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EPOETIN ALPHA IMPROVES THE RESPONSE TO THE ANTIVIRAL TREATMENT IN HCV-RELATED CHRONIC HEPATITIS

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For Peer Review

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5 RELATED CHRONIC HEPATITIS
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8 Running head: Improvement of virological response
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ABSTRACT

Background. The conventional antiviral treatment of chronic hepatitis related to hepatitis C virus often leads to anaemia. In this case it is necessary to reduce ribavirin dose or stop treatment, thus reducing the rate of sustained virological response.

Aim. To investigate whether epoetin alpha administration improves treatment adherence and leads to higher percentage of response at the end of therapy and sustained virological response.

Methods. 214 subjects with genotype 1b HCV-related chronic hepatitis underwent treatment with PEG-interferon alpha-2A 180 micrograms once weekly and ribavirin 1000–1200 mg/day; 174 were responders: 40 subjects completed treatment with no haemoglobin reduction; 134 developed anaemia during therapy. Anaemic responders were distributed randomly into two groups: group 1 continued therapy with epoetin alpha addition; group 2 continued antiviral therapy with ribavirin reduction only.

Results. The group 1 achieved better control of haemoglobin levels (13.8 ± 1.2 g/dl at the end of therapy) than the group 2 (11.5 ± 0.8 g/dl). Sustained virological response was 59.7% in group 1, compared with 34.4% observed in the group 2 ($p < 0.01$).

Conclusions. In patients with 1b HCV-related chronic hepatitis, who develop anaemia during the antiviral treatment, the administration of Epoetin Alpha increases haemoglobin levels and the rate of end-of-treatment and sustained virological response by improving treatment adherence.

KEY WORDS

Epoetin Alpha, PEG-interferon alpha-2A, Ribavirin, Genotype-1b HCV-related Chronic Hepatitis.

INTRODUCTION

Hepatitis C virus (HCV) is a significant public health problem. As a hepatotropic virus, it could lead to chronic hepatitis, cirrhosis and hepatocellular carcinoma [1]. It can also cause several extra hepatic diseases, such as mixed cryoglobulinaemia and lymphoma, on account of its “lymphotropic pattern” [2,3].

According to current guidelines, the standard treatment of HCV-related chronic hepatitis (CHC) is based on the combination of peg-interferon (peg-IFN) plus ribavirin (RBV) [4–8]. Such combination therapy may induce a normalization of transaminase levels, a sustained virological response (SVR) and histological hepatic improvement in

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3 about 65% of subjects infected with HCV genotype 1 and in about 80% of those infected with genotype 2 or 3 HCV
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5 [1,9,10].

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7 Beyond well-known predictive factors for an optimal therapeutic response (age <45 years old, duration of disease <5
8
9 years, low levels of HCV RNA, genotype 2 or 3 HCV infection, lack of fibrosis and intra hepatic iron upon hepatic
10
11 biopsy), the adherence to standard peg-IFN and RBV therapy is necessary because it is associated with higher rates of
12
13 therapeutic success [11,12]. However, several factors contribute to imperfect adherence to therapy or, in some cases, to
14
15 its interruption: therapy-induced reduction in haemoglobin levels is the most important cause of drop out during
16
17 treatment with peg-IFN and RBV [13–20]. As a result of this, RBV dose reduction is required, with a corresponding
18
19 decrease in the chance of an SVR [21].

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21 It is well known that RBV monotherapy lowers haemoglobin levels by a haemolytic mechanism, while the inhibition
22
23 of erythropoietin production during combination therapy with peg-IFN and RBV can induce anaemia [22–24].
24
25 Therefore, Epoetin Alpha may become a pivotal therapy in patients who develop anaemia during treatment with peg-
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27 IFN and RBV. In fact, patients can continue treatment with a standard optimal dose of peg-IFN and RBV and achieve
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29 positive therapeutic results with the persistence of high haemoglobin levels. This aspect plays an especially important
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31 role in patients with advanced hepatic disease, because of the possibility of achieving stabilization of hepatic disease,
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33 in terms of clinical, biochemical and histological features, with a consequent improvement in quality of life (QOL) and
34
35 an increased chance of survival [25–31].

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37 We investigated a group of patients with genotype 1b HCV-related chronic hepatitis, who were treated with PEG-
38
39 interferon alpha-2A and RBV and consequently developed anaemia. We aimed to establish if treatment with Epoetin
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41 Alpha increased the number of patients who continued therapy with the standard dose of peg-IFN and RBV and
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43 increased the number of patients who achieve a sustained virological response (SVR).
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49 METHODS

50 Study design.

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52 The study was a longitudinal prospective randomized open-label controlled study performed in cooperation with three
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54 Hepatology Units. Epoetin Alpha was administered according to Italian Agency of Drugs (A.I.F.A, Agenzia Italiana
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56 del Farmaco) indications as expressed in Law n° 648/96. All patients provided their informed consent prior to their
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58 inclusion in the study.
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Selection of Patients

Criteria for admission to the therapy protocol with peg-IFN plus RBV were [5,6]: age 18 years or older, elevated serum ALT levels on the past 6 months, anti HCV antibodies positivity, detectable HCV RNA, HCV Genotype 1b, liver histology of chronic hepatitis (Ishak score overall ≤ 13), baseline HB >13 (males) and >12 (females), serum creatinin ≤ 1.5 mg/dL, Homeostatic Model Assessment index (HOMA-IR) < 2.5 .

Exclusion criteria applied were: HBV infection, HBV-HCV co-infection, HIV infection, HCV genotype other than 1b, overall Ishak score ≥ 13 , decompensated cirrhosis, significant atherosclerotic heart disease (defined as instrumental and clinical features of coronary heart disease and chronic heart failure); starting Hb <13 (males) and <12 (females); alcohol or drug abuse, history of haematological disorders or neoplastic diseases, pregnancy, psychiatric disorders, or autoimmune diseases, Wilson's disease and haemochromatosis.

All patients underwent HBsAg, HCV-RNA and HCV genotype determination. Anti-HCV antibodies were determined by ELISA (Ortho Diagnostic Systems, Raritan, NJ, USA) [32]. Pre-treatment HCV-RNA levels were detected by polymerase chain reaction (PCR) of HCV-RNA 5' UTR using COBAS® AmpliPrep/COBAS® TaqMan® HCV Test (Roche Diagnostics Systems, Branchburg, N.J.; analytic sensitivity = 50 IU/mL) [33]. HCV genotypes were determined by INNO-LiPA (Innogenetics) assay, using Simmond's classification [34,35]. Anti-nuclear (ANA), anti-mitochondrial (AMA), anti-smooth muscle (SMA), anti-liver/kidney microsome type 1 (LKM1) autoantibodies were measured using immunofluorescence assay (IFA) and semi-quantitative ELISA immobilizing enzyme test [36]. Thyroid function was evaluated by levels of TSH, free T3 and free T4, that were determined by immunoradiometric assay (IRMA); antibodies against thyroid peroxidase and thyroglobulin were measured by IFA [37].

Patients underwent ultrasound-assisted percutaneous biopsy, obtained with Menghini modified needles (Automatic Aspiration Needle for Liver Biopsy, ACR 16G, 11 cm, manufactured by Sterylab Srl, Milan-Italy) within 6 months before the start of the study. Histological evaluation of the degree of necroinflammatory activity (grading) and fibrosis (staging) of hepatic tissue was carried out using the Ishak scoring system. Semi-quantitative evaluation of the degree and distribution of hepatic iron storage were assessed according to the classification system of Brissot et al, and intra hepatic iron was assessed using Perl's Prussian blue staining [38,39]. Serum levels of iron, saturated transferrin, total transferrin (total iron binding capacity, TIBC), unsaturated transferrin (unsaturated iron binding capacity, UIBC) and ferritin were determined, and saturation of transferrin was calculated as: serum level of iron/TIBC $\times 100$.

Genetic testing to identify haemochromatosis HFE gene mutations was performed in order to exclude subjects with hereditary haemochromatosis [40].

On the basis of clinical, blood chemistry, virological and histological data, 214 consecutive Caucasian subjects [108 male and 106 female, mean age 48 years old (range 37–52), mean body weight 68 kg (range 53–91)] with genotype 1b

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3 HCV-related chronic hepatitis, mean Ishak overall score 10.1 ± 1.9 were enrolled and eligible to receive antiviral
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5 treatment with PEG-interferon alpha-2A 180 micrograms/week + weight based ribavirin (WBR) 1000-1200 mg/day.

6
7 **Baseline characteristic (week 0) are presented in table 1.**
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11
12 Therapeutic protocol and efficacy assessment.

13
14 The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki (6th Revision, 2008).
15
16 According to current guidelines [1] at time 0, all patients started therapy with subcutaneous PEG-interferon alpha-2A,
17
18 180 micrograms once weekly, plus oral WBR in two separate doses (the total dose was 1200 mg daily for patients
19
20 weighing >75 kg, and 1000 mg daily for those weighing <75 kg). Two weeks after the start of therapy and
21
22 subsequently on a monthly basis, patients were evaluated clinically, and serum levels of transaminases, sodium,
23
24 potassium, TSH, free T3, free T4, antibodies against thyroid peroxidase and thyroglobulin, glycaemia, azotaemia,
25
26 creatininaemia, INR determination, haemachrome, and indexes of body iron were assessed.
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29 In compliance with international guidelines and recommendations of the American Association for the Study of Liver
30
31 Disease (AASLD) and the Italian Association for the Study of the Liver (AISF), HCV-RNA levels were measured in
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33 all patients at weeks 12, 24, 48 and 72 [1,6]. Patients with a HCV-RNA reduction of at least 2 log₁₀ at week 12 in
34
35 comparison with the pre-treatment HCV-RNA values were classified as early virological responders (EVR) and
36
37 continued therapy. Patients who did not reach this target were classified as non-responders and stopped therapy.

38
39 Early virological responders who showed a reduction of haemoglobin serum levels >2 g/dl compared with the
40
41 haemoglobin pre-treatment value, were 1:1 randomly distributed into 2 groups by computer-based randomization
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43 software: subjects in groups 1 were admitted to continue PEG-interferon alpha-2A and WBR dosage (1000-1200
44
45 mg/day) plus subcutaneous Epoetin Alpha, 10.000 IU twice weekly; subjects in group 2 were admitted to continue
46
47 PEG-interferon alpha-2A with a weight based reduction of RBV dose (800-1000 mg/day). Administration of Epoetin
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49 Alpha in group 1 followed the acquisition of patients informed consent, as clearly indicated by Italian Law n°
50
51 684/1996 concerning the extension of the *indications-to-use* of epoetins, which must be based on the most recent and
52
53 proved evidences in the scientific literature.

