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Plasma levels of growth-related oncogene (CXCL1-3) associate with fibrosis and platelet counts in HCV-infected patients

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Abstract

Background—Fibrosis progression in hepatitis C virus (HCV)-infected patients varies greatly between individuals. Chemokines recruit immune cells to the infected liver and may thus play a role in the fibrosis process.

Aim—To investigate plasma levels of a diverse chemokine panel in relation to liver fibrosis.

Methods—African-American and Caucasian HCV genotype 1 infected patients were treated with peginterferon (pegIFN) and ribavirin (RBV) for 48 weeks (VIRAHEP-C cohort). Plasma levels of 13 cytokines were studied at baseline (n=386). Subsequently, GROα levels were assessed in a sub cohort (n=99) at baseline, and at 4 and 12 weeks after start of pegIFN/RBV treatment.

Results—Increased severity of fibrosis (Ishak fibrosis score 0–2 versus 3–6) was associated with increased plasma IP-10 (CXCL10) and IL-8 (CXCL8) levels, and decreased plasma levels of the chemokine growth-related oncogene (GRO, CXCL1-3). Plasma GRO levels were also positively correlated with platelet counts, and were higher in African-American as compared to Caucasian patients. In response to pegIFN/RBV treatment, GROα levels increased in Caucasian but not African-American patients from week 4 onwards.

Conclusion—The association with severity of fibrosis and platelet count positions plasma GRO as a potential biomarker for liver fibrosis in HCV-infected patients. The secretion of GRO by platelets may explain the correlation between GRO plasma level and platelet count. The ethnic

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difference in GRO levels both pre-treatment and in response to pegIFN/RBV might be driven by a genetic polymorphism in GROα associated with higher plasma levels and more common in the African-American population.

Keywords

HCV; fibrosis; GRO; platelets; ethnicity; interferon; ribavirin

Introduction

Hepatitis C virus (HCV) is a blood-borne virus, infecting approximately 170 million people in the world. HCV establishes a chronic infection in the liver in about 80% of individuals infected. The severity of the disease can vary from asymptomatic chronic infection to liver fibrosis and cirrhosis that can result in hepatocellular carcinoma or liver failure [1]. The rate of progression from an early stage of fibrosis to cirrhosis varies greatly between individuals and is known to be influenced by duration of HCV infection, alcohol consumption and HIV or HBV co-infection [2].

Liver fibrosis is defined as an accumulation of extracellular matrix proteins and a modified liver architecture. Specialized liver macrophages called Kupffer cells react on viral infections by producing chemoattractants for lymphocytes (i.e. chemokines) and by activating hepatic stellate cells that start to produce excess extracellular matrix proteins [3]. Both the recruited lymphocytes and the hepatic stellate cells secrete pro-inflammatory cytokines and chemokines, leading to a low-grade inflammation that may cause tissue damage. These chemokines and cytokines together with extracellular matrix proteins are important mediators of liver fibrosis progression, since increased expression of chemokines and cytokines in the liver and peripheral blood has been reported in relation to progressive liver fibrosis and cirrhosis [3, 4].

Over the last decade, HCV-infected patients have been treated with peginterferon (pegIFN) and ribavirin (RBV), which results in 40–50% sustained virological response (SVR) in HCV genotype 1 infected patients [5]. The treatment response rate in patients of different ethnicities is known to vary, which is especially true for African-American patients showing a lower SVR rate as compared to Caucasian patients [6]. Although not fully understood this may at least partially be attributed to genetic polymorphisms, such as the rs12979860 polymorphism in the promoter region of the *IL28B* gene that is associated with SVR to pegIFN/RBV therapy [7].

Ethnicity may also influence the outcome of the HCV-related liver disease. A lower rate of cirrhosis has been reported in African-American patients as compared to other ethnicities in some studies [8, 9]. Here we investigated factors contributing to a more severe fibrosis in the Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C (VIRAHEP-C) cohort that was set up to identify factors that determine why African-Americans infected with HCV genotype 1 respond less well to therapy with pegIFN/RBV compared to Caucasians [6]. The primary aim of the study was to investigate if plasma levels of certain chemokines are associated with the liver fibrosis stage in HCV-infected patients. Multiple chemokines and cytokines were assessed in peripheral blood and their association with liver fibrosis was

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analyzed in concert with clinical characteristics and blood parameters routinely determined in HCV-infected patients. We report on a novel association for the neutrophil recruiting chemokine growth-related oncogene (GRO, CXCL1-3) with liver fibrosis scores, platelet counts and ethnicity.

