministry of Health, Labor and Welfare. Patients were considered to have RBV-induced anemia if the hemoglobin level decreased to less than 100 g/L. In such cases, a reduction in the dose of RBV was required. Patients aged 65 years or older had a significantly higher frequency of RBV dose reduction during the treatment period than those aged less than 65 years old (HCV genotype 1: group A *vs* group B, 41.2% *vs* 49.0%, P = 0.032, genotype 2: group C *vs* group D, 28.6% *vs* 54.1%, P < 0.001). Some patients also had PEG-IFN α-2b-induced psychological adverse effects or a decrease in white blood cell and platelet counts. In such cases, a reduction in the dosage of PEG-IFN α-2b was required. Both PEG-IFN α-2b and RBV were discontinued if the hemoglobin level, white blood cell count, or platelet count fell below 85 g/L, 1 × 10^9/L, and 25 × 10^9/L, respectively. The treatment was discontinued if severe general fatigue, hyperthyroidism, interstitial pneumonia, or severe hemolytic disorders developed, continuation of treatment was judged not to be possible by the attending physician, or if the patient desired discontinuation of treatment.

Determination of baseline HCV RNA level and HCV genotype

The pretreatment, baseline, serum HCV RNA level was measured by a quantitative HCV RNA polymerase chain reaction (PCR) assay (COBAS Amplicor HCV Monitor Test v 2.0 using the 10-fold dilution method; Roche Diagnostics, Tokyo, Japan), which has a lower limit of quantitation of 5000 IU (13500 copies)/mL (5 kIU/mL) and an outer limit of quantitation of 5100000 IU/mL (5100 kIU/mL). The HCV genotype was determined by type-specific primers of the core region of the HCV genome. The protocol for genotyping was carried out as previously described[3].

Efficacy of treatment

End of treatment (EOT) response and SVR were defined as serum HCV RNA undetectable at the end of treatment and at 24-wk follow-up after the end of treatment, respectively. EOT response and SVR were defined as non-detectable HCV-RNA as measured by qualitative COBAS Amplicor HCV Monitor Test v 2.0, with the results labeled as positive or negative. The lower limit of detection was 50 IU/mL (0.5 kIU/mL). The analysis of EOT and SVR was performed on an intention-to-treat basis.

Statistical analysis

Continuous data are expressed as mean ± SD. The statistics were carried out using a commercially available software package (BMDP Statistical Software Inc., Los Angeles, CA, USA) for the IBM 3090 system computer. The χ^2 test, Fisher's exact test and Kruskal-Wallis test were used to determine the differences in baseline clinical characteristics, safety, efficacy of the combination therapy, adherence to the total dose, and the association between the adherence and SVR. Logistic regression analysis was used to identify the association between age and SVR. A P < 0.05 was considered significant.

RESULTS

EOT response rate by intention-to-treat analysis

Among patients with genotype 1, the EOT response rate was significantly higher in group A (497 of 685, 72.5%) than in group B (129 of 253, 45.0%) (P < 0.001). Among patients with genotype 2, there was no significant difference between groups C (239 of 252, 94.8%) and D (55 of 61, 90.1%).

SVR rate by intention-to-treat analysis

Of 1251 patients, 631 (50.4%) achieved SVR in the intention-to-treat analysis. The SVR rate was significantly higher for genotype 2 (249 of 313, 79.6%) than for genotype 1 patients (382 of 938, 40.7%) (P < 0.001). Among patients with genotype 1, the SVR rate was significantly higher in group A (324 of 685, 47.3%) than in group B (58 of 253, 22.9%) (P < 0.001). Among patients with genotype 2, SVR was also significantly higher in group C (209 of 252, 82.9%) than in group D (40 of 61, 65.6%) (P = 0.004). The rate of SVR was significantly higher for females (113 of 128, 88.3%) than for males (96 of 124, 77.4%) in group C only (Figure 1). Furthermore, we analyzed whether or not the SVR rate differed according to the age at which the combination treatment of PEG-IFN α-2b plus RBV was started. The results showed that the SVR rate decreased significantly with age for both genotype 1 and 2. SVR was achieved by 5.6%-26.3% of genotype 1 patients aged 70 years or older, and by 57.1%-100% of genotype 2 patients aged 70 years or older (Figure 2).

We previously reported a minimum acceptable dose of at least 80% or more of the target dosage of PEG-IFN α-2b and 60% or more of the target dosage of RBV for the successful treatment of Japanese patients with genotype 1[8]. Therefore, we analyzed the SVR rates in patients with genotype 1 by the dosage they actually received during treatment (a total dose of at least 80% or more of PEG-IFN α-2b and 60% or more of RBV) (Table 3). The number who received at least this minimum acceptable dosage during treatment were 278 (40.6%) of 685 patients in group A and 62 (24.5%) of 253 in group B, significantly lower in group B than in group A (P < 0.001). Compared with patients who received less than the minimum acceptable dosage, in patients who received at least this minimum dosage, the SVR rates increased from 34.2% to 66.5% in group A patients and from 15.7% to 45.2%

Table 3 The comparison of the rate of sustained virological response of patients with genotype 1 receiving a dose of 80% or more of pegylated interferon α-2b plus 60% or more of ribavirin and the reduced dosage group *n* (%)

	Male		Female		Total	
	n	SVR	*n*	SVR	*n*	SVR
Group A						
Minimum acceptable	168	116 (69.0)	110	69 (62.7)	278	185 (66.5)
Reduced	206	73 (35.4)	201	66 (32.8)	407	139 (34.2)
Total	374	189 (50.5)	311	135 (43.4)	685	324 (47.3)
Group B						
Minimum acceptable	31	15 (48.4)	31	13 (41.9)	62	28 (45.2)
Reduced	91	18 (19.8)	100	12 (12.0)	191	30 (15.7)
Total	122	33 (27.0)	131	25 (19.1)	253	58 (22.9)

Minimum acceptable: patients who received 80% or more of the target dose of pegylated interferon (IFN) α-2b and 60% or more of ribavirin (RBV). Reduced: Patients who received less than 80% of pegylated IFN α-2b and less than 60% of RBV. SVR: Sustained virological response.

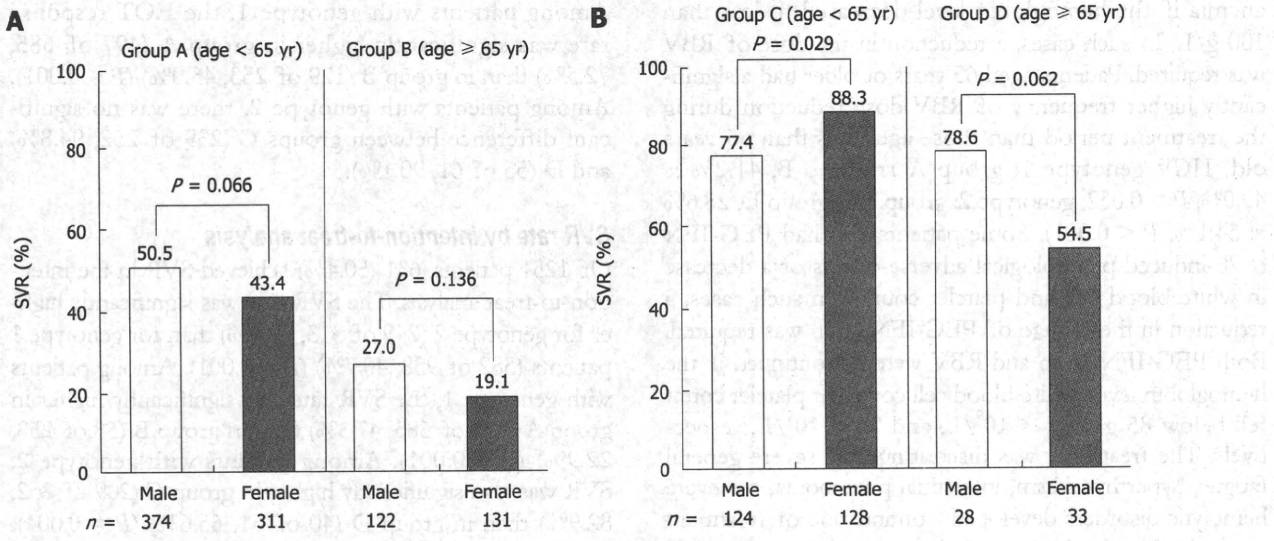

Figure 1 Virological response to the combination treatment by age and sex of patients with genotype 1 (A) and genotype 2 (B). SVR: Sustained virological response.

($P < 0.001$) in group B patients. No significant difference between groups C and D was observed. On comparing patients whose platelet count was under 10×10^{10}/L, the SVR rate for genotype 1 was significantly lower in group B (2 of 36, 5.6%) than in group A (16 of 56, 28.6%) ($P < 0.001$). Among the patients with genotype 2, SVR was not significantly different between group C (9 of 16, 56.3%) and group D (2 of 7, 28.6%).

In a comparison of the SVR rate in patients with or without one or more previous courses of IFN plus RBV, there was no significant difference between the genotypes (genotype 1: 118 of 310, 38.1% *vs* 264 of 628, 42.0%, genotype 2: 44 of 72, 61.1% *vs* 141 of 241, 58.5%). Furthermore, we compared the EOT response rate and SVR rate of cirrhosis patients whose liver fibrosis was F4, and found no significant difference between groups A (EOT: 16 of 30, 53.3%, SVR: 7 of 30, 23.3%) and B (EOT: 6 of 17, 35.3%, SVR: 2 of 17, 11.8%). In addition, no significant difference was found between groups C (EOT: 8 of 10, 80.0%, SVR: 6 of 10, 60.0%) and D (EOT: 9 of 12, 75.0%, SVR: 5 of 12, 41.7%).

