DOI: 10.18044/MedInform.201411.35

ISSUE 1, 2014

Could Inosine pranobex and Ribavirin in combination restore immune competence in chronic HCV advanced liver disease?

Nina Nikolova[1], Krasimir Antonov[1], Lyudmila Mateva[1], Zahariy Krastev[1]

1. University Hospital „St. Ivan Rilski“, Clinic of Gastroenterology, Medical University – Sofia Bulgaria

Abstract

The management of patients with advanced chronic HCV liver disease is still a problem. There is insufficient data about the role of immunomodulatory agents. The aim of the present study was to evaluate the benefit of prolonged co-administration of Inosine pranobex (Isoprinosine) and Ribavirin in patients with HCV advanced liver disease and to assess the dynamics of the IP-10 serum levels and thus – changes in the immune status of patients. Methods: Five patients (2 males, 3 females; median age 63), with chronic HCV genotype 1 infection were studied (with failed standard bi-therapy). All patients received Ribavirin and Isoprinosine. HCVRNA was measured at baseline and at day 40, month 3, 6 and 12 during treatment. IP-10 levels – at baseline and at day 10, 20, 40, 60, month 3, 6 and 12. Results: IP-10 levels were elevated in all patients at the baseline. An increase at day 10 or 20 was found in 4/5 patients and reduction at day 40 or 60 in same 4 patients. At month 12 the levels were significantly reduced (P=0.043). There was also an initially slight increase of ALT and subsequent reduction (P=0.043). The count of platelets increased in 4/5 patients. No effect on viral load was found. Conclusion: The therapeutic regime is well tolerated, no severe adverse events were documented. An improvement of the disease severity and liver inflammation were observed, with no effect on the viral load. We found reduction of IP-10 levels at month 12 – a sign of immune competence restoration.

Keywords: HCV; immunomodulator; Inosine pranovex; Ribavirin, IP-10

Background

Despite the exponential development of hepatology and increased possibilities for the treatment of chronic HCV infection, management of patients with advanced liver disease is still a problem.

Immunomodulation therapy is an important area in the treatment of infectious diseases and is becoming more popular. An immunomodulator is a substance which directly affects the specific immunological function or changes one or more components of the immunoregulatory network to achieve indirect effects on specific immune function (1).

Inosine pranobex (Isoprinosine) is a purine analogue with immunomodulating and antiviral ability, including IFN-gamma production and enhanced T-cell immune response (2,3). Various studies discuss its possible role in the treatment of chronic HBV infection (4,5,6). Isoprinosine is used in protracted forms of acute hepatitis A and B, as well as CMV-induced hepatitis (7). According to Z. Krastev, one day Isoprinosine intake reduces the serum levels of IP-10 and increases the lymphocyte count in healthy people (8).

There are insufficient data about the role of immunomodulatory agents in chronic HCV infections. There are few reports (from the 90s) about using Inosine pranobex in chronic hepatitis C (9, 10).

Ribavirin is a guanosine (ribonucleic) analogue used to block the synthesis of viral RNA and thereby to limit the viral mRNA. It is a nucleoside inhibitor. The antiviral properties of ribavirin have been described as follows: it inhibit inosine monophosphate dehydrogenase, interferes with viral RNA capping reactions; inhibits the viral polymerase and induces an error, catastrophe resulting from the accumulation of lethal mutations in the viral genome (11,12).

Apart from direct antiviral effects, ribavirin was reported to have immunomodulatory

effects on different constituents of the immune system. Ribavirin induces a switch in T-helper

(Th) cell phenotype from type 2 to type 1 (humoral immune responses to cell–mediated) (13,14,15). The exact mechanism is unclear. Th1 lymphocytes secrete IFN-gamma and Il -2 among other cytokines (16). Ribavirin enhances the specific interferon sensitive gene (ISG) expression by amplifying the IFN-a-JAK/STAT pathway and that enhances IFN-a anti-viral activity against HCV (17,18).

According to Rotman et al. Ribavirin monotherapy decreases IP-10 serum levels, but it has no effect on ISG expression in PBMC (Peripheral blood mononuclear cell) (19). The available data on the effect on viremia and sustained response of 4-6 weeks pretreatment with Ribavirin before the standard bi-therapy with Peg-interferon are controversial (19, 20, 21, 22, 23, 24, 25, 26, 27). Administration of the drug within 6 months after a standard bi-therapy does not improve response (28).