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 [6]. Only patients that classified themselves by ethnicity as either African-American or Caucasian American were included. Patients also needed to be aged between 18 and 70 years, be treatment naïve, and have detectable HCV RNA and histologic evidence of chronic HCV. The study enrolled 401 patients from eight clinical centers in the United States and pegIFN/RBV therapy started between July 2002 and December 2003. Patients were treated with 180μg pegylated interferon α-2a (Pegasys, Roche Pharmaceuticals, Nutley, NJ) per week and 1000–1200 mg RBV (Copegus, Roche Pharmaceuticals, Nutley, NJ) per day for up to 48 weeks. All participants provided written informed consent, including consent for genetic testing.

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 [6]. The *IL28B* polymorphic marker rs1297860 was genotyped in 297 patients who consented for genetic analysis, as described earlier [10].

Liver histology

All patients had undergone liver biopsy within 18 months of screening and the biopsies were graded as previously described [11, 12]. Necroinflammatory changes were graded from 0 to 18 according to the histologic activity index (HAI), which is the sum of periportal necrosis (0–10), lobular inflammation (0–4) and portal inflammation (0–4). A HAI score of more than 8 means that the inflammation is moderate or marked. Fibrosis was graded from 0 to 6 according to the ISHAK fibrosis scale, where a fibrosis score of 3 or more was regarded as severe fibrosis in accordance with a previous study from the VIRAHEP-C cohort [11]. Steatosis was graded depending on percentage of cells with fat from 0 to 4, where 1 equals 5%.

Plasma cytokine quantification

Plasma samples from 386 patients from the VIRAHEP-C cohort were available for analysis at baseline (Table 1). Milliplex human cytokine/chemokine panel I, II and III assays (Millipore) were used to assess plasma levels of 13 cytokines and chemokines, including GRO, interleukin 8 (IL-8), IL-6, IL-9, IL-10, IL-18, IL-29, IL-28a, IFN-γ, MIG, I-TAC, SDF-1a/b and MIP-1β. Each sample was analyzed in duplicate and at least three control samples were included on each plate to assess possible plate effects and inter-assay variability. Duplicate samples that varied much were rerun if samples were available. Intra-

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and inter-assay variability was calculated for each reliably measured individual analyte. BioPlex Manager 6.1 software was used to generate the standard curves for each analyte and derive protein concentrations in each sample. Plasma interferon gamma-induced protein 10 (IP-10, CXCL10) levels (Quantikine human IP-10 assay from R&D systems) in the VIRAHEP-C cohort that were determined and reported previously [10] were also included in the data analysis of this study.

A smaller sub cohort of 99 patients, comprising 49 SVR and 50 non-SVR patients, with similar numbers of Caucasian and African-American patients in each response group, were included in a sub cohort for on-therapy assessment of GROα (CXCL1) (Table 1). Plasma expression levels of GROα in samples collected at baseline, and at 4 and 12 weeks after start of treatment were derived from analyses using a human 7 cytokines BioPlex assay (Bio-Rad).

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 t-test, paired t-test, Chi-squared test, ordinary linear regression, ANOVA, Tukey's honest significance test and Pearson correlation. The Bonferroni method was applied to correct for multiple comparisons and the cut-off for a statistically significant test was set at p 0.01. 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 0.01 for a variable to be included in the final model. A mixed model was used to assess differences in change over time for different patient groups.

Results

Multiplex plasma cytokine analysis in HCV patients

In total 386 patients from the VIRAHEP-C study were included in the investigation of cytokine plasma levels in untreated HCV patients. Baseline patient characteristics for the cohort are shown in Table 1. Thirteen cytokines were selected based on literature findings and biological associations and the plasma concentrations of these were investigated by multiplex protein assays. Eight of these cytokines could be reliably measured at baseline: GRO (CXCL1, CXCL2, CXCL3), IL-8 (CXCL8), MIP-1β (CCL4), IL-18, MIG (CXCL9), SDF-1a/b (CXCL12), I-TAC (CXCL11) and IL-29 (IFNλ1). The other cytokines in the multiplex assay (IFN-γ, IL-6, IL-9, IL-10 and IL-28a) were found out of range and have been excluded from further analyses because the large majority (55–91%) of the samples had concentrations below the detection limit. The intra-assay variability for the reliably measured analytes were 3.2 % for GRO, 3.7% for MIG, 4.7% for IL-18, 4.8% for IL-8 and MIP-1β, 5.0% for SDF-1a/b, 5.3% for I-TAC and 7.9% for IL-29. Similarly the inter-assay variability varied between 4.5 and 7.0%.