Discontinuation of PEG-IFN α-2b plus RBV treatment and adverse effects

Of 1251 patients, 314 (25.1%) did not complete PEG-IFN α-2b plus RBV treatment due to adverse effects or other reasons. The discontinuation rate was significantly higher in patients with genotype 1 (273 of 938, 29.1%) than in those with genotype 2 (41 of 313, 13.1%) ($P < 0.001$) (Tables 4 and 5). Furthermore, the rate of discontinuation due to adverse effects was significantly higher in patients with genotype 1 (135 of 938, 14.4%) than in those with genotype 2 (23 of 313, 7.3%) ($P < 0.010$). The rates of discontinuation due to lack of treatment efficacy and for economic reasons (loss of job, inability to pay the medical costs) were also significantly higher in patients with genotype 1 (55 of 938, 5.9%, 15 of 938, 1.6%) than in those with genotype 2 (1 of 313, 0.3%, 0 of 938, 0%) ($P < 0.001$ and $P = 0.025$, respectively).

For genotype 1 patients, the discontinuation rate was significantly higher in group B (106 of 253, 42.9%) than in group A (167 of 685, 24.4%) ($P < 0.001$), and the rate of discontinuation due to adverse effects was also significantly

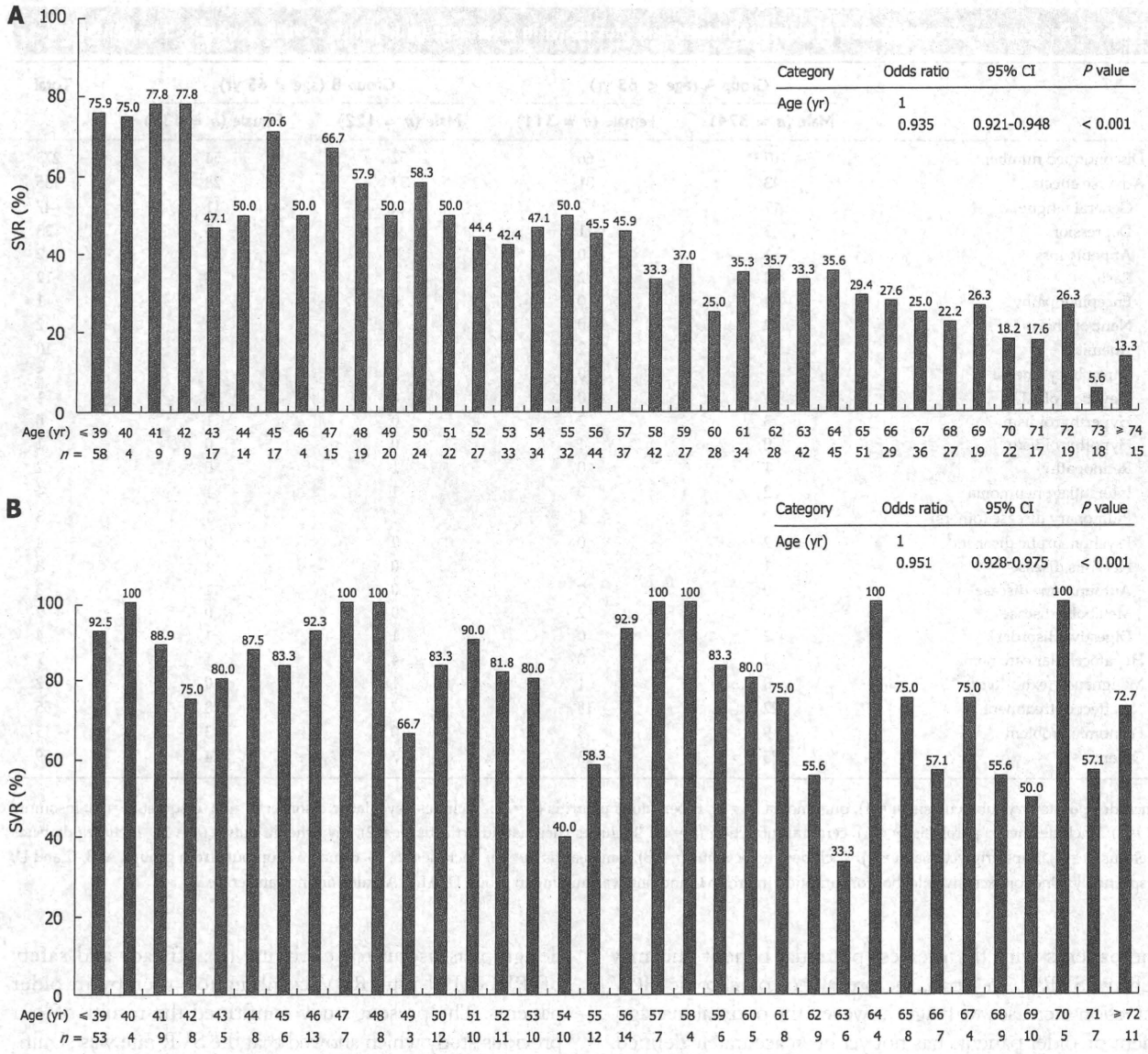

Figure 2 Virological response to the combination treatment by age of patients with genotype 1 (A) and genotype 2 (B). SVR: Sustained virological response; CI: Confidence interval.

higher in group B (61 of 253, 24.1%) than in group A (74 of 685, 10.8%) ($P < 0.001$). General fatigue was the most frequent adverse effect, and was significantly more frequent in group B than in group A ($P < 0.001$). However, in these group 1 patients, RBV was reduced due to anemia in 12.5% (3 of 24) of group A and in 30.4% (7 of 23) of group B. Furthermore, rash and thrombocytopenia were significantly more frequent in group B than in group A ($P = 0.014$ and $P = 0.007$, respectively). In group A, depression was significantly more frequent in females than in males ($P = 0.012$). In genotype 2 patients, treatment discontinuation did not differ between group C (33 of 252, 13.1%) and group D (8 of 61, 13.1%), and the rate of discontinuation due to adverse effects did not differ between these groups (17 of 252, 6.7%, 6 of 61, 9.8%, respectively).

The mean time to discontinuation in group A (21.6 ± 11.9 wk) was not significantly different from group B (21.5 ± 12.6 wk), and the mean time in group C (11.0 ± 6.8 wk) was also not significantly different from group D (11.6 ± 6.0 wk). There was no significant difference between male and female patients in each group (male: 21.0 ± 12.4 *vs* female: 22.1 ± 11.8 in group 1, male: 11.3 ± 7.1 *vs* female: 10.9 ± 6.1 in group 2).

HCC was not seen in genotype 2 patients; only in patients with genotype 1 (29.5 ± 9.9 wk) and was more frequent in group B (5 of 253, 2.0%) than in group A (2 of 685, 0.3%) ($P = 0.008$).

DISCUSSION

In a large, national, multicenter Greek study involving 993 treated and 734 untreated patients with chronic hepatitis C, patients with cirrhosis, showed a protective effect of treatment even among those without SVR. For patients without cirrhosis, the beneficial effect of IFN α treatment was particularly evident in older patients; patients with the worst prognosis if left untreated. Therefore, IFN α-based treatment should be offered to older persons, as these are

Table 4 Reasons for discontinuation of pegylated interferon plus ribavirin treatment by hepatitis C virus genotype 1 patients

	Group A (age < 65 yr)		Group B (age ≥ 65 yr)		Total
	Male (*n* = 374)	Female (*n* = 311)	Male (*n* = 122)	Female (*n* = 131)	
Discontinued number	101	66	52	54	273
Adverse effects	43	31	33	28	135
General fatigue	17	7	12	11	47
Depression	3	11	4	5	23
Appetite loss	1	0	1	0	2
Rash	3	2	3	4	12
Encephalopathy	1	0	0	0	1
Neutropenia	2	0	0	0	2
Anemia	3	2	4	1	10
Thrombocytopenia	1	0	3	1	5
Elevation of ALT	1	0	0	0	1
Hyperthyroidism	3	2	0	1	6
Hypothyroidism	0	1	0	0	1
Retinopathy	1	0	1	0	2
Interstitial pneumonia	2	0	1	1	4
Pulmonary disease (others)[1]	0	1	1	1	3
Psychoneurotic disorder[2]	2	0	2	0	4
Nervous disease[3]	1	1	0	1	3
Autoimmune disease[4]	0	2	0	1	3
Metabolic disease[5]	0	2	0	0	2
Digestive disorder[6]	2	0	1	1	4
Hepatocellular carcinoma	2	0	4	1	7
Malignancy (extra-liver)	0	1	1	0	2
No effect of treatment	22	18	7	8	55
Economic problem	9	3	0	3	15
Others[7]	25	13	7	14	59

[1]Includes pulmonary tuberculosis (*n* = 1), pneumonia (*n* = 1), tuberculous pleuritis (*n* = 1); [2]Includes psychiatric disorder (*n* = 2), disquiet (*n* = 1), insomnia (*n* = 1); [3]Includes nerve paralysis (*n* = 1), cerebral infarction (*n* = 1); [4]Includes rheumatoid arthritis (*n* = 2), myasthenia gravis (*n* = 1); [5]Includes diabetes mellitus (*n* = 1), hypertriglycemia (*n* = 1); [6]Includes cholecystitis (*n* = 3), pancreatitis (*n* = 1); [7]Includes 25, 13, 6 and 13 drop-outs from groups A, B, C and D, respectively: One for excessive alcohol consumption in group C and one was nursing in group D. ALT: Alanine aminotransferase.

the patients with the greatest potential benefit and may achieve SVR[16]. In Japan, the prevalence of chronic HCV infection increases with age, however, the optimal management of older patients has not yet been accurately defined. Whether or not to treat patients older than 65 years with antiviral treatment is highly debated, especially in terms of cost/benefit ratio. In addition, the natural history of chronic hepatitis C in elderly patients is not accurately known, as the presence of comorbidity can affect illness progression and life expectancy. HCV became more prevalent in Japan decades before the United States[17]. Japanese patients with chronic hepatitis C treated with IFN are currently 10 to 15 years older than corresponding patients in the United States and European countries, where patients treated with antiviral treatment tend to average 45 years of age[18-20]. Therefore, our results can serve as a world-wide model for the treatment of older chronic hepatitis C patients.

