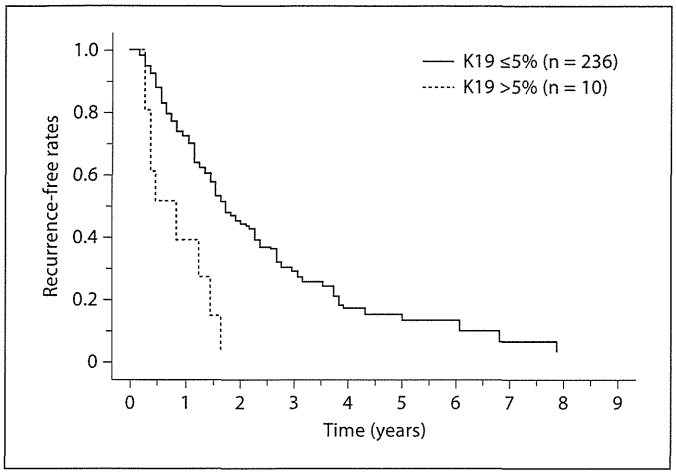

Fig. 3. The cumulative recurrence-free survival rate in patients with K19-positive (>5%) HCC was significantly lower than that in patients with K19-negative HCC (p = 0.0001).

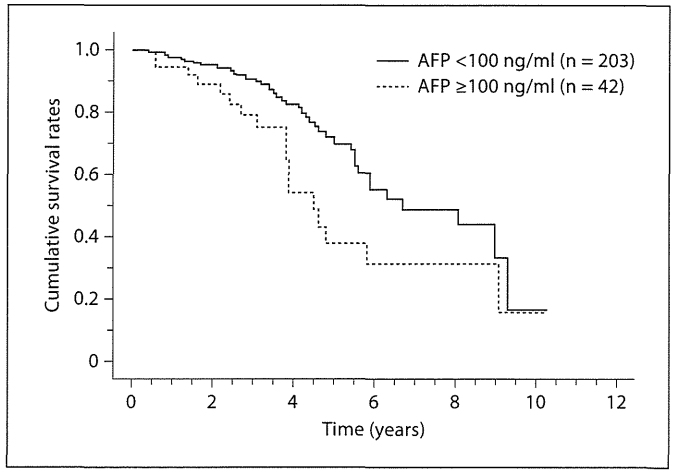

Fig. 4. The cumulative overall survival rate in patients with AFP ≥100 ng/ml was significantly lower than that in patients with AFP <100 ng/ml (p = 0.026).

3 of 5 patients with K19-positive HCC (60%) showed vascular invasion at the local tumor progression. Nine of 10 patients (90.0%) with K19-positive HCC had recurrences after initial treatment and 6 of 10 (60.0%) were detected within 1 year of initial curative RFA. On the other hand, 147 of 236 patients (62.2%) with K19-negative HCC had recurrences, and only 58 patients (24.5%) had recurrences within 1 year after RFA. There were no patients with K19-negative HCC who showed vascular invasion at the local tumor progression. Patients with K19-positive HCC were more likely to have an early recurrence of HCC (<1 year after RFA) than patients with K19-negative HCC (p = 0.012). The typical cases are shown in figure 2. The median recurrence-free survival in patients with K19-positive HCC was 194 days (range 93–635), while in patients with K19-negative HCC it was 446 days (range 65–2,978). Patients with K19-positive HCC had a significantly shorter recurrence-free survival than patients with K19-negative HCC (p = 0.0001) (fig. 3). The recurrence type, local tumor progression or distant intrahepatic recurrence differed between K19-positive and -negative patients. Local tumor progression was significantly higher in K19-positive patients than in K19-negative patients (p < 0.0001). Table 3 shows the results of univariate and multivariate analyses of prognostic factors for recurrence-free survival. In the multivariate analysis, K19 expression, the number of HCC nodules and total bilirubin ≥2 mg/dl were significant independent risk factors for HCC recurrence in all patients.

The percentage of distant metastasis and major portal invasion (VP3–4) was significantly higher in K19-positive than in K19-negative patients (p < 0.0001). Distal metastasis was detected in the lung (2 patients) and lymph node (1 patient), and major portal invasion was detected in 3 patients.

Risk Factors for Poor Prognosis

There was no patient who received liver transplantation in this study. Fifty-seven of 246 patients (23.1%) died during the follow-up period. The cause of death was progression of HCC in 37 patients, hepatic failure in 16 patients and causes unrelated to the liver in 4 patients. The overall survival rates for all patients were 97.2, 88.7 and 63.4% at 1, 3 and 5 years, respectively. A serum AFP level ≥100 ng/ml (p = 0.034), a total bilirubin level ≥2 mg/dl (p < 0.0001) and female sex (p = 0.018) were identified as risk factors for a poor prognosis in HCC in both univariate and multivariate analyses (table 4). Patients with high serum AFP levels (≥100 ng/ml) had significantly lower overall survival rates than patients with low serum AFP levels (p = 0.026) (fig. 4).

On the other hand, age (≥65 years), albumin concentration (≤3.5 g/dl), prothrombin time (≤70%), DCP (≥100 mAU/ml), tumor size, the number of HCC nodules and K19 expression were not significant risk factors for poor prognosis in the univariate analysis (table 4).

Table 3. Risk factors associated with recurrence-free survival in 246 patients with HCC after complete ablation by RFA

Risk factor	Univariate			Multivariate		
	RR	95% CI	p	RR	95% CI	p
Age <65 years	1.43	1.02–2.02	0.037	1.28	0.90–1.81	0.163
Sex, female	1.24	0.90–1.71	0.162			
Total bilirubin ≥2 mg/dl	2.50	1.02–6.25	0.034	2.70	1.08–6.66	0.032
Albumin ≤3.5 g/dl	1.12	0.81–1.56	0.492			
PT ≤70%	1.28	0.73–2.22	0.394			
AFP ≥100 ng/m	1.42	0.95–2.12	0.087			
DCP ≥100 mAU/ml	1.08	0.68–1.69	0.790			
Tumor size >3.0 cm	1.08	0.70–1.69	0.713			
2 or 3 tumor nodules	2.29	1.58–3.33	<0.0001	2.28	1.56–3.32	<0.0001
K19 positive (>5%)	3.57	1.75–7.14	0.0004	3.44	1.72–7.14	0.0005

RR = Risk ratio; CI = confidence interval; PT = prothrombin time.

Table 4. Risk factors associated with poor prognosis in 246 patients with HCC after complete ablation by RFA

Risk factor	Univariate			Multivariate		
	RR	95% CI	p	RR	95% CI	p
Age <65 years	1.19	0.68–2.09	0.527			
Sex, female	2.03	1.18–3.46	0.009	1.92	1.11–3.30	0.018
Total bilirubin ≥2 mg/dl	12.5	4.54–33.3	<0.0001	10.0	3.70–33.3	<0.0001
Albumin ≤3.5 g/dl	1.25	0.71–2.17	0.450			
PT ≤70%	1.49	0.59–3.84	0.674			
AFP ≥100 ng/ml	1.88	1.06–3.44	0.030	1.88	1.05–3.33	0.034
DCP ≥100 mAU/ml	1.06	0.53–2.12	0.880			
Tumor size >3.0 cm	1.12	0.44–1.78	0.730			
2 or 3 tumor nodules	1.23	0.67–2.26	0.492			
K19 positive (>5%)	1.29	0.46–3.57	0.632			

RR = Risk ratio; CI = confidence interval; PT = prothrombin time.

Risk Factors for Exceeding the Milan Criteria after RFA

Patients with K19-positive HCC exceeded the Milan criteria within 16.8 months. Multivariate analyses showed that K19 expression, high levels of DCP (≥100 mAU/ml), tumor number and total bilirubin ≥2 mg/dl were significant risk factors for tumor status exceeding the Milan criteria after curative RFA (table 5; fig. 5).

Complications

Most patients had mild pain or discomfort during RFA. Intraperitoneal hemorrhage and biloma were not seen in any patient. None of the patients developed dissemination of HCC, or skin or peritoneal metastases. There was no fatal complication.

