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Research Article

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Clinical milestones for the prediction of severe anemia by chronic hepatitis C patients receiving telaprevir-based triple therapy

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Background & Aims: Anemia is a common adverse effect of telaprevir (TVR) in combination with pegylated interferon (PegIFN)α and ribavirin (RBV) therapy. It occurs at a higher incidence with the TVR relative to PegIFNα and RBV alone. We herein evaluate the baseline and on-treatment predictors of the development of severe anemia by chronic hepatitis C virus (HCV) patients receiving TVR-based triple therapy.

Methods: This prospective, multicenter study consisted of 292 patients (median age: 62 years) infected with HCV genotype 1. All received 12 weeks of TVR in combination with 24 weeks of PegIFNα2b and RBV. The definition of severe anemia during antiviral treatment is hemoglobin (Hb) <85 g/L.

Results: 101 (34.6%) patients developed severe anemia during the treatment period. Multivariable logistic regression analysis of possible pretreatment predictors of the development of severe anemia extracted baseline Hb <135 g/L (Hazard ratio [HR], 2.53; $p = 0.0013$), estimated glomerular filtration rate <80 ml/min/1.73 m^2 (HR, 1.83; $p = 0.0265$), and inosine triphosphatase (ITPA) CC genotype (rs1127354) (HR, 2.91; $p = 0.0024$). For patients with ITPA CC (n = 227), multivariable logistic regression analysis of possible pretreatment and on-treatment predictors of the development of severe anemia extracted Hb level at week 2 (HR, 0.96; $p = 0.0085$) and the initial four weeks of weight-adjusted TVR (HR, 1.05; $p = 0.0281$).

Conclusions: Anemia remains a risk for all patients treated with TVR-based triple therapy. However, ITPA polymorphism (rs1127354) is useful for predicting the development of severe anemia and will be helpful in the management of treatment.

Keywords: Hepatitis C virus; Anemia; Telaprevir; Inosine triphosohatase; Pegylated interferon; Ribavirin.

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Abbreviations: HCV, hepatitis C virus; PegIFN, pegylated interferon; RBV, ribavirin; SVR, sustained virological response; TVR, telaprevir; ITPA, inosine triphosphatase; SNP, single nucleotide polymorphism; Hb, hemoglobin; eGFR, estimated glomerular filtration rate; RVR, rapid virological response; HR, hazard ratio; CI, confidence interval; AUROC, area under the receiver operating characteristic curve.

Introduction

Chronic hepatitis C virus (HCV) infection can result in serious health problems such as decompensated cirrhosis and hepatocellular carcinoma [1–3]. The standard of care regimen that includes pegylated interferon (PegIFN)α and ribavirin (RBV) has been the first line for the past decade, however, the rate of sustained virological response (SVR) that can be achieved using this regimen is only 40–52% for patients infected with HCV genotype 1 [4–6].

Of a number of direct-acting antivirals under investigation, nonstructural 3/4A protease inhibitors, including telaprevir (TVR) and boceprevir, have shown promising treatment outcomes in various clinical trials in combination with the current standard of care. The SVR rate is improved to over 70% for HCV genotype 1 patients treated with TVR-based triple therapy [7–9]. Notably, the SVR rate rises to over 80% for prior relapsers [7,10]. However, many adverse effects have been reported, with anemia being one of the most serious. Treatment requires careful management with RBV dose reduction. Because anemia has been shown to occur at a higher incidence with the TVR regimen relative to PegIFNα and RBV alone [7], it is important to understand the characteristics of severe anemia development prior to antiviral treatment.

Research Article

A genome-wide association study identified the inosine triphosphatase (*ITPA*) gene single nucleotide polymorphism (SNP) as being significantly associated with RBV-induced anemia [11,12]. Recently, Chayama *et al.* reported in a clinical trial that *ITPA* SNP (rs1127354) is associated with anemia in TVR-based triple therapy and that RBV dose reductions were required significantly earlier for patients with the ITPA CC genotype when compared with the ITPA CA and AA genotypes [13]. However, further improvement of the ability to predict the development of severe anemia will contribute to increasing the likelihood of achieving an SVR by patients whose treatment might otherwise have to be stopped.

The aim of this multicenter, prospective study was to evaluate the baseline and on-treatment predictors of the development of severe anemia (Hb <85 g/L) by chronic hepatitis C patients treated with TVR-based triple therapy.

Patients and methods

Patients

The Kyushu University Liver Disease Study (KULDS) Group consists of the Kyushu University Hospital and its affiliated hospitals in the northern Kyushu area of Japan. This prospective study consisted of 292 Japanese patients with chronic HCV infection aged 20 years or older who received TVR in combination with PegIFNα2b and RBV between December 2011 and October 2012. Exclusion criteria were: (1) positivity for antibody to human immunodeficiency virus or positivity for hepatitis B surface antigen; (2) clinical or biochemical evidence of hepatic decompensation (ascites, bleeding varices, or encephalopathy); (3) baseline hemoglobin (Hb) <120 g/L (female, <60 years), <110 g/L (female, ⩾60 years), <130 g/L (male, <60 years), and <120 g/L (male, ⩾60 years); (4) baseline serum creatinine >1.2 mg/dl (male) and >0.9 mg/dl (female); (5) other causes of liver disease (autoimmune hepatitis, or primary biliary cirrhosis); (6) excessive active alcohol consumption (a daily intake of more than 40 g of ethanol) or drug abuse; (7) suspected hepatocellular carcinoma at entry; or (8) treatment with antiviral or immunosuppressive agents prior to enrollment. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of each participating hospital. Informed consent was obtained from all patients before enrollment.

Clinical and laboratory assessment

Clinical parameters were measured by standard laboratory techniques at a commercial laboratory (SRL Laboratory, Tokyo, Japan). Body mass index was calculated as weight in kilograms/height in square meters. The estimated glomerular filtration rate (eGFR) was calculated based on the Modification of Diet in Renal Disease (MDRD) formula. Aspartate aminotransferase to platelet ratio index (APRI) was calculated, as previously recommended for evaluating severe liver fibrosis [14].

Assessment of liver fibrosis

Liver biopsy for 183 (62.7%) of the studied patients was done by experienced hepatologists. All antiviral treatment was initiated within one month after liver biopsy. The minimum length of the liver biopsy was 15 mm and at least 10 complete portal tracts were necessary for inclusion. For each specimen, the stage of fibrosis was established according to the METAVIR score [15].

Antiviral treatment

All patients received a combination treatment of TVR (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan), PegIFNα2b (PEG-Intron; MSD, Tokyo, Japan), and RBV (Rebetol; MSD) for 12 weeks, followed by an additional 12 weeks of PegIFNα2b and RBV alone. TVR 750 mg was administrated three times a day at an 8-h interval after each meal (2250 mg/day). PegIFNα2b was injected subcutaneously once weekly at a dose of 1.5 μg/kg. RBV was given orally at a daily dose of 600–1000 mg based on body weight (600 mg for patients weighing <60 kg, 800 mg for those weighing 60–80 kg, and 1000 mg for those weighing >80 kg). The above durations and dosages are those approved by the Japanese Ministry of Health, Labor, and Welfare. In case of a 1.5 to 2.0 times elevation from baseline serum creatinine, the TVR dose was reduced to 1500 mg/day (750 mg twice a day at a 12-h interval after each meal). When serum creatinine was elevated to over 2.0 times the baseline level, TVR was discontinued. If a marked anorexia was developed, the TVR dose could be reduced to 1500 mg/day. If a progressive grade 3 rash developed (severe, involving more than 50% of the body surface, or rash with the appearance of substantial systemic signs of symptoms), TVR was discontinued. However, the patients continued to receive PegIFN and RBV in all of these situations. All treatment was discontinued for patients with less than 2 $\log_{10}$ HCV RNA decrease from baseline to week 12.

Management of anemia

Severe anemia during antiviral treatment was defined as Hb <85 g/L. Complete blood count was checked every week for the first 12 weeks and then at weeks 16, 20, and 24. The management of anemia started with RBV dose reduction. Specifically, the RBV dose was reduced by 200 mg for patients receiving 600 or 800 mg and by 400 mg for those receiving 1000 mg when Hb decreased to <120 g/L and by an extra 200 mg when it lowered to <100 g/L. If Hb decreased to <90 g/L, the TVR dose was reduced to 1500 mg/day. Erythropoietin use was not allowed during treatment, but blood transfusion was allowed when necessary. Discontinuation of TVR-based triple therapy due to severe anemia was primarily based on the discretion of the physicians at each hospital.

HCV RNA level and HCV genotype

Clinical follow-up of HCV viremia was done by real-time reverse transcriptase PCR assay (COBAS® TaqMan® HCV assay) (Roche Diagnostics, Tokyo, Japan), with 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 $\log_{10}$ IU/ml) [16]. HCV RNA levels were measured at baseline, regularly during treatment, at early discontinuation, and at follow-up visits after the end of treatment. Virological response was categorized as follows: rapid virological response (RVR) was an undetectable HCV RNA at week 4 and SVR was an undetectable HCV RNA at week 24 after the end of treatment. HCV genotype determination was by sequence determination in the 5′-non-structural region of the HCV genome followed by phylogenetic analysis [17].

Genetic testing

Human genomic DNA was extracted from peripheral blood. Genotyping of the *ITPA* (rs1127354) genes was performed using the ABI TaqMan allelic discrimination kit (7500 Real Time PCR System; Applied Biosystems, Carlsbad, CA, USA). Heterozygotes (CA) or homozygotes (AA) of the minor allele (A) are described as having the *ITPA* minor allele, whereas homozygotes for the major allele (CC) are described as having the *ITPA* major allele [12].

Statistical analysis

Statistical analyses were conducted using SPSS statistics 19.0 (IBM SPSS Inc, Chicago, IL, USA). Baseline continuous data are expressed as median (first-third quartiles) or mean (±standard deviation), and categorical variables are reported as frequencies and percentages. Univariate analyses were done using the Chi-square, Fisher's exact, or Mann-Whitney U tests as appropriate. Variables with p <0.05 in univariate analysis were evaluated using multivariate logistic regression to identify variables significantly associated with the development of severe anemia. The results are expressed as hazard ratios (HR) and their 95% confidence interval (CI). The significance of trends in values was determined with the Cochran-Armitage trend test.

Area under the receiver operating characteristic curve (AUROC) analysis was done to evaluate the relationship between the Hb level and development of severe anemia. The cut-off values were selected from the receiver operating characteristic (ROC) curve to maximize the total sensitivity and specificity. A p value less than 0.05 was regarded as statistically significant in all analyses.

Results

Patient characteristics and the development of severe anemia

The baseline characteristics of the 292 studied patients as classified by the development of severe anemia are shown in Table 1.

Table 1. Baseline risk factors for the development of severe anemia by chronic hepatitis C patients treated with telaprevir-based triple therapy.

