ORIGINAL ARTICLE

UDC: 616.9:616.36-002
DOI: 10.2298/VSP1506505V

Association between risk factors, basal viral load, virus genotype and the degree of liver fibrosis with the response to the therapy in patients with chronic hepatitis C virus infection

Povezanost faktora rizika, bazalnog nivoa virusa, genotipa virusa i stepena fibroze jetre sa odgovorom na terapiju kod bolesnika sa hroničnom hepatitis C virusnom infekcijom

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Abstract

Background/Aim. Hepatitis C is an important sociomedical problem worldwide due to frequent progression to chronic disease, occurrence of liver cirrhosis and hepatocellular carcinoma. Standard pegylated interferon alfa 2a plus ribavirin therapy results in resolution of infection only in 50% of patients. The aim of this study was to determine the association of various factors with response to the therapy in patients with chronic heptitis C virus (HCV) infection. Age and sex of patients, inoculation risk factors, histopathological changes in the liver, viral load and HCV genotype were analyzed. **Methods.** The study included a group of 121 patients with chronic HCV infection. The treatment was carried out 24 weeks for virus genotype 2 and 3, and 48 weeks for genotype 1 and 4. The degree of histopathological changes in the liver was determined by hematoxylin and eosin staining, whereas polimerase chain reaction was used for HCV genotyping. **Results.** In the group of non-responding patients genotype 1 was represented with 100%, while in the other groups, although predominantly present, its percentage was lower. Unresponsiveness to therapy and relapse of disease were associated with higher viral load and advanced fibrosis. Intravenous use of psychoactive substances, as a risk factor, was present in a high percentage in the group of patients with sustained response, while blood transfusion and dialysis were leading risk factors in the group of relapse responders and non-responders. **Conclusion.** The results of our study showed that the treatment outcome of chronic HCV infection was associated with baseline HCV ribonucleic acid, HCV genotype, route of infection and the degree of histopathological changes in the liver.

Key words:
hepatitis c; hepatitis, chronic; treatment outcome; risk factors; genotype; histological techniques; disease transmission, infections.

Apstrakt

Uvod/Cilj. Hepatitis C virusna (HCV) infekcija predstavlja veliki medicinski, ekonomski i socijalni problem u svetu. Standardna terapija pegilovanim interferonom alfa 2a i ribavirinom dovodi do rezolucije bolesti kod samo oko 50% bolesnika. Cilj ovog rada bio je da se utvrdi povezanost faktora rizika od nastanka infekcije, genotipske zastupljenosti virusa i stepena patohistoloških promena jetre sa odgovorom na terapiju kod bolesnika sa hroničnom HCV infekcijom. **Metode.** Ispitivanjem je obuhvaćena grupa od 121 bolesnika sa hroničnom HCV infekcijom. Lečenje je sprovedeno tokom 24 nedelje za genotip virusa 2 i 3, i tokom 48 nedelja za genotip 1 i 4. Za određivanje genotipa virusa korišćena je metodologija lančane reakcije polimeraze. Stepen patohistoloških promena jetre određivan je standardnom hematoksilin-eozin metodom. **Rezultati.** U ispitivanoj grupi bolesnika najzastupljeniji HCV genotip bio je genotip 1. U grupi bolesnika bez odgovora na terapiju genotip 1 bio je zastupljen sa 100%, dok je u ostalim grupama, iako dominantno prisutan, njegov procenat bio znatno niži. Najveći broj virusnih čestica registrovan je u grupi bolesnika sa nepovoljnim odgovorom na terapiju. Najviši procenat bolesnika bez fibroze (F0) ili sa niskim stepenom fibroze (F1) nalazio se u grupi bolesnika sa povoljnim odgovorom na terapiju, dok se najveći broj bolesnika sa izraženom fibrozom (F3 i F4) nalazio među bolesnicima sa nepovoljnim odgovorom na terapiju. Intravenska upotreba psihoaktivnih supstanci kao faktor rizika bila je prisutna u visokom procentu

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kod bolesnika sa povoljnim odgovorom, dok su transfuzija krvi i dijaliza bili vodeći faktor rizika za bolesnike kod kojih je došlo do relapsa HCV i kod onih bolesnika koji nisu odgovorili na terapiju. **Zaključak.** Rezultati ove studije pokazuju da je ishod lečenja hronične HCV infekcije povezan sa bazalnim nivoom HCV ribonukleinske kiseline u momentu postavljanja dijagnoze, genotipom HCV virusa, načinom infekcije i stepenom oštećenja parenhima jetre.

