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IP-10 PROTEIN AT CHRONIC HEPATITIS C AND ITS ROLE IN FORECASTING TREATMENT OUTCOMES

Geyvandova N. I., Lipov A. V., Yagoda A. V., Frolova A. A.

Stavropol State Medical University, Russian Federation

ПРОТЕИН IP-10 ПРИ ХРОНИЧЕСКОМ ВИРУСНОМ ГЕПАТИТЕ С И ЕГО РОЛЬ В ПРОГНОЗИРОВАНИИ РЕЗУЛЬТАТОВ ТЕРАПИИ

Н. И. Гейвандова, А. В. Липов, А. В. Ягода, А. А. Фролова

Ставропольский государственный медицинский университет, Российская Федерация

Clinical values were identified for serum levels of γ interferon-induced protein (IP-10) to be employed in forecasting SVR to AVT for CHC, which was carried out involving 107 patients (50 females and 57 males); mean age – 43.0±11 yrs. 75 of the patients were diagnosed with genotype 1 HCV (G1), while 32 patients were identified as having non-1 genotype HCV (G2 or G3). The IP-10 levels in blood serum were detected with the ELISA method. Patients with CHC had significantly higher blood IP-10 levels compared to healthy people, and that depended on the aminotransferases' activity as well as on the stage of hepatic fibrosis. A standard AVT with pegIFN-α medications and ribavirin given to 52 patients with G1 (SVR 54.9 %) and 27 – with G2 or G3 (SVR 75 %), showed that the patients who had reached SVR had their initial levels of IP-10 below that of non-responders. The prognostic level of IP-10 was detected, above which the likelihood of ending up at SVR in case of AVT with pegIFN-α and ribavirin was questionable: ≤ 403 pg/ml for G1 and ≤ 433 pg/ml for G2 or G3. In 18 patients who were given triple AVT – pegIFN-α + ribavirin + viral proteases inhibitors (SVR – 77.7 %) the initial levels of IP-10 at various outcomes of AVT were the same. Therefore, in case of CHC, the levels of blood IP-10 are elevated and depend on cytolysis activity and the degree of hepatic fibrosis. The levels of IP-10 protein in case of CHC may be used as a predictor regarding the standard AVT outcomes.

Key words: chronic hepatitis C, chemokines, antiviral therapy, prognosis

Определялось клиническое значение сывороточных уровней интерферон-γ индуцированного протеина (IP-10) в прогнозировании устойчивого вирусологического ответа (SVR) на противовирусную терапию (ПВТ) хронического вирусного гепатита С (ХВГС) у 107 больных (50 женщин и 57 мужчин), средний возраст – 43,0±1,1 лет. У 75 был диагностирован генотип 1 HCV (G1), у 32 пациентов – не-1 генотип HCV (G2 или G3). Содержание IP-10 в сыворотке крови определяли методом ELISA. Содержание IP-10 в крови больных ХВГС значительно превышало показатели здоровых и зависело от активности аминотрансфераз и стадии фиброза печени. Стандартная ПВТ препаратами ПегИФН-α и рибавирином у 52 пациентов с G1 (SVR 54,9 %) и 27 – с G2 или G3 (SVR 75 %) показала, что у больных, достигших SVR, исходный уровень IP-10 был ниже, чем у нон-респондеров. Определен прогностический уровень IP-10, выше которого вероятность достижения SVR при ПВТ ПегИФН-α и рибавирином сомнительна: ≤ 403 пг/мл при G1 и ≤ 433 пг/мл при G2 или G3. У 18 больных, получивших тройную ПВТ: ПегИФН-α/рибавирин + ингибиторы вирусных протеаз (УВО – 77,7 %), исходное содержание IP-10 при разных результатах противовирусной терапии не различалось. Таким образом, при ХВГС уровень в крови IP-10 повышен и зависит от активности цитолиза и выраженности стадии печеночного фиброза. Содержание белка IP-10 при ХВГС может быть использовано в качестве предиктора исходов стандартной ПВТ.

Ключевые слова: хронический вирусный гепатит С, хемокины, противовирусная терапия, прогноз

Chronic hepatitis C (CHC) is rated among the top socially meaningful issues in medicine nowadays. Hepatitis C virus is believed to have infected 130–170 mln people worldwide, and it is listed as the cause behind 350,000–500,000 deaths annually [23]. CHC is also named among the major causes for hepatic cirrhosis and hepatocellular carcinoma as well as HCV-infection, and prevails among other etiological factors resulting in liver transplantation [6].

