Clinical studies on hepatitis B, C, and E virus infection
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CHAPTER 4

IP-10 in Chronic Hepatitis C Patients Treated with High-Dose Interferon


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ABSTRACT

Introduction
Interferon-γ-Inducible-Protein-10 (IP-10) serum levels are associated with IL28B genotype and may predict response to interferon/ribavirin-based therapy in chronic hepatitis C patients. Our aim was to relate IP-10 levels before and during treatment to treatment outcome, viral HCV-RNA kinetics and IL28B genotype.

Patients and methods
A cohort of chronic hepatitis C patients was treated for 6 weeks with high-dose interferon, followed by standard peginterferon/ribavirin for 24 or 48 weeks. IP-10 and HCV-RNA levels were frequently determined before, during and after treatment.

Results
IP-10 levels increased from log2.56 at baseline to log3.48 pg/mL at Day1 and diminished thereafter gradually. IP-10 levels at any time point were not statistically different between patients with or without SVR. Patients with IL28B CC genotype had significantly lower baseline IP-10 levels (p = 0.019) and a higher increase of IP-10 levels from baseline to Day1 than patients with IL28B non-CC genotypes (p = 0.015). Patients with HCV-RNA decline ≥2.28log10 at Day1 had significantly lower baseline IP-10 levels (p=0.016) and a higher increase of IP-10 levels from baseline to Day1 (p = 0.047) than patients with HCV-RNA decline of <2.28log10 at Day 1.

Conclusions
In patients treated with high induction-dose interferon, IP-10 levels at any time point were not predictive for SVR. Low baseline IP-10 levels and a higher increase of IP-10 levels from baseline to Day1 were associated with IL28B CC genotype and HCV-RNA decline ≥2.28log10 at Day 1. This suggests that for prediction of SVR in our cohort the added value of IP-10 to IL28B genotype and early viral kinetics is limited.
INTRODUCTION

Hepatitis C virus (HCV) infection is a leading cause of chronic hepatitis affecting over 170 million people worldwide.\(^1\) After being exposed to HCV, a chronic infection develops in approximately 80% of cases.\(^2\) Chronic hepatitis C (CHC) is characterized by liver inflammation due to pro-inflammatory cytokines and infiltration of specific and non-specific T-lymphocytes.\(^3\) The damage inflicted leads to liver fibrosis and may ultimately cause liver cirrhosis, hepatocellular carcinoma and death.\(^3\)

After an infection with HCV the innate immune system initiates a nonspecific immune response through type I interferon, leading to the activation of the intracellular pathway resulting in the induction of multiple interferon-stimulated genes (ISG’s). Type I interferon has also immunomodulatory effects by activating and modulating the function of different kinds of leukocytes, including natural killer (NK) cells, macrophages, dendritic cells (DC) and T-lymphocytes. This results in a strong specific CD4+/CD8+ T-cell response leading ideally to the clearance of HCV.\(^4\) In most cases however, a chronic HCV infection is established, in which the HCV specific immune responses are weaker and less specific than in acute resolving HCV infection.\(^5\)

The gene encoding the non-ELR CXC chemokine interferon-y–inducible protein-10 (IP-10 or CXCL10) is an ISG that is induced by interferon-y and tumor necrosis factor (TNF) alpha. It is produced by different kinds of cells such as endothelial cells, fibroblasts, mesangial cells, monocytes, neutrophils and hepatocytes. After binding to its receptor CXCR3, IP-10 functions as a chemotactic cytokine for T-lymphocytes, monocytes and NK-cells and induces adhesion of activated memory/effector T-cells.\(^6\) Levels of IP-10 are higher in patients with chronic HCV infection than in healthy controls.\(^7\)

Multiple inflammatory chemokines and cytokines have been suggested as markers for treatment outcome because of their regulatory function in the HCV-specific immune response. Most of these cytokines are modulated by exogenous interferon and play a critical role in viral clearance.\(^8\) In patients who develop a sustained viral response (SVR) after interferon-based therapy, the baseline activation of the immune system tends to be lower prior to treatment than in patients who do not achieve SVR.\(^8\)\(^-\)\(^10\) This difference of baseline activation of the immune system might be influenced by single nucleotide polymorphisms (SNP’s) on chromosome 19 near the interleukin-28B gene (IL28B), encoding interferon-λ. IL28B gene polymorphisms are highly associated with treatment outcome in CHC patients treated with interferon-based therapy.\(^11\) Most data have been published on two of these gene polymorphisms, SNP's rs12979860 and rs8099917, associated with SVR after peginterferon and ribavirin therapy.\(^12\)\(^-\)\(^13\)