Characteristic	All patients n = 292	Severe anemia n = 101	Non-severe anemia n = 191	p value*
Age (yr)	62 (54-66)	64 (57-68)	60 (53-65)	<0.0001
Men, n (%)	135 (46.2)	33 (32.7)	102 (53.4)	0.0007
Body mass index (kg/m^2)	23.3 (21.6-25.6)	22.9 (20.9-25.1)	23.4 (21.8-25.7)	0.0408
Alanine aminotransferase (IU/L)	50 (33-93)	50 (30-93)	50 (34-93)	0.3499
Serum albumin (g/L)	40 (37-42)	39 (36-42)	40 (38-43)	0.0054
Estimated glomerular filtration rate (ml/min/1.73 m^2)	80 (72-92)	76 (70-90)	83 (74-94)	0.0024
α-fetoprotein (ng/ml)	5.4 (3.5-11.0)	5.4 (3.3-10.2)	5.5 (3.6-11.7)	0.1753
Hemoglobin (g/L)	136 (127-147)	132 (124-140)	141 (133-153)	<0.0001
Platelet count (x10^9/L)	154 (120-190)	150 (118-200)	156 (121-189)	0.9279
HCV RNA level ($\log_{10}$ IU/ml)	6.5 (6.0-6.9)	6.4 (6.1-6.8)	6.5 (6.0-6.9)	0.5359
APRI <2.0/≥2.0, n (%)	234/58 (80.1/19.9)	77/24 (32.9/41.4)	157/34 (67.1/58.6)	0.2292
Stage of fibrosis				
F0-2/F3-4, n (%)	117/66 (63.9/36.1)	43/28 (36.8/42.4)	74/38 (63.2/57.6)	0.4505
Not determined, n (%)	109	30	79	
ITPA SNPs (rs1127354) CC/CA or AA, n (%)	227/65 (77.7/22.3)	90/11 (39.6/16.9)	137/54 (60.4/83.1)	0.0004
Treatment-naïve/experienced, n (%)	90/202 (30.8/69.2)	32/69 (35.6/34.2)	58/133 (64.4/65.8)	0.8169

Data are expressed as number (%) or median (first-third quartiles).
All demographic and clinical data are those at the start of antiviral treatment.
Severe anemia is diagnosed by hemoglobin level <85 g/L during antiviral treatment.
HCV, hepatitis C virus; ITPA, inosine triphosphatase; SNP, single nucleotide polymorphism; APRI, aspartate aminotransferase to platelet ratio index.
*Comparison between severe anemia and non-severe anemia.

HCV genotype 1b was detected in 290 (99.3%) patients and genotype 1a in the other two. Severe anemia (Hb <85 g/L) was developed during the treatment period by 101 of the 292 (34.6%) patients. The percentages of patients experiencing on-treatment severe anemia are shown in Fig. 1. No patient experienced severe anemia before week 2, and only two patients developed severe anemia after week 12. The onset of severe anemia was most frequently seen from weeks 8 to 12. The allele of the *ITPA* SNP (rs1127354) was determined for each patient. The percentages of patients with the *ITPA* CC, CA, and AA genotypes were 77.7% (n = 227), 19.2% (n = 56), and 3.1% (n = 9), respectively. There were no significant differences in sex (male 45.4% and 49.2%), age (median 62 and 59 years), BMI (23.2 and 23.7 kg/m^2), eGFR (80 and 83 ml/min/1.73 m^2), baseline Hb level (137 and 139 g/L), or initial four-week RBV dosage (9.7 and 10.5 mg/kg) between the *ITPA* CC and CA/AA genotypes.

Baseline factors associated with the development of severe anemia

Univariate analysis extracted older age (p <0.0001), female sex (p = 0.0007), lower BMI (p = 0.0408), lower serum albumin (p = 0.0054), lower eGFR (p = 0.0024), lower baseline Hb level (p <0.0001), and *ITPA* CC (p = 0.0004) as significantly associated with the development of severe anemia during treatment (Table 1). Prior therapeutic experience was not associated with the development of severe anemia (p = 0.8169). In multivariable logistic regression analysis of possible pretreatment predictors of the development of severe anemia, significant independent pretreatment predictors were baseline Hb <135 g/L (HR, 2.53; 95% CI, 1.43–4.51; p = 0.0013), eGFR <80 ml/min/1.73 m^2 (HR, 1.83; 95% CI, 1.07–3.16; p = 0.0265), and *ITPA* CC (HR, 2.91; 95% CI, 1.44–6.32; p = 0.0024). No independent predictive relationship was found between age, sex, BMI, or serum albumin. The rates of severe anemia development stratified by *ITPA* genotype (CC and CA/AA), eGFR level (≥80 and <80 ml/min/1.73 m^2), and baseline Hb level (≥135 and <135 g/L) are shown in Fig. 2.

Hb levels during antiviral treatment stratified by *ITPA* SNPs are shown in Fig. 3A. Hb levels from week 2 to week 24, except at week 12, for patients with *ITPA* CC were significantly lower than those of patients with *ITPA* CA/AA. Similarly, Hb decrement and the decrease ratio throughout the initial 12 weeks stratified by *ITPA* SNPs are shown in Fig. 3B and C, respectively. Hb decrement and the decrease ratio from week 2 to week 8 for patients with *ITPA* CC were significantly lower than for patients with *ITPA* CA or AA.

Baseline factors associated with Hb decrease by over 50 g/L

Hb decline over 50 g/L during the treatment period was found for 128 of the 292 (43.8%) patients. Univariate analysis extracted younger age (p = 0.0011), male sex (p = 0.0011), lower eGFR (p = 0.0161), higher baseline Hb level (p <0.0001), and *ITPA* CC (p = 0.0009) as significantly associated with the decline of Hb to ≥50 g/L. In multivariable logistic regression analysis, baseline Hb ≥135 g/L (HR, 2.73; 95% CI, 1.55–4.86; p = 0.0005), eGFR <80 ml/min/1.73 m^2 (HR, 1.74; 95% CI, 1.04–2.93; p = 0.0355), and *ITPA* CC (HR, 3.36; 95% CI, 1.78–6.63; p = 0.0001) were independently associated with an Hb decline of over 50 g/L.

Relationship between pretreatment or on-treatment variables and the development of severe anemia stratified by ITPA SNPs

Table 2 shows the development of severe anemia according to the *ITPA* SNPs. Univariate analysis of patients with *ITPA* CC (n = 227) extracted older age (p = 0.0004), female sex (p = 0.0011), lower serum albumin (p = 0.0083), lower eGFR (p = 0.0041), lower baseline Hb level (p <0.0001), lower Hb level

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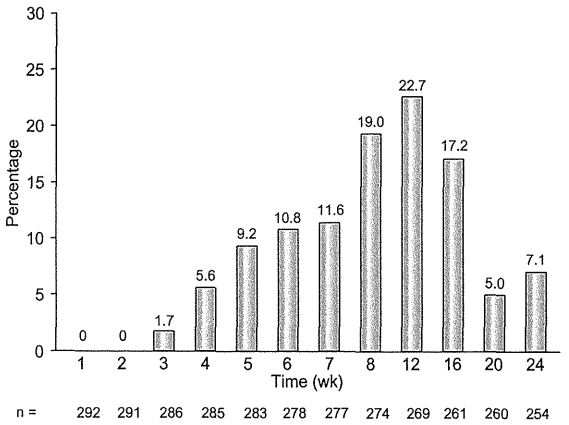

Fig. 1. The percentage of patients experiencing on-treatment severe anemia (hemoglobin <85 g/L). No patient experienced severe anemia before week 2, and the onset of severe anemia was most frequently observed from weeks 8 to 12.

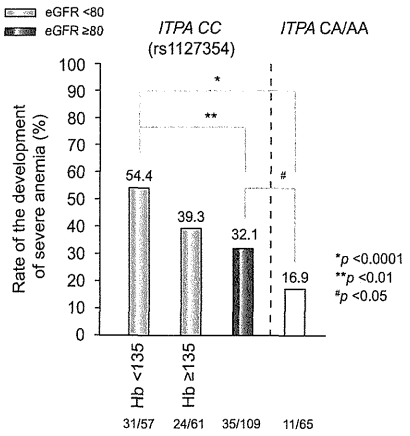

Fig. 2. The percentages of patients who developed severe anemia stratified by *ITPA* SNPs (rs1127354), baseline estimated glomerular filtration rate (eGFR), and hemoglobin (Hb) level. The percentages of *ITPA* CC patients with severe anemia were significantly increased with lower eGFR and Hb levels. Severe anemia was developed by only 16.9% of the *ITPA* CA/AA patients (the Cochran-Armitage trend test: p <0.0001).

at week 2 (p <0.0001), and higher initial four week weight-adjusted dosage of TVR (p = 0.0455) as significantly associated with the development of severe anemia. In multivariable logistic regression analysis of possible pretreatment and on-treatment predictors of the development of severe anemia, significant independent predictors were Hb level at week 2 (HR, 0.96; 95% CI, 0.93–0.98; p = 0.0085) and the initial four weeks of weight-adjusted TVR (HR, 1.05; 95% CI, 1.01–1-10; p = 0.0281). The percentages of *ITPA* CC patients experiencing on-treatment severe anemia stratified by the initial four weeks of TVR (25 mg/kg/day) are shown in Fig. 4A. The rates of severe anemia of the TVR ⩾25 mg/kg/day group were significantly higher than those of the TVR <25 mg/kg/day group at weeks 7, 8, 12, 16, and 24.

In contrast, univariate analysis of patients with *ITPA* CA/AA (n = 65) extracted only lower baseline Hb level (p = 0.0022) and lower Hb level at week 2 (p = 0.0081) as significantly associated with the development of severe anemia. No predictive relationship was found between age, sex, eGFR, or the initial weight-adjusted dosages of RBV or TVR and the development of severe anemia. The percentages of *ITPA* CA/AA patients experiencing on-treatment severe anemia are shown in Fig. 4B. Severe anemia was developed only between weeks 7 and 16, unlike patients with *ITPA* CC.

ROC curve analysis of the Hb level at week 2 and the development of severe anemia by patients with ITPA CC

The adequacy of the multivariate model was confirmed by a ROC curve analysis. This analysis was performed to determine the optimal threshold values for the Hb level at week 2 for predicting the development of severe anemia by the 227 patients with *ITPA* CC. The corresponding AUROC was 0.70 (p <0.0001) for *ITPA* CC and the cut-off value for the Hb level at week 2 was 116 g/L (sensitivity 79.0%, specificity 57.0%).

ROC curve analyses of the Hb level at baseline and week 2 and the development of severe anemia by patients with ITPA CA/AA

ROC curve analyses were performed to determine the optimal threshold values for the Hb levels at baseline and week 2 for predicting the development of severe anemia by the 65 patients with *ITPA* CA/AA. The corresponding AUROCs were 0.75 (p = 0.0089) for Hb level at baseline (cut-off values 122 g/L; sensitivity 98.0%, specificity 55.0%) and 0.68 (p = 0.0538) for Hb level at week 2, which indicates that the Hb level at baseline is more effective than that at week 2 for predicting the development of severe anemia by patients with *ITPA* CA/AA.

Treatment efficacy

The overall rates of RVR and SVR were 75.0% (219 of 292) and 82.2% (240 of 292), respectively. The RVR and SVR rates of patients with *ITPA* CC were 74.9% (170 of 227) and 81.5% (185 of 227), respectively. For patients with *ITPA* CC, there was no significant difference in the initial four weeks of weight-adjusted TVR between the RVR (28.7 [24.4–33.1] mg/kg/day) and non-RVR groups (28.7 [23.9–31.7] mg/kg/day) (p = 0.2467). However, the SVR rates for the initial four weeks of the TVR ⩾25 group (83.5%, 137 of 164) were higher than those of the TVR <25 (mg/kg/day) group (76.2%, 48 of 63), but did not reach significance (p = 0.2106).

Premature discontinuation of treatment or blood transfusion due to anemia

Of the 292 patients, 38 (13.0%) had TVR-based triple therapy discontinued during the treatment period. Of these 38 patients, 8 (21.1%) had treatment discontinued because of severe anemia between weeks 8 and 16. Of these 8 patients, 5 (62.5%) were women, 6 (66.7%) were aged over 60 years, and 6 (66.7%) were *ITPA* CC. On the other hand, 23 (7.9%) received blood transfusion without treatment discontinuation. Of these 23 patients, 13 (56.5%) were women, 16 (69.6%) were aged over 60 years, and 18 (78.3%) were *ITPA* CC.