Interferon-gamma-inducible protein 10 kDa (IP-10 or CXCL10) is a chemokine produced by endothelial cells, activated T cells (promoting a Th1 response) and hepatocytes during HCV infection (29). IP-10 has been studied in the last years as a predictor of viral responses in the treatment of HCV infection, such that high baseline levels usually above 150 pg/ml are associated with a weak response (30,31,32,33).

Zoulim et al. followed 95 chronic HCV infected patients receiving Ribavirin for 11 months. A biochemical response was established. In 10% of the patients there was HCV RNA clearance (34). The data show only one study for the combination therapy with Inosine pranobex and Ribavirin (more than 20 years ago) in patients with HIV infection. The combination was well tolerated but led to lymphopenia and did not exhibit HIV-suppressive or immunorestorative effects (35).

The data about Ribavirin monotherapy in patients with chronic HCV infection are contradictory and insufficient. Single reports have been published about the dynamics of IP-10 in such patients. Our results show a decrease of levels during standard bi-therapy with virologic response (33). No data are available for the role of co-administration of Isoprinosine and Ribavirin in patients with chronic HCV infection, as well as in cases of advanced disease.

Aim

The aim of our study was to evaluate the benefit of prolonged co-administration of Inosine pranobex and Ribavirin in patients with chronic HCV advanced liver disease, who are inappropriate for IFN therapy.
We also aimed to follow the dynamics of the IP-10 serum levels and to evaluate the change in the immune status of patients.

Methods

Patients and treatment

Five patients (2 males and 3 females; median age 63) were studied. All of them were with chronic HCV infection, genotype 1, (long-term history of disease and with previous failed courses of standard IFN based bi-therapy). HDV, HCV and HIV co-infections were excluded in all patients. (tabl.1). All patients have been continuously taking Ribavirin in doses according to body weight, and Inosine pranobex in an alternative scheme.
Serum HCVRNA levels, aminotransferases and routine blood parameters were measured at the start of the therapy and at day 40, month 3, 6 and 12 of the treatment.

The serum levels of IP-10 were measured at baseline and at day 10, 20, 40, 60, month 3, 6 and 12.

Table 1. Baseline characteristics of the studied patients

Number of pts	5
Age – median, range	63 /60-71/
Male	2
Female	3
Cirrhosis (Child A)	3 patients
high grade esophageal varices (3-4gr)	1 patient
virological response at last therapy (standard bi-therapy) Relapse Non response	 2 3

Methods

HCV RNA quantification was determined by the standard real-time PCR method.

Genotyping of HCV was performed by using INNO-LiPA HCV II (Innogenetics NV, Ghent, Belgium).
IP-10 quantification of human IP-10 was performed by using BioLegend ®, LEGEND MAX^{TM} Human CXCL10/IP-10 ELISA kit, on serum samples. All samples were stored at -70°C until assayed. Standard laboratory methods were used for the assessment of blood chemistry parameters.

Standard statistical analyses were performed using SPSS® v. 17.0. Descriptive methods. Individual characteristics between groups were evaluated by means of Wilcoxon and Mann-Whitney. Non-parametric correlation methods such as Kendall and Spearmen were used. All reported P values are two-sided, and P values less than 0.05 were considered significant.

Informed consent was obtained from each participating patient.

Results

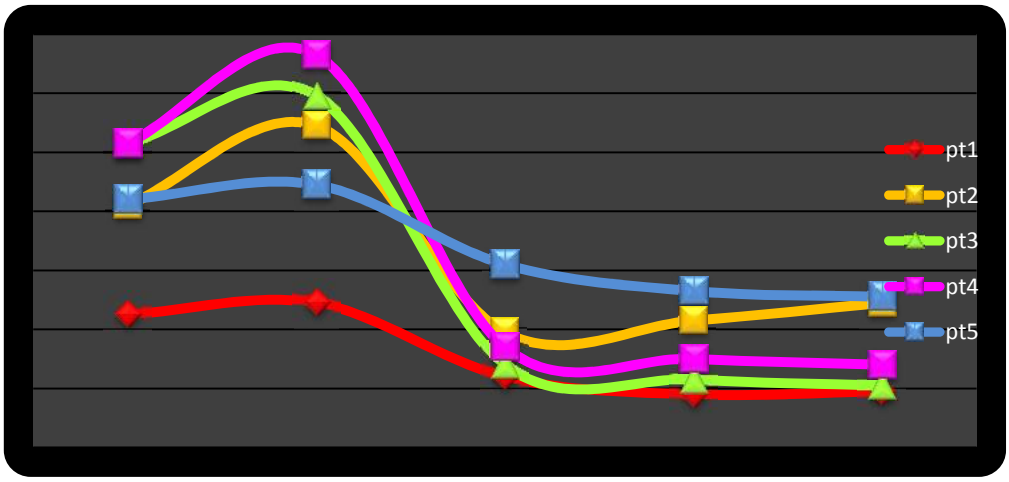
The baseline serum levels of HCVRNA, ALT, and IP-10 are shown in Table 2. All patients have high baseline levels of IP-10 (>150pg/ml) and elevated aminotrasferases.