Ključne reči:
hepatitis c; hepatitis, hronični; lečenje, ishod; faktori rizika; genotip; histološke tehnike; bolest, prenošenje.

Introduction

Hepatitis C virus (HCV) infection is a major medical, social and economic problem in the world. It is assumed that about 180 million people worldwide have chronic HCV infection [1, 2]. The discovery of HCV in 1989 clarified the etiology of a large number of posttransfusion hepatitis with unknown cause [3]. Until 1990, the most important route of infection was transfusion of blood and blood products, and today that is the intravenous use of psychoactive substances. Most patients with acute HCV infection have no distinct symptoms and the diagnosis is usually made accidentally, finding elevated activities of serum aminotransferases on routine biochemical testing. The outgo of acute HCV infection depends on many factors, such as virus genotype and the strength of host immune response. Nearly 75% of patients with acute hepatitis C develop chronic disease [4]. The progression of disease is associated with alcohol abuse [5], the presence of diabetes [6], age of the patient [7], co-infection with HIV and/or other primary hepatotropic viruses [8]. About 10–20% of patients with chronic hepatitis C will develop liver cirrhosis [4] and hepatocellular carcinoma occurs in about 1–5% of cases [9]. Standard treatment of hepatitis C using pegylated interferon alfa 2a (PEG-IFNα-2a) and ribavirin (RBV) is successful in only half of patients. Moreover, therapy is costly and has diverse side-effects (flu-like symptoms, depression, anemia, leucopenia, nausea, cough, rash, etc.). Thus, identifying factors that influence the outcome of therapy is of great importance.

The aim of this study was to determine the association of various factors with response to PEG-IFNα-2a plus RBV combined therapy in patients with chronic HCV infection.

Methods

This prospective study was carried out in the Department of Infectious Diseases, Clinical Center of Kragujevac, between 2005 and 2009. This study group consisted of 121 patients with chronic hepatitis C. Written informed consent was obtained from all patients according to the Declaration of Helsinki, and the local Ethics Committee approved the study. Anamnesis, biochemical analysis, liver biopsy, quantification of viral load and genotyping were acquired for each patient before the beginning of the treatment. Histopathological data were obtained by standard hematoxylin-eosin (HE) staining of biopsy specimens and liver damage was scored according to Knodell et al. [10]. The patients were treated with PEG-IFNα-2a (180 μg/week) and RBV (800–1200 mg/day body weight-adjusted) in a period of 24 weeks for genotype 2 and 3 and 48 weeks for genotype 1 and 4. Sustained virological response (SVR) was defined as an undetectable HCV ribonucleic acid (RNA) six months after completing the therapy. Non-responsiveness (NR) to the therapy was defined as detectable HCV RNA during and at end of the therapy. Reappearance of viral RNA after completing the therapy in patients whose serum HCV RNA was undetectable during or at the end of the treatment was categorized as relapse (relapse responders – RR).

All the results were statistically examined with the commercial SPSS program (version 19.0, SPSS Inc., Chicago, IL). Central tendency, variability and frequency were analyzed, according to the type of data collected, stratified by the subgroups of the patients of interest. Mann-Whitney *U*-test and Kruskal-Wallis-test were used for comparative analysis between the groups of nonparametric data. Contingency tables were used to analyze the relationship between two or more variables.

