coRibavirin-induced Anemia in Patients with Chronic Hepatitis C Virus Infection

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Anemia associated with ribavirin (RBV) and interferon therapy in patients with chronic viral hepatitis C is a hemolytic, multifactorial adverse effect, in direct correlation with the RBV dose. Additionally, the anemic syndrome is worsened by the interferon-induced bone marrow suppression of erythroid precursors. The resulting anemia is associated with low production of erythropoietin, similar to anemia seen in HIV-infected patients or those with cancer. Our study was performed on a group of 28 patients with viral hepatitis C treated with 3 MU interferon alfa 3 times a week and RBV 1000-1200 mg/day for 12 months. A normochromic normocytic anemia was observed, requiring a reduction of the RBV dose to 600 mg/day if the hemoglobin was below 10 g/dL and interrupted if the hemoglobin level was below 8.5 g/dL. In this case, reversibility can occur within a time interval of 4-8 weeks after discontinuation of treatment.

Keywords: ribavirin, chronic viral hepatitis C, anemia

Cytopenia is one of the most common and severe side effects of antiviral therapy [1]. Also, HCV infection can induce hemorrhagic anemia, leucopenia and thrombocytopenia. It is a well known fact that HCV persists and actively replicates in lymphocytes, leading to extrahepatic dissemination of the virus, thus persisting in the salivary glands, kidneys and bone marrow; subsequently, these areas become secondary sources of viral reinfection and contribute to the inhibition of the body’s immune response [2].

Both interferon (PEG-IFN) and RBV can cause important noticeable hematologic alterations which can trigger or even worsen a precarious hematologic status, compromising the patients adherence to therapy [3]. Patients with chronic viral hepatitis C treated with IFN / PEG-IFN and RBV have multiple types of anemia: hemorrhagic anemia, nutritional deficiency anemia, simple chronic anemia or anemia resulting from bleeding, thus defining the concept of mixed anemia [4]. However, RBV is responsible for producing the strongest effect, in comparison to interferon-mediated bone marrow inhibition. RBV causes dose-dependent haemolytic anemia which is reversible after 4-8 weeks of discontinuous administration of the drug [5]. Hemolytic anemia is the major complication of RBV treatment resulting from the accumulation of RBV triphosphate in the red blood cells, which interferes with cellular functions [6-8].

RBV causes varying degrees of hemolysis in most patients, although dose reduction is only required in 7 to 9% of patients undergoing standard antiviral therapy. Therefore, a recorded medical history of hemolytic anemia, with Hgb <11 g/dL and Ht <33%, are considered exclusion criteria for RBV administration [9].

Experimental part
Material and method

The study was performed on a group of 28 patients with viral hepatitis C admitted to the Second Medical Clinic of the Emergency County Hospital of Craiova who received 3 MU of interferon alfa 3 times a week and RBV 1000-1200 mg/day for 12 months.

Patients were selected for treatment initiation in accordance with the following criteria: anti-HCV antibodies were present; detectable levels of viremia; histological lesions of chronic moderate or severe chronic hepatitis (HAI index> 6, Ishak score); ages 18-70; without any associated serious conditions (heart failure, preexisting psychiatric conditions, epilepsy, hemoglobinopathy, anemia, haemophilia, difficult to manage type I diabetes, autoimmune diseases); normal and slightly altered hematological and biochemical values: Hb>13g% for men and Hb>12g% for women; leukocyte count> 4000 / mmc; granulocyte count>1500/mmmc; platelet count>100,000/ mmc; creatinine<1.2 mg%; bilirubin<2.5 mg%; albumin>3 g%; patients who were tested negative for pregnancy or patients who had the possibility of effective contraception during treatment.

Considering the absolute contraindications of antiviral medication, patients with the following illnesses were excluded from the beginning of our study: clinical signs of chronic decompensated liver disease (edema, jaundice, ascites, chronic renal failure); HCV-related cirrhosis; HCV related malignancies; ages 18-70; without any associated serious conditions (heart failure, preexisting psychiatric conditions, epilepsy, hemoglobinopathy, anemia, haemophilia, difficult to manage type I diabetes, autoimmune diseases); normal and slightly altered hematological and biochemical values: Hb>13g% for men and Hb>12g% for women; leukocyte count> 4000 / mmc; granulocyte count>1500/mmmc; platelet count>100,000/ mmc; creatinine<1.2 mg%; bilirubin<2.5 mg%; albumin>3 g%; patients who were tested negative for pregnancy or patients who had the possibility of effective contraception during treatment.