Antiviral therapy (AVT) for CHC has gone through significant evolution – monotherapy with interferon-α (IFN-α) medications in the 1980s, arrival of ribavirin (RBV), pegylated IFNs (PegIFN), and finally combination with direct-acting antiviral (DAA) drugs. The year 2014 witnessed non-interferon schemes coming into clinical practice. Improved AVT modes have led to a significant increase in the rate of sustained virologic response (SVR) [2, 10, 11, 17]. Yet, the newer generation medications are extremely costly, which imposes certain limitation on their use, and which keeps IFN-α medications still relevant. Given this, choice of optimal treatment modalities in view of all therapy predictors comes out as of crucial importance. The well-known predictors for SVR at a standard AVT include HCV genotype, viremia level, as well as the carrier factors, namely the stage of hepatic fibrosis,

the race, hepatic steatosis, insulin resistance (if present), IL-28B genotype, the blood levels of vitamin D, and (IP-10) γ-interferon-inducible protein, CXCL10 [4, 15].

IP-10 protein (CXCL10/IP-10) is a chemokine playing an important role in eliminating HCV infection. The protein is produced by various cells – hepatocytes, T-lymphocytes, natural killers (NK), and monocytes [9, 26]. IP-10 is produced by hepatocytes as a response to hepatitis C infection [22], while the circulating levels of protein correlate with the expression of IP-10 matrix RNA (mRNA) in the hepatic tissue [3]. Involved in immune inflammation, IP-10 as a chemokine attracts to the hepatic tissue not so many of neutrophils but, more actively, T-lymphocytes, NKs and monocytes [1, 16].

In cases of acute hepatitis C, patients with higher plasma levels of IP-10 were not capable of spontaneous HCV clearance and were viewed as candidates for early antiviral therapy, while the low levels of protein were associated with potential recovery [8]. In patients suffering from CHC who were co-infected with HIV, the initial level of IP-10>400 pg/ml was a highly specific and sensitive predictor for SVR (sustained virologic response) in case of standard AVT [19]. High blood levels of IP-10 in case of CHC are also known to be in correlation with the viremia level and the severity of hepatic fibrosis [20]. The issue related to the inefficiency of IP-10 hyperproduction for elimination of HCV still remains unclear. There reports available telling about possible limitation of T-cells' functional activity under continuous impact that chronic HCV infection has on immunity due to CXCL10/IP-10 circulating in the patients' blood in its alternated type (antagonistic for the receptor) [5, 18].

Clarifying the role played by increased production of IP-10 in the clinical presentation of CHC may prove useful in terms of developing forecast for the disease course. Detecting of IP-10 levels in patients with CHC may serve an important predictor for selecting not only an efficient yet also a cost-efficient AVT mode.

Aim of study: to identify the clinical values for IP-10 serum levels in terms of forecasting sustained virologic response to antiviral therapy offered to patients with chronic hepatitis C.

Material and Methods. 107 patients with CHC were studied (50 females and 57 males; mean age – 43.0±1.1). Most of them (75) were infected with genotype (G) 1 HCV (G1): 73 patients – 1b, 2 patients – 1a+1b. Non-1 genotype HCV (G 2 or 3) was diagnosed in 32 patients: 25 had Genotype 3 of the virus, while another 7 revealed genotype 2 HCV. The level of viral load was measured in IU/ml, the mean value for viremia was lg 6.6±0.1. The study included no patients displaying signs of being infected with hepatitis B virus – HBsAg, HBV-DNA. The average AlAT activity was 82.98±8.11 U/l, AspAT – 64.72±5.54 U/l. All the patients underwent liver elastometry (SuperSonic Imagine Aixplorer, France); advanced stages of fibrosis (F 3 and F 4, METAVIR) were detected in 32 patients (29.9 %), while complete hepatic cirrhosis (HC) was diagnosed in 16 patients. All the patients with HC had their liver function compensated; patients with sub- and decompensated pathology were not included in the study.

A standard AVT with PegIFN and ribavirin medications (PegIFN/RBV) was given to 52 patients with G1 HCV, and to 27 patients with non-1 genotype. The standard therapy course was 48 weeks for genotype 1 HCV, and 24 weeks – for G 2 or 3. The medication doses were administered subject to the current standards [7]. 18 patients with Genotype 1 HCV were given the triple therapy mode – pegIFN-α + ribavirin + viral protease inhibitors (while 12 patients were given telaprevir, 3 – boceprevir, 3 – simeprevir). In the group of cases with hepatitis C that were given three antiviral drugs, 7 of the patients had previous history of failed treatment with the standard combination of antiviral medications.

The control group included 18 basically healthy persons with no markers of viral hepatitis.

The IP-10 levels in the blood serum were identified with the ELISA method employing commercial test systems (Bioscience, Austria).

