ORIGINAL ARTICLE

Gut and Liver, Vol. 10, No. 3, May 2016, pp. 446-455

Association of *IL28B* Genotypes and Baseline Serum Interferon-γ-Inducible-Protein-10 Levels with Treatment Response in Hepatitis C Virus Patients in China

Renwen Zhang, Cuiping Shao, Na Huo, Minran Li, and Xiaoyuan Xu

Department of Infectious Diseases, Peking University First Hospital, Beijing, China

Background/Aims: Several studies have demonstrated that serum interferon-γ-inducible-protein-10 (IP-10) levels at baseline and single nucleotide polymorphisms (SNPs) near the *IL28B* gene were associated with viral response and treatment outcomes. Our purpose was to assess the combination of pretreatment IP-10 levels with *IL28B* SNPs as predictors of treatment response to pegylated interferon α-2a plus ribavirin in patients infected with genotype 1 hepatitis C virus in China. **Methods:** Seventy-two patients with chronic hepatitis C without fibrosis/cirrhosis were enrolled in the study. The virologic parameters and baseline serum IP-10 levels were determined. *IL-28B* genotypes were determined by sequencing. **Results:** In this cohort, serum baseline IP-10 levels lower than 426.7 pg/mL could predict rapid virological response/sustained virological response (SVR). Patients carrying favorable *IL28B* SNP genotypes had higher SVRs than did those carrying unfavorable variants (*IL28B rs*12979860, p=0.002; *IL28B rs*8099917, p=0.020). Combining both baseline IP-10 and *IL28B* SNPs could improve the prediction of SVR in favorable allele carriers of *IL28B*, *rs*12979860 CC and *rs*8099917 TT. Serum baseline IP-10 levels and *IL28B* genotypes were independent predictors of SVR. **Conclusions:** Our study shows that the combination of baseline serum IP-10 levels and the determination of *IL28B* SNPs increase the predictability of SVR rates in this cohort. **(Gut Liver 2016;10:446-455)**

Key Words: Interferon-γ-inducible-protein-10; *IL28B*; Viral response; Pegylated interferon α-2a plus ribavirin

INTRODUCTION

According to a latest estimation, more than 185 million people around the world have been infected with hepatitis C virus (HCV), and among them, 350,000 people have died every year.[1,2] Liver cirrhosis or hepatocellular carcinoma (HCC) will be eventually developed in one-third of those chronically infected with HCV.[3] The development of direct-acting antivirals (DAAs) has markedly improved the response rate for chronic HCV infection. However, due to the high costs and difficult availability of DAAs drugs, the standard treatment for chronic hepatitis C (CHC) in China is the therapy with the combination of pegylated interferon α and ribavirin (RBV), which leads to sustained virological response (SVR) rates of 54% to 80%.[4-8] Pegylated interferon α-2a plus ribavirin (PegIFNα-2a/RBV) are still retain pivotal roles in the management of chronic HCV infection, and predicting responses to PegIFNα-2a/RBV therapy will remain important. Response rates to PegIFNα-2a/RBV are associated with both host and viral factors.

The evaluation of hepatic gene expression is impractical and costly, so its clinical application has been greatly limited. However, serum markers may offer similar prognostic information. Multiple studies have shown that the level of the interferon-stimulated gene (ISG), interferon-γ-inducible-protein-10 (IP-10), is associated with treatment outcomes in patients with chronic HCV infection.[9-11] IP-10, a member of the CXC chemokine family, is expressed in the liver of CHC patients and selectively recruits the activated T cells to the inflammation sites.[12] Recent studies have demonstrated that low plasma concentrations of IP-10 in CHC patients is closely associated with the antiviral efficacy. Successful antiviral therapy is associated with the increase in circulating CXC-chemokine receptor 3 (CXCR3) CD8+ T cells and the reduction of IP-10 in serum.[13] In addition, other investigations have reported that serum IP-10 levels at baseline tends to be higher in African-American patients with HCV infection than in white patients.[14,15] Another study has sug-

Correspondence to: Xiaoyuan Xu
Department of Infectious Diseases, Peking University First Hospital, No. 8, Xishiku Street, Xicheng District, Beijing 100034, China
Tel: +86-010-83575787, Fax: +86-010-83572266, E-mail: xiaoyuanxu6@163.com
Received on April 2, 2015. Revised on May 19, 2015. Accepted on May 28, 2015. Published online October 19, 2015
pISSN 1976-2283 eISSN 2005-1212 http://dx.doi.org/10.5009/gnl15162

gested that serum IP-10 level may be lower in Asian patients infected with HCV.[16] Therefore, serum IP-10 concentrations in HCV-infected patients may different according to the races. In 2009, genome-wide association studies have shown that single nucleotide polymorphisms (SNPs) near the *IL28B* gene were associated with viral response and outcomes of treatment. Two of these SNPs are reportedly highly predictive of a favorable treatment response: *rs*12979860 and *rs*8099917. They were found to strongly predict treatment response with CC (*rs*12979860) and TT (*rs*8099917) genotypes being highly predictive of SVR.[17,18]

Better predictors of SVR would be helpful to identify patients with the best chance of response before the initiation of combination antiviral therapy. Recently, several studies about the predictive value of the combination of *IL28B* SNPs and serum IP-10 levels at baseline showed the promising results for HCV-infected patients.[19,20] Until now, few studies have reported the association between pretreatment serum IP-10 concentrations with *IL28B* SNPs and treatment efficacy with PegIFNα-2a/RBV in Asian populations infected with HCV, especially in the patients infected with genotype 1 HCV in China.