For this study, we selected and retained the blood panel during routine check-ups: full blood count (complete counts, morphological examination of the blood smear, reticulocyte count), myelogram (sternal puncture with morphological examination of the smears) and the red blood cells osmotic fragility test. Analysers with 18 parameters were used: Celltac Nihon Kohden, Coulter AcT diff, Abacus Junior.

We used statistical methods for data analysis which allowed us to draw conclusions about the statistical significance and/or associations. We used the Excel and
SPSS programs to perform statistical processing. The data obtained was correlated with additional data (anamnestic, serological and histological) from the patient records or the observation sheets. This information was entered into a Microsoft Office Excel database and processed statistically.

We performed a statistical study of haematological parameters in patients who showed a sustained virological response (SVR) at the end of treatment compared to non-SVR patients. The study was conducted with the approval of the Ethics Committee of the University of Medicine and Pharmacy of Craiova. A written consent was obtained from each patient included in the study.

We evaluated and compared the safety of the therapeutic agents included in the therapeutic protocols by performing the appropriate analysis sets and the subsequent statistical analysis the main haematological parameters involved in the antiviral treatment management.

These results were correlated with sustained virological response, which evaluates the effectiveness of the treatment.

Results and discussions

The patients in this group were equally represented by males and females (14:14), aged 23-69 years, with a mean age of 50.48 ± 10.96 years. Analyzing the distribution of the patients by age group, we noticed that the majority of patients were over 40 years old (15 patients, 53.5%).

The proportion of men was higher in the 31-40 years (B: F = 6: 4) and the 61-70 years age group (6: 0), while women had a larger presence in the 41-50 (B: F = 3: 6) and 51-60 years (2: 8) age group.

Hemoglobin

Hb values, prior to initiation of therapy, were between 11.90 and 16.80 g/dL, with an average value of 14.82 ± 1.21 g/dL (fig. 1).

At the end of the 12 months of treatment, Hb values ranged between 9.80 and 14.20 g/dL, with an average of 11.19 ± 1.49 g/dL (table 1, fig. 2).

Over 95% of patients experienced a decrease in hemoglobin levels by at least 1 g/dL; while 6% of the men and 8% of the women experienced a 5 g/dL or larger decrease in Hb levels, suggesting that women are significantly more susceptible to reducing RBV doses.

The maximum decrease in Hb values is noted progressively during treatment, with the highest decrease being recorded during the last month (table 3).

Patients with higher hemoglobin values before treatment initiation showed greater decreases in hemoglobin levels compared to those with lower baseline values (fig. 3).

A drop in Hb levels under 10 g/dL, which required an interferon and/or RBV dose reduction, occurred in 4 out of the 28 patients (14.28%) of the group.

Reticulocyte count

The number of reticulocytes before treatment was between 2.6 and 18‰, with an average of 9.73 ± 2.77‰.

Three months after initiation of therapy, reticulocyte counts ranged from 2.4 to 17‰ with an average of 2.31 ± 1.26‰. At the end of the treatment period, the number of reticulocytes was between 1.6 and 14.5‰, with an average value of 7.06‰ (table 4, fig. 4).

| Table 1 | THE MONTLY EVOLUTION OF THE MEAN Hb VALUES |
|---------|-------------------------------------------|
| Responders | Number of patients | M0 | M1 | M2 | M3 | M6 | M12 | M18 |
| Avg | 14.35 | 12.74 | 12.39 | 12.05 | 11.98 | 11.45 | 14.19 |
| Std.Dev. | 1.28 | 1.18 | 1.56 | 1.81 | 1.68 | 1.65 | 1.26 |
| Nonresponders | Number of patients | 15 | 15 | 10 | 15 | 10 | 15 | 15 |
| Avg | 14.30 | 12.60 | 12.00 | 12.02 | 11.94 | 10.94 | 14.00 |
| Std.Dev. | 1.14 | 1.06 | 1.79 | 2.10 | 1.34 | 1.34 | 1.21 |

| Table 2 | Hb LEVELS FOR MEN AND WOMEN |
|---------|------------------------------|
| Minimum | Group | Group |
| Hb levels | Men n (%) | Women n (%) |
| g/dL | | |
| < 10.0 | 1 (3.5) | 3 (10.7) |
| 10.0-11.0 | 2 (7.1) | 4 (14.2) |
| 11.0-12.0 | 4 (14.2) | 4 (14.2) |
| 12.0-13.0 | 3 (10.7) | 2 (7.1) |
| > 13.0 | 4 (14.2) | 1 (3.5) |
Mean corpuscular haemoglobin (MCH)
During treatment, MCH mean values increased steadily in all groups until the sixth month of treatment followed by a slightly downward curve; these results correlated with the observed macrocytosis on microscopically examined smears (table 5, fig. 5).

