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High MIG (CXCL9) plasma levels favors response to peginterferon and ribavirin in HCV-infected patients regardless of DPP4 activity

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Abstract

Background & Aims—Sustained virological response (SVR) following peginterferon (pegIFN) and ribavirin (RBV) treatment in hepatitis C virus (HCV) infected patients has been linked with the *IL28B* genotype and lower peripheral levels of the CXCR3-binding chemokine IP-10 (CXCL10). To further improve the understanding of these biomarkers we investigated plasma levels of the other CXCR3-binding chemokines and activity of the dipeptidyl peptidase IV (DPP4, CD26) protease, which cleaves IP-10, in relation to treatment response.

Methods—African-American and Caucasian HCV genotype 1 infected patients (n=401) were treated with pegIFN/RBV for 48 weeks (ViraHep-C cohort). Pretreatment plasma levels of MIG (CXCL9), I-TAC (CXCL11) and the type III interferon IL29 were investigated by Luminex and DPP4 activity by using a luciferase assay.

Results—Patients achieving SVR had higher baseline MIG plasma levels and lower DPP4 activity than non-SVR patients. MIG was higher in Caucasians, *IL28B* CC (rs1297860) genotype carriers and patients with higher ALT levels. MIG correlated with IP-10 in SVR patients, but not in non-SVRs. A high DPP4 activity correlated with higher IP-10 levels, while DPP4 activity was not associated with MIG or I-TAC levels.

Conclusions—The associations of MIG with SVR status and *IL28B* genotype imply that higher MIG plasma levels could reflect a beneficial immunological state for response to pegIFN/RBV treatment. The correlation between MIG and IP-10 observed only in SVR patients may contribute to a better treatment response whereas this MIG/IP-10 balance might be disrupted in non-SVR patients due to increased DPP4 cleavage of IP-10 into a dysfunctional form.

Keywords

HCV; MIG; DPP4; IP-10; Interferon

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Introduction

Hepatitis C virus (HCV) infects approximately 170 million people in the world and establishes a chronic infection in the liver in about 80% of individuals infected. Patients infected with HCV genotype 1 achieve sustained virological response (SVR) in 40–50% of cases following treatment with peginterferon (pegIFN) and ribavirin (RBV). Recently direct acting anti-viral drugs with substantially increased response rates have been introduced for treatment of HCV [1]. However, the high burden of HCV in resource-poor settings and the use of pegIFN for treatment of hepatitis B virus (HBV) infection justify further investigations of biomarkers and host biology in relation to pegIFN/RBV treatment.

The SVR rate in HCV patients depends on both viral and host factors, like HCV genotype and patient ethnicity [2, 3]. Single nucleotide polymorphisms (SNP) upstream of the interleukin 28B (*IL28B)* gene have been strongly associated with treatment response and account for some of the difference between ethnicities [4, 5]. Patients with CC genotype in rs12979860 SNP are more likely to achieve SVR than patients with one or two T alleles [4]. IL28B (IFN-λ3) is a type III interferon with anti-viral activity, which is located in close proximity to the other IFN-λs, IL29 (IFN- λ1), IL28A (IFN-λ2) and IFNL4 (IFN-λ4) on chromosome 19 [6]. Another predictor of treatment response is pre-treatment interferon gamma-induced protein 10 (IP-10, CXCL10) plasma level, which is higher in patients that will not respond to pegIFN/RBV therapy than in patients achieving SVR [7, 8]. The combination of baseline IP-10 levels and *IL28B* genotype further improves predictability of SVR [9–11].

The chemokine IP-10 is involved in regulating trafficking of leukocytes. IP-10 as well as the related chemokines monokine induced by interferon (MIG, CXCL9) and interferon inducible T cell alpha chemoattractant (I-TAC, CXCL11) belong to the non-ELR CXC chemokines and are induced by T-helper-1 cytokines like IFN-γ. These chemokines bind to the CXCR3 receptor, primarily expressed on T, natural killer (NK) and NKT cells, but also on other leukocytes such as B cells and plasmacytoid dendritic cells (pDCs) [12]. CXCR3 ligands are increased in the liver in patients with chronic HCV infection and can be produced by hepatocytes, stellate cells, endothelial cells and infiltrating leukocytes [13–15]. CXCR3 ligands are believed to not only recruit HCV-specific T cells, but also non-specific leukocytes, which may contribute to the chronic liver inflammation seen in HCV-infected patients [16].

