



## Autoimmune manifestation of hepatitis C virus infection as a risk for late virological relapse after pegylated interferon and ribavirin therapy

Autoimunska manifestacija infekcije virusom hepatitisa C kao rizik od kasnog virusološkog relapsa posle terapije pegilovanim interferonom i ribavirinom

Maja Ružić\*<sup>†</sup>, Milotka Fabri\*<sup>†</sup>, Tomislav Preveden\*<sup>†</sup>, Katarina Bačulov<sup>‡</sup>,  
Maria Pete\*<sup>†</sup>, Anja Stojšin\*<sup>†</sup>

Clinical Center of Vojvodina, \*Clinic for Infectious Diseases, <sup>‡</sup>Center for Laboratory  
Medicine, Novi Sad, Serbia; University of Novi Sad, <sup>†</sup>Faculty of Medicine,  
Novi Sad, Serbia

### Abstract

**Introduction.** We are aware of the risk of late virological relapse (LVR) years after sustained viral response (SVR) by pegylated interferon and ribavirin alfa (PegIFN $\alpha$  + RBV) of chronic hepatitis C viral (HCV) infection. We presented three patients with LVR, treated by PegIFN $\alpha$  and ribavirin 5 years after the SVR was established. **Case report.** We analysed 129 (38.8% female, 61.2% male, mean age 37.02  $\pm$  SD 11.99) patients treated for chronic HCV with PegIFN $\alpha$  + RBV, with at least 5 years from the establishment of SVR. In addition to the biochemical parameters of liver function, the qualitative HCV RNA polymerase chain reaction (PCR) and the quantitative PCR HCV RNA test were made. Five years after establishing SVR in 2.3% (3/129) of patients, the relapse of HCV infection was registered by qualitative and quantitative PCR HCV RNA assay and all of these patients had additional autoimmune diseases: vasculitis, autoimmune hepatitis, and vasculitis of central nervous system. **Conclusion.** The existence, but low rate of LVR HCV infection was confirmed, dominantly in patients with additional autoimmune diseases. Due to this SVR after therapy by PegIFN $\alpha$  + RBV should be considered as an indicator of successful HCV suppression, not its complete eradication.

### Key words:

hepatitis c; interferon alfa-2b; ribavirin; treatment outcome; recurrence; autoimmune diseases.

### Apstrakt

**Uvod.** Iskustvo u lečenju hepatitis C virus (HCV) infekcije pegilovanim interferonom alfa (PegIFN $\alpha$ ) i ribavirinom (RBV) ukazuje na postojanje rizika od kasnog virusološkog relapsa“ (*late virological response* – LVR) – ponovna detekcija HCV ribonukleinske kiseline (RNA) u serumu godinama nakon uspostavljanja stabilnog virusološkog odgovora – *sustained virological response* (SVR). Prikazana su tri bolesnika sa LVR lečena PegIFN $\alpha$  i ribavirinom pet godina nakon uspostavljanja SVR. **Prikaz bolesnika.** Analizirano je ukupno 129 bolesnika (38,8% ženskog, 61,2% muškog pola, prosečna starost 37,02  $\pm$  11,99 godina) lečenih od hroničnog HCV PegIFN $\alpha$  + RBV, kod kojih je prošlo najmanje pet godina od uspostavljanja SVR. Pored biohemijskih parametara funkcije jetre, rađen je kvalitativni lančane reakcije polimeraze (PCR) HCV RNA test, odnosno kvantitativni PCR HCV RNA test. Pet godina od uspostavljanja SVR kod tri (2,3%) bolesnika je kvalitativnim i kvantitativnim testom PCR HCV RNA utvrđen relaps HCV infekcije. Sva tri bolesnika imala su i pridružene autoimunske bolesti: vaskulitis, autoimunski hepatitis i vaskulitis centralnog nervnog sistema. **Zaključak.** Potvrđeno je postojanja LVR HCV infekcije prvenstveno kod bolesnika sa pridruženim autoimunskim bolestima. Stoga SVR nakon terapije PegIFN $\alpha$  + RBV treba shvatiti kao pokazatelja uspešne HCV supresije, a ne potpune eradikacije HCV.

