Hepat Mon. 2015 March; 15(3): e27148. DOI: 10.5812/hepatmon.27148
Published online 2015 March 20. **Research Article**

An Index to Predict Ribavirin-Induced Anemia in Asian Patients With Chronic Genotype 1 Hepatitis C

Sheng-Hung Chen [1,2,3]; Cheng-Yuan Peng [2,3,*]; Hsueh-Chou Lai [1,3,4]; Wen-Pang Su [3]; Chia-Hsin Lin [3]; Yu-Fen Li [5]; Po-Heng Chuang [3]; Ching-Hsiang Chen [3]

[1]Institute of Clinical Medical Science, School of Medicine, China Medical University, Taichung, Taiwan
[2]School of Medicine, China Medical University, Taichung, Taiwan
[3]Division of Hepatogastroenterology, Department of Internal Medicine, China Medical University Hospital, Taichung, Taiwan
[4]College of Chinese Medicine, China Medical University, Taichung, Taiwan
[5]Institute of Biostatistics, China Medical University, Taichung, Taiwan

*Corresponding Author: Cheng-Yuan Peng, Division of Hepatogastroenterology, Department of Internal Medicine, China Medical University Hospital, China Medical University, Taiwan. Tel: +88-6422052121, E-mail: cypeng@mail.cmuh.org.tw

Received: January 22, 2015; **Revised:** February 10, 2015; **Accepted:** February 21, 2015

Background: Single-nucleotide polymorphisms (SNP) in the inosine triphosphate pyrophosphatase (ITPA) gene correlate with ribavirin (RBV)-induced anemia in patients with chronic hepatitis C (CHC) receiving combination therapy. Managing anemia is an early priority in the treatment process.
Objectives: The aim was to develop a predictive index based on ITPA SNP status to identify CHC patients at risk of anemia.
Patients and Methods: A total of 418 eligible East Asian patients diagnosed with CHC genotype 1 (G1) received combination therapy in this study. Participant DNA was genotyped for a functional ITPA SNP (C/C, A/A or C/A) on chromosome 20 at rs1127354. A predictive index was constructed by incorporating independent factors identified for severe anemia events (hemoglobin < 10 g/dL). Areas under the receiver-operating characteristic curves (AUCs) represented the diagnostic accuracies of the predictive index in randomly assigned development and validation cohorts.
Results: Multiple logistic regressions identified age (≥ 50 y: OR = 9.7, 95% CI = 5.0 - 18.6), ITPA rs1127354 (C/C: OR = 3.3, 95% CI = 1.8 - 5.8) and baseline hemoglobin (< 14.0 g/dL: OR 6.4, 95% CI = 3.3 - 12.1; 14.0 - 14.9: OR = 2.4, 95% CI = 1.2 - 4.6) as predictors of severe anemia throughout the treatment. For severe anemia, the predictive index incorporating age, ITPA SNP status and baseline hemoglobin yielded diagnostic accuracies (AUCs) of 0.830 (95% CI = 0.783 - 0.871) in the development (n = 324) and 0.902 (0.826 - 0.925) in the validation (n = 81) cohorts.
Conclusions: In patients with CHC G1 and receiving combination therapy, ITPA SNP-based index was an accurate and practical solution for prediction of severe anemia.

Keywords: Polymorphism; Inosine Triphosphate; Hepatitis C; Ribavirin; Anemia

1. Background

Chronic hepatitis C (CHC) is a major health care burden and a leading cause of end-stage liver disease and hepatocellular carcinoma (HCC) worldwide. Although non-uniform distributions of CHC in certain areas complicate the establishment of global and regional epidemiology, the global prevalence of CHC has been estimated as 2.35%, affecting approximately 160 million people (1).

In Taiwan, seroprevalences of hepatitis C virus (HCV) antibody positivity were estimated as 4.4% to 8.6% (2, 3). Upwards of 53% of CHC patients are infected with HCV genotype 1 (G1) (4). Either in most resource-limited areas worldwide or in most of the Asian countries, pegylated interferon (pegIFN) plus ribavirin (RBV) combination therapy remains the first-line standard of care (SOC) for CHC. The National Health Insurance program of Taiwan reimburses the cost of SOC for CHC. However, severe RBV-induced hemolytic anemia severely complicates CHC patients during SOC by causing suboptimal tolerance, poor compliance and early withdrawal from therapy (5). The risks of anemia have been shown to be higher in Asian than non-Asian patients (6). Concerns about ribavirin-induced anemia and related issues still remain central to patient.

Extensive accumulation of RBV in erythrocytes causes membrane oxidative damage, premature hemolysis and subsequent anemia (7). Anemia during CHC treatment was considered multifactorial. The haptoglobin phenotype, pretreatment platelet level (8), impaired renal function (9), high dose/body weight ratio, old age and female sex (10) have been previously demonstrated to be associated with anemia. Recent genome-wide association studies have advanced to a strong association between RBV-induced anemia and single-nucleotide polymorphisms (SNPs) in the inosine triphosphate pyrophosphatase (ITPA) gene on chromosome 20 in CHC patients on SOC (11).

Identifying patients at high risk of anemia is crucial

for improving patient compliance and SOC outcomes for CHC patients (12). Novel treatment strategies or algorithms based on a combination of relevant pharmacogenetics and host factors may facilitate patient counseling prior to anemia events (13). However, limited studies included predictive modeling of severe anemia (hemoglobin, Hb < 10 g/dL) based on an index incorporating the strong predictor, ITPA SNP status (14, 15).

2. Objectives

Therefore, we estimated the effect of ITPA SNP status on severe anemia and treatment responses to construct a clinically practical predictive index for severe anemia during CHC combination therapy.

3. Patients and Methods

3.1. Ethics

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Board of China Medical University Hospital. Informed consent was obtained from each patient included in the study.