Discussion

This prospective, multicenter study was carried out to evaluate the baseline and on-treatment predictors of the development of

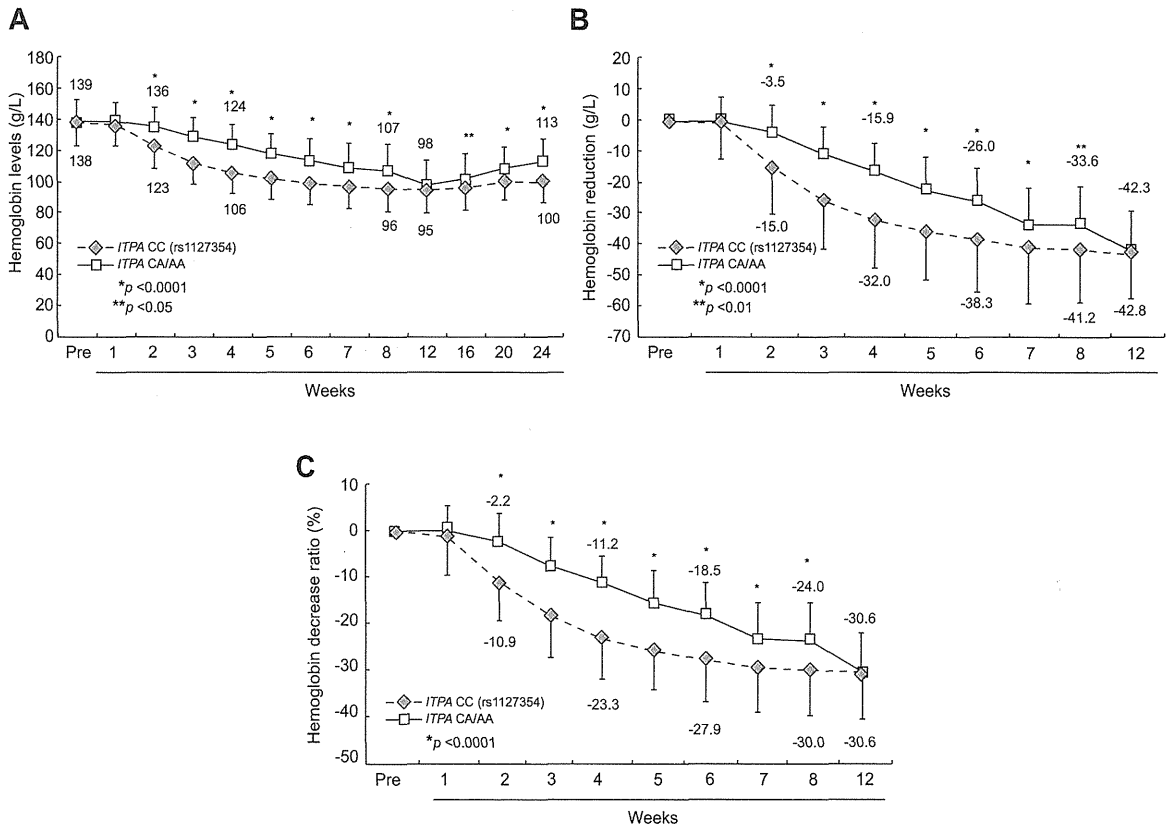

Fig. 3. Mean hemoglobin (Hb) levels, Hb decrement, and Hb decrease ratio during antiviral treatment stratified by *ITPA* SNPs. (A) Hb levels from week 2 to week 24, except at week 12, for patients with *ITPA* CC were significantly lower than those of patients with *ITPA* CA/AA. (B) Hb decrement from week 2 to week 8 for patients with *ITPA* CC was significantly lower than for those with *ITPA* CA/AA. (C) The Hb decrease ratio from week 2 to week 8 for patients with *ITPA* CC was also significantly lower than for those with *ITPA* CA/AA.

severe anemia (Hb <85 g/L) by patients treated with TVR in combination with PegIFNα2b and RBV. Several pretreatment factors, including lower baseline Hb level, lower eGFR, and *ITPA* CC (rs1127354) genotype, were independently associated with the development of severe anemia. Moreover, analysis of patients with the *ITPA* CC genotype that included baseline and on-treatment parameters found the Hb level at week 2 and the initial four week, weight adjusted dosage of TVR to be independent, significant predictors of the development of severe anemia. These findings will help increase the rate of successful completion of treatment by allowing doctors to take steps to predict severe anemia according to the *ITPA* polymorphism.

The availability of protease inhibitors has profoundly changed the management of chronic hepatitis C by achieving higher rates of SVR. However, adverse events are experienced by almost all patients. The most frequently reported adverse effects associated with TVR have been hematological disorders (anemia, thrombocytopenia and leukocytopenia), skin disorders (pruritus and rash), gastrointestinal disorders (nausea and diarrhea), general fatigue, and elevated serum levels of uric acid, bilirubin, and creatinine [7–9,18,19]. Moderate and severe anemia has been shown to develop more frequently in TVR-based triple therapy than in PegIFNα2b and RBV alone [7] and by Japanese more often than by Americans/Europeans [18,19] because Japanese patients with chronic hepatitis C average more than 10 years older than those in Western countries [20–22]. However, these findings were based on clinical trials, thus this is the first study to show the predictors of development of severe anemia as evaluated in clinical practice.

Severe anemia leads to hypoxia in organs, and this condition may be a sign of hyperdynamic circulation, tachycardia, and left heart strain, which have the potential risk of heart failure. Our results showed that the development of severe anemia began at week 3, with a peak frequency at week 12. Therefore, it is important to adjust the dosage as needed in order for patients to be able to complete the overall treatment duration.

We investigated the baseline characteristics associated with both the development of severe anemia and Hb decline by over 50 g/L. *ITPA* CC genotype and lower eGFR level were extracted as the independent risk factors in common. In the analysis of Hb decline, a higher baseline Hb level was extracted as an independent factor of higher Hb decline during treatment. This finding can be interpreted in relation to the more adequate RBV adherence of the patients with high baseline Hb levels. The above show the importance of TVR-based triple therapy strategy adjustment according to the *ITPA* SNPs (rs1127354). Of the patients with *ITPA* CC, 39.6% developed severe anemia during treatment. Their Hb levels after week 2 were significantly lower than those

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Table 2. Baseline and initial on-treatment risk factors for the development of severe anemia according to the *ITPA* SNPs (rs1127354) of chronic hepatitis C patients treated with telaprevir-based triple therapy.

Characteristic	*ITPA* CC, n = 227			*ITPA* CA/AA, n = 65		
	Severe anemia, n = 90	Non-severe anemia, n = 137	*p* value	Severe anemia, n = 11	Non-severe anemia, n = 54	*p* value
Age (yr)	64 (57-68)	61 (53-65)	0.0004	63 (55-68)	58 (53-65)	0.0900
Men, n (%)	29 (32.2)	74 (54.0)	0.0011	4 (36.4)	28 (51.9)	0.3462
Body mass index (kg/m^2)	22.8 (20.9-25.0)	23.4 (21.8-25.6)	0.0939	23.1 (20.3-25.4)	23.7 (21.8-26.9)	0.4305
Alanine aminotransferase (IU/L)	49 (29-95)	53 (34-94)	0.1157	56 (36-79)	45 (33-97)	0.3216
Serum albumin (g/L)	39 (36-42)	40 (38-43)	0.0083	39 (34-43)	39 (38-43)	0.1973
Estimated glomerular filtration rate (ml/min/1.73 m^2)	76 (69-91)	82 (74-95)	0.0041	74 (67-89)	84 (74-93)	0.2634
α-fetoprotein (ng/ml)	5.4 (3.3-11.0)	5.7 (3.5-12.5)	0.3010	5.6 (3.9-9.9)	5.2 (4.1-10.7)	0.2180
Hemoglobin at baseline (g/L)	132 (125-140)	141 (132-153)	<0.0001	121 (117-140)	141 (133-149)	0.0022
Hemoglobin at week 2 (g/L)	114 (110-123)	125 (117-136)	<0.0001	124 (110-143)	138 (131-144)	0.0081
Platelet count (x10^9/L)	144 (113-197)	153 (117-190)	0.9107	166 (147-222)	159 (131-187)	0.2659
Stage of fibrosis						
F0-2/F3-4, n (%)	37/25 (39.4/50.0)	57/25 (60.6/50.0)	0.2205	6/3 (26.1/18.8)	17/13 (73.9/81.3)	0.5895
Not determined	28	55		2	24	
Initial 4 week ribavirin dose (mg/kg/day)	10.0 (7.6-11.0)	9.4 (7.8-10.8)	0.8369	9.8 (7.0-11.4)	10.5 (8.6-11.4)	0.7143
Initial 4 week telaprevir dose (mg/kg/day)	29.9 (25.8-33.2)	28.0 (23.9-31.3)	0.0455	27.1 (23.4-31.2)	28.7 (22.6-33.2)	0.8818

Data are expressed as number (%) or median (first-third quartiles).
ITPA, inosine triphosphatase; SNP, single nucleotide polymorphism.
Severe anemia is defined as hemoglobin level <85 g/L.

of patients with *ITPA* CA/AA. Multivariable logistic regression analysis of pretreatment and early on-treatment predictors of development of severe anemia found lower Hb level at week 2 to be an independent factor. Thus, the degree of Hb decline in the first two weeks profoundly influenced the development of severe anemia because the Hb level reduced continuously until week 12. ROC analysis showed that the AUROC was 0.70 (p <0.0001), confirming that the Hb level at week 2 (cut-off value: 116 g/L) is a moderately effective predictive marker of severe anemia. Moreover, we showed that severe anemia is associated with the initial four-week weight-adjusted dosage of TVR but not RBV. Considering that the initial dosage of TVR had a small impact on treatment outcome as we have shown, reduction may be needed in clinical practice to prevent the development of severe anemia. Obviously, further studies are needed to clarify the association between treatment outcome and TVR adherence.

In contrast, 16.9% of the patients with *ITPA* CA/AA developed severe anemia during treatment. At week 12, the Hb level and Hb decline from baseline of patients with *ITPA* CA/AA were almost equal to those of patients with *ITPA* CC. The reason is thought to be RBV adherence; there was a significant difference between *ITPA* CC (7.4 ± 2.2 mg/kg/day) and CA/AA groups (9.1 ± 2.4 mg/kg/day) (p <0.0001) in the initial 12-week dosage of RBV, but no difference in TVR. Although no close relationship was found between age, sex, BMI, or treatment adherence and the odds of subsequent severe anemia onset, only a lower Hb level was found to influence severe anemia. Moreover, ROC analysis showed an AUROC of 0.75, confirming that the baseline Hb level (cut-off value: 122 g/L) is a moderately effective predictive marker of severe anemia for patients with *ITPA* CA/AA. According to these findings, a treatment strategy tailored to the patient *ITPA* polymorphism would help individualize the clinical management of TVR-based triple therapy.

Several *ITPA* gene mutations have been described that are related to *ITPA* deficiency [23,24], which leads to an accumulation of inosine triphosphatase in red blood cells and increased toxicity of purine analogue drugs [25,26], although the exact mechanisms through which TVR in combination with PegIFNα2b and RBV causes severe anemia remains obscure. Two functionally deficient variants (rs1127354 and rs7270101) of *ITPA* were recently found to protect against anemia. However, the splicing variant SNP rs7270101 is monoallelic in the Japanese population [12], thus, only the *ITPA* variant rs1127354 was tested in this study.

