TREATMENT HISTORY: FACTORS THAT AFFECT THE OUTCOME OF HEPATITIS C
VIRUS TREATMENT WITH INTERFERON-ALPHA 2A/B AND RIBAVIRIN

ISTORIJA TERAPIJE: FAKTORI KOJI SU UTICALI NA ISHOD TRETMANA PEGILOVANIM INTERFERONOM ALFA 2A/B I RIBAVIRINOM KOD PACIJENATA SA HEPATITISOM C

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Summary

Introduction. Until the 1990s, there was no available treatment for chronic hepatitis C, but during this decade the benefits of interferon-alpha therapy were reported. At the end of the 1990s, the pegylated interferon-alpha 2a/b has significantly altered the treatment, whereas direct acting antivirals have significantly affected the treatment. The aim of this study was to show the most significant predictive factors of therapy response among patients with chronic hepatitis C treated with pegylated interferon-alpha 2a/b and ribavirin. Material and Methods. A non-randomized retrospective study included 292 patients with chronic hepatitis C treated at the Clinic for Infectious Diseases, Clinical Center of Vojvodina, from 2008 to 2015. Results. The study showed that therapeutic response was not affected by sex, serum viral load, or if the therapy was applied for the first time or repeated. A sustained virological response was statistically significantly more frequent in younger patients, as well as in patients without extrahepatic manifestations. Cases with higher progression of fibrosis were associated with lower chance for sustained virological response. Genotype 1 showed to be a predictor of adverse response to therapy, and genotype 3 as a predictor of sustained virological response. Steatosis was significantly less frequent in patients with genotype 1 with sustained virological response. Patients with a shorter duration of infection were more prone to sustained virological response. Conclusion. A positive response to pegylated interferon-alpha 2a/b and ribavirin was found in 70.20% of patients with chronic hepatitis C. Elderly age, late detection of the infection, hepatitis C virus 1 genotype, fibrosis progression, presence of hepatic steatosis, and extrahepatic manifestations were risk factors for poor treatment outcome. Key words: Treatment Outcome; Interferon-alpha; Ribavirin; Hepatitis C; Drug Users; Risk Factors

Sažetak


Kljucne reći: ishod lečenja; interferon alfa; ribavirin; hepatitis C; narkomani; faktori rizika

Introduction

Hepatitis C virus (HCV) is the leading cause of chronic liver disease worldwide [1, 2]. Epidemic transmission of HCV in the twentieth century has reached its peak by mid-1980s among intravenous drug users [3]. After an incubation period of 2 weeks to 6 months, approximately 80% of people do not exhibit any symptoms. After the acute phase of infection, about 15–45% of infected persons spontaneously clear HCV within 6 months, while 55 - 85% of infected people develop chronic HCV infection [4, 5]. Research studies have shown a faster rate of disease progression among people who got infected at older...
PCR-based testing, etc. expensive and complex polymerase chain reaction (PCR)

HCV by 2030. However, in order to achieve this, there are instances, WHO set the goal of global elimination of HCV by 65% by 2030 [10]. The World Health Organization (WHO) recommends therapy regimens based on direct acting antivirals (DAAs) of the newest generation [10–15]. With the appearance of first-generation direct acting antivirals (kinetic UV test, Olympus AU 400 at the Laboratory Medicine, Basel, Switzerland); Ear Array HCV Genotyping test (Roche Diagnostics and laboratory tests; specific auto-antibodies) was verified by clinical checkup of Vojvodina. Metavir classification for staging hepatitis C liver disease was used; Pathohistological analysis was conducted at the Pathology and Histology Center of the Clinical Center of Vojvodina. Median serum alanine aminotransferase (ALT), steatosis, age of patients, co-infection with HBV, HIV, alcohol consumption, therapy adherence, etc [16, 17]. The therapy success rate most likely depends on genetic barriers within the virus itself on one, and the human host on the other side.

The aim of this research was to show the impact of the most significant predictive factors of the therapy response among patients with chronic hepatitis C treated with a combination of interferon and ribavirin.

Material and Methods

A non-randomized retrospective study included 292 patients with CHC treated at the Clinic for Infectious Diseases of the Clinical Center of Vojvodina from January 2008 to December 2015. All patients fulfilled the criteria of the National Health Insurance Fund of the Republic of Serbia for a combination antiviral treatment regimen (PegIfn–Alfa 2/Rbv) for chronic HCV infection: 18 years old or above, anti–HCV positive longer than six months, high level serum alanine aminotransferase (ALT), hepatic fibrosis confirmed by liver biopsy or fibro scan, did not use alcohol or psychoactive substances over a longer period than one year. The patients with multiple HCV genotypes were not included in the study.