Baseline IP-10 levels may be a prognostic marker for the outcome of interferon-based therapy in HCV infection.\(^14\)\(^,\)\(^23\) There are several studies that describe a relation between low baseline IP-10 levels and higher rates of rapid viral response (RVR, HCV-RNA undetectable after 4 Weeks of treatment)\(^14\)\(^,\)\(^15\)\(^,\)\(^20\)\(^,\)\(^23\) and SVR after treatment with peginterferon and ribavirin.\(^14\)\(^-\)\(^22\) However, whether the IP-10 level really is a predictor for SVR and/or RVR remains a subject of discussion.

From 2002 to 2005 a cohort of CHC patients (treatment naïve patients with HCV genotype 1 or 4 and patients of all genotypes with failure to interferon-based therapy) was treated with a high induction dose of interferon combined with ribavirin,
followed by peginterferon and ribavirin.\textsuperscript{24} Our aim was to investigate in this cohort of patients whether IP-10 levels before and during treatment with this high-dose of interferon were related to treatment outcome, IL28B genotype and HCV-RNA kinetics.

**PATIENTS AND METHODS**

**Patients and treatment regimen**
From 2002 to 2005, a cohort-study was performed in which 100 CHC patients were included (treatment naïve patients with HCV genotype 1 or 4 and patients of all genotypes who failed previous therapy with either classical interferon alone, or a combination of (peg)interferon and ribavirin). Results of this study have been reported in 2008.\textsuperscript{24} All patients were treated for 6 weeks with high-dose interferon-alpha 2b (Merck Pharmaceuticals, USA), combined with ribavirin (weight-based: 1000 mg/day in patients weighing <75 kg, and 1200 mg/day in patients weighing >75 kg), followed by 24 or 48 weeks of peginterferon alpha 2b (1.5 μg/kg once a week) and ribavirin (weight-based 1000–1200 mg/day). All patients were also treated with amantadine hydrochloride 200 mg/day (Symmetrel®, Novartis, Basel, Switzerland). Figure 1. describes the study design.

During the first six weeks of treatment the following interferon-induction scheme was used: Weeks 1 and 2: 18 MU/ day in three divided doses; Weeks 3 and 4: 9 MU/d in 3 divided doses; Weeks 5 and 6: 6 MU/d in 2 divided doses. Patients with a decline in HCV-RNA ≥3log\textsubscript{10} at Week 4 (and TMA-undetectable at week 24) were randomized to stop treatment at 24 Weeks or to continue to 48 weeks. Patients with a decline in HCV-RNA <3log\textsubscript{10} at Week 4 were treated for 48 Weeks. Treatment was stopped in all patients with detectable HCV-RNA at Week 24. All patients were followed for 24 Weeks after completion of therapy.

Plasma samples were stored at -80° C at baseline, Days 1 and 3, Weeks 1, 2, 3, 4, 6, 8, every 4 weeks until the end of treatment, and after cessation at Weeks 4, 12 and 24. The study was approved by the institutional review board. Written informed consent was obtained from each patient.

**Patient and sample selection for measurements**
All patients who completed the whole treatment course or who had to stop treatment before Week 24 or 48 because of stopping criteria, were included in our study to determine IL28B genotype and to measure IP-10 and HCV-RNA levels at baseline, Day 1, Week 1, 2, 4 and 6, at end of treatment (EOT) and at end of follow-up (EFU). Patients who stopped treatment prematurely (dropouts) between Day 0 and Week 24 (for other reasons than the above mentioned stopping criteria), and patients of whom baseline plasma samples were not available were excluded. Of the 100 included patients in the original study, 85 patients were included in this study. Reasons for exclusion of the remaining 15 patients were drop-out due to side effects of the treatment (n = 12), drop-out because of non-medical reasons (n = 1), and lack of available plasma samples (n = 2). From six of the included 85 patients Day 1 plasma samples were missing. For that reason, change in IP-10 levels from baseline to Day 1 could not be calculated and therefore these patients were excluded.
HCV-RNA measurement
HCV-RNA was quantitatively measured using a bDNA assay (VERSANT® HCV 3.0 assay; Siemens, Germany); linear dynamic range $6.15 \times 10^2$ to $7.7 \times 10^6$ IU/mL. A qualitative HCV-RNA measurement was performed when the quantitative test was negative, using transcription-mediated amplification (TMA) (VERSANT® HCV qualitative assay, Siemens, Germany; lower limit of detection (LLD) 5 IU/mL). HCV genotypes were determined using the TruGene® HCV genotyping assay and the Open-Gene® automated DNA sequencing system (Bayer Diagnostics, Berkeley, California, USA).