Table 2. Baseline levels (Median) of HCV RNA, ALT and IP-10 in studied patients**.**

	Baseline levels Median, range
HCVRNA IU/ml	895 000 /66500-2 840 000/
ALT IU/ml	84 /45-103/
IP-10 pg/ml	365 /274-501/

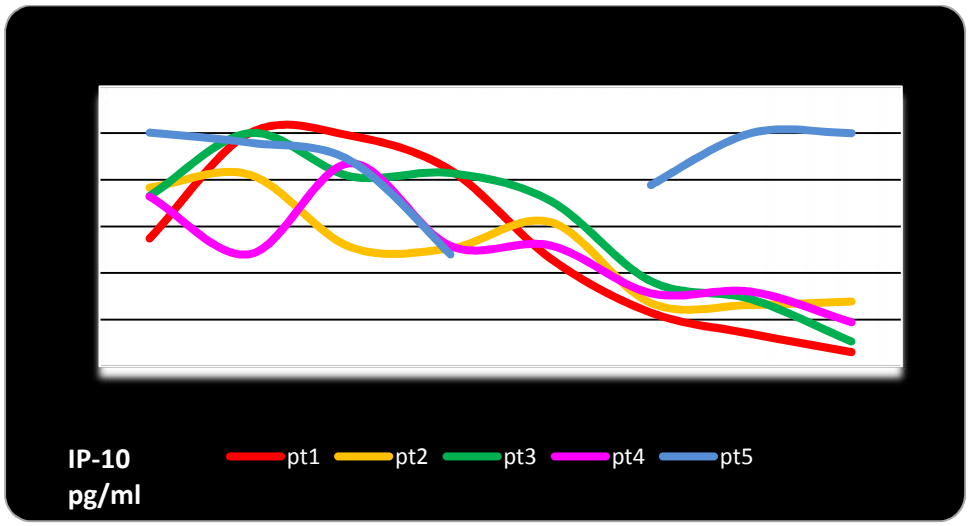
During treatment there was an initially slight increase of ALT (at day 40) in all patients (P=0.043). In 4/5 patients, ALT levels decreased and became within the normal range at month 3 (P=0.043). In the last patient ALT levels decreased but never reached the normal range (Figure 1.). There was a statistical difference between the basal ALT and decreased levels at month 12 (P=0.042) in all patients. GGT decreased in these 4 patients but remained unchanged in the last one. The same patient had cirrhosis and severe portal hypertension /high-risk esophageal varices, ligated several times/.

Figure 1. ALT levels during the treatment



During the follow up the levels of IP-10 increased (from 7 to 82%) at day 10-20 in 4 from 5 patients (in 3 patients the increase was at day 10, and in 1 at day 20). After that there was a tendency for the reduction of IP-10 (in the same 4 patients) at day 40-60 and it was statistically significant at month 3 (P 0.043). At month 12 the levels were reduced with more that 50% compared to the basal levels (from 64 to 89%) (P=0.043). In the last patient there was a reduction at day 60 but generally remained stable during the treatment period (Figure 2.)

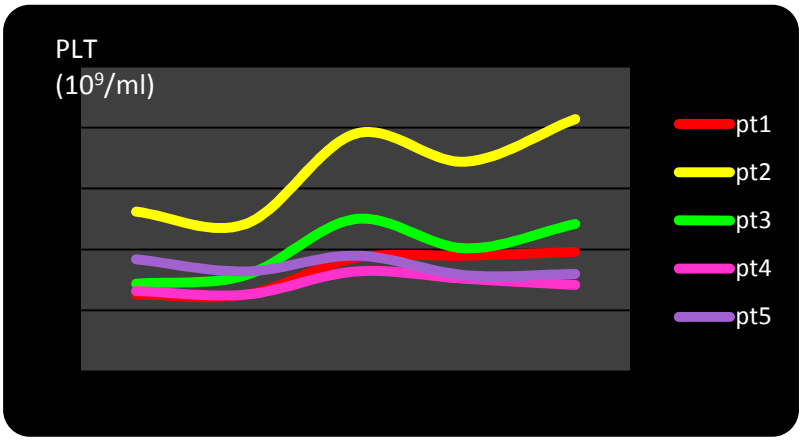
Figure 2. IP-10 levels during Isoprinosine – Ribavirin therapy in the studied patients



