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Association of Serum Cytokine Levels with Treatment Response to Pegylated Interferon and Ribavirin Therapy in Genotype 1 Chronic Hepatitis C Patients

Running title: Cytokines and antiviral therapy in HCV

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Abstract

Background. We sought to clarify the associations among serum cytokines, amino acid substitutions in the interferon sensitivity determining region (ISDR) and core region, and treatment outcome of pegylated interferon and ribavirin therapy in genotype 1 hepatitis C virus (HCV)-infected patients.

Methods. A total of 8 serum cytokines were quantified before, during, and after treatment in 79 genotype 1 chronic HCV patients. Viral ISDR and core region variants were determined by direct sequencing.

Results. High levels of IL-12 and IL-18 and more than two mutations in the ISDR were associated with a sustained virological response (SVR). Conversely, high baseline IL-10 levels and glutamine at amino acid 70 of the HCV core protein (Gln70) were significantly associated with a nonresponse to treatment, and patients with Gln70 had significantly higher IL-10 levels. In multivariate analysis, low IL-10, high IL-12, and high IL-18 levels were independently associated with a SVR. These cytokines were all decreased 4 weeks into treatment and remained low in patients with a SVR.

Conclusion. Serum IL-10, IL-12, and IL-18 levels are predictive of the response to HCV treatment with pegylated interferon and ribavirin and are associated with amino acid substitutions in the ISDR and core region.

Keywords: IL-10, IL-12, IL-18, pegylated interferon, ribavirin, SVR, ISDR, core

Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease worldwide. More than half of patients with acute HCV infections develop chronic hepatitis, which leads to liver cirrhosis and/or hepatocellular carcinoma (HCC) in at least 20% of cases [1-2]. HCC is ranked fourth in men and fifth in women as a cause of death from malignant neoplasms in Japan [3-4]. Since approximately 70-80% of Japanese HCC patients are infected with HCV, viral eradication is important to decrease the incidence of HCC. Interferon-based therapy can reduce HCV to undetectable levels and improve prognosis. The primary aim of antiviral therapy in HCV patients is a sustained virological response (SVR), which is defined as undetectable serum HCV RNA 24 weeks after completion of therapy. Despite recent advances, however, approximately 50% of patients with genotype 1 HCV infection do not achieve a SVR by antiviral therapy [5-6].

Cytokines play an important role in the pathogenesis, progression, and treatment outcome of HCV infection. As the control of cytokine production is highly complex and the effects of cytokines are widespread throughout multiple regulatory networks, it would seem that screening for multiple biomarkers could best clarify the immunopathogenesis of the disease and predict responses to antiviral therapy. However, such analysis is difficult using ELISA, which requires each biomarker be tested individually. In the present study, we used a new broad-spectrum bead-based multiplex immunoassay to simultaneously test multiple factors in the sera of patients with chronic hepatitis C. Wan *et al.* recently reported that some cytokines are elevated in non-SVR HCV patients using this bead system, but only 17 patients with genotype 1 were evaluated [7]. Thus, the association between multiple cytokines and treatment outcome are largely unknown.

The objective of this study was to determine which cytokines in patients with genotype 1 chronic hepatitis C relate to the clinical and virologic characteristics of hepatitis and how they affect the HCV response to pegylated interferon (PEG-IFN) and ribavirin therapy.

Patients and Methods

Subjects.

Seventy-nine consecutive patients with genotype 1 chronic hepatitis C were

included in this study. Diagnosis of chronic hepatitis C was based on the following criteria as reported previously [8]: 1) presence of serum HCV antibodies and detectable viral RNA; 2) absence of detectable hepatitis B surface antigen; and 3) exclusion of other causes of chronic liver disease. No patients had a history of or developed decompensated cirrhosis or hepatocellular carcinoma. The baseline characteristics of patients are shown in Table 1. A group of 26 healthy individuals with hepatitis B- and C-negative serology and normal transaminases was used as the control. All subjects were negative for the antibody to the human immunodeficiency virus. The protocol of this study was approved by the ethics committee of Shinshu University School of Medicine and all patients provided written informed consent.

Laboratory Testing.

Antibodies to HCV were measured in serum samples via third-generation enzyme-linked immunosorbent assays (EIA-3; Abbott Laboratories, North Chicago, IL). Serum levels of HCV RNA were determined using the COBAS AMPLICOR assays (Roche Diagnostic Systems, Tokyo, Japan), which amplify HCV RNA by reverse-transcription-polymerase chain reaction. The lower limit of the assay was 50 IU/mL. HCV genotypes were determined using INNO-LiPA HCV II (Innogenetics, Gent, Belgium). All patients in our test cohort were infected with genotype 1b. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and other relevant biochemical tests were performed using standard methods [9].

Antiviral Therapy.