Our purpose was to assess the combination of pretreatment IP-10 levels with *IL28B* SNPs as predictors of treatment response to PegIFNα-2a/RBV in this cohort.

MATERIALS AND METHODS

1. Patients

A total of 72 patients with CHC were enrolled in the Department of Infectious Diseases, Peking University First Hospital, from October 2011 to September 2013 for this study. The diagnostic criteria of CHC were according to the Guideline of Prevention and Treatment of Hepatitis C.[21] We assessed fibrosis stages of patients by transient elastography (Fibroscan; Echosens, Paris, France) combined with related clinical parameters. Fibroscan (Echosens) is a noninvasive method for measuring liver stiffness. In all patients included in the study, stiffness values by Fibroscan were less than 7 kPa. All the patients were treatment-naive adults, and had compensated liver disease without fibrosis/cirrhosis. All the patients included in the study were genotype 1b, which detected by direct sequencing.[22] Patients chronically infected with hepatitis B virus or human immunodeficiency virus, or with other liver disease such as autoimmune hepatitis and primary biliary cirrhosis as well as with HCC were excluded from this study.

The study was in compliance with the ethical guidelines of 1975 Declaration of Helsinki and was approved by the Medical Ethics Committee of First Hospital at Peking University prior to the study. All the enrolled patients gave their written informed consent.

2. Antiviral therapy and evaluation

All the enrolled patients with genotype 1 HCV infection received PegIFNα-2a/RBV at the dose of 180 μg per week plus RBV at the dairy dose of 600 to 1,000 mg based on body weights for 48 weeks. The dose of PegIFNα-2a or RBV was reduced according to the recommendations based on the clinical condition of individual patients.

The viral responses were defined as follows: rapid virological response (RVR), HCV RNA undetectable at week 4 after the therapy; early virological response (EVR), by either virus negativity or more than 2 log drop from the baseline level at week 12; end of treatment response (ETR), defined as HCV negativity at the end of treatment (EOT); SVR, HCV RNA undetectable at week 24 after EOT.

3. Laboratory assessment

Before treatment, all the patients underwent a 12-hour overnight fast before blood tests. Alanine aminotransferase (ALT), aspartate aminotransferase, total and direct bilirubin (TBIL and DBIL), total protein (TP), and albumin (ALB) were determined by an automatic biochemical analyzer.[23] HCV antibodies were analyzed by microsomal chemiluminescence (Abbott Diagnostics Division, Santa Clara, CA, USA) and HCV genotyping was performed by restriction fragment length polymorphism analysis.[24] Serum HCV RNA was measured using a real-time polymerase chain reaction (PCR) assay (COBAS Taqman HCV Test; Roche Molecular Systems Inc., Pleasanton, CA, USA). HCV RNA concentrations were measured at baseline, during regular treatment and at follow-up visit after the end of treatment.

4. Serum IP-10 measurements

Serum IP-10 concentrations were measured in samples collected at baseline (prior to treatment) using the Quantikine human IP-10 immunoassay (R&D Systems, Minneapolis, MN, USA). All blood samples were stored at –80°C for future assays. All samples were diluted 1:10 with Calibrator Diluent RD6Q solution and analyzed in duplicate. The linear dynamic range for IP-10 measurement by this assay is 7.8 to 500 pg/mL.

5. Testing for *IL28B* SNPs

The sequences were obtained from the NCBI Entrez SNP Database (http://www.ncbi.nlm.nih.gov/sites/entrez). The SNPs *rs*12979860 and *rs*8099917 in the region of the *IL28B* gene were analyzed by StepOnePlus Real-time PCR System (Applied Biosystems, Foster City, CA, USA), as described by Beinhardt *et al*.[19] Genomic DNA was isolated from ethylene diamine tetraacetic acid whole-blood samples with the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany).

6. Statistical analysis

Frequency was compared between the groups using the chi-square test. Results were expressed as mean±standard deviation and compared between the groups using analysis of variance and the Student t-test, or nonparametric Mann-Whitney U test

when it was used to identify significance differences between patients with different SNPs. Statistically significant difference was considered at a two-tailed p-value less than 0.05. Receiver operating characteristic (ROC) curve analysis was applied to obtain a certain optimal IP-10 levels to predict the treatment efficacy in genotype 1 HCV-infected patients. Multiple binary logistic regression was used to identify the risk factors related to SVR. All statistical analysis was conducted using Graphpad Prism 5.0 (Graphpad Software Inc., La Jolla, CA, USA).

RESULTS

1. Patients characteristics

After being enrolled in the present study, all the patients including 40 males and 32 females received PegIFNα-2a/RBV therapy for 48 weeks. All the patients were Chinese. Among them, 40 patients had RVR, 57 patients had EVR, 52 patients had ETR and 41 patients had SVR. Their viral responses of the patients at week 4, at week 12, at the end of the therapy, at week 24 after the end of treatment were shown in Table 1.

