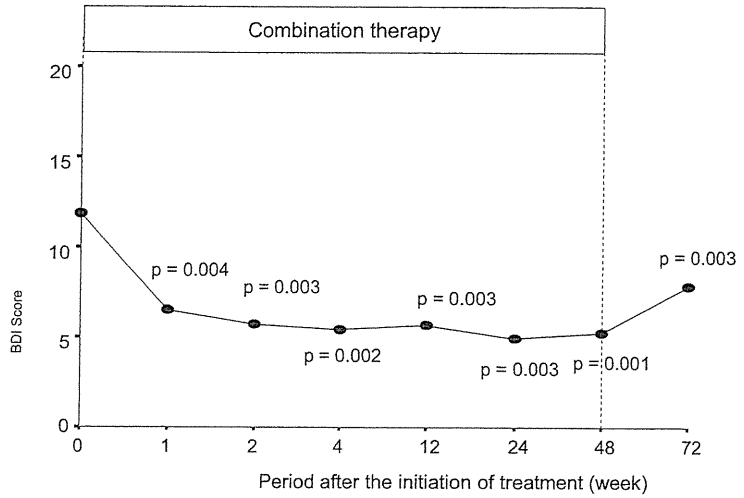


**Figure 1. The change of BDI score after the initiation of combination therapy. P-values at 1, 2, 4, 12, 24, 48, and 72 weeks indicate the statistical difference compared with the BDI-2 score at the initiation time of combination therapy by the use of Mann-Whitney U test.**

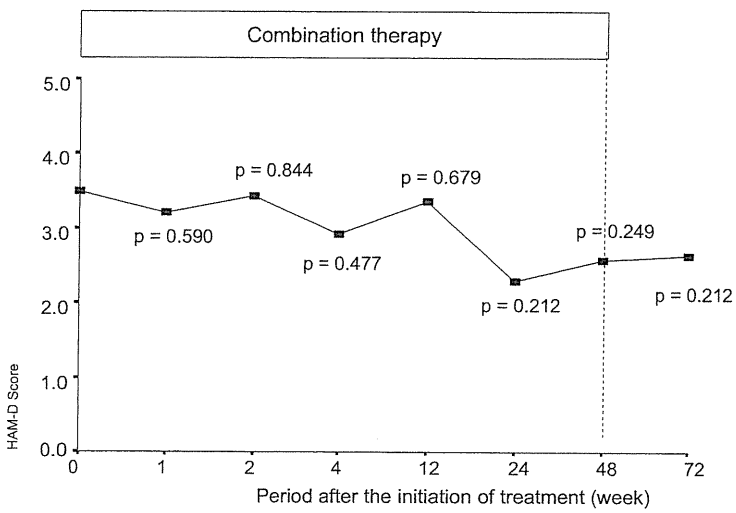


**Figure 2. The change of Ham-D score after the initiation of combination therapy. P-values at 1, 2, 4, 12, 24, 48, and 72 weeks indicate the statistical difference compared with the HAM-D score at the initiation time of combination therapy by the use of Mann-Whitney U test.**

start of IFN therapy and to four patients during IFN therapy. Anti-anxiety drugs, such as etizolam, alprazolam, were given to four patients at the start of IFN therapy and to five patients during IFN therapy.

The changes of WBC, hemoglobin, and platelet count after the initiation of combination therapy are shown in Fig. 3. WBC and hemoglobin levels were decreased during combination therapy. On the other hand, the platelet count decrease was statistically significant at 1, 2, and 4 weeks after the initiation of combination therapy compared to that at the initiation time of treatment. After that, the platelet count recovered to the base line at 12, 24, and 48 weeks after the initiation of combination therapy.

## Discussion

In the present study, we have described the efficacy and safety of combination therapy of IFN-beta and ribavirin for patients for whom IFN therapy was discontinued due to depression induced by IFN-alpha. The patients with HCV genotype 1b and HCV-load of $\geq$100 KIU/mL were enrolled. We could evaluate the relationship between IL-28 or HCV core mutation and SVR in the combination therapy of IFN-beta and ribavirin for genotype 1b and high virus load. The present study was limited to exclude the subjects with Ham-D score of more than 18. Patients with Ham-D score of more than 18 were defined as severe depression state. It is possible that high score of Ham-D enhance the dropout

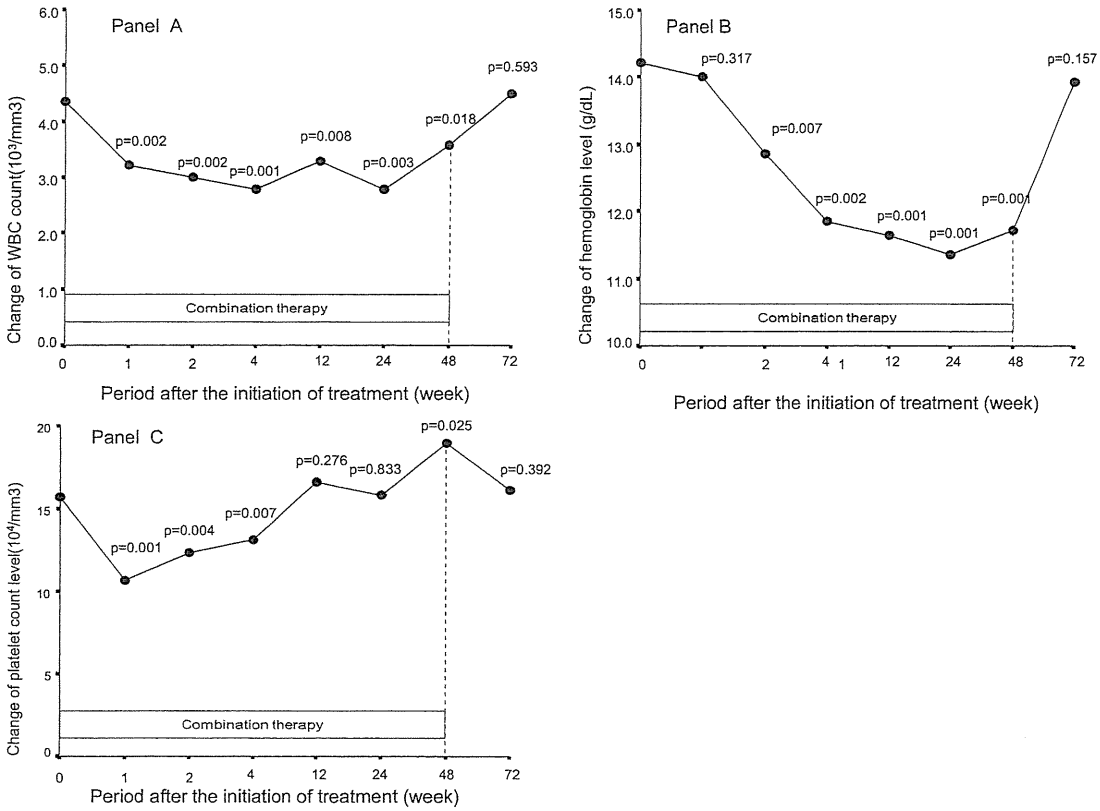


**Figure 3. The change of complete blood cell count after the initiation of combination therapy. Panel A; The change of white blood cell count. Panel B; The change of hemoglobin level. Panel C; The change of platelet count.**

due to combination therapy and aggravation of depressive state. Thus, we excluded the patients with Ham-D score of more than 18 in the present study. Moreover, the number of 14 patients enrolled was a small size. Another limitation is that the present study was not a randomized controlled study. Several findings from the present study have direct implications for combination therapy of IFN-beta and ribavirin for chronic hepatitis C in the future. First, the drop-out rate due to depressive state in combination therapy of IFN-beta and ribavirin was low. This result was similar to that in the previous study (14). The result by this prospective study confirmed that combination therapy of IFN-beta and ribavirin reduced the aggravation of depressive state compared with combination therapy of peginterferon-alpha and ribavirin.

Second, 5 out of 14 patients treated with combination therapy of IFN-beta and ribavirin had SVR. The SVR rate in the present study was almost the same to that in the previous study.

Third, SVR had a tendency to occur in patients with negativity of HCV RNA at 12 and/or 24 weeks after the initiation of combination therapy. All of the patients with positive HCV RNA at 24 weeks after the initiation of combination therapy showed non-SVR. This result agreed with our previous report (14). Thus, positive HCV RNA at 24 weeks after the initiation of combination therapy of IFN-beta and ribavirin suggests that the possibility of SVR is low. Next, patients with a high platelet count tended to show SVR. In general, a high platelet count suggests slight fibrosis of liver. Thus, the result raises the possibility that slight hepatic fibrosis enhance the efficacy of combination therapy.