It has been well documented that the combination therapy of PEG-IFN α-2b plus RBV is more effective than previous IFN monotherapy in chronic hepatitis C patients[7,8]. There have been four studies on the efficacy of PEG-IFN plus RBV therapy in patients 65 years or older with genotype 1, which revealed low rates of SVR (31.1%-51.9%)[21-24]. However, these studies were too small (11-93 patients) for conclusive recommendations to be made. Because the present study was a large multicenter design, it is useful for clarifying the efficacy and safety of PEG-IFN plus RBV combination therapy in older patients. The present study confirmed the results of our previous study which showed that the SVR rate was significantly higher for genotype 2 than for genotype 1 patients[8]. Another important result was that the ability to take at least a minimum acceptable dosage during treatment increased the SVR rate by about three times in older patients with genotype 1. This result also confirmed previous studies which indicated the importance of giving at least the minimum acceptable treatment dosage in patients infected with HCV genotype 1, especially older patients[23,24].

Secondly, we compared discontinuation of treatment by genotype and sex. In genotype 1 patients, adverse effects were seen more often in older than in younger patients. This was the most important reason why the rate of treatment discontinuation was higher in older than in younger patients, and affected the outcome of PEG-IFN α-2b plus RBV combination therapy. General fatigue was the most common adverse effect in older patients. Because older patients often have impaired renal function, they have increased blood levels of RBV[25,26]. They are also inclined to be anemic and to have general fatigue. However, only a small number of older patients in the present study had reduced RBV due to anemia. Therefore, general fatigue is probably a direct adverse effect of PEG-IFN α-2b. We previously reported that herbal medicine

Table 5 Reasons for discontinuation of pegylated interferon plus ribavirin treatment by hepatitis C virus genotype 2 patients

	Group C (age < 65 yr)		Group D (age ≥ 65 yr)		Total
	Male (n = 124)	Female (n = 128)	Male (n = 28)	Female (n = 33)	
Discontinued number	18	15	4	4	41
Adverse effects	6	11	3	3	23
General fatigue	1	3	1	0	5
Depression	0	2	0	0	2
Appetite loss	0	0	0	0	0
Rash	2	1	0	2	5
Encephalopathy	0	0	0	1	1
Neutropenia	0	2	0	0	2
Anemia	0	0	2	0	2
Thrombocytopenia	2	0	0	0	2
Elevation of ALT	0	0	0	0	0
Hyperthyroidism	0	1	0	0	1
Hypothyroidism	0	1	0	0	1
Retinopathy	0	0	0	0	0
Interstitial pneumonia	0	0	0	0	0
Pulmonary disease(others)	0	0	0	0	0
Psychoneurotic disorder	0	0	0	0	0
Nervous disease[1]	1	1	0	0	2
Autoimmune disease	0	0	0	0	0
Metabolic disease	0	0	0	0	0
Digestive disorder	0	0	0	0	0
Hepatocellular carcinoma	0	0	0	0	0
Malignancy (extra-liver)	1	0	0	0	1
No effect of treatment	1	0	0	0	1
Economic problem	0	0	0	0	0
Others[2]	10	4	1	1	16

[1]Includes nerve paralysis (n = 1), tetany (n = 1); [2]All patients were drop out. ALT: Alanine aminotransferase.

relieved the adverse effects of IFN, including general fatigue[27]. Herbal medicine may be useful for mitigating general fatigue during PEG-IFN α-2b plus RBV combination treatment, especially in older patients.

The rate of discontinuation was lower in patients with genotype 2 than in patients with genotype 1, and there was no difference between the older and the younger patients with genotype 2. These results are possibly a consequence of the shorter term of treatment in genotype 2 and the many genotype 1 patients who discontinued due to lack of efficacy.

Two of the characteristics of older patients in the present study were that both hemoglobin and platelet count were significantly lower than in younger patients. The SVR rate was significantly lower when the platelet count was less than 10×10^{10}/L. Furthermore, the older genotype 1 patients were often forced to discontinue treatment due to thrombocytopenia and the occurrence of HCC. These findings appear to result from advanced liver fibrosis in older chronic hepatitis C patients. Therefore, the possibility of HCC during long-term IFN treatment in older patients must be considered.

We previously reported that older female patients had a low response to IFN-α monotherapy[9], and other investigators have reported that older female patients have a poor response to PEG-IFN α-2b plus RBV[22,28]. Although our data showed that sex was not related to SVR, the reason for this finding was not fully elucidated. In any case, studies have conclusively shown that it is important to begin treatment with PEG-IFN α-2b plus RBV combination therapy as soon as possible. Our data suggest that age may be a more important factor than sex for increasing the rate of SVR. Resistance to treatment in older patients may be due to IFN-immunomodulation, advanced liver fibrosis, or reduced dosage.

To maximize adherence to the optimal treatment regimen, the treatment schedule can be modified or other therapeutic modalities added, such as hematopoietic growth factors[29] or the new thrombopoietin-receptor agonist, eltrombopag, for the antiviral treatment of older patients with chronic hepatitis C[30]. A further individualized treatment protocol based on viral kinetics might be more practical[31].

In conclusion, PEG-IFN α-2b plus RBV treatment was effective in the treatment of older chronic hepatitis C patients when they received at least the minimum acceptable treatment dosage. However, there were frequent adverse effects and treatment discontinuation. It is necessary to control for adverse effects that might interrupt treatment and to begin this combination therapy as soon as possible, especially in older patients.

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COMMENTS

Background

Whether or not to treat patients older than 65 years with antiviral treatment is highly debated, especially in terms of cost/benefit ratio. However, there is little data concerning the response and safety of combination treatment for a large number of older patients with chronic hepatitis C virus infection. Therefore, in an attempt to ameliorate these problems, the authors decided to treat older patients with pegylated interferon (PEG-IFN) α-2b plus ribavirin (RBV) combination therapy.

Research frontiers

The combination treatment of PEG-IFN α-2b plus RBV improved the sustained virological response rate in chronic hepatitis C patients. However, the current issue is whether or not to treat older patients because of low response and high dropout rate.

Innovations and breakthroughs

There have been four studies on the efficacy of PEG-IFN plus RBV therapy in patients 65 years or older with genotype 1. However, these studies were too small (11-93 patients) for conclusive recommendations to be made. This study is very useful for clarifying the efficacy and safety of PEG-IFN plus RBV combination therapy in older patients, because of its large scale, multicenter design.

Applications

The study demonstrated that PEG-IFN α-2b plus RBV treatment was effective in chronic hepatitis C patients 65 years or older who completed treatment with at least the minimum required treatment dosage. Furthermore, this study suggested that the combination treatment and beginning this therapy as soon as possible are important, especially in older patients.

Peer review

The study has been well conducted and includes a large number of patients. Results have been described in a lucid and informative manner and are of clinical relevance.

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RESEARCH ARTICLE Open Access

Excellent superiority and specificity of COBAS TaqMan HCV assay in an early viral kinetic change during pegylated interferon alpha-2b plus ribavirin treatment

Eiichi Ogawa[†1], Norihiro Furusyo*[†1,2], Kazuhiro Toyoda[1], Hiroaki Taniai[1], Shigeru Otaguro[1], Mosaburo Kainuma[1], Masayuki Murata[1], Yasunori Sawayama[1] and Jun Hayashi[1,2]

Abstract

Background: An early virological response (EVR) after the start of interferon (IFN) treatment for chronic hepatitis C leads to a successful virological outcome. To analyze an association between sustained virological response (SVR) and EVR by comparing TaqMan with Amplicor assays in HCV genotype 1-infected patients treated with pegylated (PEG)-IFN alpha-2b plus ribavirin (RBV).

Methods: We retrospectively analyzed a total of 80 HCV genotype 1 patients (39 SVR and 41 non-SVR patients), who received an enough dosage and a complete 48-week treatment of PEG-IFN alpha-2b plus RBV. Serum HCV RNA levels were measured by both TaqMan and Amplicor assays for each patients at Weeks 2, 4, 8 and 12 after the start of the antiviral treatment.

Results: Of the 80 patients with undetectable HCV RNA by Amplicor, 17 (21.3%) patients were positive for HCV RNA by TaqMan at Weeks 12. The quantification results showed that no significant difference in the decline of HCV RNA level between TaqMan and Amplicor 10-fold method assays within the initial 12 weeks of the treatment was found. However, the qualitative analysis showed significant differences of the positive predictive rates for SVR were found between TaqMan (100% at weeks 4 and 100% at weeks 8) and Amplicor (80.0% and 69.6%, respectively).

Conclusions: The COBAS TaqMan HCV assay is very useful for monitoring HCV viremia during antiviral treatment to predict a SVR in HCV genotype 1 patients.

Background

The hepatitis C virus (HCV) infection is a main cause of viral chronic hepatitis in Japan, where approximately 1.8 million patients have HCV infection. The prognosis of liver fibrosis made by wound-healing response to chronic liver injury may lead to cirrhosis [1,2].

The most current antiviral treatment for chronic HCV infection is pegylated interferon (PEG-IFN) alpha in combination with ribavirin (RBV), which has been used in worldwide [3,4]. The combination treatment has resulted in a higher rate of sustained virological response (SVR) than standard interferon (IFN) monotherapy [5,6]. The predictor of IFN treatment response such as HCV genotype, HCV RNA level, age, sex, the stage of liver fibrosis, the duration and dose of antiviral treatment and so on has been reported previously [7-9]. Otherwise, the predictive factor of antiviral efficacy after the start of antiviral treatment has been studied by HCV dynamics, which the duration of virological clearance of HCV RNA is associated with the therapeutic efficacy. Especially, an early viral response (EVR), a virological clearance by antiviral treatment within the initial 12 weeks, is significantly correlated with SVR in the treated patients [10-12].