The baseline characteristics of the treated CHC patients with/without RVR were listed in Table 2. HCV RNA viral loads (log_{10}, IU/mL) of patients with RVR were significantly lower than those without RVR (HCV RNA, 5.56±1.18 vs 6.34±0.74; p=0.002). The baseline characteristics of the treated CHC patients with/without EVR had no significant difference (p>0.05) (data not shown). Serum ALT levels of patients with SVR were significantly higher than those without SVR (p=0.017). Patients carrying favorable *IL28B* SNPs genotype (*rs*12979860 CC and *rs*8099917 TT) had higher SVR than those carrying unfavorable variants (*rs*12979860 CT/TT and *rs*8099917 TG/GG) (*IL28B rs*12979860, p=0.002; *IL28B rs*8099917, p=0.020). Ages of patients with RVR/EVR/SVR were similar to those without SVR (p>0.05). No significant differences in gender, HCV RNA load, TBIL, DBIL, TP, ALB, body weight, and body mass index between patients with and without SVR were observed (p>0.05). The demographics of 72 CHC patients with/without SVR enrolled in the study were shown in Table 3.

Table 1. The Viral Responses of the Enrolled Patients Receiving Treatments

Viral response type	No.	Proportion, %
RVR/non-RVR	40/32	55.6/44.4
EVR/non-EVR	57/15	79.2/20.8
ETR/non-ETR	52/20	72.2/27.8
SVR/non-SVR	41/31	56.9/43.1

RVR, rapid virological response; EVR, early virological response; ETR, end of treatment response; SVR, sustained virological response.

2. Baseline characteristics of serum IP-10 levels

Pretreatment serum IP-10 levels were higher in female patients (481.62±45.03 pg/mL) than those of male patients (433.32±40.34 pg/mL), but the difference was not statistically significant (p>0.05) (Fig. 1A). We define HCV RNA ($log_{10,}$ IU/mL) higher than 6 as high virus loads and that lower than 6 as low virus loads. IP-10 levels at baseline were higher in patients with high virus loads (476.17±38.88 pg/mL) than in patients with low virus loads (428.44±47.25 pg/mL), but difference was not statistically significant either (p>0.05) (Fig. 1B).

Lower baseline serum IP-10 levels were observed in homozygous carriers of the favorable CC at *rs*12979860 (median, 387.5 pg/mL vs 551.0 pg/mL; p=0.0028) (Fig. 2A), TT at *rs*8099917 (median, 388.8 pg/mL vs 552.4 pg/mL; p=0.0065) (Fig. 2B), as

Table 2. The Demographics of 72 Chronic Hepatitis C Patients with Rapid Virological Response (RVR) and without RVR

Demographic	RVR	Non-RVR	p-value
No. of patients	40	32	-
Gender, male/female, %	55.00/45.00	56.25/43.75	0.916
Age, yr*	45.25±15.91	47.31±14.70	0.574
HCV RNA, log_{10}, IU/mL*	5.56±1.18	6.34±0.74	0.002
Body weight, kg*	66.61±12.68	69.41±10.56	0.321
BMI, kg/m^{2}*	23.42±3.29	24.28±2.51	0.225
ALT, IU/L†	54.0 (11.0, 416.0)	44.0 (10.0, 199.0)	0.316
AST, IU/L†	45.0 (16.0, 248.0)	33.0 (12.0, 222.0)	0.505
TBIL, μmol/L*	13.54±6.81	13.24±5.06	0.847
DBIL, μmol/L†	12.65 (5.0, 48.2)	4.13 (0.69, 13.80)	0.309
TP, g/L*	74.91±6.47	76.34±6.02	0.367
ALB, g/L*	42.87±3.59	43.07±4.38	0.831

HCV, hepatitis C virus; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; TP, total protein; ALB, albumin.
*Mean±SD; †Median (minimum, maximum).

Table 3. The Demographics of 72 Chronic Hepatitis C Patients with Sustained Virological Response (SVR) and without SVR

Demographic	SVR	Non-SVR	p-value
No. of patients	41	31	-
Gender, male/female, %	63.40/36.60	45.20/54.80	0.123
Age, yr*	45.27±15.57	47.35±15.14	0.571
HCV RNA, log_{10}, IU/mL*	5.73±1.19	6.13±0.86	0.125
Body weight, kg*	69.96±12.26	65.06±10.70	0.081
BMI, kg/m^2*	24.03±3.16	23.50±2.75	0.458
ALT, IU/L†	60.0 (11.0, 416.0)	40.0 (10.0, 139.0)	0.017
AST, IU/L†	49.0 (16.0, 248.0)	32.0 (16.0, 159)	0.045
TBIL, μmol/L*	13.93±6.71	12.60±5.08	0.387
DBIL, μmol/L†	3.71 (0.69, 22.09)	3.71 (0.48, 13.80)	0.398
TP, g/L*	74.68±7.06	76.77±4.68	0.187
ALB, g/L*	43.01±4.29	42.85±3.26	0.875
*IL-28B rs*12979860 CC	35	15	0.002
*IL-28B rs*12979860 CT/TT	6	16	
*IL-28B rs*8099917 TT	34	17	0.020
*IL-28B rs*8099917 TG/GG	7	14	

HCV, hepatitis C virus; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; TP, total protein; ALB, albumin.
*Mean±SD; †Median (minimum, maximum).