All patients received body weight-adjusted PEG-IFNα-2b (PegIntron, Schering-Plough K.K., Tokyo, Japan; 45 kg or less, 60 μg/dose; 46 to 60 kg, 80 μg/dose; 61 to 75 kg, 100 μg/dose; 76 to 90 kg, 120 μg/dose; 91 kg or more, 150 μg/dose), and ribavirin (Rebetol, Schering-Plough K.K.; 60 kg or less, 600 mg/day; 61 kg to 80 kg, 800 mg/day; 81 kg or more, 1,000 mg/day) for 48 weeks, as reported previously [10].

Definition of Viral Kinetic Response and Treatment Outcome.

An early virological response (EVR) was defined as undetectable serum HCV

RNA by 12 weeks of therapy. A SVR was classified as serum HCV RNA that was undetectable 24 weeks after completing therapy. Post-treatment relapse was defined as a reappearance of serum HCV RNA after treatment in patients whose HCV RNA level was undetectable during or at the completion of therapy. A nonresponse was defined as a decrease in HCV RNA of <2 log copies/mL at week 12 and detectable HCV RNA during the treatment course.

Detection of amino acid substitutions in the core and NS5A regions.

The sequence of 1-191 amino acids (aa) in the core protein of genotype 1b HCV was determined, and substitutions at aa70 of arginine (Arg70) or glutamine (Gln70) were evaluated [11] with the use of HCV-J as a reference [12]. The sequence of 2209-2248 aa in the NS5A region of genotype 1b HCV containing the interferon-sensitivity determining region (ISDR) was also determined, and the number of aa substitutions in the ISDR was defined as wild-type (0), intermediate-type (1), or mutant-type (≥2) [13]. All aa substitutions in the core region and ISDR were determined by direct sequencing.

Detection of Cytokines.

Thirteen cytokines (IL-2, IL-4, IL-6, IL-10, IL-12p40, IL-12p70, IL-18, and vascular endothelial growth factor [VEGF]) were quantified using Luminex® Multiplex Cytokine Kits (Procarta Cytokine assay kit) for serum samples obtained before the start of treatment, 4 weeks after the start of treatment, and 24 weeks after treatment completion. All collected samples were immediately stored at -70°C before testing.

Statistical Analysis.

The Mann-Whitney *U* test and Kruskal-Wallis test were used to analyze continuous variables where appropriate. The Friedman test was used to evaluate changes in serum cytokine levels over time. Spearman's rank order correlations were used to evaluate the relationship between pairs of markers. The chi-square test with Yate's correction was used for the analysis of categorical data. In cases where the number of subjects was less than 5, Fisher's exact test was used. A P value of ≤ 0.05 was considered statistically significant. To predict treatment outcome, cutoff points for

continuous variables were decided by receiver-operating characteristic (ROC) curve analysis. Multivariate analysis was performed using a stepwise logistic regression model. Statistical analyses were performed using SPSS software version 18.0J (SPSS, Chicago, IL).

Results

Detection and quantification of serum markers in patients with chronic hepatitis C and controls.

Of the 79 patients receiving PEG-IFN and ribavirin therapy, 31 (39%) were sustained responders with accompanying normalization of ALT levels. Among the 48 patients without a SVR, 23 had a relapse and 25 did not respond to treatment. Patients with a SVR had a higher male ratio compared to patients without (P = 0.001) (Table 1). Before treatment, the median AST in the SVR group was significantly lower than that in the non-SVR group (36 vs. 48 IU/L; P = 0.012). Substitutions of aa 70 in the core region (P = 0.028) and in the ISDR (P = 0.026) were both significantly associated with treatment outcome.

Serum samples obtained prior to antiviral therapy were examined for the presence of 8 cytokines by multiplex assays. Of these, 6 could be reliably quantified in a large majority of samples. As shown in Figure 1, the median baseline serum concentrations of 4 cytokines [IL-10 (4.8 vs. 4.3 pg/mL; P = 0.032), IL-12p40 (20.4 vs. 8.5 pg/mL; P < 0.001), IL-12p70 (12.8 vs. 1.0 pg/mL; P < 0.001), and IL-18 (21.9 vs. 14.5 pg/mL; P = 0.008)] were significantly higher in patients with chronic hepatitis C than in healthy controls. Conversely, serum levels of IL-4 (7.3 vs. 7.9 pg/mL; P = 0.011) and VEGF (57.5 vs. 78.0 pg/mL; P = 0.025) were significantly lower in patients with HCV infection compared with controls.

Effects of anti-viral therapy on serum cytokine levels.

The median baseline serum levels of 4 cytokines [IL-12p40 (24.1 vs. 17.2 pg/mL; P = 0.003], IL-12p70 (15.9 vs. 12.6 pg/mL; P < 0.001), IL-18 (27.9 vs. 17.7 pg/mL; P = 0.001), and VEGF (93.0 vs. 39.7 pg/mL; P < 0.001)] were significantly higher in patients who achieved a SVR than in those who did not (Figure 2). In contrast, SVR patients

showed significantly lower baseline IL-10 concentrations (4.1 pg/mL) than non-SVR patients (7.3 pg/mL; $P = 0.002$).