Percentage of K19 Stain

We also analyzed another percentage of K19 stain (>1%). Thirteen of 246 patients had K19-positive (>1%) HCC and 12 of 13 patients with K19-positive (>1%) HCC had recurrences beyond the Milan criteria. Nine of 12 (75.0%) were detected with recurrence of HCC within 1 year of initial curative RFA. The final results were the same for K19 positivity (>5 and >1%, respectively). The

Table 5. Risk factors associated with exceeding the Milan criteria in 246 patients with HCC after complete ablation by RFA

Risk factor	Univariate			Multivariate		
	RR	95% CI	p	RR	95% CI	p
Age <65 years	1.63	1.08–2.45	0.018	1.17	0.75–1.83	0.463
Sex, female	1.16	0.78–1.72	0.457			
Total bilirubin ≥2 mg/dl	2.94	1.05–8.33	0.039	3.57	1.25–10.0	0.017
Albumin ≤3.5 g/dl	0.97	0.64–1.47	0.857			
PT ≤70%	0.89	0.41–1.96	0.763			
AFP ≥100 ng/ml	2.17	1.38–3.44	0.0008	1.56	0.96–2.50	0.077
DCP ≥100 mAU/ml	2.32	1.42–3.70	0.0007	2.08	1.26–3.44	0.004
Tumor size >3.0 cm	1.03	0.61–1.72	0.914			
2 or 3 tumor nodules	2.98	1.91–4.64	<0.0001	3.05	1.91–4.88	<0.0001
K19 positive (>5%)	3.70	1.81–7.69	0.0003	2.47	1.19–5.18	0.016

RR = Risk ratio; CI = confidence interval; PT = prothrombin time.

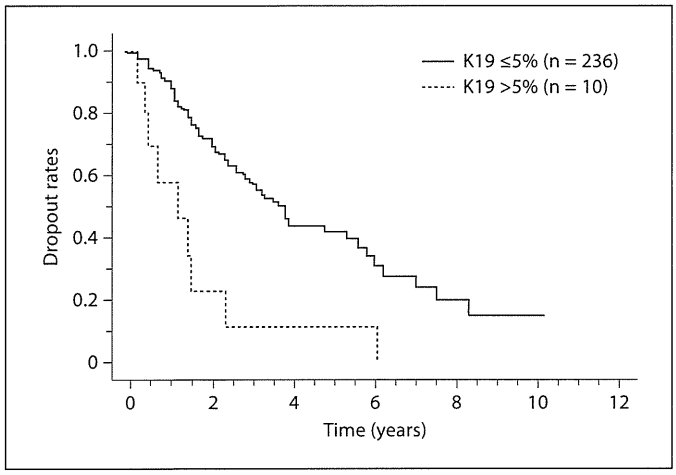

Fig. 5. The cumulative rate of exceeding the Milan criteria in patients with K19-positive HCC was significantly higher than that in patients with K19-negative HCC ($p < 0.0001$).

rate of recurrence and dropout from the Milan criteria were significantly higher in the patients with K19-positive (>1%) than in the patients with K19-negative HCC (data not shown).

Discussion

RFA therapy for HCC has been shown to achieve excellent results in appropriately selected patients [2–5]. However, recurrence of tumors is a serious impediment to improving the prognosis for patients treated with curative RFA. Therefore, several factors have been investigated as potential predictive markers for recurrence after curative RFA [7–9]. Recently, K19 was proposed as an independent prognostic factor for HCC [11–14]. However, these investigations were performed on surgically resected cases only and not on tumor biopsies. Although tumor biopsy is controversial because of potential complications such as tumor seeding [22], it would be beneficial to clinicians and patients to predict the individual tumor characteristics from a biopsy. Until now, the relationship between K19 expression and tumor recurrence after RFA treatment has not been assessed. Therefore, we have investigated the relationship between K19 expression in tumor biopsies and the clinicopathological findings in HCC. In this study, we investigated K19 expression in biopsy specimens taken just prior to the RFA session, and K19 expression (>5%) was demonstrated in 10 of 246 patients (4.1%). Because most of our patients were in early stage (within the Milan criteria) and 108 of 246 patients (43.9%) had well-differentiated HCC, the positive rate of K19 stain in our study was lower than that in surgical specimens.

We also analyzed another percentage of K19 stain (>1%) and the final results were the same for K19 positivity (>5 and >1%, respectively). K19 expression (>1%) was a statistically significant independent predictor for recurrence of HCC after RFA. Although the amount of tissue obtained by tumor biopsy is small compared to resected material, present data suggest that even biopsy can provide meaningful data on tumor recurrence irrespective of the percentage of K19 positivity (1 or 5%) (online sup-

plementary tables 1 and 2; for supplementary material see www.karger.com/doi/10.1159/000328448).

K19 positivity was not an independent predictor of the overall rate of survival, and serum AFP (≥100 ng/ml), total bilirubin (≥2 mg/dl) and female sex were significant independent predictors of survival. It is suggested that the level of total bilirubin affects the liver function of the patient, and liver function is one of the most important prognostic factors for survival of HCC patients.

The average age of our patients in this study was 68 ± 8 years, and no patients received liver transplantation in this study. However, liver transplantation is the most desirable treatment for HCC worldwide. Because of the prolonged waiting time for liver transplantation, RFA has been considered a safe and effective bridging therapy to liver transplantation. In addition, pretransplant RFA in patients with HCC has been considered for downstaging of HCC, thus improving the patient's survival [6, 7, 23]. In this study, K19 expression of HCC was a significant independent predictor for exceeding the Milan criteria ($p = 0.016$). In fact, 9 of 10 patients with K19-positive HCC exceeded the Milan criteria within 16.8 months. Therefore, if RFA is considered as a bridging therapy session prior to liver transplantation, it would be useful to obtain information on K19 expression in tumor tissue by performing a tumor biopsy before RFA. Therefore, careful observation for early detection of recurrence should be considered if K19-positive HCC patients are awaiting liver transplantation.

Compared to surgical specimens, biopsies taken prior to RFA may present some difficulties with regard to histological investigation. Needle biopsies of the nodules are less often indicated when typical vascular imaging of HCC is obtained, compared to hypovascular nodules. Needle tract seeding should also be considered. Needle biopsy has played an important role in making a diagnosis in the past. Recently, more reliance has been placed on the vascular imaging profile, because of its sensitivity and specificity without the risk of tumor dissemination. In addition, in comparison to recent advances in imaging, the information obtained from liver biopsy is lacking, as these only provide simple histological characterization, such as tumor differentiation [24]. Moreover, the positive predictive value of the vascular profile on dynamic imaging for diagnosis of HCC exceeds 95% [25]. Therefore, the current tendency is to consider needle biopsy as non-essential for diagnosis. However, in this study, K19-positive HCC showed exactly the same imaging findings as K19-negative HCC, suggesting that it is difficult to distinguish between these tumor types by imaging profile alone. In addition, K19-positive, moderately and/or poorly differentiated HCC showed similar cytological and structural abnormalities to K19-negative HCC, indicating that K19 positivity is unpredictable without staining. In figure 2, we present an impressive comparison of the features of K19-positive and -negative HCC, showing that, although the histology was similar, the prognosis for these patients was completely different. From these findings, it is clear that immunohistochemistry for K19 is the only way of demonstrating its positivity. Fortunately, staining for K19 on paraffin sections is common in diagnostic pathology, and it is not a problem to add this to routine hematoxylin and eosin (H&E) staining. Moreover, even for a general pathologist with no liver specialization, evaluating K19 expression should not be difficult, as long as care is taken not to count bile ducts, which may be associated with the remains of portal tracts. Taken together, these finding could indicate that it may be beneficial to check tumors for K19 positivity prior to RFA. Further research is warranted in larger groups to validate these findings and outweigh the potential additional clinical benefit compared to the potential risk of tract seeding during percutaneous biopsy.

Although biopsy has an important role in understanding the biological characteristics of HCC [26], tumor seeding by needle biopsy should be avoided. In practice, this is a major concern with needle biopsy of tumors. A review of tumor seeding following therapeutic procedures in HCC indicated that seeding occurred in 0–12.5% of cases (median 0.95%, mean 2.5%) [22]. As the time between biopsy and the treatment procedure was not specified, it is difficult to identify the factors that could have caused seeding. In the present study, tumor biopsies were performed just before RFA, using a needle-guiding technique, and tumor seeding was not observed. The same puncture line was used for both tumor biopsy and RFA, allowing complete ablation of the tumor using the tumor biopsy route. This may be one of the reasons it was possible in this study to biopsy the tumors without dissemination or bleeding. After treatment by RFA, the tumor cannot be investigated for histological features and K19 expression; therefore, we recommend taking a biopsy just before RFA for predicting tumor behavior using K19 expression. This would be valuable to both the clinician and the patient.

The mechanism of K19-positive HCC remains unclear. The facts that K19-positive cells are present in HCCs and that these positive cells form a spectrum suggest that K19-positive HCC may have originated from hepatic progenitor cells. These hepatic progenitor cells,

which are liver-specific adult stem cells, have potential stem cell features such as proliferation and differentiation. Once a tumor takes on these phenotypes, K19-positive HCC can still preserve these stem cell phenotypes. Therefore, this could be a possible reason why K19-positive HCC shows aggressive behavior in comparison with K19-negative HCC. In fact, previous publications and our study confirm these features [27].