Quantifying serum HCV RNA level helps to evaluate the efficacy and monitoring of antiviral treatment. Both qualitative and quantitative HCV RNA assay using

* Correspondence: furusyo@gim.med.kyushu-u.ac.jp
[1] Department of General Internal Medicine, Kyushu University Hospital, Fukuoka, Japan
[†] Contributed equally
Full list of author information is available at the end of the article

COBAS Amplicor system (Amplicor) has been used well in clinical [13]. Recently, COBAS TaqMan HCV assay (TaqMan) equipped with highly detection sensitivity and widely measuring range has been developed, and this has enabled to measure qualitative and quantitative analysis simultaneously [14,15]. The aim of the present study is to analyze an association between SVR and EVR by comparing TaqMan with Amplicor assays in patients with HCV genotype 1 infection who has been treated with PEG-IFN alpha-2b plus RBV.

Methods

Patients

A retrospective study of 80 Japanese genotype 1-infected chronic hepatitis C patients who were treated with 48-week PEG-IFN alpha-2b plus RBV was done from January 2005 to July 2007, in order to analyze an association between sustained virological response (SVR) and early virological response (EVR) by comparing TaqMan with Amplicor assays. To exactly consider an association between antiviral response and viral kinetics, we selected all 80 patients who received both PEG-IFN alpha-2b 80% or over and RBV 60% or over of the target dosage of 48-week [16]. Using COBAS Amplicor HCV Monitor Test v2.0 (qualitative Amplicor), the 80 selected patients were negative for HCV RNA during the treatment and no patients with positivity HCV RNA during the treatment were included in this study.

SVR was defined as serum HCV RNA undetectable at 24 weeks after the end of treatment. Patients who had undetectable HCV RNA within the initial 12 weeks of treatment were considered to have had an EVR. Of the 80 patients, 39 achieved SVR at 24 weeks after the end of treatment, and 41 had clearance of HCV viremia during the treatment but achieved non-SVR after the end of the treatment.

All patients were satisfied the following criteria. Criteria for exclusion were: (i) clinical or biochemical evidence of hepatic decompensation, advanced cirrhosis identified by large esophageal varices (F2 or F3), history of gastrointestinal bleeding, ascites, encephalopathy, or hepatocellular carcinoma; (ii) hemoglobin level < 115 g/L, white blood cell count < 3 × 10^9/L, and platelet count < 50 × 10^9/L; (iii) concomitant liver disease other than hepatitis C (hepatitis B surface antigen positive or human immunodeficiency virus antibody positive); (iv) excessive active alcohol consumption (> 60 g/day converted into ethanol) or drug abuse; (v) severe psychiatric disease; and (vi) antiviral or corticosteroid therapy within 12 months prior to the enrolment. Patients who fulfilled the above criteria were recruited at the Department of General Internal Medicine, Kyushu University Hospital.

The diagnosis of chronic hepatitis and cirrhosis was based on a liver biopsy in each patient. All patients were diagnosed with chronic active hepatitis with piecemeal necrosis or fibrosis formation of portal-portal bridging. No significant differences were observed between these group patients at entry.

Clinical and biochemical characteristics of the enrolled 80 patients with chronic HCV infection are summarized in Table 1. The mean age of SVR patients was significantly lower (54.8 ± 9.3 years) than that of non-SVR patients (59.8 ± 9.7 years) (P = 0.0133). No significant differences were found in the means for body mass index, alanine aminotransferase, γ-glutamyl-transpeptidase or platelet count between SVR and non-SVR patient groups.

The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and the International Conference on Harmonization of guidelines for good clinical practice.

Therapeutic protocol

All patients were treated with a weight-based, 1.5 μg/kg weekly dose of subcutaneous PEG-IFN alpha-2b (PegIntron A; Schering-Plough, Osaka, Japan). In combination with PEG-IFN alpha-2b, RBV (Rebetol; Schering-Plough) was given orally at a daily dose of 600-1,000 mg based on bodyweight (600 mg for patients weighing < 60 kg, 800 mg for those weighing 60-80 kg, and 1,000 mg for those weighing > 80 kg). The length of the combined treatment was 48 weeks. The above duration and dosage are those approved by the Japanese Ministry of Health, Labor and Welfare. Patients were considered to have RBV-induced anemia if the hemoglobin level decreased to < 100 g/L. In such cases, a reduction in the dose of RBV was required. Some patients also had PEG-IFN alpha-2b induced psychological adverse effects or a decrease of white blood cell and platelet count. In such cases, a reduction in the dosage of PEG-IFN alpha-2b was required. Both PEG-IFN alpha-2b and RBV were discontinued if the hemoglobin level, white blood cell count, or platelet count fell below 85 g/dL, 1 × 10^9/L, and 2.5 × 10^9/L, respectively.

Definition of SVR and EVR

SVR and EVR were defined as non-detectable HCV RNA as measured by the COBAS Amplicor HCV Monitor Test v2.0 (qualitative Amplicor) (Roche Diagnostics, Tokyo, Japan), and the results were labeled as positive or negative. The lower limit of detection was 50 IU/mL (0.5 kIU/mL: 1.7 log IU/mL) [17]. Moreover, we compared serum HCV RNA negativity by the qualitative Amplicor assay with that by TaqMan assay during the initial 12 weeks of treatment.

Determination of HCV RNA level

During the treatment period (the initial 12 weeks: at week 2, week 4, week 8, and week 12), we retrospectively determined serum HCV RNA level by both COBAS TaqMan

Table 1: Characteristics of 80 genotype 1-infected chronic hepatitis C patients treated with pegylated interferon alpha 2b plus ribavirin combination, classified by the treatment response

Characteristics	Total No. = 80	SVR No. = 39	Non-SVR No. = 41	P value
Male No. (%)	36 (45.0)	17 (43.6)	19 (46.3)	0.9821
Age (years)	57.4 ± 9.8	54.8 ± 9.3	59.8 ± 9.7	0.0133
Body Mass Index (kg/m^2)	23.0 ± 3.1	23.1 ± 3.0	23.6 ± 3.4	0.7821
Alanine aminotransferase (IU/L)	89.9 ± 65.4	93.2 ± 62.8	86.5 ± 65.7	0.7832
γ-glutamyltranspeptidase (IU/L)	59.2 ± 54.0	41.2 ± 34.2	77.3 ± 33.1	0.0548
Albumin (g/dL)	4.2 ± 0.4	4.2 ± 0.4	4.0 ± 0.4	0.7105
White blood cell count (10^9/L)	5.6 ± 1.4	5.3 ± 1.4	5.8 ± 1.7	0.4666
Hemoglobin (g/L)	131 ± 14	133 ± 14	129 ± 14	0.2310
Platelet count (10^9/L)	161 ± 51	167 ± 45	156 ± 55	0.1576
Creatinine (mg/dL)	0.71 ± 0.14	0.69 ± 0.16	0.70 ± 0.15	0.3376
Creatinine clearance (mL/min)	98.8 ± 27.6	100.2 ± 28.7	99.3 ± 32.3	0.4102
Histological cirrhosis No. (%)	6 (7.5)	2 (5.1)	4 (9.7)	0.6758

HCV, hepatitis C virus
Data is shown as the mean ± standard deviation.
SVR means a sustained virological response, which is defined as serum HCV RNA undetectable at 24 weeks after the end of treatment.

HCV assay (TaqMan) (Roche Diagnostics) and COBAS Amplicor HCV Monitor Test v2.0 using the 10-fold dilution method (Amplicor 10-fold method) (Roche Diagnostics) in each patient. The TaqMan has a lower limit of quantitation of 15 IU/mL and an outer limit of quantitation of 6.9 × 10^7 IU/mL (1.2 to 7.8 log IU/mL referred to $\log_{10}$ units/mL) [14,15]. Therefore, TaqMan assay is able to do both qualitative and quantitative analysis for HCV RNA. The Amplicor 10-fold method has a lower limit of quantitation of 5,000 IU (5 kIU/mL) and an outer limit of quantitation of 5,100,000 IU (5,100 kIU/mL) [18]. To compare serum HCV RNA level by TaqMan assay with Amplicor 10-fold method assay, we transformed the level by Amplicor 10-fold method assay (kIU/mL) into the logarithmic level (log IU/mL). Therefore, the range of Amplicor 10-fold method is 3.7 to 6.7 log IU/mL.

Determination of HCV genotype

HCV genotype was determined using type-specific primers from the core region of the HCV genome. The protocol for genotyping was carried out as described earlier [19].

Statistical analysis

Statistical analysis was done with BMDP statistical software for the IBM 3090 system computer (BMBD Statistical Software, Inc., Los Angeles, CA, USA) for the IBM (Yorktown Heights, NY) 3090 computer system. Continuous data were expressed as mean values, mean ± standard deviation (SD), or values ± standard error (SE) of the mean. The paired t-test, unpaired t-test, Mann-Whitney U test or Kruskal-Wallis non-parametric analysis of variance was used to compare HCV dynamics. A P value less than 0.05 was regarded as statistically significant.

Results

The correlation of pretreatment HCV RNA levels between TaqMan and Amplicor 10-fold method assays

The relationship of pretreatment HCV RNA levels between TaqMan and Amplicor 10-fold method assays was studied in 39 SVR and 41 non-SVR patients infected with genotype 1. The levels by TaqMan ranged from 4.4 to 7.2 log IU/mL (median 6.1 log IU/mL) and those by Amplicor 10-fold method ranged from 5.0 to 6.7 log IU/mL (median 6.0 log IU/mL). We found a significantly positive correlation in the pretreatment HCV RNA level between TaqMan and Amplicor 10-fold method assays ($r = 0.849$, $P < 0.0001$).