Significantly higher baseline levels of 3 cytokines [IL-4 (7.8 vs. 7.0 pg/mL; $P = 0.001$), IL-12p40 (24.1 vs. 14.6 pg/mL; $P < 0.001$), and VEGF (65.5 vs. 43.0 pg/mL; $P = 0.025$)] were observed in patients with a virological response compared to those without. Conversely, IL-10 levels (4.3 vs. 7.9 pg/mL; $P < 0.001$) were significantly lower in virological responders compared to nonresponders.

Several demographic (age and sex) and clinical (ALT, AST, and viral load) findings were examined for their correlation with serum cytokines in patients with HCV infection, but no significant associations were observed. However, serum IL-12p40 levels were significantly correlated with serum IL-18 ($P = 0.004$, $r = 0.325$) (Figure 3A) and VEGF ($P = 0.024$, $r = 0.253$) (Figure 3B). There was also a significant correlation between IL-18 and VEGF ($P < 0.001$, $r = 0.394$) (Figure 3C).

Prediction of treatment outcome in patients with chronic hepatitis C.

ROC curve analyses were performed to determine the optimal cutoff values for serum cytokines in predicting treatment outcome for genotype 1 HCV-infected patients. The ROC curve for serum IL-10 was obtained via calculations using the values obtained from 25 nonresponders and 54 patients with a virological response. The ROC curves for serum IL-12p40, IL-18, and VEGF were obtained from 31 patients who achieved a SVR and 48 non-SVR patients. Selection of optimal cutoff point values was based on the cytokine level at which accuracy was maximal. The optimal cutoff value, sensitivity, specificity, positive predictive value, negative predictive value, and calculated AUC for the 4 cytokines are listed in Table 2. The AUC values were consistently high and ranged between 0.70 (IL-12p40) and 0.86 (IL-10).

In addition, ROC curves for serum IL-10, IL-12p40, IL-18, and VEGF at 4 weeks after the start of treatment were obtained (Table 2). The AUCs for these 4 cytokines (0.62-0.86) were also high, but lower than those at baseline.

Correlation between core region and ISDR amino acid substitutions and cytokine production.

Since core region and ISDR substitutions have been associated with treatment outcome both in this study and elsewhere, we analyzed whether substitutions in these regions were correlated with baseline serum cytokine concentrations as well. Before treatment, median IL-10 levels in patients with Gln70 (7.5 pg/mL) were significantly higher than those in patients with Arg70 (4.3 pg/mL; $P = 0.045$). The prevalence of higher serum IL-10 (≥ 5.0 pg/mL at baseline) was significantly greater in the nonresponse group than in the response group [25/25 (100%) vs. 11/50 (22%); $P < 0.001$]. The frequency of the combination of higher IL-10 and HCV with and without core Gln70 was 14/25 (56%) and 3/50 (6%), respectively, which was statistically significant ($P < 0.001$).

Serum levels of IL-12p70 were significantly correlated with the number of substitutions in the ISDR (Kruskal-Wallis; $P = 0.027$). In addition, median baseline serum IL-12p70 levels were significantly higher in patients with mutant-type ISDR than in those with wild or intermediate types (15.6 vs. 12.7 pg/mL; $P = 0.009$).

Factors independently associated with a SVR

Several factors found in association with a SVR from PEG-IFN and ribavirin therapy were evaluated for their independence by multivariate analysis (Table 3). Male (odds ratio 10.93 [95% confidence interval 2.18-54.87], $P = 0.004$), AST ≥ 40 IL/L (0.95 [0.91-0.99], $P = 0.013$), IL-10 ≥ 5.0 pg/mL (0.82 [0.70-0.96], $P = 0.014$), IL-12p40 ≥ 17.4 pg/mL (1.07 [1.01-1.14], $P = 0.024$), and IL-18 ≥ 15.4 pg/mL (1.09 [1.02-1.15], $P = 0.006$) were independent risk factors related to a SVR. Conversely, core region or ISDR substitutions were not significant independent associations in this study.

Serum cytokine changes during and after treatment.

We next measured cytokine levels 4 weeks after the initiation of therapy and 6 months after its completion (Table 4). The levels of IL-10 ($P < 0.001$, Friedman test), IL-12p40 ($P = 0.008$), and IL-18 ($P < 0.001$) were significantly decreased in samples collected from patients who achieved a SVR. The reduction in serum cytokine levels from baseline to 4 weeks of treatment was determined and compared between SVR and non-SVR groups, and showed that the ratio of IL-10 had a significant negative association with both an EVR ($P = 0.024$) and SVR ($P = 0.001$).

Discussion

In this study, we measured the levels of 8 cytokines in patients with genotype 1 chronic hepatitis C and analyzed their association with the outcome of PEG-IFN and ribavirin therapy using a newly developed bead-array multiplex system. Serum IL-10, IL-12p40, IL-12p70, and IL-18 were higher in patients with HCV infection than in healthy subjects. In addition, cytokines IL-10, IL-12p40, and IL-18 all decreased during treatment and remained low in patients with a SVR. These findings suggest that cytokines may in fact compromise host immune responses to the virus.