Finally, SVR in combination therapy of IFN-beta + ribavirin was associated with IL-28B in the present study. None of the seven patients with genotype TG or GG at the genetic variation in rs8099917 near the IL28B gene had SVR. The results suggested that only patients with genotype TT might have the possibility of getting SVR. On substitution of core amino acid (aa) 70, two of eight patients with mutant type of core aa 70 showed SVR. The result shows that patients with mutant type of core aa 70 have the possibility of getting SVR. Several authors have reported that virus clearance in combination therapy of peginterferon-alpha and ribavirin is associated with HCV mutations in the core region and IL-28B (21-26). The present study confirmed that IL-28B was related with SVR for HCV patients with genotype 1b and high virus load.

IFN-beta is not convenient for treatment compared to intramuscular or subcutaneous injection. However, IFN-beta-related side effects are mild and few compared to those of IFN-alpha. IFN-beta-induced mental disorders are mild compare to those induced by IFN-alpha. Out of 7,250 HCV patients treated with IFN in our hospital, 960 (13.2%) were

given IFN-beta. The mechanism of the better tolerability of IFN-beta and ribavirin is unclear. However, the following mechanism might be considered: 1) IFN-beta is not recombinant IFN but produced from human white blood cell. Thus, IFN-beta has a tendency not to produce some immune complex relating to IFN-related side effects. 2) IFN-beta might have different intracellular mechanisms compared to IFN-alpha. Although the receptor of IFN alpha and beta are common, intracellular mechanisms could differ. Our results described above suggest that combination therapy of IFN-beta and ribavirin is one possible method for patients who have HCV-genotype 1, high virus load and depressive state of Ham-D scale of <18. In conclusion, the combination therapy of IFN-beta and ribavirin is a possible therapy selection for the patients for whom interferon therapy was discontinued due to depression induced by interferon-alpha.

**The authors state that they have no Conflict of Interest (COI).**

**Acknowledgement**

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Hepatology Research

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***Original Article***

# Development rate of chronic kidney disease in hepatitis C virus patients with advanced fibrosis after interferon therapy

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*Aim:* The aim of this retrospective cohort study is to assess the development incidence and predictive factors for chronic kidney disease (CKD) after the termination of interferon therapy in hepatitis C virus (HCV) positive Japanese patients with liver cirrhosis.

*Methods:* A total of 650 HCV positive, liver cirrhotic patients who were treated with interferon and showed an estimated glomerular filtration rate (eGFR) of ≥60 mL/min per 1.73 $m^2$ after the termination of interferon therapy were enrolled. CKD was defined as an eGFR of <60 mL/min per 1.73 $m^2$. End-stage-CKD was defined as an eGFR of <15 mL/min/1.73 $m^2$. The primary goal is the new development of CKD and end-stage-CKD.

*Results:* Eighty-five patients developed CKD, and six patients progressed to end-stage-CKD. The development rate of CKD was 5.2% at the 5th year, 14.5% at the 10th year and 30.6% at the 15th year. Multivariate Cox proportional hazards analysis showed that CKD occurred when patients had age increments of 10 years (hazard ratio: 2.32; 95% confidence interval [CI] 1.61–3.35; $P < 0.001$), eGFR decrements of 10 mL/min per 1.73 $m^2$ (hazard ratio: 1.66; 95% CI 1.27–2.16; $P < 0.001$), hypertension (hazard ratio: 2.00; 95% CI 1.13–3.53; $P = 0.017$), diabetes (hazard ratio: 1.79; 95% CI 1.02–3.14; $P = 0.042$), and non-clearance of HCV (hazard ratio: 2.67; 95% CI 1.34–5.32; $P = 0.005$). The development rate of end-stage-CKD was 0.4% at the 5th year, 1.6% at the 10th year and 2.8% at the 15th year.

*Conclusions:* The annual incidence for CKD among cirrhotic patients with HCV was determined to be about 1.0–1.5%. In addition, the annual incidence for end-stage-CKD is one order of magnitude lower than that of CKD.

**Key words:** chronic kidney disease, hepatitis C virus, liver cirrhosis

**Abbreviations:** ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; CI, confidence interval; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; IFN, interferon; SVR, Sustained virological response.

## INTRODUCTION

HEPATITIS C VIRUS (HCV) is a major risk for hepatocellular carcinoma (HCC).[1–4] In addition, chronic HCV infection has been associated with a variety of extrahepatic complications such as essential mixed cryoglobulinemia, lymphoproliferative disorders, autoimmune thyroiditis, sialadenitis, cardiomyopathy, and diabetes.[5–8]

Data supporting a link between hepatitis C infection and chronic kidney disease (CKD) have been reported.[9–15] CKD, a disease entity including mild to end-stage renal diseases due to any etiology, was recently defined as an estimated glomerular filtration rate (eGFR) <60 mL/min per 1.73 $m^2$ and/or the presence of proteinuria.[16] CKD is currently considered a serious worldwide public health problem.[16,17] Tsuji *et al.* have reported that HCV infection enhance the onset of end-stage renal disease.[18,19] Dalrymple *et al.* have

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showed that HCV-positive patients had a 40% higher likelihood for developing renal insufficiency compared with seronegative subjects.[20] We had reported that patients with severe fibrosis had high possibility of progressed kidney damage.[11,12] Although there is growing evidence to support the concept that HCV infection is a risk factor for CKD, there have been a few interventional studies confirming this issue. This issue needs to be confirmed with a long-term follow-up of patients.

With this background in mind, the retrospective cohort study was initiated to investigate the cumulative incidence and risk factors of aggravation of renal function after prolonged follow-up in HCV-infected and cirrhotic patients treated with interferon (IFN) monotherapy or combination therapy of IFN and ribavirin. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

## METHODS

### Patients

A TOTAL OF 982 HCV positive and cirrhotic patients with infection were treated with IFN monotherapy or combination therapy of IFN and ribavirin between September 1990 and December 2007 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. Out of 982 patients, 650 satisfied the following criteria: (i) an estimated glomerular filtration rate (eGFR) of ≥60 (mL/min per 1.73 $m^2$); (ii) features of cirrhosis diagnosed by laparoscopy and/or liver biopsy before the initiation of IFN therapy; (iii) positivity for serum HCV-RNA before the initiation of IFN therapy; (iv) age of ≥40 years; (v) period of ≤ 1year on IFN therapy; (vi) negativity for hepatitis B surface antigen (HBsAg), antinuclear antibodies, or antimitochondrial antibodies in serum, as determined by radioimmunoassay or indirect immunofluorescence assay; (vii) no evidence of HCC nodules as shown by ultrasonography and/or computed tomography; and (viii) no underlying systemic disease, such as systemic lupus erythmatosus, rheumatic arthritis. Next, we excluded from the study all the patients with a history of alcohol abuse or advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites.

Alcohol abuse is a pattern of drinking that involves one or more of the following problems within a one-year period: (i) failure to carry out major responsibilities at work, school, or home; (ii) drinking in physically dangerous situations, such as while driving; (iii) legal problems related to using alcohol; and (iv) continued drinking despite ongoing problems in relationships with other people that are related to alcohol use.[21]

The primary outcome was the new development of CKD and/or end-stage CKD. CKD was defined as the first time when eGFR of <60 mL/min per 1.73 $m^2$ persisted for up to 3 months. End-stage CKD was defined as the first time when eGFR of <15 mL/min per 1.73 $m^2$ persisted for up to 3 months. Serum creatinine level was also measured using an enzymatical method, and the eGFR was estimated from the Japanese Society of Nephrology CKD Practice Guide: eGFR (mL/min per 1.73 $m^2$) = 194 × (serum creatinine level [mg/dL])$^{-1.094}$ × (age [y])$^{-0.287}$ The product of this equation was multiplied by a correction factor of 0.739 for women. CKD's stages were defined from estimated eGFR of <60 mL/min per 1.73 $m^2$ or dipstick proteinuria (≥+1) as follows: stage 1, eGFR ≥90 and proteinuria (≥+1); stage 2, 90 > eGFR ≥60 and proteinuria (≥+1); stage 3, 60 > eGFR ≥ 30; stage 4, 30 > eGFR ≥ 15; and stage 5, eGFR of <15. In the present study, patients with stage 3–5 were regarded as having CKD regardless of the absence of other markers of kidney damage.[22,23]

The physicians in charge explained the methods and side effects of IFN therapy, the storage of serum samples, and the use of stored serum samples to each patient and/or patient's family before IFN therapy. Informed consent was obtained from 650 patients before the initiation of IFN therapy. All of the studies were performed retrospectively by collecting and analyzing data from the patient records. This study had been approved by the Institutional Review Board of our hospital.