In conclusion, we successfully evaluated the positivity of K19 in biopsy specimens. K19-positive HCCs showed significantly more frequent recurrence after curative RFA than K19-negative tumors and positive staining of K19 in the cytoplasm of HCC is closely associated with early intrahepatic recurrence (<1 year) and dropout from the Milan criteria. On imaging, K19-positive HCC showed only typical HCC findings and it was difficult to distinguish between K19-positive and -negative HCC. Taken together, these findings could indicate that >5% K19 positivity in tumor biopsy tissue is important for predicting tumor recurrence, which is not possible by imaging. Because of the high risk of tumor recurrence in K19-positive HCC, close observation for early detection of recurrence should be required.

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Disclosure Statement

The authors have nothing to disclose. They have no affiliation with the manufacturers of the drugs used in this study and have not received funding from the manufacturers to support this research.

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Original article

Relationship between polymorphisms of the inosine triphosphatase gene and anaemia or outcome after treatment with pegylated interferon and ribavirin

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Background: A genome-wide association study revealed an association between variants of the inosine triphosphatase (*ITPA*) gene and ribavirin (RBV)-induced anaemia. The aim of this study was to replicate this finding in an independent Japanese cohort and to define a method to allow pretreatment prediction of anaemia in combination with other factors.

Methods: Genotype 1b chronic hepatitis C patients (n=132) treated with pegylated interferon (PEG-IFN)-α and RBV for 48 weeks were genotyped for *ITPA* rs1127354 and examined for anaemia and treatment outcome.

Results: Variants of the *ITPA* gene protected against severe anaemia throughout the 48-week treatment period and were associated with lower incidence of anaemia-related RBV dose reduction. A combination of the *ITPA* genotype with baseline haemoglobin (Hb) and creatinine clearance (CLcr) levels predicted severe anaemia with high accuracy (90% sensitivity and 62% specificity). Among a subset of patients with the *IL28B* genotype of TT at rs8099917, patients with variants of the *ITPA* gene were associated with a higher rate of receiving >80% of the expected RBV dose, a higher rate of sustained virological response (SVR), and a lower rate of relapse.

Conclusions: The variants of the *ITPA* gene, which could protect against haemolytic anaemia and RBV dose reduction, were associated with a high rate of SVR by standard PEG-IFN and RBV therapy in a subset of Japanese patients with the favourable TT genotype at rs8099917 of *IL28B*. A combination of *ITPA* genetic polymorphisms with baseline Hb and CLcr levels further improves the predictive accuracy of severe anaemia.

Introduction

Treatment with pegylated interferon (PEG-IFN) combined with ribavirin (RBV) is the most effective standard treatment for chronic HCV infection. Successful eradication of HCV is associated with a reduced risk of developing hepatocellular carcinoma. However, the rate of sustained virological response (SVR) is approximately 50% in patients with HCV genotype 1 [1,2]. The probability of SVR decreases when the patients become intolerant to therapy and receive <80% of the planned dose of PEG-IFN and/or RBV [3]. One of the major reasons for intolerance to therapy is severe haemolytic anaemia induced by RBV [1]. The degree of haemolytic anaemia caused by RBV varies among individuals, and no reliable baseline predictors exist for this severe anaemia.

Recently, a genome-wide association study revealed that a single nucleotide polymorphism (SNP) at rs6051702 is strongly associated with RBV-induced haemolytic anaemia at week 4 of treatment [4]. This SNP was linked to two functional SNPs (rs1127354 and rs7270101) in the inosine triphosphatase (*ITPA*)

gene on chromosome 20, which had previously been well-characterized in studies of patients with ITPase deficiency [5–8]. Subsequent studies confirmed independently that variants of the *ITPA* gene are protective against haemolytic anaemia during the early weeks of treatment [9,10]. Furthermore, Thompson *et al.* [9] showed that the variants are protective against anaemia over the entire 48-week course of therapy and are associated with reduced requirement for an anaemia-related dose reduction of RBV. Notably, despite these protective effects, variants in the *ITPA* gene were not associated with treatment outcome [4,9] or showed only a marginal association [10].

In the present study, we aimed to replicate the association between *ITPA* genetic polymorphisms and RBV-induced anaemia in the early weeks, as well as throughout the entire course, of therapy in an independent Japanese cohort. In addition, for the general application of these genetic associations in clinical practice, we aimed to define a pretreatment prediction for severe anaemia in combination with other clinical covariates.

Methods

Patients

Data were collected retrospectively from a total of 132 genotype 1b chronic hepatitis C patients who were treated with PEG-IFN-α and RBV at Musashino Red Cross Hospital (Tokyo, Japan) and at Nagoya City University Graduate School of Medical Sciences (Nagoya, Japan). The inclusion criteria were: genotype 1b, HCV RNA titre >100 KIU/ml by quantitative PCR (Cobas Amplicor HCV Monitor version 2.0; Roche Diagnostic Systems, Indianapolis, IN, USA), no coinfection with HBV or HIV, no other causes of liver disease such as autoimmune hepatitis and primary biliary cirrhosis, and availability of DNA for the analysis of the genetic polymorphism of *ITPA*. Patients received PEG-IFN-α2a (180 μg) and -α2b (1.5 μg/kg) subcutaneously every week and were administered a daily weight-adjusted dose of RBV (600 mg for patients weighing <60 kg, 800 mg for patients weighing 60–80 kg, and 1,000 mg for patients weighing >80 kg) for 48 weeks. Dose reduction of RBV was considered by physicians based on the clinical conditions of the individual patients or the recommendations on the package inserts: dose reduction from 800 mg and 1,000 mg to 600 mg or from 600 mg to 400 mg for haemoglobin levels <10 g/dl and drug discontinuation when haemoglobin levels drop to <8.5 g/dl. No patient received erythropoietin or other growth factors for the treatment of anaemia. PEG-IFN and RBV was stopped prematurely in 22 patients: in 15 patients due to non-virological response and in 7 patients due to adverse events. Written informed consent was obtained from each patient and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethics review committees.

Laboratory and histological tests

Blood samples were obtained before therapy and at 1, 2, 4, 6, 8, 12, 16, 20, 24, 36 and 48 weeks after the start of therapy, and were analysed for haematological tests, blood chemistry and HCV RNA. Genetic polymorphisms in an SNP located in exon 2 (rs1127354) and in intron 2 (rs7270101) of the *ITPA* gene were determined using ABI TaqMan Probes (Applied Biosystems, Carlsbad, CA, USA) [4]. Since a recent paper studying Japanese patients showed no variants in rs7270101 [10] and our preliminary genotyping data for 100 Japanese patients also showed no variations in rs7270101, rs1127354 was used for further analysis (major allele =C and minor allele =A). Genetic polymorphisms in the *IL28B* gene (rs8099917), an SNP recently identified to be associated with hepatitis C treatment response [11–14], was also determined by a DigiTag2 assay [15]. Viral factors affecting therapeutic efficacy was determined. A stretch of 40 amino acids in the NS5A region of HCV, designated as the interferon sensitivity-determining region (ISDR) [16,17] and amino acid substitutions at positions 70 of the core region (Core70) [18] were determined by direct sequencing after amplification by reverse transcription and PCR as reported previously. Arginine at Core70 was defined as the wild type, and glutamine or histidine was defined as the mutant type. Baseline creatinine clearance (CLcr) levels were calculated using the formula of Cockcroft and Gault [19]: for males, CLcr= ([140 - age in years] × body weight in kg)/(72× serum creatinine in mg/dl) and for females, CLcr=0.85× ([140 - age in years]× body weight in kg)/(72× serum creatinine in mg/dl). Fibrosis was evaluated on a scale of 0–4: F0 indicates no fibrosis, F1 indicates mild fibrosis, F2 indicates moderate fibrosis, F3 indicates severe fibrosis and F4 indicates cirrhosis according to the Metavir scoring system [20]. The end of treatment response was defined as an undetectable HCV RNA level by qualitative PCR with a lower detection limit of 50 IU/ml (Amplicor; Roche Diagnostic Systems) at the end of therapy. SVR was defined as an undetectable HCV RNA level 24 weeks after the completion of therapy. A relapse was defined as the reappearance of HCV RNA after the completion of therapy.

