

Research article

Open access

Role of host and viral factors and genetic variation of IL28B on therapy outcome in patients with chronic hepatitis C genotype 1b from Serbia

Snežana Jovanović-Ćupić^{1*}, Nina Petrović¹, Milena Krajnović¹, Maja Bundalo¹, Nikola Kokanov¹, Ana Božović¹, Gorana Stamenković²

¹University of Belgrade, Institute for Nuclear Sciences-Vinča, Laboratory for Radiobiology and Molecular Genetics, P.O. Box 522, Belgrade, Serbia

²University of Belgrade, Institute for Biological Research-Siniša Stanković, 11000 Belgrade, Serbia

DOI: 10.31383/ga.vol3iss1pp36-41

Abstract

Viral and host factors in hepatitis C virus (HCV) infection can influence on therapy outcome to pegylated interferon/ribavirin (PEG-IFN/RBV) and progression of liver fibrosis. Although novel direct-acting antivirals (DAAs) represent promising successful treatment of hepatitis C infection, the majority of patients are deprived of this therapy because of its expensiveness and therefore they remain untreated. Also, the efficacy of this novel treatment may be affected by the presence of resistance-associated substitutions (RASs). This study was designed to describe associations between baseline host and viral factors, progression of liver fibrosis and response to therapy with pegylated interferon/ribavirin (PEG-IFN/RBV) in patients with chronic hepatitis C (HCV) genotype 1b. Pre-treatment of 100 patients with chronic hepatitis C genotype 1b was analyzed, and related to outcome of therapy. TaqMan assay was used to determine SNP rs12979860 in all patients. In our study there was significant correlation between age and response to therapy. Also, we found associations between a known route of transmission and age, gender, stage of liver fibrosis and therapy outcome. With respect to SNP rs12979860, the frequency of the CC genotype in the group with a sustained virologic response (SVR) was significantly higher than in the group of non-responders (NR). In contrast, there was no correlation between IL28B polymorphism and progression of liver fibrosis.

*Correspondence

E-mail: sjcupic@gmail.com

Received

May, 2019

Accepted

May, 2019

Published

June, 2019

Copyright: ©2019 Genetics & Applications, The Official Publication of the Institute for Genetic Engineering and Biotechnology, University of Sarajevo

Keywords

Hepatitis C virus (HCV), genotype 1b, IL28B polymorphism, response to therapy, liver fibrosis

Introduction

Hepatitis C virus (HCV) is a global health problem affecting more than 150 million people worldwide and approximately 8 to 11 million people in Europe (WHO, 1999; Cornberg et al., 2011). Patients with chronic hepatitis C infection have a higher risk of developing liver cirrhosis and hepatocellular carcinoma (Maasoumy & Wedemeyer, 2012). Pegylated interferon (PEG-IFN) and ribavirin (RBV) is the standard of care for treating HCV infection in Serbia. New DAA therapy, such as sofosbuvir, daclatasvir and the sofosbuvir/ledipasvir combination, has increased the rate of SVR and eliminated persistent HCV infection in > 90% of patients. Although, current DAA therapy significantly increased the SVR, and showed better tolerance over a shorter time, it is still limited by viral resistance and high cost, especially in countries with limited resources (Bartenschlager et al., 2018). Since the treatment response is influenced by genetic variations in patients, as well as in the HCV genome, recognition of biological markers that may predict the response and hence treatment outcome would be helpful also in the era of DAA. Therefore, identification of the determinants of response to treatment has high priority, even though many viral, genetic and host factors interact in the prediction of therapy outcome in patients with HCV infection (Hadziyannis et al., 2004). In this study we analyzed relationships between age, gender, alanine aminotransferase (ALT) concentration, stage of liver fibrosis, route of transmission and pre-treatment viral load with therapy outcome in patients with chronic hepatitis C genotype 1b. Previously it was shown that single nucleotide polymorphisms (SNPs) near the interleukin (IL) 28B gene were strongly associated with response to PEG-IFN/RBV therapy in patients with genotype 1 (Ge et al., 2009; Suppiah et al., 2009) as well as the risk of fibrosis progression, cirrhosis and hepatocellular carcinoma (HCC), especially in NR (Fabris et al., 2011; de la Fuente et al., 2017). The aim of this study was to evaluate the effects of host and viral factors during antiviral treatment with PEG-IFN/RBV in patients chronically infected with HCV genotype 1b with special emphasis on the effects of potentially

important prognostic factors such as IL28B rs12979860 polymorphism on therapy outcome.