The data used in the study included:
- Medical history data: gender, age, path of infection, duration of infection, medical records of patients with CHC who were not treated with PegIFN–Alfa 2/Rbv for the first time;
- Laboratory data: pre-therapy serum ALT level (kinetic UV test, Olympus AU 400 at the Laboratory Diagnostics Center of the Clinical Center of Vojvodina);
- Liver biopsy (LB): the degree of fibrosis and steatosis (percutaneous LB using Hepafix needle of 1.2 or 1.4 mm; B Braun Melsungen AG, Germany);
- Pathohistological analysis was conducted at the Pathology and Histology Center of the Clinical Center of Vojvodina. Metavir classification for staging hepatitis C liver disease was used;
- The presence of extrahepatic manifestations (arthritis, diabetes mellitus, thyroiditis, organ nonspecific auto-antibodies) was verified by clinical checkup and laboratory tests;
- Genotype of the virus was determined by Linear Array HCV Genotyping test (Roche Diagnostics Systems, Basel, Switzerland);

<table>
<thead>
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<th>Abbreviations</th>
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<tr>
<td>HCV – hepatitis C virus</td>
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<tr>
<td>PEG-IFN – pegylated interferon</td>
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<td>CHC – chronic hepatitis C</td>
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<td>SVR – sustained virological response</td>
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<td>HBV – hepatitis B virus</td>
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<td>HIV – human immunodeficiency virus</td>
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<td>DAAs – direct acting antivirals</td>
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<td>WHO – World Health Organization</td>
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<td>PegIFn-Alfa2/Rbv – PEG-INF-alpha 2a/b with ribavirin</td>
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<tr>
<td>RNA – ribonucleic acid</td>
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<td>BMI – body mass index</td>
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<tr>
<td>ALT – alanine aminotransferase</td>
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<tr>
<td>LB – liver biopsy</td>
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<tr>
<td>VL – viral load</td>
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<tr>
<td>PCR – polymerase chain reaction</td>
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<tr>
<td>F0 – without liver fibrosis</td>
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<tr>
<td>F1 – mild fibrosis</td>
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<tr>
<td>F2 – moderate fibrosis</td>
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<tr>
<td>F3 – severe fibrosis</td>
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<tr>
<td>F4 – cirrhosis</td>
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<td>EHMs – extrahepatic manifestations</td>
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Age, predominantly among men, those who consume alcohol as well as hepatitis B virus (HBV), hepatitis D virus (HDV) or human immunodeficiency virus (HIV) positive patients [6]. Because the infection remains asymptomatic until decades after infection, chronic hepatitis C (CHC) is often undiagnosed despite being the most common cause of chronic liver disease. HCV infection is usually diagnosed based on abnormal liver function tests. The second most common way of diagnosis is to test patients who inject drugs or use intranasal drugs, recipients of blood transfusion, persons who engage high-risk sexual behavior, etc [7]. Anti-HCV prevalence in the general population of Vojvodina is 1.5% and the diagnosis of HCV infection is usually made by detecting anti-HCV antibodies among blood donors [8].

Until the early 1990s, there was no available treatment for CHC, but during this decade the benefits of interferon-alfa therapy were reported, and development of pegylated interferon (PEG-IFN)-alfa 2a and 2b at the end of the 1990s has significantly altered the treatment for CHC. The treatment for CHC has advanced significantly in the last few years with the appearance of first-generation direct acting antivirals (DAAs) in 2011 [9]. In May 2016, the World Health Assembly adopted the first “Global Health Sector Strategy on Viral Hepatitis, 2016 - 2021”. The strategy has a vision of eliminating viral hepatitis as a public health problem with reducing new viral hepatitis infections by 90% and reducing deaths due to viral hepatitis by 65% by 2030 [10]. The World Health Organization (WHO) recommends therapy regimens based on DAAs of the newest generation [10–15]. With the emergence of therapies able to cure HCV in almost all instances, WHO set the goal of global elimination of HCV by 2030. However, in order to achieve this, there are a number of barriers such as high cost of DAAs, expensive and complex polymerase chain reaction (PCR)-based testing, etc.