### Ključne reči:

hepatitis c; interferon alfa-2b; ribavirin; lečenje, ishod; recidiv; autoimunske bolesti.

## Introduction

Hepatitis C viral (HCV) infection is the most common cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC) and the leading cause of liver transplantation worldwide<sup>1,2</sup>. Therapy with pegylated interferon alfa 2a/2b (PegIFN $\alpha$ ) and ribavirin was successful in 50% to 80% of the patients who achieved sustained viral response (SVR), and more than 98% of them were considered cured<sup>3</sup>. However, over a longer follow-up period, the occurrence of a „late virological relapse“ (LVR), redetection of HCV ribonucleic acid (RNA) in the serum years after achievement of SVR was recorded<sup>4</sup>. Little is known about the diagnostic criteria, LVR incidence and risk factors of its occurrence.

We presented three patients with LVR, treated with PegIFN $\alpha$  and ribavirin 5 years after the SVR was established.

## Case report

We analyzed 129 patients with chronic hepatitis C (CHC) with established SVR within the period of 5 years who had been treated by PegIFN $\alpha$ -2a and ribavirin during 2014 at the Clinic for Infectious Diseases of the Clinical Center of Vojvodina. The patients underwent a qualitative HCV RNA polymerase chain reaction (PCR) test, COBAS AMPLICOR Hepatitis C test, version 2.0 (Roche Diagnostics systems, Basel, Switzerland). In the patients who obtained indeterminate or positive results, transitional viremia was excluded by the quantitative PCR HCV RNA test [COBAS AmpliPrep/COBAS TaqMantest (Roche Diagnostics systems, Basel, Switzerland)], where the values of the detected flickers were expressed in the International Units/mL (U/mL), with lower limit of detection of 15 U/mL. Gene sequencing of 5' NTR type-specific PCR or commercial kits (InnoLipa, Innogenetics, Genotyping Linear Array hepatitis C virus test, Roche Diagnostics) were performed for HCV genotyping.

Out of the 129 patients, 50 (38.8%) were females and 79 (61.2%) were males. At the initiation of the therapy, the

mean age was 37.02 years [19–66 years; standard deviation (SD) 11.99]. Average estimated duration of the HCV infection was 10 years (95% CI 10.00-14.00). Mean activity of alanine aminotransferase (ALT) before beginning of the therapy amounted to 98.00 U/mL (95% CI 80.00 to 122.00) (normal range 7–56 U/L). Before the treatment, a majority of the patients 100/129 (77.5%), had a high viral load detected (> 400,000 U/mL) and the average value was 1,142,100.00 (95% CI; 999,973.09 to 1,485,084.68 U/mL). Characteristics of CHC are shown in Table 1.

Out of 129 patients included in the study, 5 (3.9%) were treated with recombinant interferon alpha 2/2b (IFN $\alpha$ 2a/2b) before the combined therapy with PegIFN $\alpha$  2a and ribavirin was applied. During the treatment, the dose of PegIFN $\alpha$  was corrected in 29 (22.5%) patients (mostly due to neutropenia, or thrombocytopenia), and the dose of ribavirin was corrected in 19 (14.7%) patients (most commonly for anemia). Other side effects of the treatment were noted in 21 (16.3%) patients. Five years after SVR, in 3 (2.3%) patients relapse of the HCV infection was suspected with the qualitative HCV RNA PCR assay, and then confirmed by the quantitative PCR HCV RNA test. Clinical characteristics of CHC, a course of the treatment and the disease course in these 3 patients are given in Table 2.