Results

Characteristics of the patients

The study group of 121 patients comprised of 80 (66%) males and 41 (34%) females with the average age 41.9 ± 14 years. The number of males was significantly higher than the number of females ($p = 0.004$). The route of infection was intravenous use of psychoactive substances (IVU PAS) in 41 (33.84%) patients, blood transfusion in 23 (19%), dialysis in 15 (12.4%), sexual contact in 3 (2.48%), professional exposure in 2 (1.65%), perinatal transmission in 1 (0.83%) patient, and for 36 (29.7%) patients the route of virus transmission was unknown (Figure 1A). The median viral load (HCV RNA titer) in the whole group was 3,839.500 IU/mL. HCV genotype 1 was dominant (83 of 121 patients, 68.6%), represented in statistically higher number than other genotypes ($p < 0.001$). Genotype 3 was registered in 33 (27.2%) patients, genotype 4 in 3 (2.6%) and genotype 2 in 2 (1.65%) patients (Figure 1B). Liver biopsy specimens were obtained from 104 patients and scored according to Knodell et al. [10]. The degree of fibrosis was evaluated as: no fibrosis (F0), recorded in 11 (10.6%) patients, F1 found in 48 (46.1%), F2 in 25 (24.0%), F3 in 14 (13.47%) and F4 in 6 (5.77%) patients (Figure 1C).

Regarding the response to the therapy, data were obtained for 95 subjects. SVR was achieved in 69 (72.63%) patients, 9 (9,47%) patients were NR to the therapy and 17 (17.89%) patients were RR (Figure 1D). According to treatment response all data were organized in three groups: SVR, RR and NR.

Viral factors influencing response to the therapy

The lowest median HCV RNA levels were registered in the patients with favorable response to the therapy (SVR – 2,378.250 IU/mL), higher in RRs (4,968,000 IU/mL) and the highest in NRs (6,021.000 IU/mL) (Figure 2).

Although dominant in all four groups, genotype 1 was the most frequent in NRs (9 of 9 patients; $p < 0.001$). The second most frequent was genotype 3, present in high percent in the group of SVRs (25 of 69 patients – 36.23%; $p < 0.001$), while lesser in RR (4 of 17 – 23.53% patients). Genotype 4 was found only in the SVR group in 3 (4.35%) of 69 patients. One patient with genotype 2 the group of RRs was registered in (5.88%) (Table 1).

Host factors influencing response to the therapy

Except for the SVR group ($p = 0.026$), there was no statistically significant difference in the age of the patients, as well as in the percent of the males and the females among the study groups (Table 1).

IVU PAS as the route of viral inoculation was present in statistically higher percentage in the group of the patients with SVR (43.48%; $p = 0.026$), while blood transfusion and