Dipeptidyl-peptidase IV (DPP4) specifically cleaves N-terminal dipeptides with a proline or alanine at the second position, including IP-10 and the other CXCR3 binding chemokines [17]. DPP4, also named CD26, exists both in a cell surface-bound form and a circulating form in plasma. It is expressed on T cells, fibroblasts, endothelial and epithelial cells. During various inflammatory diseases and chronic infections, e.g. HCV, DPP4 activity in plasma is elevated [18, 19]. In HCV-infected patients failing pegIFN/RBV treatment, baseline plasma DPP4 activity and soluble DPP4 levels were reported to be higher than in patients achieving SVR [20–22]. This has been suggested to lead to the increase of a short antagonistic form of IP-10 in non-SVR patients [20].

We determined baseline plasma levels of the CXCR3 binding chemokines and activity of the DPP4 enzyme in patients treated with pegIFN/RBV from the Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C cohort (ViraHep-C). Here we report on higher baseline MIG plasma levels and lower DPP4 activity in SVR patients and relate this to previous findings regarding IP-10 and *IL28B* genotype in the cohort in order to further improve our understanding of how these biomarkers contribute to treatment response.

Material and Methods

Patients

The ViraHep-C study is a multicenter study of pegIFN/RBV therapy in African-American and Caucasian patients with HCV genotype 1 infection [2]. Patients were included that classified themselves by ethnicity as African-American or Caucasian-American, were between 18 and 70 years, were treatment naïve and had detectable HCV RNA and histologic evidence of chronic HCV. The study enrolled 401 patients from eight clinical centers in the United States. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutional review boards from the participating institutions and by a central board assembled by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). All participants provided written informed consent prior to their participation in the ViraHep-C study, including consent for genetic testing [2].

Treatment

Patients were treated with 180μg pegylated interferon-α-2a (pegIFN) (Pegasys, Roche Pharmaceuticals, Nutley, NJ) per week and 1000–1200 mg ribavirin (RBV) (Copegus, Roche Pharmaceuticals, Nutley, NJ) per day for up to 48 weeks. SVR was defined as serum HCV RNA below the detection limit 24 weeks after end of treatment. Patients with detectable HCV RNA after 24 weeks of treatment were considered as nonresponders. Non-SVR patients include nonresponders and patients with virologic breakthrough at treatment week 24 to 48 or virologic relapse 24 weeks after end of treatment.

Baseline blood assessments

Blood counts, including platelet count and serum levels of alanine transaminase (ALT), total bilirubin, albumin, ferritin, fasting triglycerides, hematocrit and hemoglobin were assessed at the local clinical laboratories. HCV RNA testing was done as previously reported [2]. The *IL28B* polymorphic marker rs1297860 was genotyped as described earlier in 297 patients who consented for genetic analysis [9].

Liver histology

All patients had undergone liver biopsy within 18 months of screening and the biopsies were graded as previously described [23, 24]. Necroinflammatory changes were graded from 0 to 18 according to the histologic activity index (HAI), fibrosis from 0 to 6 according to the ISHAK fibrosis scale and steatosis from 0 to 4.

Plasma cytokine quantification

Plasma samples from 386 patients from the ViraHep-C cohort were available for analysis at baseline (Supplementary Table 1). MIG, I-TAC and IL29 plasma levels were assessed with the Milliplex human cytokine/chemokine panel III assay (Millipore). Each sample was analyzed in duplicate and control samples were included to assess possible plate effects. BioPlex Manager 6.1 software was used to generate the standard curves and derive protein concentrations. Plasma IP-10 levels determined (Quantikine human IP-10 assay from R&D systems) and reported previously [9] were also included in the data analysis of this study.

DPP4 activity assessment

DPP4 enzyme activity was determined with a luciferase based DPPIV-Glo™ protease assay (Promega) in 348 baseline plasma samples. Samples diluted 1/250 in phosphate buffered saline (PBS) were mixed 1:1 with DPPIV-Glo reagent, incubated for 30 min and then recorded for luminescence for 0.1 s. Relative luminescence units were derived by dividing units per sample with units for the PBS controls.

Statistical analysis

Cytokine concentrations and blood parameters that were not adhering to normal distribution were log-transformed before statistical testing. Standard statistical analyses were performed in R (version 3.0.1), using publicly available packages. For the univariate analyses of single variables, statistical tests included Student's t-test, ANOVA, Tukey's honest significance test, ordinary linear regression and Pearson correlation. The Bonferroni method was applied to correct for multiple comparisons and the cut-off for statistical significance was set at $p \leq 0.05$. Generalized linear binomial models and linear models were used for multivariate prediction of categorical variables and continuous variables, respectively. Step-wise selection (upwards and downwards) was performed with a cut-off of $p \leq 0.05$ for a variable to be included in the final model.