Previous standard therapy by PEG-INF-alpha 2a/b in combination with ribavirin (PeglnF-Alfa2/Rbv) is still current therapy in Serbia. Therapy success is defined by the non-presence of HCV-ribonucleic acid (RNA) in the serum 6 months after completing therapy, sustained virological response (SVR). Unfortunately, this therapy has not shown definite success. This therapy achieves a SVR rate of 50% to 80%. About 99% of treated patients remain PCR HCV RNA negative. Many studies have been conducted in order to improve therapy outcomes and to identify predictive factors for successful therapy response. Some of the predictive factors are fibrosis score, body mass index (BMI), steatosis, age of patients, co-infection with HBV, HIV, alcohol consumption, therapy adherence, etc [16, 17]. The therapy success rate most likely depends on genetic barriers within the virus itself on one, and the human host on the other side.

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- Genotype of the virus was determined by Linear Array HCV Genotyping test (Roche Diagnostics Systems, Basel, Switzerland);
- Serum viral load (VL) and active virus replication were determined by real-time PCR on the COBAS® AmpliPrep/COBAS® TaqMan® (Roche Diagnostics Systems, Basel, Switzerland), analytic sensitivity 5x103 to 1x104 copies HCV/ml.

The patients were divided into three groups based on the response to antiviral treatment: 1) patients with SVR - PCR HCV RNA below the level of detection at the end of treatment and 6 months later; 2) non-responders (NR) – patients who didn’t respond to antiviral treatment - PCR HCV RNA positive after 12-week treatment or at the end of the treatment; 3) relapse (RL) patients with the activation of the virus during the period of monitoring - PCR HCV RNA negative at the end of the treatment, but positive at the end of the monitoring period. The influence of social, demographic and clinical characteristics (gender, age, path of infection, duration of infection, fibrosis degree, steatosis, presence of extrahepatic manifestations, repeated treatments, virus genotype and serum VL) on the success of antiviral therapy for CHC was examined.

The data were analyzed by descriptive and inferential statistics using the GraphPad Prism Software. The difference in the frequency of attributive characteristics was tested by $\chi^2$ and differences between the values in these groups by unpaired t-test. The $p < 0.05$ was taken as statistically significant. The results were shown in tables and graphs.

**Results**

A total of 292 patients included 190 (65.07%) male and 102 (34.93%) female patients, with a male to female ratio of 1.86 : 1. The patients’ age ranged from 19 to 64 years. The average age of patients was 38.54 years (SD ± 11.30). The average age of males (37.78) was by 2.18 years lower than in females (39.96). The average age of patients was 38.54 years (SD ± 11.30). The average age of males (37.78) was by 2.18 years lower than in females (39.96). The SVR was achieved in 71.05% (135/190) of males and 68.63% (70/102) of females (Table 1). Gender did not show to be a statistically significant predictive factor for reaching SVR ($\chi^2$ test, $p = 0.665$).

In the group of patients over 40 years of age (average age 49.95) SVR was achieved in 44.85% (61/136) whereas in the group of patients younger than 40 years (average age 29.69) SVR was achieved in 92.31% (144/156). These results showed a high statistical significance of SVR at younger age ($\chi^2$ test, $p = 0.0001$).

The most frequent paths of infection were intravenous drug addiction (52.05%) and tattoo and piercing (19.86%). The path of infection could not be determined in 12.33% of patients (Graph 1).

The HCV genotype 1 was found in 183 patients (62.67%), genotype 2 in 98 (33.56%), genotype 3 in 10 (3.43%), and genotype 4 only in one patient (0.34%). The SVR was achieved in 61.20% (112/183) of patients with genotype 1; 85.71% (84/98) of patients with genotype 3; 80% (8/10) of patients with genotype 2, and in one patient (1/1) with genotype 4. The SVR was statistically significantly higher in patients with HCV genotype 3 compared with patients with genotype 1 ($\chi^2$ test, $p < 0.0001$).

Before the beginning of treatment, normal levels of ALT were found in 0.34% (17/292) of patients. The rest of patients had elevated ALT levels, from 1 to 10 times (average 2.86) higher comparing to the upper reference limit. The ALT activity was not considered as a factor that influenced the treatment response.

The assessment of the stage of hepatic fibrosis in patients after LB showed that there were 31 patients (10.62%) without liver fibrosis (F0), 114 patients (39.04%) with mild fibrosis (F1), 79 patients (27.05 %) with moderate fibrosis (F2), 46 patients (15.75%) with severe fibrosis (F3), and 22 patients (7.54%) with liver cirrhosis (F4). There were 224 (76.71%) patients with F0 - F2 fibrosis. The SVR was achieved in 75.45% (169/224) of patients in the group F0 - F2 and in 52.94% (36/68) in the group F3 - F4 (Graph 2). Therefore, the stage of liver fibrosis showed to be a significant predictive factor for achieving SVR ($\chi^2$ test, $p = 0.0004$).