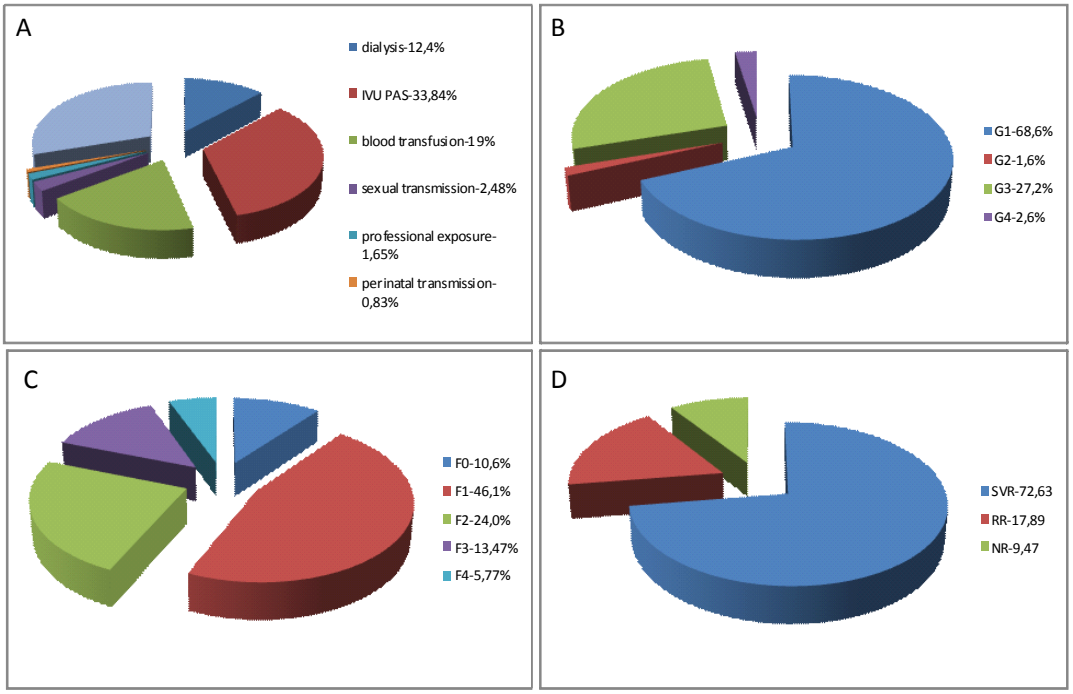

Fig. 1 – Characteristics of the patients: (A) Rate of hepatitis C virus (HCV) transmission route; (B) HCV genotypes; (C) Stage of fibrosis, and (D) Responsiveness to the therapy.
IVU PAS – intravenous use of psychoactive substances; G – genotype; F – fibrosis; SVR – sustained virological response; RR – relapse responder; NR – non-responsiveness.

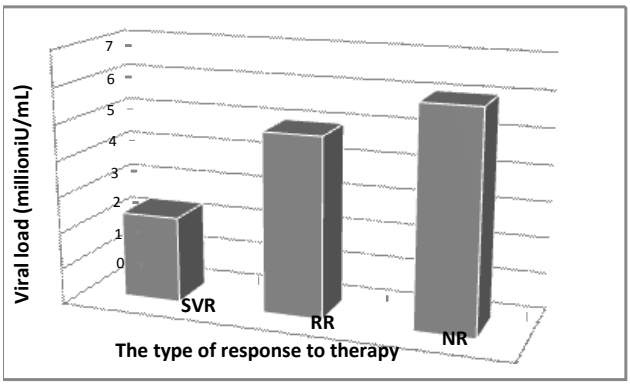

Fig. 2 – Median viral load related to the response to the therapy.
SVR – sustained virological response; RR relapse responder; NR – non-responsiveness;
SVR – sustained virological response; RR – relapse responder; NR – non-responsiveness; IVU PAS – intravenous use of psychoactive substances; *$p < 0.05$; **$p < 0.01$.

dialysis were the leading risk factors in the group of RRs (transfusion – 23.53%, dialysis – 17.65%) and NEs (33.3% both) (Table 1). Dialysis as the route of infection was most frequent in the group of NRs ($p = 0.023$).

Analysis of the relation between the degree of liver fibrosis and responsiveness to the therapy showed that the majority of the patients with F0 were in the group of SVRs (7 patients), and only one patient in the RR group. F1 and F2 were prevailing in the SVR (49.21% and 26.98%, respectively), in the RR group F1, F2 and F3 were represented in the similar percent (35.71%; 28.57%; 28.57%, respectively) and in the group of NRs the most frequent were F3 and F4 (33.3% each) (Table 1). F3 stage was significantly more immanent in the RR and NR patients ($p < 0.05$) and F4 in non-responders ($p < 0.05$).

whereas the lowest viral load was found in the SVRs. This data is in agreement with previous findings that baseline viral load correlates with treatment outcome, regardless the virus genotype [11].

In our study, genotype 1 was dominant, represented in all the groups, and the genotype 3 was second most frequent. All NR patients were infected with HCV-1. Indeed, this genotype is a more aggressive strain and most difficult to treat [12]. Clinical studies have shown that standard PEG-IFNα-2a plus RBV therapy is quite successful in case of genotype 3 [13]. Similarly, we found genotype 3 significantly more immanent in the group of SVRs. Genotype 4 is considered to be associated with progression to cirrhosis and worse response to the therapy [14, 15], although recent clinical trials have demonstrated the opposite results [16]. The results of our

Table 1

Response to pegylated interferon alfa-2a plus ribavirin therapy depending on the patients characteristics, route of hepatitis C virus (HCV) infection, HCV genotypes, and the stage of liver fibrosis