Results

MIG and IP-10 differentially associated with treatment response

Potential associations of baseline CXCR3 ligand levels with response to pegIFN/RBV treatment were assessed by comparing patients that achieved SVR 24 weeks after end of treatment with those that did not (Supplementary Table 1). As previously reported IP-10 levels were significantly higher in non-SVR (median=492) as compared to SVR patients (median=322) (Figure 1A) [9]. MIG plasma levels, on the contrary, were significantly lower in non-SVR (median=733.3) as compared to SVR patients (median=892.3) (Figure 1B). Although borderline to reach statistical significance, I-TAC levels were found not being different after correcting for multiple comparisons (median=222.9 in non-SVR and 190.8 in SVR) (Figure 1C).

MIG plasma levels associated with *IL28B* genotype, ethnicity and ALT levels

In a univariate analysis, we investigated the association between MIG plasma levels and baseline parameters measured in the HCV-infected patients. The variables most strongly

associated with MIG were ALT, ethnicity and *IL28B* genotype (Table 1). There was a positive correlation between MIG and ALT levels (r=0.40, $p=2.5\times10^{-14}$) (Figure 2A). MIG plasma levels were higher in patients of Caucasian ethnicity (median=976.4) or those possessing the *IL28B* CC alleles (median=1180) as compared to patients of African-American ethnicity (median=638.5) or with the *IL28B* CT/TT alleles (median=692.9 and 544.1 respectively) (Figure 2B and C). In addition liver biopsy inflammation score (HAI), SVR status and peripheral levels of the CXCR3 ligands IP-10 and I-TAC, associated with MIG levels.

Increased IL29 serum levels have been reported in patients with the *IL28B* CC alleles [25] and in *in vitro* experiments IL29 induced the release of MIG [26, 27]. We therefore investigated plasma levels of IL29 to find out whether that could explain the connection between MIG and *IL28B* genotype. However, despite a statistically significant but weak correlation between MIG and IL29 plasma levels (r=0.17, p=0.001) no association between IL29 levels and *IL28B* genotype was detected (p=0.87).

In a multivariate linear regression model serum ALT levels ($p=4.7\times10^{-9}$), ethnicity ($p=1.2\times10^{-7}$) and *IL28B* genotype ($p=8.3\times10^{-6}$) contributed significantly to explaining MIG plasma levels. An interaction between IP-10 plasma levels and *IL28B* genotype also contributed significantly (p=0.007) to the multivariate model explaining MIG levels. MIG and IP-10 correlated in patients with the *IL28B* CC alleles (r=0.51, $p=3.5\times10^{-7}$), but not in those with CT or TT alleles (r=0.12, p=0.09) (Figure 3A).

MIG and IP-10 levels associate in SVR, but not in non-SVR patients

Since *IL28B* genotype is a good predictor of response to treatment we further investigated whether MIG and IP-10 levels correlate differently depending on response status. Indeed, MIG and IP-10 were significantly correlated in SVR patients (r=0.4, $p=3.1\times10^{-7}$), but not in non-SVR patients (r=0.11, p=0.09) (Figure 3B). Of note, SVR status of the patients did not affect the associations between the other CXCR3 binding chemokines (Supplementary Figure 1).

DPP4 protease activity is higher in non-SVR patients and associate with IP-10, but not MIG

We hypothesized that the lack of correlation between MIG and IP-10 in non-SVR patients could be due to an increased presence of non-functional cleaved IP-10 in these patients. To test this hypothesis, the activity of the DPP4 protease, known to cleave IP-10 [20], was measured in baseline plasma samples. DPP4 activity was significantly higher in non-SVR (median=92.5) compared to SVR patients (median=82.1), consistent with our hypothesis (Figure 4A). DPP4 activity and IP-10 levels were significantly correlated (r=0.31, $p=5.8\times10^{-9}$) and patients with higher IP-10 levels had higher DPP4 activity (Figure 4B). In addition to IP-10, DPP4 may also cleave the other CXCR3 chemokines MIG and I-TAC [17]. However, no statistically significant correlations between DPP4 activity and plasma levels of MIG (r=0.05, p=0.93) (Figure 4C) or I-TAC (r=0.12, p=0.06) (data not shown) were detected.