A limitation of this study is that it consists of only Japanese participants. As is characteristic of Japanese chronic hepatitis C patients, almost all are infected with HCV genotype 1b (patients infected with genotype 1a are very rare), the rates of obesity and patients under 60 years are much lower than those found in other ethnic groups, and the overwhelming majority of our patients had the *ITPA* CC genotype. Further studies of other ethnic groups and patients with the *ITPA* CA and AA genotypes are needed to clarify the findings. Nevertheless, we provided significant information because the study included patients of older age who are considered to be at high risk of development of anemia. Second, the patients received only 24 weeks of total therapy (TVR-based triple therapy of 12 weeks followed by only 12 weeks of dual-therapy), which is not the same as the response-guided therapy that is standard elsewhere, but it is the approved regimen of the Japanese Ministry of Health, Labor, and Welfare. However, almost all patients who developed severe anemia (98.0%, 99 of 101) first experienced it during the initial 12 weeks of treatment. Therefore, the frequency of severe anemia was little affected by the treatment duration. Third, Japanese patients received lower dose RBV than is standard elsewhere, and TVR dose reduction due to adverse effects has not been reported previously. In fact, currently there is insufficient evidence to allow

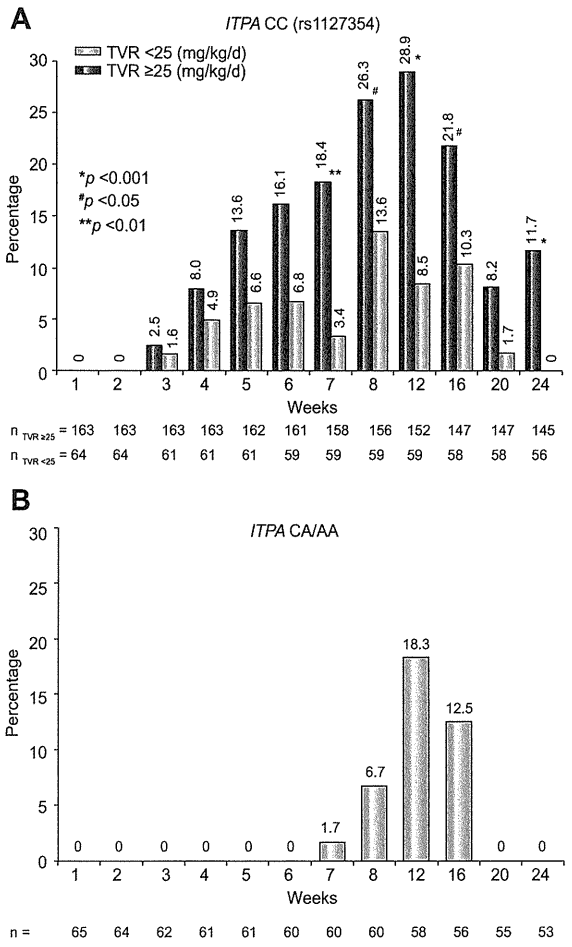

Fig. 4. The weekly percentages of patients who experienced on-treatment severe anemia stratified by *ITPA* SNPs. (A) The percentage of *ITPA* CC patients experiencing on-treatment severe anemia stratified by the initial four weeks of telaprevir (TVR) (≥25 or <25 mg/kg/day). (B) The percentage of *ITPA* CA/AA patients who experienced on-treatment severe anemia.

for the development of criteria for TVR dose reduction. Last, the number of patients with *ITPA* CA or AA was relatively small, therefore, the findings for this group are not conclusive.

In conclusion, chronic hepatitis C patients treated with TVR in combination with PegIFNα2b and RBV are at high risk of developing severe anemia, therefore, an intense monitoring program for all patients should be followed. Our finding that *ITPA* polymorphism (rs1127354) is effective for the prediction of the development of severe anemia and will be helpful in the management of patients undergoing TVR-based triple therapy.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Telaprevir can be successfully and safely used to treat older patients with genotype 1b chronic hepatitis C

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Background & Aims: This study was performed to evaluate the efficacy of a triple therapy in older Japanese patients; telaprevir (TVR) was added to pegylated interferon α2b and ribavirin.

Methods: This prospective study enrolled 120 genotype 1b patients with chronic hepatitis C who received 12 weeks of triple therapy followed by a 12-week dual therapy that included pegylated interferon α2b and ribavirin. Patients were categorized according to age: group A, 64 patients aged >60 and group B, 56 patients aged ⩽60. Serum HCV RNA levels were monitored by COBAS TaqMan HCV test.

Results: The rates of undetectable HCV RNA at week 4 (rapid virological response, RVR) were 73.4% in group A and 73.2% in group B. No significant difference in sustained virological response (SVR) was found between groups A (76.6%) and B (83.9%) ($p = 0.314$). The SVR rates for patients with interleukin 28B (*IL28B*) (rs8099917) TT allele (89.4% and 91.9% for groups A and B) were significantly higher than for those with the *IL28B* TG/GG allele (41.2% and 68.4%, respectively) (both $p < 0.05$). Multivariate analysis extracted *IL28B* TT and RVR as independent factors associated with SVR. Adverse effects resulted in treatment discontinuation by 12.5% in each group. Hemoglobin decrease significantly differed between groups A and B: the decrease to ⩾100 g/L, to 85 – <100 g/L, and to <85 g/L, was 9.4%, 40.6%, and 50% in group A patients, respectively, and 41.1%, 25%, and 33.9% in group B patients, respectively ($p = 0.0006$).

Conclusions: TVR-based triple therapy can be successfully used to treat older patients with genotype 1b chronic hepatitis C.

Introduction

Chronic hepatitis C virus (HCV) infection affects approximately 170 million people worldwide and often causes cirrhosis and hepatocellular carcinoma (HCC) [1,2]. The ultimate goal of treatment for chronic hepatitis C is achieving sustained virological response (SVR), defined as undetectable HCV RNA in serum 6 months after the termination of treatment. A 48-week combination of pegylated interferon α (PegIFN-α) plus ribavirin (RBV) is successful for only about 45% of chronic hepatitis C patients infected with HCV genotype 1. Patients for whom treatment is unsuccessful are termed difficult-to-treat [3–6].

In Japan, the majority of patients are infected with HCV genotype 1 and they are older than patients in the United States and Europe. Such older patients have poor virological response to antiviral treatment [7–9], especially postmenopausal women [6]. Several studies have shown low SVR rates for older patients who received dual therapy with PegIFN-α and RBV [7,9,10]. It has been hypothesized that older patients have a decreased blood count and reduced cardiovascular, pulmonary, and kidney func-

Keywords: Chronic hepatitis C; Older patients; Telaprevir; Pegylated interferon α; Ribavirin.

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Abbreviations: TVR, telaprevir; RVR, rapid virological response; SVR, sustained virological response; IL28B, interleukin 28B; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; PegIFN-α, pegylated interferon α; RBV, ribavirin; NS3/4A, non-structural 3/4A; DAA, direct-acting antiviral agent; KULDS, The Kyushu University Liver Disease Study; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGTP, γ-glutamyl-transpeptidase; eGFR, estimated glomerular filtration rate; PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism; ITPA, inosine triphosphate pyrophosphatase; cEVR, complete early virological response; EOT, end-of-treatment response.

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tion, and thus are less resistant to the influence induced by treatment. This also leads to more adverse events and poorer drug adherence. Treatment strategies tailored to the needs of these difficult-to-treat chronic hepatitis C patients are necessary.

In the United States, Canada, the European Union, and Japan, telaprevir (TVR), an HCV non-structural 3/4A (NS3/4A) protease inhibitor, has recently been approved for the treatment of chronic hepatitis C genotype 1 and represents a new class of small molecules that are direct-acting antiviral agents (DAA) for reflecting HCV replication [11–14]. TVR-based triple therapy, combined with PegIFN-α and RBV, has resulted in an improved SVR rate, when compared to PegIFN-α monotherapy and PegIFN-α plus RBV dual therapy [15–19].

Earlier studies of TVR-based regimens for chronic hepatitis C have not shown any correlation between age and virological outcome. Furthermore, there are no data regarding differences in factors predictive of SVR by older and younger patients. For this reason, we conducted a prospective, multicenter study to investigate the efficacy and safety of TVR-based triple therapy for older patients with chronic hepatitis C.

Patients and methods

Patients

Since 2004, the Kyushu University Liver Disease Study (KULDS) Group has conducted a prospective, multicenter study to investigate the efficacy and safety of antiviral treatment in chronic hepatitis C patients [1,4,9]. Our more recent research, performed between December 2011 and November 2012, included 406 Japanese patients with HCV genotype 1b aged ⩾20 years, who received TVR-based triple therapy. The current study was restricted to the 120 patients (age range 25–73 years) for whom data is currently available for their 24-week post-therapy follow-up. The older group (group A) consists of 64 patients aged >60 years and the younger group (group B) of 56 patients aged ⩽60 years.

According to previous treatment response, relapse was defined as undetectable HCV RNA during and at the end of previous treatment, with HCV RNA positivity representing later on. Non-response was defined as detectable HCV RNA for more than 24 weeks. The study included 27 patients (22.5%) who were treatment naïve, 64 (53.3%) with prior relapse, 25 (20.8%) with prior non-response, and 4 (3.3%) with unknown response.

Exclusion criteria were: positivity for antibody to human immunodeficiency virus or positivity for hepatitis B surface antigen; clinical or biochemical evidence of hepatic decompensation (ascites, bleeding varices, or encephalopathy); other causes of liver disease; excessive active alcohol consumption (>40 g/day of ethanol) or drug abuse; suspected HCC or active cancer at entry; chronic renal failure or creatinine clearance of ⩽50 ml/min; very poorly controlled heart diseases, pulmonary disorders, diabetes, or thyroid diseases; depression, or its history, history of suicide attempt; pregnancy in progress or planned during the study period of either partner; or treatment with antiviral or immunosuppressive agents prior to enrollment. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of our hospital. Informed consent was obtained from all patients before enrollment. The study was registered as a clinical trial on the University Hospital Medical Information Network (ID 000009711).

Clinical assessment

Clinical parameters included serum albumin, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl-transpeptidase (γGTP), estimated glomerular filtration rate (eGFR), whole blood cell count, and HCV RNA. Blood samples were taken for all patients at baseline, days 3 and 7, every week thereafter to week 24, and at the end of follow-up. Liver biopsy at entry for 76 (63.3%) of the 120 patients was done by two or more experienced hepatologists. For each specimen, the stage of fibrosis (F0-4) and grade of activity (A0-3) were established according to Metavir score [20].

Determination of HCV markers

The baseline and follow-up tests for HCV viremia were done by real-time polymerase chain reaction (PCR) assay (COBAS TaqMan HCV test v2.0, Roche Diagnostics, Tokyo, Japan), with a detectability of ⩾15 IU/ml and the linear dynamic range of 1.2–7.8 log IU/ml. HCV genotype and the core amino acid substitution at position 70 of the HCV genome were determined before treatment for all patients. HCV genotype was determined by sequence determination in the 5′ non-structural region of the HCV genome followed by phylogenetic analysis [21]. Amino acid substitution at position 70 of the core region was analyzed by direct sequencing, as reported previously [22].

Interleukin 28B and inosine triphosphate pyrophosphatase

Human genomic DNA was extracted from peripheral blood. Genotyping by the single-nucleotide polymorphism (SNP) of the interleukin 28B (*IL28B*) (rs8099917) gene was done using the TaqMan Allelic Discrimination Demonstration Kit (7500 Real-Time PCR System; Applied Biosystems, Foster City, CA) [21]. Patients were genotyped as TT, TG, or GG at the polymorphic site. Similarly, genotyping by the SNP of the inosine triphosphate pyrophosphatase (*ITPA*) (rs1127354) gene was done using the TaqMan Allelic Discrimination Demonstration Kit. Patients were genotyped as CC, CA, or AA at the polymorphic site [23].

Therapeutic protocol

All patients received 12-week triple therapy that included TVR (2250 mg/day) (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan), PegIFN-α-2b (60–150 μg/week) (PEG-Intron; MSD, Tokyo, Japan) and RBV (600–1000 mg/day) (Rebetol; MSD) followed by a 12-week dual therapy that included PegIFN-α-2b and RBV. TVR 750 mg was administered orally three times a day at an 8-h interval after each meal. PegIFN-α-2b was injected subcutaneously once weekly at a dose of 1.5 μg/kg. RBV was given orally at a daily dose of 600–1000 mg based on body weight (600 mg for patients weighing <60 kg, 800 mg for those weighing 60–80 kg, and 1000 mg for those weighing >80 kg). The above durations and dosages are those approved by the Japanese Ministry of Health, Labor, and Welfare.

Efficacy of treatment

Successful treatment was SVR, defined as undetectable serum HCV RNA at 24 weeks after the end of treatment. Early virological response during the first 12 weeks of treatment was categorized as follows: rapid virological response (RVR), undetectable HCV RNA at week 4; complete early virological response (cEVR), detectable HCV RNA at week 4 but undetectable at week 12. End-of-treatment response (EOT) was defined as undetectable HCV RNA at the end of treatment. Relapse was defined as an EOT response but non-SVR.