The levels of HCVRNA fluctuated during treatement. There was no signifficant difference between basal HCVRNA and that at month 12 (P>0.05).

For the same period a reduction of Hgb was established with a statistical difference between the basal Hgb and this at month 12 (P=0.043). Anemia was found in 3 patients. A positive correlation was found between baseline PLT and IP-10 (P=0.037, r=0.900). There was no correlation between basal levels of IP-10 and GGT, HCVRNA, ALT, Hgb. During therapy the count of PLT elevated, only in the last patient PLT remained low (Figure 3.).

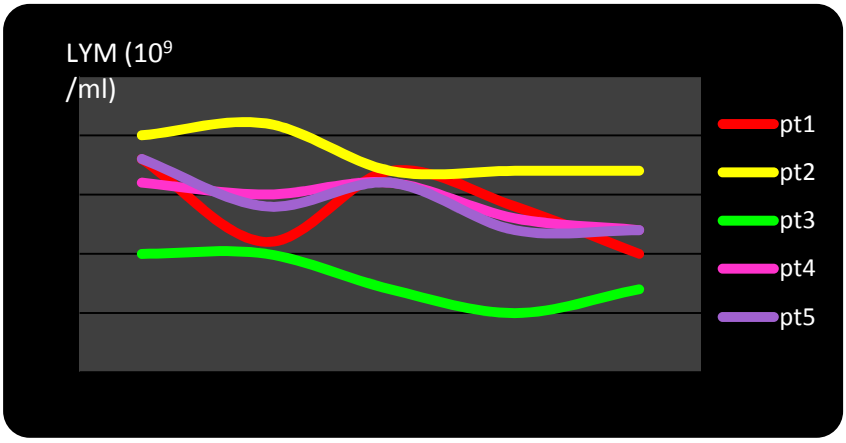
Figure 3. Count of PLT during the treatment



All patients had normal values of albumin before treatment and remained without significant changes during follow-up.

In two patients levels of uric acid were slightly elevated. In one patient (female) lymphopenia appeared with no infection or other complications (Figure 4.). The elevation of uric acid levels is due to the catabolic metabolism of the inosine moiety in this product in humans to uric acid. Ribavirin can also provoke an increase of uric acid associated with its metabolism and hemolysis (Summary of product characteristics).

Figure 4. Count of Lymphocytes during the treatment



Discussion

In this study we followed up a small group of patients with advanced chronic HCV liver disease and with no currently available therapy. Our aim was to reduce the liver inflamation and to stabilise the liver disease by modulating the immune system.

We decided to combine these two medications because of their immunomodulating effects. In the last years, together with the direct antiviral effects, ribavirin has been reported to have immunomodulatory effects. Ribavirin induces a switch in T-helper (Th) cell phenotype from type 2 to type 1 (13,14,15). According to our data Isoprinosine has the ability to activate the cytokine cascade from the first day of intake (3) and in this way resembles the effects of IFN like in standard bi-therapy.

We chose to follow up IP-10 as a surrogate marker of the IFN–gamma system and as a predictor for an IFN-based therapy response. Ribavirin could provoke lymphopenia (3%) (Summary of product characteristics) but at the same time Isoprinosine elevates the lymphocyte count and decreases IP-10 levels (8).

We observed a stable reduction of aminotrasferases and no severe adverse events because of the combined therapy, so we decided to continue this therapeutic regimen and to follow up the patients for more than one year.

This combination of Isoprinosine and Ribavirin restores the immune competence and significantly reduces IP-10 levels to such values that subsequent IFN therapy would be successful. But it has no effect on viral load. This is a probably an indirect sign of the immunomodulating effects of the two medications. The decreasing of aminotrasferases levels is related to the beneficial effect on the disease. The changes of HCV viremia ±0.5 log are not significant and are due to the natural fluctuation of HCVRNA without therapy (36).