IP-10 measurement
IP-10 levels were measured using a solid base sandwich ELISA (lower limit of detection 4.46 pg/ml, dynamic quantitative assay range 7.8 – 500 pg/mL; Quantikine human CXCL10/IP-10 immunoassay, R&D Systems). Plasma samples were tested in duplicate in a dilution of 1:5 (according to manufacturer’s description). A first evaluation of the test results showed that in many cases IP-10 levels, especially at Day 1, were above the upper limit of the assay range of 500 pg/mL. By using Bland-Altman plots comparing duplicate measurements, we retested all plasma samples with an initial test value >730 pg/mL (with 1:5 dilution) after a second dilution step of 1:5, resulting in a dilution of 1:25 for calculation of IP-10 levels.

IL28B genotyping
IL28B single nucleotide polymorphism (SNP) genotyping (rs12979860) was performed by High Resolution Melting Curve Analysis (HRMCA) on a LightCycler480 (Roche Applied Science) using custom-designed primers and LC480 High Resolution Melting Master (Roche Applied Science). Results were analyzed with the LC480 HRMCA module implemented in the LC480 Software.
**Assessment of treatment outcome**

The following definitions were used to categorise treatment outcomes:

**SVR**: Undetectable HCV-RNA at end of follow-up (24 Weeks after end of treatment);

**RVR**: Undetectable HCV-RNA at Week 4 during treatment;

**Non-response**: Detectable HCV-RNA (TMA positive) at all time points during treatment and at end of follow-up;

**Relapse**: Undetectable HCV-RNA (TMA negative) at end of treatment but detectable HCV-RNA at end of follow-up;

**Non-SVR**: All patients who did not achieve SVR.

**Drop-out**: Any patient who stopped treatment prematurely between Day 0 and Week 24/48 or who was lost to follow up during 24 Weeks thereafter.

**Statistical analysis**

IP-10 values were logarithmically transformed to achieve a normal distribution. Graphic representation was performed using GraphPad Prism version 5 for Windows (GraphPad Software, San Diego, California, USA) and SPSS version 19.2 for Windows (SPSS Inc., Chicago, Illinois, USA). Data were analyzed on per protocol basis. We used the Bland-Altman plots, Student’s t-test, the Mann-Whitney U-test, chi-square and Fisher’s Exact test where appropriate. Differences were considered statistically significant when p was < 0.05. A receiver operating characteristic (ROC)-analysis was performed to determine at Day 1 which level of HCV-RNA decline gave the best prediction for SVR.

**RESULTS**

**Baseline characteristics and treatment outcome**

Baseline characteristics of the 85 patients included in the study are shown in Table 1. Thirty-six of the 85 patients (42 %) achieved SVR, whereas 49 (58 %) did not. Treatment naïve patients, patients with RVR, IL28B CC genotype or a low METAVIR fibrosis stage (F0-F1-F2) were significantly more likely to achieve SVR. The group of patients with genotype 2, 3 or 5 had a higher SVR rate than patients with genotype 1 or 4. Statistically this was not significant, but there was a trend (p = 0.09). IP-10 levels at baseline were lower in patients with SVR compared to patients without SVR, but this difference was not statistically significant (Table 1.). There was no statistically significant difference in baseline IP-10 levels between patients with partial response or patients with null response (data not shown). There were 26 patients with IL28B genotype CC of which 17 (65 %) had SVR and 9 (35 %) did not. Of the 59 IL28B non-CC genotype patients 19 (32 %) had SVR (p = 0.008) (Table 1.).