Morphological examination of the blood smear
On smears prepared from capillary or venous blood, we found the same erythrocytic morphological changes in most patients, with a similar evolution, regardless of the type of treatment administered: anisocytosis, either with macrocytosis or with microcitosis; poikilocytosis (ovalocytes), microsferocytosis - in patients with RBV-induced hemolytic anemia (fig. 6).
Morphological examination of the bone marrow

90% of patients accepted the sternal puncture performed for haematological pretreatment evaluation. Subsequently, a second sternal puncture was performed randomly in a small number of patients, either in M3 or M6 or M12; we found different degrees of cellular hypoplasia, with increased fibrous component in 40% of patients, and increased fat content in elderly patients [10].

RBV causes different degrees of hemolysis in almost all patients. Therefore, patients with pre-existing haemolysis or anemia (Hb <10g /dL) should not be treated with RBV [11]. Similarly, patients with significant coronary or cerebrovascular disease should not be treated with RBV due to the fact that treatment-induced anemia can induce ischaemia. Acute myocardial infarction and fatal stroke have been reported in patients receiving PEG-IFN and RBV [12].

RBV is eliminated from the body by the kidneys. Patients with kidney disease can experience severe, life-threatening haemolysis. Patients with creatinine levels higher than 2 mg / dL (which qualifies as renal dysfunction) should not be treated with RBV.

Over 95% of patients presented decreased Hb levels, by at least 1g / dL; 6% men and 8% women presented a drop in Hb levels by at least 5 g/dL, suggesting that women are significantly more susceptible to reductions in RBV doses as recommended in current guidelines for the management of treatment-induced anemia.

Baseline values of Hb are reattained within 4 to 8 weeks after discontinuation. Reticulocytosis follows the same timeline, but in reverse.

Upon completion of treatment, Hb values return to normal at varying intervals, but values at the end of the monitoring period are below pretreatment values. Patients with higher Hb values before the treatment showed greater decreases in Hb values in comparison to those with lower baseline Hb. The maximum decrease in Hb values is noted progressively during treatment, with the highest decrease in the last month.

Reticulocytosis occurs within the first 4 weeks of treatment. This complication is directly related to the pharmacological properties of the molecule and, in particular, its accumulation in erythrocytes. The occurrence of haemolytic anemia is very common.

The aspect of the bone marrow returned to normal 3 months after treatment in the majority of patients who accepted sternular puncture, with the exception of 2 female patients, 60 years of age, which presented a cellular hypoplastic appearance and predominance of fibrous and fatty components and in which the peripheral blood cell count remained at the lower limits of normal and at the end of the surveillance period.

It should also taken into account that lowering the dose of RBV affects the survival rates, so early steps to correct anemia are required to maintain the recommended doses. In addition to RBV induced anemia, IFN inhibits bone
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Conclusions

Anemia affects the tolerability and efficacy of HCV
treatment being associated with asthenia and decreased
quality of life, as shown by other studies [6, 17]. It would be
preferable for clinicians to consider both relative and
absolute decreases in hemoglobin levels as part of adverse
reactions such as asthenia, dyspnoea, etc [18].

The main adverse effect of RBV was a regenerative-
type normocytic normochromic anemia. The levels of
reticulocytosis encountered in our study are directly related
to the pharmacological properties of the RBV molecule
and, in particular, its accumulation in erythrocytes. The
RBV dose should be reduced to 600 mg / day if the
hemoglobin is below 10 g/dL and discontinued when the
hemoglobin levels fall below 8.5 g/dL. Blood panels
performed at 2, 4, 6, 8 weeks of treatment followed by 4
week intervals have the effect of detecting significant
decreases in hemoglobin and adjusting the doses of
antiviral agents for an optimum treatment regimen.
Baseline values are reobtained within 4 to 8 weeks of
treatment discontinuation.

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