Similar to IFN – soon after the beginning of therapy there was an elevation of IP-10 serum levels (37) and here we found an initial increase /10-20 day/ of IP-10 and a subsequent tendency to reduction.

Conclusion

The long-term combined intake of Isoprinosine and Ribavirin is well tolerated without severe adverse events. This therapy leads to:

- Reducing the severity of the disease and liver inflammation (assessed by achieving a biochemical response, PLT increasing);

- Significant reduction (>50% from the baseline) of IP-10 for a 12-month treatment period which is a sign for a restoring of the immune competence of the organism;

- Lack of effect on viral load;

References

1. Fauci AS, Rosenberg SA, Sherwin SA et al. NIH conference. Immunomodulators in clinical medicine. Ann Intern Med 1997;106:2:421-433.

2. Milano S, Dieli M, Millott S, et al. Effect of isoprinosine on IL-2, IFN-gamma and IL-4 production in vivo and in vitro. Int J Immunopharmacol 1991;13:1013-1018.

3. Petrova M, Jelev D, Ivanova A, et al. Isoprinosine affects serum cytokine levels in healthy adults. Journal of interferon & cytokine research : the official journal of the international society for interferon and cytokine research 2010;30:4.

4. Kasimov IZ, Efficiency of isoprinosine in the complex treatment of acute virus hepatitis B (Russian). Lik Sprava 2004;7:74-77.

5. Krastev Z, Antonov K, Jelev D. The prevention of an expected hepatic flare in HBe negative patients after lamivudine discontinuation. Clinic of Gastroenterology, St. Ivan Rilsky University Hospital, Medical University, Sofia, Bulgaria; J Gastrointestin Liver Dis 2006;15:4:389-391.

6. Nikolova N, Jelev D, Antonov K, et al. Inosine pranobex can enhance HBeAg-loss during long-term Tenofovir treatment, University Hospital "St.Ivan Rilski" – Sofia, Clinic of gastroenterology, Bulgaria; poster presentation, EASL, Lyon, 2013.

7. Nicoara E, Crisan A. Possibilities to modulate the immune response during infections. TMJ 2003; 53:3-4.

8. Krastev Z. Personal communication.

9. Pardo M, Carreño V. Lack of efficacy of inosine pranobex in the treatment of chronic hepatitis C, Journal of Hepatology 1994;21:2:278.

10. Pár A, Beró T, Brasch G, Gógl A, et al. Isoprinosine therapy in chronic hepatitis C (multicenter placebo-controlled double-blind prospective study). Orv Hetil 1993;9:134:19:1015-1019.

11. Graci JD, Cameron CE. Mechanisms of action of ribavirin against distinct viruses. Rev, Med Virol 2006;16:37-48.

12. Crotty S, Cameron C, Andino R. Ribavirin's antiviral mechanism of action: lethal mutagenesis. J Mol Med 2002;80:86–95.

13. Hultgren C, Milich DR, Weiland O, et al. The antiviral compound ribavirin modulates the T helper (Th) 1/Th2 subset balance in hepatitis B and C virus-specific immune responses. J Gen Virol 1998; 79:2381-2391.

14. Ogbomo H, Michaelis M, Altenbrandt B, et al. A novel immunomodulatory mechanism of ribavirin in suppressing natural killer cell function. Biochemical Pharmacology 2008;15:79:2:188-197.

15. Kast R. Ribavirin in Cancer Immunotherapies: Controlling Nitric Oxide Augments Cytotoxic Lymphocyte Function, Neoplasia 2003;5:1:3–8.

16. Jankovic D, Liu Z, Gause WC. Th1 and Th2 cell commitment during infectious disease. Trends Immunol 2001; 22, 450 – 473.

17. Stevenson NJ, Murphy AG, Bourke NM, et al. Ribavirin Enhances IFN-a Signalling and MxA Expression: A Novel Immune Modulation Mechanism during Treatment of HCV. PLoS One 2011;6:11:e27866.

18. Werner JM, Serti E, Chepa-Lotrea X, et al. Ribavirin Pretreatment Improves the IFN-γ Response of Natural Killer Cells to IFN-based Therapy of Hepatitis C Virus Infection. Gastroenterol 2014; 52:5:41.

19. Rotman Y, Noureddin M, Feld JJ et al., Effect of ribavirin on viral kinetics and liver gene expression in chronic hepatitis C, Gut. 2014 Jan;63:1:161-9.