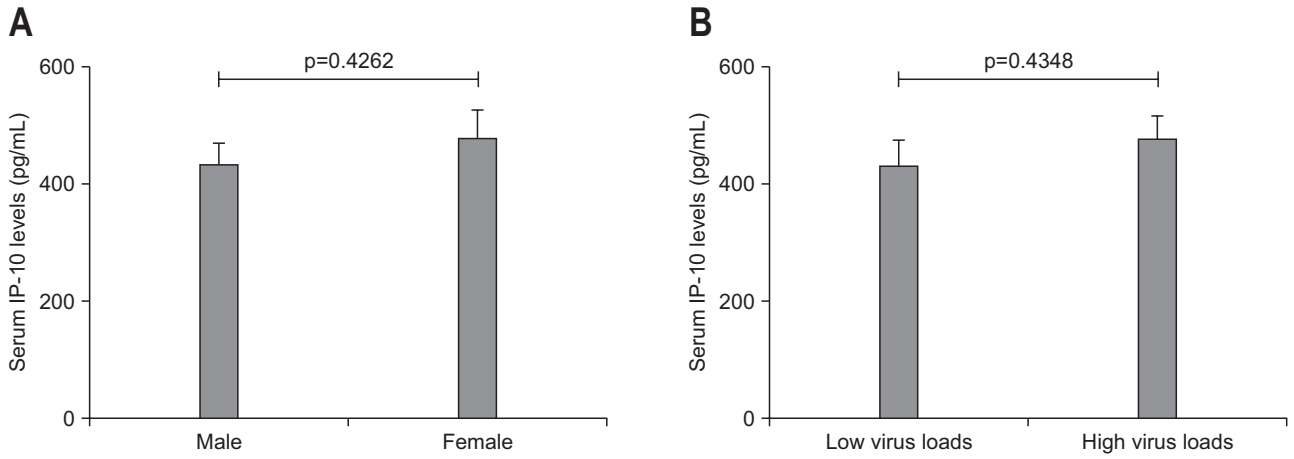

Fig. 1. Pretreatment serum interferon-γ-inducible-protein-10 (IP-10) levels according to patient characteristics. (A) Gender (male and female). (B) Hepatitis C virus loads (HCV RNA [log_{10}, IU/mL] higher than 6 as high virus loads and that lower than 6 as low virus loads).

compared with patients carrying unfavorable genotype, respectively.

3. Serum IP-10 levels and treatment responses

Mean IP-10 levels at baseline were significantly lower in patients with RVR than in those without RVR (RVR, 372.13±186.23 pg/mL vs 599.24±264.69 pg/mL; p=0.001) (Fig. 3A). Pretreatment IP-10 levels were also significantly lower in patients with EVR than in those without EVR (EVR, 420.51±215.18 pg/mL vs 583.49±294.94 pg/mL; p=0.024) (Fig. 3B). Baseline IP-10 levels were elevated in the genotype 1 HCV infected patients without SVR after the completion of therapy (SVR, 384.33±206.10 pg/mL vs 544.09±256.43 pg/mL, p=0.007) (Fig. 3D). However, no significant difference in basal IP-10 levels was detected between the genotype 1 HCV infected patients with and without ETR (ETR, 422.27±221.87 pg/mL vs 536.33±273.63 pg/mL; p=0.083) (Fig. 3C).

4. ROC curve analysis of serum IP-10 levels

ROC curve analysis was conducted to achieve the optimal

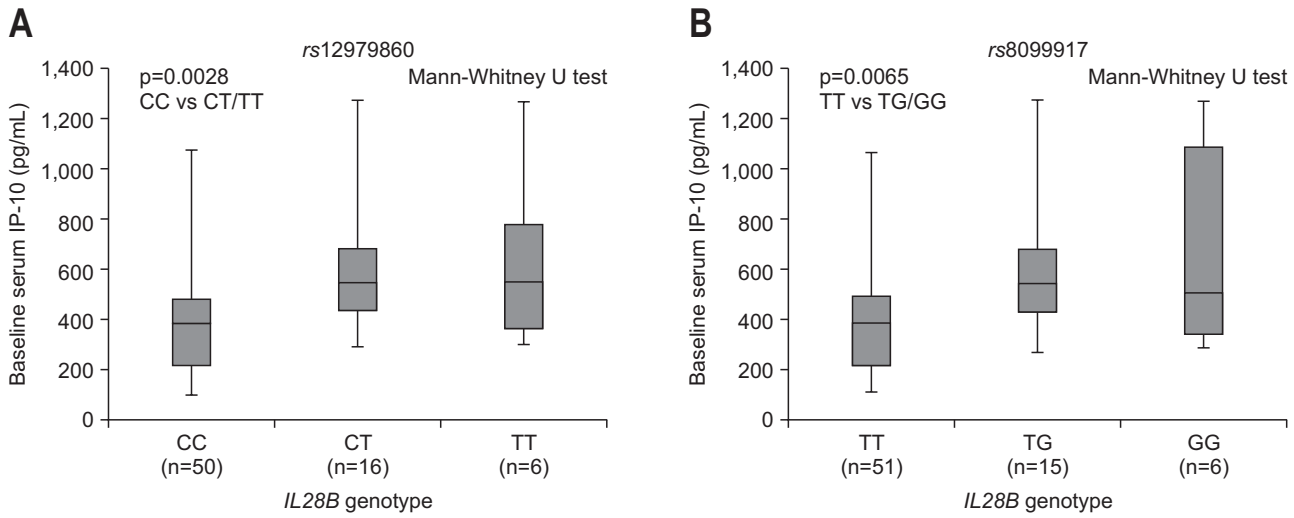

Fig. 2. Baseline serum interferon-γ-inducible-protein-10 (IP-10) levels in relation to the *IL28B* variant. (A) Baseline serum IP-10 levels in relation to *IL28B rs*12979860. (B) Baseline serum IP-10 levels in relation to *IL28B rs*8099917.