Statistical analysis

We analysed the association between an SNP of the *ITPA* gene (rs1127354) and the following: the incidence of haemoglobin (Hb) reduction of >3.0 g/dl at week 4 and the incidence of severe anaemia (Hb<10 g/dl) at week 4 or at any time point during the therapy; the time-dependent decrease in Hb levels throughout

the treatment period; the time-dependent requirement for RBV dose reduction throughout the treatment period; and the rate of virological response or relapse. Associations between pretreatment variables and anaemia were analysed by multivariable regression. The association between the *ITPA* polymorphisms and anaemia or treatment outcome was analysed by Fisher's exact test. The association between the *ITPA* polymorphisms and the time-dependent reduction in Hb levels or the requirement for RBV dose reduction was analysed by Kaplan–Meier survival analysis. SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA) was used for these analyses.

Table 1. Clinical characteristics of the study population

Characteristic	Value
Age, years	57.5 (±9.5)
Sex, male/female	50/82
Baseline platelet count, 10^9/l	150.4 (±55.8)
Baseline Hb, g/dl	14.0 (±1.5)
Baseline creatinine clearance, ml/min	94.8 (±24.1)
Baseline liver fibrosis, F0–2/F3–4	102/30
Initial ribavirin dose	
600 mg/day, *n* (%)	91 (69)
800 mg/day, *n* (%)	38 (29)
1,000 mg/day, *n* (%)	3 (2)
Dose reduction of ribavirin, *n* (%)	58 (43)
Hb reduction at week 4, g/dl	2.2 (±1.4)
Hb reduction >3.0 g/dl at week 4, *n* (%)	37 (28)
Severe anaemia at week 4, *n* (%)[a]	21 (16)
Severe anaemia at any time point, *n* (%)[a]	57 (43)
ITPA rs1127354, AA/CA/CC	4/33/95
ISDR mutation ≤1, *n*/total *n* (%)	96/114 (84)
Core70 mutant type, *n*/total *n* (%)	42/105 (40)

Continuous variables were described as mean (±SD) and categorical variables were described as frequency and percentage. [a]Severe anaemia defined as haemoglobin (Hb)<10 g/dl. Core70, amino acid substitutions at position 70 of the core region; ISDR, interferon sensitivity-determining region; *ITPA*, inosine triphosphatase gene.

Results

ITPA rs1127354 minor genotype alleles AA and CA were protective for anaemia during drug therapy

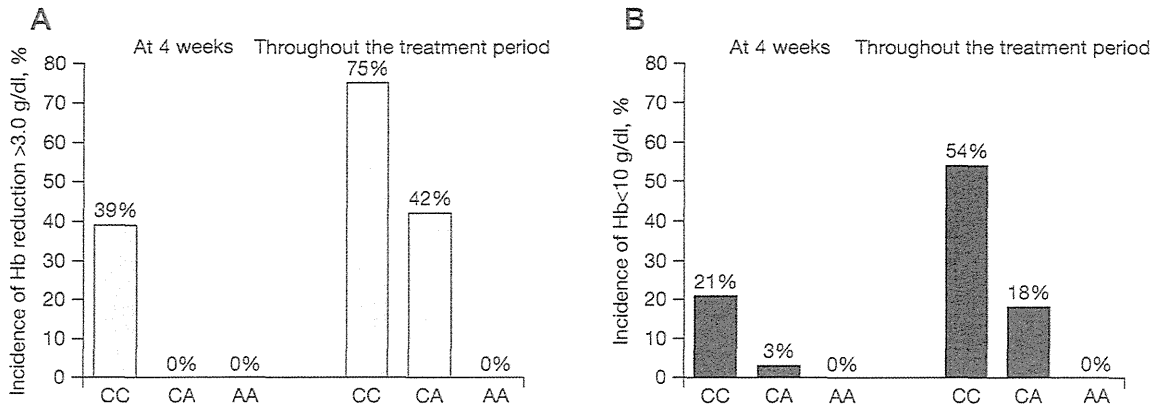
The baseline characteristics are listed in Table 1. Genotyping of rs1127354 revealed that 4 patients were homozygous for the minor allele (AA), 95 were homozygous for the major allele (CC) and 33 were heterozygous (CA). The frequency of the minor allele A was 0.16. The *ITPA* genotype was not associated with any baseline factors including age, gender, Hb levels, CLcr, platelet counts, liver fibrosis, mutations in the ISDR and Core70 (Table 2). The mean value of Hb reduction at week 4 was 2.2 g/dl and a reduction of >3.0 g/dl developed in 37 patients (28%) at week 4. Severe anaemia (Hb<10 g/dl) developed in 21 (16%) patients at week 4 of therapy and in 57 (43%) patients at any time point during the entire 48 weeks of therapy. Figure 1A and 1B shows the percentages of patients with anaemia according to the rs1127354 genotypes. At week 4, Hb reduction of >3.0 g/dl developed in 37 patients (39%) with the CC genotype, which is in contrast to 0 patients with the CA or AA genotypes (Figure 1A). Severe anaemia developed in 20 (21%) patients with the CC genotype, which is in contrast to only 1 (3%) patient with the CA genotype and 0 patients with the AA genotype (CC versus AA/CA, P=0.008; Figure 1B). Throughout the course of the 48-week therapy, Hb reduction of >3.0 g/dl developed in 71 (75%) patients with the CC genotype in contrast to 14 (42%) patients with the CA genotype and 0 patients with the AA genotype (CC versus AA/CA, P=0.0001). Severe anaemia was observed in 51 (54%) patients with the CC genotype, which is in contrast to 6 (18%) patients with the CA genotype and 0 patients with the AA genotype (CC versus AA/CA, P<0.0001). The mean reduction of Hb levels and the time course of therapy are shown in Figure 2. Patients with genotypes AA and CA showed less Hb reduction at weeks 2, 4, 6, 8 and 12 during drug therapy compared to those with the

Table 2. Clinical characteristics of patients according to *ITPA* genotype

Characteristic	rs1127354 AA/CA	rs1127354 CC	*P*-value
Age, *n* (%)	56.0 (10.9)	58.1 (8.8)	0.316
Sex, male/female	17/20	33/62	0.239
Baseline platelet count, 10^9/l	153.3 (±48.5)	149.2 (±58.5)	0.711
Baseline Hb, g/dl	14.3 (±1.4)	13.8 (±1.5)	0.132
Baseline creatinine clearance, ml/min	93.4 (±23.3)	95.3 (±24.5)	0.692
Baseline liver fibrosis, F0–2/F3–4	33/4	69/26	0.063
ISDR mutation ≤1, *n*/total *n* (%)	26/30 (87)	70/84 (83)	0.777
Core70 mutant type, *n*/total *n* (%)	11/27 (41)	31/78 (40)	1.000

Continuous variables were described as mean (±SD) and categorical variables were described as frequency and percentage. Core70, amino acid substitutions at position 70 of the core region; Hb, haemoglobin; ISDR, interferon sensitivity-determining region.

Figure 1. *ITPA* rs1127354 genotypes and anaemia during drug therapy



The percentage of patients with (A) haemoglobin (Hb) reduction of >3.0 g/dl or (B) Hb concentrations of <10 g/dl at week 4 and at any time point throughout the treatment period is shown for rs1127354 genotypes. Severe anaemia was less frequent in patients with the rs1127354 genotypes AA and CA (Hb reduction >3.0 g/dl at any time point: CC versus AA/CA, P=0.0001; Hb concentrations <10 g/dl at week 4: CC versus AA/CA, P=0.008; and Hb concentrations <10 g/dl at any time point: CC versus AA/CA, P<0.0001). *ITPA*, inosine triphosphatase gene.

CC genotype (P<0.0001 for weeks 2, 4 and 6; P=0.02 for weeks 8 and 12). These results show that the AA and CA genotypes are significantly associated with less absolute reduction in Hb levels, especially during the early weeks of therapy, and are protective against the development of severe anaemia. The sensitivity and specificity of the *ITPA* genotype for the prediction of severe anaemia (Hb<10 g/dl) throughout the course of treatment was 89% (51/57) and 41% (31/75), respectively.