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Association of GRO, IL-8 and IP-10 with ISHAK fibrosis score

Potential associations of cytokine levels with fibrosis severity were assessed by comparing patients with high (3) versus low (<3) ISHAK fibrosis score. We also included gender, ethnicity, and other measured baseline blood parameters in this analysis. Eight out of the 23 variables in the analysis were significantly associated with severe fibrosis after correction for multiple comparisons (Table 2). The chemokines IP-10, GRO and IL-8 were statistically significantly different in patients with a high ISHAK fibrosis score compared to those with a low score. The other variables in the analysis found to be associated with fibrosis (platelet count, age, ALT, bilirubin and ferritin) were identified and reported earlier in the VIRAHEP-C cohort [11]. When including all significant variables in a multivariate generalized linear model, only platelet count ($p=6\times10^{-6}$) and ALT levels (p=0.008) appear to significantly contribute to the model explaining severe versus non-severe fibrosis.

Plots of the plasma IP-10, GRO and IL-8 levels in function of ISHAK scores (0, 1–2, 3–4 and 5–6) show increasing levels of IP-10 and IL-8 and decreasing levels of GRO with higher ISHAK score (Figure 1). Whereas the association between increased peripheral IP-10 levels and more severe fibrosis is well established in the literature [13, 14], the relationship between fibrosis and GRO or IL-8 is much less investigated. Therefore, we focused our further analyses on GRO and IL-8, which share the possibility to bind the CXCR2 receptor that is expressed on neutrophils.

Association of GRO with ethnicity and platelet count

The association between GRO or IL-8 and various patient parameters connected to liver disease were assessed. Factors associated with IL-8 in univariate analyses were albumin, HAI inflammation and ISHAK fibrosis score (Supplementary Table), however in a multivariate analysis none of the variables were good predictors for plasma levels of IL-8 (data not shown). In a univariate analysis of associations between GRO plasma levels and baseline parameters measured in the HCV-infected patients the variable most strongly associated with GRO was ethnicity (Table 3). Indeed, African-American patients had higher plasma GRO levels as compared to Caucasian patients (Supplementary Figure A). GRO was also significantly associated with the blood parameters platelet count, ALT levels, bilirubin, hemoglobin and the liver biopsy parameters for fibrosis (ISHAK) and inflammation (HAI) (Table 3 and Supplementary Figure). Multivariate linear regression modeling to predict GRO plasma levels revealed a model with significant contributions of ethnicity ($p=2.2\times10^{-16}$) and platelet count ($p=2.3\times10^{-9}$). Although there was no significant interaction between ethnicity and platelet count in prediction of GRO levels (p=0.087), a trend towards a stronger correlation between GRO and platelet count was observed in Caucasian (r=0.58) as compared to African-American patients (r=0.34) (Figure 2).

High GROα levels in African-Americans

A polymorphism in the GROα (CXCL1) gene has been associated with higher plasma levels of GROα [15] and this polymorphism is more common in the African-American population [16]. Since the antibody used in the analysis of the whole cohort bound three proteins, GROα (CXCL1), β (CXCL2), γ (CXCL3), we investigated if our association between ethnicity and GRO levels would be seen in a GROα specific assay in a sub cohort from the VIRAHEP-C

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study. This sub cohort included 99 patients and was representative of the patients in the whole cohort (Table 1). Our results confirm that the GROα levels were significantly higher in African-American versus Caucasian patients ($p=4\times10^{-13}$).

Increased GROα levels in response to pegIFN/RBV treatment in Caucasians

To investigate whether GROα levels are affected by pegIFN/RBV treatment, plasma levels at week 4 and week 12 after the start of treatment were also assessed in the sub cohort of 99 patients. During the first 4 weeks of treatment with pegIFN/RBV, GROα levels increased in Caucasian but not in African-American patients (Figure 3). However, GROα levels did not further change between week 4 and week 12 after start of treatment. The interaction between ethnicity and time was highly significant ($p=1.9\times10^{-7}$) in a mixed model comparing subject-specific time profiles. Of note, the GROα response to pegIFN/RBV treatment did not differ between patients that achieved SVR and those that did not, or between patients with a low (<3) or high (3) ISHAK score (data not shown).