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55 For all patients who underwent antiviral therapy in our Hepatology Unit, QOL was evaluated using the Linear
56
57 Analogue Self Assessment (LASA) scale by both activity and energy score, at the beginning of the study and at weeks
58
59 4, 12, 24, 36 and 48 [31].

60
The primary efficacy endpoint was sustained virologic response (SVR) defined as undetectable serum HCV RNA
levels (<50 IU/mL) 24 weeks after the end of therapy (week 72).

Randomization and Statistical analysis

Randomization process was performed through SAS® Software (Chicago, IL, USA). Results are presented as means \pm standard deviation (SD), range and frequencies. Fisher's Exact test and Mann Whitney U Test for independent samples were applied. Two-tailed P values <0.01 were considered statistically significant. The impact of baseline factors and exposure to ribavirin were evaluated by multiple logistic regression (MLR) analyses. A predictive model was developed for primary endpoint (SVR). Baseline factors considered for inclusion in the models were: age, body weight, body mass index, HOMA-IR, haemoglobin and HCV RNA level at week 0 and week 12 as continuous variables; gender and histological stage as categorical variables. Drug exposure was converted to the percentage of optimal dose and was included in the models as a continuous variable.

RESULTS

All patients were negative for ANA, AMA, SMA and LKM1 autoantibodies. Thyroid function, as evaluated by levels of TSH, free T3 and free T4, was also normal. Autoantibodies against thyroid peroxidase and thyroglobulin were absent at the beginning of therapy. However, in 8 patients, they were positive during treatment, and decreased progressively after the end of antiviral therapy, except in one patient in group who continued to have mild thyroiditis. Prior to treatment, all patients had a mean haemoglobin level of 13.8 ± 0.9 g/dl. Four weeks after the start of therapy, we noted a reduction in haemoglobin and consequent asthenia, insomnia and loss of concentration.

At week 12, no significant statistical difference was found between the three groups, concerning the following parameters: total bilirubin, platelets count, haemoglobin, ferritin and albumin serum levels. At week 12, 174/214 patients (81.3%) achieved an EVR, while 40/214 (18.7%) dropped out because they were non-responders, and stopped therapy. Forty out of 174 EVR patients (23%) did not show decrease of haemoglobin serum levels > 2 g/dL during the study period and fully completed the treatment protocol (control group). Reduction of haemoglobin levels > 2 g/dL (mean value: 11.4 ± 1.3 g/dl) compared with the pre-treatment values has been achieved in 134/174 (77%) EVR patients. They also had worsening treatment compliance, such that about 60% of responders would have preferred to stop therapy. They were randomly distributed into 2 groups: 67 subjects in group 1 received subcutaneous Epoetin Alpha, 10,000 IU twice weekly, in addition to PEG-interferon alpha-2A 180 micrograms/week + weight based ribavirin (WBR) 1000-1200 mg/day; 67 subjects in group 2 received PEG-interferon alpha-2A 180 micrograms/week and reduced doses of RBV (800-1000 mg/day).

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3 The 2 groups of study, as well as the control group, were followed up monthly during the 48 weeks of treatment up to
4 six months after treatment interruption. In group 1 42/67 subjects (62.7%) showed undetectable HCV-RNA serum
5 levels at week 48 and they were classified as ETR (end of treatment responders); 25/67 (37.3%) showed HCV-RNA
6 levels at week 48 and they were classified as ETR (end of treatment responders); 25/67 (37.3%) showed HCV-RNA
7 serum levels still detectable. In group 2 ETR subjects were 35/67 (52.5%); subjects with detectable HCV-RNA serum
8 levels were 32/67 (47.8%).

9
10
11 Six months after the end of treatment (week 72), Sustained Virological Response (SVR) was assessed. Forty subjects
12 out of 67 in group 1 (59.7%) achieved a SVR; 2/67 subjects (3%) had a relapse of disease during follow up, detected
13 through HCV-RNA assay and elevated transaminase levels. In group 2, 23/67 subjects (34.4%) achieved a SVR; 12/67
14 subjects had a relapse (17.9%). In control group 26/40 (65%) were ETR and 24/40 (60%) achieved a SVR; 2/40 (5%)
15 were relapsers (Figure 1).
16
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19 During the course of treatment, no one of the patients showed Hb serum levels <10 g/dl. However, in 19/67 (28.3%) of
20 the EVR-patients, therapy was suspended for a period of 3 weeks because of worsening asthenia and QOL, and
21 incomplete adherence to therapy.
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24
25 The difference in SVR rate was statistically significant comparing group 2 with group 1 and with control group (p
26 <0.01). Otherwise no statistically significant difference has been found comparing SVR rates between group 1 and
27 control group, as well as no difference has been found in ETR rate between all the groups of study (Table 3).
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30
31 **Patients in group 2, who received a reduction in RBV dosage, did not achieve increase of haemoglobin level and**
32 **showed a SVR rate lower than that of patients in group 1** who maintained the standard dose of PEG-interferon alpha-
33 2A and RBV by the addition of Epoetin Alpha, and showed a significant increase of haemoglobin serum levels (Figure
34 2). Therefore, only in group 1 treated with Epoetin Alpha was the “80/80/80 rule” respected, i.e. administration of at
35 least 80% of the dose of peg-IFN and RBV for at least 80% of the time of treatment [42].
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39 The evaluation of QOL by the LASA scale showed that mean scores of patients treated with Epoetin Alpha (group 1)
40 were significantly superior to those in group 2 concerning tolerability, adherence to treatment, few side effects, and
41 improved QOL. In particular, good results in the changes in activity and energy score were observed in group 1 at 36
42 weeks (energy score change 18 ± 17.3 and activity score change 20 ± 18.5) compared with the results obtained in group 2
43 (energy score change 12.2 ± 21.6 and activity score change 7 ± 18.7) ($P < 0.05$ for energy score change and $P < 0.01$ for
44 activity score change). **In the QOL assessment, rates of asthenia, loss of concentration, and insomnia, were also**
45 **evaluated and a comparison between the three groups was performed, with no statistical difference found between**
46 **group 1 and group 3 [11/67 (16.4%) vs 7/40 (17.5%)]. Rates of asthenia, loss of concentration, and insomnia in group**
47 **2 were significantly higher (65/67; 97.5%) when compared with those in group 1 and group 3 ($p < 0.001$).**
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3 In group 1, serum ferritin decreased from a mean pre-treatment level of 800 ng/ml to 320 ng/ml after treatment (Figure
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5 3). In contrast, in group 2, we observed a slight reduction in mean ferritin level from a pre-treatment value of 790
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7 ng/ml to 580 ng/ml after treatment (Figure 3).

8
9 No patient in group 1 showed adverse effects of Epoetin Alpha administration. No severe psychiatric side-effects, such
10
11 as major depression or psychosis have been reported during the antiviral treatment administration as well as in the
12
13 follow-up. In 13 patients of group 1 such as in 11 subjects of group 2, symptoms of anxiety or mild depression
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15 occurred but they were successfully controlled by Psychological Support Programmes, or administration of
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17 antidepressant drugs.

20 21 MLR Analyses.

22
23 Significant predictors of SVR are presented in table 2. In our regression model, factors significantly associated with
24
25 achievement of SVR were RBV exposure ($p < 0.001$) and Epoetin alpha addition ($p < 0.001$).

28 29 DISCUSSION

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31 Combination treatment with Peg-IFN and RBV represents the gold standard in the treatment of chronic hepatitis C.
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33 During HCV infection, factors associated with a favourable response to peg-IFN and RBV therapy are viral genotypes
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35 2 or 3, low levels of viremia before treatment, young age, absence of obesity and insulin resistance, and a low grade of
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37 fibrosis. In patients with CHC it is extremely important to ensure complete adherence to therapy with peg-IFN and
38
39 RBV in order to achieve therapeutic success (greater percentage of SVR). Indeed, early interruption of therapy or
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41 reduction of peg-IFN or RBV dose because of side effects, leads to a significant decrease in SVR [21, 41– 43].

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43 After 12 weeks of therapy, in patients with an EVR, which is a reduction in HCV RNA of at least 2 log₁₀ in
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45 comparison with baseline, adherence to the “80/80/80 rule” is highly predictive of an SVR. This rule, which means
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47 taking 80% of the dose of peg-IFN and RBV for at least 80% of the duration of therapy, yields a greater SVR [41–45].

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49 Thus, exposure to an optimal treatment regimen, which consists of the full dose of drugs and prolonged treatment, is
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51 important in achieving a successful treatment target, as it results from our logistic regression model for SVR (primary
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53 endpoint). This target was obtained by the addition of epoetin alpha, whereas the development of anaemia leads to
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55 reduce the dose of RBV, as Standard of Care (SOC). In fact, HCV related genotype 1b CH patients who receive 80%
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57 of the full dose of peg-IFN and RBV for 48 weeks show a higher SVR than patients with the same virological features
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59 of hepatic disease who receive a reduced dose of drugs. This is because the maintenance of optimal drug levels allows
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the patient to achieve a strong and complete inhibition of viral replication [45].

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3 During therapy with peg-IFN and RBV, alterations of the haematological profile and, in particular, evidence of
4 neutropenia, thrombocytopenia and anaemia are critical factors that play an important role in the reduction of duration
5 and dose of therapy. Anaemia caused by peg-IFN is additional to that induced by RBV [15]. Therefore, anaemia
6 represents a frequent side effect that may lead to a reduction in exposure time and dose of antiviral drugs, with
7 consequent incomplete control of viral replication and clinical, biochemical and histological progression of hepatic
8 disease [18,20,22,23,46].