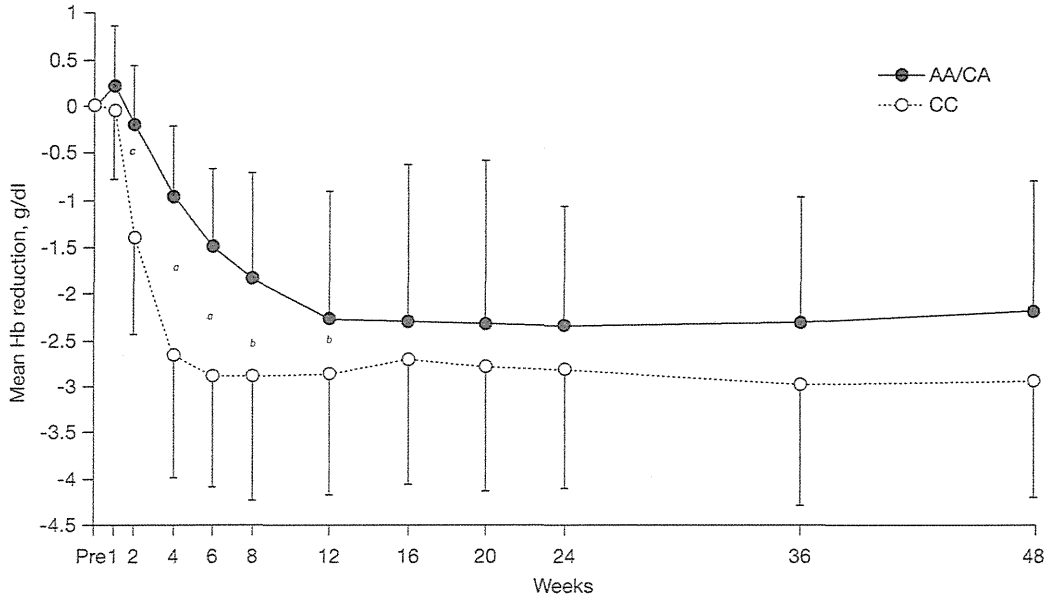
ITPA rs1127354 minor genotypes AA and CA were protective against the requirement for RBV dose reduction

The dose of RBV was reduced in 58 (43%) patients. Severe anaemia was the indication for dose reduction in 45 of the 58 (78%) patients. In the remaining 13 patients, the RBV dose was reduced because of other adverse events such as fatigue, skin eruption or loss of appetite. Figure 3 shows the time to the first RBV dose reduction during the 48 weeks of therapy. A dose reduction of RBV for any reason was less frequent and delayed in patients with the AA and CA genotypes compared to those with the CC genotype (Figure 3A; P=0.048). The difference was more significant for anaemia-related RBV dose reduction (Figure 3B; P=0.004).

Other factors associated with severe anaemia during therapy

Since 18% of the patients with the protective *ITPA* genotype of CA developed severe anaemia, we analysed the patients for other predictive factors of severe anaemia. By univariable analysis, the rs1127354 CC genotype, female gender, older age, and lower baseline Hb levels, platelet counts and CLcr levels were associated with severe anaemia. Next, multivariable regression models with backward selection were used to identify the independent predictors of severe anaemia. Covariates included age, sex, fibrosis stage, baseline Hb levels, CLcr levels and platelet counts, and the rs1127354 genotype. The multivariable regression analysis showed that the rs1127354 CC genotype, a baseline Hb of <14 g/dl and a baseline CLcr of ≤95 ml/min were independent predictors of severe anaemia at week 4 and at any time point during the 48 weeks of therapy (Table 3). Figure 4 shows the percentage of patients with Hb concentrations of <10 g/dl at any time point during therapy for the subgroups of patients stratified by rs1127354 genotype, baseline Hb levels and baseline CLcr levels. Among patients with the rs1127354 CC genotype, the risk of developing severe anaemia was more prominent in those with a baseline Hb<14 g/dl and a baseline CLcr≤95 ml/min (88%) compared to those with a baseline Hb≥14 g/dl and a baseline CLcr >95 ml/min (P<0.0001) or those with a baseline Hb<14 g/dl or a baseline CLcr≤95 ml/min (P=0.0036). Notably, the incidence of severe anaemia was only 12% in patients with the rs1127354 CC genotype if the baseline Hb was ≥14 g/dl and the CLcr was >95 ml/min. By contrast, there was a moderate risk of severe anaemia (33%) even in patients with the rs1127354 protective genotypes AA or CA when the baseline Hb was <14 g/dl and the baseline CLcr was ≤95 ml/min. Thus, patients who have >30%

Figure 2. *ITPA* rs1127354 genotypes and the quantitative Hb reduction from baseline



The mean reduction of haemoglobin (Hb) levels along the time points of treatment is shown for the rs1127354 genotypes. Solid and dotted lines indicate patients with the AA/CA and CC genotypes, respectively. The error bars indicate standard deviation. The AA/CA genotype had less of a reduction in the mean Hb levels at weeks 2–12 during therapy compared to the CC genotype. [a]*P*<0.001; [b]*P*=0.02. *ITPA*, inosine triphosphatase gene; Pre, pretreatment.

risk of severe anaemia had the following characteristics: rs1127354 CC genotype, baseline Hb<14 g/dl and CLcr≤95 ml/min; rs1127354 CC genotype and baseline Hb<14 g/dl or CLcr≤95 ml/min; and rs1127354 AA or CA genotype, baseline Hb<14 g/dl and CLcr≤95 ml/min. The sensitivity and specificity of the combination of these three factors for the prediction of severe anaemia (Hb<10 g/dl) throughout the course of treatment was 89% (51/57) and 64% (48/75). Compared to the *ITPA* genotype alone, specificity improved from 41% to 64% with the same sensitivity (89%), indicating that the combination of the *ITPA* genotype, baseline Hb levels and baseline CLcr levels could improve the prediction accuracy. The AA/CA genotypes of rs1127354 were protective against the requirement for RBV dose reduction even after standardization by baseline Hb and CLcr (Figure 3C). The predictive model for anaemia and recommendations for monitoring and treatment were made for clinical practice application (Table 4).

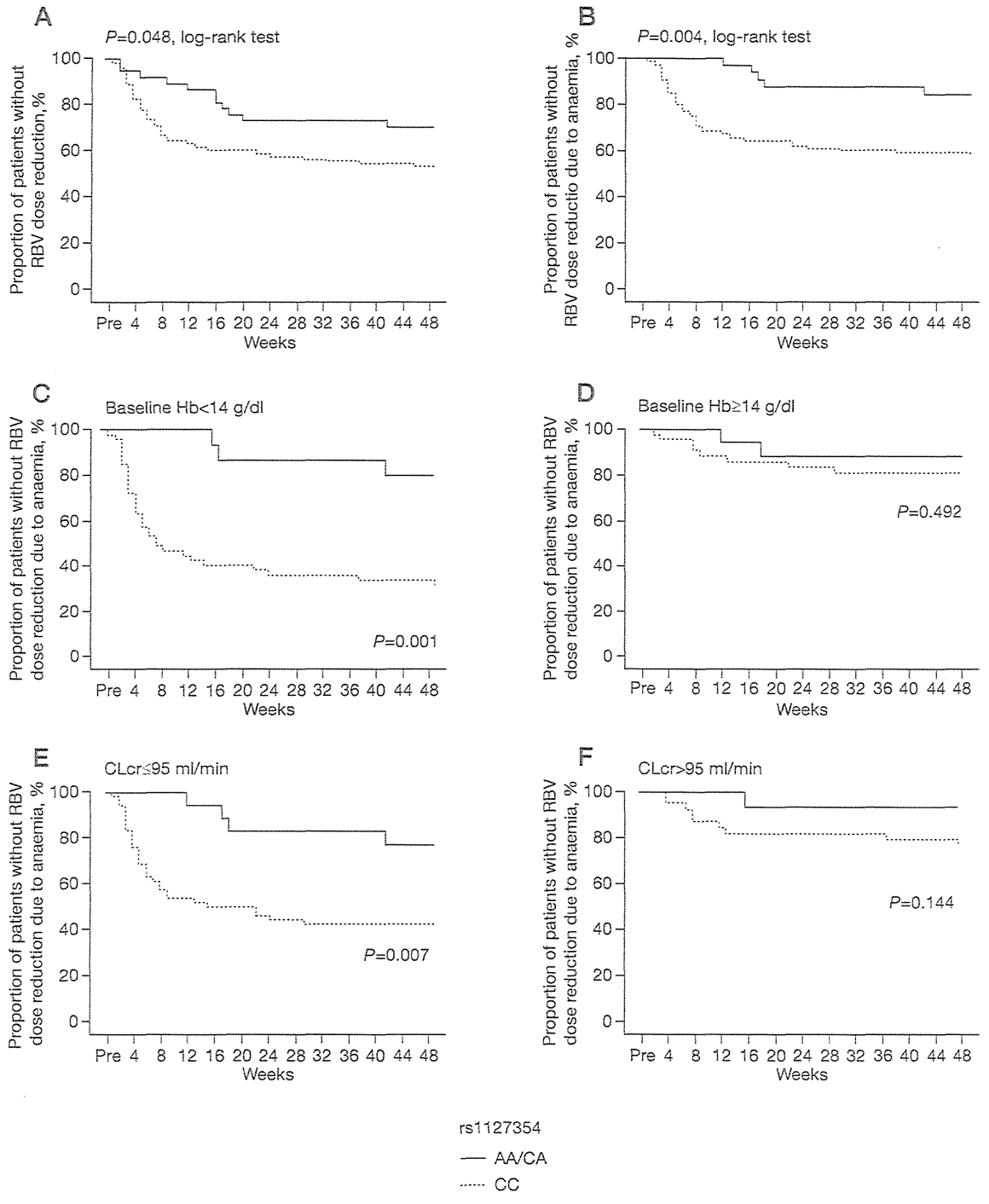
ITPA rs1127354 minor genotypes AA and CA were associated with higher adherence to RBV, higher rate of SVR and lower rate of relapse

