HAEMATOLOGICAL ADVERSE EFFECTS OF INTERFERON AND RIBAVIRIN TREATMENT IN PATIENTS WITH CHRONIC C VIRUS HEPATITIS

CITTO IULIAN TAISESCU1*, GABRIELA ILIESCU2*, OANA TAISESCU3*, ANCA PREDESCU4*, ANA MARINA ANDREI5*, BOGDAN CĂTĂLIN1*, CRISTIAN SILOȘI6*, VIOREL BICIUȘCĂ7*

1Department of Physiology, University of Medicine and Pharmacy, 2 Petru Rareș, 200349, Craiova, Romania
2Clinical Laboratory, County Clinical Emergency Hospital Craiova. I Tabaci, 200642, Craiova, Romania
3Department of Anatomy, University of Medicine and Pharmacy, 2 Petru Rareș, 200349, Craiova, Romania
4Clinical Laboratory, Clinical Hospital of Neuropsychiatry Craiova, 99 Calea București, 200473, Craiova, Romania
5Department of Physiology, University of Medicine and Pharmacy, 2 Petru Rareș, 200349, Craiova, Romania
6Department of Surgery, University of Medicine and Pharmacy, 2 Petru Rareș, 200349, Craiova, Romania
7Department of Semiology, University of Medicine and Pharmacy, 2 Petru Rareș, 200349, Craiova, Romania

*corresponding author: taisescu@yahoo.com

All authors have contributed equally in preparing this manuscript and thus share first authorship.

Manuscript received: November 2014

Abstract

Haematological abnormalities were frequently found in chronic C hepatitis virus infection, but especially as complications of interferon-based antiviral therapy. The study of hematologic adverse reactions was initiated on a group of 30 patients with chronic hepatitis C, hospitalized in the IInd Clinic Medical Emergency Hospital Craiova, monitored and treated with peginterferon alfa-2b (PEG-IFN), 1.5 µg/kg/wk plus ribavirin (RBV) 1000-1200 mg/day for 12 months, within four years. The haemoglobin level decreased significantly when combining IFN with RBV. RBV induces anaemia, and in addition IFN inhibits the activity of hematopoietic bone marrow. Erythrocyte morphology provided information on viral activity, modifying with liver lesion progression and bone marrow infection, and the low number of reticulocytes confirmed both the toxic mechanism of IFN and a low regenerating bone marrow capacity.

Keywords: haematological abnormalities, interferon, ribavirin, C virus infection

Introduction

According to the World Health Organization (WHO), 3% of world population is infected with hepatitis C virus (HCV) and the number of new cases continues to rise, more than 80% of cases leading to chronic hepatitis, liver cirrhosis and hepatocellular carcinoma [1,2]. All studies from recent years show that adherence to treatment is a decisive factor in achieving a sustained virological response [3]. Monitoring haematological adverse reactions is a major goal of therapy with interferon (IFN) and ribavirin (RBV), as these changes may provide data on the mechanisms responsible for the dysfunction of hematopoietic organs, allowing anticipation and correction [4]. This paper reports the haematological adverse reactions occurring in patients undergoing treatment with IFN and RBV for chronic HCV.

Materials and Methods

The study was initiated on a group of 30 patients with chronic HCV hospitalized in the IInd Medical Clinic, Emergency County Hospital Craiova, monitored and treated with peginterferon alfa-2b (PEG-IFN), 1.5 µg /kg/wk plus ribavirin (RBV) 1000-1200 mg/day for 12 months, within four years. The study was performed with the
approval of the Ethics Committee of the University of Medicine and Pharmacy of Craiova and a written informed consent was obtained from each patient included in the study. Once the diagnosis of HCV established, for the initiation of antiviral therapy we selected patients with: anti-HCV present; detectable levels of viremia; histological lesions of moderate or severe chronic active hepatitis (histological activity index (HAI) > 6 Ishak score); aged 18-70 years; without serious underlying conditions (heart failure, pre-existing psychiatric disorders, epilepsy, haemoglobinopathy, anaemia, haemophilia, type I diabetes, difficult to control autoimmune diseases).

