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Interferon-gamma Inducible Protein 10 - biomarker for treatment outcome in chronic hepatitis C

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Abstract

The predictive value of two easy-to-determine serum biomarkers: IP-10 (Interferon-gamma inducible protein-10 kD) and sCD26 (soluble CD26) on the virological response of interferon-based therapy was assessed in 119 patients with chronic hepatitis C. Patients who obtained sustained virological response (SVR) had significantly decreased baseline IP-10 concentrations as compared to non-responders (mean IP-10 = 338 ± 200 pg/mL vs. 472.7 ± 254 g/mL; p=0.003). Correlation of this inexpensive serological marker with IL2B genotype and baseline HCV viral load can be used in the selection of patients who might benefit from interferon-based therapies, which are currently available in developing economies. On the contrary, there was no correlation between the baseline concentration of sCD26 and the outcome of treatment. IP-10 might be a suitable biomarker to predict virological success after pegylated interferon alpha and ribavirin treatment.

Keywords: chronic hepatitis C, treatment, IP-10, sCD26, IL28B polymorphism.

1. Introduction

Chronic hepatitis C (CHC) is one of the main causes of end-stage liver disease, cirrhosis and hepatocellular carcinoma. The successive introduction of direct acting antivirals (DAA), targeting the key viral proteins involved in the replication of hepatitis C virus (HCV)

has increased the therapeutical success rates to more than 95% (JACOBSON & al [1], ZEUZEM & al [2], KOHLER & al [3]). However, due to the financial constraints related to the present prohibitive cost of DAA, dual therapy with pegylated interferon alpha and ribavirin (PEG-IFN/RBV) is still in use (GHEORGHE & al [4]). As such, it has become increasingly important to stratify patients according to their likelihood to obtain a sustained virological response, the best indicator of treatment success and virological cure. There are several traditional predictors of treatment success, some of them virus-related (HCV genotype, baseline viral load, on-treatment viral kinetics), and others host-related (sex, age, race, degree of liver fibrosis and single nucleotide polymorphism upstream of the IL28B gene, associated with responsiveness to interferon therapy especially in HCV genotype 1 patients) (GE & al [5]). In addition, two easy-to-determine serologic markers have been associated with interferon-stimulated genes' activation: IP-10 (Interferon-gamma inducible protein 10 kD or CXCL-10) and sCD26 (soluble CD26).

IP-10 is an alpha chemokine induced by interferon gamma in monocytes, fibroblasts, endothelial cells, involved in cell migration and homing of immune cells with important effects on T-lymphocyte dysfunction, inflammation and apoptosis in viral hepatitis. In chronic hepatitis C, hepatocytes produce large amounts of this alpha chemokine, and the IP-10 receptor is upregulated on lymphocytes. The systemic and intrahepatic IP-10 levels are well correlated (ASKARIEH & al [6] and both are linked to important necroinflammatory and fibrotic liver modifications in CHC; as such, their level might predict the response rate to interferon-based therapy (ZEREMSKI & al [7].

Soluble CD26 (also known as DPP IV) is a dipeptidyl peptidase IV that cleaves a peptide from the N-terminus of polypeptides with proline or alanine at the penultimate position (DENNEY & al [8]). This peptidase cleaves IP-10 into an antagonistic form; accordingly, this cleavage has been suggested to influence the treatment outcome in patients with chronic HCV infection. A recent study has reported that patients failing therapy have higher baseline plasma sCD26 activity, indicating that a systemic accumulation of the truncated antagonistic form of IP-10 inhibits the migration of $CXCR3^{+}$ activated T lymphocytes to the infected liver (CASROUGE & al [9]). The serum level of sCD26 has been associated with a rapid decline in viral replication during the first days of IFN-based treatment, suggesting a better chance of achieving therapeutic success (SÖDERHOLM & al [10]).

We conducted a prospective study in order to assess the predictive value of baseline seric levels of IP-10 and sCD26 on the outcome of therapy in HCV infected patients, entering a first therapeutic regimen with pegylated-IFN and ribavirin (PEG-IFN/RBV).

2. Materials and Methods

Patients. Between October 2012 and January 2014, 119 patients diagnosed with CHC, with detectable viral replication, were enrolled in a prospective cohort study conducted in a tertiary care facility from Bucharest, Romania. Treatment was initiated with a combination of pegylated IFN-a2a (180 μg/week) or pegylated IFN-a2b (1.5 μg/kg/week), plus ribavirin (800–1200 mg/day, according to the body weight). Informed consent was obtained from all the patients, and the study was approved by the hospital Bioethic Committee.