The association of the rs1127354 genotype with the adherence to RBV or treatment outcome was analysed. When analysed in the entire population, the percentage of patients receiving >80% of the expected RBV dose, which was reported to be a threshold for an enhanced response to therapy [3], was not significantly different among the rs1127354 genotypes. Treatment outcomes such as the end-of-treatment response, SVR and relapse were also not different among the rs1127354 genotypes (Table 5). By contrast, SVR was closely associated with the *IL28B* genotype [11–14,21]: the rate of SVR was 0% (0/51) for *IL28B* minor type (TG/GG genotype at rs8099917) and 48% (39/81) for *IL28B* major type (TT genotype at rs8099917). This finding confirms that *IL28B* genotype is a significant factor for the prediction of SVR. Thus, we performed a subset analysis on subgroup of patients with the favourable *IL28B* genotype (TT at rs8099917). As a result, patients with the rs8099917 TT genotype and the rs1127354 AA or CA genotypes had a significantly higher rate of receiving >80% of the expected RBV dose (P=0.016), a higher rate of SVR (P=0.031), as well as a lower rate of relapse (P=0.046) compared to patients with the rs8099918 TT and rs1127354 CC genotype (Table 5).

Discussion

In the present study, we confirmed that variants of the *ITPA* gene protect against severe haemolytic anaemia not

Figure 3. *ITPA* rs1127354 genotypes and the time-dependent incidence of RBV dose reduction



The time to the first reduction of the ribavirin (RBV) dose (A) due to any reason or (B) due to anaemia is shown stratified by the rs1127354 genotypes. Solid and broken lines indicate patients with the AA/CA and CC genotypes, respectively. The AA/CA genotype protected against the requirement for RBV dose reduction. (C–F) Patients were standardized according to the baseline haemoglobin (Hb) and creatinine clearance (CLcr). Even after standardization by baseline Hb and CLcr, the AA/CA genotype protected against the requirement for RBV dose reduction. *ITPA*, inosine triphosphatase gene; Pre, pretreatment.

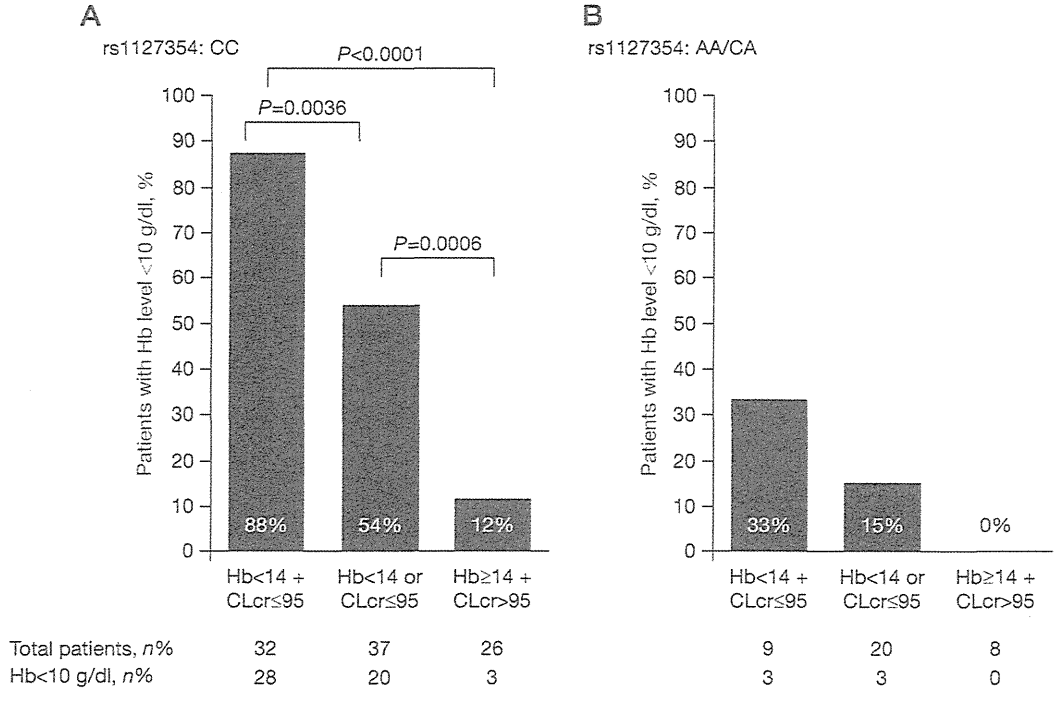
only at the early stage of treatment, but also throughout the 48-week course of treatment in a Japanese cohort of genotype 1b chronic hepatitis C patients treated with PEG-IFN and RBV. We also replicated a previous study [9] that showed that the *ITPA* genotype is significantly associated with a time-dependent reduction of the RBV dose. Furthermore, we found that a combination of the *ITPA* genotype and the baseline Hb and CLcr levels improve the accuracy of predicting RBV-induced severe anaemia. Previous reports on the IDEAL [4] or Vira-Hep-C [9] studies did not find any association between the *ITPA* genotype and treatment outcome; however, we were able to demonstrate the association of the *ITPA* genotype with a higher adherence to RBV, a higher rate

Table 3. Multivariable regression analysis of factors associated with severe anaemia during therapy[a]

Predictor	OR	95% CI	*P*-value
At week 4			
Baseline Hb<14 g/dl	7.18	1.90–27.09	0.004
Baseline creatinine clearance ≤95 ml/min	5.30	1.39–20.26	0.015
ITPA rs1127354: CC	10.17	1.25–82.85	0.030
At any time point			
Baseline Hb<14 g/dl	7.67	3.07–19.12	<0.0001
Baseline creatinine clearance ≤95 ml/min	5.51	2.21–13.73	<0.0001
ITPA rs1127354: CC	9.66	3.11–29.95	<0.0001

[a]Severe anaemia was defined as haemoglobin (Hb)<10 g/dl. *ITPA*, inosine triphosphatase gene.

Figure 4. Combination of the *ITPA* rs1127354 genotype, baseline Hb level and baseline CLcr level is predictive of severe anaemia during the therapy



Patients with rs1127354 genotype (A) CC and (B) AA/CA were further stratified by the baseline haemoglobin (Hb) and creatinine clearance (CLcr) levels. The percentage of patients with Hb concentrations of <10 g/dl (severe anaemia) at any time point during therapy is shown for the subgroups of patients. Patients with baseline Hb levels of <14 g/dl and CLcr levels of <95 ml/min had a higher incidence of severe anaemia among patients with the rs1127354 genotype CC (Hb<14 g/dl and CLcr≤95 ml/min versus Hb≥14 g/dl and CLcr>95 ml/min, *P*<0.0001; Hb<14 g/dl and CLcr≤95 ml/min versus Hb<14 g/dl or CLcr≤95 ml/min, *P*=0.0036). *ITPA*, inosine triphosphatase gene.

Table 4. Prediction model for severe anaemia and recommendation for monitoring and treatment

ITPA genotype (rs1127354)	Baseline Hb and CLcr	Risk of anaemia	Recommendation	
			Monitoring	Treatment option
CC	Hb<14 g/dl and CLcr≤95 ml/min	High	Intensive	Consider erythropoietin
	Hb<14 g/dl or CLcr≤95 ml/min	Intermediate	Intensive	Early dose reduction of RBV
	Hb≥14 g/dl and CLcr>95 ml/min	Low	As usual	–
AA/CA	Hb<14 g/dl and CLcr≤95 ml/min	Intermediate	Intensive	Early dose reduction of RBV
	Hb<14 g/dl or CLcr≤95 ml/min	Low	As usual	–
	Hb≥14 g/dl and CLcr>95 ml/min	Absent	As usual	May consider higher RBV dose

CLcr, creatinine clearance; Hb, haemoglobin; *ITPA*, inosine triphosphatase gene; RBV, ribavirin.