From the monitoring plan we selected and retained for this study the haematological module: complete blood count (complete number of elements, morphological examination of blood smear, reticulocyte count), myelogram (sternal puncture with morphological examination of smears after May-Grünwald Giemsa-MGG staining) and erythrocyte osmotic resistance test. The analysers used for evaluation were Nikon Kohden Celltac, Coulter Ac-T diff and Abacus Junior.

To perform statistical analysis we used methods that allowed us to draw conclusions regarding the differences and/or joint statistically significant values. The data were correlated with additional data (anamnesis, serological and histological markers) obtained from patient records or observation sheets, were included into in a Microsoft Office Excel database and processed for the statistical analysis.

Results and Discussion

Demographic variables
Of the 30 patients studied, 17 (56.6%) were females and 13 (43.3%) were males. The mean age of patients was 45.97 ± 10.96 years, with limits between 21 and 66 years. Only 7 patients (25%) were aged less than 40 years. Most patients (15 patients, 53.57%) were aged between 41 and 50 years, men being the majority in this age group, while women were the majority in the age group 51-60 years.

Haematological findings
Considering that the main objective of getting antiviral treatment is obtaining sustained virological response (SVR), we performed a statistical study of haematological parameters in patients that showed SVR at end of treatment compared with patients without SVR. Analysis of these parameters showed no statistically significant changes between responders and non-responders (Table I, Figure 1).

Although anaemia was observed from the first month of treatment, the mean values of reticulocytes have not increased accordingly, showing the inhibitory effect of IFN on bone marrow.

Evolution of the average values of haemoglobin during antiviral therapy, depending on the type of therapeutic response

<table>
<thead>
<tr>
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<th>M3</th>
<th>M6</th>
<th>M12</th>
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<tr>
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<td>14.95</td>
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<td>11.90</td>
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<td></td>
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<td>1.34</td>
<td>1.28</td>
<td>1.12</td>
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<td>Average</td>
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<td>12.00</td>
<td>12.20</td>
<td>11.70</td>
<td>11.60</td>
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<td></td>
<td>Std. Dev</td>
<td>1.76</td>
<td>1.52</td>
<td>1.61</td>
<td>1.15</td>
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<td>1.15</td>
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*Legend: M (0-18) – month

Figure 1.
Variation of mean haemoglobin level in patients, depending on the type of therapeutic response to interferon and ribavirin
The lowest average values of reticulocytes were recorded in the third month of treatment, both in responders and in non-responders. The average values of reticulocytes increased significantly only at the end of treatment proving the regenerative capacity of the bone marrow (Table II, Figure 2).

During treatment, the mean corpuscular volume (MCV) increased steadily in all groups until the sixth month of treatment, after which followed a slightly downward curve, but all over the upper limit of normal (ULN). Return to the normal value of MCV was observed at 6 months after the discontinuation of treatment.

### Table II

<table>
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<th>M0*</th>
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<tr>
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<tr>
<td>Average</td>
<td>9.66</td>
<td>9.09</td>
<td>1.88</td>
<td>2.08</td>
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</table>

*Legend: M0 - month

![Figure 2. Variation of average values of reticulocytes in patients treated with interferon and ribavirin](image)

No statistically significant changes of MCV were observed between responders and non-responders (Table III, Figure 3). These results were correlated with macrocytosis observed on smears examined microscopically. Macrocytosis is often accompanied by higher values of bilirubin and, especially thrombocytopenia in 50% of patients with HCV.