### Laboratory investigation

Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL, USA). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, version 2.0; Roche, Tokyo, Japan). HBsAg was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI, USA). Diagnosis of HCV infection was based on detection of serum HCV antibody and positive HCV RNA. HCV genotype and HCV RNA level were determined by the serum samples stored at −80°C before the initiation of IFN therapy.

Height and weight were recorded at baseline and the body mass index was calculated as weight (in kg)/height (in $m^2$). The criteria for the diagnosis of diabetes include: (i) casual plasma glucose ≥200 mg/dL; (ii) fasting plasma glucose (FPG) ≥126 mg/dL; and (iii) 2 h post-glucose (oral glucose tolerance test) ≥200 mg/dL.[24]

Patients were regarded as hypertension by the confirmation of blood pressure ≥140 mmHg systolic and/or ≥90 mmHg diastolic on at least three visits. Blood pressure was measured by a physician with a mercury sphygmomanometer, with subjects sitting and relaxed for at least 10 min.

### Evaluation of liver cirrhosis

Liver status of the 650 patients was 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-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.[25]

### Follow-up

The starting time of follow up was 3 months after the termination of IFN therapy. After that, patients were followed-up monthly to tri-monthly in our hospital. Physical examination and biochemical tests were conducted at each examination together with regular check-ups. Blood samples were taken for routine analyses. These included transaminase activities, total cholesterol, uric acid, glucose, complete blood cell count, serum HCV RNA, and creatinine level. Fifty-seven patients were lost to follow-up. Because the appearance of worsening renal function was not identified in the 57 patients, they were considered as censored data in statistical analysis.[26] Moreover, patients retreated with antiviral agents were regarded as withdrawals at the time of starting the retreatment of antiviral agents.

### Statistical analysis

Clinical differences between sustained virological response (SVR) group and non-SVR group were evaluated by Wilcoxon rank sum test or Fisher's exact test. The cumulative development rate of CKD and end-stage CKD was calculated from 3 months after the termination of IFN treatment using the Kaplan–Meier method. Independent factors associated with the development rate of CKD and end-stage CKD were analyzed by the Cox proportional hazard model. The following 17 variables were analyzed for potential covariates for incidence of aggravation of renal function: age, sex, body mass index, eGFR, HCV RNA level, HCV genotype, alanine aminotransferase, aspartate aminotransferase, platelet count, type of IFN, combination of ribavirin, efficacy of IFN therapy, triglyceride, total cholesterol, uric acid, hypertension, diabetes, and frequencies of using contrast medium in computed tomography. HCV RNA level and HCV genotype were measured by the serum samples stored −80 °C before the initiation of IFN therapy. Yearly frequencies of using contrast medium in computed tomography were determined by clinical records. The remaining 15 variables were determined at the starting time of follow up after IFN therapy. A *P*-value of less than 0.05 was considered significant. Data analysis was performed using SPSS 11.5 for Windows (SPSS, Chicago, IL, USA).

## RESULTS

### Patients' characteristics

TABLE 1 SHOWS the characteristics of the 650 HCV-positive and cirrhotic patients treated with IFN monotherapy or combination therapy of IFN and ribavirin. There were several differences in clinical backgrounds between the SVR group and the non-SVR group. However, there was no significant difference in eGFR between SVR group and non-SVR group. The sustained virological response (SVR) rate was 30.6% (169/553) in IFN monotherapy and 42.2% (41/97) in combination therapy of IFN and ribavirin. Thus, the number of patients with SVR was 210. The mean follow-up period after the termination of anti-virus drugs was 6.5 years.

### Incidence of CKD in cirrhotic patients with HCV

A total of 85 subjects (56 men and 29 women) developed CKD during the follow-up period. Of these, 14 were SVR and 67 were non-SVR. The cumulative development rate of CKD was determined to be 4.9% at the 5th year, 14.5% at the 10th year and 30.6% at the 15th year by the use of the Kaplan–Meier method (Fig. 1).

The factors associated with the development of CKD in all 650 patients treated with IFN are shown in Table 2. Multivariate Cox proportional hazards analysis showed that CKD development after the termination of IFN therapy occurred when patients had age increments of 10 years (hazard ratio: 2.32; 95% confidence interval [CI] 1.61–3.35; $P < 0.001$), eGFR decrements of 10 mL/min pe 1.73 $m^2$ (hazard ratio: 1.66; 95% CI 1.27–2.16; $P < 0.001$), hypertension (hazard ratio: 2.00; 95% CI 1.13–3.53; $P = 0.017$), diabetes (hazard ratio: 1.79; 95% CI 1.02–3.14; $P = 0.042$), and non-SVR (hazard ratio:

**Table 1** Patients characteristics

| Characteristic | Total | SVR | Non-SVR | *P** |
|---|---|---|---|---|
| *n* | 650 | 210 | 440 | |
| Sex (male/female) | 405/245 | 134/76 | 271/169 | 0.604 |
| Age (years) | 57.4 ± 11.7 | 57.0 ± 11.9 | 57.6 ± 12.8 | 0.185 |
| Height (cm) | 162.8 ± 9.1 | 163.3 ± 9.2 | 162.1 ± 9.1 | 0.270 |
| Body weight (kg) | 63.1 ± 13.7 | 63.6 ± 13.9 | 62.1 ± 13.7 | 0.387 |
| Body mass index | 23.6 ± 3.1 | 23.7 ± 3.2 | 23.6 ± 3.2 | 0.654 |
| Blood pressure (systolic, mmHg) | 132 ± 17 | 130 ± 17 | 133 ± 18 | 0.334 |
| Blood pressure (diastolic, mmHg) | 78 ± 12 | 78 ± 11 | 79 ± 11 | 0.929 |
| Hypertension (+/−) | 152/498 | 48/162 | 104/336 | 0.844 |
| HCV-genotype (1b/2a/2b/others) | 389/159/56/46 | 92/84/19/15 | 297/75/37/31 | <0.001 |
| HCV RNA level (KIU/mL) | 659 ± 508 | 435 ± 476 | 728 ± 532 | <0.001 |
| eGFR | 85.2 ± 15.5 | 86.2 ± 15.9 | 84.7 ± 15.7 | 0.141 |
| Fasting plasma glucose (mg/dL) | 100 ± 31 | 99 ± 25 | 102 ± 34 | 0.888 |
| Diabetes | 149/501 | 42/168 | 107/333 | 0.232 |
| Total cholesterol (g/dL) | 156 ± 30 | 158 ± 38 | 154 ± 30 | 0.486 |
| Triglyceride (mg/dL) | 104 ± 46 | 108 ± 56 | 102 ± 45 | 0.764 |
| Uric Acid (mg/dL) | 5.6 ± 2.1 | 5.5 ± 2.1 | 5.7 ± 2.2 | 0.433 |
| AST (IU/L) | 62 ± 50 | 39 ± 19 | 73 ± 55 | <0.001 |
| ALT (IU/L) | 68 ± 72 | 36 ± 20 | 80 ± 80 | <0.001 |
| Platelet ($\times 10^4/mm^3$) | 11.6 ± 4.7 | 12.2 ± 5.0 | 11.3 ± 4.5 | 0.040 |
| Frequencies of contrast imaging per year (≥1/<1) | 252/398 | 28/182 | 224/216 | <0.001 |
| IFN monotherapy†/combination therapy‡ | 553/97 | 169/41 | 384/56 | 0.026 |

*Clinical differences between SVR group and Non-SVR group were evaluated by Wilcoxon rank sum test or Fisher's exact test.
†Outbreak of IFN monotherapy: recombinant IFNα 2a, 73 cases; recombinant IFNα 2b, 52 cases; natural IFNα, 278 cases; natural IFNβ, 150 cases; total dose of IFN = 572 ± 165 megaunit.
‡Outbreak of combination therapy: recombinant IFNα 2b+ribavirin, 29 cases, total dose of IFN = 502 ± 182 megaunit, total dose of ribavirin = 160 ± 68 g; peg IFNα 2b+ribavirin, 68 cases, total dose of peg IFN = 4.10 ± 1.08 mg, total dose of ribavirin = 202 ± 56 g.
Data are number of patients, median (range) or mean ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; IFN, interferon; SVR, sustained virological response.