Table 5. Treatment response and ribavirin adherence in terms of *ITPA* rs1127354 genotype

Response	rs1127354		*P*-value
	AA/CA, *n*/total *n* (%)	CC, *n*/total *n* (%)	
All patients			
Ribavirin adherence >80%	19/37 (51)	40/95 (42)	0.436
End-of-treatment response	19/37 (51)	58/95 (61)	0.332
Sustained virological response	13/37 (35)	26/95 (27)	0.401
Relapse	6/19 (32)	32/58 (55)	0.112
Subgroup of patients with IL28B rs8099917 TT			
Ribavirin adherence >80%	14/18 (78)	28/63 (49)	0.016
End of treatment response	16/18 (89)	50/63 (79)	0.501
Sustained virological response	13/18 (79)	26/63 (41)	0.031
Relapse	3/16 (19)	24/50 (48)	0.046

ITPA, inosine triphosphatase gene.

of SVR and a lower rate of relapse among a subset of Japanese patients with the favourable *IL28B* genotype (TT at rs8099917).

Haemolytic anaemia induced by RBV is one of the major adverse events of PEG-IFN and RBV therapy leading to dose reduction of RBV or premature termination of therapy [1]. RBV is essential for improving SVR by prevention of relapses and a breakthrough [22], and a reduction of the RBV dose can lower the response rates considerably. It was reported that the maintenance of >80% of the expected RBV dose is associated with an increased SVR [23]. Thus, the prediction and prevention of RBV-induced haemolytic anaemia is clinically important. Previously, no reliable means were available to predict RBV-induced anaemia before therapy, but a recent genome-wide association study identified a strong association between two functional SNPs (rs1127354 and rs7270101) in the *ITPA* gene on chromosome 20 [4] and severe anaemia at week 4 of treatment. This genetic association has been replicated recently by two studies [9,10]. However, the effect of these variants on the long-term development of anaemia or on the requirement for RBV dose reduction has been reported by only one study to date [9]. Therefore, validation of these results by an independent cohort with respect to different geographical areas, age, gender or race is needed. Although the clinical background of our cohort was different from that of the US cohort [9], such as their race, older age (mean age of 57.5 years versus the median age of 48.5 years), and higher predominance of females (62% versus 35%), we were still able to replicate the results that the rs1127354 genotypes AA and CA are protective against anaemia throughout the 48-week course of treatment, especially within the 12 weeks following the initial treatment. We also replicated the association of this genotype with less requirement for RBV dose reduction. These results indicate that the *ITPA* genotype is universally an important determinant of RBV-induced haemolytic anaemia.

For the general application of these genetic associations in clinical practice, we aimed to further improve the accuracy of prediction by combining other clinical covariates. Among the patients with the rs1127354 CC genotype, the risk of developing severe anaemia was as high as 88% in those with baseline Hb levels of <14 g/dl and baseline CLcr levels of ≤95 ml/min, which is in contrast to only 12% in patients with Hb levels of ≥14 g/dl and CLcr levels of >95 ml/min. The rs1127354 AA and CA genotypes were protective against anaemia, but an exception occurred when patients (33%) with a baseline Hb level of <14 g/dl and a CLcr level of ≤95 ml/min developed severe

anaemia. The combination of these three factors may therefore be useful in clinical practice, since it improved the specificity of prediction from 41% to 64% with the same sensitivity (89%) compared to examining just the *ITPA* genotype. These findings may have the potential to support individualized treatment strategies. Patients with the rs1127354 CC genotype, especially those with a baseline Hb level of <14 g/dl and a baseline CLcr level of ≤95 ml/min, require intensive monitoring for anaemia during therapy, and an early dose reduction of RBV or support by erythropoietin may be indicated for safety. By contrast, patients with the AA and CA genotypes, excluding those with a baseline Hb level of <14 g/dl and a baseline CLcr level of ≤95 ml/min, may be candidates for therapy with a higher RBV dose, which may lead to higher rates of SVR. The prediction of RBV-induced anaemia will remain an important issue even in the near future, since direct antiviral agents require RBV and PEG-IFN in combination in order to achieve higher SVR rates for genotype 1 [24,25] and this combination will remain a standard therapy for other genotypes.

In a previous study, there was no clear association between ITPase deficiency and treatment outcome [4,9,10], even after a detailed subset analysis that excluded patients in whom RBV had been reduced for indications other than anaemia or after stratification by the *IL28B* genotype [9]. Thompson *et al.* [9] speculated that the lack of association may derive from several reasons such as an underpowered error due to the small number of patients, a high incidence of RBV dose reduction unrelated to anaemia, and the possibility that the ITPase deficiency may reduce antiviral efficacy. In the present study, we also failed to show associations between the *ITPA* genotype and treatment outcomes among the entire cohort. However, when patients were stratified by the *IL28B* genotype, which is now recognized as the major determinant of treatment outcome [11–14,21], the AA and CA genotypes at rs1127354 were linked to a higher adherence to RBV, a lower rate of relapse and a significantly higher rate of SVR. One of the reasons for this discrepancy may be the lower incidence of anaemia-unrelated RBV dose reduction in our study compared to the participants of the Vira-Hep-C study (22% versus 48%) [9]. The effect of the *ITPA* genotype on RBV adherence and treatment outcome may be less apparent in patients who reduced their RBV dose in the absence of anaemia. Another possibility is that the difference in mean age may have some effect on this association between the *ITPA* genotype and treatment outcome since older age has been reported to compromise drug adherence or treatment outcomes [26,27]. Our results indicated that, although IL28B genotype is the major determinant of SVR, the *ITPA* genotype may be used supplementary to predict the treatment outcome in patients with a favourable *IL28B* genotype (TT at rs8099917), as long as the RBV dose is not reduced in the absence of anaemia. Further studies involving larger populations in different geographical areas or races may be necessary to confirm this speculation.

In conclusion, variants of the *ITPA* gene, which could protect against haemolytic anaemia and RBV dose reduction, were associated with a high rate of SVR by standard PEG-IFN and RBV therapy in a subset of Japanese patients with the favourable *IL28B* genotype. A combination of the *ITPA* genetic polymorphism with baseline Hb and CLcr levels further improved the predictive accuracy of severe anaemia. These findings may have the potential to support selection of the optimum and personalized treatment strategy for individual patients.

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Disclosure statement

The authors declare no competing interests.

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Early Decrease in α-Fetoprotein, but Not Des-γ-Carboxy Prothrombin, Predicts Sorafenib Efficacy in Patients with Advanced Hepatocellular Carcinoma

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Key Words

Antitumor response · Chemotherapy · Des-γ-carboxy prothrombin · α-Fetoprotein · Hepatocellular carcinoma · Sorafenib · Tumor markers

Abstract

Objectives: The aim of this study was to investigate the relationships between early changes in the tumor markers α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP), and antitumor response in the early period following administration of sorafenib in patients with advanced hepatocellular carcinoma (HCC). ***Methods:*** Forty-eight advanced HCC patients were evaluated. AFP and DCP were measured at baseline, and after 2 and 4 weeks, and the antitumor responses were evaluated according to the RECIST criteria 4 weeks after starting sorafenib therapy. The ratios of each tumor marker were compared by stratifying the patients into the partial response (PR) + stable disease (SD) group or the progressive disease (PD) group. ***Results:*** Both 2 and 4 weeks after starting sorafenib therapy, the AFP ratio in the PR + SD group (n = 32) was significantly lower than in the PD group (n = 16; p = 0.002, p = 0.002). DCP was elevated in both the PR + SD group and the PD group 2 weeks and 4 weeks after starting sorafenib therapy. ***Conclusions:*** Evaluation of AFP ratios 2 and 4 weeks after starting sorafenib therapy may be useful for predicting antitumor response. On the other hand, early elevation of DCP does not necessarily suggest treatment failure by sorafenib, as DCP elevation can occur despite therapeutic efficacy.

Introduction

Sorafenib is a molecularly targeted multikinase inhibitor that suppresses both signal transduction of tumor growth and angiogenesis by inhibiting Raf kinase, and VEGF and PDGF receptor kinase [1]. The SHARP Study and the Asia-Pacific Study [2, 3], two large-scale, phase III, clinical studies, demonstrated that sorafenib significantly prolongs time to progression (TTP) and improves overall survival (OS) in patients with advanced hepatocellular carcinoma (HCC), and confirmed its efficacy in improving prognosis in these patients for the first time as a systemic chemotherapeutic agent. Accordingly,

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sorafenib has been recognized as the only standard systemic chemotherapeutic agent for patients with advanced HCC for whom resection and local therapy are not indicated [4–6].

α-Fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP) are well-known and widely used serological tumor markers in the screening and diagnosis of HCC [7–11]. These tumor markers are also useful as indicators of the therapeutic effect by evaluating serial changes in these values before and after tumor resection and local ablation therapy. Although numerous studies have reported the relationships between the changes in tumor markers during treatment and antitumor response [12–19], there have been no comprehensive reports evaluating the relationship between prognosis and serial changes in AFP and DCP during treatment with sorafenib. Even in the SHARP Study and the Asia-Pacific Study, this relationship was not evaluated, despite the lack of systemic chemotherapeutic agents other than sorafenib that improve prognosis in advanced HCC.