Figure 1 shows pretreatment levels of HCV RNA classified by viral response (SVR or non-SVR). In TaqMan assay, the 39 SVR patients had significantly lower pretreatment HCV RNA level (median 5.89 log IU/mL) than the 41 non-SVR patients (median 6.25 log IU/mL) ($P = 0.0191$). However, Amplicor 10-fold method assay showed no significant difference of pretreatment HCV RNA level between the SVR (median 5.91 log IU/mL) and non-SVR (median 6.09 log IU/mL) patients ($P = 0.0929$). Therefore, pretreatment HCV RNA by TaqMan assay may be a predictive factor for SVR, but not by Amplicor

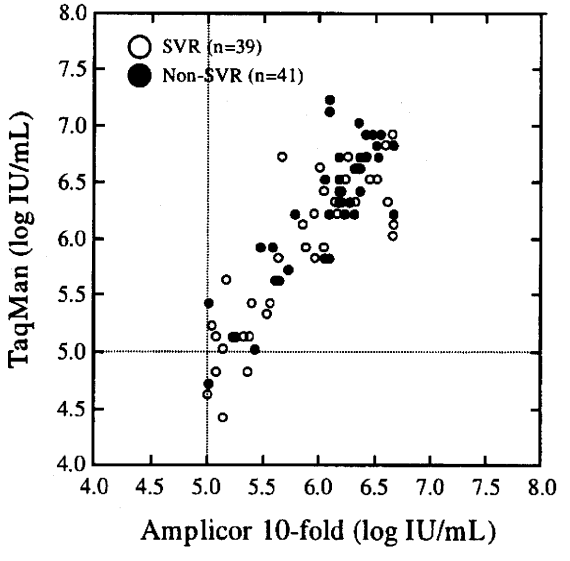

Figure 1 Correlation of pretreatment hepatitis C virus (HCV) RNA levels between TaqMan and Amplicor 10-fold method assays, classified by sustained virological response (SVR) and non-SVR. Open and closed circles mean SVR and non-SVR patients, respectively.

10-fold method assay among patients with HCV genotype 1.

The comparison with TaqMan and Amplicor 10-fold method assays in HCV dynamics within the initial 12 weeks of PEG-IFN plus RBV combined treatment

The relationship of HCV dynamics within the initial 12 weeks of the combined treatment between TaqMan and Amplicor 10-fold method assays was studied in 39 SVR and 41 non-SVR patients. Figure 2 shows the difference in the logarithmic decline from pretreatment HCV RNA level within the initial 12 weeks of the treatment between SVR and non-SVR patients by TaqMan and Amplicor 10-fold method assays. The logarithmic declines of HCV RNA levels by TaqMan in SVR patients (-3.54, -5.19, and -5.65 at weeks 2, 4, and 8, respectively) were significantly higher than those in non-SVR patients (-2.23, -3.42, and -4.68 at weeks 2, 4, and 8, respectively) ($P < 0.0001$, $P < 0.001$, and $P = 0.0012$, respectively), except for at weeks 12 (-5.89 and -5.75, $P = 0.3936$). Similarly, the logarithmic declines of HCV RNA levels by Amplicor 10-fold method assay in SVR patients (-2.95, -4.36, and -5.47 at weeks 2, 4, and 8, respectively) were significantly higher than non-SVR patients (-2.01, -3.09, and -4.11 at weeks 2, 4, and 8, respectively) ($P = 0.00281$, $P = 0.00021$, and $P = 0.0006$, respectively), except for at weeks 12 (-5.91 and -6.09, $P = 0.0929$). No significant difference in monitoring the HCV dynamics during the initial 12 weeks administration of the combined treatment was found between TaqMan and Amplicor 10-fold method assays.

The comparison with TaqMan and qualitative Amplicor assays in HCV RNA negativity within the initial 12 weeks of PEG-IFN plus RBV combined treatment

The undetectable HCV RNA by TaqMan was found in all of 39 SVR patients (100%) but only in 24 of 41 non-SVR patients (58.5%), while that by qualitative Amplicor was found in all of 39 SVR and 41 non-SVR patients. TaqMan reduced the numbers of undetectable HCV RNA in the group of non-SVR.

Figure 3 shows the distribution of timing of HCV RNA undetectable after the initiation of treatment at weeks 2, 4, 8, and 12 in comparison with TaqMan and qualitative Amplicor assays. Among 39 SVR patients, the distributions of timing of detectable HCV RNA were 17.9% (7/39), 43.6% (17/39), 0% (0/39), and 38.5% (15/39) by TaqMan and 17.9% (7/39), 33.3% (13/39), 30.8% (12/39), and 17.9% (7/39) by qualitative Amplicor assay at 2, 4, 8, and 12 weeks, respectively. There was no significant difference in timing of undetectable HCV RNA between TaqMan and qualitative Amplicor assays in the group of SVR. Among 41 non-SVR patients, the distribution of timing of detectable HCV RNA by TaqMan was 58.5% (24/41) at 12 weeks, and those by qualitative Amplicor assay were 2.4% (7/41), 9.8% (4/41), 21.9% (9/41), and 65.8% (27/41) at 2, 4, 8, and 12 weeks, respectively. Patients of non-SVR had apparent delayed timing of undetectable HCV RNA, and the tendency was more frequently found by TaqMan than by qualitative Amplicor assay.

The comparison with TaqMan and qualitative Amplicor assays for positive predictive value (PPV) rates of SVR in patients with EVR

The PPV rates for SVR were calculated within the initial 12 weeks during the combined treatment, based on HCV RNA negativity by TaqMan and qualitative Amplicor assays, respectively. Figure 4 shows significant differences of the initial 12-week PPV rates for SVR were found between TaqMan assay (at weeks 4 and 8, 100% and 100%, respectively) and qualitative Amplicor (80.0% and 69.6%, respectively) assays. To predict SVR when patients have clearance of viremia, TaqMan assay is more useful than qualitative Amplicor assay in analysis of the early stage (within 8 weeks) of HCV dynamics.

Discussion

Measurement of serum HCV RNA level is absolutely imperative for the treatment of chronic HCV infection, because this is an effective predictive and indicative factor for the response of IFN therapy and duration of therapy [7,9,10,20]. A more useful analysis is the time to first undetectability and its relation to the end of treatment response, relapse rate and SVR [12]. Therefore, an advanced technique for detecting serum HCV RNA during the antiviral treatment needs a high degree of accu-

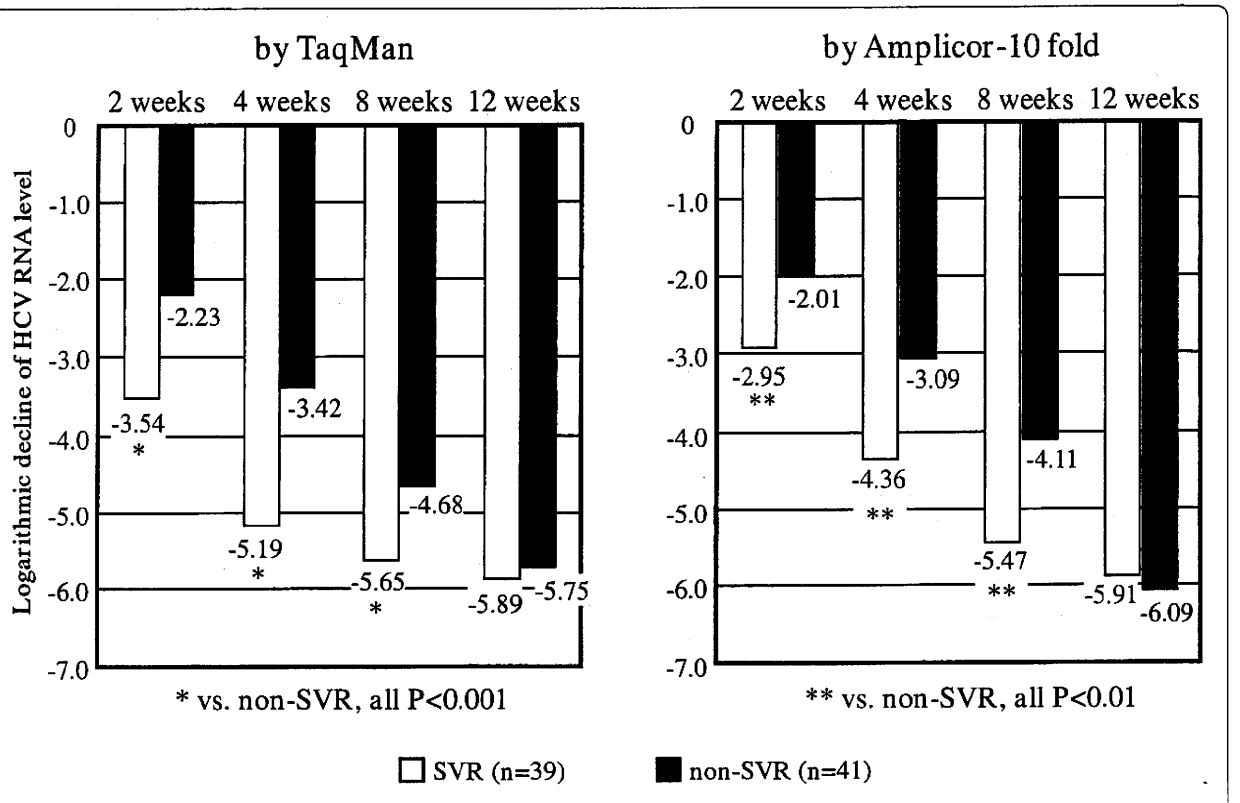

Figure 2 The logarithmic declines from pretreatment hepatitis C virus (HCV) RNA by TaqMan and Amplicor 10-fold method assays within the initial 12 weeks during pegylated interferon alpha plus ribavirin treatment. White and black bars mean sustained virological response (SVR) and non-SVR patients, respectively.

racy. The ideal molecular analysis for HCV RNA level has to be sensitive, accurate and have a broad dynamic range to monitor viral load changes during antiviral therapy. TaqMan assay is useful for simultaneously analyzing qualitative and quantitative HCV RNA level in serum [14,15]. The present study included only patients infected with chronic HCV genotype 1, who received a full of 48-week treatment of PEG-IFN plus RBV combination with target dosages of both drugs (PEG-IFN alpha-2b 80% or over and RBV 60% or over of the above prescribed dosage). We believed that analyzing the exact response factors for antiviral treatment should be considered only under this enough dosage of the combined treatment [16]. The present study showed the superiority of TaqMan assay over Amplicor assay for analyzing the early prediction of virologic response to antiviral therapy.

