

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

CITTO IULIAN TAISESCU^{1#*}, GABRIELA ILIESCU^{2#}, OANA TAISESCU^{3#}, ANCA PREDESCU^{4#}, ANA MARINA ANDREI^{5#}, BOGDAN CĂTĂLIN^{1#}, CRISTIAN SILOȘI^{6#}, VIOREL BICIUȘCĂ^{7#}

¹Department of Physiology, University of Medicine and Pharmacy, 2 Petru Rareș, 200349, Craiova, Romania

²Clinical Laboratory, County Clinical Emergency Hospital Craiova, 1 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 Semiology, 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/kgbw/week 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.

Rezumat

Anomaliile hematologice apar ca urmare a infecției cronice cu virusul hepatitei C, dar mai ales ca și complicații ale terapiei antivirale bazate pe interferon (IFN). Studiul reacțiilor adverse hematologice a fost efectuat pe un lot de 30 de pacienți cu hepatită cronică virală C internă, monitorizați și tratați cu peginterferon alfa-2b (PEG-IFN) 1,5 µg/kgc/săptămână plus ribavirină (RBV) 1000-1200 mg/zi, timp de 12 luni, în Clinica a II-a Medicală a Spitalului Clinic de Urgență Craiova, într-un interval de patru ani. Hemoglobina a scăzut semnificativ în cazul tratamentului asociat IFN cu RBV, atingând cele mai scăzute valori medii către sfârșitul tratamentului. Pe lângă anemia indusă de RBV, IFN inhibă activitatea hematopoietică a măduvei osoase. Morfologia eritocitară a furnizat informații asupra activității virale, modificându-se odată cu progresia leziunii hepatice dar și cu infecția măduvei osoase, de asemenea, numărul scăzut de reticulocite confirmă efectul toxic al interferonului și, totodată, capacitatea scăzută de regenerare medulară.

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 /kgbw/week 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.

Table I

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

		M0*	M1	M2	M3	M6	M12	M18
Responders	No. Pac	18	18	15	18	18	18	18
	Average	14.95	12.10	12.45	11.90	11.90	11.40	14.20
	Std. Dev	1.71	1.26	1.34	1.28	1.12	1.10	3.21
Non-responders	No. Pac	12	12	10	12	10	12	12
	Average	14.85	12.00	12.20	11.70	11.60	11.00	14.00
	Std. Dev	1.76	1.52	1.61	1.15	1.20	1.15	1.07

*Legend: M (0-18) – month

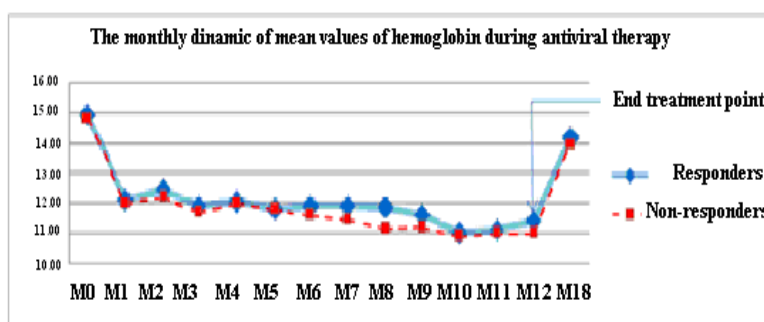


Figure 1.

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).