9
10 The use of adjuvant agents (erythropoietin and granulocyte colony stimulating factor) to reduce the frequency of dose
11 reductions because of haematological side-effects has been proven to be effective but there are few reports of what
12 effect the use of these adjuvant therapies is having on sustained virological response (SVR).
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15 Our researches showed that patients in group 1, who were treated throughout with the initial dose of RBV, along with
16 Epoetin Alpha after anaemia onset, showed a greater rate of SVR than patients in group 2, who were treated with a
17 reduced dose of RBV, without Epoetin Alpha, after anaemia onset. In particular, the rate of SVR in group 1 was of
18 59.7% compared with 34.4% ($p < 0.01$) achieved in group 2 (Table 3). Group 1 SVR rate was similar to SVR rate in
19 our control group (early responders who experienced no haemoglobin reduction during all therapy and survey period)
20 with no statistical difference.
21

22
23 Our results are in accordance with some recent studies [47–49]. Shiffman et al. observed a lower rate of relapse and an
24 higher rate of SVR in patients treated with a higher starting dose of RBV (15.2 mg/kg/day) plus Epoetin Alpha
25 compared with those treated with a lower dose of RBV (13.3 mg/kg/day) with or without Epoetin Alpha [50].
26 Moreover, in a retrospective observational cohort study of predictors of SVR to peg-IFN and RBV in routine medical
27 practice at 121 Department of Veterans Affairs facilities, Backus et al. identified, by univariate and multivariate
28 analyses, the use of Epoetin Alpha as an independent predictor of SVR [51].
29

30
31 In the Casch WJ et al. paper the aim of the study was to assess the clinical impact on sustained virological response of
32 adjuvant therapies (erythropoietin and granulocyte colony stimulating factor) during combination therapy with Peg-
33 IFN and ribavirin for chronic hepatitis C; the authors have shown that, in CH genotype 1 related, the SVR can be
34 improved with the use of erythropoietins [52].
35

36
37 It may be assumed that there is no cost–benefit relationship for the use of Epoetin Alpha in patients with cirrhosis,
38 because of the high cost of Epoetin Alpha therapy, as with other haematological growth factors. However, some
39 studies have used decision analysis to estimate the cost-effectiveness of adjunctive therapy with erythropoietic growth
40 factor (EGF) compared with that of standard care in the treatment of hepatitis C. The use of EGFs appears to be cost-
41 effective in patients with anaemia who are receiving combination therapy for HCV genotype 1, 2 or 3. Compared with
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3 standard care, adjuvant therapy with EGF, may increase the probability of achieving an SVR, increase unadjusted
4 lifespan, and improve quality-adjusted lifespan at an acceptable cost [52, 53-55].

7 The results of previous studies [27,28,30,37,49,50,52,56–58] and the present study have shown that the introduction of
8
9 Epoetin Alpha therapy in patients treated with PEG-interferon alpha-2A and RBV for CH related to genotype 1b HCV
10 infection leads to an improved adherence to treatment, and, consequently, to a significant improvement of SVR.

13 Moreover, the reduction in ferritinaemia observed in group 1 may have contributed to the better therapeutic response
14 to peg-IFN and RBV treatment. The favourable trend in ferritinaemia in group 1 may have been the consequence of
15 decreased hepatic necroinflammation, secondary to antiviral therapy, as well as the major movement of iron deposits
16 towards the erythropoietic medulla, secondary to Epoetin Alpha administration.

21 In conclusion, our data suggest that in patients with genotype 1b HCV-related CH, who develop anaemia during
22 therapy with PEG-interferon alpha-2A and RBV, the administration of Epoetin Alpha is justified because it ensures
23 normal levels of haemoglobin and increases the SVR rate, assuring exposure to the optimal RBV dosage, improving
24 patient's adherence to treatment and their quality of life. However, more data are necessary to clarify when Epoetin
25 Alpha treatment should be started, the duration of treatment, the optimal dosage, the effect on end-of-treatment and
26 sustained virologic response rates, and its costs.
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42 REFERENCES

- 44 1. Strader DB, Wright T, Thomas DL et al. American Association for the Study of Liver Diseases. Diagnosis,
45 management, and treatment of hepatitis C. *Hepatology*. 2004 Apr;39(4):1147-71.
 - 46 2. Zignego AL, Giannini C, Ferri C. Hepatitis C virus-related lymphoproliferative disorders: an overview. *World J*
47 *Gastroenterol*. 2007 May 7;13(17):2467-78. Review.
 - 48 3. Craxì A, Laffi G, Zignego AL. Hepatitis C virus (HCV) infection: a systemic disease. *Mol Aspects Med*. 2008
49 Feb-Apr;29(1-2):85-95
- 50
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2
3 4. Manns MP, McHutchison JG, Gordon SC et al. Peginterferon alpha-2b plus ribavirin compared with interferon
4 alpha-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001;358:958-956.
5
6
7
- 8
9 5. Fried MW, Shiffman ML, Reddy KR et al. Peginterferon alpha-2a plus ribavirin for chronic hepatitis C virus
10 infection. *N Engl J Med* 2002;347:975-982.
11
12
- 13
14 6. Hadziyannis SJ, Sette HJr, Morgan TR et al. PEGASYS International Study Group. Peginterferon-alpha2a and
15 ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose.
16 *Ann Intern Med* 2004; 140:346-355.
17
18
- 19
20 7. Shiffman ML, Di Bisceglie AM, Lindsay KL et al. Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis
21 Trial Group. Peginterferon alpha-2a and ribavirin in patients with chronic hepatitis C who have failed prior
22 treatment. *Gastroenterology*. 2004 Apr;126(4):1015-23.
23
24
25
26
27
28
- 29
30 8. Leiner S. Peginterferon and ribavirin for hepatitis C. *N Engl J Med*. 2007 Mar22;356(12):1270
31
32
33
- 34
35 9. Hadziyannis SJ, Koskinas JS. Differences in epidemiology, liver disease and treatment response among HCV
36 genotypes. *Hepatology Research*. 2004 Jul;29(3):129-135.
37
38
39
- 40
41 10. Formann E, Jessner W, Bennett L et al. Twice-weekly administration of peginterferon-alpha-2b improves viral
42 kinetics in patients with chronic hepatitis C genotype 1. *Journal of viral hepatitis*. 2003 Jul;10(4):271-6.
43
44
45
- 46
47 11. Saracco G, Rizzetto M. Predictors of response to interferon therapy. *Digestive diseases and sciences*. 1996
48 Dec;41(12 Suppl):115S-120S.
49
50
- 51
52 12. Neuman MG, Benhamou JP, Martinot M., et al. Predictors of sustained response to alpha interferon therapy in
53 chronic hepatitis C. *Clinical biochemistry*. 1999 Oct;32(7):537-45
54
55
56
- 57
58 13. Manns MP. Adherence to combination therapy: influence on sustained virologic response and economic impact.
59 *Gastroenterology clinics of North America*. 2004 Mar;33(1 Suppl):S11-24.
60

- 1
2
3 14. Russo MW, Fried MW. Side effects of therapy for chronic hepatitis C. *Gastroenterology*. 2003 May;124(6):1711-
4
5 9.
6
7
8
9
- 10
11 15. De Franceschi L, Fattovich G, Turrini F et al. Hemolytic anemia induced by ribavirin therapy in patients with
12 chronic hepatitis C virus infection. *Hepatology* 2000;31:997-1004.
13
14
15
- 16
17 16. Rendo P, Rosso A, Gomez F et al. Anaemia in patients with chronic hepatitis C treated with ribavirin and peg-
18 interferon. *Antiviral Therapy* 2000;5(suppl 1):C96).
19
20
21
- 22
23 17. Dieterich DT, Spivak JL. Hematologic disorders associated with hepatitis C virus infection and their management.
24 *Clinical infectious diseases*. 2003 Aug 15;37(4):533-41.
25
26
27
- 28
29 18. Peck-Radosavljevic M, Wichlas M, Homoncik-Kraml M et al. Rapid suppression of haematopoiesis by standard
30 or pegylated interferon alpha. *Gastroenterology* 2002;123:141-151.
31
32
33
- 34
35 19. Golia P, Talal AH, Jacobs IM et al. A preliminary study of growth factor versus dose reduction for peg-interferon
36 alpha-2b and ribavirin associated neutropenia and anemia in HCV infected patients. *Gastroenterology*
37 2003;124:A766.
38
39
40
- 41
42 20. Lindahl K, Schvarcz R, Bruchfeld A et al. Evidence that plasma concentration rather than dose per kilogram body
43 weight predict ribavirin induced anemia. *Journal of viral hepatitis*. 2004;11:84-87.
44
45
46
47
- 48
49 21. Reddy KR, Shiffman ML, Morgan TR et al. Impact of ribavirin dose reductions in hepatitis C virus genotype 1
50 patients completing peginterferon alpha 2a/ribavirin treatment. *Clin Gastroenterol Hepatol* 2007;5:124-129.
51
52
53
- 54
55 22. Ong JP, Younossi ZM. Managing the hematologic side effects of antiviral therapy for chronic hepatitis C: anemia,
56 neutropenia, and thrombocytopenia. *Cleve Clin J Med*. 2004 May;71 Suppl 3:S17-21. Review.
57
58
59
- 60 23. Kowdley KV. Hematologic side effects of interferon and ribavirin therapy. *J Clin Gastroenterol*. 2005 Jan;39(1
Suppl):S3-8. Review.

- 1
2
3
4
5 24. Schmid M, Kreil A, Jessner W et al. Suppression of haematopoiesis during therapy of chronic hepatitis C with
6
7 different interferon alpha mono and combination therapy regimens. *Gut*. 2005 Jul;54(7):1014-20.
8
9
- 10
11 25. Sharvadze L, Tsertsvadze T, Gochitashvili N et al. Ifn/Rbv treatment induced anemia and its correction with
12
13 epoetin alpha in patients with hepatitis C. *Georgian medical news*. 2006 Aug;(137):62-5.
14
15
- 16
17 26. Talal AH, Weisz K, Hau T et al. A preliminary study of erythropoietin for anemia associated with ribavirin and
18
19 interferon-alpha. *The American journal of gastroenterology* 2001 Sep;96(9):2802-4.
20
21
- 22
23 27. Dieterich DT, Wasserman R, Bräu N et al. Once-weekly epoetin alpha improves anemia and facilitates
24
25 maintenance of ribavirin dosing in hepatitis C virus-infected patients receiving ribavirin plus interferon alpha. *The*
26
27 *American journal of gastroenterology*. 2003 Nov;98(11):2491-9.
28
29
- 30
31 28. Bräu N. Epoetin alpha treatment for acute anaemia during interferon plus ribavirin combination therapy for
32
33 chronic hepatitis C. *Journal of viral hepatitis*. 2004 May;11(3):191-7. Review.
34
35
- 36
37 29. Balan V, Schwartz D, Wu GY et al. HCV Natural History Study Group. Erythropoietic response to anemia in
38
39 chronic hepatitis C patients receiving combination pegylated interferon/ribavirin. *The American journal of*
40
41 *gastroenterology* 2005 Feb;100(2):299-307.
42
43
- 44
45 30. Afdhal NH, Dieterich DT, Pockros PJ et al. Proactive Study Group. Epoetin alpha maintains ribavirin dose in
46
47 HCV-infected patients: a prospective, double-blind, randomized controlled study. *Gastroenterology*. 2004
48
49 May;126(5):1302-11.
50
51
- 52
53 31. Pockros PJ, Shiffman ML, Schiff ER et al. PROACTIVE Study Group. Epoetin alpha improves quality of life in
54
55 anemic HCV-infected patients receiving combination therapy. *Hepatology*. 2004 Dec;40(6):1450-8.
56
57
- 58
59 32. Lee S, McHutchinson J, Francis B et al. Improved detection of antibodies to hepatitis C virus using a second
60
61 generation ELISA. *Adv Exp Med Biol*. 1992;312:183-9.