3.2. Patients

From September 2005 to September 2013, consecutive East Asian patients with CHC G1 were screened and enrolled in a prospective cohort to analyze antiviral treatment responses. We defined CHC G1 as positive result for serum anti-HCV antibody (Abbott Laboratories, Abbott Park, IL, USA) for more than six months with detectable serum HCV G1 RNA (Cobas Amplicor HCV Monitor 2.0; Roche Diagnostics, Branchburg, NJ, USA). Patients with a history of any of the following conditions were excluded from the cohort; age < 20 years or > 75 years, treatment discontinued in less than four months after starting SOC with no severe anemia event, hepatitis B virus coinfection, human immunodeficiency virus coinfection, HCC, alcoholic liver disease, primary biliary cirrhosis, primary sclerosing cholangitis, Wilson's disease, autoimmune hepatitis, hemochromatosis, previous IFN-based therapy, baseline Hb < 13 g/dL in men, Hb < 12 g/dL in women, nucleoside- or nucleotide-analog therapies other than RBV, decompensated cirrhosis and end-stage renal disease. Eligible patients were randomly assigned to the development and validation cohorts at a ratio of 4:1.

3.3. Combination Therapy

Patients received pegIFN αα-2a (Pegasys, Hoffmann-La Roche, Basel, Switzerland) at a dosage of 180 μg/week or pegIFN α-2b (Peg-Intron, Schering-Plough, Kenilworth, NJ, USA) at a dosage of 1.5 μg/kg/week subcutaneously. Oral RBV was prescribed for all patients at a daily dose of 1000 mg (body weight < 75 kg) or 1200 mg (body weight ≥ 75 kg) for 24 weeks or 48 weeks based on the baseline HCV RNA (< versus ≥ 6 $\log_{10}$ copies/mL) and virological response at week 4 (16, 17). Baseline and mean doses of RBV (mg/kg/day) on treatment were calculated for each patient. Erythropoietin (EPO) was considered if Hb fell below 10 g/dL during the treatment.

3.4. Treatment Monitoring

Blood biochemistry (Beckman Coulter, CA, USA) and complete blood count analyses (Sysmex HST-series, Kanogawa, Japan) were performed in the central laboratory at the medical center. HCV RNA load was quantified at baseline and monitored at weeks 4, 12, 24 and 48.

3.5. Clinical Endpoints

Severe anemia was defined as Hb level < 10 g/dL. Sustained virological response (SVR) was defined as undetectable HCV RNA at or before the end of treatment and at 24 weeks after the end of treatment. Monthly distributions of patients with severe anemia (hemoglobin < 10 g/dL) on treatment were compared between C/C versus "A/A or C/A" genotypes.

3.6. ITPA SNP Genotyping

Participant genomic DNA was extracted from peripheral blood mononuclear cells using a Qiagen DNA blood mini kit (Qiagen, Valencia, CA, USA). Genotyping for one functional missense ITPA variant in exon 2 at rs1127354 on chromosome 20 (18, 19) was performed using 20 μL of FastStart universal probe master mix (Roche Diagnostics, Branchburg, NJ, USA) containing 500 nmol rs1127354 forward primer (*5'-TCTTGGAACAGGTCGTTCAGATTCTA-3'*), 500 nmol rs1127354 reverse primer (*5'-AGGAAGACAGAGAAATCCAACCATC-3'*), 250 nmol C allele probe (*FAM-AGTTTCCATGCACTTTGGTGG-BBQ*) and 250 nmol A allele probe (*YAKAGTTTACATGCACTTTGGTGGC-BBQ*). The reaction mixture was denatured at 95°C for 10 minutes before thermal cycling at 95°C for 15 seconds and 60°C for 1 minute for 40 cycles. Genotypes were analyzed using StepOneTM software Version 2.0 (Life Technologies, Carlsbad, CA, USA). Another reported ITPA SNP at rs7270101 is non-polymorphic in Asians. Therefore, patients were not genotyped for ITPA SNP rs7270101 (18). The cohort was also genotyped for interleukin 28B (IL28B) polymorphisms at rs8099917 and rs12979860, as previously described (20).

3.7. Statistical Analysis

Both Kolmogorov-Smirnov and Shapiro-Wilk tests showed that ($P < 0.05$) none of the continuous variables in this study had a normal distribution. Therefore, continuous variables were expressed as median (interquartile range, IQR) and estimated using Mann-Whitney U test. Categorical variables were estimated using the chi-square test or Fisher's exact test. Univariate logistic regression identified the variables ($P < 0.25$) associated with severe anemia for subsequent multiple regressions.

By expressing the likelihood of severe anemia as a probability ranging from 0 to 1, an index for identifying severe anemia was constructed by incorporating significant independent associated factors and the beta coefficients acquired through the final multiple logistic regression for severe anemia (14). The diagnostic performance of the predictive index was evaluated by the area under the receiver operating-characteristic curves (AUCs). The odds ratios (ORs) of significant associations were determined based on a 95% confidence interval (CI). The data was analyzed using SAS Version 9.3 (SAS Institute, Inc., Cary, NC, USA). A 2-sided $P < 0.05$ indicated statistical significance.

4. Results

4.1. Patient Characteristics

A total of 453 patients were screened. Excluded patients included those with baseline hepatitis B virus coinfection (n = 9), HCC (n = 5), a history of IFN-based therapy (n = 11), men with baseline Hb < 13 g/dL or women < 12 g/dL (n = 8) and end-stage renal disease (n = 2). Therefore, the CHC G1 cohort consisted of 418 eligible participants who intended to receive combination therapy. ITPA data were missing for four patients. Nine patients were also excluded, because the treatment was discontinued within four months from the treatment baseline for reasons other than severe anemia.

Thus, 405 patients entered the complete analysis. The cohort was randomized into a development cohort (n = 324) and a validation cohort (n = 81). Among them, 367 (90.6%) of 405 patients had liver histology data. A total of 148 (36.5%) patients received combination therapy for 24 weeks and 257 (63.5%) patients for 48 weeks. Patients per protocol (n = 357) entered the analysis for SVR (41 patients discontinued before the intended EOT and seven patients were lost to follow-up for SVR visit).