Materials and methods

Samples were collected from a total 100 patients with chronic hepatitis C genotype 1b, before the start of PEG-IFN/RBV therapy. A virologic response was defined as SVR (absence of HCV RNA in serum 6 months after end of treatment) and non-response (NR) (detection of serum HCV RNA 6 months after cessation of treatment). Histological activity grade and fibrosis stage were evaluated according to the METAVIR scoring system (Bedossa & Poynard, 1996) as follows: F0 (no fibrosis), F1 (mild fibrosis without septa), F2 (moderate fibrosis with few septa), F3 (severe fibrosis with numerous septa without cirrhosis) and F4 (cirrhosis). Our study was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade, and all patients provided written consent.

Total ribonucleic acid (RNA) was extracted from 100 µL of serum using the Ribo-Sorb-100 (HCV Quant) RNA/DNA Extraction Kit (Sacace Biotechnologies, Como, Italy) according to the manufacturer's protocol. Concentration of HCV RNA was determined by real-time PCR (Applied Biosystems 7500, Foster City, United States) using the commercially available R-TMQ HCV Kit (Sacace Biotechnologies, Como, Italy) according to the manufacturer's instructions (sensitivity limit-250 IU/mL). Genotyping of HCV was performed with a combination of type-specific primers as described previously (Okamoto et al., 1992, 1994).

Genotyping of IL28B

Genomic DNA was extracted from serum using a commercially available kit QIAamp UltraSens (Qiagen, GmbH, Germany) according to the manufacturer's instructions. The IL28B SNP rs12979860 was analyzed using Custom® SNP Genotyping Assays (Applied Biosystems). The allele specific primers were designed as reported previously (Alestig et al., 2011). Genotyping was performed on ABI-7500 real-time PCR (Applied Biosystems, Foster City, United States) in 25 µL reaction volume containing 10 ng DNA, 12.5 µL

TaqMan® Universal PCR Master Mix and 1.25 µL (40x) Custom® SNP Genotyping Assays.

Statistical analysis

The results are presented as mean $\pm$ standard deviation (SD) or number (percentage). Differences in the frequency distribution between two or more categorical variables were evaluated by Pearson's χ^2 test. Means of normally distributed continuous variables between sustained virologic responders and non-responders were compared by the unpaired t-test, while the non-parametric Mann-Whitney U Test was used for means of skewed continuous variables. Relations between ALT levels and RNA viral load were evaluated by the Spearman rank order correlation test. In all tests, p-values of less than 0.05 were considered statistically significant. All statistical analyses were performed using the Sigma Plot 10.0 licensed statistical analysis software package.

Results and Discussion

Baseline characteristics for the 100 patients divided according to therapy outcome (SVR, n = 47, NR, n = 53) are shown in Table 1. There was no significant difference between the genders and response to the therapy (Table 2). The mean age of all our patients was 44.3 ± 12.7 . Patients with SVR were significantly younger than those with NR (Table 1). Both groups had elevated (ALT) levels (upper limit of normal is 40 U/L) with a statistically insignificant difference (Table 1). Moreover, the ALT concentration was not correlated with pre-treatment viral load (Spearman's, $p = 0.653$, data not shown), stage of fibrosis or therapy outcome (Table 1).