SVR status associates with baseline viral load, DPP4 activity, MIG and IP-10 levels

Association between SVR status after treatment and baseline patient characteristics and blood parameters, including cytokine levels and DPP4 activity, were investigated. Seven out of the 23 variables were significantly associated with SVR after correction for multiple comparisons (Supplementary Table 2). Variables associated with SVR were ethnicity, *IL28B* genotype, ferritin and viral load as well as the above reported IP-10 and MIG levels and DPP4 activity. When including all significant variables in a multivariate generalized linear model, viral load (p=0.003), MIG levels (p=0,008), DPP4 activity (p=0.009) and IP-10 levels (p=0.017) significantly contributed to the model.

Discussion

This is the first report in HCV-infected pegIFN/RBV-treated patients demonstrating higher baseline MIG plasma levels in patients achieving SVR compared to non-SVR patients. The lack of a difference in MIG levels depending on treatment response in earlier studies may be explained by the much smaller number of patients included in those studies and/or the difference in patient ethnicity in this study compared to previous studies [7, 8]. Interestingly, it has been described that HBV-infected patients treated with pegIFNα-2a who achieved SVR had higher MIG plasma levels at baseline than non-SVR patients [28]. Thus higher MIG plasma levels at baseline may be an indicator of a favorable response to pegIFN treatment both in HCV-and HBV-infected patients.

MIG plasma levels were higher in patients with CC alleles in the *IL28B* rs12979860 SNP and in patients of Caucasian ethnicity, both of which are strong predictors for treatment response [2, 4]. This strengthens the hypothesis that higher MIG levels in the periphery reflect a beneficial immunological state for response to pegIFN/RBV treatment. The strong associations between SVR, *IL28B* genotype and ethnicity make it difficult to distinguish whether the MIG association with one or several of these variables also indirectly causes the observed association with the other variables. The association of MIG with SVR status may depend on ethnicity as MIG levels in Caucasian and African-American patients analyzed separately were not significantly different in SVR and non-SVR patients (Supplementary Table 3). The association of MIG with *IL28B* genotype on the other hand was independent of ethnicity as MIG levels in both Caucasians and African-Americans were significantly different depending on *IL28B* genotype (Supplementary Figure 2).

In our study no association between plasma levels of the type III interferon, IL29, and *IL28B* genotype was detected. This observation disaffirms our hypothesis that the higher MIG levels in patients with the *IL28B* CC genotype would be related to higher IL29 levels. In fact, the association between mRNA expression and protein levels of IFN-λ1-3 and the *IL28B* genotype is contradictory and only seen in few of the studies done [5, 25, 29–31].

IP-10 and MIG differ in their promoter region and are therefore differently regulated. Both are induced by IFN-γ, but IP-10 is also induced by IFN-α/β [12]. IFN-α is released as a first line of defense in response to viral infections like HCV and induces the secretion of IP-10 [32]. CXCR3+ lymphocytes, like NK cells and T-helper 1 cells, are recruited to the liver where their release of IFN-γ induces the production of MIG and more IP-10 and

subsequently additional recruitment of lymphocytes to the liver [12]. This temporal cooperation between IP-10 and MIG levels suggests that the baseline correlation between IP-10 and MIG in patients responding to treatment probably reflects an immune system where the normal balance between these CXCR3 ligands is intact. MIG and IP-10 induced recruitment of CXCR3+ HCV-specific T cells to the liver in response to pegIFN treatment may be important for clearance of the infection. This could explain why higher MIG levels and the correlation between IP-10 and MIG levels associate with a successful response to treatment. Reports from studies in HBV-infected patients describing a positive correlation between IP-10 and MIG [28] and an association of higher IP-10 levels with response to IFN treatment [33, 34] together suggest a balanced ratio between IP-10 and MIG in these patients.

DPP4 activity was higher in non-SVR patients, in line with previous reports [20–22]. This observation, together with the demonstrated correlation between DPP4 activity and IP-10 levels, indicates that IP-10 levels in non-SVR patients are influenced by DPP4 activity. Since there was no correlation between DPP4 activity and MIG levels, the DPP4 cleavage of MIG may not be as pronounced or not have the same effect as DPP4 cleavage of IP-10. Indeed, MIG is N-terminally cleaved by DPP4 at a four times lower rate than IP-10 [17]. Although DPP4 cleavage of IP-10 and MIG result in similarly impaired chemotactic activities, the DPP4-truncated form of IP-10 has a reduced binding capacity to CXCR3 and an antagonistic effect on chemotactic activity, while DPP4-cleaved MIG has similar binding ability and calcium signaling as uncleaved protein [17]. Structural differences between IP-10 and MIG might be responsible for these differences. In IP-10 and I-TAC, the N-terminal is involved in the interaction with the receptor, while MIG has an extended C-terminal that is important for receptor binding and chemotactic activity [35].