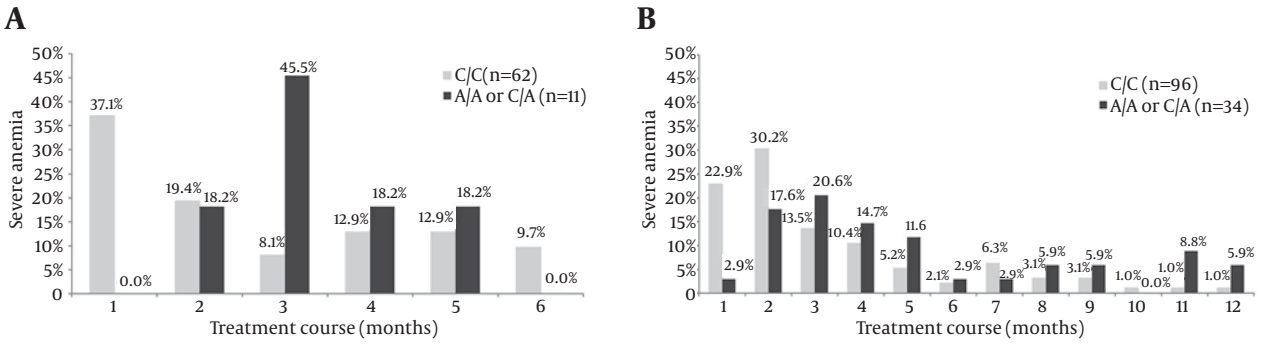
The median patient age was 53.0 (13.0) years and 49.6% of patients were men. The median baseline Hb was 14.4 (1.9) g/dL. The C/C genotype was present in 66.4% of patients and 33.6% had the A/A or the C/A allelic variants ("A/A or C/A" as a combo variable) at rs1127354. The development cohort (n = 324) and validation cohort (n = 81) did not differ significantly in a univariate analysis.

4.2. Predictive Index

Monthly distributions of severe anemia events are demonstrated in Figure 1. Among patients who received 24-week treatment and developed severe anemia, a significantly higher percentage of patients with C/C genotype did so during the first month on treatment than those with "A/A or C/A" genotype (37.1% versus 0.0%, P = 0.0135). Likewise, among patients who received 48-week treatment and developed severe anemia, a significantly higher percentage of patients with C/C genotype did so during the first month on treatment than those with "A/A or C/A" genotype (22.9% versus 2.9%, P = 0.0079). In total, 166 patients (166/324, 51.2%) in the development cohort developed severe anemia on treatment. Multiple logistic regressions identified age (≥ 50 y: OR = 9.7, 95% CI = 5.0 - 18.6), ITPA rs1127354 (C/C: OR = 3.3, 95% CI = 1.8 - 5.8) and baseline hemoglobin (< 14.0 g/dL: OR 6.4, 95% CI = 3.3 - 12.1; 14.0 - 14.9: OR = 2.4, 95% CI = 1.2 - 4.6) as predictors of severe anemia on treatment (Tables 1 and 2). There was no significant dose effect between ITPA SNP status (C/A versus A/A, P = 0.5387) and severe anemia. Therefore, the predictive index derived from the development cohort incorporating age (y), ITPA SNP status and baseline Hb (g/dL) was expressed as $1/(1 + \exp(-x))$, where $x = -3.2835 + 2.2709 \times A + 1.1822 \times I + 1.8848 \times H1 + 0.8684 \times H2$ (A = 1 if age ≥ 50; A = 0 if age < 50; I = 1 if ITPA SNP = C/C; I = 0 if ITPA SNP = "A/A or C/A"; H1 = 1 if Hb < 14.0; H1 = 0 if Hb ≥ 14.0; H2 = 1 if Hb = 14.0 - 14.9; H2 = 0 if Hb < 14.0 or ≥ 15.0).

To dichotomize the anemia status (with versus without severe anemia) in the development, the cutoff value of 0.5 was optimal when maximizing the values of "sensitivity + specificity - 1" (21). The predictive index yielded a diagnostic accuracy (AUC) of 0.830 (95% CI = 0.783-0.871) in the development (n = 324) and 0.902 (0.826-0.925) in the validation (n = 81) cohorts to dichotomize the anemia status, respectively (Figure 2).

Figure 1. Monthly Distributions of Patients With Severe Anemia (Hemoglobin < 10 g/dL) on Treatment



A) Among patients who received 24-week treatment and developed severe anemia, a significantly higher percentage of patients with C/C genotype did so during the first month than those with "A/A or C/A" genotype (P = 0.0135). B); also, among patients who received 48-week treatment (P = 0.0079).

4.3. Treatment Responses

In total, 135 (135/357 per protocol, 37.8%) patients achieved RVR. Seventy-three of them (73/135, 54.1%) had a median baseline HCV RNA of 5.29 (0.76) $\log_{10}$ copies/mL and received 24-week treatment. The remaining 62 patients (62/135, 45.9%) received 48-week treatment. Liver pathology was acquired in 69 of 73 (94.5%) patients with RVR and 24-week treatment. Among them, METAVIR fibrosis stages 0 - 2 were noted in 54 (54/69, 78.3%), and 3 - 4 in 15 (15/69, 21.7%).

In total, 255 (255/357 per protocol, 71.4%) patients achieved SVR. The RVR (OR = 8.2; 95% CI = 3.5–19.1; P < .0001), T/T IL28B genotype at rs8099917 (OR = 3.0; 95% CI = 1.2 - 7.4; P = 0.0156), the METAVIR fibrosis stages 0-2 (OR = 1.9; 95% CI = 1.0 - 3.5; P = 0.0450), baseline HCV RNA (OR = 0.4; 95% CI = 0.2 - 0.7; P = 0.0008) and 48-week treatment duration (OR = 1.1; 95% CI = 1.0 - 1.1; P = 0.0003) were independently associated with SVR. Anemia events and EPO treatment were not independently associated with SVR.