The major route of HCV transmission was unknown (48%), subsequent to blood transfusion (23%) or intravenous drug use (IVDU, 21%). The correlations between the known route of transmission and response to therapy, gender and stage of liver fibrosis were statistically significant. Thus, patients with a positive history of blood product transfusion were more frequently NR than with SVR, while IVDU was more common among SVR than NR patients ($p < 0.001$, data not shown). Additionally, in the group of IVDU males were significantly more common than females (18% vs. 3%; $p < 0.001$, data

not shown), while women were significantly more frequent than men in the group with a positive history of blood product transfusion (16% vs. 7%; $p < 0.001$, data not shown). With respect to IL28 rs12979860 genotype distribution, more than half of patients (56%) carried the CC genotype and the remainder (44%) had CT/TT genotypes. Therapy response rates divided according to the IL28B genotype are presented in Table 2. The frequency of the CC genotype in the group with SVR was significantly higher than for the NR group, while patients with the other genotypes (CT/TT) were more frequently NR (Table 2).

The genotype CC was associated with the patient's age. Thus, subjects with the rs12979860 CC genotype were markedly younger than those with CT/TT genotypes (Table 2). Moreover, by multiple logistic regressions, age (below 40 years) and rs12979860 CC genotype were shown to be the strongest prediction factors for SVR ($p < 0.001$, data not shown). In contrast, the rs12979860 genotype was not correlated with level of ALT, pre-treatment viral load and fibrosis stage. Currently, PEG-IFN/RBV is the standard routine treatment for chronic hepatitis C infection in Serbia. We found a significant correlation between age and response to therapy. Moreover, older patients had a lower SVR rate to PEG-IFN/RBV therapy than younger ones which confirms previous results (Antonucci et al., 2007; Jovanovic-Cupic et al., 2014, 2016).

Connections between HCV transmission and therapy outcome were analyzed in the recent studies. Our results showed a significant relationship between a known route of transmission and response to the therapy. The major known route of HCV transmission was blood transfusion (23/100), followed by intravenous drug use IVDU (21/100) and post-operative (4/100). The high percentage of patients infected through blood transfusion may be the consequence of migration, inadequate screening or poor prophylaxis, which is characteristic of countries with limited resources. Moreover, there was a significant association between age and route of transmission. Thus, younger patients were more frequent among the IVDU group than among those infected by blood transfusion. As in previous investigations, men were more common in the

Table 1. Baseline clinical characteristics of HCV genotype 1b patients according to therapy outcome

Clinical and pathological characteristics of patients	Sustained responders (n=47)	Non-responders (n=53)	HCV patients overall value $\pm$ SD	HCV patients overall p
Age (years) ^a	41.0 $\pm$ 13.9	47.3 $\pm$ 10.8	44.3 $\pm$ 12.7	0.013* ^d
Gender, n (male/female)	24/23	28/25	52/48	0.864 ^d
ALT level (U/L) ^a	90.3 $\pm$ 62.7	48.64 $\pm$ 6.68	84.8 $\pm$ 55.6	0.315 ^d
HCV RNA level (log IU/mL) ^c	9.5 $\pm$ 32.7	23.4 $\pm$ 97.4	16.7 $\pm$ 75.5	0.973 ^d
Stages of fibrosis ^b	n (%)	n (%)	All n (%)	
F0-F2	27 (27)	29 (29)	56 (56)	
F3-F4	20 (20)	24 (24)	44 (44)	0.788 ^d

^aData expressed as mean $\pm$ SD; ALT alanine aminotransferase; ^bStage of fibrosis expressed by METAVIR score (fibrosis 0-2 and 3 to 4); ^cExpressed HCV RNA level $\times 10^5$; * Statistically significant; p-values < 0.05 were considered statistically significant; ^d Mann-Whitney U-test

Table 2. The IL28 rs12979860 polymorphism distribution according to response to therapy and baseline characteristics of patients with chronic hepatitis C infection and genotype 1b