Discussion

Plasma levels of three chemokines associated significantly with liver fibrosis in the VIRAHEP-C cohort. This is to our knowledge the first report associating lower plasma GRO levels with fibrosis in HCV-infected patients, while the higher peripheral levels of IP-10 and IL-8 in HCV-infected patients with severe fibrosis confirm earlier reports [13, 14, 17, 18]. Many chemokines are up-regulated in the infected liver in response to the HCV virus and the pro-inflammatory milieu, where they are involved in regulating inflammation, fibroproliferation and angiogenesis [4]. The role and significance of altered levels of chemokines in peripheral blood of HCV infected patients is, however, less well understood. They may be byproducts of the excess production in the inflamed liver or have a regulatory function, either by mediating a gradient for leukocyte recruitment or by inducing downregulation of chemokine receptors on leukocytes in the periphery. Irrespective of their functional role in the periphery, it seemed reasonable to explore whether peripheral chemokines may serve as biomarkers reflecting the fibrosis stage in the liver of HCV-infected patients [4].

IP-10, IL-8 and GRO all belong to the CXC chemokine family. IP-10 (CXCL10) and the two related chemokines MIG (CXCL9) and I-TAC (CXCL11) are induced by Th1 cytokines, bind the CXCR3 receptor and recruit T cells. IL-8 (CXCL8) and GRO proteins are homologous for only about 40%, but they both bind to the CXCR2 receptor and recruit neutrophils. However, IL-8 can in addition also bind the CXCR1 receptor [19]. The three GRO proteins, GROα (CXCL1), β (CXCL2) and γ (CXCL3) are close homologs, with an amino acid similarity of approximately 90 % [20]. GRO proteins are produced by monocytes and neutrophils, and differences in the upstream regulatory region of the GRO encoding genes may result in diverse cell type expressions [20]. GROα can be produced by liver cells, like hepatocytes and hepatic stellate cells, upon stimulation with IL-1α, TNF or IL-17 [21, 22]. Expression of GRO is also induced by TLR signaling [23]. The HCV antigens HCV core and NS3 signal through TLR2 and Nischalke et al showed that these HCV antigens induce secretion of GROα in monocytes from whole blood [15].

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The increased levels of both IP-10 and IL-8 in patients with more severe fibrosis have been suggested to promote liver damage. In HCV-infected livers IP-10 secretion is induced by virus activation of pathogen recognition receptors and by increased levels of type I interferons [4]. The elevated levels of IP-10 and the other CXCR3 binding chemokines in the liver of HCV-infected patients [13, 24, 25] attracts CXCR3+ cells from the periphery, most of them not HCV-specific, and these inflammatory cells can cause damage to the surrounding tissue and could lead to fibrosis [25, 26]. The levels of IL-8 are also increased in the liver of HCV-infected patients [27, 28]. IL-8 is secreted by neutrophils recruited to the liver in response to the viral infection and can also be induced by the HCV virus [4]. IL-8 may recruit neutrophils, monocytes and macrophages to the liver, and the enhanced presence of macrophages has been correlated with increased severity of fibrosis in liver disease patients [29]. However, our finding that IL-8 is only weakly associated with liver damage parameters in HCV-infected patients is in line with findings from other studies [29, 30].

The opposite directions of the association with liver fibrosis score for peripheral GRO versus IL-8 levels may suggest that these chemokines have different functions during the progression of fibrosis. Peripheral GROα levels are increased in HCV infected patients as compared to healthy individuals [18] and this may be due to HCV-induced secretion of GROα [15]. However, only one other research group has reported on peripheral GRO levels related to fibrosis in HCV-infected patients. Costantini et al found similar serum levels of GROα in cirrhotic and non-cirrhotic HCV-infected patients [18, 31]. The relatively small numbers of patients in these studies and the GROα analysis that was performed in serum instead of plasma samples might have led to different results as compared to our study. Indeed, the latter could influence the results since activated platelets may release pre-stored chemokines, including GRO, upon collection of serum in tubes [32]. Interestingly, elevated GROα mRNA levels in the liver have been reported in HCV-infected patients with severe fibrosis [15, 27]. Also in mice models do increased GROα levels in the liver coincide with the onset of liver fibrosis [33]. We hypothesize that this together with our reported decrease in peripheral GRO levels reflect a chemokine gradient favoring recruitment of GRO-binding leukocytes out of the liver during early phases of fibrosis and into the liver in patients with more severe fibrosis. If so, higher GRO levels in the periphery might serve as a protection against further progression of fibrosis. Additional studies with paired liver biopsy and plasma samples from HCV-infected patients are necessary to discern this potential mechanism further. Also, in order to assess whether GRO is involved in the progression of fibrosis, the chemokine should be analyzed in a cohort of patients with the same fibrosis stage but with different fibrosis progression rates.