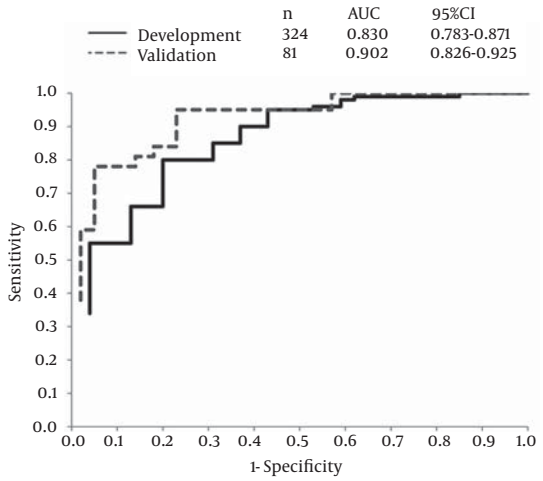


Figure 2. Receiver Operating Characteristic Curves of the Predictive Index for the Development and Validation Cohorts

Table 1. Baseline Characteristics of the Development Cohort (n = 324) [a,b]

Variables	Anemia (n = 166)	Non-Anemia (n = 158)	P Value
Age, y			< 0.0001
< 50	17 (10.2)	83 (52.5)	
≥ 50	149 (89.8)	75 (47.5)	
Gender			< 0.0001
Female	100 (60.2)	56 (35.4)	
Male	66 (39.8)	102 (64.6)	
Body mass index, kg/m^2	23.75 (3.8)	25 (3.9)	0.0011
IL28B SNP (rs8099917)			0.5189
T/G or G/G	24 (14.5)	19 (12.0)	
T/T	142 (85.5)	139 (88.0)	
IL28B (rs12979860)			0.5566
C/T or T/T	27 (16.3)	22 (13.9)	
C/C	139 (83.7)	136 (86.1)	
ITPA (rs1127354)			0.0002
A/A or C/A	37 (22.3)	66 (41.8)	
C/C	129 (77.7)	92 (58.2)	
Platelet, 1000/μL	155.0 (68.0)	168.0 (82.0)	0.0007
Creatinine, mg/dL	0.77 (0.30)	0.81 (0.27)	0.3375
Baseline hemoglobin, g/dL			< 0.0001
< 14	85 (51.2)	32 (20.3)	
14 - 14.9	47 (28.3)	37 (23.4)	
≥ 15	34 (20.5)	89 (56.3)	
Alanine aminotransferase, IU/L	85.0 (84.0)	86.5 (74.0)	0.3627
METAVIR fibrosis stage			0.4233
0 - 2	100 (69.9)	109 (74.1)	
3 - 4	43 (30.1)	38 (25.9)	
HCV RNA, $\log_{10}$ copies/mL	6.89 (0.94)	6.89 (0.89)	0.9754
Interferon use			0.2744
< 100% intended dose	27 (16.3)	19 (12.0)	
Ribavirin dose, mg/kg/d	14.9 (2.9)	15.6 (2.1)	0.0037
Erythropoietin use			< 0.0001
No	47 (28.3)	117 (74.1)	
Yes	119 (71.7)	41 (25.9)	
Treatment durations, week			0.7906
24	54 (32.5)	54 (34.2)	
48	94 (56.6)	84 (53.2)	
Discontinued	18 (10.8)	20 (12.7)	

[a] Data are presented as Median (IQR) or No. (%).
[b] Continuous variables were estimated using Mann-Whitney U test. Categorical variables were estimated using chi-square test.

Table 2. Multiple Logistic Regressions for Severe Anemia [a]

Variables	β Coefficient	Standard Error of β Coefficient	Odds Ratio (95% CI)	P Value
Age, y				< 0.0001
< 50 (19 - 49) [b]			1.000	
≥ 50 (50 - 73) [b]	2.2709	0.3325	9.688 (5.049,18.588)	
ITPA (rs1127354)				< 0.0001
A/A or C/A			1.000	
C/C	1.1822	0.2977	3.262 (1.820, 5.845)	
Baseline hemoglobin, g/dL				< 0.0001
< 14 (12.0 - 13.9) [b]	1.8848	0.3308	6.352 (3.322, 12.147)	
14-14.9	0.8684	0.3365	2.383 (1.232, 4.609)	0.0099
≥ 15 (15.0 - 17.7) [b]			1.000	

[a] Data are presented for Hemoglobin < 10 g/dL and Development Cohort (n = 324).
[b] Range of variables, intercept = -3.2835.

In patients with either ITPA C/C or "A/A or C/A" genotypes, the mean RBV dose exposures were not significantly different between patients with SVR versus non-SVR. The IL28B SNP (rs12979860) was not included in the final multiple regression because of collinearity with the other IL28B SNP (rs8099917) (Table 3).

In addition, through univariate analyses, the ITPA SNP status was significantly associated with RBV dose reduction in the development cohort (n = 324) (Mann-Whitney U test, P = 0.0035). Patients (n = 103/324, 31.8%) with "A/A or C/A" genotype received 100% (8%) intended RBV doses. In contrast, patients (n = 221/324, 68.2%) with C/C genotype received 97% (1.4%) intended RBV doses.

However, ITPA SNP status was not significantly associated with EPO use (chi-square test, P = 0.1617). Forty-five of 103 patients (45/103, 43.7%) with "A/A or C/A" genotype received EPO treatment. In contrast, 115 of 221 patients (115/221, 52.0%) with C/C genotype received EPO treatment.

5. Discussion

The frequency of severe anemia was 50.1% (203/405). This incidence was higher than that reported (39.3%) in a previous study of 466 East Asian patients diagnosed with CHC (5). The higher anemia frequency in our study can be attributed to the higher RBV dosage in patients infected with HCV G1. Consistent with Hb kinetics reported in previous studies (5, 12, 19, 22-25), the anemia events in our study accumulated during the first few months of treatment and remained relatively stable thereafter (Figure 1).