Clinical and pathological characteristics of patients	Sustained virological responders IL28B rs12979860 (n=47)		Non-responders IL28B rs12979860 (n=53)		HCV patients overall value $\pm$ SD	p
	CC (n=44)	CT/TT (n=3)	CC (n=12)	CT/TT (n=41)		
Age (years) ^a	40.7 $\pm$ 13.6	46.0 $\pm$ 20.9	48.7 $\pm$ 11.0	47.0 $\pm$ 10.9	44.3 $\pm$ 12.7	0.013*
Gender, n (male/female)	24/20	0/3	5/7	23/18	52/48	0.641
ALT level (U/L) ^c	91.1 $\pm$ 64.4	63.3 $\pm$ 13.3	72.4 $\pm$ 40.3	82.1 $\pm$ 51.0	84.8 $\pm$ 55.7	0.560
HCV RNA level (log IU/mL) ^c	9.9 $\pm$ 33.8	3.5 $\pm$ 7.7	9.8 $\pm$ 35.4	23.0 $\pm$ 10.5	16.7 $\pm$ 73.5	0.947
Stages of fibrosis ^b	n (%)	n (%)	n (%)	n (%)	n (%)	
F0-F2	26 (26)	2 (2)	7 (7)	22 (22)	57 (57)	
F3-F4	18 (18)	1 (1)	5 (5)	19 (19)	43 (43)	0.183

^aData expressed as mean $\pm$ SD; ALT alanine aminotransferase; ^bStage of fibrosis expressed by METAVIR score (fibrosis 0-2 and 3 to 4); ^cvalues expressed as median-range; * Statistically significant; p-values < 0.05 were considered statistically significant; ^d Mann-Whitney U-test

IVDU group, while women were significantly more frequent among the patients with a positive history of blood transfusion (Pawlotsky et al., 1995; Cornberg et al., 2011). With respect to previous results SVR rate was reported to be significantly higher in patients with the favorable (CC) genotype of IL28B than in those with unfavorable (CT/TT) genotypes (Halfon et al., 2011, Lazarevic et al., 2013) as confirmed here. Therefore, 44% of our patients with the CC genotype achieved SVR compared to 2% of those with the CT genotype and only 1% of those with the TT genotype. The main limitations of this study are the relatively small number of patients and a study design matched for genotype and therapy outcome. Considering host factors, we have demonstrated that patient age (below 40 years) and the CC genotype of IL28B could be important predictive factors for SVR. According to our knowledge, this is the second study to provide detailed results on the effect of polymorphism on therapy outcome of patients

with chronic infection genotype 1b from Serbia. Combined therapy with PEG-IFN/RBV, remains an important and relevant therapy option. Thus, patients with genotype 1 achieved SVR rates up to 79%, while the rate was 89% for those with genotype 2 or 3. This rate increases for patients with favorable IL28B genotypes (Huang et al., 2017). Previous data have shown that IFN-induced SVR is associated with a lower incidence of hepatocellular carcinoma compared with DAA therapy. Also, the effects of novel DAAs are still unclear, especially in patients with cirrhosis and/or hepatocellular carcinoma. In this interferon-free era, the previous combined therapy remains a safe and effective option for selected HCV patients (Huang et al., 2017). The efficacy of treatment using new DAA therapy for patients with chronic infection may be affected by the presence of resistance-associated substitutions (RASs) (Bartenschlager et al., 2018).

Conclusions

This study represents an overview of the current state of patients with chronic hepatitis C genotype 1b in Serbia and their response to the combined therapy with PEG-IFN/RBV. The response to novel DAA therapy remains to be explored, especially in developing countries. One of the main issues is that a vast number of patients do not respond to this therapy and show the progression of liver fibrosis. On the other side, decreasing the cost of DAA therapy is an important challenge for its introduction in basic clinical routine.

Conflict of interest

The authors declare that they have no conflicts of interest.

