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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 with high-dose interferon for six weeks, 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 pg/ml at baseline to log3.48 pg/ml at Day 1 and gradually diminished thereafter. IP-10 levels at any time point were not statistically different between patients with or without sustained viral response (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 Day 1 than patients with IL28B non-CC genotypes ($p = 0.015$). Patients with HCV-RNA decline $\geq 2.28\log_{10}$ at Day 1 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 $< 2.28\log_{10}$ 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 Day 1 were associated with IL28B CC genotype and HCV-RNA decline $\geq 2.28\log_{10}$ at Day 1. This suggests that, in our cohort, for prediction of SVR the added value of IP-10 to IL28B genotype and early viral kinetics is limited.

KEYWORDS

HCV, IL28B, interferon-based therapy, interferon-gamma-inducible protein-10, IP-10

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 characterised by liver inflammation due to pro-inflammatory cytokines and infiltration of specific and non-specific T lymphocytes.[2] 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 (ISGs). 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 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-γ-inducible protein-10 (IP-10 or CXCL10) is an ISG that is induced by interferon-γ and tumour necrosis factor 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 (SNPs) 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, SNPs 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 four 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.

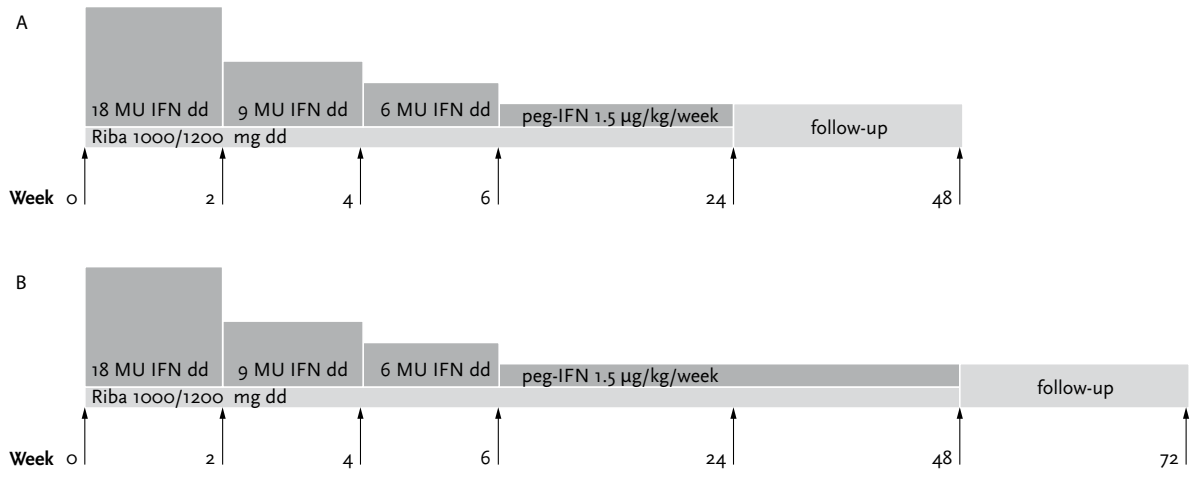
From 2002-2005 a cohort of CHC patients (treatment-naive patients with HCV genotype 1 or 4 and patients of all genotypes with failure to respond to interferon-based therapy) was treated with a high induction dose of interferon combined with ribavirin, followed by peginterferon and ribavirin.[24] Our 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 2002-2005, a cohort study was performed in which 100 CHC patients were included (treatment-naive 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). The results of this study were reported in 2008.[24] All patients were treated for six 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 regimen was used: Weeks 1 and 2: 18 MU/ day in three divided doses; Weeks 3 and 4: 9 MU/day in three divided doses; Weeks 5 and 6: 6 MU/day in two divided doses. Patients with a decline in HCV-RNA ≥ $3\log_{10}$ at Week 4 (and transcription-

Figure 1. *Study design.[24] Patients with a decline in HCV-RNA ≥ $3\log_{10}$ at Week 4 (and TMA undetectable at Week 24) were randomised to stop treatment at 24 weeks (A) or to continue to 48 weeks (B). Patients with a decline in HCV-RNA < $3\log_{10}$ at Week 4 were treated for 48 weeks (B). Treatment was stopped in all patients with detectable HCV-RNA at Week 24 (A). All patients were followed for 24 weeks after completion of therapy*



HCV = hepatitis C virus; IFN = interferon-alpha-2b; Riba = ribavirin.