2.67; 95% CI 1.34–5.32; $P. = 0.005$). The cumulative development rate for CKD based on difference of efficacy of the IFN therapy is shown in Figure 2. In addition to non-SVR, the four factors of aging, low eGFR, hypertension, and diabetes are high risk of developing the CKD. The development rates for CKD based on difference of age, eGFR, blood pressure, and blood glucose level at the starting time of follow-up are shown in Figure 3.

## Incidence of end-stage CKD in cirrhotic patients with HCV

A total of six subjects (five male and one female) developed end-stage CKD during the follow-up period. The cumulative development rate of end-stage CKD was determined to be 0.4% at the 5th year, 1.6% at the 10th year and 2.8% at the 15th year by the use of the Kaplan–

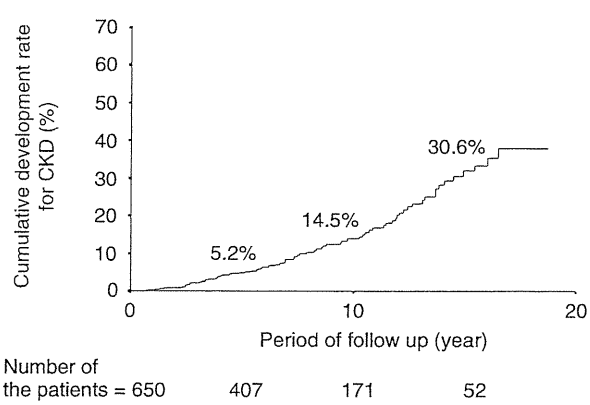


**Figure 1** Cumulative development rate for chronic kidney disease (CKD) in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon.

**Table 2** Predictive factors for chronic kidney disease (CKD) development

| Variables | Univariate analysis | | Cox-regression | |
|---|---|---|---|---|
| | HR (95% CI) | *P* | HR (95% CI) | *P* |
| Age, per 10 years | 2.30 (1.72–3.12) | <0.001 | 2.32 (1.61–3.35) | <0.001 |
| Sex (female/male) | 0.90 (0.57–1.40) | 0.628 | | |
| Body mass index (≥25/<25) | 1.35 (0.72–2.50) | 0.347 | | |
| HCV load(KIU/mL, ≥1000/<1000) | 1.39 (0.80–2.38) | 0.173 | | |
| Genotype (1/2) | 1.19 (0.78–1.89) | 0.436 | | |
| AST (IU/L, ≥50/<50) | 1.63 (0.92–2.94) | 0.097 | | |
| ALT (IU/L, ≥50/<50) | 2.01 (1.13–3.57) | 0.016 | | |
| **Platelet ($\times 10^4$/mm$^3$, ≥15/<15)** | 0.70 (0.25–1.94) | 0.487 | | |
| eGFR, per decrease of 10 mL/min/1.73 m$^2$ | 2.00 (1.56–2.56) | <0.001 | 1.66 (1.27–2.16) | <0.001 |
| Uric acid (mg/dL, ≥7.0/<7.0) | 1.43 (0.81–2.47) | 0.225 | | |
| Triglyceride (mg/dL, ≥150/<150) | 1.61 (0.62–3.70) | 0.336 | | |
| Cholesterol (mg/dL, ≥220/<220) | 1.22 (0.48–3.12) | 0.678 | | |
| Diabetes (+/–) | 2.76 (1.79–4.22) | 0.001 | 1.79 (1.02–3.14) | 0.042 |
| Hypertension (+/–) | 2.82 (1.80–4.39) | <0.001 | 2.00 (1.13–3.53) | 0.017 |
| Combination of ribavirin (+/–) | 0.75 (0.36–1.58) | 0.453 | | |
| Kind of IFN (beta/alpha) | 0.91 (0.53–1.57) | 0.729 | | |
| Efficacy (non-SVR/SVR) | 2.10 (1.21–3.58) | 0.008 | 2.67 (1.34–5.32) | 0.005 |
| Frequencies of contrast imaging per year (≥1/<1) | 1.83 (1.17–2.87) | 0.009 | | |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; HR, hazards ratio; IFN, interferon; SVR, sustained virological response.

Meier method (Fig. 4). The factors associated with the incidence of end-stage CKD in all 650 patients are shown in Table 3. There were no significant factors associated with the incidence of end-stage CKD as shown in Table 3.

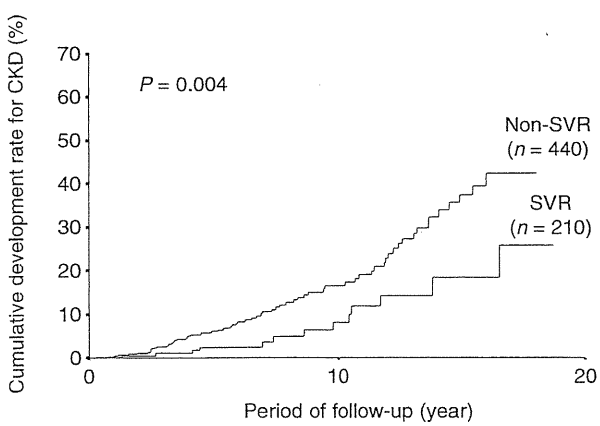


**Figure 2** Cumulative development rate for chronic kidney disease (CKD) based on the difference of efficacy in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon.

## DISCUSSION

WE HAVE DESCRIBED the development incidence for CKD and end-stage CKD after the termination of IFN therapy in HCV positive and liver cirrhotic patients treated with IFN. In the present study, the liver cirrhotic patients were enrolled to evaluate the new onset of CKD or end-stage CKD. Moreover, kidney damage has been reported in patients treated with IFN.[27] To exclude kidney damage originated from IFN-related side effects, patients with eGFR of ≥60 (mL/min per 1.73 m$^2$) for 3 months after the termination of IFN were enrolled in the present study. Our results indicate that the annual incidence for CKD as defined by a GFR of less than 60 mL/min per 1.73 m$^2$ for a prolonged follow-up after the termination of IFN therapy in HCV positive and cirrhotic patients is about 1.0–1.5% based on the development incidence for CKD at the 5th year and the 10th year. In addition, the annual incidence for end-stage CKD is one order of magnitude lower than that of a total of CKD.

Imai *et al.* have reported that about 20% of the Japanese adult population have stage 3 to 5 CKD by the use of database for 527 594 (male, 211 034; female, 316 560) participants obtained from the general adult population aged over 20 years who received annual

