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Quantitative analysis of serum chemokines associated with treatment failure of direct-acting antivirals in chronic hepatitis C

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ABSTRACT

Although serum chemokine levels have been reported to influence the outcome of interferon-based treatment in patients with chronic hepatitis C, their effect on the hepatitis C virus (HCV) response to direct-acting antiviral agents (DAAs), which can achieve high rates of a sustained virological response (SVR), is largely unknown. To clarify this relationship, 9 chemokines (eotaxin, GRO-α, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, RANTES, and SDF-1α) were quantified before, during, and after DAA treatment using serum samples obtained from 57 patients with chronic hepatitis C. All baseline median chemokine levels were significantly higher in patients with chronic hepatitis C than in healthy subjects ($P < 0.05$). In particular, lower MIP-1β (≤71.5 pg/mL) and higher RANTES (> 671.5 pg/mL) levels were significantly associated with patients who failed to clear HCV RNA ($P = 0.0039$ and 0.013, respectively). Prediction of a clinical response based on a combination of these chemokines demonstrated high sensitivity (82%), specificity (85%), negative predictive value (95%), and area under the curve (0.833). The non-SVR rate (56.3%; 9 of 16) was significantly higher in patients with low MIP-1β and high RANTES compared with other combinations. Moreover, baseline MIP-1β and RANTES were both additive and independent for predicting a non-SVR. Apart from an increase in eotaxin, all chemokines became decreased in patients with a SVR. In conclusion, a combination of serum MIP-1β and RANTES levels may be predictive of a treatment response to DAAs in Japanese patients with chronic hepatitis C.

1. Introduction

Approximately two million people (1.6–2% of the population) are chronically infected with the hepatitis C virus (HCV) in Japan. Chronic HCV infection can lead to cirrhosis, hepatic failure, and hepatocellular carcinoma (HCC) [1,2]. Direct-acting antiviral agent (DAA) regimens have become the standard of care for chronic hepatitis C and have enabled high rates of a sustained virological response (SVR) [3–5]. HCV relapse after therapy cessation is the most common reason for treatment failure, although the mechanisms and predictors of treatment failure are poorly understood. Innate and adaptive immune functions were highly activated in patients receiving DAAs who achieved a SVR relative to relapsers [6,7], suggesting a role of host immunity in modulating the HCV response to therapeutic agents. The identification of chemokine biomarkers predictive of treatment outcome could therefore help individualize treatment durations required to achieve a SVR.

Chemokines are important determinants in the pathogenesis, disease progression, and treatment outcome of HCV infection [8–11]. Serum chemokines have been examined in patients with chronic hepatitis C and their changes studied in HCV-infected patients receiving DAAs [12–15]. However, the role of chemokines in estimating DAA therapy outcome remains unknown, especially in Japanese patients. This study aimed to clarify the serum levels of chemokines in patients with chronic hepatitis C in relation to the HCV response to DAA treatment.

2. Patients and methods

2.1. Subjects

A total of 333 patients with chronic hepatitis C had received DAA therapy at Shinshu University Hospital (Matsumoto, Japan) between 2014 and 2016. Of them, 15 patients were classified as having treatment failure by positive HCV RNA at 12 weeks after the end of treatment, with 4 being excluded due to unavailable serial stored serum. Among the 318 patients achieving a SVR, 46 with preserved serum

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obtained at all time points were enrolled. Five healthy subjects were included as controls. The diagnosis of chronic hepatitis C was based on the presence of serum HCV antibodies and detectable HCV RNA, as previously reported [16]. Serum HCV RNA levels were measured using COBAS TaqMan HCV Test assays (Roche Diagnostic Systems, Tokyo, Japan) with a detection limit of 1.2 log IU/mL. HCV genotypes were determined as described previously [17]. Subjects with a history of or who developed decompensated cirrhosis or hepatocellular carcinoma or other causes of liver disease were excluded. No subject had detectable hepatitis B surface antigen or antibody to the human immunodeficiency virus. This study was reviewed and approved by the Institutional Review Board of Shinshu University Hospital (approval number: 3244) and conducted according to the principals of the Declaration of Helsinki. Written informed consent was obtained from all participating subjects.