A cut-off of < / ≥ 600 pg/mL was used (chosen based on earlier literature16) to define high and low IP-10 levels at baseline. In the group of patients with baseline IP-10 levels ≥ 600 pg/mL. Treatment-experienced patients had lower SVR-rates that treatment-naïve patients. However, these differences were not statistically significant (Table 2.).

**Baseline IP-10 levels and response parameters**

Mean log IP-10 levels at baseline were significantly lower in patients achieving RVR than in patients without RVR (2.43 pg/mL / 2.62 pg/mL, p = 0.016) (Table 3.). This was also the case in patients with IL28B CC genotype versus patients with IL28B non-CC
Table 1. Baseline characteristics of patients treated with high-dose induction interferon followed by peginterferon and ribavirin for 24 or 48 weeks according to SVR

<table>
<thead>
<tr>
<th></th>
<th>SVR</th>
<th>Non-SVR</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>36</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Male (%)/ female (%)</td>
<td>28/8</td>
<td>38/11</td>
<td>0.98</td>
</tr>
<tr>
<td>Mean age, years (range)</td>
<td>44/63</td>
<td>46/67</td>
<td>0.37</td>
</tr>
<tr>
<td>Baseline HCV-RNA (log)</td>
<td>5.97</td>
<td>5.77</td>
<td>0.28</td>
</tr>
<tr>
<td>Naïve / non-naïve (%) *</td>
<td>24/12</td>
<td>22/27</td>
<td>0.046</td>
</tr>
<tr>
<td>Genotype (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>23</td>
<td>34</td>
<td>0.65</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>12</td>
<td>0.43</td>
</tr>
<tr>
<td>2/3/5</td>
<td>7/8</td>
<td>3</td>
<td>0.09</td>
</tr>
<tr>
<td>RVR / non-RVR (%)</td>
<td>19/17</td>
<td>5/44</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IL28B genotype CC / non-CC (%)</td>
<td>17/19</td>
<td>9/40</td>
<td>0.008</td>
</tr>
<tr>
<td>Baseline IP-10 (log pg/mL) (+/- SEM)</td>
<td>2.53 / 0.04</td>
<td>2.59 / 0.05</td>
<td>0.34</td>
</tr>
<tr>
<td>Liver biopsy (%)</td>
<td>32</td>
<td>46</td>
<td>0.001</td>
</tr>
<tr>
<td>Fibrosis stage Metavir F3/F4 (%)</td>
<td>12/15</td>
<td>31/40</td>
<td></td>
</tr>
</tbody>
</table>

* Non-naïve: earlier treatment with either classical interferon alone, or combination therapy with (peg)interferon and ribavirin

Table 2. SVR versus non-SVR in naïve and treatment experienced patients with baseline IP-10 levels of < or ≥ 600 pg/mL

<table>
<thead>
<tr>
<th>IP-10 baseline (pg/mL)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 600 pg/mL</td>
<td></td>
</tr>
<tr>
<td>Naïve</td>
<td>5/8</td>
</tr>
<tr>
<td>Non-naïve</td>
<td>2/7</td>
</tr>
<tr>
<td>≥ 600 pg/mL</td>
<td></td>
</tr>
<tr>
<td>Naïve</td>
<td>19/38</td>
</tr>
<tr>
<td>Non-naïve</td>
<td>10/32</td>
</tr>
</tbody>
</table>

genotypes (2.45 pg/mL / 2.62 pg/mL, p = 0.019) (Table 3). Statistically there was a trend towards lower baseline mean log IP-10 levels in HCV genotype non-1 patients (compared to HCV genotype 1 patients, p = 0.098) (Table 3). For all other parameters shown in Table 3. there was no statistically significant difference in baseline IP-10 levels. Because it is well-known that IP-10 levels and IL28B are related, we performed a multivariate analysis showing that IL28B CC genotype was an independent predictor of RVR (Table 4). This multivariate analysis showed a trend towards lower baseline IP-10 levels in patients achieving RVR (p = 0.079).
### Table 3. Baseline IP-10 levels and various response parameters