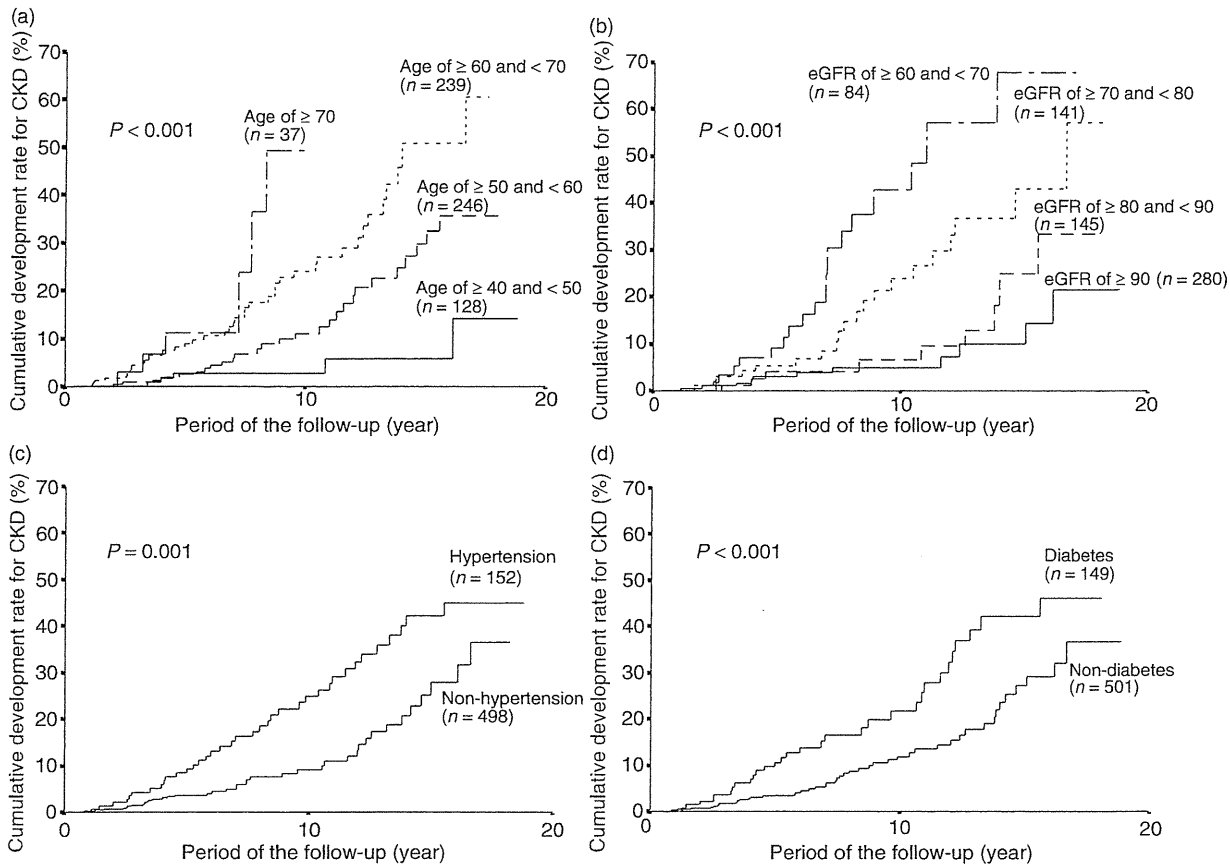


**Figure 3** Cumulative development rate for chronic kidney disease (CKD) in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon: (a) Cumulative development rate for CKD based on difference of age; (b) Cumulative development rate for CKD based on the difference of estimated glomerular filtration rate (eGFR); (c) Cumulative development rate for CKD based on the difference of blood pressure; (d) Cumulative development rate for CKD based on the difference of glucose level.

health check programs in 2000–2004, from seven different prefectures in Japan. Next, the prevalence of CKD stage 3 in the study population, stratified by age groups of 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, and 80–89 years, were 1.4%, 3.6%, 10.8%, 15.9%, 31.8%, 44.0%, and 59.1%, respectively. Moreover, they provided that the prevalence of stage 4+5 was <0.2%. Our results agreed with Imai's report in the fact that end-stage CKD patients were few.

The present study was limited by a retrospective cohort trial. This cohort is over 10 years; hence, many patients had complications, such as diabetes and hypertension. However, the development of CKD was mainly evaluated based on the clinical characteristics at the initiation of follow-up. Second limitation of the study was that we defined CKD according to eGFR alone. Gener-

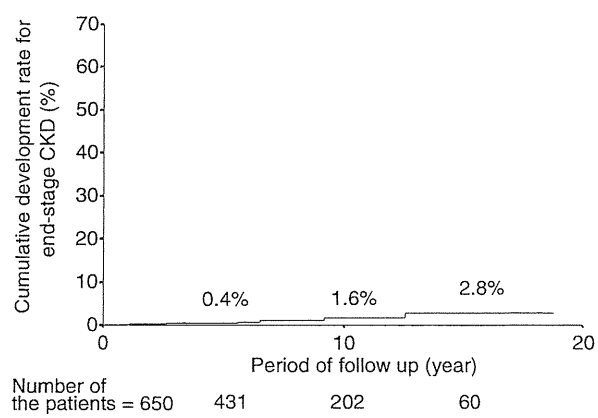


**Figure 4** Cumulative development rate for end-stage chronic kidney disease (CKD) based on the difference of efficacy in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon.

**Table 3** Predictive factors for end-stage chronic kidney disease (CKD) development

| Variables | Univariate analysis | |
|---|---|---|
| | HR (95%CI) | *P* |
| Age, per 10 years | 2.13 (0.86–5.30) | 0.104 |
| Sex (female/male) | 0.24 (0.03–1.92) | 0.182 |
| Body mass index (≥25/<25) | 0.80 (0.16–4.10) | 0.782 |
| HCV load (KIU/mL, ≥1000/<1000) | 1.58 (0.37–6.67) | 0.535 |
| Genotype (1/2) | 2.74 (0.66–11.50) | 0.167 |
| AST (IU/L, ≥50/<50) | 1.45 (0.18–11.76) | 0.730 |
| ALT (IU/L, ≥50/<50) | 1.89 (0.45–7.93) | 0.382 |
| **Platelet ($\times 10^4/mm^3$, ≥15/<15)** | 0.67 (0.16–2.86) | 0.586 |
| eGFR, per decrease of 10 mL/min/1.73 $m^2$ | 1.70 (0.89–3.23) | 0.105 |
| Uric acid (mg/dL, ≥7.0/<7.0) | 1.27 (0.23–6.96) | 0.784 |
| Triglyceride (mg/dL, ≥150/<150) | 1.33 (0.15–11.87) | 0.802 |
| Cholesterol (mg/dL, ≥220/<220) | 1.03 (0.12–8.67) | 0.980 |
| Diabetes (+/–) | 1.89 (0.45–7.93) | 0.382 |
| Hypertension (+/–) | 2.83 (0.70–11.41) | 0.143 |
| Combination of ribavirin (+/–) | 0.88 (0.10–7.66) | 0.908 |
| Kind of IFN (beta/alpha) | 2.08 (0.52–8.37) | 0.300 |
| Efficacy (non-SVR/SVR) | 3.25 (0.40–26.4) | 0.269 |
| Frequencies of contrast imaging per year (≥1/<1) | 3.72 (0.70–19.72) | 0.123 |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; HR, hazards ratio; IFN, interferon; SVR, sustained virological response.

ally, a recent definition of CKD also includes proteinuria.[28,29] Although the use of both eGFR and proteinuria might lead to a more accurate classification of CKD, we could not assess proteinuria in this study. Third, prescribed agents during the follow-up were not considered in the present study. However, therapy intervention is very important for protecting new development for CKD. In future, the intervention therapy for protecting the development of CKD should be evaluated. Finally, in the present study, patients were treated with different types of antivirus therapy (IFN monotherapy or combination therapy of IFN and ribavirin) for different durations (4 weeks to 52 weeks). This heterogeneity makes it slightly difficult to interpret the results of the study. On the other hand, the strengths of the present study are a long-term follow-up in the large numbers of patients included.

The present study shows several findings with regard to development incidence for CKD or end-stage CKD after the termination of IFN therapy for HCV positive and cirrhotic patients. First, SVR is effective for protecting the development incidence for CKD in HCV patients with liver cirrhosis. Though the role of HCV in the pathogenesis of aggravation of renal function remains speculative, the following possible mechanism have been reported: (i) systemic immune response to HCV infection mediated by cryoglobulins, HCV-antibody immune complexes, or amyloid deposition;[8,30,31] (ii) toll-like receptors increased expression in glomeruli induce immune response;[32] and (iii) insulin resistance and hyperinsulinemia cause excess intrarenal production of insulin-like growth factor-1 and transforming growth factor β, thus induce oxidative stress.[33] In addition, patients with liver cirrhosis might have the possibility of kidney damage such as hypovolemia due to fluid loss or hemorrhage, hepatorenal syndrome, and drug-induced renal failure. Second, in addition to non-SVR, the present study suggests that aging, low eGFR, hypertension, and diabetes enhanced the development of worsening renal function in cirrhotic patients with HCV infection after the termination of IFN. The repeated use of contrast imaging of computed tomography might worsen renal function. However, in the present study, SVR, aging, low eGFR, hypertension, and diabetes were the main predictive factors for the development of CKD compared to the repeated use of contrast imaging of computed tomography. The result that aging, hypertension and diabetes were associated with the development of worsening renal function agreed with several studies.[16–19]

In the present study, the predictive factors for end-stage CKD (stage 5) were not similar to those for CKD 3–5. The possible reason for this discrepancy is as follows. First, the number of patients who had progressed to end-stage CKD was six. Because of so few patients, we could not show the statistical significance in the predictive factors for end-stage CKD. Second, development of end-stage CKD might be robust to the several factors at the initiation of the follow-up. Development of end-stage CKD might be associated with the accidents during follow-up, such as the repeated use of contrast medium and hypovolemia due to bleeding. In fact, four of six patients who progressed to end-stage CKD had been given the repeated use of contrast medium. Next, whether HCV eradication in patients whose renal function progressed to stage of CKD 3–5 improves the mortality due to cardiovascular disease and stroke is a very important issue. However, this problem was not evaluated in the present study. This should be clarified by further examination.

In conclusion, our study suggests that the annual incidence for CKD among cirrhotic patients with HCV was determined to be about 1.0–1.5%. In addition, the annual incidence for end-stage CKD is one order of magnitude lower than that of CKD.