Steatosis was found in 18.15% (53/292) of patients with CHC. In this group of patients, the SVR was achieved in 52.83% (28/53). In the group of patients without steatosis, the SVR was achieved in 74.06% (177/239). This difference (18.15% vs. 74.06%) was highly statistically significant ($\chi^2$ test, $p = 0.0022$).

Among patients with HCV genotype 1, the SVR was achieved in 40% (14/35) of patients with steatosis, compared to 66.22% (98/148) of those without steatosis. Steatosis showed to have a statistically significant (Fisher’s test, $p = 0.0013$) impact on the treatment outcome in genotype 1. Among patients with HCV genotype 3, the SVR was achieved in 76.47% (13/17) of patients with steatosis, compared to 87.65% (71/81) of those without steatosis. In HCV genotype 3, steatosis showed to be statistically insignificant (Fisher’s test, $p = 0.2164$) regarding the treatment outcome.

Extrahepatic manifestations were determined in 43.49% (127/292) of patients. Out of them, 63.78% (81/127) achieved SVR. Among 56.51% (165/292)
The serum VL in patients with CHC ranged from 11421 to 11110000 IU/ml, average 6297698.09 (SD ± 10.41). The average duration of HCV infection was 14.47 years (SD ± 10.16) in patients with SVR and 18.57 (SD ± 8.87) in patients who had poor treatment outcomes. The average duration of HCV infection before treatment ranged from to 2 to 50 years, 16.38 years on average (SD ± 10.41). The average duration of HCV infection was 14.47 years (SD ± 10.16) in patients with SVR and 18.57 (SD ± 8.87) in patients who had poor treatment outcomes. The difference between these two groups was statistically significant in regard to the treatment outcome (unpaired t-test, p = 0.0012).

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The serum VL in patients with CHC ranged from 11421 to 11110000 IU/ml, average 6297698.09 (SD ± 11781304.79). The influence of VL on the treatment outcome in patients with CHC was statistically insignificant (unpaired t-test, p = 0.3034).

### Discussion

The study shows that CHC affects males more than females, that gender does not affect the response to PegIFN-Alfa2/Rbv therapy, as well as the serum VL. The study has also shown that there was no difference in the treatment response between patients being treated for hepatitis C for the first time or have previously been treated. It was found that intravenous drug use was the most frequent (52.05%) transmission path. Statistically significantly higher SVR was found in younger patients, patients without extrahepatic manifestations, as well as in patients with shorter duration of untreated illness. Findings from this study suggest that early stages of liver fibrosis were associated with a greater chance for SVR. Genotype 1 has been identified as a predictor of unfavorable treatment response, and genotype 3 as a positive predictor of SVR to PegIFN-Alfa2/Rbv therapy in CHC patients. Absence of liver steatosis in patients with SVR suggests that it may play an important role in determining response rates to PegIFN-Alfa2/Rbv treatment.

In 2016, WHO defined the latest recommendations about the importance of treating CHC, treatment protocols, and desired effects of the therapy [10]. In settings where DAAs are not available, the combination of PegIFN-Alfa2/Rbv remains acceptable. However, a significant number of patients who did not achieve SVR nor had side effects show that the research of predictive factors (by the virus and a host) of the treatment outcome in CHC patients is still a subject of interest. In 2012, Nathan Ford and his associates conducted a meta-analysis to assess treatment success in less developed and middle developed countries. Their study showed that SVR was achieved in 52 % of patients and that in patients infected by HCV genotype 1, after 48 weeks of treatment with PegIFN-Alfa2/Rbv, SVR ranged from 45–48% [18]. The results of our study showed that SVR was achieved in 70% of patients and in 61% of patients infected with HCV genotype 1.

The male to female ratio in our sample of patients suffering from CHC showed male predominance. However, there were no significant differences between
male and female participants in regard to the therapeutic response. The results of the study are in accordance with previous studies about gender as a possible predictive factor for treatment success with PegIFN-Alfa2/Rbv [6, 16].

The researches show increasing incidence of HCV infection in patients aged 18 – 30 years, because they visit doctors more often [19]. The reported data are in accordance with the fact that 53.42% of patients included in our study were younger than 40 years of age. Our study found that the average age of patients was 38.54 years, as well as that the younger patients achieved SVR statistically significantly more often. A previous study reported that patients infected at an older age (>40 years) could have faster progression of liver disease [4]. Unfavorable treatment outcomes of older patients with CHC can be explained by a greater prevalence of comorbid states at old age, as well as higher cumulative exposition to hepatotoxins from the surroundings during their lives [20].