Characteristics of the patients	The patients grouped according to the treatment response		
	SVR (n = 69)	RR (n = 17)	NR (n = 9)
Age (years)	37*	46	48
Gender, n (%)			
male	46 (66.7)	10 (55.6)	6 (66.7%)
female	23 (33.33)	7 (44.4)	3 (33.33%)
Route of infection, n (%)			
IVU PAS	30 (43.48)*	2 (11.76)	1 (11.11)
transfusion	14 (20.29)	4 (23.53)	3 (33.33)
dialysis	4 (5.80)	3 (17.65)	3 (33.33)*
sexual transmission	0 (0)	1 (5.88)	0 (0)
professional exposure	1 (1.45)	0 (0)	0 (0)
perinatal transmission	1 (1.45)	0 (0)	0 (0)
unknown	19 (27.54)	7 (41.18)	2 (22.22)
HCV genotypes (G), n (%)			
G1	41 (59.42)	12 (70.59)	9 (100.0)**
G2	0 (0.0)	1 (5.88)	0 (0.0)
G3	25 (36.23)**	4 (23.53)	0 (0.0)
G4	3 (4.35)	0 (0.0)	0 (0.0)
Rate of fibrosis (F) stage, n (%)	SVR (n = 63)	RR (n = 14)	NR (n = 6)
F0	7 (11.11)	1 (7.14)	0 (0)
F1	31 (49.21)	5 (35.71)	1 (16.7)
F2	17 (26.98)	4 (28.57%)	1 (16.7)
F3	5 (7.94)	4 (28.57)*	2 (33.3%)*
F4	3 (4.76)	0 (0%)	2 (33.3)*

SVR – sustained virological response; RR – relapse responder; NR – non-responsiveness; IVU PAS – intravenous use of psychoactive substances; *$p < 0.05$; **$p < 0.01$.

Discussion

The ultimate goal of PEG-IFNα-2a plus RBV therapy is the resolution of HCV infection. Considering that only half of treated patients achieve SVR, it is of great importance to reveal factors that may influence the outcome of the therapy. In the present study in the group of 121 patients host and viral factors that can affect the response to PEG-IFNα-2a plus RBV therapy were analyzed.

The results of the study showed that the largest number of virus particles was registered in the group of NRs, study are in accordance with the latest, given that all patients infected with HCV-4 responded to the therapy.

The majority of studies point to age as predictive factor in reaching SVR [17]. In our study we also found this correlation. There was no statistically significant difference in the percent of males and females among the study groups. Similarly, previous studies have shown that gender have no influence in achieving SVR [18].

Up to 1990s the leading risk factor for HCV infection was blood transfusion. Ever since 1992, blood from donators has been screened for the presence of HCV. Nowadays the

most common route of infection is VU PAS, followed by transfusion of blood and blood derivates, long-term hemodialysis, organ transplantation, sexual contact, perinatal transmission, nosocomial transmission, tattoos or body piercings and professional exposure. The results of our study are in accordance with this data since the most numerous were the patients infected through intravenous use of drugs, the second most frequent risk factor were transfusion and dialysis, and in a small percent of patients sexual contact, professional exposure and perinatal transmission. Analyzing data we found that in the group of SVRs the most frequent routes of HCV transmission were IVV PAS and blood transfusion, while in the group of RRs and NRs dialysis and transfusion were the leading risk factors.

Chronic HCV infection gives rise to liver injury that can lead to formation of scar tissue, *ie* fibrosis. As inflammation continues, liver lesions are more massive and more liver tissue is replaced with nonfunctional connective tissue. About 10–20% of chronically infected patients can develop cirrhosis and liver cancer. Fibrosis is not an irreversible process. In patients who achieve SVR to the therapy, fibrosis stabilization and retraction occur [19]. However, the presence of progressive fibrosis predicts a lower response rate [20]. In the present study the majority of patients with stage F0 were in the group of SVRs and with increase of the stage of fibrosis the response rate was decreasing. The patients with advanced fibrosis (F2 and F3) were the most prevailing in the group of RRs, and the patients with high stage fibrosis (F4) in the group of NRs.

Conclusion

The results of this study showed that the majority of patients on pegylated interferon alfa-2a plus ribavarin responded to the therapy (71.88%). Hepatitis C virus genotype, viral load, age of the patients and the stage of fibrosis were related to the response to the therapy. Our study did not confirm the association between the gender of the patients and the treatment outcome. Intravenous use of psychoactive substances as the route of infection was the most frequent in the group of responders with sustained virological response, and transfusion and dialysis in the group of the patients with poor response to the therapy (relapse responders and non-responders).