## ACKNOWLEDGMENTS

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# Amino Acid Substitutions in Hepatitis C Virus Core Region Predict Hepatocarcinogenesis Following Eradication of HCV RNA by Antiviral Therapy

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Substitution of amino acid (aa) 70 and/or 91 in the core region of HCV genotype 1b (HCV-1b) is an important predictor of hepatocarcinogenesis, but its impact on the development of hepatocellular carcinoma (HCC) following eradication of HCV RNA by antiviral therapy is not clear. 1,273 patients with HCV-related chronic liver disease, with sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of interferon monotherapy or interferon plus ribavirin combination therapy, were included in a follow-up study to evaluate the impact of aa substitution in the core region on hepatocarcinogenesis. Twenty six patients developed HCC during the follow-up. The cumulative rates of new HCC were 3.2%, 4.8%, and 8.6% at the end of 5, 10, and 15 years, respectively. The rates in patients infected with HCV-1b/Gln70(His70) [glutamine (histidine) at aa 70] were significantly higher than in patients infected with HCV-1b/Arg70 (arginine at aa 70) ($P = 0.007$; log-rank test) and HCV-2a/2b ($P < 0.001$; log-rank test). The rates in patients infected with HCV-1b/Arg70 were not significantly higher than in those infected with HCV-2a/2b ($P = 0.617$; log-rank test). Multivariate analysis identified HCV-1b/Gln70(His70) (HR 10.5, $P < 0.001$), advanced fibrosis (HR 9.03, $P = 0.002$), and old age (HR 3.09, $P = 0.066$) as determinants of hepatocarcinogenesis. In conclusion, aa substitution in the core region of HCV-1b at the start of antiviral therapy is an important predictor of HCC following eradication of HCV RNA. This study emphasizes the importance of detection of aa substitutions in the core region before antiviral therapy. ***J. Med. Virol. 83:1016–1022, 2011.*** © 2011 Wiley-Liss, Inc.

**KEY WORDS:** HCV; genotype; sustained virological response; hepatocellular carcinoma; core region; glutamine



## INTRODUCTION

Infection with hepatitis C virus (HCV) is often persistent and can progress to chronic hepatitis, cirrhosis of the liver, and hepatocellular carcinoma (HCC) [Niederau et al., 1998; Kenny-Walsh, 1999]. At present, interferon (IFN), in combination with ribavirin, is the mainstay for treatment of HCV infection. In Japan, HCV genotype 1b (HCV-1b) and high viral loads account for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis C [Tsubota et al., 2005].

Despite numerous lines of epidemiological evidence of an association between HCV infection and the development of HCC, it remains controversial whether the virus itself plays a direct role or an indirect role in the pathogenesis of HCC [Koike, 2005]. It has become evident that the HCV core region is potentially oncogenic in transgenic mice, but the clinical impact of the core region on hepatocarcinogenesis is still unclear [Moriya et al., 1998]. Previous reports indicated that amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of patients infected with HCV-1b are pretreatment predictors of poor virological response to pegylated IFN (PEG-IFN)/ribavirin combination therapy and triple therapy of

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telaprevir/PEG-IFN/ribavirin [Akuta et al., 2005, 2007a, 2010; Donlin et al., 2007], and also affect hepatocarcinogenesis [Akuta et al., 2007b; Fishman et al., 2009; Hu et al., 2009; Nakamoto et al., 2010]. These reports support the oncogenic potential of the core region from the clinical aspect. However, hepatocarcinogenesis still occurs even after eradication of HCV RNA by antiviral therapy [Ikeda et al., 2003, 2005; Tokita et al., 2005; Kobayashi et al., 2007; Hirakawa et al., 2008], though whether substitutions of aa 70 and/or 91 in the core region also affect hepatocarcinogenesis following eradication of HCV RNA await further investigation.

The present study included 1,273 patients with HCV-related chronic liver disease, with sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of antiviral therapy (IFN monotherapy or IFN plus ribavirin combination therapy). The aims of this study were to evaluate the impact of aa substitutions in the core region detected at the start of antiviral therapy on hepatocarcinogenesis following eradication of HCV RNA.

## PATIENTS AND METHODS

### Patients

Among 4,570 consecutive patients infected with HCV, in whom antiviral therapy (IFN monotherapy or IFN plus ribavirin combination therapy) was initiated between February 1987 and June 2010 at the Toranomon Hospital, 1,273 were selected for the present study. We included patients who fulfilled the following criteria: (1) Patients positive for anti-HCV (by a third-generation enzyme immunoassay, Chiron Corp., Emerville, CA) and for HCV RNA by qualitative or quantitative analysis, before antiviral therapy. (2) Patients with sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of antiviral therapy, based on HCV RNA qualitative analysis (Amplicor, Roche Diagnostics, Manheim, Germany) or by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). (3) Patients without HCC, before and during IFN therapy. (4) Patients infected with a single genotype of HCV-1b, 2a, or 2b. (5) Patients negative for hepatitis B surface antigen (by radioimmunoassay, Dainabot, Tokyo). (6) Patients free of coinfection with the human immunodeficiency virus. (7) Lifetime cumulative alcohol intake <500 kg (mild to moderate alcohol intake). (8) Patients free of other types of hepatitis, and without hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease. (9) Each signed a consent form of the study protocol that had been approved by the human ethics review committee.

Table I summarizes the profile and laboratory data at the start of antiviral therapy of 1,273 patients with sustained virological response. They included 783 males and 490 females, aged 15–83 years (median, 53 years). The median follow-up time, from the end of antiviral therapy until the last visit, was 1.1 years (range, 0.0–18.0 years).

### Laboratory Investigations

Blood samples were frozen at −80°C within 4 hr of collection and were not thawed until used for testing. HCV genotype was determined by PCR using a mixed primer set derived from nucleotide sequences of the NS5 region [Chayama et al., 1993]. HCV RNA was quantitated by branched DNA assay version 2.0 (Chiron Corp.), AMPLICOR GT HCV Monitor version 2.0 using the 10-fold dilution method (Roche Molecular Systems, Inc., Pleasanton, CA), or COBAS TaqMan HCV test (Roche Diagnostics). A high viral load was defined as branched DNA assay value of

TABLE I. Clinical Profile and Laboratory Data at the Start of Antiviral Therapy

| | |
|---|---|
| Demographic data | |
| Number of patients | 1,273 |
| Sex (male/female) | 783/490 |
| Age (years)* | 53 (15–83) |
| Body mass index (kg/m$^2$)* | 22.7 (14.4–38.0) |
| Laboratory data | |
| Serum aspartate aminotransferase (IU/L)* | 48 (11–1,386) |
| Serum alanine aminotransferase (IU/L)* | 68 (10–2,009) |
| Total cholesterol (mg/dl)* | 168 (79–328) |
| Fasting plasma glucose (mg/dl)* | 93 (69–290) |
| HCV genotype (1b/2a/2b)* | 664/433/176 |
| Level of viremia (high viral load/low viral load) | 838/415 |
| Treatment regimen | |
| IFN monotherapy/IFN plus ribavirin | 545/728 |
| Histological findings | |
| Stage of fibrosis (F1/F2/F3/F4) | 508/224/62/47 |
| Amino acid substitutions in the HCV genotype 1b | |
| Core aa 70 [arginine/glutamine (histidine)] | 348/127 |
| Core aa 91 (leucine/methionine) | 321/156 |

The enrolled patients had sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of antiviral therapy.
Data are numbers and percentages of patients, except those denoted by asterisk (*), which represent the median (range) values.

≥1.0 Meq/ml, AMPLICOR GT HCV Monitor ≥100 × $10^3$ IU/ml, or COBAS TaqMan HCV test ≥5.0 log IU/ml. Low viral load was defined as branched DNA assay value of <1.0 Meq/ml, AMPLICOR GT HCV Monitor <100 × $10^3$ IU/ml, or COBAS TaqMan HCV test <5.0 log IU/ml. The lower limit of HCV RNA qualitative analysis (Amplicor, Roche Diagnostics, Manheim) was 100 copies/ml, and that of COBAS TaqMan HCV test was 1.2 log IU/ml. Samples with undetectable HCV RNA at 24 weeks after cessation of antiviral therapy by qualitative analysis or COBAS TaqMan HCV test were defined as HCV RNA-negative.