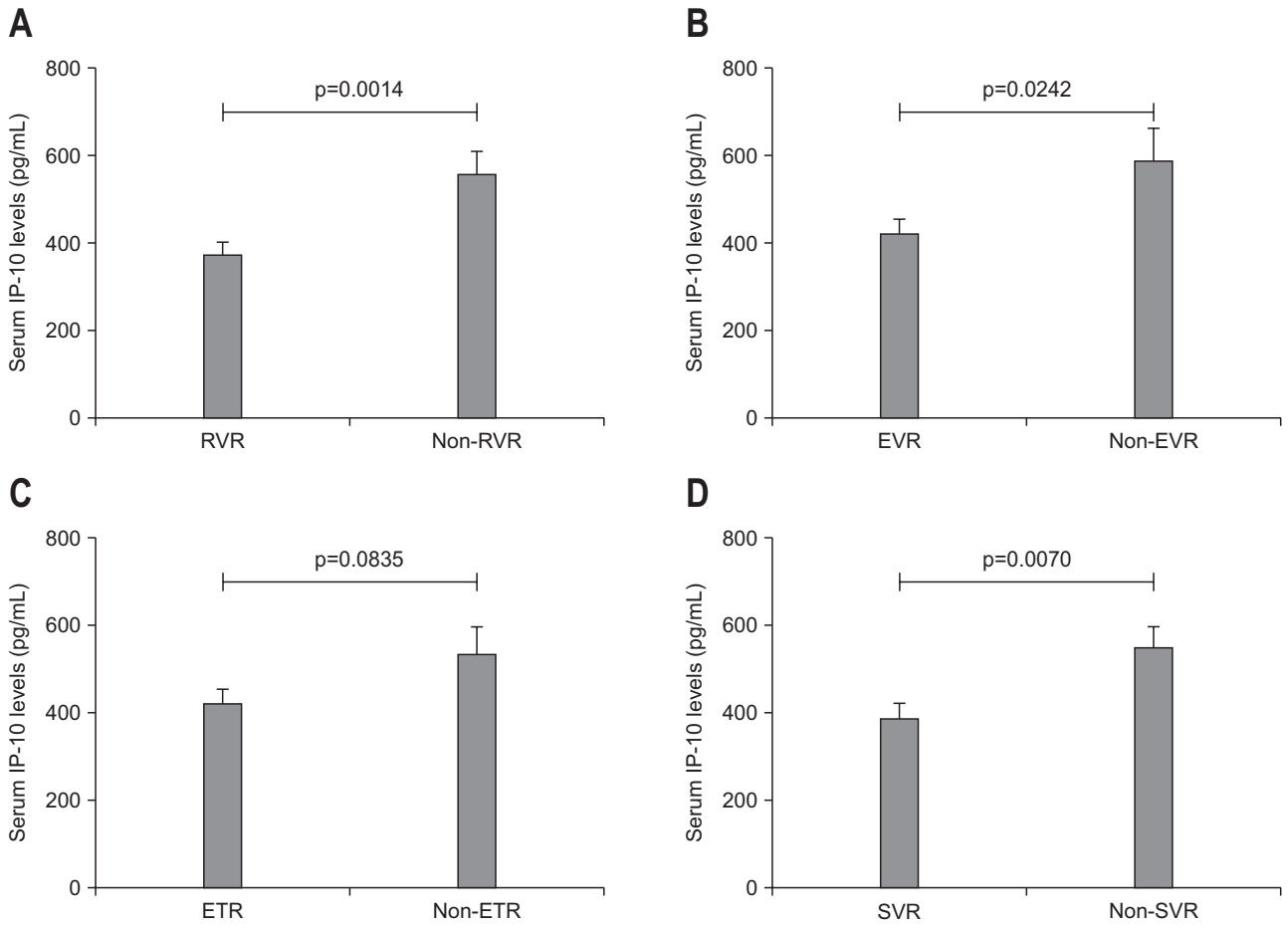

Fig. 3. Pretreatment serum interferon-γ-inducible-protein-10 (IP-10) levels according to different viral responses. (A) Rapid virological response (RVR) vs non-RVR. (B) Early virological response (EVR) vs non-EVR. (C) End of treatment response (ETR) vs non-ETR. (D) Sustained virological response (SVR) vs non-SVR.

Table 4. Receiver Operating Characteristic Analysis of Interferon-γ-Inducible-Protein-10 Levels in Genotype 1 Hepatitis C Virus-Infected Patients

Variable	RVR	EVR	SVR
Area	0.72	0.67	0.70
SE	0.064	0.086	0.066
95% CI	0.59–0.84	0.50–0.84	0.56–0.82
p-value	0.003	0.053	0.008
Sensitivity, %	66.67	33.33	66.67
Specificity, %	68.97	85.71	68.97
Cutoff value	426.7	303.1	426.7

RVR, rapid virological response; EVR, early virological response; SVR, sustained virological response; SE, standard error; CI, confidence interval.

cutoff value of pretreatment serum IP-10 levels to predict the treatment efficacy of PegIFNα-2a/RBV. The results indicated that IP-10 cutoff value at 426.7 pg/mL was used for distinguishing patients with high possibility of RVR/SVR from those with low possibility of RVR/SVR (Table 4, Fig. 4). Among 39 patients with pretreatment serum IP-10 levels lower than 426.7 pg/mL, 28 patients (75.7%) had RVR after week 4 treatment; and 30 patients (76.9%) had received SVR after the treatment. The calculated predictive values of IP-10 for RVR were 66.67% for sensitivity and 68.97% for specificity. For SVR, the prognostic sensitivity was 66.67% and the specificity was 68.97%. Positive predictive value (PPV) and negative predictive value (NPV) for RVR were achieved as 75.7% and 69.0%, respectively. PPV and NPV for SVR were 77.0% and 71.0%, respectively.