mediated amplification (TMA) undetectable at Week 24) were randomised to stop treatment at 24 weeks or to continue to 48 weeks. Patients with a decline in HCV-RNA < $3\log_{10}$ 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 exclusion criteria were included in our study to determine IL28B genotype and to measure IP-10 and HCV-RNA levels at baseline, Day 1, Weeks 1, 2, 4 and 6, at end of treatment and at end of follow-up. Patients who stopped treatment prematurely (dropouts) between Day 0 and Week 24 (for other reasons than the above-mentioned exclusion criteria), and patients for 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 dropout due to side effects of the treatment (n = 12), dropout 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 x 10^2 to 7.7 x 10^6 IU/ml).[25] A qualitative HCV-RNA measurement was performed when the quantitative test was negative, using TMA (VERSANT® HCV qualitative assay, Siemens, Germany; lower limit of detection (LLD) 5 IU/ml).[26] 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 the 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-Altmann 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 analysed 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 the 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. Dropout: Any patients who stopped treatment prematurely between Day 0 and Week 24/48 or who were lost to follow-up during the 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 analysed on per protocol basis. We used the Bland-Altmann 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 which level of HCV-RNA decline gave the best prediction for SVR at Day 1.

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-naive patients, patients with RVR, IL28B CC genotype or a low METAVIR fibrosis stage (F0-F1-F2)

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*

	SVR	Non-SVR	p-values
N (%)	36 (42)	49 (58)	
Male (%)/ female (%)	28 (33)/ 8 (9)	38 (46)/ 11 (13)	0.98
Mean age, years (range)	44 (25 – 63)	46 (19 – 67)	0.37
Baseline HCV-RNA (log)	5.97	5.77	0.28
Naive / non-naive (%) *	24 (28)/ 12 (14)	22 (26)/ 27 (32)	0.046
Genotype (%)			
1	23 (27)	34 (40)	0.65
4	6 (7)	12 (14)	0.43
2/3/5	7 (8)	3 (4)	0.09
RVR / non-RVR (%)	19 (22)/ 17 (20)	5 (6) / 44 (52)	< 0.001
IL28B genotype CC / non-CC (%)	17 (20) / 19 (23)	9 (11) / 40 (47)	0.008
Baseline IP-10 (log pg/ml) (±SEM)	2.53 (0.04)	2.59 (0.05)	0.34
Liver biopsy (%)	32 (41)	46 (59)	
Fibrosis stage Metavir F3/F4 (%)	12 (15)	31 (40)	0.001

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

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 with 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 a partial response or patients with no 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 literature[16]) 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-naive patients. However, these differences were not statistically significant (*table 2*).

Table 3. *Baseline IP-10 levels and various response parameters*

	Baseline IP-10 levels (mean log ± SEM, pg/ml)	p-value	
Naive / non-naive	2.54 (0.05)	2.59 (0.04)	0.51
Genotype 1 / Genotype non-1	2.60 (0.04)	2.50 (0.04)	0.098
Baseline HCV-RNA < 600,000 / ≥ 600,000 IU/ml	2.57 (0.05)	2.56 (0.04)	0.81
Fibrosis score Metavir F3-F4 / F0-F2	2.56 (0.04)	2.58 (0.05)	0.81
IL28B genotype CC / non-CC	2.45 (0.05)	2.62 (0.04)	0.019
RVR / non-RVR	2.44 (0.05)	2.61 (0.04)	0.016

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 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 with 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

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

	IP-10 baseline (pg/ml)				
	< 600 pg/ml		≥ 600 pg/ml		
	Naive	Non-naive	Naive	Non-naive	Total
SVR, n/total (%)	19/38 (50)	10/32 (31)	5/8 (63)	2/7 (29)	36/85 (42)
Non-SVR, n/total (%)	19/38 (50)	22/32 (69)	3/8 (37)	5/7 (71)	49/85 (58)
Total, n/total (%)	38/70 (54)	32/70 (46)	8/15 (53)	7/15 (47)	

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).

IP-10 levels during therapy

From baseline to Day 1 an almost tenfold 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 2-40. 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 end of treatment (2.41 pg/ml, p = 0.01) and the end of follow-up (2.35 pg/ml, p = 0.01) (*figure 2*).