The statistical processing of the results was done using the software Microsoft Office Excel 2007 with Attestat 10.5.1, IBM SPSS Statistics 21 incorporated. The values for the blood levels of IP-10 showed abnormal distribution, for which reason they were presented as a median (Me) with an interquartile range (25^{th} and 75^{th} percentiles); the two groups were compared employing Mann-Whitney U-test. The differences were accepted as statistically significant at $p<0.05$. The correlation analysis was performed with Spearman rank correlation evaluated – r_s. The ROC-analysis (Receiver Operator Characteristic) with ROC curve built was used to calculate the optimal value of the cutoff threshold.

Results and Discussion. The blood levels of IP-10 in patients with CHC was significantly above the similar indices obtained from the control group: 291 (229–435) pg/ml vs. 170.5 (145; 184.5) pg/ml; $p<0.0001$ (Fig. 1). These outcomes were predictable and matched the data obtained earlier [5, 12, 13, 20, 21]. The HCV genotype had no impact on the IP-10 levels in blood; the protein values in the groups of patients with genotype 1 and non-1 HCV were no different either. Hyperproduction of IP-10 at CHC is initiated via the stable influence that HCV has on the patient's immunity. The link between the level of viremia and IP-10 in the blood of patients with CHC was reported by A. I. Romero et al. [20]. Our study revealed a positive weak correlation between the viral load level and the blood IP-10 ($r_s= 0.24$; $p= 0.032$). An investigation of the dependence between the serum levels of IP-10 and the biochemical features of hepatitis activity – serum aminotransferases, revealed a weak yet reliable direct correlation between the aminotransferases' activity indicators: respectively, with the AlAT activity – $r_s= 0.23$ ($p = 0.035$) and the AspAT activity – $r_s= 0.29$ ($p = 0.007$). A number of earlier research showed a connection between the histological activity index (HAI) in the liver tissues and the IP-10 levels at CHC [19, 24], while the hepatocytes expressing CXCL10/IP-10 in hepatic lobules were prevailing in the inflammation areas [26]. This serves a logic proof to the dependence that we revealed between protein levels and cytolytic activity.

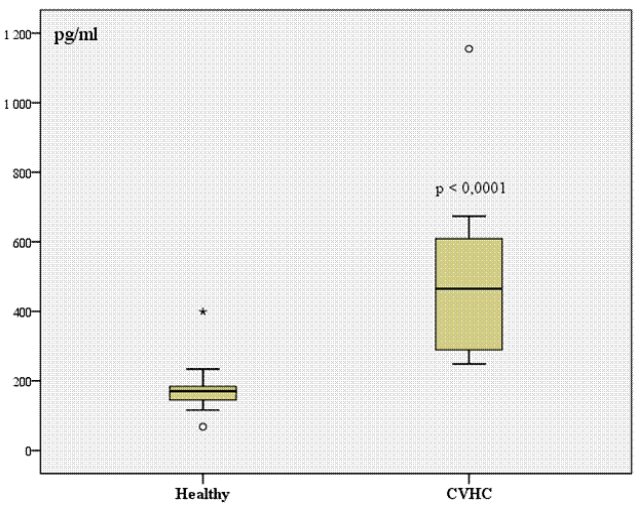

Fig. 1. Blood levels of IP-10 in patients with chronic viral hepatitis C

Top high serum levels of IP-10 were detected in patients with advanced stages of fibrosis (F 3–4) – 435 (283; 656) pg/ml (Fig. 2). The serum levels of IP-10 are known to have shown correlation not with the histological activity index (HAI) alone but also with the fibrosis stage in patients with Genotype 1 HCV [19, 24]. Besides, we found a correlation between the blood levels of IP-10 and the fibrosis stage on METAVIR – r_s= 0.373 (p=0.0009). The negative impact that IP-10 has on the course of HCV infection was described by A. Casrouge et al. [5]. They noted that the serum IP-10 in case of CHC is in its antagonistic type that may change the normal interaction with the CXCR3 receptor thus disturbing the transmission of the intracellular signal. The antagonistic type of CXCL10/IP-10 is mediated by dipeptidyl-peptidase IV (DPP4, CD26), which leads to the growth of the number of deficient types of chemokines that potentiate liver tissue infiltration with non-specific effector cells, hepatocyte lesion, and activation of stellate cells inducing fibrogenesis.

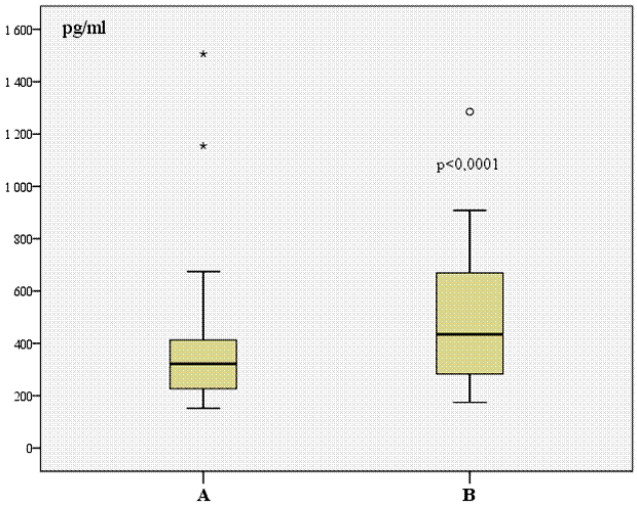

Fig. 2. Blood level of IP-10 protein in patients with chronic hepatitis C: A – fibrosis stage F 0–2; B – fibrosis stage F 3–4