The accumulated ITP was substituted for GTP during the biosynthesis of ATP and protected against RBV-induced hemolytic anemia. Therefore, the wild C/C genotype for ITPA is hemolysis-susceptible. Methods of gene expression analysis such as mRNA transcripts or western blotting were not used in our study. However, the results of SNP genotyping of ITPA that we performed correlated closely with phenotypes or predicted ITPA activities of these allelic variants (26-28). Although RBV pharmacokinetics in serum or in erythrocytes or predicted ITPA activities were not applicable in our current study (29), this index may serve as a clinically practical one based on dichotomized parameters available in clinical settings. Future studies can develop algorithms or scoring systems based on age, sex, body weight, renal function, Hb and ITPA SNP status for modifying RBV dosage to achieve an optimal steady-state concentration range or for administering EPO prior to the development of anemia, rather than reducing the RBV dose following an anemia event (30, 31). Recent studies indicated that ITPA allelic variants "A/A or C/A" at rs1127354 are less likely associated with RBV dose reduction (19, 22, 23, 32). Future studies can also use a time-to-event analysis to identify significant predictors for RBV dose reduction.

The effects of older age and female sex on severe anemia (< 11 g/dL) during the first four weeks of treatment were also reported in a study of 61 HCV G1-infected Japanese patients, in which 49 patients exhibited RBV-sensitive ITPA C/C genotype at rs1127354 and 12 patients exhibited RBV-resistant "A/A or C/A" variants (19). Therefore, older female patients require stringent monitoring of anemia during combination therapy. However, sex was not included in the predictive index through multiple logistic regressions in the current study because of collinearity with baseline Hb. The serum creatinine levels did not gain significance during univariate analysis. Therefore, it was not applicable to adopt creatinine level in multiple regression modeling for predictive index of severe anemia. In the study by Tsubota et al., estimated glomerular filtration rate was found to be one of the significant associated factors to construct a predictive model for RBV-induced anemia (14). Our future studies would recruit participants in larger sample sizes receiving more uniform treatment durations to allow time-dependent analysis of anemia events for constructing a predictive index through Cox regression analysis.

Based on our results, we suggest that subjects with a predictive index greater than 0.5 might receive EPO treatment early after the start of pegIFN and RBV combination therapy to facilitate quality of life, prevent RBV dose reduction and avoid compromised treatment responses. However, anticipated benefits associated with early implementation of EPO treatment need to be confirmed by further studies.

Treatment responses are a major concern regarding SOC for CHC. Both RVR and T/T IL28B genotype at rs8099917 remained significant predictors for SVR in our CHC G1 cohort (33). Also consistent with recent studies (18, 19, 22-24, 34), ITPA SNP status was a non-significant predictor of SVR in our study. However, the reported ITPA-SVR correlations varied (35). In a recent report by Rembeck et al. (27), ITPA allelic variants encoding reduced ITPA activity was demonstrated

Table 3. Factors Associated With Sustained Virological Response [a,b]

Variables	Univariate			Multiple	
	Sustained Virological Response		P Value	OR (95% CI)	P Value
	(+) n = 255	(-) n = 102			
Age, y	52.0 (14.0)	55.5 (14)	0.0296		
Gender			0.8408		
Female	128 (50.2)	50 (49.0)			
Male	127 (49.8)	52 (51.0)			
Body mass index, kg/m^2	23.97 (4.0)	25.04 (3.95)	0.0110		
IL28B SNP (rs8099917)			< 0.0001		0.0156
T/G or G/G	17 (6.7)	23 (22.5)			
T/T	238 (93.3)	79 (77.5)		3.032 (1.234 - 7.449)	
IL28B SNP (rs12979860)			0.0002		
C/T or T/T	21 (8.2)	23 (22.5)			
C/C	234 (91.8)	79 (77.5)			
ITPA (rs1127354)			0.9441		
A/A or C/A	89 (34.9)	36 (35.3)			
C/C	166 (65.1)	66 (64.7)			
Platelet, 1000/μL	169 (72)	145 (71)	0.0008		
Serum creatinine, mg/dL	0.77 (0.3)	0.8 (0.24)	0.1199		
Hemoglobin, g/dL	14.4 (2.0)	14.4 (1.9)	0.7231		
Alanine aminotransferase, IU/L	85 (92)	86.5 (66)	0.7509		
METAVIR fibrosis stage			0.0067		0.0450
0 - 2	174 (75.3)	56 (60.2)		1.886 (1.014 - 3.507)	
3 - 4	57 (24.7)	37 (39.8)		1.000	
HCV RNA, $\log_{10}$ copies/mL	6.71 (1.14)	7.02 (0.54)	< 0.0001	0.385 (0.220 - 0.673)	0.0008
Interferon use			0.0068		
< 100% intended dose	27 (10.6)	22 (21.6)			
Ribavirin dose, mg/kg/d	15.4 (2.6)	14.85 (2.8)	0.0461		
Erythropoietin use			0.6388		
No	133 (52.2)	56 (54.9)			
Yes	122 (47.8)	46 (45.1)			
Rapid virological response			< 0.0001		< 0.0001
No	123 (49.4)	84 (90.3)		1.000	
Yes	126 (50.6)	9 (9.7)		8.212 (3.535 - 19.079)	
Treatment durations, wk			0.0443		0.0003
24	86 (33.7)	46 (45.1)		1.000	
48	169 (66.3)	56 (54.9)		1.051 (1.023 - 1.079)	
Hemoglobin < 10, g/dL			0.8935		
No	127 (49.8)	50 (49.0)			
Yes	128 (50.2)	52 (51.0)			
Hemoglobin < 10, g/dL [c]			0.8735		
No	226 (88.6)	91 (89.2)			
Yes	29 (11.4)	11 (10.8)			
Hemoglobin decline ≥ 4, g/dL			0.9166		
No	91 (35.7)	37 (36.3)			
Yes	164 (64.3)	65 (63.7)			

[a] Data are presented as Median (IQR) or No. (%).
[b] Continuous variables were estimated using Mann-Whitney U test. Categorical variables were estimated using chi-square test.
[c] Data are presented within 4 weeks.

to correlate with SVR and non-relapse, unrelated to RBV adherence or protection against anemia, although the molecular mechanisms require further elucidation.