**Table 1**

**Characteristics of chronic hepatitis C (CHC) in the patients treated with pegylated interferon alfa 2a/2b (PegIFN $\alpha$ ) and ribavirin who achieved sustained viral response (SVR)**

Characteristics of CHC	n	%
HCV Genotype 1	82	63.5
HCV Genotype non 1	47	36.5
Without fibrosis (METAVIR 0)	21	16.3
Fibrosis light to moderate (METAVIR 1 and 2)	85	65.9
Severe fibrosis/cirrhosis (METAVIR 3 and 4)	12	9.3
Steatosis	21	16.3

HCV – hepatitis C virus.

**Table 2**

**Comparison of three patients with detected late relapse of hepatitis C virus (HCV) infection**

Variable	Patient No. 1	Patient No. 2	Patient No. 3
Female	yes	yes	no
Age > 60 years	no	no	no
BMI > 30 kg/m <sup>2</sup>	no	yes	no
HCV Genotype 1	yes	yes	yes
VL > 400,000 U/mL	yes	no	yes
Severe liver fibrosis (3 and 4)	yes	yes	yes
PegIFN 80%	yes	no	yes
RBV 80%	no	no	yes
Previously treated	no	no	yes

BMI – body mass index; VL – viral load of HCV in the serum;

PegIFN 80% – cumulative dose of pegylated interferon;

(Peg IFN) not lower than 80% of the prescribed dose; RBV 80% – cumulative dose of ribavirin not lower than 80% of the prescribed dose.

### Patient 1

A 55-year-old female patient suffered from vasculitis and was in hemodialysis (HD) program for over 3 decades. Six years after achieving SVR with 48 weeks PegIFN $\alpha$  monotherapy of the HCV infection (fibrosis 4, HCV genotype 1), a discrete increase of ALT was detected. The qualitative HCV RNA PCR test was positive, and the quantitative PCR HCV RNA resulted in 280,000 U/mL, genotype 1. Clinical reevaluation did not report any progression of liver cirrhosis. From the moment SVR was achieved, the patient was included in the HD program. Bearing in mind the excellent epidemiological situation in the HD centre, the HCV reinfection was unlikely.

### Patient 2

A 57-year-old female patient suffered from "overlap" autoimmune hepatitis (AIH)/HCV syndrome [fibrosis 2, with "interface" hepatitis, detected antinuclear antibodies (ANA) 1: 320 and antimitochondrial antibodies (AMA) 1: 320 in serum, HCV genotype 1]. After initial treatment with azathioprine and methylprednisolone, SVR was achieved on 48 week of PegIFN $\alpha$ -2a and ribavirin therapy. Even though the qualitative HCV RNA PCR was repeated annually due to continually increased ALT activity, the HCV viremia was confirmed after 5 years by the quantitative HCV RNA (82,000 U/mL, genotype 1). Clinical reevaluation of liver disease showed progression to liver cirrhosis (CP B). The risk for the HCV reinfection from the moment of achieving SVR to the moment of the HCV infection relapse was not known.

### Patient 3

A 44-year-old male patient achieved SVR after 48 weeks of PegIFN $\alpha$ -2a and ribavirin therapy of CHC (fibrosis 3, HCV genotype 1). During the follow-up period, the increased activity of aminotransferases was detected, however, the quantitative HCV RNA test was repeated several times and was negative. Six years after achieving SVR, transverse myelitis was diagnosed. Reevaluation of the liver diseases determined progression to cirrhosis (A CP score, liver fibroelastography 16.4 kPa) and the HCV relapse was confirmed by the quantitative HCV RNA (VL 1,289,000 IU/mL, genotype 1). From the moment of achieving SVR to the moment of HCV infection relapse, the risk factors for HCV reinfection were not found.

## Discussion

According to the study of Swain et al.<sup>4</sup>, Manns et al.<sup>5</sup> and Formann et al.<sup>6</sup>, the LVR rate ranges from 1% to 8%, in the follow-up period of 1 to 12 years after achieving SVR. In our research, the LVR HCV infection was rare; it was identified in 2.3% (3/129) of patients treated and followed for more than 5 years.