Therapeutic dosage assessments

The dosages of the drugs used in triple therapy mainly depended on the possibility of adverse effects: anemia, leukopenia, thrombocytopenia, malaise, and anorexia. They were accomplished by reviewing the medical records and by counting the remaining pills of each patient to determine the total dosage actually administered over the treatment period. The dosages of TVR during three different periods (within the first 4 weeks, from week 5 to week 12, and within the first 12 weeks) were calculated individually as the percentage of TVR 2250 mg/day. The dosages of PegIFN-α-2b and RBV were calculated individually as averages on the basis of body weight at baseline. The percentages of the assigned total cumulative PegIFN-α-2b and RBV dosages were calculated individually.

Safety assessments

Adverse events and hematological and chemical laboratory data were assessed up to the last visit for each patient. We mainly monitored for rash, serious skin reactions, and anemia. Also, newly occurred and deteriorated comorbidities were assessed during the study period according to the Charlson comorbidity index [24].

Statistical analysis

Statistical analyses were performed using the SAS system, version 9.1.3 (SAS Institute, Cary, NC). Continuous data of patients are expressed as median with interquartile range. Univariate analyses were performed using the Chi-square, Fisher's exact test, Mann-Whitney *U* test, Cochran-Armitage test, or Bonferroni's correction, as appropriate, with SVR as the outcome. To identify independent factors for predicting an SVR, variables that reached the $p < 0.1$ level in univariate tests were used as candidate factors for multiple logistic regression analysis. The model was reduced using AIC-based forward and/or backward stepwise selection with bootstrap validation. A p value less than 0.05 was regarded as statistically significant in all analyses.

Results

Patient characteristics

The patient characteristics are summarized by age in Table 1. Analysis of the pretreatment factors found male percentage, white blood cell count, hemoglobin, platelet count, serum albumin, γGTP, and eGFR of group A to be significantly lower than those of group B (all $p < 0.05$). No significant difference in liver histology, prior treatment response, or *IL28B* or *ITPA* genotype was found between groups A and B. Analysis of treatment factors found the percentage of patients who were able to tolerate the assigned total cumulative PegIFN-α-2b dosage ⩾80% and RBV dosage ⩾80% to be significantly lower in group A than in group B ($p = 0.023$). The percentage of patients who were able to tolerate assigned total cumulative RBV dosage ⩾80% showed a similar difference ($p = 0.014$), as would be expected by the lower hemoglobin level at baseline.

Virological outcome and response

Table 2 shows the virological responses by age. RVR, cEVR, EOT, and SVR did not significantly differ between groups A and B patients (73.4% *vs.* 73.2%, 18.8% *vs.* 19.6%, 87.5% *vs.* 89.3, and 76.6% *vs.* 83.9%, respectively). Relapse was more frequently seen in group A (12.5%) compared with group B (6.0%), but with no significant difference ($p = 0.253$). Patients with RVR had a significantly higher SVR rate (89.4%, 42 of 47 in group A: 97.6%, 40 of 41 in group B) than patients with cEVR (41.7%, 5 of 12 in group A: 54.5%, 6 of 11 in group B) ($p = 0.0002$ for group A and $p < 0.0001$ for group B).

Fig. 1 shows differences in serum HCV RNA level at treatment day 3 by age and virological response. The median serum HCV RNA level at day 3 was significantly lower for RVR patients (2.6 log IU/ml for group A patients and 2.4 log IU/ml for group B patients) than for non-RVR patients (3.3 log IU/ml and 3.2 log IU/ml, respectively) ($p < 0.0001$). A significant difference in the level at day 3 was also found between SVR (2.7 log IU/ml and 2.6 log IU/ml, respectively) and non-SVR (3.0 log IU/ml and 3.2 log IU/ml, respectively) patients. No significant differences in the level at day 3 were found between group A and B patients.

Demographic and clinical features of patients by age and SVR

Table 3 shows the differences of patient demographic and clinical features by age and SVR. The SVR rates of the patients with *IL28B* (rs8099917) TT (42 of 47, 89.4% and 34 of 37, 91.9% for group A and B patients) were significantly higher than for *IL28B* TG/GG (7 of 17, 41.2% and 13 of 19, 68.4%, respectively) ($p < 0.0001$ and $p = 0.023$). The SVR rates of the treatment naïve patients and those who had prior relapse (41 of 47, 87.2% and 39 of 44, 88.6% for group A and B patients) were higher than those of the patients who had prior non-response (5 of 14, 35.7% and 8 of 11, 72.7%, respectively), significantly different in group A ($p < 0.0001$), but with no significant difference in group B ($p = 0.180$).

We assessed the relationship between SVR and liver fibrosis only in the 76 patients who received liver biopsy at entry (Table 3). In group A, the more fibrosis progressed the more the SVR rate decreased significantly (100%, 100%, 83.3%, 100%, and 50% for the patients with F0, F1, F2, F3, and F4, respectively, $p = 0.007$). A similar trend was observed in group B (100%, 94.1%, 80%, 57.1%, and 66.7%, respectively), but with no significant difference ($p = 0.067$).

Of the 120 patients, 12 (10.0%) had to discontinue TVR due to adverse effects (n = 10), dropout (n = 1), and no virological effect (n = 1) (5, 4, and 3 patients at weeks 4, 8, and 11, respectively). Discontinuation of PegIFN-α-2b, RBV, and TVR significantly influenced an achievement of SVR in both groups (all $p < 0.0001$) (Table 3). The ability to tolerate the assigned total cumulative PegIFN-α-2b dosage ⩾80% was significantly related to the achievement of SVR only in group A patients ($p = 0.0002$). Patients in group A with SVR had a higher rate of tolerance of the assigned total cumulative RBV dosage ⩾80% than patients without SVR, but with no significant difference ($p = 0.061$).

SVR and drug adherence by age

We investigated the association between SVR and the cumulative exposure to TVR, as a percentage of the target dosage, during three different periods: within the first 4 weeks, from week 5 to week 12, and within the first 12 weeks. Within the first 4 weeks, patients with <60% of the target dosage to TVR had extremely low SVR rates (group A: 3 of 5, 60.0% and group B: 1 of 3, 33.3%), compared to those with ⩾60% of the dosage (group A: 46 of 59, 78.0% and group B: 46 of 53, 86.8%), but with no significant difference. Significant differences in SVR rates were found between patients taking <60% and ⩾60% of the target dosage to TVR in all the studied patients (4 of 8, 50% *vs.* 92 of 112, 82.1%), respectively, $p = 0.028$). No significant differences in the SVR rates were found between patients taking <60% and ⩾60% of the target dosage from week 5 to week 12 (35 of 43, 81.4% *vs.* 61 of 77, 79.2%, respectively) and within the first 12 weeks (24 of 31, 77.4% *vs.* 72 of 89, 80.9%, respectively) in all the studied patients. Classified by age, no significant differences in the rates were found among the three periods.

We investigated the association between SVR and cumulative exposures to PegIFN-α-2b and RBV for the whole 24-week treatment period, as a percentage of the target dosage of each drug. Lower exposure to PegIFN-α-2b (3 of 9, 33.3% with exposure <60%; 2 of 4, 50% with exposure 60 to <80%; and 44 of 51, 86.3% with exposure ⩾80%, $p = 0.009$) and to RBV (12 of 20, 60% with exposure <60%; 22 of 28, 78.6% with exposure 60 to <80%; and 15 of 16, 93.8% with exposure ⩾80%, $p = 0.046$) was significantly related to lower SVR rates for group A but not for group B patients.

Predictive factors correlated with an SVR

Multiple logistic regression analysis identified the only three factors that independently influenced an SVR for group A patients:

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Table 1. Patient characteristics by age.

Variables	Group A Patients aged >60 (n = 64)	Group B Patients aged ≤60 (n = 56)	*p* value*
Men, n (%)	19 (29.7)	35 (62.5)	0.0003
Age (yr)	66.0 [7.0]	53.0 [10.2]	<0.0001
Age range (yr)	61-73	25-60	
Body mass index (kg/m^2)	23.0 [3.5]	23.7 [3.8]	0.159
Baseline co-morbidities, n (%)	12 (18.8)**	7 (12.5)***	0.349
Baseline HCV RNA (log_{10} IU/ml)	6.4 [0.7]	6.6 [0.7]	0.137
HCV core amino acid substitution at position 70, wild/mutation, n	33/31	25/31	0.449
White blood cell count (×10^6/L)	4165 [1300]	4890 [2862]	0.002
Hemoglobin level (g/L)	133 [18]	144 [22]	<0.0001
Platelet count (×10^9/L)	144 [47]	159 [83]	0.003
Serum albumin (g/L)	39 [5.0]	41 [5.0]	0.001
Aspartate aminotransferase (IU/L)	52.5 [48.5]	51 [48.4]	0.283
Alanine aminotransferase (IU/L)	55 [56.7]	58.5 [65.7]	0.920
γ-glutamyl-transpeptidase (IU/L)	32 [31.5]	56 [70]	0.003
Estimated glomerular filtration rate (ml/min)	75.4 [19.6]	83.7 [20.7]	<0.0001
Liver histology			
Stage, F0-2/F3-4, n	21/21	24/10	0.069
Grade, A0-1/A2-3, n	14/28	13/21	0.657
Not determined, n	22	22	
Previous treatment response			
Treatment naïve, n (%)	12 (18.8)	15 (26.8)	0.622
Prior relapse, n (%)	35 (54.7)	29 (51.8)	
Prior non-response, n (%)	14 (21.9)	11 (19.6)	
Prior unknown response, n (%)	3 (4.7)	1 (1.8)	
IL28B SNP (rs8099917)			
TT, n (%)	47 (73.4)	37 (66.1)	0.379
TG/GG, n (%)	17 (26.6)	19 (33.9)	
ITPA SNP (rs1127354)			
CC, n (%)	51 (79.7)	41 (73.2)	0.402
CA/AA, n (%)	13 (20.3)	15 (26.8)	
Completed assigned total cumulative PegIFN-α-2b dosage ≥80% and RBV dosage ≥80%, n (%)	15 (23.4)	24 (42.9)	0.023
Completed assigned total cumulative PegIFN-α-2b dosage ≥80%, n (%)	51 (79.7)	46 (82.1)	0.733
Completed assigned total cumulative RBV dosage ≥80%, n (%)	16 (25.0)	26 (46.4)	0.014
Discontinuation of PegIFN-α-2b and RBV, n (%)	8 (12.5)	7 (12.5)	>0.999
Discontinuation of TVR, n (%)	7 (10.9)	5 (8.9)	0.714
Discontinuation of treatment, n (%)	8 (12.5)	7 (12.5)	>0.999

HCV, hepatitis C virus; *IL28B*, interleukin 28B; SNP, single-nucleotide polymorphism; *ITPA*, inosine triphosphate pyrophosphatase; PegIFN-α-2b, pegylated interferon α2b; RBV, ribavirin; TVR, telaprevir.

All patients were infected with HCV genotype 1b.

Continuous variables are expressed as median [interquartile range].

**p* Value draws a comparison between SVR and non-SVR patients.

**Group A included 12 patients with baseline comorbidities (1 with cerebrovascular disease but no paralysis, 3 with peptic ulcer disease, and 8 with diabetes mellitus), according to the Charlson comorbidity index [24] (except for liver disease).

***Group B included 7 patients with baseline comorbidities (2 with peptic ulcer disease and 5 with diabetes mellitus), according to the Charlson comorbidity index [24] (except for liver disease).

patients treatment naïve and with prior treatment relapse (odds ratio (OR) 8.403 for those with prior non-response, $p = 0.047$), *IL28B* TT genotype (OR 14.93 for non-TT genotype, $p = 0.017$) and RVR (OR 7.498 for non-RVR, $p = 0.009$) (Table 4). Similarly, multiple logistic regression analysis identified the only two factors that independently influenced an SVR for group B patients: *IL28B* TT genotype and RVR. *IL28B* TT genotype and RVR were common factors for SVR in both groups.