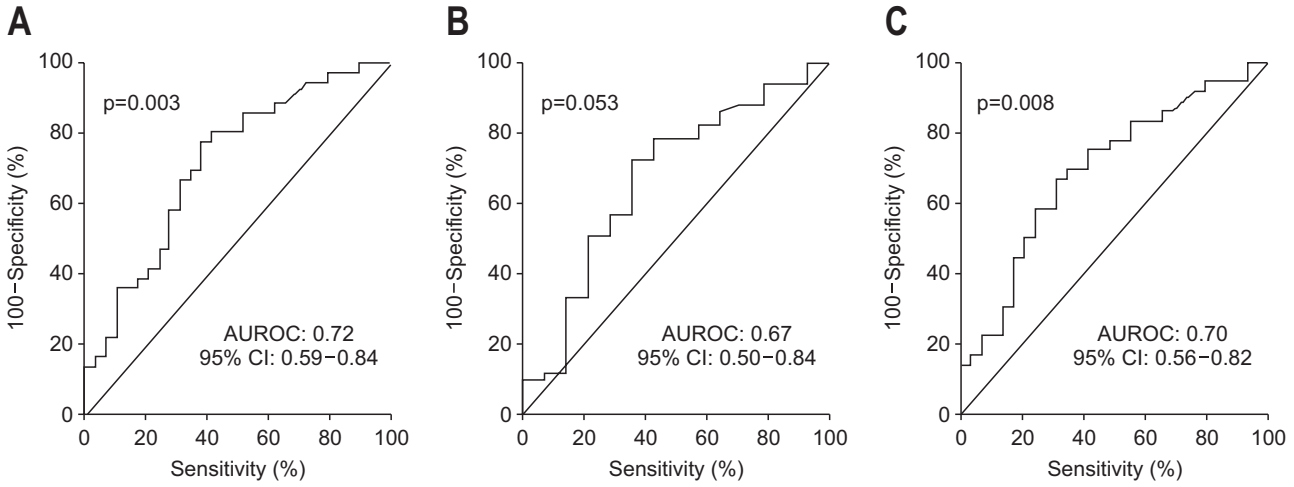

Fig. 4. Receiver operating characteristic (ROC) analysis of interferon-γ-inducible-protein-10 (IP-10) levels in genotype 1 hepatitis C virus-infected patients. (A) Pretreatment serum IP-10 levels to predict rapid virological response. (B) Pretreatment serum IP-10 levels to predict early virological response. (C) Pretreatment serum IP-10 levels to predict sustained virological response.
AUROC, area under receiver operating characteristic; CI, confidence interval.

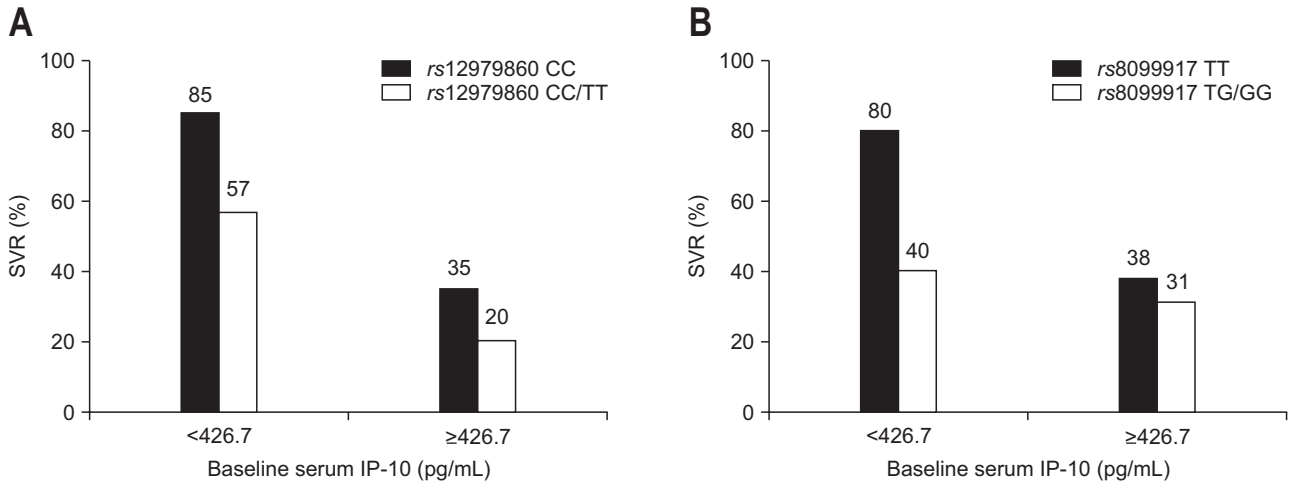

Fig. 5. Percentage of patients with sustained virological response (SVR) in relation to interferon-γ-inducible-protein-10 (IP-10) and the *IL28* genotype. (A) Percentages of patients with IP-10 levels in relation to *IL28B rs*12979860 (CC vs CT/TT). (B) Percentages of patients with IP-10 levels in relation to *IL28B rs*8099917 (TT vs TG/GG).