- 1
2
3 33. Kawai S, Yokosuka O, Imazeki F et al. Evaluation of the clinical usefulness of COBAS AMPLICOR HCV
4 MONITOR assay (ver2.0): Comparison with AMPLICOR HCV MONITOR assay (ver1.0) and HCV core protein
5 level. *J Med Virol.* 2002 Nov;68(3):343-51
6
7
8
9
10
11 34. Simmonds P. Variability of hepatitis C virus. *Hepatology* 1995;21:570-83.
12
13
14
15 35. Simmonds P, Alberti A, Halter HJ et al. A proposed system for the nomenclature of hepatitis C viral genotypes.
16 *Hepatology* 1995;22(2):418-25.
17
18
19
20
21 36. Bayer PM, Fabian B, Hübl W. Immunofluorescence assays (IFA) and enzyme-linked immunosorbent assays
22 (ELISA) in autoimmune disease diagnostics--technique, benefits, limitations and applications. *Scand J Clin Lab*
23 *Invest Suppl.* 2001;235:68-76.
24
25
26
27
28
29 37. Mikosch P, Gallowitsch HJ, Kresnik E et al. Influence of human anti-mouse antibodies on thyrotropin in-vitro
30 analysis: a comparison of 6 thyrotropin IRMA kits. *Eur J Clin Chem Clin Biochem.* 1997 Nov;35(11):881-3.
31
32
33
34
35 38. Bedossa P, Poinard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR
36 Cooperative Study Group. *Hepatology.* 1996 Aug;24(2):289-93.
37
38
39
40
41 39. Deugnier Y, Moirand R, Brissot P et al. Iron in the era of molecular biology. *Pathologie-biologie.* 1999
42 Nov;47(9):938-44.
43
44
45
46
47 40. Adams P, Brissot P, Powell LW. EASL International Consensus Conference on Haemochromatosis. *Journal of*
48 *hepatology* 2000 Sep;33(3):485-504.
49
50
51
52
53 41. Mchutchison JG, Manns M, Patel K et al. Adherence to combination therapy enhances sustained response in
54 genotype 1 infected patients with chronic hepatitis C. *Gastroenterology* 2002; 123:1061-1069.
55
56
57
58
59 42. Lindahl K, Stahle L, Bruchfeld A et al. High-dose ribavirin in combination with standard dose peginterferon for
60 treatment of patients with chronic hepatitis C. *Hepatology.* 2005 Feb;41(2):275-9.

- 1
2
3 43. Yu ML, Dai CY, Huang JF et al. Rapid virological response to peginterferon and ribavirin for hepatitis C
4 genotype 1: the role of weight-based ribavirin exposure. *Hepatology*. 2008 Sep;48(3):1019-20.
5
6
7
8
9 44. Ferenci P, Fried MW, Shiffman ML et al. Predicting sustained virological responses in chronic hepatitis C
10 patients treated with peginterferon alpha-2a (40 KD)/ribavirin. *Journal of Hepatology*. 2005 Sep;43(3):425-33.
11
12
13
14
15 45. Bain VG, Lee SS, Peltekian K et al. Canadian PEGASYS Study Group. Clinical trial: exposure to ribavirin
16 predicts EVR and SVR in patients with HCV genotype 1 infection treated with peginterferon alpha-2a plus
17 ribavirin. *Aliment Pharmacol Ther*. 2008 Jul;28(1):43-50.
18
19
20
21
22 46. McHutchison JG, Manns MP, Longo DL. Definition and management of anemia in patients infected with hepatitis
23 C virus. *Liver Int*. 2006 May;26(4):389-98. Review.
24
25
26
27
28 47. Afdhal NH. Role of epoetin alpha in maintaining ribavirin dose. *Gastroenterol Clin North Am*. 2004 Mar;33(1
29 Suppl):S25-35.
30
31
32
33
34 48. McHutchison JG, Manns MP, Brown RSJr et al. Strategies for managing anemia in hepatitis C patients
35 undergoing antiviral therapy. *Am J Gastroenterol*. 2007 Apr;102(4):880-9. Review.
36
37
38
39
40 49. Thevenot T, Di Martino V, Lunel-Fabiani F et al. Complementary treatments of chronic viral hepatitis C
41 *Gastroenterol Clin Biol*. 2006 Feb;30(2):197-214. Review.
42
43
44
45
46 50. Shiffman ML, Salvatore J, Hubbard S et al. Treatment of chronic hepatitis C virus genotype 1 with peginterferon,
47 ribavirin, and epoetin alpha. *Hepatology*. 2007 Aug;46(2):371-9.
48
49
50
51
52 51. Backus LI, Boothroyd DB, Philips BR et al. Predictors of response of US veterans to the treatment for the
53 hepatitis C virus. *Hepatology* 2007;46:37-47.
54
55
56
57
58 52. Cash WJ, Patterson K, Callender ME et al. Adjuvant therapy used in conjunction with combination therapy for
59 chronic hepatitis C improves sustained virus response rates in genotype 1 patients. *J Viral Hepat*. 2009 Oct 11.
60

- 1
2
3 53. Del Rio RA, Post AB, Singer ME. Cost-effectiveness of hematologic growth factors for anemia occurring during
4 hepatitis C combination therapy. *Hepatology*. 2006 Dec;44(6):1598-606.
5
6
7
8
9 54. Spiegel BM, Chen K, Chiou CF et al. Erythropoietic growth factors for treatment-induced anemia in hepatitis C: a
10 cost-effectiveness analysis. *Clin Gastroenterol Hepatol*. 2005 Oct;3(10):1034-42.
11
12
13
14
15 55. Devine EB, Cross JT, Kowdley KV et al. The cost of treating ribavirin-induced anemia in hepatitis C: the impact
16 of using recombinant human erythropoietin. *Curr Med Res Opin*. 2007 Jun;23(6):1463-72.
17
18
19
20
21 56. Kontorinis N, Agarwal K, Dieterich DT. Current status of the use of growth factors and other adjuvant
22 medications in patients receiving peginterferon and ribavirin. *Rev Gastroenterol Disord*. 2004;4 Suppl 1:S39-47.
23 Review.
24
25
26
27
28
29 57. Younossi ZM, Nader FH, Bai C et al. A phase II dose finding study of darbepoetin alpha and filgrastim for the
30 management of anaemia and neutropenia in chronic hepatitis C treatment. *J Viral Hepat*. 2008 May;15(5):370-8.
31
32
33
34
35 58. Thévenot T, Cadranel JF, Di Martino V et al. A national French survey on the use of growth factors as adjuvant
36 treatment of chronic hepatitis C. *Hepatology*. 2007 Feb;45(2):377-83.
37
38
39
40
41
42
43
44
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46
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Characteristics	N° (%)
Gender – n° (%)	
Male	108 (50.5)
Female	106 (49.5)
Age - years	48.0 ± 1.1
Weight - Kg	68.2 ± 1.3
Body-mass index	27.6 ± 1.2
Race	Caucasian (100)
HCV Genotype 1b	214 (100)
Serum HCV RNA level	
Mean – log ₁₀	6.40 ± 0.86
< 400.000 IU/ml	34 (16)
400.000 – 800.000 IU/ml	58 (27)
> 800.000 IU/ml	122 (57)
AST serum level - UI/l	99 ± 3.2
ALT serum level - UI/l	139 ± 3.0
Total bilirubin – mg/dl	1.01 ± 0.4
PLTs count – cell/mm ³	250 ± 19 x 10 ³
Hb level - g/dL	13.8 ± 0.9
Ferritin level - mg/dL	824 ± 20.3
Serum Albumin - mg/dL	4.4 ± 0.2
INR - %	0.9 ± 0.1
ISHAK score (overall)	10.1 ± 1.9
LASA energy score ^b	31.8 ± 16.2
LASA activity score ^b	40.2 ± 20.8

39 Abbreviations. ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; Hb: haemoglobin;
40 HCV: hepatitis C virus; HBV: hepatitis B virus; HIV: human immunodeficiency virus; PLTs: platelets;
41 INR: International Normalized Ratio; LASA: Linear Analog Self Assessment.

42 ^a Values are expressed as the mean ± standard deviation (SD). The body-mass index is the weight in
43 kilograms divided by the square of the height in meters. All liver specimens were assessed by a local
44 pathologist for histology status and reviewed by one expert pathologist, blinded about specimens group
45 origin.

46 ^b The Linear Analog Self Assessment (LASA) scale, with both activity and energy score, was performed to
47 evaluate Quality Of Life (QOL) at the beginning of the study, and at weeks 4, 12, 24, 36 and 48.
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Table 2. Significant predictors of Sustained Virological Response in the Multiple Logistic Regression Model (n = 174) including baseline factors and Ribavirin exposure.