A strong association between high baseline serum IL-10 and a nonresponse to PEG-IFN and ribavirin therapy was found in our cohort, which is consistent with previous studies [7, 14-15]. Achievement of an EVR or SVR was found to be diminished in patients who had a lower IL-10 ratio between baseline and 4 weeks of treatment. In addition, sensitivity, specificity, and AUC were all high for IL-10 using ROC curve analysis, suggesting that serum IL-10 values at baseline and 4 weeks of treatment are predictive markers for treatment nonresponse (Table 2). Although humoral immunity is said to play a minor role in recovery from HCV infection and B-cell immunity is strongest in those with persistent infection [8, 16], a strong natural killer cell- and Th1 cell-mediated immune response seems to be a key factor in protection from HCV infection. IL-10 was originally described as a cytokine synthesis inhibitory factor [17-18], but recent studies have demonstrated that IL-10 produced by Th17 cells restrains the pathologic effects of Th17 [19-20]. Furthermore, there is strong evidence of a substantial genetic component to IL-10 production [21-22]; -1082 G/G is known to be related to increased IL-10 production and this genotype is associated with a high risk of inefficient HCV clearance [23-24] and resistance to IFN treatment [25-28].

In agreement with our findings, recent studies have indicated that Gln70 substitutions in the HCV core region are associated with treatment failure [11, 29-32]. Additionally, patients with Gln70 had higher IL-10 levels compared to those with Arg70. Among the 28 HCV patients who had Gln70, all 14 nonresponders had higher IL-10 (≥ 5.0 pg/mL), while 11 of 14 responders had lower IL-10 levels (P < 0.001). This association between Gln70 and elevated IL-10 levels is intriguing. Dolganiuc *et al.*

reported that HCV core and NS3 proteins in monocytes and dendritic cells induce IL-10 [33], so further studies are needed to clarify the relationship between IL-10 and core region amino acid substitutions.

This report demonstrates the beneficial role of IL-12 in achieving a SVR during PEG-IFN and ribavirin therapy. IL-12 is a proinflammatory cytokine that promotes the differentiation of Th1 cells, suppresses Th2 function, and amplifies the cytotoxicity of cytotoxic T lymphocytes and natural killer cells [34]. Thus, production of IL-12 is directed towards the elimination of intracellular pathogens and viruses. Elevated serum IL-12 has been noted in patients with chronic HBV or HCV infection, and is even more prominent among responders to IFN-α treatment [35-36]. In our study, significantly higher serum IL-12p70 was noted in subjects carrying mutant-type ISDR than in those with intermediate- or wild-type ISDR. This correlation between IL-12 and ISDR substitutions is striking, and requires further study to verify its favorable effect during PEG-IFN and ribavirin therapy.

It is believed that the dynamics of the Th1/Th2 response determine the outcome of antiviral therapy to chronic hepatitis C [10] and that IL-18 is an important mediator of the Th1/Th2 balance. IL-18 plays a critical role in host defense against infection by intracellular microbes, but on the other hand, induces autoimmune diseases and propagates inflammation [37]. IL-18 is significantly up-regulated in patients with chronic HCV infection and is correlated with hepatic injury [38-39], indicating a key role in disease pathogenesis. However, the effect of IL-18 on antiviral therapy for chronic hepatitis C is still unclear. We found that IL-18 levels were significantly higher in patients with chronic HCV infection compared with healthy controls, but were also higher at baseline in patients who achieved a SVR than in those who did not. In addition, there was a significant correlation between IL-18 and IL-12; in the presence of IL-12, IL-18 stimulates *IFNG* expression, thus promoting the Th1-mediated immune response. Without IL-12, IL-18 stimulates Th2 responses [37]. In this study, since serum IFN-γ levels were below detection thresholds, the association of such cytokines could not be assessed.

Lastly, we observed that pretreatment serum VEGF levels were associated with a SVR. A previous study showed no association between baseline VEGF and treatment

outcome, but only 36 patients, including 19 with genotype 1, were studied [40]. Hence, it is still unclear if this angiogenesis marker plays a critical role in response to antiviral therapy in chronic HCV infection. Furthermore, VEGF was correlated with IL-12 and IL-18 in our study. In particular, IL-18 enhances the production of VEGF in rheumatoid arthritis synovial fibroblasts, suggesting that IL-18 could be an angiogenic mediator with triggering effects on VEGF production [41]. Although preoperative serum VEGF was found to be a significant predictor of tumor recurrence and overall survival in patients with HCC [42], there have been no reports regarding treatment response in patients with chronic hepatitis C during antiviral therapy.

In multivariate analysis of our cohort, low IL-10, high IL-12p40, and high IL-18 were independent factors related to a SVR in patients treated with PEG-IFN and ribavirin. Our results indicate that such 3-cytokine profiling may offer clinicians another tool in predicting treatment outcome of HCV infection. Further investigation must be done *in vitro* and using a large number of samples to validate the significance of our findings.