Active viral replication was monitored at baseline, 4 and 12 weeks after treatment initiation, as well as at 6 month after stopping treatment, by quantitative detection of HCV RNA, using COBAS AmpliPrep/COBAS TaqMan Quantitative Test, version 2.0 (Roche Diagnostics, Mannheim, Germany) with a linear range between 15 and 100.000.000 IU/mL.

Serum levels of IP-10 were quantified using a quantitative sandwich immunoassay for determination of human interferon gamma inducible protein 10 concentrations in cell culture supernatant, serum, plasma, saliva, and urine (Quantikine Human CXCL10/IP-10, R&DSystems); basically, the test uses a monoclonal antibody specific for IP-10, pre-coated onto a microplate, and an enzyme-linked polyclonal antibody in order to form the spectrophotometer detected complex. A serial diluted standard is used to construct a standard curve by plotting the mean absorbance for standard and generating the best fit curve through the points on a log/log graph. According to the manufacturer's protocol the minimum detectable dose ranged from 0.41 - 4.46 pg/mL, with a linear range between 0 - 500 pg/mL and the mean IP10 concentration for negative controls (healthy volunteers) was 96 ± 47 pg/mL.

Serum levels of soluble CD26 / DPP IV were assessed using a direct immunoassay for the quantitative detection of human sCD26 (Human sCD26 ELISA, *ALPCO)*; briefly, the protocol uses an anti-human sCD26 coating antibody adsorbed onto the microwells, while the sCD26 present in the sample and captured by the first antibody is recognized by a biotin-conjugated anti-human sCD26 antibody. A standard curve is prepared from 6 human sCD26 standard dilutions and human sCD26 sample concentration determined. According to the manufacturer's protocol the minimum detectable dose ranged from 7.3 ng/mL and mean levels of sCD26 in negative controls (healthy volunteers) was 591 ± 179 ng/ml.

Single Nucleotide Polymorphism in IL28B rs12979860 was assessed by Custom TaqMan 5' allelic discrimination assay (Assays-by-DesignSM Service for SNP Genotyping Assays, Applied Biosystems, USA) running a real time PCR on ABI 7300 instrument, with primers and fluorescent probes predesigned by the manufacturer, and allele identification performed using SDS software (Applied Biosystems Inc., USA). Basically, the method uses the allelic discrimination analysis with manual or automatic allele call, after PCR amplification of the patients extracted DNA; the sequence detection system software v1.3.1 is used in order to plot the fluorescence values based on the signals from each well. The plotted fluorescence signals indicate which alleles are in each sample.

Liver fibrosis was evaluated using two noninvasive methods: Transient Elastography - FibroScan™ and FibroMax™ (BioPredictive, France), both with good accuracy and reproducibility in differentiating between mild and significant fibrosis (CASTERA & al [11], MORRA & al [12]).

Statistical analysis was performed using IBM SPSS Statistics v.20 and GraphPad Prism 6, Continuous variables were compared using Unpaired t test or Kruskal Wallis Test for Independent variables. Categorical variables were compared using Pearson Chi2 or Fischer's Exact Test. Significance was accepted at a P value <0.05.

3. Results and Discussions

Patients' characteristics

The median age of the enrolled patients was 51.2 years, and 61.3% of the subjects were women (Table 1). The mean baseline HCV viral load was high (2.7×10^6 IU/mL = 6.45 $\log_{10}$), with more than a half of the patients having baseline viral load of more than 600 000 IU/mL (5.8 $\log_{10}$); nevertheless, 52.1% had only mild fibrosis and 30.3% had normal serum aminotransferases values. All patients were infected with HCV genotype 1b.

Table 1. Baseline characteristics of study participants

	Total = 119
Age, years (median, range)	51.2 (22 - 71)
Female gender (%)	73 (61.3)
Urban residence (%)	84 (70.6)
Baseline ALT, IU/L (median, range)	88 (16 - 269)
Patients with baseline HCV-RNA > 5.8 $\log_{10}$ IU/mL (%)	74 (62.2)
Patients with mild fibrosis (F1+ F2) (%)	62 (52.1)

Predictive value of IP-10, HCV viral load and IL28B rs12979860 polymorphism

A sustained virological response (SVR - undetectable HCV-RNA after 6 months of treatment completion), the final indicator of therapeutic success, was obtained by 44.5% of the treated patients. The median serum IP-10 value for the whole study group was 397.8 pg/mL (range 100.5 – 1251.7). In order to predict the probability of obtaining sustained virological response which is the final goal of therapy, we stratified patients related to undetectable HCV-RNA after 6 months from treatment completion (SVR). Patients who obtained SVR had significantly decreased baseline IP-10 concentrations as compared to non-responders (mean IP-10 = 338 ± 200 pg/mL *vs*. 472.7 ± 254 g/mL; p=0.003) – Figure 1.