Although previous reports yielded conflicting results regarding the significance of anemia-SVR correlation (5, 12, 36, 37), most recent studies on ITPA SNPs have not estimated anemia-SVR correlation (18, 19, 22-25, 32, 38). In our study, SVR was not associated with severe anemia or Hb decline (≥ 4 g/dL) on treatment (Table 3). Recent anemia-SVR correlations have also been reported based on the divergent results of non-genetic (5, 23, 36) and molecular studies (11, 14). Molecular studies indicated that anemia might not only be a surrogate for RBV exposure, because anemia-SVR association was attenuated when adjusting for RBV levels (39), but also represent the first molecular evidence connecting anemia-SVR association to novel genes and pathways through statistical analyses on gene expressions and mRNA signatures (11).

In conclusion, ITPA SNP status is a significant predictor of RBV-induced hemolytic anemia in patients diagnosed with CHC G1 and receiving pegIFN and RBV combination therapy. The ITPA SNP-based index is an accurate and practical predictive solution for severe anemia in clinical practice. Information on ITPA SNP status of patients may assist in maximizing the tolerability of SOC for patients with CHC.

Appendices

Appendix 1. Comparisons Between the Development and Validation Cohorts [a, b]

Variable	Development (n = 324)	Validation (n = 81)	P Value
Age, y	53.0 (12.0)	52.0 (18.0)	0.4739
Gender			0.0736
Female	156 (48.1)	48 (59.3)	
Male	168 (51.9)	33 (40.7)	
Body mass index, kg/m^2	24.24 (3.8)	24.06 (4.7)	0.6257
IL28B SNP (rs8099917)			0.8251
T/G or G/G	43 (13.3)	10 (12.3)	
T/T	281 (86.7)	71 (87.7)	
IL28B SNP (rs12979860)			0.7266
C/T or T/T	49 (15.1)	11 (13.6)	
C/C	275 (84.9)	70 (86.4)	
ITPA (rs1127354)			0.1271
A/A or C/A	103 (31.8)	33 (40.7)	
C/C	221 (68.2)	48 (59.3)	
Platelet, 1000/μL	164.0 (70.5)	167.0 (68.0)	0.8431
Creatinine, mg/dL	0.80 (0.29)	0.72 (0.26)	0.2180
Baseline hemoglobin, g/dL			0.2768
< 14	117 (36.1)	37 (45.7)	
14 - 14.9	84 (25.9)	17 (21)	
≥ 15	123 (38)	27 (33.3)	
Alanine aminotransferase, IU/L	86.0 (80.0)	94.0 (79.0)	0.2669
METAVIR fibrosis stage			0.4349
0 - 2	209 (72.1)	52 (67.5)	
3 - 4	81 (27.9)	25 (32.5)	
HCV RNA, $\log_{10}$ copies/mL	6.89 (0.92)	6.75 (1.15)	0.0528
Interferon use			0.3062
< 100% intended dose	46 (14.2)	8 (9.9)	
Ribavirin dose, mg/kg/d	15.3 (2.55)	15.5 (3.10)	0.3448
Erythropoietin use			0.0732
No	164 (50.6)	50 (61.7)	
Yes	160 (49.4)	31 (38.3)	
Anemia			0.3711
No	158 (48.8)	44 (54.3)	
Yes	166 (51.2)	37 (45.7)	
Treatment durations, week			0.1003
24	108 (33.3)	30 (37.0)	
48	178 (54.9)	48 (59.3)	
Discontinued	38 (11.7)	3 (3.7)	

[a] Data are presented as median (IQR) or No. (%).
[b] Continuous variables were estimated using Mann-Whitney U test, categorical variables were estimated using chi-square test.

Appendix 2. Diagnostic Performances of the Predictive Index for Severe Anemia [a]

	Development Cohort (n = 324)			Validation Cohort (n = 81)		
Cutoff	0.25	0.50	0.75	0.25	0.50	0.75
TP [b]	157	133	57	35	30	14
FP [b]	84	49	7	20	8	1
TN [b]	74	109	151	24	36	43
FN [b]	9	33	109	2	7	23
Sensitivity [c]	94.6 (91.1 - 98.0)	80.1 (74.1 - 86.2)	34.3 (27.1 - 41.6)	94.6 (87.3 - 100)	81.1 (68.5 - 93.7)	37.8 (22.2 - 53.5)
Specificity [c]	46.8 (39.1 - 54.6)	69.0 (61.8 - 76.2)	95.6 (92.4 - 98.8)	54.6 (39.8 - 69.3)	81.8 (70.4 - 93.2)	97.7 (93.3 - 100)
PPV [c]	65.2 (59.1 - 71.2)	73.1 (66.6 - 79.5)	89.1 (81.4 - 96.7)	63.6 (50.9 - 76.4)	79 (66 - 91.9)	93.3 (80.7 - 100)
NPV [c]	89.2 (82.5 - 95.9)	76.8 (69.8 - 83.7)	58.1 (52.1 - 64.1)	92.3 (82.1 - 100)	83.7 (72.7 - 94.8)	65.2 (53.7 - 76.7)
+LR	1.8 (1.5 - 2.1)	2.6 (2.1 - 3.3)	7.8 (3.7 - 16.5)	2.1 (1.5 - 2.9)	4.5 (2.3 - 8.5)	16.7 (2.3 - 120.7)
-LR	0.1 (0.1 - 0.2)	0.3 (0.2 - 0.4)	0.7 (0.6 - 0.8)	0.1 (0.0 - 0.4)	0.2 (0.1 - 0.5)	0.6 (0.5 - 0.8)
DOR	15.4 (7.3 - 32.2)	9.0 (5.4 - 14.9)	11.3 (5.0 - 25.7)	21 (4.5 - 98.3)	19.3 (6.3 - 59.4)	26.2 (3.2 - 211.8)

[a] Data are presented for Hemoglobin < 10 g/dL.
[b] Data are presented as No.
[c] Data are presented as %.

