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 hepatitis 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 polymerase 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.

Apstrakt


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

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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. The discovery of HCV in 1989 clarified the etiology of a large number of posttransfusion hepatitis with unknown cause. 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 outbreak 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. The progression of disease is associated with alcohol abuse, the presence of diabetes, age of the patient, co-infection with HIV and/or other primary hepatotropic viruses. About 10–20% of patients with chronic hepatitis C will develop liver cirrhosis and hepatocellular carcinoma occurs in about 1–5% of cases.

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 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. The degree of fibrosis was evaluated: no fibrosis (F0), recorded 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: 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 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.

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-respondiveness (NR) to the therapy was defined as detectable HCV RNA during and at the 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.
**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
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 ($p < 0.05$).

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. Clinical studies have shown that standard PEG-IFNα-2a plus RBV therapy is quite successful in case of genotype 3. 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, although recent clinical trials have demonstrated the opposite results. The results of our study are in accordance with the latest, given that all patients infected with HCV-4 responded to the therapy.

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, 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.

Table 1

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

SVR – sustained virological response; RR – relapse responder; NR – non-responsiveness; IVU PAS – intravenous use of psychoactive substances; *p < 0.05; **p < 0.01.
most common route of infection is VU PAS, followed by trans- 
fusion of blood and blood derivatives, long-term hemodialysis, 
organ transplantation, sexual contact, perinatal transmission, 
oscopic transmission, tattoos or body piercings and profes-
sional 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 
occurred 19. However, the presence of progressive fibrosis predicts 
a lower response rate 20. In the present study the majority of pa-
tients with stage F0 were in the group of SVRs and with in-
crease of the stage of fibrosis the response rate was decreasing.

References


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Conclusion

The results of this study showed that the majority of pa-
tients on pegylated interferon alfa-2a plus ribavirin 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 re-
sponse 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 infec-
tion was the most frequent in the group of responders with sus-
tained virological response, and transfusion and dialysis in the 
group of the patients with poor response to the therapy (relapse 
responders and non-responders).

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