### Detection of Amino Acid Substitutions in the Core Regions of HCV-1b

In the present study, aa substitutions in the core region of HCV-1b were analyzed by direct sequencing. HCV RNA was extracted from serum samples at the start of antiviral therapy and reverse transcribed with a random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo, Japan). Nucleic acids of the core region were amplified by nested PCR using the following primers. The first-round PCR was performed with CE1 (sense, 5′-GTC TGC GGA ACC GGT GAG TA-3′, nucleotides: 134–153) and CE2 (antisense, 5′-GAC GTG GCG TCG TAT TGT CG-3′, nucleotides: 1096–1115) primers, and the second-round PCR with CC9 (sense, 5′-ACT GCT AGC CGA GTA GTG TT-3′, nucleotides: 234–253) and CE6 (antisense, 5′-GGA GCA GTC GTT CGT GAC AT-3′, nucleotides: 934–953) primers. All samples were initially denatured at 95°C for 2 min. The 35 cycles of amplification were set as follows: denaturation for 30 sec at 95°C, annealing of primers for 30 sec at 55°C, and extension for 1 min at 72°C with an additional 7 min for extension. Then, 1 μl of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were the same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (Perkin-Elmer, Tokyo, Japan).

Using HCV-J (accession no. D90208) as a reference [Kato et al., 1990], the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed using 50 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [Akuta et al., 2005]. Thus, patients were classified into three HCV subgroups according to the HCV genotype and aa substitutions in the HCV-1b core region: (1) HCV-1b with Arg70, (2) HCV-1b with Gln70(His70), and (3) HCV-2a/2b.

### Liver Histopathological Examination

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan). The samples were fixed in 10% formalin and then stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. Each specimen submitted for examination contained ≥6 portal areas. Histopathological diagnosis was made by an experienced liver pathologist (HK) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on the scoring system of Desmet et al. [1994] for histopathological assessment.

### Follow-Up and Diagnosis of Hepatocellular Carcinoma

Hematological, biochemical, and virological tests were performed at least once every month until the virological response was determined. When sustained virological response was confirmed, blood tests and imaging studies (computed tomography or ultrasonography) were conducted once or twice per year in the majority of patients, except those lost to follow-up. When HCC was suspected, additional procedures, such as magnetic resonance imaging, abdominal angiography, and ultrasonography-guided tumor biopsy when necessary, were used to confirm the diagnosis.

### Statistical Analysis

The cumulative rate of new cases of HCC was calculated using the Kaplan–Meier technique, and differences between the curves were tested using the log-rank test. Differences in the proportion of new cases of HCC according to groups were analyzed according to the period between the end of antiviral therapy and appearance of HCC. Stepwise Cox regression analysis was used to determine independent predictive factors that were associated with the development of HCC. The hazard ratio (HR) and 95% confidence interval (95%CI) were also calculated. Potential predictive factors associated with the development of HCC included the following variables: sex, age, body mass index, AST, ALT, total cholesterol, fasting plasma glucose, HCV genotype, level of viremia, treatment regimen, stage of fibrosis, and HCV subgroup according to HCV genotype in combination with aa substitutions in the core region. Variables that achieved statistical significance ($P < 0.05$) on univariate analysis were entered into a multivariate Cox proportional hazard model to identify significant independent factors. Statistical comparisons were performed using The Statistical Package for Social Sciences software (SPSS, Inc., Chicago, IL). All $P$ values of less than 0.05 by the two-tailed test were considered significant.

## RESULTS

### Rate of New Cases of HCC in Patients With Sustained Virological Response

During the follow-up, 26 patients (2.0%) developed HCC. The median interval between the end of antiviral therapy and detection of HCC (latency to HCC) was 2.5 years (range, 0.0–15.9 years). The cumulative rates of new cases of HCC were 3.2%, 4.8%, and 8.6% at the end of 5, 10, and 15 years, respectively.

### HCC Rate According to HCV Genotype and Amino Acid Substitutions in the Core Region of HCV-1b

During the follow-up, 7 (5.5%), 5 (1.4%), and 12 (2.0%) patients developed HCC in the HCV-1b with Gln70(His70), HCV-1b with Arg70, and HCV-2a/2b groups, respectively. The median latency to HCC was 1.1 years (range, 0.0–14.0 years), 3.9 (range, 0.0–15.9), and 2.8 (range, 0.0–12.9), respectively, and the cumulative rates of new cases of HCC were 10.6%, 3.6%, 3.0% at the end of 5 years; 10.6%, 6.3%, 5.2% at the end of 10 years; and 62.7%, 6.3%, 7.2% at the end of 15 years, respectively. The rates were significantly different among the three HCV subgroups ($P < 0.001$; log-rank test; Fig. 1). Especially, the rates for HCV-1b with Gln70(His70) were significantly higher than those for HCV-1b with Arg70 ($P = 0.007$; log-rank test) and HCV-2a/2b ($P < 0.001$; log-rank test). However, the rates for the HCV-1b with Arg70 group were not significantly higher than those for the HCV-2a/2b group ($P = 0.617$; log-rank test).

During the follow-up, 4 (2.6%) and 7 (2.2%) patients with HCV-1b/Met91, and HCV-1b/Leu91 developed HCC, respectively. In these two subgroups, the respective median latency to HCC was 3.4 years (range, 0.0–14.0 years) and 1.1 (range, 0.0–12.4), and the cumulative rates of new cases of HCC were 1.3%, 8.6% at the end of 5 years; 5.4%, 8.6% at the end of 10 years; and 36.9%, 14.7% at the end of 15 years. The rates for the HCV-1b/Met91 group were not significantly different from those for the HCV-1b/Leu91 group ($P = 0.908$; log-rank test).

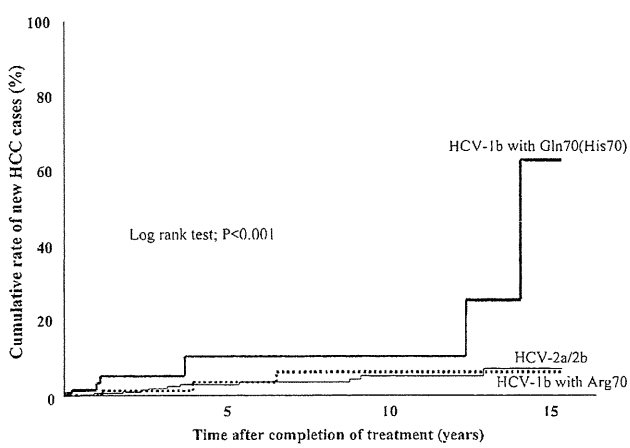


Fig. 1. Cumulative rates of new cases of HCC according to HCV genotype and amino acid substitutions in the core region of HCV-1b. The rates were significantly different among the three HCV groups ($P < 0.001$; log-rank test). Especially, the rate in patients with HCV-1b/Gln70(His70) was significantly higher than those of patients with HCV-1b/Arg70 ($P = 0.007$; log-rank test) and HCV-2a/2b ($P < 0.001$; log-rank test). Furthermore, the rate in patients with HCV-1b/Arg70 was not significantly higher than that in HCV-2a/2b ($P = 0.617$; log-rank test).

### Predictive Factors Associated With the Development of HCC in Patients of Sustained Virological Response

Next, we analyzed the predictor of HCC using data of the entire group. There were significant relationships between the rate of new cases of HCC and male sex ($P = 0.003$), severe fibrosis (F3,4) ($P < 0.001$), old age (≥55 years) ($P = 0.002$), high levels of AST (≥39 IU/L) ($P = 0.023$), and HCV-1b/Gln70(His70) (log-rank test). These five factors were entered into multivariate analysis, which then identified three parameters that independently tended to or significantly influenced the development of HCC; HCV-1b/Gln70(His70) (HR 10.5, $P < 0.001$), advanced stage of fibrosis (F3,4; HR 9.03, $P = 0.002$), and old age (≥55 years; HR 3.09, $P = 0.066$; Table II).

### Predictors of HCC in HCV-1b Patients With Sustained Virological Response

Finally we analyzed the data of 664 patients with HCV-1b to determine the predictors of HCC with sustained virological response. Univariate analysis identified three parameters that significantly correlated with the development of HCC: male sex ($P = 0.005$), old age ($P = 0.020$), and HCV-1b with Gln70(His70) ($P = 0.007$; log-rank test). These three factors were entered into multivariate analysis, which then identified HCV-1b with Gln70(His70) as the single parameter that significantly influenced the development of HCC (HR 8.19, $P = 0.034$).