In conclusion, several cytokines were seen to be elevated in patients with chronic hepatitis C using the multiplex bead assay. Serum IL-10 levels and amino acid substitutions at the 70 aa core region of HCV are useful for predicting a nonresponse to PEG-IFN and ribavirin therapy in patients with chronic hepatitis C genotype 1. A higher level of serum IL-12 is considered to be favorable for response to antiviral therapy, and is correlated with substitutions in the ISDR. Lastly, IL-18 is notably high in patients with chronic HCV infection, and is correlated with IL-12.

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Table 1. Demographic and Clinical Characteristics of Patients with Hepatitis C Virus (HCV) Infection

Characteristics	All (n = 79)	SVR (n = 31)	Non-SVR (n = 48)	*P*
Median age, *yrs* (range)	60 (17-74)	56 (28-72)	61 (17-74)	0.08
Male, *n* (%)	40 (51)	23 (74)	17 (35)	0.001
Median values (range)				
ALT (IU/L)	54 (22-389)	53 (24-172)	61 (22-389)	0.25
AST (IU/L)	44 (20-288)	36 (21-133)	48 (20-288)	0.012
HCV RNA (10^5 IU/mL)	17 (1.1 - 51)	15 (1.1-50)	19 (2.2-51)	0.13
Substitutions				
Core aa 70 (Arg70/Gln70)	47/28	22/6	25/22	0.028
ISDR of NS5A (wild/intermediate/mutant)	46/17/13	13/7/9	33/10/4	0.026

Abbreviations: HCV, hepatitis C virus; SVR, sustained virological response; AST, aspartate aminotransferase; ALT, alanine aminotransferase; aa, amino acid; ISDR, interferon-sensitivity determining region.

Table 2. Optimal Cutoff Value, Sensitivity, Specificity, AUC, and Predictive Value of Serum IL-10, IL-12p40, IL-18, and VEGF at baseline and after 4 weeks of treatment in 79 Patients with Chronic Hepatitis C

Cytokine	Collected time	Cutoff value	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	AUC (95% CI)	PPV (%)	NPV (%)
IL-10	baseline	5.0	100 (86-100)	80 (67-89)	0.86 (0.84-0.98)	69	100
	4 weeks	6.8	82 (69-91)	100 (86-100)	0.86 (0.78-0.95)	100	71
IL-12p40	baseline	17.4	81 (63-93)	52 (37-67)	0.70 (0.59-0.82)	52	81
	4 weeks	21.3	81 (63-93)	60 (45-74)	0.69 (0.57-0.81)	57	83
IL-18	baseline	15.4	97 (83-100)	46 (31-61)	0.72 (0.61-0.83)	54	96
	4 weeks	24.6	87 (70-96)	42 (28-57)	0.62 (0.50-0.75)	49	83
VEGF	baseline	57.6	77 (59-90)	69 (54-81)	0.74 (0.63-0.86)	62	83
	4 weeks	62.6	74 (55-88)	67 (52-80)	0.70 (0.58-0.82)	59	80

All AUC values were significantly higher than a 0.50 nonpredictive value ($P < 0.01$ for all comparisons). Cutoff values were determined by constructing receiver operating characteristic curves and are expressed as pg/mL. IL-10 is predictive of a nonresponse. IL-12p40, IL-18, and VEGF are predictive of a sustained virological response. Abbreviations: AUC, area under the curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Table 3. Multivariate Analysis of Factors Independently Associated with a Sustained Virological Response to Pegylated Interferon and Ribavirin Therapy in Patients Infected with HCV Genotype 1

Factors	OR	95% CI	*P*
Gender: male	10.932	2.178 – 54.780	0.004
AST ≥ 40 IU/L	0.946	0.906 – 0.989	0.013
IL-10 ≥ 5.0 pg/mL	0.823	0.704 – 0.962	0.014
IL-12p40 ≥ 17.4 pg/mL	1.071	1.009 – 1.137	0.024
IL-18 ≥ 15.4 pg/mL	1.085	1.024 – 1.150	0.006

Only variables that achieved statistical significance ($P < 0.05$) in multivariate logistic regression analysis are shown.

AST, aspartate aminotransferase; OR, odds ratio; CI, confidence interval.

Table 4.

Serum Cytokine Levels Changes During and After Treatment of Pegylated Interferon Plus Ribavirin

Cytokines	Treatment outcome	Baseline	Week 4	Week 72	*P*
IL-10	SVR	4.1 (3.3 – 25.4)	3.7 (3.1 – 19.9)	3.5 (2.9 – 9.0)	< 0.001
	Non-SVR	7.3 (3.7 – 10.8)	7.5 (3.9 – 8.8)	7.4 (3.9 – 10.9)	0.962
IL-12p40	SVR	24.1 (11.3– 99.0)	22.1 (11.6 – 75.2)	18.4 (7.8 – 76.5)	0.008
	Non-SVR	17.2 (4.6 – 57.9)	19.2 (8.1 – 50.1)	21.6 (5.8 – 77.0)	0.281
IL-18	SVR	27.9 (13.8 – 100.6)	25.1 (13.2 – 95.2)	23.3 (6.6 – 48.5)	< 0.001
	Non-SVR	17.7 (1.1 – 59.9)	31.3 (10.3 – 90.6)	17.4 (5.4 – 52.0)	< 0.001

Data are median (5^{th}-95^{th} percentile) values.