It was proved that in spite of SVR, in up to 6% followed-up patients, it was possible to detect HCV RNA in the

liver tissue, lymphocytes and monocyte/macrophage cells<sup>7-10</sup>. Those findings led to the new clinical aspects of the HCV infection – secondary occult HCV infection (OHC)<sup>11,12</sup>. In the liver tissue, mild to moderate disease activity was proved, including the presence of lymphocytic infiltration, hepatocyte necrosis and fibrosis of different degree<sup>13</sup>. OHC may be the explanation of LVR HCV, whereas SVR should be seen as successful HCV suppression rather than complete eradication of HCV after combined therapy with PegIFN and RBV<sup>14</sup>. To cure the patient means to achieve complete eradication of the virus, without presence of HCV RNA in the serum and liver tissue as well as having load of antibodies to the nucleus of HCV (anti-HCV core) reduced<sup>15</sup>.

Some studies showed that predisposition for the HCV relapse is related to female gender, older age, high body mass index (BMI), high viral load before treatment, HCV genotype 1, HCV molecular characteristics (especially HCV core region), genetic predisposition of patients (IL28), previous therapy with IFN and also cumulative dose of administered drugs, ribavirin, as most important<sup>16-18</sup>. Despite the statistical irrelevance, due to a small number of patients in addition to CHC, all three patients had some autoimmune disease in common – the patient No. 1 vasculitis, the patient No. 2 autoimmune hepatitis and the patient No. 3 central nervous system (CNS) vasculitis (Table 2). Cytotoxic T lymphocytes (CTLs) are held as the most responsible for the HCV replication control<sup>19</sup>. In addition to the inevitable antiviral effect, the IFN-based therapy has immunomodulatory effects aimed at recovery and reconstitution of T cell activity<sup>20</sup>. If this effect is achieved with elimination of HCV and if the potency of CTLs is sustained after the end of the therapy, the ability of HCV reactivation is excluded<sup>21</sup>. Patients with late virological response detected in this study had severe, recurrent and fulminant forms of autoimmune disease at times that required concomitant immunosuppressive therapy (corticosteroids in all three cases) and doubtlessly had compromised functional capacities of both humoral and cellular immunity. Although it is clear that our patients had secondary OHC<sup>9</sup>, in the second and third case, on several occasions the tested HCV viremia was negative, but with present progression of liver disease. This is consistent with the findings of Radkowski et al.<sup>10</sup> and Pham and Michalak<sup>11</sup> regarding presence of active liver disease in intrahepatic HCV RNA detection, besides achieved SVR.

However, there is a possibility of the re-HCV infection in the presented patients, especially in the patient No. 1. who was on chronic HD. Owing to the stricter blood bank screening rules, widespread use of erythropoiesis-stimulating agents instead of blood transfusions and stronger adherence to infection control practices in dialysis units, there was a reduction in the prevalence of the HCV infection in the HD patient group<sup>22</sup>. The percentage of the anti HCV positive patients dropped from 23.2% in 1999 to 12.7% in 2009 in Serbia<sup>22</sup>. In this particular HD Center our patient was the only anti-HCV positive and during 10 years of follow-up, there was no new HCV infection among the patients on HD. On account of this positive epidemiological situation, we excluded the possibility of the HCV re-infection in the patient

No. 1. Unfortunately, serum samples of the patients before treatment PegIFN were not available and we could not exclude the re-HCV infection by the molecular analysis of HCV sequences<sup>21–23</sup>.

### Conclusion

Even SVR achieved on IFN based therapy of CHC protocol cannot be considered as an absolute cure, but the rate of

its reliability is high. One should be aware of the possibility of late relaps and therefore it is necessary to monitor the patients actively after establishing SVR, especially those with autoimmune disorders within the HCV infection.

The existence of a low rate LVR HCV infection was confirmed predominantly in the patients with additional autoimmune diseases.

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