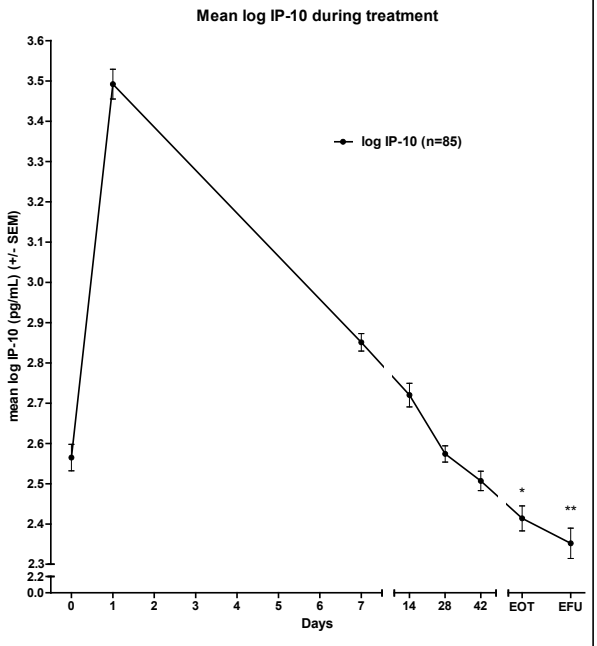
IP-10 levels during therapy and treatment outcome

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 end of follow-up, mean log IP-10

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

	RVR	Non-RVR	Confidence interval (95%)	p-value
IL28B genotype CC, N (%)	14 (58)	12 (20)	0.78 – 0.65	0.006
Non-CC, N (%)	*10 (42)*	*49 (80)*		
Log IP-10 baseline (mean, pg/ml)	2.44	2.61	0.013 – 1.267	0.079
Total, N (%)	24 (28)	61 (72)		

Figure 2. *IP-10 levels before and during treatment. A 1log$_{10}$ 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)*

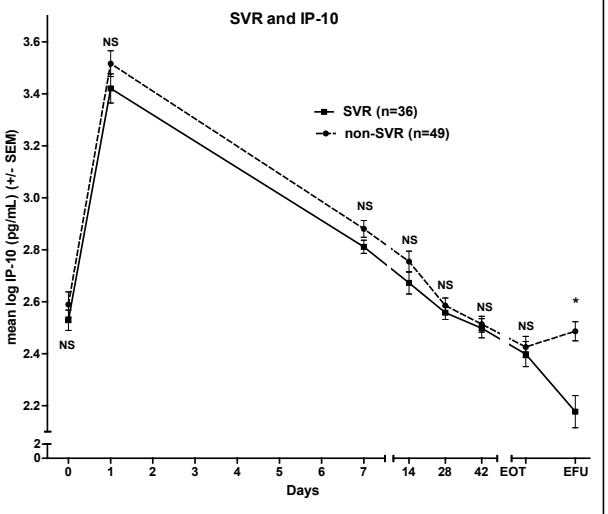


* p = 0.01 ** p = 0.01.

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)*

IP-10 baseline	N	Factor of increase D1 (mean)	p-value
< 150 ≥ 150	8 71	27 10	0.005
< 300 ≥ 300	31 48	16 9	0.001
< 375 ≥ 375	41 38	15 8	< 0.001
< 600 ≥ 600	68 11	13 4	< 0.001

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**

levels were significantly lower in patients with SVR than in non-SVR patients (2.40 pg/ml vs. 2.43 pg/ml, p < 0.0001) (*figure 3*).

IP-10 levels and HCV-RNA kinetics during therapy

A ROC curve (*figure 4*) was made to determine at which level of HCV-RNA decline at Day 1 prediction for SVR was optimal. This curve showed the best diagnostic odds ratio of 8.24 with a confidence interval of ± 1.04, corresponding with an HCV-RNA decline of ≥ 2.28$\log_{10}$, with a sensitivity of 58.3% and a specificity of 85.7% (*table 6*).

In 28 (33%) of the 85 included patients, the decline of HCV-RNA at Day 1 was ≥ 2.28$\log_{10}$, and in 57 (67%) it was not. In patients with an HCV-RNA decline of ≥ 2.28$\log_{10}$ or < 2.28$\log_{10}$ at Day 1 the baseline mean log IP-10 level was 2.45 pg/ml, and 2.62 pg/ml respectively (p = 0.016). At Day 1, IP-10 levels were slightly higher in patients with an HCV-RNA decline of ≥ 2.28$\log_{10}$ at Day 1 than in patients with a decline of < 2.28$\log_{10}$ at Day 1 (log3.51 pg/ml vs. log3.48 pg/ml), but this difference was not statistically significant (*table 7*). The increase of IP-10 levels from baseline to Day 1 was larger in patients with an HCV-RNA decline of ≥ 2.28$\log_{10}$ than in patients who did not show this decline (log1.09 pg/ml vs. log0.88 pg/ml, p = 0.047) (*table 7*).

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 vs. log0.89 pg/ml, p = 0.015) (*table 7*).