Abbreviations: SVR, sustained virological response.

Figure Legends

Figure 1. Detection of serum cytokines in patients with HCV infection and healthy subjects.

Boxes represent the interquartile range of the data. The lines across the boxes indicate the median values. The hash marks above and below the boxes indicate the 90th and 10th percentiles for each group, respectively. Serum IL-10, IL-12p40, IL-12p70, IL-18, IL-4, and VEGF levels were detected in 79 patients with HCV infection and 26 controls.

Figure 2. Serum cytokines related to antiviral therapy outcome.

Boxes represent the interquartile range of the data. The lines across the boxes indicate the median values. The hash marks above and below the boxes indicate the 90th and 10th percentiles for each group, respectively.

(A) Serum IL-10, IL-12p40, IL-12p70, IL-18, and VEGF were detected in 31 patients who achieved a sustained virological response and 48 patients who did not.

Figure 3. Correlation between serum cytokines in 79 patients with HCV infection.

(A-B) Serum IL-12p40 was significantly correlated with the level of (A) IL-18 (r = 0.325, P = 0.004) and (B) VEGF (r = 0.253, P = 0.024).

(C) Serum IL-18 was correlated with the level of VEGF (r = 0.394, P < 0.001).

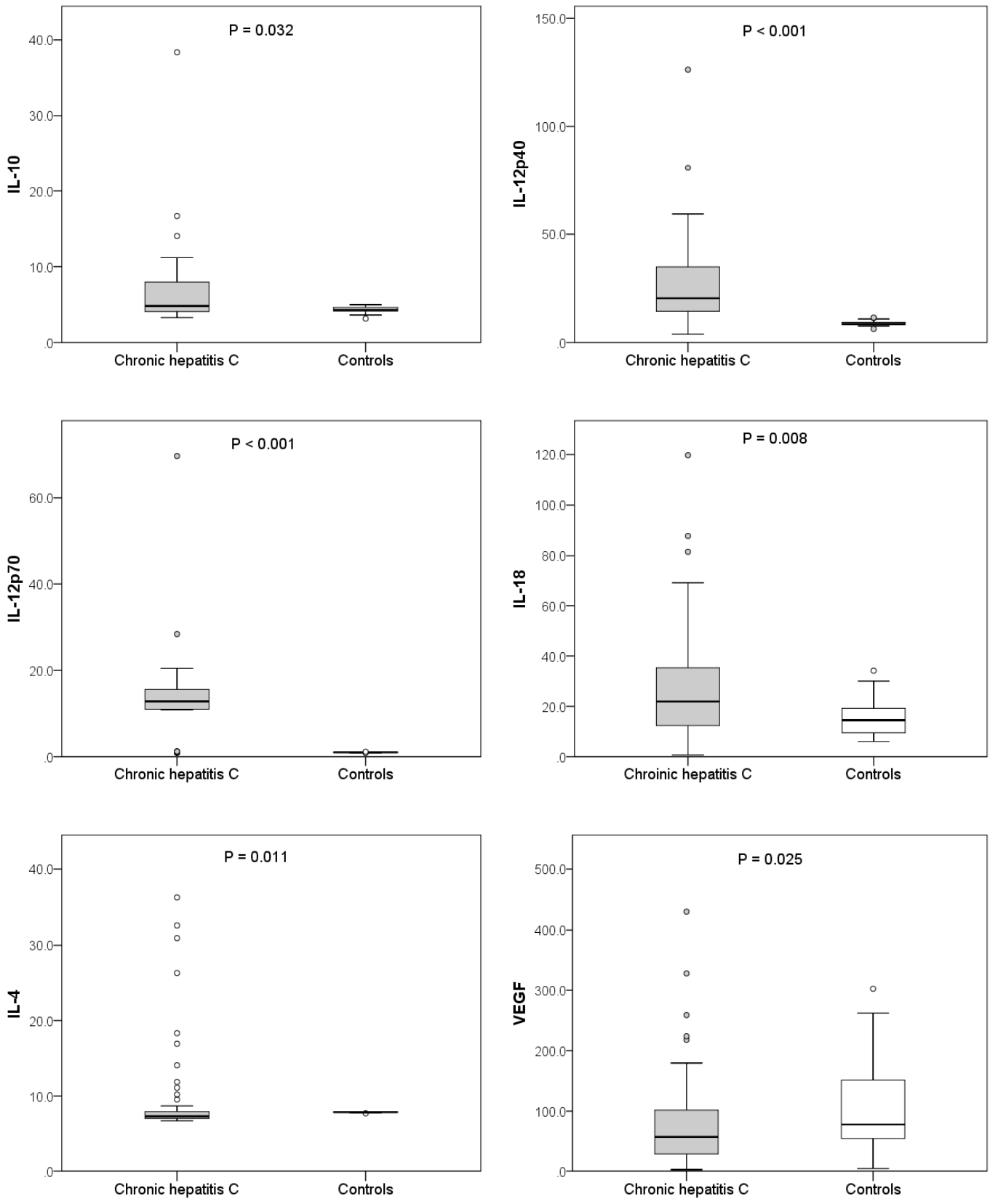
P = 0.032
IL-10
40.0
30.0
20.0
10.0
.0
Chronic hepatitis C
Controls
P < 0.001
IL-12p40
150.0
100.0
50.0
.0
Chronic hepatitis C
Controls
P < 0.001
IL-12p70
60.0
40.0
20.0
.0
Chronic hepatitis C
Controls
P = 0.008
IL-18
120.0
100.0
80.0
60.0
40.0
20.0
.0
Chroinic hepatitis C
Controls
P = 0.011
IL-4
40.0
30.0
20.0
10.0
.0
Chronic hepatitis C
Controls
P = 0.025
VEGF
500.0
400.0
300.0
200.0
100.0
0
Chronic hepatitis C
Controls