5. Association of *IL28B* genotypes and baseline serum IP-10 levels with treatment response

In 72 patients, measurement of IP-10 levels and determination of *IL28B* SNPs were available. The predictive value of the combination of IP-10 levels with *IL28B* genotypes was significant with SVR rates of 85% (28/33) in *rs*12979860 CC patients with IP-10 levels <426.7 pg/mL and 35% (6/17) in CC patients with IP-10 levels ≥426.7 pg/mL (p<0.001) (Fig. 5A). In *rs*12979860 CT/TT patients, SVR rates of patients with IP-10 levels <426.7 pg/mL was 57% (4/7), while in CT/TT patients with baseline IP-10 levels ≥426.7 pg/mL achieved SVR rates of 20% (3/15) (p=0.081) (Fig. 5A).

In patients with an IP-10 level <426.7 pg/mL plus *IL28B rs*8099917 TT genotype, 28 of 35 (80%) had achieved SVR compared with seven of 35 (20%) who did not. In contrast, only six of 16 patients (38%) with IP-10 level ≥426.7 pg/mL plus *IL28B rs*8099917 TT genotype achieved SVR (p=0.008) (Fig. 5B). In TG/GG patients, 40% (2/5) achieved SVR if IP-10 levels were <426.7 pg/mL, whereas in only 31% (5/16) SVR was observed if IP-10 levels were ≥426.7 pg/mL (p=0.717) (Fig. 5B).

Using a predictive model including both *IL28B* SNPs and baseline serum IP-10 levels with distinct chances of virology response could be clearly identified: PPV of the combination of IP-10 levels <426.7 pg/mL and *IL28B rs*12979860 CC genotype was 85%, and NPV of IP-10 level ≥426.7 pg/mL and *IL28B rs*12979860 CC was 64.7%. PPV of the combination of IP-10 levels <426.7 pg/mL and *IL28B rs*8099917 TT genotype was 80%, and NPV of IP-10 level ≥426.7 pg/mL and *IL28B rs*8099917 TT was 62.5%.

6. Parameters associated with SVR on multivariate analysis

Some parameters were significant associated with SVR in univariate analysis as shown in Table 3. When performing the multivariate logistic regression to identify factors predictive of achieving SVR, serum baseline IP-10 levels (p=0.032), CC genotype at *IL28B rs*12979860 (p=0.005), TT genotype at *IL28B rs*8099917 (p=0.048) were independent predictors of SVR.

DISCUSSION

In the present study, baseline serum IP-10 levels lower than 426.7 pg/mL can be used to predict RVR/SVR in genotype 1 HCV infected patients. We demonstrated that the combination of baseline serum IP-10 levels and *IL28B* SNPs increase predictability of SVR rates in patients with genotype 1 HCV infection. Our results have also demonstrated that baseline serum IP-10 levels are associated with RVR, EVR, and SVR, but not with ETR in genotype 1 HCV-infected patients treated with PegIFNα-2a/RBV. ROC curve analysis has shown that with serum baseline IP-10 levels, CC genotype at *IL28B rs*12979860 and TT genotype at *IL28B rs*8099917 were independent predictive factors of SVR.

Chemokines could regulate inflammation and immunity during HCV infection. They also played critical roles in the eradication of HCV during interferon (IFN)-based treatment.[25] The expression of CXC chemokines was closely linked to the outcomes of antiviral therapy in CHC patients.[12,26] IP-10 is an IFN-inducible chemokine that binds to chemokine receptor, CXCR3, on lymphoid cells for driving them to inflammatory sites.[27] Recent studies have shown that CXCR3 ligands were elevated in livers and sera of CHC patients, and IP-10 secreted by monocytes, and fibroblasts in response to IFN-γ was correlated with treatment responses, thus attracting CXCR3-expressing cells on the surface.[11,28-30] Therefore, it potentially contributes to the host immune response against HCV, as well as to the progression of liver diseases.[31] Previous study showed that serum IP-10 concentration was correlated with the severity of liver histology, stages 3/4 of fibrosis, in genotype 1 HCV infection.[32,33] Therefore, we chose the patients without fibrosis/cirrhosis to exterminate the effects of cirrhosis on serum IP-10 levels.

Besides, pretreatment plasma levels of IP-10 were elevated in genotype 1 HCV-infected patients without SVR after completion of antiviral therapy.[11,26,33,34] Previous studies have shown that pretreatment IP-10 levels may be lower in Japanese than in African Americans or Caucasian Americans. It was not surprising that the cutoff values of IP-10 levels for predicting SVR varied from 150 to 600 pg/mL in different studies.[9,15,16,33] Therefore, cutoff value of IP-10 with the best predictive performance should be "individualized" in regions with different races and HCV epidemiology. Besides, differences in the reagents and type of assay that is used from laboratory to laboratory also could explain "individualized," this may be as important as any potential ethnic variation in the levels.