TaqMan assay, the performance of a fully automated system based on the real-time PCR technology, was evaluated for nucleic acid extraction from plasma. This results in enhanced user convenience, a great reduction in labor requirements minimizing hands-on time and a decrease the risk of sample contaminations. TaqMan assay was extremely sensitive with a linear dynamic range up to 6.6 log IU/mL (0.015-69,000 kIU/mL: 1.2-7.8 log IU/mL) [14,15]. Otherwise, Amplicor 10-fold method assay had a narrower linear dynamic range up to 3.0 log IU/mL (5-5,000 kIU/mL: 3.7-6.7 log IU/mL) than TaqMan assay and qualitative Amplicor assay, the lower limit of detection was 50 IU/mL (1.7 log IU/mL) [17]. These different ranges can explain significant differences in the pretreatment HCV RNA levels and the reduction rates of HCV RNA levels during an antiviral treatment between TaqMan and Amplicor assays.

Previous studies demonstrated that PEG-IFN plus RBV treatment dramatically increased SVR rate in patients with HCV infection and thus are currently the gold standard of treatment [3,4,7]. The most significant predictors of SVR to IFN treatment for patients with chronic HCV infection are absence of severe fibrosis or cirrhosis, non-genotype 1 and pretreatment serum low HCV RNA level [7,21,22]. With regard to virological factor after the start of antiviral treatment, early clearance of HCV RNA, or rapid decline of HCV RNA level during the early treatment duration is predictive of SVR among patients treated with IFN treatment [10,12,20]. For monitoring of the antiviral response to IFN treatment, both reverse-transcription PCR and branched DNA have been developed and have become available for clinical use

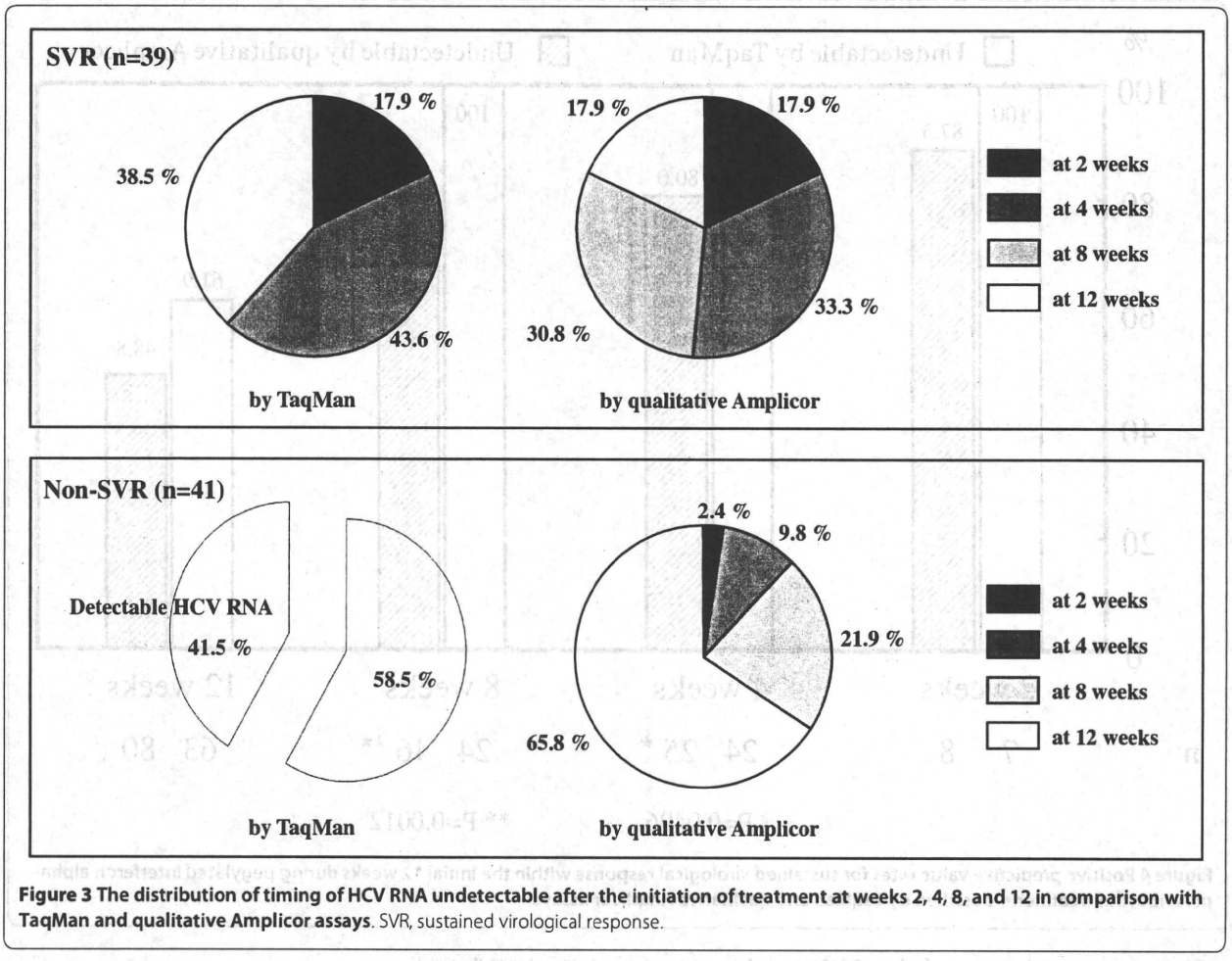

Figure 3 The distribution of timing of HCV RNA undetectable after the initiation of treatment at weeks 2, 4, 8, and 12 in comparison with TaqMan and qualitative Amplicor assays. SVR, sustained virological response.

[18,23,24]. These assays are especially useful because the early monitoring of favorable viral kinetics has a direct bearing on the possibility of a sustained response by IFN treatment. Actually, our findings showed that pretreatment HCV RNA by TaqMan assay may be a predictive factor for SVR, but not by Amplicor 10-fold method assay, and that TaqMan assay is more useful than qualitative Amplicor assay in analysis of the early stage (at the initial 4 and 8 weeks) of HCV dynamics, which is most related with SVR. Early prediction of early virologic response to IFN based treatment can help identify patients who are unlikely to have SVR and allow clinicians to discontinuation of treatment, saving patients the drug-induced adverse events and cost of additional treatment.

Our results suggested that the reduction rates of HCV RNA levels were significantly higher than those in non-SVR patients both in TaqMan and Amplicor 10-fold method assays for patients having achieved SVR. However, in our analysis, significant differences of the initial 4 and 8 week PPV for SVR were found between Amplicor and TaqMan assays. PPV for SVR at 4 and 8 week was 100% and 100% respectively in TaqMan assay, otherwise PPV for SVR was 80.0% and 69.6% respectively in qualitative Amplicor assay. In addition to EVR, within the initial 12 weeks undetectability of HCV RNA of the antiviral treatment, the critical role of rapid virological response (RVR), within the initial 4 weeks undetectability, on the SVR patients with genotype 1 and non-1 infection has been recently emphasized [7,9,16]. In fact, our findings showed more advantage using TaqMan assay on the PPV for SVR, especially at weeks 4 and 8, than Amplicor assay. Apparently, TaqMan assay is more accurate than Amplicor assay in analysis of the early stage (within 8 weeks) of HCV dynamics. PEG-IFN plus RBV treatment contributed to improve rates of SVR, but this rate is still lower for patients with HCV genotype 1 infection. Especially, most of Japanese patients with chronic hepatitis C who are candidate for antiviral treatment are relatively older than other countries. As a result, a relapse rate with EVR was a little high regardless of adequate treatment. Therefore, analysis of HCV dynamics after the antiviral treatment, especially for these patients, is very important.