Factors (units)	Group 1 (67 pz)	Group 2 (67 pz)	Group 3 (40 pz)	p-value (Odds Ratio 95% CI)
Gender – n° (%)				
Male	33 (49.2)	32 (47.8)	21 (52.5)	ns
Female	34 (50.7)	35 (52.2)	19 (47.5)	ns
Age – years	47.3 ± 1.2	46.8 ± 1.1	47.2 ± 1.3	ns
Weight – Kg	67.8 ± 1.2	68.1 ± 1.0	66.7 ± 1.1	ns
Body-mass index	27.6 ± 1.2	26.3 ± 1.0	27.4 ± 1.0	ns
HOMA-IR	1.9 ± 0.1	1.8 ± 0.3	1.9 ± 0.2	ns
ALT serum level – UI/l	133 ± 2.2	135 ± 2.4	136 ± 2.0	ns
HCV-RNA– log ₁₀ IU/mL				
at week 0	6.32 ± 0.84 †	6.29 ± 0.78 ‡	6.30 ± 0.46 §	ns
at week 12 (EVR) ^a	2.09 ± 0.32 †	2.08 ± 0.25 ‡	2.12 ± 0.23 §	ns
RBV exposure – % of the optimal dose	97.1	80.2	96.5	< 0.001
Epoetin alpha (10000 UI/twice weekly) – n° (%)	67 (100)	0 (0)	0 (0)	< 0.001
ISHAK score (overall)	9.7 ± 1.8	9.5 ± 1.9	10.1 ± 1.3	ns

Abbreviations. HOMA-IR: homeostatic model assessment; ALT: Alanine Aminotransferase; HOMA-IR: Homeostatic Model Assessment index; Hb: haemoglobin; HCV: hepatitis C virus; EVR: early virological response; RBV: ribavirin.

Group 1: full RBV dose, with Epoetin alpha addition; Group 2: RBV dose reduction, as *Standard of Care*; Group 3: controls, full RBV dose.

Values are expressed as the mean ± standard deviation (SD). The body-mass index is the weight in kilograms divided by the square of the height in meters. All liver specimens were assessed by a local pathologist for histology status and reviewed by one expert pathologist, blinded about specimens group origin.

P was calculated by multiple logistic regression analysis. ANOVA was also performed inside each group.

^a Early Virological Response is defined as unquantifiable HCV RNA level or HCV RNA reduction > 2 log₁₀ IU/mL at week 12.

†, ‡, § HCV RNA level: week 0 vs week 12 (EVR): p < 0.001 in the three groups (ANOVA).

Table 3. ETR and SVR rates comparison between the three study groups.

	G1 (67 pz)	G2 (67 pz)	G3 (40 pz)
EVR	62.7%	52.2%	65.0%
SVR	59.7%	34.3%	60.0%

ETR: G1 vs G2 p= ns; G2 vs G3 p= ns. SVR: G1 vs G2 p<0.01; G2 vs G3 p<0.01

Abbreviations: ETR: End of Treatment Response; SVR: Sustained Virological Response; G1: group 1 (epoetin alpha); G2: group 2 (reduction in RBV dose); G3: controls.

P values were calculated using Fisher's exact test. Two-tailed p value <0.01 was considered statistically significant.

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3 EPOETIN ALPHA IMPROVES THE RESPONSE TO ANTIVIRAL TREATMENT IN HCV-
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5 RELATED CHRONIC HEPATITIS
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8 Running head: Improvement of virological response
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12 Gaetano Bertino¹, Annalisa Ardiri¹, Patrizia Maria Boemi¹, Giuseppe Stefano Calvagno¹, Speranza
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ABSTRACT

Background. The conventional antiviral treatment of chronic hepatitis related to hepatitis C virus often leads to anaemia. In this case it is necessary to reduce ribavirin dose or stop treatment, thus reducing the rate of sustained virological response.

Aim. To investigate whether epoetin alpha administration improves treatment adherence and leads to higher percentage of response at the end of therapy and sustained virological response.

Methods. 214 subjects with genotype 1b HCV-related chronic hepatitis underwent treatment with PEG-interferon alpha-2A 180 micrograms once weekly and ribavirin 1000–1200 mg/day; 174 were responders: 40 subjects completed treatment with no haemoglobin reduction; 134 developed anaemia during therapy. Anaemic responders were distributed randomly into two groups: group 1 continued therapy with epoetin alpha addition; group 2 continued antiviral therapy with ribavirin reduction only.

Results. The group 1 achieved better control of haemoglobin levels (13.8 ± 1.2 g/dl at the end of therapy) than the group 2 (11.5 ± 0.8 g/dl). Sustained virological response was 59.7% in group 1, compared with 34.4% observed in the group 2 ($p < 0.01$).

Conclusions. In patients with 1b HCV-related chronic hepatitis, who develop anaemia during the antiviral treatment, the administration of Epoetin Alpha increases haemoglobin levels and the rate of end-of-treatment and sustained virological response by improving treatment adherence.

KEY WORDS

Epoetin Alpha, PEG-interferon alpha-2A, Ribavirin, Genotype-1b HCV-related Chronic Hepatitis.

INTRODUCTION

Hepatitis C virus (HCV) is a significant public health problem. As a hepatotropic virus, it could lead to chronic hepatitis, cirrhosis and hepatocellular carcinoma [1]. It can also cause several extra hepatic diseases, such as mixed cryoglobulinaemia and lymphoma, on account of its “lymphotropic pattern” [2,3].

According to current guidelines, the standard treatment of HCV-related chronic hepatitis (CHC) is based on the combination of peg-interferon (peg-IFN) plus ribavirin (RBV) [4–8]. Such combination therapy may induce a normalization of transaminase levels, a sustained virological response (SVR) and histological hepatic improvement in

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3 about 65% of subjects infected with HCV genotype 1 and in about 80% of those infected with genotype 2 or 3 HCV
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5 [1,9,10].

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7 Beyond well-known predictive factors for an optimal therapeutic response (age <45 years old, duration of disease <5
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9 years, low levels of HCV RNA, genotype 2 or 3 HCV infection, lack of fibrosis and intra hepatic iron upon hepatic
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11 biopsy), the adherence to standard peg-IFN and RBV therapy is necessary because it is associated with higher rates of
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13 therapeutic success [11,12]. However, several factors contribute to imperfect adherence to therapy or, in some cases, to
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15 its interruption: therapy-induced reduction in haemoglobin levels is the most important cause of drop out during
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17 treatment with peg-IFN and RBV [13–20]. As a result of this, RBV dose reduction is required, with a corresponding
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19 decrease in the chance of an SVR [21].

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21 It is well known that RBV monotherapy lowers haemoglobin levels by a haemolytic mechanism, while the inhibition
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23 of erythropoietin production during combination therapy with peg-IFN and RBV can induce anaemia [22–24].
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25 Therefore, Epoetin Alpha may become a pivotal therapy in patients who develop anaemia during treatment with peg-
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27 IFN and RBV. In fact, patients can continue treatment with a standard optimal dose of peg-IFN and RBV and achieve
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29 positive therapeutic results with the persistence of high haemoglobin levels. This aspect plays an especially important
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31 role in patients with advanced hepatic disease, because of the possibility of achieving stabilization of hepatic disease,
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33 in terms of clinical, biochemical and histological features, with a consequent improvement in quality of life (QOL) and
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35 an increased chance of survival [25–31].

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37 We investigated a group of patients with genotype 1b HCV-related chronic hepatitis, who were treated with PEG-
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39 interferon alpha-2A and RBV and consequently developed anaemia. We aimed to establish if treatment with Epoetin
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41 Alpha increased the number of patients who continued therapy with the standard dose of peg-IFN and RBV and
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43 increased the number of patients who achieve a sustained virological response (SVR).
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49 METHODS

50 Study design.

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52 The study was a longitudinal prospective randomized open-label controlled study performed in cooperation with three
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54 Hepatology Units. Epoetin Alpha was administered according to Italian Agency of Drugs (A.I.F.A, Agenzia Italiana
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56 del Farmaco) indications as expressed in Law n° 648/96. All patients provided their informed consent prior to their
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58 inclusion in the study.
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Selection of Patients

Criteria for admission to the therapy protocol with peg-IFN plus RBV were [5,6]: age 18 years or older, elevated serum ALT levels on the past 6 months, anti HCV antibodies positivity, detectable HCV RNA, HCV Genotype 1b, liver histology of chronic hepatitis (Ishak score overall ≤ 13), baseline HB >13 (males) and >12 (females), serum creatinin ≤ 1.5 mg/dL, Homeostatic Model Assessment index (HOMA-IR) < 2.5 .

Exclusion criteria applied were: HBV infection, HBV-HCV co-infection, HIV infection, HCV genotype other than 1b, overall Ishak score ≥ 13 , decompensated cirrhosis, significant atherosclerotic heart disease (defined as instrumental and clinical features of coronary heart disease and chronic heart failure); starting Hb <13 (males) and <12 (females); alcohol or drug abuse, history of haematological disorders or neoplastic diseases, pregnancy, psychiatric disorders, or autoimmune diseases, Wilson's disease and haemochromatosis.

All patients underwent HBsAg, HCV-RNA and HCV genotype determination. Anti-HCV antibodies were determined by ELISA (Ortho Diagnostic Systems, Raritan, NJ, USA) [32]. Pre-treatment HCV-RNA levels were detected by polymerase chain reaction (PCR) of HCV-RNA 5' UTR using COBAS® AmpliPrep/COBAS® TaqMan® HCV Test (Roche Diagnostics Systems, Branchburg, N.J.; analytic sensitivity = 50 IU/mL) [33]. HCV genotypes were determined by INNO-LiPA (Innogenetics) assay, using Simmond's classification [34,35]. Anti-nuclear (ANA), anti-mitochondrial (AMA), anti-smooth muscle (SMA), anti-liver/kidney microsome type 1 (LKM1) autoantibodies were measured using immunofluorescence assay (IFA) and semi-quantitative ELISA immobilizing enzyme test [36]. Thyroid function was evaluated by levels of TSH, free T3 and free T4, that were determined by immunoradiometric assay (IRMA); antibodies against thyroid peroxidase and thyroglobulin were measured by IFA [37].

Patients underwent ultrasound-assisted percutaneous biopsy, obtained with Menghini modified needles (Automatic Aspiration Needle for Liver Biopsy, ACR 16G, 11 cm, manufactured by Sterylab Srl, Milan-Italy) within 6 months before the start of the study. Histological evaluation of the degree of necroinflammatory activity (grading) and fibrosis (staging) of hepatic tissue was carried out using the Ishak scoring system. Semi-quantitative evaluation of the degree and distribution of hepatic iron storage were assessed according to the classification system of Brissot et al, and intra hepatic iron was assessed using Perl's Prussian blue staining [38,39]. Serum levels of iron, saturated transferrin, total transferrin (total iron binding capacity, TIBC), unsaturated transferrin (unsaturated iron binding capacity, UIBC) and ferritin were determined, and saturation of transferrin was calculated as: serum level of iron/TIBC $\times 100$.