Adverse events and comorbidities

Adverse events were observed in all patients, most of them mild to moderate. The following serious adverse events and others resulted in discontinuation of treatment. Of the studied patients, 15 (12.5%) had to discontinue treatment. In group A, the reasons for discontinuation (n = 8) were severe anemia (n = 2), malaise (n = 2), anorexia (n = 2), deterioration of diabetes mellitus

Table 2. Treatment response of patients aged >60 and ⩽60 years.

	Rate, n (%)		
	Group A Patients aged >60 (n = 64)	Group B Patients aged ≤60 (n = 56)	*p* value[a]
SVR	49 (76.6)	47 (83.9)	0.314
RVR	47[b] (73.4)	41[c] (73.2)	0.977
cEVR (not RVR)	12[b] (18.8)	11[c] (19.6)	0.901
EOT	56 (87.5)	50 (89.3)	0.761
Relapse	7 (12.5)[d]	3 (6.0)[d]	0.253

A sustained virological response (SVR) is defined as undetectable serum HCV RNA at 24 weeks after the end of treatment. A rapid virological response (RVR) is defined as undetectable HCV RNA at week 4. A complete early virological response (cEVR) is defined as detectable HCV RNA at week 4 but undetectable HCV RNA at week 12. End-of-treatment response (EOT) is defined as undetectable HCV RNA at the end of treatment. Relapse is defined as an EOT response but non-SVR. All patients were infected with HCV genotype 1b.

[a]*p* Value draws a comparison between group A and B patients.

[b]Group A patients with RVR had a significantly higher SVR rate (42 of 47, 89.4%) than the patients with cEVR (5 of 12, 41.7%) ($p = 0.0002$).

[c]Group B patients with RVR had a significantly higher SVR rate (40 of 41, 97.6%) than patients with cEVR (6 of 11, 54.5%) ($p < 0.0001$).

[d]Relapse rate is calculated by dividing the number of patients with relapse by those with EOT.

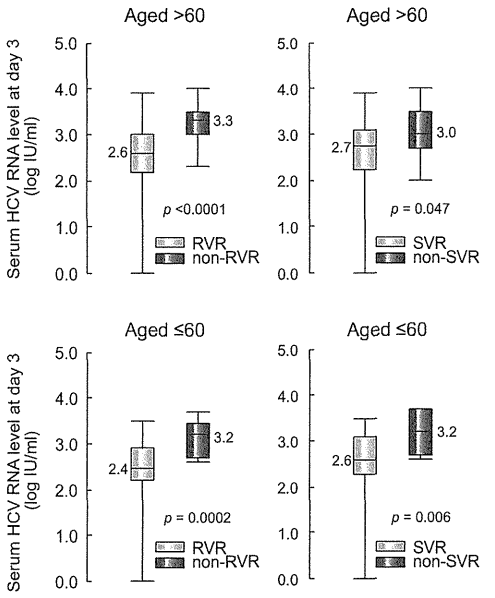

Fig. 1. Differences in serum HCV RNA levels at treatment day 3 by age and virological response. Medians are shown as horizontal bars. Boxes cover the interquartile range and tails show the minimum and maximum data. A zero of HCV RNA level indicates negativity. RVR, rapid virological response; SVR, sustained virological response.

(n = 1), and dropout (n = 1). In group B, the reasons (n = 7) were increased serum creatinine (n = 2), severe anemia (n = 1), malaise (n = 1), anorexia (1), depression (n = 1), and no virological response (n = 1). Of the 15 patients who had discontinued treatment, only one with RVR who stopped treatment at week 11 achieved an SVR, but 4 relapse and 10 had no virological response.

Drug-induced skin disorders (rashes, eruptions, and erythema) were found in 33 (51.6%) group A patients and 25 (44.6%) group B patients with no significant difference ($p = 0.449$). Severe skin disorders (grade 3), defined as skin lesions covering >50% of the body surface or rashes with bullae, ulceration of mucous membrane, epidermal detachment, target lesion or systematic signs, were found in 1 (3.0%) of the 33 subjects in group A and 4 (16%) of the 25 in group B, with no significant difference ($p = 0.081$). These patients who developed skin disorders were able to continue treatment with the prescription by topical corticosteroid or oral administration of glucocorticoids added to antihistamine therapy.

We analyzed the differences in hematological changes between the two groups. The decrease in hemoglobin significantly differed between group A and B patients: the decrease to ⩾100 g/L, to 85 to <100 g/L, and to <85 g/L, 6 (9.4%), 26 (40.6%), and 32 (50%) of group A patients, respectively, and 23 (41.1%), 14 (25%), and 19 (33.9%) of group B patients, respectively ($p = 0.0006$), as would be expected by the lower level at baseline. No significant differences in white blood cell, neutrophil, or platelet count were observed between the two groups.

Newly occurred and deteriorated comorbidities were found in two group A patients. An asymptomatic man aged 65 years was diagnosed with early gastric cancer at week 12 when a workup was done due to severe anemia. He was treated by endoscopic submucosal dissection of the lesion, completed the triple therapy and achieved an SVR. A symptomatic man aged 62 years was found to have deteriorated diabetes at week 2 and stopped triple therapy. No other comorbidities were found among the other patients.

Discussion

This study shows that there is no impact by age on the virological outcome of TVR-based triple therapy for HCV genotype 1b chronic hepatitis C. Our findings showed significant correlations between virological outcome and early virological response: SVR, RVR, and extremely rapid virological response at day 3 of the treatment for both older and younger patients. Moreover, *IL28B* genotype and prior treatment response are significant independent pretreatment factors for the achievement of SVR by older patients, but prior treatment response does not influence the achievement of SVR by younger patients. To date, no studies have assessed differences in the predictive factors for SVR between older and younger patients who underwent TVR-based triple therapy.

Research Article

Table 3. Demographic and clinical features of chronic hepatitis C genotype 1b patients who received telaprevir, PegIFN-α-2b, and ribavirin triple therapy, classified by age and sustained virological response or non-sustained response.

Characteristics	Group A; patients aged >60 (n = 64)			Group B; patients aged ≤60 (n = 56)		
	SVR (n = 49)	Non-SVR (n = 15)	*p* value*	SVR (n = 47)	Non-SVR (n = 9)	*p* value*
Men, n (%)	17 (34.7)	2 (13.3)	0.113	29 (61.7)	6 (66.7)	0.778
Average age (yr)	66 [7.0]	69 [7.0]	0.094	54 [12]	52 [5.5]	0.814
Body mass index (kg/m²)	22.6 [3.1]	23.9 [5.2]	0.130	23.5 [3.9]	24.8 [4.0]	0.058
Baseline HCV RNA (log_{10} IU/ml)	6.4 [0.6]	6.3 [0.8]	0.691	6.6 [0.9]	6.4 [0.5]	0.433
HCV core amino acid substitution at position 70, wild/mutation, n	29/20	4/11	0.027	21/26	4/5	0.989
White blood cell count (×10⁶/L)	4210 [1355]	4090 [580]	0.409	5100 [2600]	3700 [2715]	0.224
Hemoglobin level (g/L)	134 [17]	132 [22]	0.261	144 [22]	141 [33]	0.911
Platelet count (×10⁹/L)	152 [53]	115 [63]	0.002	171 [81]	142 [68]	0.064
Serum albumin (g/L)	39 [5.0]	39 [8.0]	0.430	41 [5.0]	39 [8.0]	0.072
Aspartate aminotransferase (IU/L)	48 [43.5]	70 [70]	0.007	46 [34]	70 [31.5]	0.031
Alanine aminotransferase (IU/L)	53 [65.5]	72 [74]	0.026	53 [68]	64 [41]	0.255
γ-glutamyl-transpeptidase (IU/L)	26 [24]	53 [62]	0.0004	45 [60]	103 [63.2]	0.023
Estimated glomerular filtration rate (ml/min)	75.3 [17.7]	74.4 [15.9]	0.686	83.7 [20.2]	83.1 [20.5]	0.561
Liver histology						
Stage, F0-2/F3-4, n	20/14	1/7	0.018	22/6	2/4	0.027
Stage F0, n	5	0	0.007	2	0	0.067
Stage F1, n	10	0		16	1	
Stage F2, n	5	1		4	1	
Stage F3, n	7	0		4	3	
Stage F4, n	7	7		2	1	
Grade, A0-1/A2-3, n	15/19	0/8	0.019	18/10	1/5	0.033
Not determined, n	15	7		19	3	
Previous treatment outcome						
Treatment naïve, n	12	0	0.084	13	2	0.081
Prior relapse, n	29	6		26	3	
Prior non-response, n	5	9		8	3	
Prior unknown response, n	3	0		0	1	
IL28B SNP (rs8099917)						
TT, n	42	5	<0.0001	34	3	0.023
TG/GG, n	7	10		13	6	
ITPA SNP (rs1127354)						
CC, n	39	12	0.972	35	6	0.628
CA/AA, n	10	3		12	3	
Completed assigned total cumulative PegIFN-α-2b dosage ≥80% and RBV dosage ≥80%, n (%)	14 (28.6)	1 (6.7)	0.079	19 (40.4)	5 (55.6)	0.400
Completed assigned total cumulative PegIFN-α-2b dosage ≥80%, n (%)	44 (89.8)	7 (46.7)	0.0002	40 (85.1)	6 (66.7)	0.185
Completed assigned total cumulative RBV dosage ≥80%, n (%)	15 (30.6)	1 (6.7)	0.061	20 (42.6)	6 (66.7)	0.184
Discontinuation of PegIFN-α-2b and RBV, n (%)	1 (2.0)	7 (46.7)	<0.0001	1 (2.1)	6 (66.7)	<0.0001
Discontinuation of TVR, n (%)	1 (2.0)	6 (40.0)	<0.0001	1 (2.1)	4 (44.4)	<0.0001
Discontinuation of treatment, n (%)	1 (2.0)	7 (46.7)	<0.0001	0 -	7 (77.8)	<0.0001
RVR, n (%)	42 (85.7)	5 (33.3)	<0.0001	40 (85.1)	1 (11.1)	<0.0001

SVR, sustained virological response; HCV, hepatitis C virus; *IL28B*, interleukin 28B; SNP, single-nucleotide polymorphism; *ITPA*, inosine triphosphate pyrophosphatase; PegIFN-α-2b, pegylated interferon α2b; RBV, ribavirin; TVR, telaprevir; RVR, rapid virological response.
All patients were infected with HCV genotype 1b.
Continuous variables are expressed as median [interquartile range].
*p Value draws a comparison between the SVR and non-SVR patients of each group.

We found that the median serum HCV RNA level at day 3 was significantly lower for RVR patients than for non-RVR patients in both older and younger patients. RVR patients have a high potential for achieving an SVR. After a single dose of TVR, the mean half-life is approximately 4 hours. At steady state, the effective half-life is approximately 9–11 hours. When TVR is administered at 750 mg every 8 hours, a steady state is reached from 3 to 7 days after the start of administration [11–14]. For these reasons, we investigated the correlation between the HCV RNA level at day 3 and virological response. We also showed that lower exposure (accumulated dosage <60%) to TVR within the first 4 weeks of treatment leads to a lower SVR rate; however, other accumulated dosages were not related to SVR. The quick steady state of TVR is presumed to be related to a rapid decline of the HCV RNA level, which leads to RVR and SVR, irrespective of age.

Table 4. Predictive factors associated with sustained virological response by chronic hepatitis C genotype 1b patients who received telaprevir, PegIFN-α-2b and ribavirin triple therapy, classified by age.