The finding that GRO plasma levels correlate with platelet count, the factor most strongly associated with severe fibrosis in our cohort, strengthens the picture of GRO as a marker for fibrosis stage. Indeed, platelet count as well as liver transaminase levels (ALT and aspartate transaminase (AST)) are known biomarkers of liver fibrosis and cirrhosis [34], and the multivariate model generated previously aiming to predict severe fibrosis in the VIRAHEP-C cohort included platelet count, in addition to AST, age and alkaline phosphatase [11]. GROα is stored in platelet granules and can be secreted upon activation [32, 35]. The decrease in GRO plasma levels in patients with more severe fibrosis might therefore at least partially reflect a reduced number of blood platelets (thrombocytopenia) in these patients. Apart from

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the thrombocytopenia itself, also other mechanisms could underlie the correlation between plasma GRO levels and platelet counts in chronically HCV-infected patients. The platelet growth factor thrombopoietin can induce GRO production by megakaryocytes and stimulate platelet activation and granule release [36, 37]. As thrombopoietin levels are decreased in patients with severe fibrosis [38] it may contribute to the interaction between GRO levels and platelet counts.

The observed association between plasma GRO levels and ethnicity may at least partially be attributed to a previously reported single nucleotide polymorphism (SNP) in the *CXCL1* gene encoding GROα [15]. Indeed, Nischalke et al showed that HCV-infected patients carrying the A allele of SNP rs4074 had higher serum levels of GROα. Moreover, population genotyping data show that the A allele is much more prevalent in African-Americans (90 %) as opposed to Caucasians (40 %) [16] and is thus a likely explanation for the ethnicity-related differences in plasma GRO levels observed in our study. Interestingly, the effect of the rs4074 A allele on GROα serum levels was only seen in HCV-infected patients but not in healthy individuals [15]. This may be due to the HCV antigen-induced secretion of GROα, which was also more pronounced in cells from patients with the rs4074 AA genotype [15]. The association of GRO plasma levels with both ethnicity and fibrosis might suggest a role for GRO to explain the decreased rate of liver fibrosis in African-American patients as compared to Caucasian patients [8, 9].

Our finding that GROα is upregulated in response to pegIFN/RBV treatment in Caucasians - but not in African-Americans - is intriguing. Although GRO is not known to be an interferon-stimulated gene, other proteins induced by interferon treatment might be responsible for the effect seen in Caucasian patients. One possibility is thrombopoietin, since increased levels of this protein have been described during treatment of HCV-infected patients with interferon-α and RBV [39, 40]. Thrombopoietin can potentiate GROα release from the platelet precursor megakaryocytes [36]. Whereas this could explain the increased GROα levels in response to pegIFN/RBV treatment in Caucasians, the lack of increased GROα levels in African-Americans is less clear. Possibly, the already high pre-treatment GROα levels in the latter patients may make it impossible to further increase above the maximum expression level. As the change in plasma GROα levels in response to pegIFN/RBV treatment is not associated with therapy outcome, it cannot explain the discrepancy in response outcome between African-American and Caucasian HCV-infected patients. Our results might suggest that increased GRO levels observed during treatment, in particular in patients with low baseline GRO levels, are indicative for an improvement in liver disease. Future studies that prospectively assess pre- and post-treatment GRO levels in association with fibrosis progression during and after therapy are needed to further investigate the potential value of GRO as a biomarker in liver disease.

In conclusion, we have identified plasma GRO as a novel chemokine that is associated with severity of liver fibrosis. The higher peripheral GRO levels in patients with less severe liver fibrosis suggest a protective role for GRO against liver fibrosis. The association of peripheral GRO levels with platelet count and ethnicity further adds to the understanding of GRO in relation to fibrosis in chronically HCV-infected patients. Complementary functional studies on GRO will be needed to further elucidate underlying biological mechanisms.