<table>
<thead>
<tr>
<th></th>
<th>Baseline IP-10 levels (mean log +/- SEM, pg/mL)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naïve / non-Naïve</td>
<td>2.54 (0.05) / 2.59 (0.04)</td>
<td>0.51</td>
</tr>
<tr>
<td>Genotype 1 / Genotype non-1</td>
<td>2.60 (0.04) / 2.50 (0.04)</td>
<td>0.098</td>
</tr>
<tr>
<td>Baseline HCV-RNA &lt; 600.000 / ≥ 600.000 IU/mL</td>
<td>2.57 (0.05) / 2.56 (0.04)</td>
<td>0.81</td>
</tr>
<tr>
<td>Fibrosis score Metavir F3-F4 / F0-F2</td>
<td>2.56 (0.04) / 2.58 (0.05)</td>
<td>0.81</td>
</tr>
<tr>
<td>IL28B genotype CC / non-CC</td>
<td>2.45 (0.05) / 2.62 (0.04)</td>
<td>0.019</td>
</tr>
<tr>
<td>RVR / non-RVR</td>
<td>2.44 (0.05) / 2.61 (0.04)</td>
<td>0.016</td>
</tr>
</tbody>
</table>

### Table 4. Predictors of RVR: multivariate analysis of baseline IP-10 levels and IL28B genotype

<table>
<thead>
<tr>
<th></th>
<th>RVR</th>
<th>Non-RVR</th>
<th>Confidence Interval (95%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL28B genotype CC, N (%)</td>
<td>14 (58)</td>
<td>12 (20)</td>
<td>0.78 – 0.65</td>
<td>0.006</td>
</tr>
<tr>
<td>non-CC, N (%)</td>
<td>10 (42)</td>
<td>49 (80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log IP-10 baseline (mean, pg/mL)</td>
<td>2.44</td>
<td>2.61</td>
<td>0.013 – 1.267</td>
<td>0.079</td>
</tr>
<tr>
<td>Total, N (%)</td>
<td>24 (28)</td>
<td>61 (72)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 5. Factor of increase in IP-10 levels from baseline to Day 1, different baseline IP-10 levels (dependent on the baseline IP-10 level; the lower the baseline IP-10 level, the higher the factor of increase).

<table>
<thead>
<tr>
<th>IP-10 baseline</th>
<th>N</th>
<th>Factor of increase D1 (mean)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 150</td>
<td>8</td>
<td>27</td>
<td>0.005</td>
</tr>
<tr>
<td>≥ 150</td>
<td>71</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>&lt; 300</td>
<td>31</td>
<td>16</td>
<td>0.001</td>
</tr>
<tr>
<td>≥ 300</td>
<td>48</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>&lt; 375</td>
<td>41</td>
<td>15</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>≥ 375</td>
<td>38</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>&lt; 600</td>
<td>68</td>
<td>13</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>≥ 600</td>
<td>11</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

### Table 6. Patients with a decline of HCV-RNA at Day 1 of ≥ or < 2.28log10 and SVR or non-SVR status.

<table>
<thead>
<tr>
<th>Decline HCV-RNA Day 1</th>
<th>SVR (N)</th>
<th>Non-SVR (N)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 2.28 log10</td>
<td>21</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>&lt; 2.28 log10</td>
<td>15</td>
<td>42</td>
<td>57</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>49</td>
<td>85</td>
</tr>
</tbody>
</table>

PPV = positive predictive value; NPV = negative predictive value; Sens = sensitivity; Spec = specificity

Sens 58.3% Spec 85.7%
**Figure 2.** IP-10 levels before and during treatment. An 1log10 rise at Day 1 was observed, and thereafter IP-10 levels gradually declined and were significantly lower than baseline levels at end of treatment (EOT) and end of follow-up (EFU). * p = 0.01 ** p = 0.01

**Figure 3.** IP-10 levels before and during treatment: SVR versus non-SVR EOT at 24 Weeks (n=55) or 48 Weeks (n=30); EFU at 24 Weeks after EOT (n=85). * p < 0.001
IP-10 levels during therapy

From baseline to Day 1 an almost 10-fold increase of mean log IP-10 levels was observed (from log 2.56 pg/mL to log 3.48 pg/mL) (Figure 2.). The range of the fold increase in IP-10 levels was 2 to 40. The increase was related to baseline IP-10 levels: the lower the baseline IP-10 levels, the greater the increase at Day 1 (Table 5.). Thereafter, mean log IP-10 levels diminished gradually, returning to baseline levels between Week 4 and 6 of treatment, and diminishing further to a level significantly lower than the baseline level at EOT (2.41 pg/mL, p = 0.01) and EFU (2.35 pg/mL, p = 0.01) (Figure 2.).