Figure 1

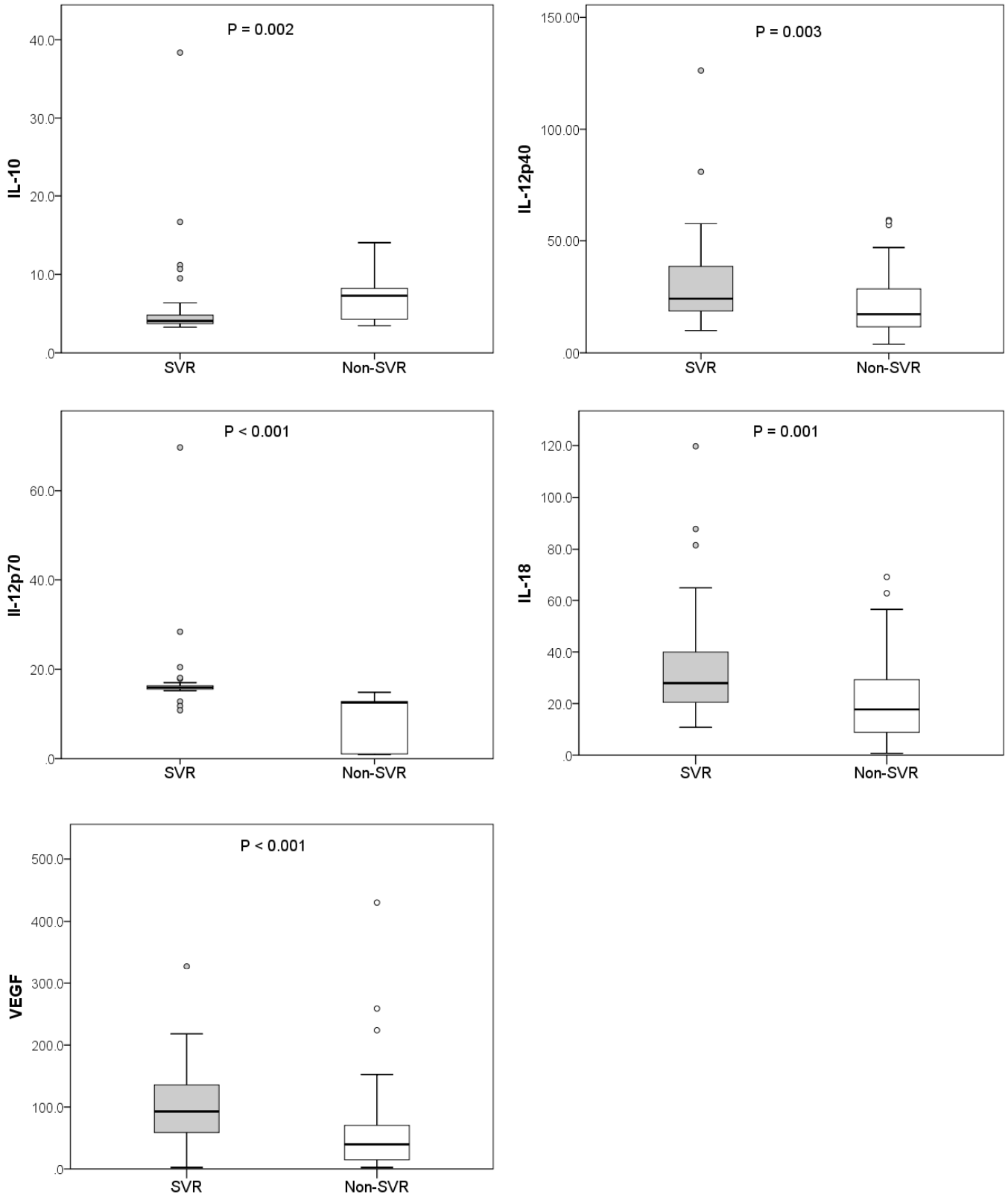
P = 0.002
IL-10
40.0
30.0
20.0
10.0
.0
SVR
Non-SVR
P = 0.003
IL-12p40
150.00
100.00
50.00
.00
SVR
Non-SVR
P < 0.001
Il-12p70
60.0
40.0
20.0
.0
SVR
Non-SVR
P = 0.001
IL-18
120.0
100.0
80.0
60.0
40.0
20.0
.0
SVR
Non-SVR
P < 0.001
VEGF
500.0
400.0
300.0
200.0
100.0
.0
SVR
Non-SVR