References

- Alestig E, Arnholm B, Eilard A, Lagging M, Nilsson S, Norkrans G et al. (2011) Core mutations, IL28B polymorphisms and response to peginterferon/ribavirin treatment in Swedish patients with hepatitis C virus genotype 1 infection. *BMC Infect Dis*, 11:124.
- Antonucci G, Longo MA, Angeletti C, Vairo F, Oliva A, Comandini UV et al. (2007) The effect of age on response to therapy with peginterferon alpha plus ribavirin in a cohort of patients with chronic HCV hepatitis including subjects older than 65 yr. *Am J Gastroenterol*, 102(7):1383-91.
- Bartenschlager R, Baumert TF, Bukh J, Houghton M, Lemon SM, Lindenbach BD et al. (2018) Critical challenges and emerging opportunities in hepatitis C virus research in an era of potent antiviral therapy: Considerations for scientists and funding agencies. *Virus Res*, 248:53-62.
- Bedossa P, Poynard T (1996) An algorithm for the grading of activity in chronic hepatitis C. *Hepatology*, 24(2):289-93.
- Cornberg M, Razavi HA, Alberti A, Bernasconi E, Buti M, Cooper C et al. (2011) A systematic review of hepatitis C virus epidemiology in Europe, Canada and Israel. *Liver Int*, 31(s2):30-60.
- Fabris C, Falletti E, Cussigh A, Bitetto D, Fontanini E, Bignulin S et al. (2011) IL-28B rs12979860 C/T allele distribution in patients with liver cirrhosis: Role in the course of chronic viral hepatitis and the development of HCC. *J Hepatol*, 54(4):716-722.
- de la Fuente S, Citores MJ, Duca A, Cisneros E, Baños I, Vilches C, Cuervas-Mons V (2017) Interleukin-28B TT genotype is frequently found in patients with hepatitis C virus cirrhosis but does not influence hepatocarcinogenesis. *Clin Exp Med*, 17(2):217-223.
- Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ et al. (2009) Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature*, 461(7262):399-401.
- Hadziyannis SJ, Sette H, Morgan TR, Balan V, Diago M, Marcellin P et al. (2004) Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med*, 140(5):346-55.
- Halfon P, Bourliere M, Ouzan D, Maor Y, Renou C, Wartelle C et al. (2011) A single IL28B genotype SNP rs12979860 determination predicts treatment response in patients with chronic hepatitis C Genotype 1 virus. *Eur J Gastroenterol Hepatol*, 23(10):931-935.
- Huang Y, Li MH, Hou M, Xie Y (2017) Peginterferon alfa-2a for the treatment of chronic hepatitis C in the era of direct-acting antivirals. *Hepatobiliary Pancreat Dis Int*, 16(5):470-479.
- Jovanović-Cupić S, Glisić S, Stanojević M, Vasiljević N, Bojić-Milinović T, Božović A, Dimitrijević B (2014) Response factors to pegylated-interferon-alfa/ribavirin treatment in chronic hepatitis C patients genotype 1b. *Archives of Biological Sciences*, 66(1):193-201.
- Jovanovic-Cupic S, Glisic S, Stanojevic M, Nozic D, Petrovic N, Mandusic V, Krajnovic M (2016) The influence of host factors and sequence variability of the p7 region on the response to pegylated interferon/ribavirin therapy for chronic hepatitis C genotype 1b in patients from Serbia. *Arch Virol*, 161(5):1189-98.
- Lazarevic I, Djordjevic J, Cupic M, Karalic D, Delic D, Svirtlih N et al. (2013) The influence of single and combined IL28B polymorphisms on response to treatment of chronic hepatitis C. *Clin Virol*, 58(1):254-257.
- Maasoumy B, Wedemeyer H (2012) Natural history of acute and chronic hepatitis C. *Best Pract Res Clin Gastroenterol*, 26(4):401-412.
- Okamoto H, Sugiyama Y, Okada S, Kurai K, Akahane Y, Sugai Y et al. (1992) Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. *J Gen Virol*, 73 (Pt3):673-9.

- Okamoto H, Mishiro S (1994) Genetic heterogeneity of hepatitis C virus. *Intervirology*, 37(2):68-76.
- Pawlotsky JM, Tsakiris L, Roudot-Thoraval F, Pellet C, Stuyver L, Duval J, Dhumeaux D (1995) Relationship between hepatitis C virus genotypes and sources of infection in patients with chronic hepatitis C. *J Infect Dis*, 171(6):1607-10.
- Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML et al. (2009) IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet*, 41(10):1100-4.
- World Health Organization (1999) Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. *J Viral Hepat*, 6(1):35-47.