### Table III

<table>
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<tr>
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<td>15</td>
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<td>Average</td>
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<td>85.68</td>
<td>85.89</td>
<td>97.11</td>
<td>106.08</td>
<td>90.41</td>
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<td>Std. dev</td>
<td>11.86</td>
<td>11.94</td>
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<td>12.75</td>
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<td>12.35</td>
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<td><strong>Non-responders</strong></td>
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<td>86.58</td>
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<td>96.92</td>
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<tr>
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<td>12.46</td>
<td>12.39</td>
<td>12.61</td>
<td>12.97</td>
<td>12.81</td>
<td>12.10</td>
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</table>

*Legend: M (0-18) - month

![Figure 3. Variation of MCV average values during antiviral therapy](image)
In most patients, the same morphological changes were found during the treatment period when it was performed either the analysis of capillary blood smears or of the venous blood, with a similar pattern regardless the type of treatment administered: anisocytosis, with macrocytosis or microcytosis and poikilocytosis (Figure 4 and 5).

Figure 4.
Peripheral blood smear before the beginning of the treatment (a, MGG staining, x400); Normal appearance after three months of treatment (b, MGG staining, x400)

Figure 5.
Peripheral blood smear - anaemia, leukopenia, normal platelets (a, MGG staining, x400); Changes in erythrocyte morphology in the sixth month, leukopenia; thrombocytopenia (b, MGG staining, x1000)

Morphological examination of bone marrow
We have found various degrees of cellular hypoplasia after treatment with IFN and RBV (Figure 6), the growth of the fibrous component in 40% of patients, to which has been added the increase of fat component in elderly patients.

Figure 6.
Smear bone marrow. (a) Appearance of bone marrow before treatment (M0) (MGG staining, x400); (b) Hypocellular bone marrow in the sixth month of treatment (M6) (MGG staining, x1000)

Haemoglobin decreased significantly during the treatment with combined IFN and RBV, reaching the lowest average values at the end of treatment. RBV induced anaemia, and in addition IFN inhibited the activity of hematopoietic bone marrow. Anaemia was not accompanied by compensatory reticulocytosis, which confirmed the inhibition of their production in the bone marrow [3]. In patients with chronic hepatitis, and in particular during antiviral therapy, examination of blood smears showed changes in erythrocyte morphology, shape and/or volume, even in the absence of anaemia [5]. Over 90% of patients taking antiviral therapy showed different levels of erythrocyte morphological changes. Thus erythrocyte morphology provides information on viral activity, modifying with liver lesion progression, and bone marrow infection.

Microcytosis accompanying pancytopenia is due in 25% of cases of inefficient haematopoiesis, while in 75% of the cases is due to bone marrow hypoplasia. [6]. Macrocytosis is produced by folic acid and/or vitamin B12 metabolism disorders. Macrocytosis, observed on smear and confirmed by MCV, occurs in 70% of patients with chronic viral hepatitis and was exacerbated by IFN [7]; there were recorded higher mean values of MCV in the sixth month of treatment and the return to normal occurred only in six months after the treatment discontinuation. Macrocytosis appeared both in cases with normoblastic marrow and those with megaloblastic type or bone marrow hypoplasia [8, 9]. Another mechanism involved in producing morphological changes of erythrocytes is hypersplenism which occurs during the evolution of chronic C virus hepatitis [10].

In most cases of patients receiving any formulation of IFN we observed a decrease in bone marrow cellularity with a high percentage of fibrous structures [11]; these aspects are normalized by the end of the treatment, especially in young patients, in which the bone has a high regeneration ability as opposed to the elderly [12]. Some authors [13] considered that macrocytosis is a bad prognostic factor for development of chronic liver disease to cirrhosis. Anaemia affects the tolerability and efficiency, is associated with fatigue and a poor quality of life, as shown in other studies [14].

Conclusions
The most important haematological adverse effect of IFN and RBV treatment was the appearance of anaemia, evidenced from the first month of therapy. Information about viral activity is provided by the morphology of erythrocytes which are changed due to liver lesion progression and bone marrow infection. The low number of reticulocytes
confirmed the toxic effect of IFN, joined by the decreased regeneration capacity of the bone marrow. The presence of anaemia with a 20% decrease of haemoglobin required the reduction of RBV dose, because we considered important to maintain the quality of patient’s life.

References