Authors' Contributions

Study concept and design: Sheng-Hung Chen, Cheng -Yuan Peng, Chia-Hsin Lin and Yu-Fen Li. Sampling and executive procedure: Sheng-Hung Chen, Cheng-Yuan Peng, Hsueh-Chou Lai, Wen-Pang Su, Po-Heng Chuang, and Ching-Hsiang Chen. Drafting of the manuscript: Sheng-Hung Chen and Cheng-Yuan Peng. Statistical analysis: Chia-Hsin Lin and Yu-Fen Li.

Funding/Support

This study was supported by a grant (DMR-98-011) from the Department of Medical Research, China Medical University Hospital, Taichung, Taiwan.

References

1. Lavanchy D. Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect.* 2011;**17**(2):107–15.
2. Chen CH, Yang PM, Huang GT, Lee HS, Sung JL, Sheu JC. Estimation of seroprevalence of hepatitis B virus and hepatitis C virus in Taiwan from a large-scale survey of free hepatitis screening participants. *J Formos Med Assoc.* 2007;**106**(2):148–55.
3. Yang JF, Lin CI, Huang JF, Dai CY, Lin WY, Ho CK, et al. Viral hepatitis infections in southern Taiwan: a multicenter community-based study. *Kaohsiung J Med Sci.* 2010;**26**(9):461–9.
4. Yu ML, Chuang WL. Treatment of chronic hepatitis C in Asia: when East meets West. *J Gastroenterol Hepatol.* 2009;**24**(3):336–45.
5. Hung CH, Lee CM, Lu SN, Wang JH, Chen CH, Hu TH, et al. Anemia associated with antiviral therapy in chronic hepatitis C: incidence, risk factors, and impact on treatment response. *Liver Int.* 2006;**26**(9):1079–86.
6. Chang CH, Chen KY, Lai MY, Chan KA. Meta-analysis: ribavirin-induced haemolytic anaemia in patients with chronic hepatitis C. *Aliment Pharmacol Ther.* 2002;**16**(9):1623–32.
7. Inoue Y, Homma M, Matsuzaki Y, Shibata M, Matsumura T, Ito T, et al. Erythrocyte ribavirin concentration for assessing hemoglobin reduction in interferon and ribavirin combination therapy. *Hepatol Res.* 2006;**34**(1):23–7.
8. Van Vlierbergh H, Delanghe JR, De Vos M, Leroux-Roel G, Basl Steering Committee . Factors influencing ribavirin-induced hemolysis. *J Hepatol.* 2001;**34**(6):911–6.
9. Hu CC, Weng CH, Lin CL, Tien HC, Kuo YL, Chien CH, et al. Predictors of changes in hemoglobin levels in patients with chronic hepatitis C treated with ribavirin plus pegylated interferon-alpha. *Ren Fail.* 2012;**34**(4):429–34.
10. Scherzer TM, Stattermayer AF, Stauber R, Maieron A, Strasser M, Laferl H, et al. Effect of gender and ITPA polymorphisms on ribavirin-induced anemia in chronic hepatitis C patients. *J Hepatol.* 2013;**59**(5):964–71.
11. Birerdinc A, Estep M, Afendy A, Stepanova M, Younossi I, Baranova A, et al. Gene expression profiles associated with anaemia and ITPA genotypes in patients with chronic hepatitis C (CH-C). *J Viral Hepat.* 2012;**19**(6):414–22.
12. Azakami T, Hayes CN, Sezaki H, Kobayashi M, Akuta N, Suzuki F, et al. Common genetic polymorphism of ITPA gene affects ribavirin-induced anemia and effect of peg-interferon plus ribavirin therapy. *J Med Virol.* 2011;**83**(6):1048–57.
13. Krishnan SM, Dixit NM. A formula to estimate the optimal dosage of ribavirin for the treatment of chronic hepatitis C: influence of ITPA polymorphisms. *Antivir Ther.* 2012;**17**(8):1581–92.
14. Tsubota A, Shimada N, Abe H, Yoshizawa K, Agata R, Yumoto Y, et al. Several factors including ITPA polymorphism influence ribavirin-induced anemia in chronic hepatitis C. *World J Gastroenterol.* 2012;**18**(41):5879–88.
15. Kurosaki M, Tanaka Y, Nishida N, Sakamoto N, Enomoto N, Matsuura K, et al. Model incorporating the ITPA genotype identifies patients at high risk of anemia and treatment failure with pegylated-interferon plus ribavirin therapy for chronic hepatitis C. *J Med Virol.* 2013;**85**(3):449–58.
16. Hsieh MY, Dai CY, Lee LP, Huang JF, Chuang WL, Hou NJ, et al. Antinuclear antibody titer and treatment response to peginterferon plus ribavirin for chronic hepatitis C patients. *Kaohsiung J Med Sci.* 2012;**28**(2):86–93.
17. Yu ML, Dai CY, Huang JF, Chiu CF, Yang YH, Hou NJ, et al. Rapid virological response and treatment duration for chronic hepatitis C genotype 1 patients: a randomized trial. *Hepatology.* 2008;**47**(6):1884–93.
18. Ochi H, Maekawa T, Abe H, Hayashida Y, Nakano R, Kubo M, et al. ITPA polymorphism affects ribavirin-induced anemia and outcomes of therapy--a genome-wide study of Japanese HCV virus patients. *Gastroenterology.* 2010;**139**(4):1190–7.
19. Suzuki F, Suzuki Y, Akuta N, Sezaki H, Hirakawa M, Kawamura Y, et al. Influence of ITPA polymorphisms on decreases of hemoglobin during treatment with pegylated interferon, ribavirin, and telaprevir. *Hepatology.* 2011;**53**(2):415–21.
20. Yu ML, Huang CF, Huang JF, Chang NC, Yang JF, Lin ZY, et al. Role of interleukin-28B polymorphisms in the treatment of hepa-