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Supplementary Material

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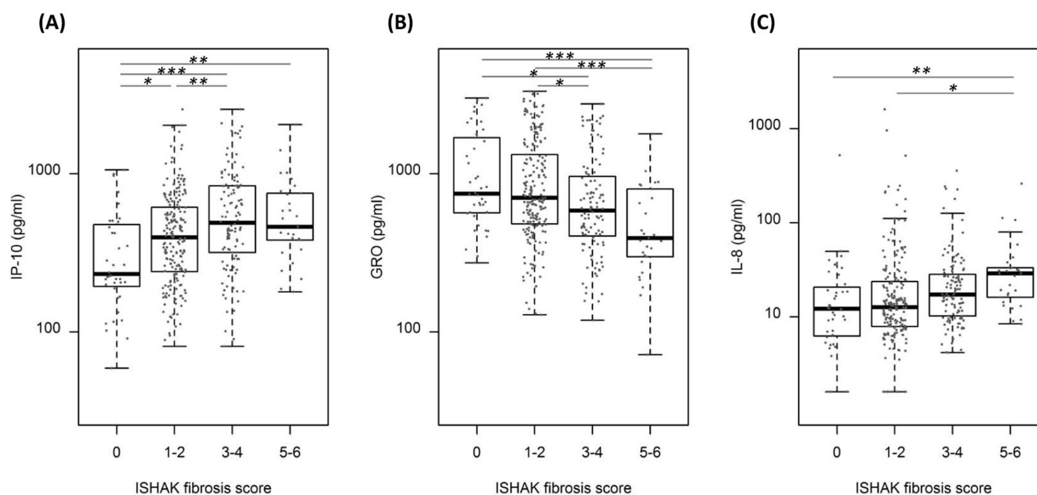

Figure 1.

Boxplots of plasma levels for the chemokines (A) IP-10, (B) GRO and (C) IL-8, which were significantly associated with severe fibrosis. Chemokine data are shown for patients stratified according to ISHAK fibrosis score: 0 (n=40), 1–2 (n=202), 3–4 (n=112) and 5–6 (n=29). P-values were derived by the Tukey's honest significance test. *<0.05, **<0.01, ***<0.001. Comparisons without a significant difference (p>0.05) are not shown.

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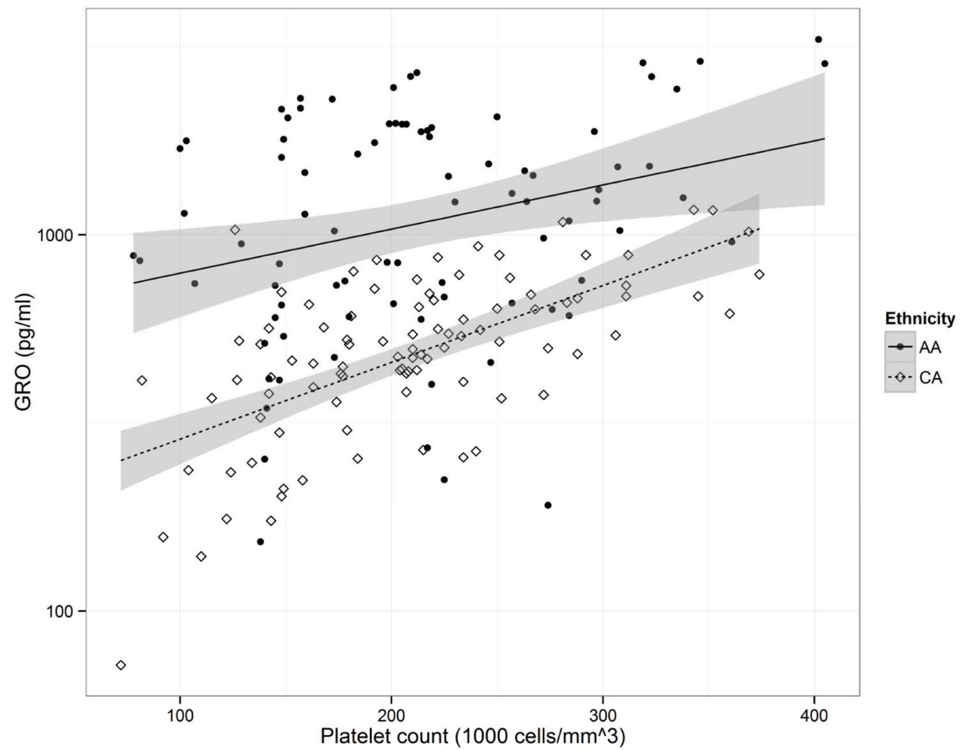

Figure 2.