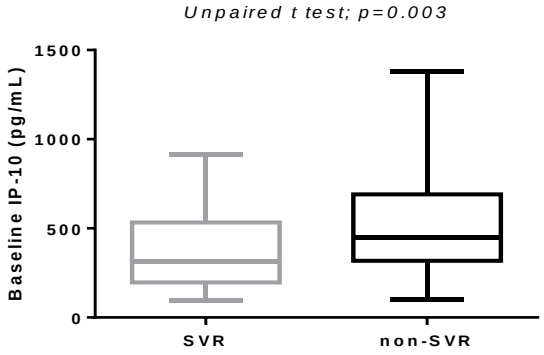

Figure 1. Baseline IP-10 levels correlated with sustained virological response (SVR)

Another investigated predictive factor - baseline HCV viral load, showed significantly decreased values for patients who obtained sustained virological response related to non-responders (mean viral load = 6.3 ± 5 *vs.* 6.6 ± 5.6 $\log_{10}$ IU/mL, p=0.001) – Figure 2.

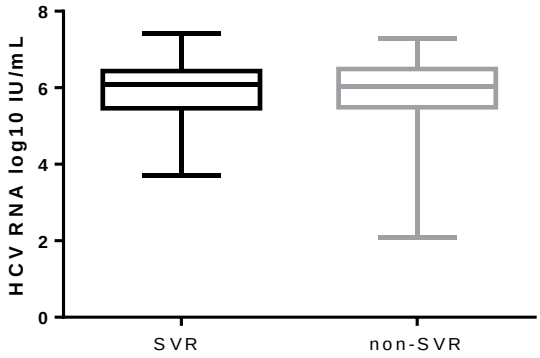

Figure 2. Baseline HCV viral load correlated with sustained virological response (SVR)

The favorable CC IL28B rs12979860 genotype, which predicts a good response to interferon based therapy, was present in only 15.9% of the subjects. Patients who obtained SVR had significantly higher percentage of favorable IL28B CC polymorphism as compared to non-SVR patients (30.2% *vs.* 3.03%; p<0.0001) – Figure 3.

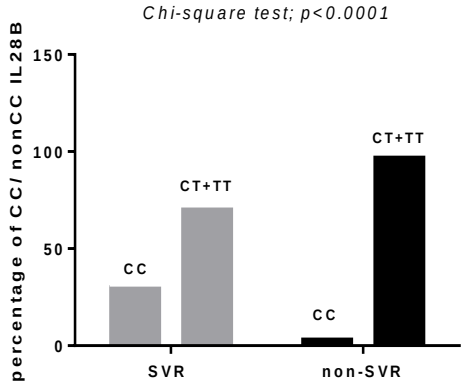

Figure 3. IL28B polymorphism correlated with sustained virological response (SVR)

Lower values of IP-10 were recorded in patients with the favorable CC IL28 genotype related to patients carrying one or two copies of the risk alleles CT or TT (mean IP-10 = 338.7 ± 200 *vs.* 472.7 ± 254 pg/mL; p=0.02) – Figure 4, while the IL28B polymorphism showed no correlations with baseline viral load or the degree of liver fibrosis.

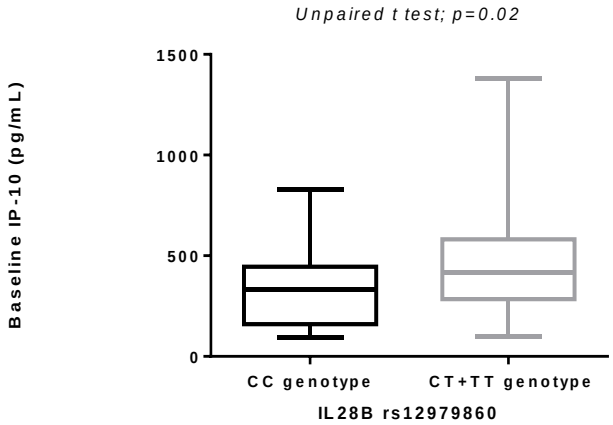

Figure 4. Baseline serum IP-10 levels related to IL28B rs12979860 genotype

The favorable CC IL28 genotype was previously associated with the likelihood of SVR, an effect related to exogenous IFN-λ activation of interferon stimulated genes (ISG) through the Jak-STAT pathway. On the contrary, patients with the unfavourable IL28 TT genotype tend to have a continuous stimulation of the IFN signaling pathway, which prevents a strong induction of ISG during interferon based treatment (NELSON HAYES & al [13], NAGGIE & al [14]).