	Group A: Patients aged >60 (n = 64)					Group B: Patients aged ≤60 (n = 56)				
	Simple		Multiple			Simple		Multiple		
	Odds ratio	*p* value	Odds ratio	95% CI	*p* value	Odds ratio	*p* value	Odds ratio	95% CI	*p* value
Age (per 1 yr)	0.876	0.101				0.975	0.609			
Sex (male to female)	3.448	0.129				0.805	0.778			
Body mass index (per 1 kg/m^2)	0.822	0.062				0.823	0.093			
γ-glutamyl-transpeptidase (per 1 IU/L)	0.971	0.003				0.998	0.709			
White blood cell count (per 1×10^6/L)	1.348	0.093				1.000	0.242			
Hemoglobin level (per 1 g/L)	1.364	0.187				1.043	0.876			
Platelet count (per 1 × 10^9/L)	1.316	0.004				1.173	0.076			
Histological stage (F3-4 to F0-2)	0.700	0.049				0.136	0.027			
Previous treatment response (treatment naïve and prior relapse to prior non-response)	12.34	0.0004	8.403	1.025-66.67	0.047	2.437	0.259			
IL28B SNP (rs8099917) (TT to TG/GG)	107.5	0.0006	14.93	1.600-142.9	0.017	4.231	0.005	25.34	1.848-1409	0.042
RVR	27.78	0.0003	7.498	1.014-65.42	0.009	45.71	<0.0001	78.35	6.618-4418	0.005

IL28B, interleukin 28B; SNP, single-nucleotide polymorphism; RVR, rapid virological response; PegIFN-α-2b, pegylated interferon α2b.

The *IL28B* gene-related SNP on chromosome 19 is the most important baseline predictor of SVR in the treatment of chronic hepatitis C patients with PegIFN-α plus RBV [6,17,18]. This study found the *IL28B* genotype to be a significant, independent pre-treatment factor for achievement of SVR in the TVR-based regimen. Several classes of DAA are under development, and it is expected that, when used in combination with PegIFN-α plus RBV, these new drugs will improve SVR and decrease the required duration of treatment [25]. The *IL28B* genotype has an influence on early viral kinetics, even during treatment with interferon-free DAA regimens. Interferon-free regimens produce no difference within 6 days after the start of treatment, but a significant difference in HCV RNA reduction after day 7 between patients with *IL28B* SNP (rs12979860) CC *vs.* the non-CC genotypes [26]. The host *IL28B* genotype has also been shown to affect the spontaneous clearance of HCV infection [27]. Thus, *IL28B* genotyping will continue to play an important role in determining the likelihood of anti-HCV treatment response.

Older patients have been reported to have a greater frequency of treatment discontinuation or to require a reduction in drug dosage during anti-HCV treatment due to laboratory abnormalities and adverse effects [9,10]. The efficacy and safety of PegIFN-α plus RBV treatment have been documented in a large-scale KULDS research of 1,251 Japanese patients [9]. The findings for HCV genotype 1 have shown that the discontinuation rates due to complications and adverse effects of older patients were almost twice those of younger patients (42.9% *vs.* 24.4% for the 48-week treatment course, respectively). The present study showed no difference in the SVR and discontinuation rates of older and younger patients. This may be attributed to the fact that the TVR-based regimen is completed in a short period, 24 weeks, and that it results in a higher RVR rate. Moreover, this study did not include the principle of response-guided therapy, but our physicians explained the patients' virological response during the treatment, thus most of the patients knew they were responding and were motivated to continue to receive the treatment.

Surprisingly, our older patient group included a higher female percentage than the younger group, however, no significant differences in virological responses were found. Post-menopause is one of the most predictive factors for failure to achieve an SVR, which links SVR to estrogen secretion [6]. Despite the good virological response in the older patients to TVR-based treatment, a higher relapse rate was found in the older patients than the younger, but the difference was not significant. Moreover, lower cumulative dosages of PegIFN-α and RBV resulted in lower SVR rates only in the older patients. Our study supports the better drug adherence to improve the chance of SVR for older patients with non-RVR and EOT.

The main adverse effects monitored in this study were rash, serious skin reactions, and anemia. Treatment discontinuation rates for these conditions were compared between older and younger patients. No significant differences in drug-induced skin disorders were found. There was a significant difference in the occurrence of severe anemia between the two groups. As expected, the older patients had higher rates of severe anemia than the younger patients, probably due to significantly lower blood counts at baseline of the older patients than the younger patients. Moreover, one man of the older group developed comorbid early gastric cancer and another had deteriorating diabetes. Care must be taken to protect against the occurrence of such disorders in older patients undergoing triple therapy.

The study has a number of limitations. First, the sample size might provide inadequate statistical power to detect definitive differences between the SVR and non-SVR data of both age groups. However, as far as we know, ours is the first study of a TVR-based regimen to show a significant clinical impact by age. Second, we studied only Japanese patients with HCV genotype 1b. Significant differences in virological response and drug mutations between HCV genotypes 1a and 1b have been reported in DAA-based treatment [28]. Among Japanese, the favorable *IL28B* genotype is found in the majority of the population (about 75%) [6,21,22]. Hence, our results may not be able to be extended to patients with other HCV genotypes or other racial cohorts. Third, our patients received a 24-week triple therapy. TVR has been approved since September 2011 for use in Japan only for the treatment of genotype 1 chronic hepatitis C with a high HCV RNA level (≥5.0 log IU/ml). Also, the duration of triple therapy is 24 weeks, with all three agents for the first 12 weeks, then PegIFN-αb plus RBV dual therapy for the remaining 12 weeks [14]. However, in Europe, the USA, and Canada, TVR must be administered with PegIFN-α and RBV for all patients for 12 weeks, followed by a response-guided regimen of either 12 or 36

Research Article

additional weeks of PegIFN-α and RBV, depending on early viral response of treatment naïve and prior relapse or prior partial and null response [11–13]. Our results show that patients with RVR can achieve high rates of SVR with 24 weeks of TVR-based triple therapy, irrespective of age. The fact that RVR is such a strong predictor for achieving an SVR may partly be explained by the fact that a 48-week course TVR-triple therapy is not necessary for patients with RVR [29]. Forth, our older patients had no severe baseline comorbidities such as heart, pulmonary, renal, and hematological diseases, namely under favorable baseline conditions, so that the drawn conclusion for the safety by TVR-based triple therapy may be limited. However, our findings that there were no serious newly occurred and deteriorated comorbidities are important. We believe the safety of this therapy is probably due to the shorter period.

In conclusion, in this prospective, multicenter study of HCV genotype 1b chronic hepatitis C patients, we found that older patients achieve a better virological outcome by TVR-based triple therapy than with the traditional dual therapy. *IL28B* genotyping and early virological response indicate the potential to achieve an SVR in these difficult-to-treat older patients.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Research Article

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Efficacy of pegylated interferon alpha-2b and ribavirin treatment on the risk of hepatocellular carcinoma in patients with chronic hepatitis C: A prospective, multicenter study☆

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Background & Aims: The effects of pegylated interferon (PegIFN) α and ribavirin (RBV) treatment of chronic hepatitis C on the incidence of hepatocellular carcinoma (HCC) have not been well established. This study investigated the impact of treatment outcome on the development of HCC by chronic hepatitis C patients treated with PegIFNα2b and RBV.

Methods: This large-scale, prospective, multicenter study consisted of 1013 Japanese chronic hepatitis C patients with no history of HCC (non-cirrhosis, n = 863 and cirrhosis, n = 150). All patients were treated with PegIFNα2b and RBV and the follow-up period started at the end of the antiviral treatment (median observation period of 3.6 years). The cumulative incidence rate of HCC was estimated using the Kaplan–Meier method, according to treatment outcome.

Results: Forty-seven patients (4.6%) developed HCC during the observation period. In the non-cirrhosis group, the 5-year cumulative incidence rates of HCC for the sustained virological response (SVR) (1.7%) and transient virological response (3.2%) (TVR: defined as relapse or breakthrough) groups were significantly lower than those of the non-virological response (NVR) group (7.6%) ($p = 0.003$ and $p = 0.03$, respectively). A significantly low rate of incidence of HCC by TVR patients in comparison with NVR patients was found for patients aged 60 years and over, but not for those under 60 years of age. In the cirrhosis group, the 5-year cumulative incidence rates of HCC for the SVR (18.9%) and TVR groups (20.8%) were also significantly lower than those of the NVR group (39.4%) ($p = 0.03$ and $p = 0.04$, respectively).

Conclusions: SVR and complete viral suppression during treatment with relapse (TVR) were associated with a lower risk of HCC development when compared with NVR.

Keywords: Hepatitis C; Pegylated interferon; Ribavirin; Hepatocellular carcinoma.

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Abbreviations: HCV, hepatitis C virus; HCC, hepatocellular carcinoma; SVR, sustained virological response; IFN, interferon; PegIFN, pegylated interferon; RBV, ribavirin; NVR, non-virological response; TVR, transient virological response; KULDS, Kyushu University Liver Disease Study; AFP, α-fetoprotein; HIV, human immunodeficiency virus; EASL, European Association for the Study of the Liver; ALT, alanine aminotransferase; HbA1c, hemoglobin A1c; EPV, events per predictor variable; HR, hazard ratio; CI, confidence interval; DAAs, direct acting antivirals.

Introduction

Hepatitis C virus (HCV) is a major human pathogen responsible for chronic hepatitis, which often progresses to cirrhosis and hepatocellular carcinoma (HCC) [1–3]. While recent advances in HCV have led to a markedly improved treatment, HCC is at present the sixth most common cancer and the third cause of cancer death worldwide [4]; moreover, its incidence is increasing due to HCV infection [5].

Previous studies have reported that patients who achieved a sustained virological response (SVR) after interferon (IFN) monotherapy demonstrated improvement in liver fibrosis and a

reduction in the incidence of decompensated liver disease and HCC compared with non-SVR patients [6–9]. In the past 10 years, a combination of pegylated IFN (PegIFN) α and ribavirin (RBV) has become the standard treatment and has resulted in an increased SVR rate [10–12]. Therefore, whether or not PegIFNα and RBV treatment is effective in preventing HCC is important, but its effect on the incidence of HCC has not been adequately studied, particularly in a large prospective study.

A recent prospective study from the United States reported that the cumulative incidence rate of HCC in an SVR group was significantly lower than in a non-virological response (NVR) group. It was also lower in a transient virological response (TVR) group than in an NVR group, although the difference did not reach statistical significance [13]. The number of aging chronic hepatitis C patients has been increasing in Japan, earlier than in other countries [14], thus investigation into the development of HCC by Japanese chronic hepatitis C patients treated with PegIFNα and RBV is highly important. Furthermore, the risk factors for the development of HCC by patients who achieve an SVR after treatment with PegIFNα and RBV have not been adequately clarified in a prospective study, although a recent report suggested that SVR reduced the risk of all-cause mortality in patients treated with PegIFNα and RBV [15]. Clarification of the demographic and clinical factors associated with HCC development, such as advanced age, lower albumin, lower platelet count and higher α-fetoprotein (AFP) level, is important.

The aim of this large-scale, multicenter, prospective study was to evaluate the relationships among pretreatment clinical factors, virological response, and development of HCC by chronic hepatitis C patients with no history of HCC, who were treated with PegIFNα2b and RBV.

Cancer

Patients and methods

Patients

The Kyushu University Liver Disease Study (KULDS) Group consists of the Kyushu University Hospital and affiliated hospitals in the Northern Kyushu area of Japan. We conducted a prospective study to investigate the efficacy and safety of PegIFNα2b and RBV for chronic hepatitis C patients. The design of the KULDS project has been described previously [12,16,17]. This prospective study consisted of 1013 Japanese patients with chronic HCV infection aged 18 years or older, treated with PegIFNα2b and RBV between December 2004 and November 2009.

The exclusion criteria were: (1) history of HCC; (2) HCC development during antiviral treatment; (3) previous PegIFNα and RBV treatment; (4) positivity for antibody to human immunodeficiency virus (HIV) or positivity for hepatitis B surface antigen; (5) clinical or biochemical evidence of hepatic decompensation at entry; (6) excessive active alcohol consumption (a daily intake of more than 40 g of ethanol) or drug abuse; (7) other forms of liver disease (e.g., autoimmune hepatitis, alcoholic liver disease, hemochromatosis); or (8) treatment with antiviral or immunosuppressive agents prior to enrollment.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of each participating hospital. Informed consent was obtained from all patients before enrollment.