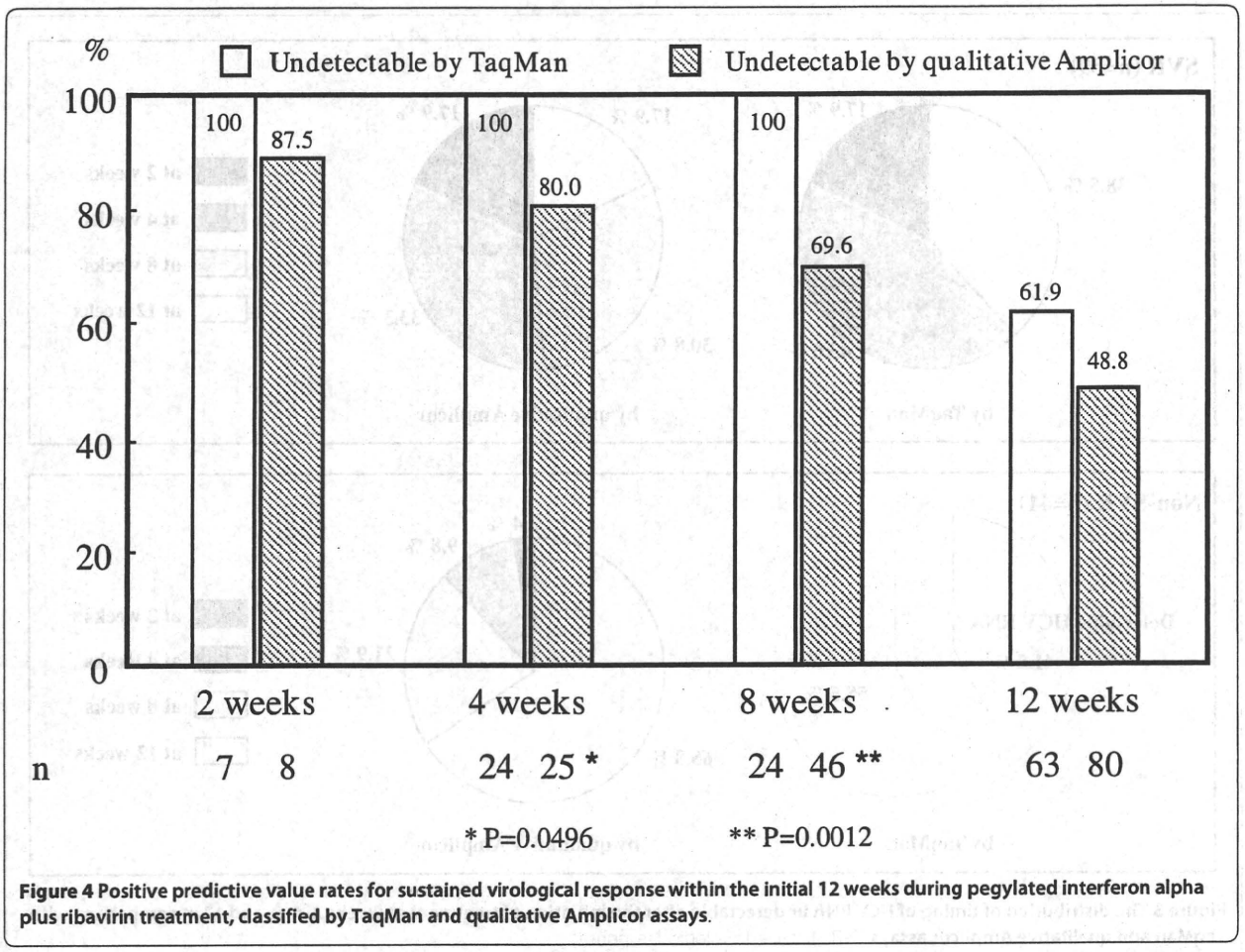

Figure 4 Positive predictive value rates for sustained virological response within the initial 12 weeks during pegylated interferon alpha plus ribavirin treatment, classified by TaqMan and qualitative Amplicor assays.

The present study showed that 21.3% of the patients with undetectable HCV RNA by qualitative Amplicor assay were positive for TaqMan assay within the initial 12 weeks during PEG-IFN plus RBV treatment. It is possible that qualitative Amplicor assay reveals late responders by the combined treatment in comparison with TaqMan assay. Berg and colleagues suggested extended treatment duration was recommended for patients with SVR as HCV RNA positive at week 12 but negative at week 24 [25]. About such patients, we need to extend the duration of the combination treatment of PEG-IFN alpha-2b plus RBV from 48 weeks to 72 weeks by an appearance of TaqMan assay.

Conclusions

In conclusion, the COBAS TaqMan HCV assay is useful for monitoring HCV viremia during antiviral treatment to predict a SVR in patients with chronically infected HCV genotype 1.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

EO performed the literature review, collected the clinical data and drafted the manuscript. NF, KT, HT, SO, MK, MM and YS collected the clinical data. NF participated in the design of the study and performed the statistical analysis. NF and JH revised the manuscript. All authors read and approved the final manuscript.

Author Details

[1]Department of General Internal Medicine, Kyushu University Hospital, Fukuoka, Japan and [2]Department of Environmental Medicine and Infectious Disease, Kyushu University, Fukuoka, Japan

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C型肝炎ウイルスの疫学的・臨床的研究

九州大学大学院 感染環境医学分野
九州大学病院 総合診療科

林 純, 古庄憲浩

はじめに

1989年にC型肝炎ウイルス（Hepatitis C virus：HCV）の塩基配列が明らかになり，1992年には一般医療機関でもHCVに対する抗体が測定されるようになった．その結果，わが国は世界的にみてもHCVの感染率が高いことが判明し，さらに非A非B型肝炎と呼ばれていた肝炎の大部分がHCV感染によるものと判明した．九州大学病院において実際に診療していた慢性肝炎の50%，肝硬変の70%，肝癌の80%はHCV感染によることは驚きであった[1]．当初，その感染経路の主なものは輸血および血液製剤によるものということであったが，輸血歴のないHCV感染者も多く存在しており，その感染経路の解明は重要な問題の一つであった．

また，肝癌患者の大部分がHCV感染者であることから，HCV感染と肝癌発症との関連が強く示唆され，その機序の解明も求められていた．一方，治療についてはHCVが測定される以前より，非A非B型慢性肝炎に対してはインターフェロン（Interferon：IFN）投与有効性が示唆されていた．

以上のことを踏まえて，著者らが行った一般住民および血液透析患者におけるHCV感染の実態調査あるいはその感染対策，およびHCV感染者に対するIFN療法の成績を中心に述べる．

1．感染経路

HCVの家族内感染については，母児間感染率は約5％で，夫婦間感染もHCVの遺伝子解析から約3％と低いことが報告されていた．しかし，性行為による感染については，夫婦間感染は少ないものの感染機会の多い特殊浴場女性従業者でのHCV抗体陽性率は6.2%と女性献血者の1.4%に比較し有意に高率であり（$p < 0.001$），その際梅毒罹患者に高率であった[2]．

本邦におけるHCVの主な感染経路を知るために，HCV抗体陽性率が高い福岡県星野村（2049例中19.7%）において疫学的検討を行った．その結果，1950-60年にかけて某医療機関に通院していた住民にHCV抗体陽性率が高く，さらに，そこでは使用した注射器および針が十分に滅菌消毒されずに，繰り返し使用されていたことが判明した[3]．本邦でdisposableの注射器および針などが使用され始めたのは1963年頃であり，一般の医療機関で使用されるようになったのは1995年頃からである．

長崎県壱岐市での調査でも同様の結果がえられ[4]，本邦におけるHCVの感染経路は母児間感染および夫婦間感染よりも，輸血を含む医療行為による感染が主流であったと考えられた．

2．一般住民の最近のHCVの感染状況の推移

上述した福岡県星野村において，1993年でのHCV抗体陽性率は50歳以上では25%以上を示していたが，年齢が若いほど低下し，19歳以下にはHCV抗体陽性者は存在していなかった．すなわち，B型肝炎ウイルスの発見を契機に1970年頃より，血液由来ウイルに対する衛生観念が発達し，HCV感染は減少の一途を辿っていると思われる．さらに，1993年の調査でHCV抗体陰性であった1,351例について追跡調

Jun HAYASHI and Norihiro FURUSYO:
Department of Medicine and Surgery, Internal Medicine, Environmental Medicine and Infections Diseases, Faculty of Medical Sciences, Kyushu University
Department of General Internal Medicine, Kyushu University Hospital
Epidemiological and Clinical Study for Hepatitis C Virus Infection

査を2003年まで行ったが，新たなHCV感染者は2例（70歳および74歳女性）のみであった．この2例はいずれも某医療機関で下部消化管内視鏡検査（生検を含む）を受けていた．C型慢性肝炎患者の大腸ポリープ摘出術に用いた内視鏡で検査を受けた一組の夫婦が，HCVに感染し急性肝炎を発症した事例が米国で報告されている．著者らの症例も他に感染機会などがないことから，同様な感染経路の可能性が考えられた[5]．いずれにしても，日本赤十字社血液センターでのスクリーニング体制の改善，disposable器材の普及などから新たなHCV感染は著明に減少していると思われる．

また，著者らが継続調査を行っている長崎県壱岐市では，1996-1999年における30歳以上のHCV抗体陽性率は17.6%で，HCV抗体陽性者のうちHCVRNA陽性は76.7%であった[6]．すなわち30歳以上のHCVRNA陽性率は13.5%であったが，2005年のHCVRNA陽性率は2.7%と激減していた[7]．この減少の原因として，この地域では九州大学病院総合診療科が1993年より行政および医師会と連携して，HCV感染者の掘り起こしおよび感染者の肝機能検査を中心とした健康管理を行ってきた結果，ほとんどの感染者が医療機関に受診し管理されているため，今回の検診に参加しなかったことが判明した．研究機関である大学病院と地域医療機関および行政が協力した啓蒙活動により，一般住民にHCV感染の重要性が理解された結果と考えられた．

3．血液透析患者におけるHCV感染

福岡および佐賀県の6施設の血液透析患者HCV抗体陽性率は418例中30.4%（20.0-34.9%）と高率であった．このうち輸血歴のない113例のHCV抗体陽性率は22.1%と高く，透析期間と比例して陽性率は上昇していた．すなわち，透析期間が2年以内では12.2%，2-4年で16.2%，5-9年で28.6%，10年以上で57.1%であった[8]．この6施設のうちの1施設について，HCV感染撲滅のため感染経路を明確にすることを目的とした継続的調査を行った．この透析施設での1989-1998年のHCV感染率は15.4%（年間感染率1.711%）で，あった．この感染率は上述の福岡県星野村の1993-2003年，0.26%（年間感染率0.023%）と比較して有意に高率であった（$p < 0.001$，リスク比59.1）[5]．HCV感染者の透析後の透析液にはHCVRNAが存在していることから[9]，透析液が流れる管とダイアライザーとの接合部位の洗浄などを行っていたが，HCV感染者の新たな発生は依然として続いていた．2000年に同時に5例のC型急性肝炎の発症が認められた．HCVの遺伝子学的解析および聞き取り調査により，HCV感染者の血液で汚染された生理食塩水のアンプルを介して感染が広がったことが判明した[10]．この原因として，この生理食塩水のアンプルがリキャップできるために起こったこと，汚染されたものを運ぶ動線が長く，また，汚染されたものを置くテーブルと，種々の薬剤を準備するテーブルが近接していることが感染のリスクを高めていると考えられた．以上のことを踏まえて，各透析ベッドの下に廃棄ボックスを設置し，薬剤の調整は別の部屋で行うことを指導した．その結果，現在2010年4月まで新たなHCV感染者は出現しておらず，この透析施設の院内感染に対する関心とたゆまざる努力の成果と考えられる．また，血液透析のHCV感染者は肝機能検査が正常にもかかわらず肝線維化マーカーは高く，血小板数も減少しており，腹部超音波検査から肝病変の進行が考えられるが[11]，HCVRNA量は低いため[12]，後述するIFN療法の効果が期待されることも判明した．