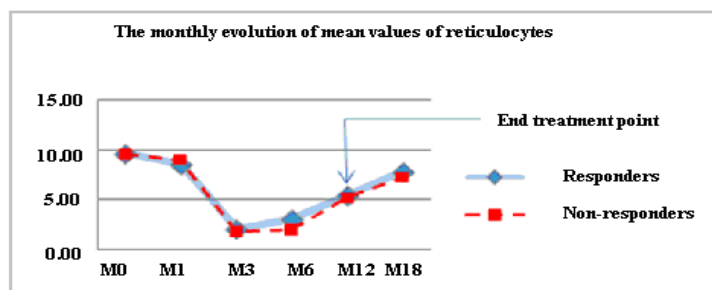
During treatment, the mean corpuscular volume (MCV) increased steadily in all groups until the sixth month of treatment, after which followed a slightly downward curve, but all over the upper limit of normal (ULN). Return to the normal value of MCV was observed at 6 months after the discontinuation of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

Table II

Monthly evolution of the average values of reticulocytes

		M0*	M1	M3	M6	M12	M18
Responders	No. Pac	18	18	18	18	18	18
	Average	9.63	8.53	2.02	3.04	5.41	7.79
	Std. Dev	2.13	1.88	0.57	0.86	0.91	1.26
Non-responders	No. Pac	12	12	12	10	12	12
	Average	9.66	9.09	1.88	2.08	5.27	7.32
	Std. Dev	1.38	1.12	0.44	0.65	0.89	1.03

*Legend: M0 - month

**Figure 2.**

Variation of average values of reticulocytes in patients treated with interferon and ribavirin

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

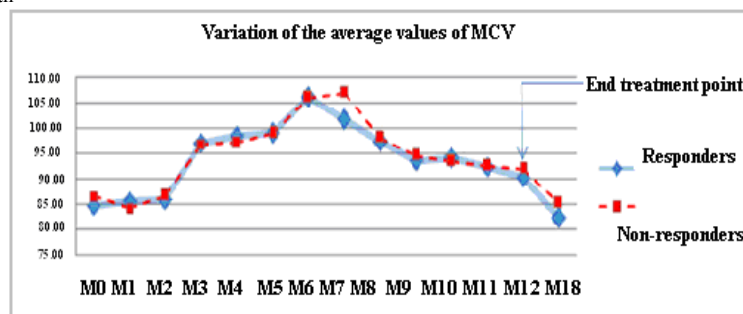
microscopically. Macrocytosis is often accompanied by higher values of bilirubin and, especially thrombocytopenia in 50% of patients with HCV.

Table III

Evolution of MCV average values during treatment

		M0*	M1	M2	M3	M6	M12	M18
Responders	No. Pac	18	18	15	18	18	18	18
	Average	84.69	85.68	85.89	97.11	106.08	90.41	82.26
	Std. dev	11.86	11.94	12.01	12.75	12.71	12.35	12.06
Non-responders	No. Pac	12	12	10	12	10	12	12
	Average	86.58	84.19	86.75	96.92	106.14	92.0	85.31
	Std. dev	12.19	12.46	12.39	12.61	12.97	12.81	12.10

*Legend: M (0-18) - month

**Figure 3.**

Variation of MCV average values during antiviral therapy

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).

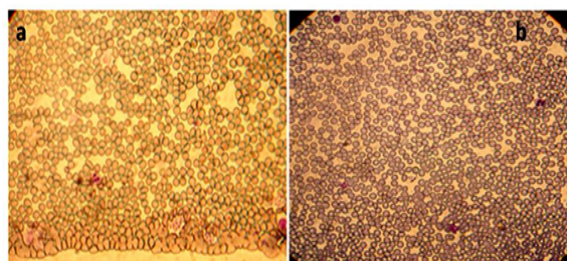


Figure 4.

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

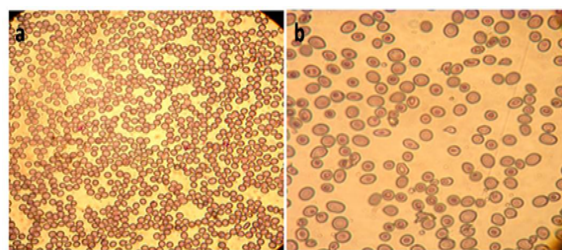


Figure 5.

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.