Figure 2

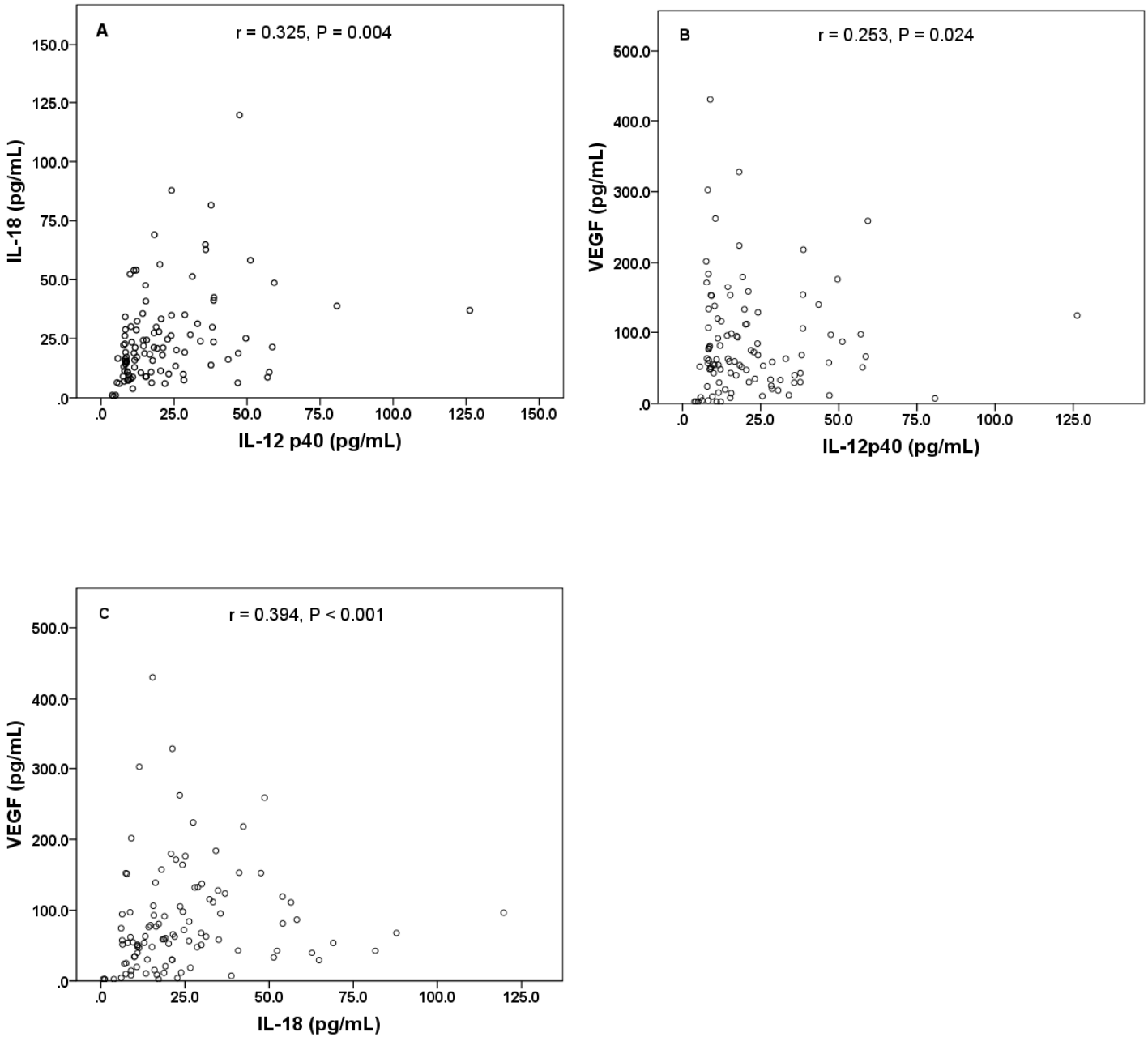
A
r = 0.325, P = 0.004
IL-18 (pg/mL)
IL-12 p40 (pg/mL)
B
r = 0.253, P = 0.024
VEGF (pg/mL)
IL-12p40 (pg/mL)
C
r = 0.394, P < 0.001
VEGF (pg/mL)
IL-18 (pg/mL)

Figure 3