Antiviral treatment and patient follow-up

All HCV genotype 1 patients received a combination treatment of PegIFNα2b (PEG-Intron; MSD, Tokyo, Japan) and RBV (Rebetol; MSD) for 48 weeks: the same regimen was prescribed for 24 weeks for genotype 2 patients. In order to investigate the incidence of HCC after treatment, the length of the follow-up period was calculated from the end of antiviral treatment to the diagnosis of HCC or last follow-up visit. Serum AFP and abdominal imaging (ultrasonographic examination, or computed tomography) were performed every 3–6 months, for each patient. The HCC diagnosis was based on histology or non-invasive criteria according to the guidelines of the European Association for the Study of the Liver (EASL) [18].

Clinical and laboratory assessment

Clinical parameters included serum albumin, alanine aminotransferase (ALT), serum AFP, hemoglobin, platelet count, hemoglobin A1c (HbA1c), HCV genotype, and HCV RNA. All were measured by standard laboratory techniques in a commercial laboratory (SRL Laboratory, Tokyo, Japan). The HbA1c levels that we report are expressed as National Glycohemoglobin Standardization Program units (%). Body mass index was calculated as weight in kilograms/height in square meters.

Assessment of liver fibrosis

Liver biopsy for 613 (60.5%) of the 1013 patients was performed by experienced hepatologists. The antiviral treatment was initiated within 1 month after liver biopsy. The minimum length of liver biopsy was 15 mm and at least 10 complete portal tracts were necessary for inclusion. For each specimen, the stage of fibrosis was established according to the METAVIR score [19]. Liver cirrhosis in patients with no liver biopsy was diagnosed by ultrasonographic findings (nodules in the hepatic parenchyma, portal vein >16 mm) (mandatory inspection) at the time of antiviral treatment initiation. Moreover, the diagnosis of liver cirrhosis was made based on at least one of the following: (1) endoscopic findings (varices, portal gastropathy); (2) serological markers (aspartate aminotransferase to platelet ratio index >2.0; the cut-off value that indicates a negative predictive value for cirrhosis is 93%) [20]; or (3) transient elastography (FibroScan value ⩾14.9 kiloPascal; the cut-off value that indicates that the negative predictive value for cirrhosis is 100%) [21]. The EASL HCV guidelines of 2011 describe the accuracy of these non-invasive tests of liver fibrosis as sufficient for identifying patients with cirrhosis [22].

Efficacy of treatment

Successful treatment was an SVR, defined as undetectable HCV RNA at 24 weeks after the end of treatment. A TVR was defined as relapse of serum HCV RNA after treatment of patients whose HCV RNA level was undetectable at the end of treatment and the reappearance of HCV RNA at any time during treatment after virological response (breakthrough). An NVR was defined as a decrease in the HCV RNA level of less than 2 $\log_{10}$ IU/ml at week 12 (null response) and a more than 2 $\log_{10}$ IU/ml decrease in the HCV RNA level from baseline at week 12, but detectable HCV RNA at weeks 12 and 24 (partial response).

HCV RNA level and HCV genotype

Clinical follow-up of HCV viremia was done by real-time reverse transcriptase PCR assay (COBAS TaqMan HCV assay) (Roche Diagnostics, Tokyo, Japan), with 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 $\log_{10}$ IU/ml). HCV genotype determination was by sequence determination in the 5′ non-structural region of the HCV genome, followed by phylogenetic analysis [23].

Statistical analysis

Statistical analyses were conducted using SPSS Statistics 19.0 (IBM SPSS Inc., Chicago, IL, USA). Baseline continuous data are expressed as median (first-third quartiles) and categorical variables are reported as frequencies and percentages. Univariate analyses were performed using the Chi-square, Fisher's Exact, Mann–Whitney U tests or analysis of variance (ANOVA) as appropriate. Variables with $p < 0.05$ in univariate analysis were evaluated using multivariate logistic regression to identify those significantly associated with the incidence of HCC. As a rule of thumb, 10 events per predictor variable (EPV) are needed when performing a logistic regression analysis. However, 5 to 9 EPV with a large sample size (over 1000) showed robust results of as much as 10 to 16 EPV [24]. Thus, our sample size and 5 to 9 EPV might be sufficient to insure the robustness of our model. Results are expressed as hazard ratios (HR) and their 95% confidence interval (CI).

The main outcome of this study was HCC incidence. Cumulative incidence curves of HCC according to response to antiviral treatment were plotted using the Kaplan–Meier method. Differences between groups were assessed using

log-rank tests. The time frame for HCC incidence was defined as the time from the end of antiviral treatment to the diagnosis of HCC. A p value less than 0.05 was regarded as statistically significant in all analyses.

Results

Patient characteristics

The baseline characteristics of the 1013 studied patients at the start of antiviral treatment, as classified by the existence of cirrhosis and treatment outcome, are shown in Table 1. HCV genotype 1 was detected in 710 patients and genotype 2 in 303. Of all patients, 151 (14.9%) discontinued antiviral treatment because of adverse effects or other reasons (e.g., poor virological response, economic reasons, or dropout). The discontinuation rate of patients with HCV genotype 1 (129 of 710, 18.2%) was significantly higher than that of those with HCV genotype 2 (22 of 303, 7.3%) (p <0.001). Of the studied patients, 557 achieved SVR (55.0%), 304, including 20 with breakthrough, were TVR (30.0%), and 152 (15.0%) were NVR. The SVR rate of patients infected with HCV genotype 1 was 43.9% (312 of 710), significantly lower than the 80.9% (245 of 303) found for patients with genotype 2 (p <0.001).

In the non-cirrhosis group (n = 863), the three treatment outcome groups differed significantly for age, sex, HCV genotype, and laboratory values associated with liver and metabolic disease (e.g., ALT, platelet count, AFP and HbA1c). The SVR group was more likely to be infected with HCV genotype 2 and to have mild liver fibrosis, but less likely to have laboratory values associated with advanced liver and metabolic disease (e.g., low platelet count, or high AFP and HbA1c level) than the TVR and NVR groups. Independent comparisons of SVR and TVR patients extracted age (p <0.001), sex distribution (p = 0.01), ALT level (p = 0.01), platelet count (p <0.001) and HCV genotype (p <0.001). Likewise, independent comparisons of TVR and NVR patients extracted only AFP level (p = 0.01).

Liver cirrhosis was diagnosed according to clinical (n = 77) and histological (n = 73) findings. In the cirrhosis group (n = 150), however, no significant differences, except for ALT and HCV genotype, were found among the clinical and biochemical parameters of the three treatment outcome groups.

SVR and TVR patients had fewer deaths from any cause (four [0.7%] and four [1.3%], respectively) in comparison to NVR patients (six [3.9%]). Similarly, the frequency of SVR and TVR patients who developed ascites and encephalopathy, symptoms of hepatic decompensation, was lower than that of NVR patients (ascites: two [0.4%], six [2.0%] and eight [5.3%], and encephalopathy: two [0.4%], two [0.7%] and five [3.3%] patients with SVR, TVR and NVR, respectively). None of the patients underwent liver transplantation during the observation period.

Risk of HCC classified by treatment outcome

Of 1013 patients who were followed for a median of 3.6 (range 0.3–7.0) years, 47 (4.6%) developed HCC during the observation period. The baseline characteristics of these patients classified by the development of HCC are shown in Table 2. By univariate analysis, the development of HCC was associated with older age, male sex, higher ALT level, lower serum albumin, lower platelet count, higher AFP level, cirrhosis, and NVR. No significant difference in the duration of HCV RNA negativity was found between the HCC (median [first-third quartiles]: 30.0 [24.0–48.5] weeks) and non-HCC group (41.0 [27.0–48.0] weeks) (p = 0.36) in patients with TVR.

Multivariable logistic regression analysis of possible predictors of HCC development is shown in Table 3. We examined eight factors (age [<60 *vs.* ⩾60 years], sex [men *vs.* women], ALT [<40 *vs.* ⩾40 IU/L], platelet count [<150 *vs.* ⩾150 × 10^9/L], AFP [<10 *vs.* ⩾10 ng/ml], serum albumin [<40 *vs.* ⩾40 g/L], liver pathophysiology [non-cirrhosis *vs.* cirrhosis] and treatment outcome [SVR *vs.* TVR *vs.* NVR]). Significant independent pretreatment predictors of HCC were age 60 years and over (HR 2.81; 95%CI 1.39–5.69; p = 0.004), male sex (HR 2.98; 95%CI 1.46–6.05; p = 0.003), low platelet count (<150 × 10^9/L) (HR 4.04; 95%CI 1.57–10.44; p = 0.004), higher AFP level (⩾10 ng/ml) (HR 2.50; 95%CI 1.09–5.78; p = 0.03), cirrhosis (HR 3.22; 95%CI 1.28–8.13; p = 0.01), and NVR (HR 3.72; 95%CI 1.69–8.18; p = 0.001). Baseline ALT level, serum albumin level, and TVR were not associated with the development of HCC.

Table 1. Pretreatment characteristics of 1013 patients with chronic hepatitis C classified by the existence of cirrhosis and treatment outcome.

Characteristic	Non-cirrhosis n = 863				Cirrhosis n = 150			
	SVR n = 504	TVR n = 255	NVR n = 104	p value*	SVR n = 53	TVR n = 49	NVR n = 48	p value*
Age (yr)	54 (46-63)	61 (55-67)	61 (53-67)	<0.001	61 (57-67)	63 (53-68)	60 (54-68)	0.94
Male, n (%)	263 (52.2)	109 (42.7)	52 (50.0)	0.05	30 (56.6)	19 (38.8)	25 (52.1)	0.18
Body mass index (kg/m²)	22.9 (20.8-25.2)	23.3 (21.3-25.7)	23.1 (21.2-25.1)	0.12	23.0 (20.4-25.6)	23.7 (21.9-26.7)	24.6 (22.8-26.9)	0.07
ALT (IU/L)	52 (34-91)	47 (33-78)	51 (31-80)	0.02	88 (69-127)	65 (53-107)	66 (48-102)	0.01
Albumin (g/L)	42 (40-44)	42 (39-44)	42 (39-44)	0.26	37 (35-39)	37 (35-40)	37 (33-39)	0.87
Platelet count (x10^9/L)	177 (144-212)	158 (129-194)	159 (130-197)	<0.001	103 (89-116)	97 (84-111)	99 (84-118)	0.26
Hemoglobin (g/L)	137 (129-148)	136 (128-147)	138 (127-149)	0.49	130 (122-140)	133 (123-142)	137 (126-147)	0.37
Ferritin (ng/ml)	156 (75-280)	174 (92-316)	213 (116-361)	0.16	200 (127-317)	202 (134-327)	250 (170-452)	0.05
α-fetoprotein (ng/ml)	4.1 (2.9-6.0)	4.8 (2.9-7.8)	5.9 (3.4-8.9)	<0.001	14.0 (9.2-36.0)	14.1 (9.3-31.3)	30.2 (15.4-42.9)	0.24
Hemoglobin A1c (%)	5.8 (5.7-6.3)	5.9 (5.7-6.4)	6.0 (5.7-6.7)	0.005	5.8 (5.4-6.4)	5.6 (5.3-6.4)	6.0 (5.4-6.6)	0.73
HCV genotype (1/2), n (%)	288/216 (57.1/42.9)	220/35 (86.3/13.7)	92/12 (88.5/11.5)	<0.001	24/29 (45.3/54.7)	43/6 (87.8/12.2)	43/5 (89.6/10.4)	<0.001

Data are expressed as number (%) or median (first-third quartiles).
SVR, sustained virological response; TVR, transient virological response; NVR, non-virological response; HCV, hepatitis C virus; ALT, alanine aminotransferase.
*Comparison among the three groups.