IP-10 levels during therapy and treatment outcome

Before and during treatment mean log IP-10 values were in general lower in SVR patients than in non-SVR patients, but this difference was not statistically significant at any time point (Figure 3.). At EFU, mean log IP-10 levels were significantly lower in patients with SVR than in non-SVR patients (2.40 pg/mL versus 2.43 pg/mL, p<0.0001) (Figure 3.).
IP-10 levels during therapy and IL28B genotype
The increase of IP-10 levels from baseline to Day 1 was significantly greater in patients with IL28B CC genotype than in patients with IL28B non-CC genotypes (log1.07 pg/mL versus log0.89 pg/mL, p=0.015) (Table 7).

DISCUSSION
In contrast to what has been described earlier, we did not find a clear association between IP-10 levels before or during treatment and SVR or non-SVR. There are also other studies that, like ours, did not confirm the association between a low baseline IP-10 level and SVR. Nevertheless, in our study baseline IP-10 levels were significantly lower in patients with RVR than in those without RVR. The association of RVR and low baseline IP-10 levels without a significant difference in baseline IP-10 between SVR and non-SVR patients was previously described in HCV genotype 1 and 4 patients and in patients with acute HCV infection. However, there are reports contradicting these findings, in which no difference was seen in baseline IP-10 levels between CHC patients with or without RVR or with or without SVR. We also found a clear relation between IL28B genotype and SVR, in line with previous data.

A possible explanation for the relationship we observed between baseline IP-10 levels and RVR and the absence of a relationship between baseline IP-10 levels and SVR may be that the high induction dose of interferon resulted in a higher rate of RVR than would have occurred with standard dose of IFN. Consequently, this higher rate of RVR with high induction IFN may not have the same predictive value for SVR as with standard (peg)IFN. It may also be that our cohort of patients was too small to show a statistical difference in baseline IP-10 levels and change in IP-10 levels during treatment between patients achieving SVR or not. In multivariate analysis the association we found between low baseline IP-10 levels and RVR seemed to be dependent on IL28B CC genotype, where IL28B CC genotype was an independent predictor of RVR. This suggests that IL28B genotype is a more important factor for prediction of RVR (and SVR) than baseline IP-10 levels.

Our findings, demonstrating a relation between IL28B genotype and IP-10 levels, confirm the results of earlier studies, showing that patients with favourable IL28B polymorphisms (CC) had lower pre-treatment IP-10 levels than patients with unfavourable IL28B genotypes (CT or TT). These studies also showed that when pre-treatment IP-10 levels are low (<600 pg/mL), the predictive value for RVR or SVR of IL28B genotype is increased (especially in patients with CT and TT genotypes). These findings together with ours, implicate the utility of combining these two markers in predicting treatment outcome. Also in patients with acute HCV infection low serum IP-10 levels increased the predictive value of IL28B polymorphisms (SNPs rs12979860 and rs8099917) with regards to the spontaneous clearance of HCV.

Treatment experienced patients had a lower SVR-rate than treatment naïve patients. In patients with a baseline IP-10 level of ≥600 pg/mL SVR rate was lower than in patients with a baseline IP-10 level of <600 pg/mL, especially in treatment experienced patients. These differences were not statistically significant, but numbers were very small (n=13). These findings confirm, what was already known, that treatment experienced patients
were less interferon-responsive than naïve patients. The higher dose of interferon did not overcome this irresponsiveness. The fact that we did not find a relation between baseline IP-10 levels and SVR, and the fact that in this cohort of patients SVR-rates were not higher than SVR-rates of comparable cohorts of patients treated with standard peginterferon and ribavirin therapy, as described in literature\textsuperscript{36-40}, supports this.