The apparent contradictory finding that non-SVR patients have higher IP-10 levels than SVRs [7–9], may be explained by increased DPP4 cleavage of IP-10 into an antagonistic form in these patients [20]. Furthermore, absence of a correlation between IP-10 and MIG in non-SVR patients is probably due to DPP4 cleavage of IP-10, but not MIG, into the non-functional form in these patients. The lack of association between the CXCR3 ligands may contribute to the deficit pegIFN response. In mice lacking either IP-10 or MIG it was evident that expression of both CXCR3 ligands was necessary for optimal recruitment of cells to inflamed tissues and clearance of herpes simplex virus (HSV) infection [36]. Since both temporal and cell-specific expression of IP-10 and MIG can influence outcome of an infection [12] further investigations of the cellular source for the CXCR3 ligands during pegIFN/RBV treatment may shed light on how the ligands collaborate in resolving HCV infection.

MIG was significantly associated with both first and second phase viral load decline, while IP-10 was significantly but inversely associated with second phase viral load decline (Supplementary Figure 3). In contrast to an earlier study [37] IP-10 was not associated with first phase viral load decline when dividing patients based on median viral load decline. However, when dividing the cohort using the earlier defined cut-off threshold of 600 pg/ml IP-10 [9, 11] a significant association with both first and second phase decline was found (data not shown). We conclude that both chemokines are connected with viral load decline.

This is further strengthened by the multivariate analysis where MIG, IP-10, DPP4 and viral load all significantly contributed to predict treatment response.

MIG plasma levels were associated with ALT levels and HAI inflammation score, indicating that MIG might have a role in the chronic liver inflammation of HCV-infected patients. This corroborates previous findings of higher peripheral and liver levels of MIG and the other CXCR3 ligands in patients with more severe liver inflammation [13–15, 38, 39]. Higher levels of CXCR3 ligands probably cause increased recruitment of non-specific CXCR3+ T cells [40] and other leukocytes to the liver contributing to induction of an inflammatory state.

In conclusion, we demonstrate that not only *IL28B* genotype and low serum IP-10 levels but also high MIG plasma levels are associated with successful treatment response. Furthermore, a high level of DPP4 enzyme activity was found in non-SVR patients. Our findings suggest that increased DPP4 cleavage of IP-10, but not MIG, in non-SVR patients disrupt the balance between the CXCR3 ligands, which results in an unfavorable immunological status that makes it less likely to respond to pegIFN/RBV treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Conflict of interest: S.J., W.T., M.T., G.F. and J.A. are employees of Janssen Research and Development. G.F., M.F. and J.A. are co-inventors of a patent regarding the use of peripheral IP-10 levels in combination with *IL28B* genotype to predict pegIFN/RBV treatment response in HCV-infected patients. J.D. is a consultant for Gilead and has received research support from Bristol Myers Squibb and Vertex. M.F. has served as a consultant for Abbott Pharmaceuticals, Bristol-Myers Squibb, Gilead, Janssen and Merck and has received research funding from Abbott Pharmaceuticals, Bristol-Myers Squibb, Genentech, Gilead, Janssen, Merck and Vertex.

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Abbreviations

SVR	Sustained virological response
pegIFN	peginterferon
RBV	ribavirin
HCV	hepatitis C virus
IL28B	interleukin 28B
IP-10	interferon gamma-induced protein 10

DPP4	dipeptidyl peptidase IV
ViraHep-C	Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C
MIG	monokine induced by interferon
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
I-TAC	interferon inducible T cell alpha chemoattractant
ALT	alanine transaminase
HBV	hepatitis B virus
SNP	Single nucleotide polymorphisms
HAI	histologic activity index.

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Key points

- Baseline plasma levels of the chemokine MIG are higher in HCV infected patients successfully treated with pegIFN/RBV, while the levels of the related chemokine and known biomarker IP-10 are lower.
- Plasma levels of MIG and IP-10 correlate in SVR, but not in non-SVR patients and only in patients with the favorable *IL28B* CC genotype.
- Non-SVR patients show higher activity of the chemokine cleaving enzyme DPP4, which is associated with levels of IP-10, but not MIG.
- Higher DPP4 activity in non-SVR patients might cause a disturbed balance of IP-10 and MIG, which may lead to a dysfunctional immune response.