Genetic testing to identify haemochromatosis HFE gene mutations was performed in order to exclude subjects with hereditary haemochromatosis [40].

On the basis of clinical, blood chemistry, virological and histological data, 214 consecutive Caucasian subjects [108 male and 106 female, mean age 48 years old (range 37–52), mean body weight 68 kg (range 53–91)] with genotype 1b

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3 HCV-related chronic hepatitis, mean Ishak overall score 10.1 ± 1.9 were enrolled and eligible to receive antiviral
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5 treatment with PEG-interferon alpha-2A 180 micrograms/week + weight based ribavirin (WBR) 1000-1200 mg/day.
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7 Baseline characteristic (week 0) are presented in table 1.
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13 Therapeutic protocol and efficacy assessment.

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15 The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki (6th Revision, 2008).
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17 According to current guidelines [1] at time 0, all patients started therapy with subcutaneous PEG-interferon alpha-2A,
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19 180 micrograms once weekly, plus oral WBR in two separate doses (the total dose was 1200 mg daily for patients
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21 weighing >75 kg, and 1000 mg daily for those weighing <75 kg). Two weeks after the start of therapy and
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23 subsequently on a monthly basis, patients were evaluated clinically, and serum levels of transaminases, sodium,
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25 potassium, TSH, free T3, free T4, antibodies against thyroid peroxidase and thyroglobulin, glycaemia, azotaemia,
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27 creatininaemia, INR determination, haemachrome, and indexes of body iron were assessed.

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29 In compliance with international guidelines and recommendations of the American Association for the Study of Liver
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31 Disease (AASLD) and the Italian Association for the Study of the Liver (AISF), HCV-RNA levels were measured in
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33 all patients at weeks 12, 24, 48 and 72 [1,6]. Patients with a HCV-RNA reduction of at least 2 log₁₀ at week 12 in
34
35 comparison with the pre-treatment HCV-RNA values were classified as early virological responders (EVR) and
36
37 continued therapy. Patients who did not reach this target were classified as non-responders and stopped therapy.

38
39 Early virological responders who showed a reduction of haemoglobin serum levels >2 g/dl compared with the
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41 haemoglobin pre-treatment value, were 1:1 randomly distributed into 2 groups by computer-based randomization
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43 software: subjects in groups 1 were admitted to continue PEG-interferon alpha-2A and WBR dosage (1000-1200
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45 mg/day) plus subcutaneous Epoetin Alpha, 10.000 IU twice weekly; subjects in group 2 were admitted to continue
46
47 PEG-interferon alpha-2A with a weight based reduction of RBV dose (800-1000 mg/day). Administration of Epoetin
48
49 Alpha in group 1 followed the acquisition of patients informed consent, as clearly indicated by Italian Law n°
50
51 684/1996 concerning the extension of the *indications-to-use* of epoetins, which must be based on the most recent and
52
53 proved evidences in the scientific literature.

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55 For all patients who underwent antiviral therapy in our Hepatology Unit, QOL was evaluated using the Linear
56
57 Analogue Self Assessment (LASA) scale by both activity and energy score, at the beginning of the study and at weeks
58
59 4, 12, 24, 36 and 48 [31].

60
The primary efficacy endpoint was sustained virologic response (SVR) defined as undetectable serum HCV RNA
levels (<50 IU/mL) 24 weeks after the end of therapy (week 72).

Randomization and Statistical analysis

Randomization process was performed through SAS® Software (Chicago, IL, USA). Results are presented as means \pm standard deviation (SD), range and frequencies. Fisher's Exact test and Mann Whitney U Test for independent samples were applied. Two-tailed P values <0.01 were considered statistically significant. The impact of baseline factors and exposure to ribavirin were evaluated by multiple logistic regression (MLR) analyses. A predictive model was developed for primary endpoint (SVR). Baseline factors considered for inclusion in the models were: age, body weight, body mass index, HOMA-IR, haemoglobin and HCV RNA level at week 0 and week 12 as continuous variables; gender and histological stage as categorical variables. Drug exposure was converted to the percentage of optimal dose and was included in the models as a continuous variable.

RESULTS

All patients were negative for ANA, AMA, SMA and LKM1 autoantibodies. Thyroid function, as evaluated by levels of TSH, free T3 and free T4, was also normal. Autoantibodies against thyroid peroxidase and thyroglobulin were absent at the beginning of therapy. However, in 8 patients, they were positive during treatment, and decreased progressively after the end of antiviral therapy, except in one patient in group who continued to have mild thyroiditis.

Prior to treatment, all patients had a mean haemoglobin level of 13.8 ± 0.9 g/dl. Four weeks after the start of therapy, we noted a reduction in haemoglobin and consequent asthenia, insomnia and loss of concentration.

At week 12, no significant statistical difference was found between the three groups, concerning the following parameters: total bilirubin, platelets count, haemoglobin, ferritin and albumin serum levels. At week 12, 174/214 patients (81.3%) achieved an EVR, while 40/214 (18.7%) dropped out because they were non-responders, and stopped therapy. Forty out of 174 EVR patients (23%) did not show decrease of haemoglobin serum levels > 2 g/dL during the study period and fully completed the treatment protocol (control group). Reduction of haemoglobin levels > 2 g/dL (mean value: 11.4 ± 1.3 g/dl) compared with the pre-treatment values has been achieved in 134/174 (77%) EVR patients. They also had worsening treatment compliance, such that about 60% of responders would have preferred to stop therapy. They were randomly distributed into 2 groups: 67 subjects in group 1 received subcutaneous Epoetin Alpha, 10,000 IU twice weekly, in addition to PEG-interferon alpha-2A 180 micrograms/week + weight based ribavirin (WBR) 1000-1200 mg/day; 67 subjects in group 2 received PEG-interferon alpha-2A 180 micrograms/week and reduced doses of RBV (800-1000 mg/day).

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3 The 2 groups of study, as well as the control group, were followed up monthly during the 48 weeks of treatment up to
4 six months after treatment interruption. In group 1 42/67 subjects (62.7%) showed undetectable HCV-RNA serum
5 levels at week 48 and they were classified as ETR (end of treatment responders); 25/67 (37.3%) showed HCV-RNA
6 serum levels still detectable. In group 2 ETR subjects were 35/67 (52.5%); subjects with detectable HCV-RNA serum
7 levels were 32/67 (47.8%).

8
9 Six months after the end of treatment (week 72), Sustained Virological Response (SVR) was assessed. Forty subjects
10 out of 67 in group 1 (59.7%) achieved a SVR; 2/67 subjects (3%) had a relapse of disease during follow up, detected
11 through HCV-RNA assay and elevated transaminase levels. In group 2, 23/67 subjects (34.4%) achieved a SVR; 12/67
12 subjects had a relapse (17.9%). In control group 26/40 (65%) were ETR and 24/40 (60%) achieved a SVR; 2/40 (5%)
13 were relapsers (Figure 1).

14
15 During the course of treatment, no one of the patients showed Hb serum levels <10 g/dl. However, in 19/67 (28.3%) of
16 the EVR-patients, therapy was suspended for a period of 3 weeks because of worsening asthenia and QOL, and
17 incomplete adherence to therapy.

18
19 The difference in SVR rate was statistically significant comparing group 2 with group 1 and with control group (p
20 <0.01). Otherwise no statistically significant difference has been found comparing SVR rates between group 1 and
21 control group, as well as no difference has been found in ETR rate between all the groups of study (Table 3).

22
23 Patients in group 2, who received a reduction in RBV dosage, did not achieve increase of haemoglobin level and
24 showed a SVR rate lower than that of patients in group 1 who maintained the standard dose of PEG-interferon alpha-
25 2A and RBV by the addition of Epoetin Alpha, and showed a significant increase of haemoglobin serum levels (Figure
26 2). Therefore, only in group 1 treated with Epoetin Alpha was the “80/80/80 rule” respected, i.e. administration of at
27 least 80% of the dose of peg-IFN and RBV for at least 80% of the time of treatment [42].

28
29 The evaluation of QOL by the LASA scale showed that mean scores of patients treated with Epoetin Alpha (group 1)
30 were significantly superior to those in group 2 concerning tolerability, adherence to treatment, few side effects, and
31 improved QOL. In particular, good results in the changes in activity and energy score were observed in group 1 at 36
32 weeks (energy score change 18 ± 17.3 and activity score change 20 ± 18.5) compared with the results obtained in group 2
33 (energy score change 12.2 ± 21.6 and activity score change 7 ± 18.7) ($P < 0.05$ for energy score change and $P < 0.01$ for
34 activity score change). In the QOL assessment, rates of asthenia, loss of concentration, and insomnia, were also
35 evaluated and a comparison between the three groups was performed, with no statistical difference found between
36 group 1 and group 3 [11/67 (16.4%) vs 7/40 (17.5%)]. Rates of asthenia, loss of concentration, and insomnia in group
37 2 were significantly higher (65/67; 97.5%) when compared with those in group 1 and group 3 ($p < 0.001$).

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3 In group 1, serum ferritin decreased from a mean pre-treatment level of 800 ng/ml to 320 ng/ml after treatment (Figure
4
5 3). In contrast, in group 2, we observed a slight reduction in mean ferritin level from a pre-treatment value of 790
6
7 ng/ml to 580 ng/ml after treatment (Figure 3).

8
9 No patient in group 1 showed adverse effects of Epoetin Alpha administration. No severe psychiatric side-effects, such
10
11 as major depression or psychosis have been reported during the antiviral treatment administration as well as in the
12
13 follow-up. In 13 patients of group 1 such as in 11 subjects of group 2, symptoms of anxiety or mild depression
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15 occurred but they were successfully controlled by Psychological Support Programmes, or administration of
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17 antidepressant drugs.

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21 MLR Analyses.

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23 Significant predictors of SVR are presented in table 2. In our regression model, factors significantly associated with
24
25 achievement of SVR were RBV exposure ($p < 0.001$) and Epoetin alpha addition ($p < 0.001$).

26 27 28 29 DISCUSSION

30
31 Combination treatment with Peg-IFN and RBV represents the gold standard in the treatment of chronic hepatitis C.
32
33 During HCV infection, factors associated with a favourable response to peg-INF and RBV therapy are viral genotypes
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35 2 or 3, low levels of viremia before treatment, young age, absence of obesity and insulin resistance, and a low grade of
36
37 fibrosis. In patients with CHC it is extremely important to ensure complete adherence to therapy with peg-IFN and
38
39 RBV in order to achieve therapeutic success (greater percentage of SVR). Indeed, early interruption of therapy or
40
41 reduction of peg-IFN or RBV dose because of side effects, leads to a significant decrease in SVR [21, 41– 43].