Correlation between plasma levels of GRO and platelet count at baseline. Caucasian (CA) patients are shown as unfilled diamonds and African-American (AA) patients as filled circles. Linear regressions with 95% confidence intervals are shown as a dashed line for Caucasians ($p=1.4\times10^{-18}$) and a solid line for African-Americans ($p=2\times10^{-06}$).

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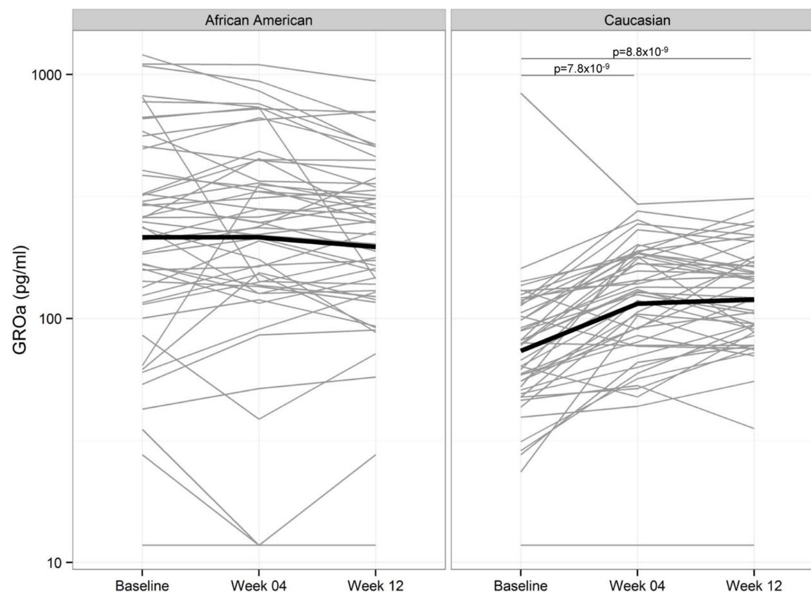

Figure 3.

GROα plasma levels at baseline, week 4 and week 12 after start of pegIFN/RBV treatment grouped according to ethnicity. Each line represents one individual patient's GROα levels over time. The thick black lines show the mean for each ethnic group. P-values were generated by pairwised student's t-test. Comparisons without a significant difference ($p>0.05$) are not shown.

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Table 1

Patient characteristics

Characteristics		Whole cohort (n=386)	Sub cohort for several time points (n=99)
Ethnicity	Caucasian, n (%)	197 (51)	48 (48.5)
Gender	Female, n (%)	135 (35)	41 (41.4)
Age (years)	mean (range)	47.8 (23–70)	47.8 (30–64)
SVR status	SVR, n (%)	157 (40.7)	49 (49.5)
IL28B genotype	CC, n (%)	89 (30)	15 (19)
	CT, n (%)	149 (50.2)	48 (60.8)
	TT, n (%)	59 (19.9)	16 (20.3)
HCV RNA log10 (IU/ml)	mean (range)	6.3 (3.1–7.7)	6.3 (3.6–7.4)
Platelet count (10^3 cells/mm^3)	mean (range)	214.8 (60–489)	214.4 (70–489)
Alanine transaminase (ALT) (IU/L)	mean (range)	90.9 (14–508)	88.1 (21–409)
Total bilirubin (mg/dl)	mean (range)	0.70 (0.1–2.9)	0.75 (0.1–2.9)
Albumin (g/dl)	mean (range)	4.1 (2.3–5)	4.1 (2.9–4.8)
Hemoglobin (g/dl)	mean (range)	14.6 (10.7–18)	14.5 (10.8–17.8)
Ferritin (mg/dl)	mean (range)	279.8 (5.4–1860)	314.6 (20.1–1860)
ISHAK fibrosis score 3	n (%)	141 (36.7)	40 (40.4)
HAI inflammation >8	n (%)	183 (47.7)	46 (46.5)
	median (range)	8 (2–14)	8 (2–14)
Steatosis 5 %	n (%)	129 (33.6)	38 (38.4)

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Table 2

Univariate analysis of non-invasive parameters associated with ISHAK fibrosis score <3 or 3