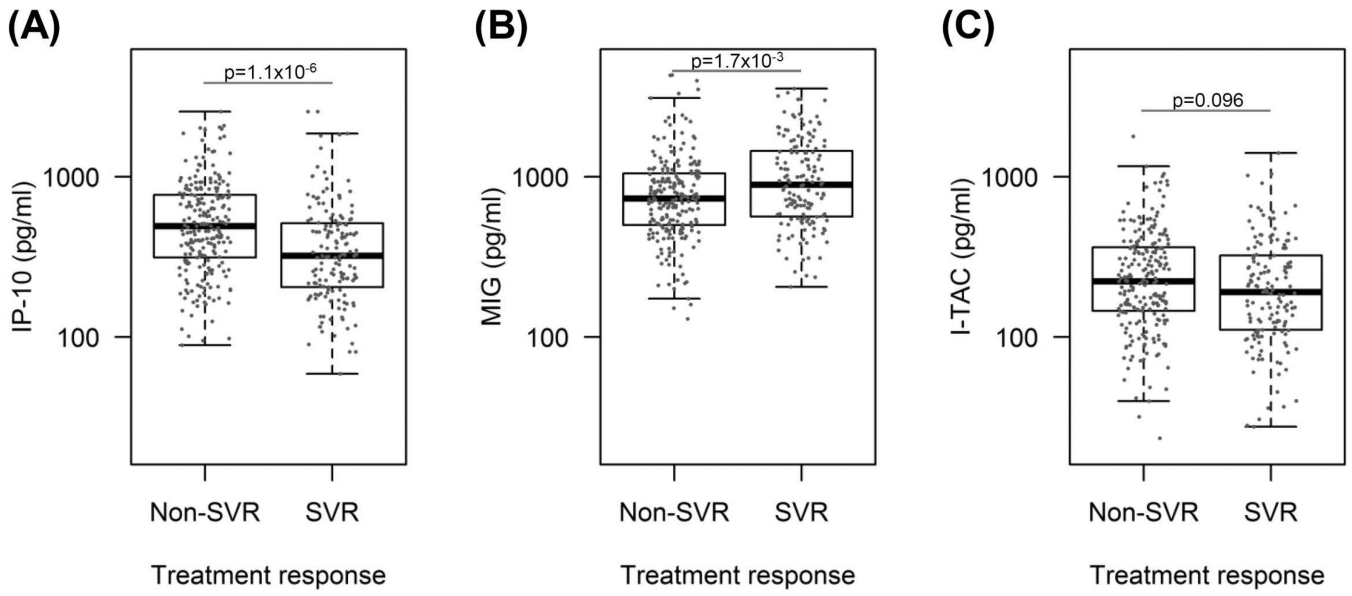

Figure 1.

Boxplots of plasma levels for the chemokines (A) IP-10, (B) MIG and (C) I-TAC stratified according to response to pegIFN/RBV treatment. P-values were derived by Student's t-test and were adjusted for multiple comparisons (Bonferroni, n=3).

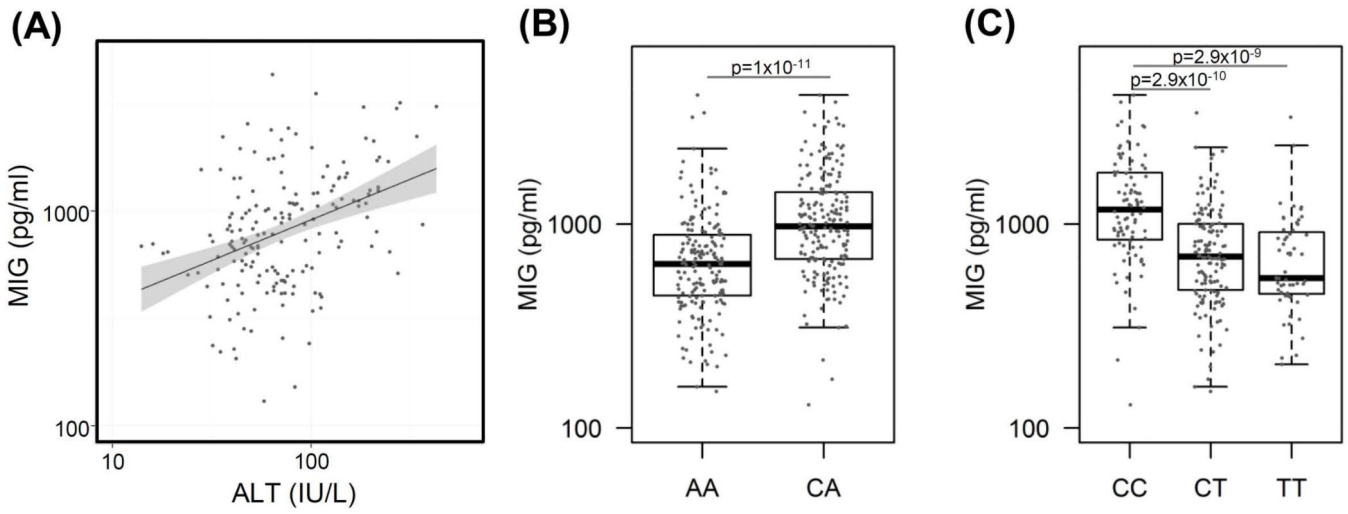

Figure 2.