42
43 After 12 weeks of therapy, in patients with an EVR, which is a reduction in HCV RNA of at least 2 log₁₀ in
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45 comparison with baseline, adherence to the “80/80/80 rule” is highly predictive of an SVR. This rule, which means
46
47 taking 80% of the dose of peg-IFN and RBV for at least 80% of the duration of therapy, yields a greater SVR [41–45].

48
49 Thus, exposure to an optimal treatment regimen, which consists of the full dose of drugs and prolonged treatment, is
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51 important in achieving a successful treatment target, as it results from our logistic regression model for SVR (primary
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53 endpoint). This target was obtained by the addition of epoetin alpha, whereas the development of anaemia leads to
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55 reduce the dose of RBV, as Standard of Care (SOC). In fact, HCV related genotype 1b CH patients who receive 80%
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57 of the full dose of peg-IFN and RBV for 48 weeks show a higher SVR than patients with the same virological features
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59 of hepatic disease who receive a reduced dose of drugs. This is because the maintenance of optimal drug levels allows
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the patient to achieve a strong and complete inhibition of viral replication [45].

1
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3 During therapy with peg-IFN and RBV, alterations of the haematological profile and, in particular, evidence of
4 neutropenia, thrombocytopenia and anaemia are critical factors that play an important role in the reduction of duration
5 and dose of therapy. Anaemia caused by peg-IFN is additional to that induced by RBV [15]. Therefore, anaemia
6 represents a frequent side effect that may lead to a reduction in exposure time and dose of antiviral drugs, with
7 consequent incomplete control of viral replication and clinical, biochemical and histological progression of hepatic
8 disease [18,20,22,23,46].

9
10 The use of adjuvant agents (erythropoietin and granulocyte colony stimulating factor) to reduce the frequency of dose
11 reductions because of haematological side-effects has been proven to be effective but there are few reports of what
12 effect the use of these adjuvant therapies is having on sustained virological response (SVR).

13
14 Our researches showed that patients in group 1, who were treated throughout with the initial dose of RBV, along with
15 Epoetin Alpha after anaemia onset, showed a greater rate of SVR than patients in group 2, who were treated with a
16 reduced dose of RBV, without Epoetin Alpha, after anaemia onset. In particular, the rate of SVR in group 1 was of
17 59.7% compared with 34.4% ($p < 0.01$) achieved in group 2 (Table 3). Group 1 SVR rate was similar to SVR rate in
18 our control group (early responders who experienced no haemoglobin reduction during all therapy and survey period)
19 with no statistical difference.

20
21 Our results are in accordance with some recent studies [47–49]. Shiffman et al. observed a lower rate of relapse and an
22 higher rate of SVR in patients treated with a higher starting dose of RBV (15.2 mg/kg/day) plus Epoetin Alpha
23 compared with those treated with a lower dose of RBV (13.3 mg/kg/day) with or without Epoetin Alpha [50].
24 Moreover, in a retrospective observational cohort study of predictors of SVR to peg-IFN and RBV in routine medical
25 practice at 121 Department of Veterans Affairs facilities, Backus et al. identified, by univariate and multivariate
26 analyses, the use of Epoetin Alpha as an independent predictor of SVR [51].

27
28 In the Casch WJ et al. paper the aim of the study was to assess the clinical impact on sustained virological response of
29 adjuvant therapies (erythropoietin and granulocyte colony stimulating factor) during combination therapy with Peg-
30 IFN and ribavirin for chronic hepatitis C; the authors have shown that, in CH genotype 1 related, the SVR can be
31 improved with the use of erythropoietins [52].

32
33 It may be assumed that there is no cost–benefit relationship for the use of Epoetin Alpha in patients with cirrhosis,
34 because of the high cost of Epoetin Alpha therapy, as with other haematological growth factors. However, some
35 studies have used decision analysis to estimate the cost-effectiveness of adjunctive therapy with erythropoietic growth
36 factor (EGF) compared with that of standard care in the treatment of hepatitis C. The use of EGFs appears to be cost-
37 effective in patients with anaemia who are receiving combination therapy for HCV genotype 1, 2 or 3. Compared with
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3 standard care, adjuvant therapy with EGF, may increase the probability of achieving an SVR, increase unadjusted
4 lifespan, and improve quality-adjusted lifespan at an acceptable cost [52, 53-55].

7 The results of previous studies [27,28,30,37,49,50,52,56–58] and the present study have shown that the introduction of
8
9 Epoetin Alpha therapy in patients treated with PEG-interferon alpha-2A and RBV for CH related to genotype 1b HCV
10 infection leads to an improved adherence to treatment, and, consequently, to a significant improvement of SVR.

13 Moreover, the reduction in ferritinaemia observed in group 1 may have contributed to the better therapeutic response
14 to peg-IFN and RBV treatment. The favourable trend in ferritinaemia in group 1 may have been the consequence of
15 decreased hepatic necroinflammation, secondary to antiviral therapy, as well as the major movement of iron deposits
16 towards the erythropoietic medulla, secondary to Epoetin Alpha administration.

21 In conclusion, our data suggest that in patients with genotype 1b HCV-related CH, who develop anaemia during
22 therapy with PEG-interferon alpha-2A and RBV, the administration of Epoetin Alpha is justified because it ensures
23 normal levels of haemoglobin and increases the SVR rate, assuring exposure to the optimal RBV dosage, improving
24 patient's adherence to treatment and their quality of life. However, more data are necessary to clarify when Epoetin
25 Alpha treatment should be started, the duration of treatment, the optimal dosage, the effect on end-of-treatment and
26 sustained virologic response rates, and its costs.
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38 CONFLICT OF INTERESTS: The authors declare that they have no conflict of interest.
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42 REFERENCES

- 44 1. Strader DB, Wright T, Thomas DL et al. American Association for the Study of Liver Diseases. Diagnosis,
45 management, and treatment of hepatitis C. *Hepatology*. 2004 Apr;39(4):1147-71.
- 46 2. Zignego AL, Giannini C, Ferri C. Hepatitis C virus-related lymphoproliferative disorders: an overview. *World J*
47 *Gastroenterol*. 2007 May 7;13(17):2467-78. Review.
- 48 3. Craxi A, Laffi G, Zignego AL. Hepatitis C virus (HCV) infection: a systemic disease. *Mol Aspects Med*. 2008
49 Feb-Apr;29(1-2):85-95
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 4. Manns MP, McHutchison JG, Gordon SC et al. Peginterferon alpha-2b plus ribavirin compared with interferon
4 alpha-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001;358:958-956.
5
6
7
- 8
9 5. Fried MW, Shiffman ML, Reddy KR et al. Peginterferon alpha-2a plus ribavirin for chronic hepatitis C virus
10 infection. *N Engl J Med* 2002;347:975-982.
11
12
- 13
14 6. Hadziyannis SJ, Sette HJr, Morgan TR et al. PEGASYS International Study Group. Peginterferon-alpha2a and
15 ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose.
16
17 *Ann Intern Med* 2004; 140:346-355.
18
19
- 20
21 7. Shiffman ML, Di Bisceglie AM, Lindsay KL et al. Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis
22 Trial Group. Peginterferon alpha-2a and ribavirin in patients with chronic hepatitis C who have failed prior
23 treatment. *Gastroenterology*. 2004 Apr;126(4):1015-23.
24
25
26
27
28
- 29
30 8. Leiner S. Peginterferon and ribavirin for hepatitis C. *N Engl J Med*. 2007 Mar22;356(12):1270
31
32
33
- 34
35 9. Hadziyannis SJ, Koskinas JS. Differences in epidemiology, liver disease and treatment response among HCV
36 genotypes. *Hepatology Research*. 2004 Jul;29(3):129-135.
37
38
39
- 40
41 10. Formann E, Jessner W, Bennett L et al. Twice-weekly administration of peginterferon-alpha-2b improves viral
42 kinetics in patients with chronic hepatitis C genotype 1. *Journal of viral hepatitis*. 2003 Jul;10(4):271-6.
43
44
45
- 46
47 11. Saracco G, Rizzetto M. Predictors of response to interferon therapy. *Digestive diseases and sciences*. 1996
48 Dec;41(12 Suppl):115S-120S.
49
50
- 51
52 12. Neuman MG, Benhamou JP, Martinot M., et al. Predictors of sustained response to alpha interferon therapy in
53 chronic hepatitis C. *Clinical biochemistry*. 1999 Oct;32(7):537-45
54
55
56
- 57
58 13. Manns MP. Adherence to combination therapy: influence on sustained virologic response and economic impact.
59
60 *Gastroenterology clinics of North America*. 2004 Mar;33(1 Suppl):S11-24.

- 1
2
3 14. Russo MW, Fried MW. Side effects of therapy for chronic hepatitis C. *Gastroenterology*. 2003 May;124(6):1711-
4
5 9.
6
7
8
9
- 10
11 15. De Franceschi L, Fattovich G, Turrini F et al. Hemolytic anemia induced by ribavirin therapy in patients with
12 chronic hepatitis C virus infection. *Hepatology* 2000;31:997-1004.
13
14
15
- 16
17 16. Rendo P, Rosso A, Gomez F et al. Anaemia in patients with chronic hepatitis C treated with ribavirin and peg-
18 interferon. *Antiviral Therapy* 2000;5(suppl 1):C96).
19
20
21
- 22
23 17. Dieterich DT, Spivak JL. Hematologic disorders associated with hepatitis C virus infection and their management.
24 *Clinical infectious diseases*. 2003 Aug 15;37(4):533-41.
25
26
27
- 28
29 18. Peck-Radosavljevic M, Wichlas M, Homoncik-Kraml M et al. Rapid suppression of haematopoiesis by standard
30 or pegylated interferon alpha. *Gastroenterology* 2002;123:141-151.
31
32
33
- 34
35 19. Golia P, Talal AH, Jacobs IM et al. A preliminary study of growth factor versus dose reduction for peg-interferon
36 alpha-2b and ribavirin associated neutropenia and anemia in HCV infected patients. *Gastroenterology*
37 2003;124:A766.
38
39
40
- 41
42 20. Lindahl K, Schvarcz R, Bruchfeld A et al. Evidence that plasma concentration rather than dose per kilogram body
43 weight predict ribavirin induced anemia. *Journal of viral hepatitis*. 2004;11:84-87.
44
45
46
47
- 48
49 21. Reddy KR, Shiffman ML, Morgan TR et al. Impact of ribavirin dose reductions in hepatitis C virus genotype 1
50 patients completing peginterferon alpha 2a/ribavirin treatment. *Clin Gastroenterol Hepatol* 2007;5:124-129.
51
52
53
- 54
55 22. Ong JP, Younossi ZM. Managing the hematologic side effects of antiviral therapy for chronic hepatitis C: anemia,
56 neutropenia, and thrombocytopenia. *Cleve Clin J Med*. 2004 May;71 Suppl 3:S17-21. Review.
57
58
59
- 60 23. Kowdley KV. Hematologic side effects of interferon and ribavirin therapy. *J Clin Gastroenterol*. 2005 Jan;39(1
Suppl):S3-8. Review.