The Table offers a view on the levels of the IP-10 levels at the start of AVT in patients with various treatment modes and outcomes. The standard AVT with PegIFN/RBV was given to 52 patients with Genotype 1 HCV (SVR was achieved in 55.7 % of cases). All the patients that reached SVR had their initial levels of IP-10 prior to the therapy lower than in non-responders. The prognostic IP-10 levels for patients with G1 (above which the likelihood of reaching SVR under a standard AVT mode was questionable) was 403 pg/ml. The AUC (Area Under Curve) index was 0.72, which proved good clinical reliability. At the same time the odds ratio (OR) was 6.24 (1.75–22.17) at p= 0.0047. Our study confirms the predictor role of lower initial levels of IP-10 in terms of reaching SVR in case of employing PegIFN/RBV to treat patients with CHC G1. M. Zeremski et al., for instance, reported that the serum IP-10 levels median prior to the start of treatment given to patients with hepatitis C co-infected with HIV and who further reached SVR, was much lower than in those who later proved to be non-responders [25]. Similar outcomes were yielded after giving PegIFN/RBV treatment to patients with CHC with no HIV [12, 14, 20].

Table

Blood IP-10 levels (pg/ml) in patients with CHC undergoing various AVT schemes, prior to start of treatment, Me (Q1-Q3)

Groups of patients	SVR presence	Lack of SVR	Reliability of difference
Genotype 1: PegIFN/RBV (n=52)	249 (227S–290) (n=29)	433 (237S–606) (n=23)	p=0.002
Genotype 1: PegIFN/RBV + proteases' inhibitors (n=18)	288.5 (219S–606) (n=14)	352.5 (234.5S–561.5) (n=4)	p=0.43
Genotype 2 or 3: PegIFN/RBV (n=27)	277 (202.5S–370.5) (n=20)	435 (285S–435) (n=7)	p=0.009

During that, the prognostic value of the initial IP-10 levels in treating CHC employing the triple therapy mode remains basically unstudied.

To do so, we offered treatment to 18 patients suffering from CHC G1, where the treatment included a combination of PegIFN/RBV with viral proteases' inhibitors (11 patients were given telaprevir, 3 – boceprevir). SVR was achieved in 14 cases (77.7 %); the initial IP-10 levels were not different in patients with differing outcomes of the AVT (see Table). It should be borne in mind that the group in question included 8 patients with a previous history of failed therapy based on PegIFN/RBV, as well as another 9 patients with advanced stages of fibrosis (F 3–4). IP-10, therefore, can not be taken as a predictor for reaching SVR in treating CHC cases with a combination of PegIFN/RBV and viral proteases' inhibitors.

The impact of IP-10 on SVR likelihood at G2 or G3 has basically never been studied. Only one study reflects some data on potential use of IP-10 as a prognostic criterion in such patients [14]. In our case SVR was detected in 20 patients of non-1 genotype HCV (74 %). This category of patients with CHC reached SVR in case of the initial serum levels of IP-10 ≤ 433 pg/ml (AUC= 0.8). Thus, identifying the levels of the IP-10 protein when selecting the most effective and yet cost-efficient AVT for CHC cases may be of importance. Low levels of IP-10, along with other predictors for SVR may be used to select the standard therapy mode – PegIFN/RBV.

Conclusions. Cases of CHC have higher levels of the blood IP-10 compared to healthy people. The IP-10 protein levels depend on the serum aminotransferases' activity and the severity of hepatic fibrosis. In patients with CHC the serum levels of the IP-10 protein may be used as a predictor for the outcomes of the standard antiviral therapy with PegIFN-α and ribavirin.

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About authors:

Geyvandova Natalia, MD, PhD, Professor, Department for Hospital Therapy; tel.: +79624452246; e-mail: ngeyvandova@yandex.ru

Lipov Andrey, MD, Postgraduate Student, Department for Hospital Therapy; tel.: +79286507264; e-mail: andrey_lipov89@mail.ru

Yagoda Alexander, MD, PhD, Professor, Head of Department for Hospital Therapy; tel.: +79064907330; e-mail: alexander.yagoda@gmail.com

Frolova Anastasia, MD, Doctor of Functional Diagnostics; tel.: +79187472499; e-mail: nastyaboblova@mail.ru