Variable	Estimate (confidence interval)	p-value
Platelet count	52.80 (39.36 : 66.24)	**3.63E-12**[*]
ALT[•]	−0.56 (−0.76 : −0.37)	**9.19E-07**[*]
Age	−3.93 (−5.45 : −2.42)	**1.24E-05**[*]
IP-10[•]	−0.47 (−0.68 : −0.26)	**3.36E-04**[*]
GRO[•]	0.48 (0.27 : 0.70)	**3.78E-04**[*]
Bilirubin	−0.17 (−0.25 : −0.09)	**4.03E-04**[*]
Ferritin[•]	−0.63 (−0.93 : −0.33)	**1.23E-03**[*]
IL-8[•]	−0.55 (−0.84 : −0.26)	**5.34E-03**[*]
I-TAC[•]	−0.30 (−0.52 : −0.09)	1.41E-01
Albumin	0.11 (0.03 : 0.20)	2.50E-01
MIG[•]	−0.21 (−0.40 : −0.02)	6.11E-01
IL-29[•]	−0.25 (−0.47 : −0.02)	6.74E-01
SVR status (SVR or non-SVR)	−0.09 (−0.19 : 0.01)	1.00E+00
Hemoglobin	−0.18 (−0.45 : 0.10)	1.00E+00
Hematocrit	−0.42 (−1.19 : 0.35)	1.00E+00
MIP-1β[•]	0.18 (−0.15 : 0.51)	1.00E+00
SDF-1α/β[•]	0.10 (−0.10 : 0.30)	1.00E+00
HCV RNA level[•]	−0.08 (−0.22 : 0.07)	1.00E+00
Gender (F or M)	0.05 (−0.05 : 0.15)	1.00E+00
Ethnicity (AA or CA)[†]	0.05 (−0.05 : 0.16)	1.00E+00
Triglycerides[•]	−0.06 (−0.21 : 0.10)	1.00E+00
IL-18[•]	−0.06 (−0.31 : 0.18)	1.00E+00
IL28b genotype (CC or CT/TT)	−0.01 (−0.12 : 0.10)	1.00E+00

Estimates reflect differences between low compared to high ISHAK score groups and indicate either difference in means (for the continuous variables) or differences in proportions (for categorical variables). P-values are derived from t-tests or Chi-square tests for continuous and categorical variables, respectively.

[*] p-value <0.01, adjusted for multiple comparisons by Bonferroni correction

[•] data log-transformed before statistical data analysis

[†] AA=African-American, CA=Caucasian

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Table 3

Univariate analysis of liver disease variables associated with plasma levels of GRO

Analyte	Estimate (confidence interval)	p-value
Ethnicity (AA or CA)†	−1.21 (−1.39 : −1.04)	**4.31E-33***
Platelet count	0.006 (0.005 : 0.008)	**3.96E-15***
ALT•	−0.35 (−0.46 : −0.24)	**7.63E-09***
Bilirubin	−0.72 (−1.02 : −0.43)	**3.16E-05***
ISHAK (< or 3)	−0.48 (−0.70 : −0.27)	**2.55E-04***
HAI (or > 8)	−0.46 (−0.66 : −0.25)	**4.14E-04***
Hemoglobin	−0.16 (−0.24 : −0.09)	**7.95E-04***
IL28B genotype (CC or CT/TT)	0.44 (0.18 : 0.69)	1.46E-02
Steatosis (< or 5%)	−0.31 (−0.54 : −0.09)	1.15E-01
Hematocrit	−0.04 (−0.07: −0.01)	1.47E-01
Triglycerides•	−0.13 (−0.28 : 0.02)	1.00E+00
HCV RNA level•	−0.10 (−0.25 : 0.04)	1.00E+00
Gender (F or M)	−0.14 (−0.36 : 0.09)	1.00E+00
Ferritin•	−0.03 (−0.11 : 0.04)	1.00E+00
SVR status (SVR or non-SVR)	−0.09 (−0.31 : 0.12)	1.00E+00
Albumin	−0.08 (−0.41 : 0.26)	1.00E+00
Age	−0.003 (−0.016 : 0.011)	1.00E+00

Estimate is the coefficient derived from a linear model

*p-value <0.01, adjusted for multiple comparisons by Bonferroni correction

•data log-transformed before statistical data analysis

†AA=African-American, CA=Caucasian