2.2. DAA therapy

Patients with HCV genotype 1 received a 24 week regimen of daclatasvir and asunaprevir (n = 140) [3] or a 12 week regimen of ledipasvir and sofosbuvir (n = 106) [5]. Patients with HCV genotype 2 were given a 12 week regimen of sofosbuvir and ribavirin (n = 87) [4]. A SVR was defined as undetectable HCV RNA at 12 weeks after the end of treatment. The SVR rates of each regimen were 94% (132/140), 96% (102/106), and 96% (84/87), respectively. The presence of known resistant associated variants (RAVs) in HCV-NS5A was tested at baseline in all genotype 1b subjects and at 12 weeks after the end of treatment in all genotype 1b non-SVR patients. Only patients with a wild-type NS5A region that did not contain these variants were treated using daclatasvir and asunaprevir as SVR rates are reportedly lower in patients with variants under this regimen [3]. At baseline, 2 and 3 patients respectively harbored L31M and Y93H and were treated with ledipasvir and sofosbuvir. All patients completed their treatment course. Serum samples were obtained just prior to the start of treatment, at 4 weeks after treatment commencement, and at 12 weeks post-treatment and were stored at −30 °C until testing.

2.3. Detection of serum chemokines by multiplex bead assays

We performed multianalyte profiling of chemokines using the Procarta Plex Human Chemokine Panel 1 (Affymetrix, eBioscience, Vienna, Austria) for quantification of 9 chemokines: eotaxin (CCL11), GRO-α (CXCL11), IL-8 (CXCL8), IP-10 (CXCL10), MCP-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCL4), RANTES (CCL5), and SDF-1α (CXCL12). The assays were conducted according to the manufacturer's instructions as described previously [18–20]. All samples were analyzed in 1 run.

2.4. Statistical analysis

Data were analyzed using IBM SPSS Statistics 24 software (IBM, Tokyo, Japan). Categorical variables were compared using the χ^2 test or Fisher's exact test and continuous variables were compared with the Mann-Whitney *U* test. Receiver operating characteristic (ROC) curve analysis was performed to determine optical cutoff values for treatment outcome. Cutoff values were determined by the Youden index. The Friedman test and Wilcoxon signed rank test were employed to analyze the differences among continuous variables at baseline, 4 weeks after the start of treatment, and 24 weeks post-treatment. Logistic regression analysis was performed to evaluate the effects of MIP-1β and RANTES on treatment outcome. Association strength was estimated by calculating the odds ratio (OR) and 95% confidence interval (CI). A *P* value of less than 0.05 was considered statistically significant.

3. Results

The clinical profile of the 56 patients is summarized in Table 1.

Table 1
Demographic and Clinical Characteristics of 57 Patients with Chronic Hepatitis C.

Characteristic	All (n = 57)	SVR (n = 46)	Non-SVR (n = 11)	*P* value
Median age (years)	71 (66–74)	71 (65–74)	71 (67–75)	0.808
Male, n (%)	27 (47)	22 (48)	5 (46)	0.887
Previous interferon treatment, n (%)	22 (39)	17 (37)	5 (46)	0.603
Treatment regimen, n (%)				0.716
Daclatasvir + asunaprevir	30 (53)	23 (50)	7 (64)	
Ledipasvir + sofosbuvir	14 (25)	12 (26)	2 (18)	
Sofosbuvir + ribavirin	13 (23)	11 (24)	2 (18)	
Genotype 1/2	44/13	35/11	9/2	0.909
HCV RNA (logIU/mL)	6.2 (5.9–6.6)	6.2 (6.0–6.6)	5.9 (5.3–6.5)	0.213
Alanine aminotransferase (U/L)	40 (24–55)	40 (24–51)	52 (27–73)	0.903
Albumin (g/dL)	4.1 (3.9–4.3)	4.2 (3.9–4.3)	4.0 (3.8–4.2)	0.376
Hemoglobin (g/dL)	14.1 (12.9–15.5)	14.3 (13.0–15.6)	12.9 (12.1–14.0)	0.020
Platelets ($\times 10^4$/mm^3)	15.6 (11.8–19.4)	16.3 (12.0–20.5)	9.4 (5.8–16.1)	0.279
α-fetoprotein (ng/mL)	4.2 (2.9–7.3)	3.9 (2.5–6.3)	6.3 (5.4–9.0)	0.056