Our study is the first to describe IP-10 kinetics in CHC patients treated with high-dose interferon and amantadine. We found an almost 10-fold increase of IP-10 levels at Day 1 after the start of treatment, which was dependent of baseline IP-10 levels (4-fold when baseline IP-10 level was $\geq 600$ pg/mL to 27-fold when baseline IP-10 level $< 150$ pg/mL). A rise in IP-10 levels dependent of baseline IP-10 levels shortly (24 hours) after the start of treatment with peginterferon and ribavirin was also described in HCV/HIV co-infected patients.\textsuperscript{32} In this study a 3-fold rise was seen in patients with a baseline IP-10 level of $> 600$ pg/mL versus a 9-fold rise in patients with a baseline IP-10 level of $< 150$ pg/mL. Another study showed a dose-dependent 2- to 5-fold rise in IP-10 level, 2 days after the start of a low dose versus a normal dose of peginterferon in CHC patients.\textsuperscript{30} As interferon up-regulates ISG’s, including IP-10, one may expect that the IP-10 expression induced after a high dose of interferon is greater than after a lower dose. Our data support this suggestion, and it may be that high-dose interferon induces such a high level of IP-10 expression that other factors such as the baseline IP-10 level are less important as a predictor for RVR and SVR.

We also found that, after the initial rise, of IP-10 levels, the levels gradually declined to below the baseline value at end of treatment and at end of follow-up, and was significantly lower in patients achieving SVR. This was previously described,\textsuperscript{20-22,28} and may indicate that when HCV-RNA levels are declining, IP-10 is down-regulated.

It is unlikely that the addition of amantadine to the treatment regimen of our cohort of patients did influence SVR and IP-10 levels, since SVR rates were not different in patients with or without addition of amantadine, as was shown in several studies.\textsuperscript{41,42}

In our study, a first phase viral decline (HCV-RNA decline of $\geq 2.28\log_{10}$ at Day 1) was associated with lower baseline IP-10 levels, which is supported by earlier studies.\textsuperscript{18,32} One of these studies showed that a first phase decline of HCV-RNA of $> 1\log_{10}$ at Day 1 of treatment with peginterferon/ribavirin was associated with lower IP-10 levels at baseline.\textsuperscript{18} In HIV/HCV co-infected patients a similar pattern has been described, with a negative correlation between baseline IP-10 levels and the degree of HCV-RNA decline at Day 2 of treatment with peginterferon/ribavirin.\textsuperscript{32} In contrast to earlier experience with interferon-based therapy, one study with peginterferon monotherapy combined with danoprevir showed that baseline IP-10 levels were positively correlated with a decline of HCV-RNA at Day 1 of treatment and that IP-10 levels at Day 7 and Day 14 were significantly lower than at baseline.\textsuperscript{35} The association we found between this large first phase decline of HCV-RNA $\geq 2.28\log_{10}$ and a significantly higher increase of IP-10 levels from baseline to Day 1 of treatment has not been described before. This may be due to the high induction dose of interferon applied in our study, inducing strong up-regulation of ISG’s responsible for a rapid decline of HCV-RNA. Our finding that the increase of IP-10 levels from baseline to Day 1 was larger in patients with IL28B CC genotype than in IL28B non-CC patients, suggests that induction of IP-10 is dependent of the IL28B genotype. This is also supported by our findings in multivariate analysis, where IL28B CC genotype was an independent predictor of RVR, but baseline IP-10 level was not.
A limitation to our study is the fact that our data were valid for patients with HCV genotype 1 and 4 because only limited numbers of patients with genotype 2, 3 and 5 were included in our study.

In conclusion, there was no significant difference in IP-10 levels between patients with or without SVR, but baseline IP-10 level was significantly lower in patients with RVR versus non-RVR. IP-10 levels changed markedly after one day of treatment with high induction-dose interferon. The factor of increase of IP-10 levels from baseline to Day 1 was higher, when the baseline IP-10 level was lower. There was a clear relation between IP-10 levels at baseline and Day 1 of treatment and a decline of HCV-RNA of ≥ 2.28log_{10} at Day 1. Baseline and dynamic IP-10 levels early during treatment seem to be closely related to early viral kinetics and IL28B genotype. At present all-oral DAA combination treatment will result in eradication of HCV in most patients, and predictive markers for response become of less importance. However, in the future some patients like HCV genotype 3 and some difficult-to-treat patients such as end-stage liver cirrhotics will fail to achieve SVR. Immunological markers may help to understand why some patients fail also with DAA therapy.

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