In addition, IP-10 is also synthesized as a part of the innate immune response to the virus infection itself, without the intervention of interferon production (THOMAS & al [15]). Our results are concordant with those obtained previously by other groups reporting elevated pretreatment levels of IP-10 in plasma and of IP-10 mRNA in hepatocytes, associated with nonresponse to interferon-based therapy, both in patients mono-infected with HCV genotype 1 (ASKARIEH & al [6]) and in HIV-HCV coinfected patients (ZEREMSKI & al [16], SULTANA & al [17]). Altogether, these results suggest a potential use of low IP-10 serum levels in the early identification of patients who are likely to respond to the interferon-based therapy, especially in association with the profile of IL28B.

Serum soluble CD26 implication.

We also tested serum baseline sCD26 values in HCV infected patients, since this peptidase cleaves IP-10 into an antagonistic form, and thus can influence the treatment outcome in patients with chronic HCV infection. The median serum value of sCD26 for the study group was 667.5 ng/mL (range - 167, 1504). Our results did not show any significant correlation between the baseline serum concentrations of sCD26 and SVR rate (p=0.7) – Figure 5, nor with the median HCV viral load levels (p=0.7), the percentage of patients with severe fibrosis (p=0.5) and IL28B genotype (p=0.8).

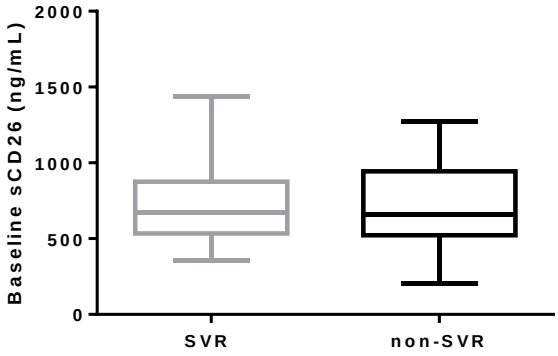

Figure 5. Baseline serum sCD26 values in responders/non-responders

No significant association had been found between sCD26 and IP-10 concentration (p=0.5) – Figure 6. This might be related to the fact that sCD26 is present both in a soluble and a membrane-bound form; the activity of the last form seems to influence more the systemic accumulation of the truncated antagonistic product of IP-10, as well as T cell activation, migration of CXCR3+ CD8 T cells to the infected liver and subsequent progression of hepatic lesions (ANSORGE & al [18]).

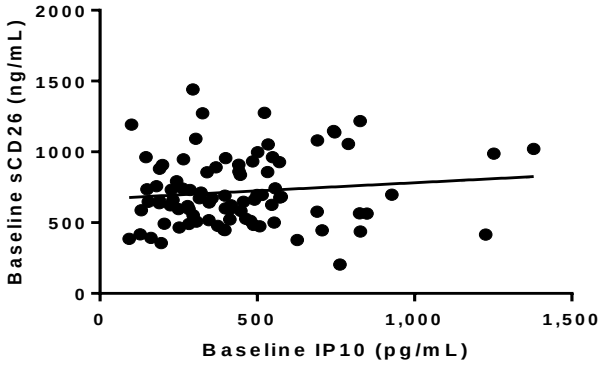

Figure 6. Correlation of sCD26 with baseline IP-10 values

The synergic predictive value of the simultaneous determination of pretreatment IP-10 and IL28B genotype has been also shown for the first phase of HCV RNA decline, associated with the rapidity of virologic response to treatment (LAGGING & al [19], REIBERGER & al [20]). Furthermore, the prognostic value of IP-10 seems to be maintained even during IFN-free regimens, with rapidly decreasing concentrations in the first week after treatment initiation, consolidated by a more modest decline in the following weeks (LIN & al [21]). We suggest that using an algorithm combining low IP-10 serum value (in our study 338 pg/mL - the mean value for responders), the favorable CC IL28B genotype and low baseline HCV viral load can increase the chances to detect patients who can still benefit from the affordable PEG-IFN/RBV therapy.

4. Conclusions

A low pretreatment concentration of IP-10 is a strong prognostic marker for the achievement of a virological response in patients treated with PEG-IFN/RBV. Correlation of

this inexpensive serological marker with IL28B genotype and baseline HCV viral load can be used in the selection of patients who might benefit from the interferon-based therapies, which are currently available in developing economies. Long-term studies will be needed to decide if this cytokine can be also used as a reliable pre-treatment predictor during interferon free therapies, based on combinations of the direct acting antivirals

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