Values are expressed as the median (interquartile range). SVR, sustained virological response; HCV, hepatitis C virus.

Median age was 71 years and 27 (47%) were male. Patients achieving a SVR had a significantly higher hemoglobin concentration than patients who did not. There were no remarkable differences between responders and nonresponders for other clinical parameters. Among the 5 patients with pretreatment RAVs, 4 achieved a SVR and 1 patient with L31M relapsed.

The serum levels of eotaxin (62.0 vs. 30.8 pg/mL; $P = 0.001$), GRO-α (23.1 vs. 5.1 pg/mL; $P < 0.001$), IL-8 (27.1 vs. 0.5 pg/mL; $P < 0.001$), IP-10 (316.9 vs. 18.6 pg/mL; $P < 0.001$), MCP-1 (142.5 vs. 33.4 pg/mL; $P < 0.001$), MIP-1α (15.1 vs. 3.3 pg/mL; $P < 0.001$), MIP-1β (73.1 vs. 57.3 pg/mL; $P = 0.031$), RANTES (674.1 vs. 47.4 pg/mL; $P < 0.001$), and SDF-1α (1095.6 vs. 478.5 pg/mL; $P < 0.001$) were all significantly higher in chronic hepatitis C patients than in healthy subjects (Fig. 1).

Median MIP-1β levels at enrollment were significantly lower in patients with a non-SVR (59.7 pg/mL; interquartile range [IQR]: 51.0–68.7) than in SVR patients (82.3 pg/mL; IQR: 59.3–116.1; $P = 0.015$) (Fig. 2). Conversely, median baseline serum levels of RANTES were significantly higher in patients with a non-SVR (930.4 pg/mL; IQR: 690.0–3912.5 vs. 655.8 pg/mL; IQR: 476.6–1008.7; $P = 0.020$). The remaining 7 chemokines did not differ noticeably between patients with and without a SVR (Table 2).

To determine the optical cutoff values of MIP-1β and RANTES for predicting non-SVR patients, ROC curve analysis revealed threshold levels for MIP-1β and RANTES of 71.5 pg/mL and 671.5 pg/mL, respectively, with calculated areas under the ROC curve (AUCs) of 0.738 (95% CI: 0.602–0.874) and 0.728 (95% CI: 0.552–0.903), respectively (Table 3). In patients with MIP-1β ≤ 71.5 pg/mL, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for predicting a non-SVR were 90.9%, 63.0%, 37.0%, 96.7%, and 68.4%, respectively (Table 3). There was a significant association between patients with low MIP-1β and a non-SVR (OR = 17.1, 95% CI: 2.0–145.2; $P = 0.0039$). In patients with RANTES > 671.5 pg/mL, the respective sensitivity, specificity, PPV, NPV, and accuracy were 90.9%, 56.5%, 33.3%, 96.3%, and 63.2%. Higher RANTES associated significantly with a non-SVR (OR 13.0, 95% CI: 1.5–110.1; $P = 0.013$).

Modeling a non-SVR as a function of serum MIP-1β (above or at/below 71.5 pg/mL) and RANTES (above or at/below 671.5 pg/mL) in