4．肝炎および肝癌発症機序

1）HCV感染例における肝機能異常

福岡県星野村でのHCV感染306例について，年1回行なわれる肝機能検査の10年間の成績をまとめて検討すると，39.5%が肝機能持続正常例，41.5%が間欠異常例，19.0%が持続異常例であった．また，持続正常例には女性が多く，持続異常例には男性が多かった[13]．

2）肝炎発症とウイルス側因子

細胞障害性Tリンパ球（CTL）が認識するHCVコア領域に注目し，福岡県星野村における肝機能持続

正常例と持続異常例について，クローニング・シークエンスを行った．前者ではアミノ酸レベルでは変異がみられず，後者では変異が多くみられた．また，観察中，肝機能が正常から異常になった例では，今までなかったコア領域の変異が出現するようになった．すなわち，HCV 感染による肝障害と，HCV のコア領域でみたウイルスの quasispecies との関連性が示唆された[13)14)]．

3）肝炎発症と宿主側因子

①免疫：C 型慢性肝炎患者の末梢血のリンパ球では IL-1β および TNF-α の産性能が亢進し[15)]，また，T 細胞の活性化を示すとされる可溶性 IL-2 レセプターが血清中で高値を示している[16)]．さらに，HCV 陰性の患者に比較して，CD4 +細胞では IFN-γ +細胞が有意に増加しており，CTL の前駆細胞とされる CD28 + CD8 + IFN-γ +細胞が増加していることも判明し，HCV 感染例における肝炎に CTL が強くかかわっていることが考えられた[17)18)]．

②生活習慣：福岡県星野村における HCV 感染者について生活習慣因子と HCV genotype，ウイルス量を変数として多変量解析を行った．HCV 感染者では，重労働 2 時間以上，男性，飲酒歴が肝機能に影響を及ぼす因子として抽出されたが，HCV 非感染者では飲酒歴のみしか抽出されなかった[19)]．HCV 感染者にとっては過激な運動は推奨できないと思われた．

4）肝癌発症機序

福岡県星野村での HCV 感染 411 例において，12 年間，肝癌発症について経過観察した．肝癌発症は 15.6% であった．肝機能持続正常群 144 例からは，5 年目までに 0 例，12 年目までに 74 歳と 80 歳男性 2 例，1.4% であったが，肝機能間欠異常群 137 例からは，5 年目に 5.1%，12 年目までに 12.4% で，肝機能持続異常群 130 例からは，5 年目までに 17.7% で，12 年目までに 27.8% であった．肝機能異常群に有意に多くの肝癌発症がみられたことから，C 型慢性肝炎における肝癌発症には，持続する炎症が重要な役割を果たしていると考えられた．それ以外の詳細な機序については肝細胞の再生，サイトカインや増殖因子による増殖ストレスが誘因となること，また様々の癌遺伝子の異常発現や変異産物の蓄積などにより異常クローンが発生することなどが考えられている．また，肝機能が正常であっても，高齢の HCV 感染者は肝癌の発症があり，HCV 感染者の診療上注意を要することと考えられた[5)14)]．

5．HCV 感染者に対する IFN 療法

1）PEG-IFNα-2b +リバビリン併用療法

わが国では 1992 年に C 型慢性肝炎に対して IFN の投与が保険適応となった．当初，IFNα は 24 週[20)]，β は 6-8 週[21)]投与の単独投与で，その効果は IFN 投与終了後 24 週でも HCVRNA の持続的に陰性化している有効例は約 25% で，genotype 1 型/HCVRNA 高値の例では，さらに低く約 5 % であった．また，40 歳過ぎの女性の有効率は低値であった[22)]．

このため，新たな IFN 療法として 2001 年より Th2 の産生を抑制することによって Th1/Th2 の比を高めるリバビリンと IFN の併用療法が行われ[23)]，さらに 2004 年より，週 1 回の投与の Pegylated（PEG）-IFNα-2b とリバビリン併用の 48 週投与が開始され，現在，この治療法が主流となっている．この成績については九州大学大学院感染環境医学分野と病態修復内科学分野，病態機能内科学分野，病態制御内科分野，およびそれぞれの関連病院とで組織した九州大学関連肝疾患研究会（Kyushu University Liver Disease Study：KULDS）での成績を述べる[24)]．有効率は genotype 1 型では 939 例中 40.7% と genotype 2 型の 313 例中 79.6% に比較し有意に低値であったが（ITT 解析），以前の IFN 単独投与に比較すると飛躍的な上昇であった．有効に寄与する独立因子を多変量解析を用いて検討したところ，genotype 1 型では男性，低年齢，HCVRNA 量低値，γ-GTP 低値，アルブミン高値，空腹時血糖低値，血小板数高値であった．その他の因子としては，ウイルスのコア領域や IFN 感受性決定領域および宿主の IFN-λ に関連する IL-28 遺伝子多型などとの関連が報告されている．genotype 2 型では IFN 治療歴が無いことおよび HCVRNA

量低値であった．近年，HCVRNAの測定感度がさらに改善されたことにより，治療開始8週目までにHCVRNAが陰性化した例の殆どは有効例となることが理解されるようになった[25]．

治療薬の減量について体重規定総投与量からみると，genotype 2では減量とその効果に有意な差はみられなかったが，genotype 1型ではPEG-IFNα-2bは80%以下，RBVは60%以下の量では，有効率の有意な低下がみられた[26]．

IFN療法の有効例では肝生検組織像も改善することを既に証明していたが[27]，多くの症例対して肝線維化測定機器FibroScan（Echosens, Paris, France）を用いて，非侵襲的に長期に肝病態の推移を検討した．FibroScan値はC型慢性肝炎の肝生検組織像のStage分類およびGrade分類と正の相関を示していた[28]．FibroScan値の推移から有効例では長期にわたって組織学的改善が考えられ，無効例では一時期的な改善はみられたが最終的に悪化していることが示された[29]．

2）IFNの肝癌発症抑制効果

IFN投与患者の観察期間平均5.7年での肝癌発症は，IFNα投与群では260例中6.5%，β投与群では91例中4.4%で，福岡県星野村HCV感染者を年齢補正した肝機能持続異常群における5年の肝癌発症率17.7%に比較し低い値であった．特に，ウイルスが持続的に陰性化した有効例や，肝機能が正常化した例に肝癌発症が少なかった[30]．肝癌細胞株Huh7およびHepG2を用いたin vitroの実験では，IL15を介してIFNが癌の発育を抑制していたことから[31)32]，IFN療法によるHCVの持続的な排除が望めないような症例に対しては，肝癌発症抑制効果を期待して，IFNの少量長期投与が推奨されている．

6．HCV感染とインスリン抵抗性

C型慢性肝炎患者に対して食事負荷試験（クッキーテスト：糖質75g，脂肪28.5g，蛋白質7g，計585Kcal）を行い[33]，HOMA-IRだけでなくインスリン面積，インスリン面積×グルコース面積を指標としてインスリン抵抗性を検討した．いずれの指標でも非糖尿病C型慢性肝炎患者では，コントロール（非糖尿病高血圧患者）に比較して高値であった[34]．また，HCVRNA量は種々のインスリン抵抗性の指標と有意な関連がみられるだけでなく，高分子量アディポネクチンとは逆相関がみられ，HCV感染とインスリン抵抗性との関連が強く示唆された[35]．

さらに，C型慢性肝炎に対するpegIFNα-2b + ribavirin併用療法の効果とインスリン抵抗性との関連を検討した．genotype 1型での有効率は血清空腹時インスリン高値例あるいはHOMA-IR高値例に有意に低かったが（$p < 0.0001$），genotype 2型では有意な差はみられなかった．しかし，インスリン面積，インスリン面積×グルコース面積でみるとgenotype 2型でもインスリン抵抗性が存在する例では有効率が有意に低かった．

さらに，pegIFN α-2b + ribavirin併用療法を受けたC型慢性肝炎患者のインスリン抵抗性を経時的に観察すると，HCVが完全に排除された有効例では血清空腹時インスリン値の低下あるいはHOMA-IRの低下がみられ，インスリン抵抗性の改善が認められた[33]．

7．HCVと成人T細胞白血病ウイルス（HTLV-1）との重複感染

福岡県を含む九州はHTLV-1の高浸淫地区であるが，HCV感染者にHTLV-1が重複感染すると，HCV単独感染に比較して，肝癌の発症頻度が高く，また，若年で発症する傾向がみられることが，著者らの調査で判明した．すなわち，長崎県壱岐市における継続調査では，10年間の肝癌発症率はHCV/HTLV-1重複感染159例中20.7%で，HCV単独感染491例中7.9%に比較して有意に高率であった（$p < 0.001$）．これを45歳以下の年齢から観察できた例と，55歳以上の年齢から観察できた例に分けて検討した．前者ではHCV/HTLV-1重複感染32例中21.9%で，HCV単独感染184例中4.3%に比較して有意に高率であったが（$p < 0.01$），後者ではHCV/HTLV-1重複感染127例中20.5%で，HCV単独感染307例中10.1%と差はみられなかった．すなわち，HCV/HTLV-1重複感染例ではHCV単独感染例より肝癌の発