The Annual Epidemiological Report for 2016 of the European Center for Disease Prevention and Control, suggested that in Europe hepatitis C is more frequent in males (male to female ratio: 1.8 : 1). The HCV seroprevalence was found to be 51.3% in 25 and 44 years olds, 8.0% in patients under the age of 25 years, and 40.7% in patients over 45 years [21]. In patients with CHC in the Republic of Serbia age distribution corresponds to that in Southern Europe with two peaks. The first peak occurs in patients over 55 years of age (HCV transmission through blood transfusion, before introducing a regular screening of blood donors) and the second peak occurs in patients over 35 years of age (infected by contaminated set for intravenous drug use) [22, 23]. In our study, the average age of patients was 38.54 years. The research confirmed the Public Service Announcement that the intravenous addiction was the most frequent path of transmission (52.05%).

In a study including almost 440 patients treated with PegIFN-Alfa2/Rbv, Delić and colleagues demonstrated SVR in 70.5% of patients [24]. The effect of the combined treatment was much better in patients under the age of 40 years, without liver cirrhosis, not affected by HCV genotype 1, and if the recommended regimen of antiviral therapy for CHC was respected.

Mild increases in ALT levels (30 - 100 IU/l) are commonly found and could be associated with viral hepatitis [25]. Our study showed that 99.66% of patients had increased ALT levels, by one to ten times than the upper normal limit before therapy. Although the ALT level before therapy was not considered as a factor that affects the treatment response, the study draws attention to the need for anti-HCV testing of all patients with increased ALT levels.

The fibrosis may progress or regress during the treatment with PegIFN-alpha2/Rbv. In patients who did not achieve SVR, regression of fibrosis can be mostly explained by the action of interferon. Progression of fibrosis can be explained as a consequence of homogenization quasi species under pressure of immunological system and selective overgrowing of aggressive virus alternatives [26, 27]. The present study has found fibrosis stage F0 - F2 in 76.71% of patients with hepatitis C, and SVR was achieved in 52.94%. It has statistically been confirmed that patients with lower fibrosis progression had a bigger chance of achieving SVR, and fibrosis was found to be a significant predictive factor for treatment response in patients with hepatitis C.

Steatosis is more frequently associated with obesity, diabetes mellitus, CHC, metabolic syndrome. In their research, Preveden and colleagues found steatosis in 35% of patients with CHC and statistically confirmed its negative effects to the efficiency of antivirus therapy in patients with hepatitis C genotype 3 [28]. Our findings showed steatosis in 18% of patients with CHC and statistically confirmed significant absence of steatosis in patients with hepatitis C genotype 1 with SVR.

Adaptation of HCV for replication outside hepatocytes causes proliferation of B lymphocytes and appearance of extrahepatic manifestations (EHMs). EHMs are frequent and polymorphous [29]. The study focuses on four extrahepatic manifestations (arthritis, diabetes mellitus, thyreoiditis, and presence of organically nonspecific auto antibodies). The presence of EHMs was found in 43% of patients in the study. Although 60% of patients with SVR did not have EHMs, effects of EHMs on the treatment response were not statistically confirmed.

The study included 85.27% naïve patients (who received PegIFN-Alfa2/Rbv for the first time). The SVR of 72.29% was achieved in this group of patients. In the group of previously treated patients, SVR of 58.14% was achieved. The difference in the treatment response was not statistically confirmed so the data of this study are consistent with the published data [13, 14].

In our study, HCV genotype 1 (62.67%) and HCV genotype 3 (33.56%) were most frequent. Similar findings of wide spreading of HCV genotype 1 and 3 were observed in a study conducted in Europe [2]. The results of the study also confirmed significant influence of HCV genotype on treatment response: SVR was achieved in 61.20% of patients with genotype 1 and in 85.71% of patients with genotype 3. The SVR achieved in this study (70.20%) is consistent with publish data [18, 24, 27].

Our research showed that patients with shorter duration of infection before therapy aimed towards SVR, whereas those with longer infection duration aimed towards unfavorable treatment response. Other research studies reported that the duration of infection before treatment represents a significant predictive factor of treatment response [5, 6]. Nevertheless, our study did not confirm that the serum VL before therapy significantly affected the treatment response.

**Conclusion**

The knowledge about demographic and clinical characteristics of patients which may have major effects on treatment outcomes is useful for researchers.
References