Acknowledgements

This study was funded by the Faculty of Medical Sciences, University of Kragujevac (JP 22-10) and the Ministry of Education, Science and Technological Development of the Republic of Serbia (III41010).

R E F E R E N C E S

1. *Lavanchy D.* The global burden of hepatitis C. Liver Int 2009; 29(Suppl 1): 74−81.
2. *Shepard CW, Finelli L, Alter MJ.* Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005; 5(9): 558−67.
3. *Esteban JI, Sauleda S, Quer J.* The changing epidemiology of hepatitis C virus infection in Europe. J Hepatol 2008; 48(1): 148−62.
4. *Afdhal NH.* The Natural History of Hepatitis C. Semin Liver Dis 2004; 24 Suppl 2: 3−8.
5. *Poynard T, Bedossa P, Opolon P.* Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 1997; 349(9055): 825−32.
6. *Zein CO, Levy C, Basu A, Zein NN.* Chronic hepatitis C and type II diabetes mellitus: a prospective cross-sectional study. Am J Gastroenterol 2005; 100(1): 48−55.
7. *Fried MW, Shiffman ML, Reddy RK, Smith C, Marinos G, Gonçales FL,* et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002; 347(13): 975−82.
8. *Operskalski EA, Kovacs A.* HIV/HCV co-infection: pathogenesis, clinical complications, treatment, and new therapeutic technologies. Curr HIV/AIDS Rep 2011; 8(1): 12−22.
9. *Thompson C, Rogers G, Hewson P, Wright D, Anderson R, Cramp M,* et al. Surveillance of cirrhosis for hepatocellular carcinoma: systematic review and economic analysis. Health Technol Assess 2007; 11(34): 1−206.
10. *Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N,* et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981; 1(5): 431−5.
11. *Berg T, Sarrazin C, Herrmann E, Hinrichsen H, Gerlach T, Zachoval R,* et al. Prediction of treatment outcome in patients with chronic hepatitis C: significance of baseline parameters and viral dynamics during therapy. Hepatology 2003; 37(3): 600−9.
12. *Pozzato G, Moretti M, Crocé LS, Sasso F, Kaneko S, Unoura M,* et al. Interferon therapy in chronic hepatitis C virus: evidence of different outcome with respect to different viral strains. J Med Virol 1995; 45(4): 445−50.
13. *Sarin SK, Kumar CK.* Treatment of patients with genotype 3 chronic hepatitis C- current and future therapies. Liver Int 2012; 32(1 Suppl): 141−5.
14. *Zein NN, Rakela J, Krawitt EL, Reddy KR, Tominaga T, Persing DH.* Hepatitis C virus genotypes in the United States: epidemiology, pathogenicity, and response to interferon therapy. Collaborative Study Group. Ann Intern Med 1996; 125(8): 634−9.
15. *Attaullah S, Khan S, Ali I.* Hepatitis C virus genotypes in Pakistan: a systemic review. Virol J 2011; 8: 433.
16. *Kamal SM, Nasser IA.* Hepatitis C genotype 4: What we know and what we don't yet know. Hepatology 2008; 47(4): 1371−83.
17. *Antonucci G, Angeletti C, Vairo F, Longo MA, Girardi E.* Age and prediction of sustained virological response to hepatitis C virus (HCV) infection treatment based on 28-day decrease in HCV RNA levels. J Infect Dis 2009; 200(9): 1484−5.
18. *Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R,* et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001; 358(9286): 958−65.
19. *Shiratori Y, Imazeki F, Moriyama M, Yano M, Arakawa Y, Yokosuka O,* et al. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. Ann Intern Med 2000; 132(7): 517−24.
20. *Karino Y, Toyota J, Sugawara M, Miyazaki K, Kuwata Y, Yamazaki K,* et al. Hepatitis C virus genotypes and hepatic fibrosis regulate 24-h decline of serum hepatitis C virus RNA during interferon therapy in patients with chronic hepatitis C. J Gastroenterol Hepatol 2003; 18(4): 404−10.

Received on March 21, 2014.
Revised on May 20, 2014.
Accepted on June 9, 2014.