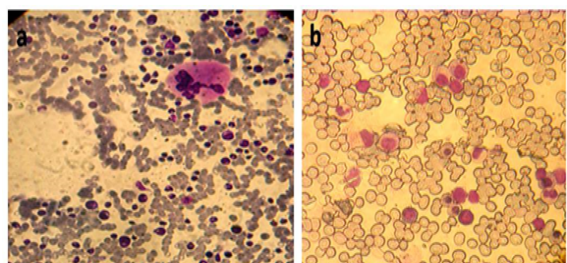


Figure 6.

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

1. Strader D.B., Seeff L.B., Hepatitis C: a brief clinical overview. *ILAR J.*, 2001; 42: 107-116.
2. Stănculescu C., Hâncu M., Pisoschi C., Baniță M., Fusaru A.M., Comănescu V., Correlation between NOS2 expression and apoptosis in chronic hepatitis C - An immunohistochemical study. *Annals of RSCB*, 2010; XV(1): 136-141.
3. Manns M.P., McHutchison J.G., Gordon S.C., Rustgi V.K., Shiffman M., Reindollar R., Goodman Z.D., Koury K., Ling M., Albrecht J.K., Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. *Lancet*, 2001; 358: 958-965.
4. Hadziyannis S.J., Sette H.Jr., Morgan T.R., Balan V., Diago M., Marcellin P., Ramadori G., Bodenheimer H.Jr., Bernstein D., Rizzetto M., Zeuzem S., Pockros P.J., Lin A., Ackrill A.M., PEGASYS International Study Group.. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann. Intern. Med.*, 2004; 140(5): 346-355.
5. Streiff M.B., Mehta S., Thomas D.L., Peripheral blood count abnormalities among patients with hepatitis C in the United States. *Hepatology*, 2002; 35: 947-952.
6. Tanriverdi O., Ger E., Uyunoglu S., Serez M.K., Ure U.B., Ergen K., Kumbasar A.B., Determination of the Role of Mean Corpuscular Volume Level on the Diagnosis of Alcoholic Liver Cirrhosis and Investigation of Its Effect on the Prognosis. *Firat Tıp Dergisi*, 2008; 13(1): 49-52.
7. Vashakidze E., Kiladze I., Gegeshidze T., The syndrome of hemopoetic organs damage in patients with chronic viral hepatitis. *Georgian Medical News*, 2005; 202(119): 49-51.
8. Rusu E., Jinga M., Rusu F., Ciurtin C., Enache G., Dragomir A., Cristescu V., Stoica V., Costache A., Cheta D., Radulian G., Statin therapy in patients with diabetes and hepatitis C. *Farmacia*, 2013; 61(6): 1204-1215.
9. Bold A., Iliescu G., Mateescu G., Biciușcă V., Interferon effect about morphological red blood cells in chronic hepatitis C. Abstract, 23rd Meeting of Adriatic Society of Pathology, 27-29 June 200, Dubrovnik, Croatia.
10. Herbert P.C., Van Der Linden P., Biro G., Hu L.Q., Physiologic aspects of anemia. *Crit. Care Clin.*, 2004; 20: 187-212.
11. Thomas H., Foster G., Platis D., Mechanisms of action of interferon and nucleoside analogues. *J. Hepatol.*, 2003; 39(1): S93-98.
12. Abou El Azam A.R., El-Bate H., Abo-Ali L., Mansour N., Ghoraba H., Salem M.L., Correlation of viral load with bone marrow and hematological changes in pale patients with chronic hepatitis C virus. *Archiv. Virol.*, 2012; 157(8): 1579-1586.
13. Borawska M.H., Markiewicz-Żukowska R., Sawicka D., Naliwajko S.K., Socha K., Omeljaniuk W., Car H., Effects of chrysin on haematological parameters in rats. *Farmacia*, 2014; 62(2): 390-399.
14. Azzam H., Ahmed F., Peterson B., Rovner D., Taylor N., Jacobson I.M., Delayed recovery of pegylated interferon and ribavirin induced anemia. *Hepatology*, 2003; 38(4 Suppl1): 751a.