Quantitation of serum IP-10 levels may differentiate antiviral response. This study has indicated that, pretreatment serum IP-10 levels were associated with RVR, EVR, and SVR, but not with ETR in genotype 1 HCV-infected patients during the treatment with PegIFNα-2a/RBV. Our results were consistent with those reported by Matsuura *et al*.,[16] which suggest that pretreatment serum IP-10 levels were associated with treatment efficacy of PegIFNα-2a/RBV and the early viral kinetics of HCV in PegIFNα-2a/RBV therapy. The difference between two studies is that in our experiments the interference of genotypes is excluded, and we have only selected patients with genotype 1 HCV infection. Additionally, some studies have investigated the associations among IP-10 level, RVR, EVR, and ETR.[16,35] Feld *et al*.[35] have observed the difference in pretreatment IP-10 levels between patients with and without EVR, ETR or SVR in the whole cohort or in patients with genotype 1 infection only. Due to the treatment regimen, in fact, in HCV patients treated with IFN, a low pretreatment IP-10 levels was significantly associated with low basal viral load, RVR, and SVR, which do not affect the role of the pretreatment IP-10 levels in treatment efficacy.[9-11,14]

We have demonstrated that patients with lower pretreatment

serum IP-10 level were much more likely to achieve RVR/SVR. In order to achieve the optimal cutoff value of serum IP-10 levels for prognosticating the curative effect of antiviral therapy, we have conducted a ROC analysis. According to the results, serum IP-10 levels at baseline were correlated with the efficacy of antiviral therapy at week 4 and at week 24 after EOT. Among 39 patients with serum IP-10 levels lower than 426.7 pg/mL at baseline, 28 patients (75.7%) presented RVR after week 4 treatment; 30 patients (76.9%) presented SVR after the treatment. For SVR, the prognostic sensitivity was 66.67% and the specificity was 68.97%. Darling *et al*.[15] have reported that pretreatment serum IP-10 was an independent predictive factor for SVR in PegIFNα-2a/RBV therapy. SVR rates revealed significant difference according to pretreatment serum IP-10 levels. Other studies have shown that *IL28B* genotype and pretreatment serum IP-10 concentrations were associated with early viral kinetics of HCV, the first phase decline or RVR, as well as SVR in PegIFNα-2a/RBV therapy.[20,36]

In the present study, lower baseline serum IP-10 levels were observed in homozygous carriers of the favorable CC at *rs*12979860, TT at *rs*8099917, as compared with patients carrying unfavorable genotype, respectively. It implicates that IP-10 levels were related with *IL28B* SNPs. As well known, baseline hepatic ISGs levels significantly affect the outcomes of IFN-based therapy in CHC, that is, "preactivation" of ISGs lead to an unfavorable virological responses, which might be associated with either unfavorable *IL28B* genotype or HCV genotype 1.[37] Maybe IP-10 was just a marker of ISG preactivation and it could reflect *IL28B* genotypes, but the mechanism still needed to be further investigated.

Polymorphisms of *IL28B* gene are highly associated with SVR in patients with CHC treated with peginterferon and ribavirin. Regardless of race, carriage of the C allele in *rs*12979860 increases treatment response rates with those who have the CC genotype having the highest SVR rates. *IL28B* SNPs is the strongest pretreatment predictor of SVR in chronic genotype 1 HCV infection.[17] Wang *et al*.[38] have reported that IP-10 levels of patients with genotype 1 or 2 HCV in China were independently predictive for SVR with cutoff values of 250.60 pg/mL at baseline. IP-10 levels at baseline were predictive of SVR and improved predictive performances of *IL28B* genotypes for SVR. But the predictive value of IP-10 only in genotype 1 HCV patients in China is not yet clear.

As for the predictive performance, further analyses found that combination of baseline IP-10 level helped in predicting SVR in patient with favorable *IL28B* genotype. Using a predictive model including both *IL28B* SNPs and baseline serum IP-10 levels with distinct chances of virology response could be clearly identified: PPV of the combination of IP-10 levels <426.7 pg/mL and *IL28B rs*12979860 CC genotype was 85%, and NPV of IP-10 level ≥426.7 pg/mL and *IL28B rs*12979860 CC was 64.7%. PPV of the combination of IP-10 levels <426.7 pg/mL and *IL28B rs*8099917 TT genotype was 80%, and NPV of IP-10 level ≥426.7 pg/mL and *IL28B rs*8099917 TT was 62.5%. However, PPV and NPV of baseline serum IP-10 levels for SVR were 77.0% and 71.0%, respectively. Therefore, when combining both baseline IP-10 and *IL28B rs*12979860/*rs*8099917 SNPs, the prediction of SVR seems to be better than when using predictive factor alone, it could improve the prediction of SVR in favorable allele carriers of *IL28B*, *rs*12979860 CC and *rs*8099917 TT. Combination of IP-10 level with *IL28B* genotypes at baseline makes an improvement of predictive performance of SVR.

Factors independently associated with a favorable treatment include serum HCV-RNA levels lower than 2 million copies/mL (approximately 800,000 IU/mL), body weight lower than 75 kg, age younger than 40 years old, the absence of bridging fibrosis or cirrhosis in liver biopsy before treatment, *IL28B* SNPs and a favorable initial viral kinetic response.[6,7,39,40] According to the above-mentioned response rates, serum baseline IP-10 levels and *IL28B* genotypes were independent predictors of SVR.

A limitation of this study was the relative small number of enrolled patients. Furthermore, our findings need to be confirmed by further studies using much larger sample size.

In conclusion, our study shows that the combination of baseline serum IP-10 levels and the determination of *IL28B* SNPs increase predictability of SVR rates in patients infected with genotype 1 HCV and treated with PegIFNα-2a/RBV in China.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

This work was supported by the 12th Five-Year Plan (NO 2012ZX10002 003-004-003, NO 2013 ZX10002004-001-003); the National Natural Science Foundation of China (grant number: 81373056).

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