20. Fulop B, Mihm U, Schlosser B, et al. Antiviral efficacy of ribavirin monotherapy followed by standard combination treatment in chronic hepatitis C. Journal of Hepatology 2010; 52:59–182.

21. Mihm U, Welker M, Teuber G, et al. HCV viral kinetics during ribavirin monotherapy: results of a randomized partially double blind placebo controlled clinical trial. Journal of Hepatology 2011; 54:61–208.

22. Yen C, Chang J, Lee T, et al. Ribavirin monotherapy increases sustained response rate in relapsers of end treatment virologic responders. World J Gastroenterol 2005;11:11:1663-1667.

23. Hultgren C, Milich DR, Weiland O, et al. The antiviral compound ribavirin modulates T1/Th2 subset balance in hepatitis B and C virus specific immune responses. J Gen Virol 1998;79:10: 2381 –2391.

24. Querenghi F, Yu Q, Billaud G, et al. Evolution of hepatitis C virus genome in chronically infected patients receiving ribavirin monotherapy. J Viral Hepatol 2001;8,120–131.

25. Reichard O, Sonnerborg A, and Weiland O. HCV RNA titers prior to, during, and after oral RBV treatment. J Med Virol 1993;41,99–102.

26. Di Bisceglie AM, Hoofnagle JH, and Krawczynski K . Changes in HCV antigen in liver with antiviral therapy. Gastroenterology 1993;105,858–862.

27. Brok J, Gluud L, Gluud C. Ribavirin monotherapy for chronic hepatitis C, Published Online: 19 OCT 2005, Editorial Group: Cochrane Hepato-Biliary Group.

28. Shiffman M, Hofmann Ch, Sterling K, et al. Controlled Trial to Determine Whether Continued Ribavirin Monotherapy in Hepatitis C Virus–Infected Patients Who Responded to Interferon-Ribavirin Combination Therapy Will Enhance Sustained Virologic Response. The Journal of Infectious Diseases 2001;184:405–409.

29. Zeremski M, Petrovic LM, Chiriboga L, et al. Intrahepatic levels of CXCR3 associatedchemokines correlate with liverinflammation and fibrosis in chronic hepatitis C. Hepatology 2008;48:5:1440-1450.

30. Askarieh G, Alsi E, Pugnale P, et al. Systemic and Intrahepatic Interferon-Gamma-InducibleProtein 10 kDa Predicts the First-Phase Decline in Hepatitis C Virus RNA and Overall Viral Response toTherapy in Chronic Hepatitis C. Hepatology 2010;51:5:1523-1530

31. Fattovich G, Covolo L, Bibert S, et al.; ITAHEC Study Group. IL28B polymorphisms, IP-10 and viral load predict virological response to therapy in chronic hepatitis C, Aliment Pharmacol Ther. 2011;33:10:1162-1172.

32. Derbala M, Rizk NM, Al-Kaabi S, et al. The predictive value of IL28B rs12979860, rs11881222 and rs8099917 polymorphisms and IP-10 in the therapeutic response of Egyptian genotype 4 patients, Virology. 2013;444:292-300.

33. Nikolova N, Antonov K, Jelev D, et al. The cytokine IP-10 in chronic HBV and HCV infection. J of Imab 2013;19:3:442-447.

34. Zoulim F, Haem J, Ahmed SS, et al. Ribavirin monotherapy in patients with chronic hepatitis C: a retrospective study of 95 patients. J Viral Hepat. 1998;5:3:193-8.

35. Schulof RS, Parenti DM, Simon GL, et al.. Clinical, virologic, and immunologic effects of combination therapy with ribavirin and isoprinosine in HIV-infected homosexual men. Acquir Immune Defic Syndr. 1990;3:5:485-492.

36. Nikolova N, Antonov K, Jelev D, et al. Natural course of HCVRNA. The paradigm of 5 log? Abstract 53 (poster presentation), Falk Symposium 2012;186:5-6.

37. Reiberger T, Aberle JH, Kundi M, et al. IP-10 correlates with hepatitis C viral load, hepatic inflammation and fibrosis and predicts hepatitis C virus relapse or non-response in HIV-HCV coinfection. Antivir Ther. 2008;13:8:969-976.

Corresponding author:

Nina Nikolova
15 Akad. Ivan Geshov Blvd., Sofia 1431, Bulgaria
University Hospital St. Ivan Rilski
Clinic of Gastroenterology
tel: 0886 774 258; e-mail: nnikolova234@gmail.com