titis C virus genotype 2 infection in Asian patients. *Hepatology.* 2011;**53**(1):7–13.
21. Youden WJ. Index for rating diagnostic tests. *Cancer.* 1950;**3**(1):32–5.
22. Domingo P, Guardiola JM, Salazar J, Torres F, Mateo MG, Pacho C, et al. Association of ITPA gene polymorphisms and the risk of ribavirin-induced anemia in HIV/hepatitis C virus (HCV)-coinfected patients receiving HCV combination therapy. *Antimicrob Agents Chemother.* 2012;**56**(6):2987–93.
23. Eskesen AN, Melum E, Moghaddam A, Bjoro K, Verbaan H, Ring-Larsen H, et al. Genetic variants at the ITPA locus protect against ribavirin-induced hemolytic anemia and dose reduction in an HCV G2/G3 cohort. *Eur J Gastroenterol Hepatol.* 2012;**24**(8):890–6.
24. Naggie S, Rallon NI, Benito JM, Morello J, Rodriguez-Novoa S, Clark PJ, et al. Variants in the ITPA gene protect against ribavirin-induced hemolytic anemia in HIV/HCV-coinfected patients with all HCV genotypes. *J Infect Dis.* 2012;**205**(3):376–83.
25. Osinusi A, Naggie S, Poonia S, Trippler M, Hu Z, Funk E, et al. ITPA gene polymorphisms significantly affect hemoglobin decline and treatment outcomes in patients coinfected with HIV and HCV. *J Med Virol.* 2012;**84**(7):1106–14.
26. Fiorina L, Paolucci S, Papadimitriou S, Baldanti F. Comparison of three different methods for the evaluation of IL28 and ITPA polymorphisms in patients infected with HCV. *J Virol Methods.* 2012;**184**(1-2):103–5.
27. Rembeck K, Waldenstrom J, Hellstrand K, Nilsson S, Nystrom K, Martner A, et al. Variants of the inosine triphosphate pyrophosphatase gene are associated with reduced relapse risk following treatment for HCV genotype 2/3. *Hepatology.* 2014;**59**(6):2131–9.
28. Takahashi H, Mizuta T, Oeda S, Isoda H, Nakashita S, Kawaguchi Y, et al. An automated rapid detection system using the quenching probe method for detecting interleukin 28B and inosine triphosphatase single nucleotide polymorphisms in chronic hepatitis C. *J Viral Hepat.* 2013;**20**(4):e124–6.
29. Inoue Y, Homma M, Matsuzaki Y, Shibata M, Matsumura T, Ito T, et al. Liquid chromatography assay for routine monitoring of cellular ribavirin levels in blood. *Antimicrob Agents Chemother.* 2004;**48**(10):3813–6.
30. Ackefors M, Gjertsen H, Wernerson A, Weiland O. Concentration-guided ribavirin dosing with darbepoetin support and peg-IFN alfa-2a for treatment of hepatitis C recurrence after liver transplantation. *J Viral Hepat.* 2012;**19**(9):635–9.
31. Brochot E, Castelain S, Duverlie G, Capron D, Nguyen-Khac E, Francois C. Ribavirin monitoring in chronic hepatitis C therapy: anaemia versus efficacy. *Antivir Ther.* 2010;**15**(5):687–95.
32. Sakamoto N, Tanaka Y, Nakagawa M, Yatsuhashi H, Nishiguchi S, Enomoto N, et al. ITPA gene variant protects against anemia induced by pegylated interferon-alpha and ribavirin therapy for Japanese patients with chronic hepatitis C. *Hepatol Res.* 2010;**40**(11):1063–71.
33. Chayama K, Hayes CN, Abe H, Miki D, Ochi H, Karino Y, et al. IL28B but not ITPA polymorphism is predictive of response to pegylated interferon, ribavirin, and telaprevir triple therapy in patients with genotype 1 hepatitis C. *J Infect Dis.* 2011;**204**(1):84–93.
34. Thompson AJ, Clark PJ, Singh A, Ge D, Fellay J, Zhu M, et al. Genome-wide association study of interferon-related cytopenia in chronic hepatitis C patients. *J Hepatol.* 2012;**56**(2):313–9.
35. Dai CY, Yu ML, Chuang WL. Association between response to pegylated interferon/ribavirin therapy and ribavirin levels. *Hepatology.* 2015;**61**(1):408–9.
36. Giusto M, Rodriguez M, Navarro L, Rubin A, Aguilera V, San-Juan F, et al. Anemia is not predictive of sustained virological response in liver transplant recipients with hepatitis C virus who are treated with pegylated interferon and ribavirin. *Liver Transpl.* 2011;**17**(11):1318–27.
37. Sulkowski MS, Shiffman ML, Afdhal NH, Reddy KR, McCone J, Lee WM, et al. Hepatitis C virus treatment-related anemia is associated with higher sustained virologic response rate. *Gastroenterology.* 2010;**139**(5):1602–11.
38. Thompson AJ, Santoro R, Piazzolla V, Clark PJ, Naggie S, Tillmann HL, et al. Inosine triphosphatase genetic variants are protective against anemia during antiviral therapy for HCV2/3 but do not decrease dose reductions of RBV or increase SVR. *Hepatology.* 2011;**53**(2):389–95.
39. Holmes JA, Roberts SK, Ali RJ, Dore GJ, Sievert W, McCaughan GW, et al. ITPA genotype protects against anemia during peginterferon and ribavirin therapy but does not influence virological response. *Hepatology.* 2014;**59**(6):2152–60.