Patient characteristics significantly associated with MIG plasma levels in a multivariate model. (A) Correlation between plasma levels of MIG and serum levels of ALT at baseline. Linear regression with 95% confidence intervals (grey shaded area) are shown. Boxplots of MIG plasma levels are grouped according to (B) ethnicity and (C) *IL28B* genotype of the patients. AA=African-American and CA=Caucasian-American. P-values were derived by Student's t-test or Tukey's honest significance test respectively. P-values were adjusted for multiple comparisons (Bonferroni). Comparisons without a significant difference ($p>0.05$) are not shown in (C).

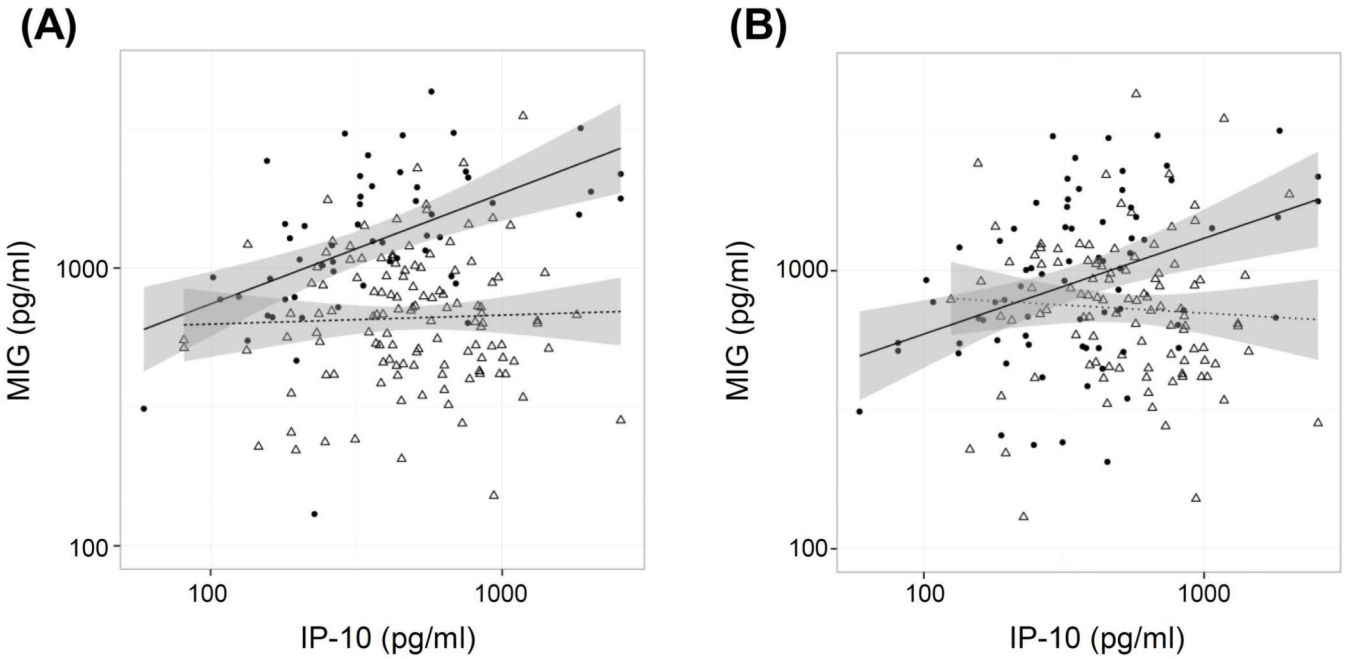

Figure 3.

Correlation between plasma levels of MIG and IP-10 at baseline for (A) patients with the *IL28B* CC (filled circles) or CT/TT alleles (empty triangles) and (B) SVR (filled circles) and non-SVR patients (empty triangles) in response to pegIFN/RBV treatment. Linear regressions with 95% confidence intervals (grey-shaded area) are shown as a solid line for *IL28B* CC and SVR patients and a dashed line for *IL28B* CT/TT and non-SVR patients.