DISCUSSION

In contrast to what has been described earlier,[14-22,27] 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.[28-30] 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.[14,20,31] 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[18,32] or with or without SVR.[28,33]

We also found a clear relation between IL28B genotype and SVR, in line with previous data.[11,13-15]

Figure 4. *ROC curve for the prediction of SVR on the basis of the HCV-RNA decline at Day 1. Best diagnostic test performance at a decline of HCV-RNA of ≥ 2.28$\log_{10}$ at Day 1. Diagnostic odds ratio 8.24 (CI ± 1.04). Sensitivity 58.3% and specificity 86.7%*

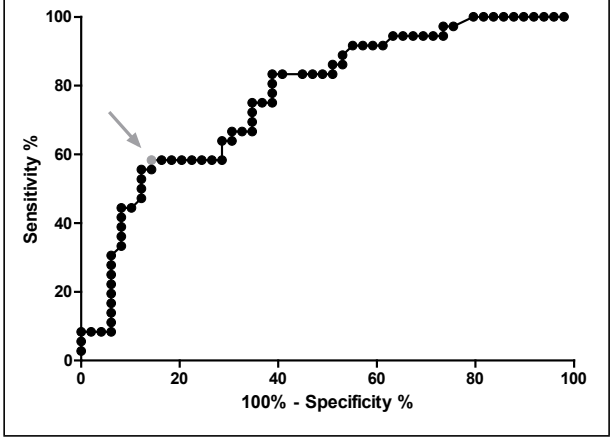


Table 6. *Patients with a decline of HCV-RNA at Day 1 of ≥ or < 2.28$\log_{10}$ and SVR or non-SVR status.*

Decline HCV-RNA Day 1	SVR (N)	Non-SVR (N)	Total	
≥ 2.28 $\log_{10}$	21	7	28	PPV 75.0%
< 2.28 $\log_{10}$	15	42	57	NPV 73.7%
Total	36	49	85	
	Sens 58.3%	Spec 85.7%		

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

Table 7. *Increase of IP-10 levels from baseline to Day 1 after start of treatment according to different response parameters*

	IP-10 levels (mean log, pg/ml)		
	Baseline	**Day 1**	**Δ To-D1 (p-value)**
SVR / non-SVR	2.52 / 2.59	3.42 / 3.52	0.93 / 0.96 (p = 0.75)
RVR / non-RVR	2.44 / 2.61	3.47 / 3.50	1.03 / 0.91 (p = 0.19)
D1 HCV-RNA decline ≥2.28$\log_{10}$ / < 2.28$\log_{10}$	2.45 / 2.62	3.51 / 3.48	1.07 / 0.89 (p = 0.047)
IL28B genotype CC / non-CC	2.45 / 2.62	3.54 / 3.47	1.09 / 0.88 (p = 0.015)

Δ To-D1 = Difference between IP-10 levels from baseline to Day 1 after start of treatment.

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 a 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 a 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).[12,14,15,23,34] 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,[14-16] 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.[35]

Treatment-experienced patients had a lower SVR rate than treatment-naive 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 the numbers were very small (n = 13). These findings confirm, what was already known, that treatment-experienced patients were less interferon responsive than naive patients. The higher dose of interferon did not overcome this lack of response. 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,[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 tenfold increase of IP-10 levels at Day 1 after the start of treatment, which was dependent of baseline IP-10 levels (fourfold when baseline IP-10 level was ≥ 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.[32] In this study a threefold rise was seen in patients with a baseline IP-10 level of > 600 pg/ml versus a ninefold rise in patients with a baseline IP-10 level of < 150 pg/ml. Another study showed a dose-dependent two- to five-fold rise in IP-10 level, two days after the start of a low dose versus a normal dose of peginterferon in CHC patients.[30] As interferon upregulates ISGs, 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 decreased to below the baseline value at the end of treatment and at end of follow-up, and were significantly lower in patients achieving SVR. This has previously been described,[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 influenced 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.[41,42]

In our study, a first phase viral decline (HCV-RNA decline of ≥ 2.28$\log_{10}$ at Day 1) was associated with lower baseline IP-10 levels, which is supported by earlier studies.[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.[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.[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.[35] The association we found between this large first phase decline of HCV-RNA ≥ 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 upregulation of ISGs 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 are 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 $\geq 2.28\log_{10}$ 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 an all-oral direct-acting antiviral (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 such as those with HCV genotype 3 and some difficult-to-treat patients such as those with end-stage liver cirrhosis will fail to achieve SVR. Immunological markers may help to understand why some patients also fail on DAA therapy.

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