## DISCUSSION

Previous studies reported that the risk factors for hepatocarcinogenesis after elimination of HCV RNA

TABLE II. Results of Multivariate Analysis (Cox Proportional Hazard Model) for Factors Associated With Hepatocarcinogenesis in Patients With Sustained Virological Response

| Factors and categories | Hazard ratio (95%CI) | *P*-Value |
|---|---|---|
| HCV group | | |
| HCV-2a/2b | 1 | |
| HCV-1b with Arg70 | 1.15 (0.24–5.56) | 0.863 |
| HCV-1b with Gln70(His70) | 10.5 (2.89–38.2) | <0.001 |
| Fibrosis stage | | |
| F1,2 | 1 | |
| F3,4 | 9.03 (2.32–35.2) | 0.002 |
| Age (years) | | |
| <55 | 1 | |
| ≥55 | 3.09 (0.93–10.3) | 0.066 |

were severe fibrosis, male sex, and old age at the start of IFN treatment [Ikeda et al., 2003, 2005; Tokita et al., 2005; Kobayashi et al., 2007; Hirakawa et al., 2008]. In the present study, multivariate analysis identified HCV-1b with Gln70(His70), advanced fibrosis stage, and old age as determinants of HCC in patients with a sustained virological response. The present study is the first report to indicate that aa substitution in the core region at the start of antiviral therapy also influences hepatocarcinogenesis following eradication of HCV RNA. This result should be interpreted with caution since races other than the Japanese and patients infected with HCV-1a were not included. Any generalization of the results should await confirmation by studies of patients of other races and those infected with HCV-1a.

Despite numerous lines of epidemiological evidence linking HCV infection to the development of HCC, it remains controversial whether HCV itself plays a direct or indirect role in the pathogenesis of HCC [Koike, 2005]. Evidence suggests that the HCV core region is potentially oncogenic in the transgenic mice [Moriya et al., 1998], though the clinical impact of the core region on hepatocarcinogenesis remains unclear. Previous reports indicated that aa substitutions in the core region of HCV-1b are pretreatment predictors of poor virological response to antiviral therapy [Akuta et al., 2005, 2007a, 2010; Donlin et al., 2007], and also are etiological factors in HCC [Akuta et al., 2007b; Fishman et al., 2009; Hu et al., 2009; Nakamoto et al., 2010]. Importantly, the present study indicated that aa substitution in the core region at the start of antiviral therapy also affects the development of HCC even after the eradication of HCV RNA, and this is the first report to suggest the persistent oncogenic potential of the core region regardless of HCV RNA persistence. Previous reports identified the PA28γ-dependent pathway as one of the mechanisms of HCV-associated hepatocarcinogenesis. Moriishi et al. [2003, 2007] reported that knockout of the PA28γ gene induces accumulation of HCV core protein in the nuclei of hepatocytes of HCV core gene transgenic mice and disrupts the development of both hepatic steatosis and HCC. Furthermore, the HCV core protein also enhances the binding of liver X receptor α (LXRα) and retinoid X receptor α (RXRα) to the LXR-response element in the presence of PA28γ [Moriishi et al., 2007]. Thus, it seems that PA28γ plays a crucial role in the development of HCV-associated steatosis and HCC. However, these basic studies were performed under the state of HCV RNA persistence [Moriya et al., 1998; Moriishi et al., 2003, 2007; Koike, 2005], and further studies should be performed to investigate the oncogenic potential of aa substitution in the core region detected at the start of antiviral therapy on hepatocarcinogenesis following eradication of HCV RNA.

The association between HCV genotype and the risk of HCC is not clear. A study of Italian cohort indicated that the rate of HCC in patients infected with HCV-1b was significantly higher than that of patients infected with HCV-2a/2c [Bruno et al., 2007]. On the other hand, the present study of Japanese patients indicated that the rates in patients infected with HCV-1b were not significantly higher than those in those infected with HCV-2a/2b. The discrepancy between the present result and the above Italian study may be explained by differences in host factors [Montes-Cano et al., 2010], and/or differences in viral factors, such as the distribution of HCV-1b with Arg70 or Gln70(His70), and geographic diversities of HCV-1b [Nakano et al., 1999].

Previous studies showed that the 12- and 24-week regimen of telaprevir/PEG-IFN/ribavirin achieved sustained virological response rates of 35–60% and 61–69% in patients infected with HCV-1, respectively [Hézode et al., 2009; McHutchison et al., 2009; Akuta et al., 2010]. Furthermore, the PROVE3 study also showed that the 24- and 48-week regimen of triple therapy achieved sustained virological response rates of 51% and 53%, respectively, in patients infected with HCV-1 who had been unsuccessfully treated with PEG-IFN/ribavirin [McHutchison et al., 2010]. While it is anticipated that larger numbers of HCV-1 patients will achieve sustained virological response in response to telaprevir/PEG-IFN/ribavirin, a larger proportion of patients could develop HCC following eradication of HCV RNA by antiviral therapy. Hence, our study indicated that aa substitutions in the core region of HCV-1b should be detected before eradication of HCV RNA by antiviral therapy. Especially, even if patients of HCV-1b with Gln70(His70) could achieve sustained virological response, blood tests and imaging studies should be conducted at regular intervals in this high risk group for early detection and treatment of HCC.

Genetic variations near the IL28B gene are pretreatment predictors of poor virological response to the combination therapy of PEG-IFN/ribavirin and triple therapy of telaprevir/PEG-IFN/ribavirin [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Akuta et al., 2010; Rauch et al., 2010], but their impact on hepatocarcinogenesis are unknown at this stage. In this study, 387 of 1,273 patients were evaluated for HCC according to genetic variation in rs8099917 (data not shown). A preliminary study based on a small number of patients showed that the HCC rate in genotype TT of treatment sensitive type (2.2%) was not significantly different from that in genotype non-TT of treatment resistant type (1.6%). Unfortunately, we could analyze the effect of rs8099917 on HCC following eradication of HCV RNA by antiviral therapy. Further studies of larger patient populations should be performed to investigate the relationship between genetic variations near the IL28B gene and HCC.

The limitations of the present study were that viral factors associated with hepatocarcinogenesis were incompletely investigated. Ogata et al. [2003] reported that HCV-1b strains might be associated with HCC

on the basis of the secondary structure of the amino-terminal portion of the HCV NS3 protein. Giménez-Barcons et al. [2001] reported that high amino acid variability within the NS5A of HCV might be associated with HCC in patients with HCV-1b-related cirrhosis. In the present study, the clinical impact of other regions on hepatocarcinogenesis could not be investigated, except for aa 70 and 91 in the HCV core region. The results should also be interpreted with caution since patients infected with HCV-1a were not included. Other limitations include lack of analysis of the effects of life-style related diseases (such as diabetes, insulin resistance or non-alcoholic steatohepatitis) on hepatocarcinogenesis, except for fasting plasma glucose and total cholesterol [Sumida et al., 2010a,b]. The impact of viral factors and life-style related diseases on hepatocarcinogenesis should also be investigated in future studies.

In conclusion, aa substitution in the core region of HCV-1b at the start of antiviral therapy is an important predictor of hepatocarcinogenesis following eradication of HCV RNA. This study emphasizes the importance of detection of aa substitutions in the core region before antiviral therapy.

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# Amino Acid Substitution in HCV Core Region and Genetic Variation near the *IL28B* Gene Affect Viral Dynamics during Telaprevir, Peginterferon and Ribavirin Treatment

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**Key Words**
Hepatitis C virus · Core region · *IL28B* · Telaprevir · Peginterferon · Ribavirin · Viral dynamics

**Abstract**
***Objectives:*** Genetic variation near the *IL28B* gene and substitution of aa 70 and 91 in the core region of HCV-1b are useful as predictors of treatment efficacy to telaprevir/pegylated interferon (PEG-IFN)/ribavirin, but its impact on viral dynamics is not clear. ***Methods:*** This study investigated predictive factors of viral dynamics during 12- or 24-week regimen of triple therapy in 80 Japanese adults infected with HCV-1b. ***Results:*** After 24 h of commencement of treatment, the proportion of patients with Arg70 and Leu91 substitutions in the core region who showed ≥3.0 log drop in HCV RNA level was significantly higher than that of patients with Gln70 (His70) and/or Met91. At 8 and 12 weeks, HCV RNA loss rate of patients with rs8099917 genotype TT near *IL28B* gene was significantly higher than that of patients with non-TT. Multivariate analysis identified substitution of aa 70 and 91 as a predictor of ≥3.0 log fall in HCV RNA level at 24 h (Arg70 and Leu91) and SVR (Arg70), and rs8099917 (TT) as a predictor of HCV RNA loss at 12 weeks and SVR. ***Conclusions:*** This study identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of viral dynamics during triple therapy. 

## Introduction

Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [1, 2]. At present, treatments based on interferon (IFN), in combination with ribavirin, are mainstay for combating HCV infection. In Japan, HCV genotype 1b (HCV-1b) in high viral loads (>100 kIU/ml) accounts for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis

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