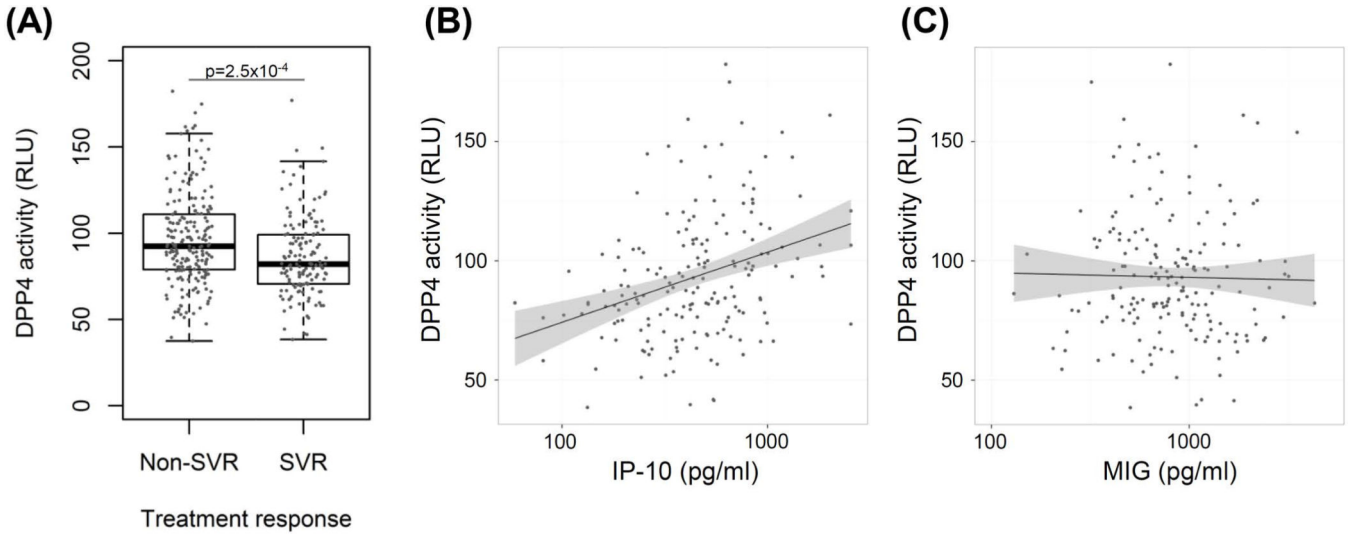

Figure 4.

(A) Boxplots of plasma DPP4 protease activity according to pegIFN/RBV treatment response. The p-value was derived by Student's t-test. Correlation between DPP4 activity and baseline plasma levels of (B) IP-10 and (C) MIG. Linear regression with 95% confidence intervals (grey-shaded area) are shown.

Table 1

Univariate analysis of liver disease variables and the other CXCR3 ligands associated with plasma levels of MIG

Analyte	Estimate	(confidence interval)	p-value
ALT •	0.37	(0.29 : 0.46)	2.5×10^{-14}*
Ethnicity	0.65	(0.48 : 0.82)	1.0×10^{-11}*
IL28B genotype CC vs CT/TT	−0.78	(−0.99 : −0.58)	1.6×10^{-11}*
HAI (< or > 8)	0.40	(0.22 : 0.58)	2.6×10^{-4}*
I-TAC •	0.18	(0.09 : 0.26)	7.8×10^{-4}*
SVR	0.32	(0.14 : 0.51)	1.1×10^{-2}*
IP-10 •	0.15	(0.07 : 0.24)	1.1×10^{-2}*
Platelet count	−0.0015	(−0.0028 : −0.0002)	4.7×10^{-1}
ISHAK fibrosis stage (< or > 3)	0.21	(0.02 : 0.40)	5.2×10^{-1}
Age	0.01	(0.001 : 0.022)	1
Total bilirubin	0.23	(−0.02: 0.49)	1
Albumin	0.20	(−0.10 : 0.50)	1
Viral load •	0.06	(−0.07 : 0.18)	1
Ferritin •	−0.03	(−0.09 : 0.03)	1
Triglycerides •	−0.05	(−0.17 : 0.08)	1
Hemoglobin	0.01	(−0.05 : 0.08)	1
Gender	−0.04	(−0.23 : 0.15)	1
Hematocrit	0.005	(−0.03 : 0.02)	1
Steatosis (>5% fat)	0.02	(−0.18 : 0.21)	1

Estimate is the coefficient derived from a linear model

* p-value <0.05, adjusted for multiple comparisons (Bonferroni)

• data log-transformed before statistical data analysis