Accordingly, we investigated cumulative TTP and OS stratified by antitumor effects based on image analysis, and assessed the relationship between antitumor effects and changes in AFP and DCP in the early period of sorafenib administration in patients with advanced HCC.

Patients and Methods

Patient Eligibility

Between July 2009 and December 2010, a total of 52 patients with advanced HCC were consecutively started on sorafenib (Nexavar®; Bayer Health Care Pharmaceuticals, West Haven, Conn., USA) therapy at the Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital. Inclusion criteria for this study were as follows: HCC was diagnosed either by needle biopsy or by the combination of typical radiological findings on dynamic multidetector row computed tomography (MDCT) and elevated AFP serum levels, according to the American Association for the Study of Liver Diseases [20]; patients were classified as having advanced HCC if they were not eligible for or had disease progression after surgical or locoregional therapies; Eastern Cooperative Oncology Group performance status score of 0–1; Child-Pugh liver function class A or B (≤7); adequate hepatic function (albumin level >2.5 g/dl, total bilirubin level <3.0 mg/dl, and alanine and aspartate aminotransferase levels <5 times the upper limit of normal); dynamic MDCT was obtained at baseline and after 4 weeks of sorafenib treatment in order to assess the therapeutic effects.

Of 52 patients, 48 patients meeting the inclusion criteria were enrolled. HCC stage was diagnosed according to the criteria of the Liver Cancer Study Group of Japan [21]. This study was approved by the Ethics Committee of the Musashino Red Cross Hospital and was performed in compliance with the Helsinki Declaration.

Sorafenib Therapy

The starting dosage of sorafenib was 800 mg/day p.o. However, out of concern regarding the possibility of having to discontinue sorafenib treatment at an early stage due to adverse events, the initial dosage was set at 400 mg/day for patients aged ≥80 years, and those with a body weight ≤40 kg or a history of treatment for varices or ascites. Sorafenib therapy was continued until the occurrence of potentially fatal adverse events.

Image-Based Evaluation of Antitumor Effects

Dynamic MDCT images were taken at baseline and after 4 weeks of sorafenib treatment. Tumor responses were defined as the time point response [(in accordance with the Response Evaluation Criteria In Solid Tumors (RECIST; version 1.1)] [22] 4 weeks after sorafenib administration where the confirmation of response was not required. Patients in whom the effect was rated as partial response (PR) or stable disease (SD) were pooled in the PR + SD group, while patients showing progressive disease (PD) comprised the PD group. MDCT images were obtained every 2–6 weeks after the first MDCT image, which was obtained 4 weeks after the start of sorafenib administration.

Measurement and Evaluation of Serum AFP and DCP

The HCC tumor markers analyzed were serum AFP and DCP at baseline, and 2 and 4 weeks after starting sorafenib administration. Because DCP levels are influenced by vitamin K and warfarin, patients ingesting these agents were excluded from DCP analysis. For each patient, the baseline concentration of each tumor marker was assigned a value of 1, and the ratios for each tumor marker 2 and 4 weeks after the start of administration were calculated.

Statistics

Statistical analyses were performed using Stat View J software (version 5; SAS Institute, Cary, N.C., USA). TTP and OS after the start of sorafenib administration were analyzed by the Kaplan-Meier method, while comparisons between the two patient groups were performed by log-rank test. Tumor marker levels were analyzed by Wilcoxon signed-rank test, and comparisons of the ratios for the tumor markers between the two patient groups were performed by the Mann-Whitney U test. A value of $p < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient Baseline Characteristics

Table 1 shows baseline characteristics of the 48 HCC patients enrolled in this study. The study cohort consisted of 38 males and 10 females, with a mean age of 69.9 ± 10.0 years. Six patients had never been treated for HCC, while the remaining 42 patients had previously undergone therapy. None of these previous treatments had involved molecularly targeted therapy. The starting dosage of sorafenib in this study was 800 mg/day in 26 patients and 400 mg/day in 22 patients. Criteria for starting sorafenib at 400 mg/day were as follows: (a) age ≥80 years

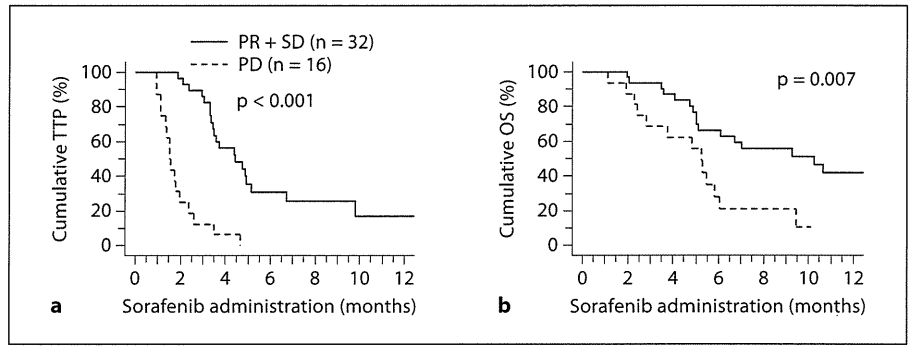

Fig. 1. Comparison of cumulative TTP (**a**) and OS (**b**) in the PR + SD and PD groups according to RECIST.

(n = 8); (b) body weight ≤40 kg (n = 2), and (c) history of treatment for varices or ascites (n = 12). The median baseline AFP level was 572 ng/ml (range, 2.3–148,000), and the median baseline DCP level was 424 mAU/ml (range, 15–305,000). The mean observation period was 7.2 ± 4.5 months.

Antitumor Responses 4 Weeks after the Start of Sorafenib Therapy

According to RECIST, 4 weeks after the start of sorafenib therapy, there were no complete responses, 2 PR, 30 SD, and 16 PD. The response rate was 4.2%, and the disease control rate was 66.7%.

Cumulative TTP and OS in the PR + SD and PD Groups

Cumulative TTP in the two groups according to RECIST is shown in figure 1a. The median observation period was 3.2 months. The median TTP was significantly longer in the PR + SD group than in the PD group (4.4 vs. 1.5 months; hazard ratio, 0.14; 95% CI, 0.06–0.29; $p < 0.001$).

Cumulative OS in the two groups according to RECIST is shown in figure 1b. The median observation period was 5.7 months. The median OS was significantly longer in the PR + SD group than in the PD group (10.3 vs. 5.2 months; hazard ratio, 0.36; 95% CI, 0.17–0.78; $p = 0.007$).

Comparison of Actual and Relative Levels of AFP at Baseline, and 2 and 4 Weeks after the Start of Sorafenib Therapy (Stratified by Antitumor Response)

AFP was not measured in 9 and 1 patients 2 and 4 weeks after starting sorafenib administration, respectively. Accordingly, AFP was analyzed in 39 and 47 patients 2 and 4 weeks after starting sorafenib administration, respectively.

Table 1. Baseline characteristics of the 48 HCC patients enrolled in this study

Mean age, years	69.9 ± 10.0
Male/female	38/10
HBV/HCV/NBNC	6/30/12
ECOG PS (0/1)	29/19
Child-Pugh score (5/6/7)	24/21/3
HCC stage (III/IVA/IVB)	11/18/19
Initial therapy/therapy for recurrence	6/42
Sorafenib starting dosage (800/400 mg)	26/22
Median serum AFP level, ng/ml	572
Range	2.3–148,000
Median serum DCP level, mAU/ml	424
Range	15–305,000
Mean observation period, months	7.2 ± 4.5

Numbers of patients are shown unless indicated otherwise. HBV/HCV = Hepatitis B/C virus; NBNC = non-HBV, non-HCV; ECOG = Eastern Cooperative Oncology Group; PS = performance status.

Data comparing actual AFP levels at baseline, and 2 and 4 weeks after starting sorafenib administration, both for the total patients and when stratified by antitumor response according to RECIST, are shown in table 2. Among the total number of patients, AFP showed no statistically significant differences between baseline and 2-week treatment levels, but in the PD group, AFP levels after 2 weeks of treatment were significantly elevated versus baseline levels ($p = 0.013$). Similarly, in the total number of patients, AFP showed no statistically significant differences between baseline and 4-week treatment levels, but in the PD group, AFP was significantly higher after 4 weeks of treatment compared with baseline levels ($p = 0.002$). In the PR + SD group, the median actual AFP level 4 weeks after starting sorafenib administration was higher than that at 2 weeks; however, there were no sig-