- 1
2
3
4
5 24. Schmid M, Kreil A, Jessner W et al. Suppression of haematopoiesis during therapy of chronic hepatitis C with
6
7 different interferon alpha mono and combination therapy regimens. *Gut*. 2005 Jul;54(7):1014-20.
8
9
- 10
11 25. Sharvadze L, Tsertsvadze T, Gochitashvili N et al. Ifn/Rbv treatment induced anemia and its correction with
12
13 epoetin alpha in patients with hepatitis C. *Georgian medical news*. 2006 Aug;(137):62-5.
14
15
- 16
17 26. Talal AH, Weisz K, Hau T et al. A preliminary study of erythropoietin for anemia associated with ribavirin and
18
19 interferon-alpha. *The American journal of gastroenterology* 2001 Sep;96(9):2802-4.
20
21
- 22
23 27. Dieterich DT, Wasserman R, Bräu N et al. Once-weekly epoetin alpha improves anemia and facilitates
24
25 maintenance of ribavirin dosing in hepatitis C virus-infected patients receiving ribavirin plus interferon alpha. *The*
26
27 *American journal of gastroenterology*. 2003 Nov;98(11):2491-9.
28
29
- 30
31 28. Bräu N. Epoetin alpha treatment for acute anaemia during interferon plus ribavirin combination therapy for
32
33 chronic hepatitis C. *Journal of viral hepatitis*. 2004 May;11(3):191-7. Review.
34
35
- 36
37 29. Balan V, Schwartz D, Wu GY et al. HCV Natural History Study Group. Erythropoietic response to anemia in
38
39 chronic hepatitis C patients receiving combination pegylated interferon/ribavirin. *The American journal of*
40
41 *gastroenterology* 2005 Feb;100(2):299-307.
42
43
- 44
45 30. Afdhal NH, Dieterich DT, Pockros PJ et al. Proactive Study Group. Epoetin alpha maintains ribavirin dose in
46
47 HCV-infected patients: a prospective, double-blind, randomized controlled study. *Gastroenterology*. 2004
48
49 May;126(5):1302-11.
50
51
- 52
53 31. Pockros PJ, Shiffman ML, Schiff ER et al. PROACTIVE Study Group. Epoetin alpha improves quality of life in
54
55 anemic HCV-infected patients receiving combination therapy. *Hepatology*. 2004 Dec;40(6):1450-8.
56
57
- 58
59 32. Lee S, McHutchinson J, Francis B et al. Improved detection of antibodies to hepatitis C virus using a second
60
61 generation ELISA. *Adv Exp Med Biol*. 1992;312:183-9.

- 1
2
3 33. Kawai S, Yokosuka O, Imazeki F et al. Evaluation of the clinical usefulness of COBAS AMPLICOR HCV
4 MONITOR assay (ver2.0): Comparison with AMPLICOR HCV MONITOR assay (ver1.0) and HCV core protein
5 level. J Med Virol. 2002 Nov;68(3):343-51
6
7
8
9
10
11 34. Simmonds P. Variability of hepatitis C virus. Hepatology 1995;21:570-83.
12
13
14
15 35. Simmonds P, Alberti A, Halter HJ et al. A proposed system for the nomenclature of hepatitis C viral genotypes.
16 Hepatology 1995;22(2):418-25.
17
18
19
20
21 36. Bayer PM, Fabian B, Hübl W. Immunofluorescence assays (IFA) and enzyme-linked immunosorbent assays
22 (ELISA) in autoimmune disease diagnostics--technique, benefits, limitations and applications. Scand J Clin Lab
23 Invest Suppl. 2001;235:68-76.
24
25
26
27
28
29 37. Mikosch P, Gallowitsch HJ, Kresnik E et al. Influence of human anti-mouse antibodies on thyrotropin in-vitro
30 analysis: a comparison of 6 thyrotropin IRMA kits. Eur J Clin Chem Clin Biochem. 1997 Nov;35(11):881-3.
31
32
33
34
35 38. Bedossa P, Poinard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR
36 Cooperative Study Group. Hepatology. 1996 Aug;24(2):289-93.
37
38
39
40
41 39. Deugnier Y, Moirand R, Brissot P et al. Iron in the era of molecular biology. Pathologie-biologie. 1999
42 Nov;47(9):938-44.
43
44
45
46
47 40. Adams P, Brissot P, Powell LW. EASL International Consensus Conference on Haemochromatosis. Journal of
48 hepatology 2000 Sep;33(3):485-504.
49
50
51
52
53 41. Mchutchison JG, Manns M, Patel K et al. Adherence to combination therapy enhances sustained response in
54 genotype 1 infected patients with chronic hepatitis C. Gastroenterology 2002; 123:1061-1069.
55
56
57
58
59 42. Lindahl K, Stahle L, Bruchfeld A et al. High-dose ribavirin in combination with standard dose peginterferon for
60 treatment of patients with chronic hepatitis C. Hepatology. 2005 Feb;41(2):275-9.

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43. Yu ML, Dai CY, Huang JF et al. Rapid virological response to peginterferon and ribavirin for hepatitis C genotype 1: the role of weight-based ribavirin exposure. *Hepatology*. 2008 Sep;48(3):1019-20.
 44. Ferenci P, Fried MW, Shiffman ML et al. Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alpha-2a (40 KD)/ribavirin. *Journal of Hepatology*. 2005 Sep;43(3):425-33.
 45. Bain VG, Lee SS, Peltekian K et al. Canadian PEGASYS Study Group. Clinical trial: exposure to ribavirin predicts EVR and SVR in patients with HCV genotype 1 infection treated with peginterferon alpha-2a plus ribavirin. *Aliment Pharmacol Ther*. 2008 Jul;28(1):43-50.
 46. McHutchison JG, Manns MP, Longo DL. Definition and management of anemia in patients infected with hepatitis C virus. *Liver Int*. 2006 May;26(4):389-98. Review.
 47. Afdhal NH. Role of epoetin alpha in maintaining ribavirin dose. *Gastroenterol Clin North Am*. 2004 Mar;33(1 Suppl):S25-35.
 48. McHutchison JG, Manns MP, Brown RSJr et al. Strategies for managing anemia in hepatitis C patients undergoing antiviral therapy. *Am J Gastroenterol*. 2007 Apr;102(4):880-9. Review.
 49. Thevenot T, Di Martino V, Lunel-Fabiani F et al. Complementary treatments of chronic viral hepatitis C *Gastroenterol Clin Biol*. 2006 Feb;30(2):197-214. Review.
 50. Shiffman ML, Salvatore J, Hubbard S et al. Treatment of chronic hepatitis C virus genotype 1 with peginterferon, ribavirin, and epoetin alpha. *Hepatology*. 2007 Aug;46(2):371-9.
 51. Backus LI, Boothroyd DB, Philips BR et al. Predictors of response of US veterans to the treatment for the hepatitis C virus. *Hepatology* 2007;46:37-47.
 52. Cash WJ, Patterson K, Callender ME et al. Adjuvant therapy used in conjunction with combination therapy for chronic hepatitis C improves sustained virus response rates in genotype 1 patients. *J Viral Hepat*. 2009 Oct 11.

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3 53. Del Rio RA, Post AB, Singer ME. Cost-effectiveness of hematologic growth factors for anemia occurring during
4 hepatitis C combination therapy. *Hepatology*. 2006 Dec;44(6):1598-606.
5
6
7
8
9 54. Spiegel BM, Chen K, Chiou CF et al. Erythropoietic growth factors for treatment-induced anemia in hepatitis C: a
10 cost-effectiveness analysis. *Clin Gastroenterol Hepatol*. 2005 Oct;3(10):1034-42.
11
12
13
14
15 55. Devine EB, Cross JT, Kowdley KV et al. The cost of treating ribavirin-induced anemia in hepatitis C: the impact
16 of using recombinant human erythropoietin. *Curr Med Res Opin*. 2007 Jun;23(6):1463-72.
17
18
19
20
21 56. Kontorinis N, Agarwal K, Dieterich DT. Current status of the use of growth factors and other adjuvant
22 medications in patients receiving peginterferon and ribavirin. *Rev Gastroenterol Disord*. 2004;4 Suppl 1:S39-47.
23 Review.
24
25
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27
28
29 57. Younossi ZM, Nader FH, Bai C et al. A phase II dose finding study of darbepoetin alpha and filgrastim for the
30 management of anaemia and neutropenia in chronic hepatitis C treatment. *J Viral Hepat*. 2008 May;15(5):370-8.
31
32
33
34
35 58. Thévenot T, Cadranel JF, Di Martino V et al. A national French survey on the use of growth factors as adjuvant
36 treatment of chronic hepatitis C. *Hepatology*. 2007 Feb;45(2):377-83.
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Characteristics	N° (%)
Gender – n° (%)	
Male	108 (50.5)
Female	106 (49.5)
Age - years	48.0 ± 1.1
Weight - Kg	68.2 ± 1.3
Body-mass index	27.6 ± 1.2
Race	Caucasian (100)
HCV Genotype 1b	214 (100)
Serum HCV RNA level	
Mean – log ₁₀	6.40 ± 0.86
< 400.000 IU/ml	34 (16)
400.000 – 800.000 IU/ml	58 (27)
> 800.000 IU/ml	122 (57)
AST serum level - UI/l	99 ± 3.2
ALT serum level - UI/l	139 ± 3.0
Total bilirubin – mg/dl	1.01 ± 0.4
PLTs count – cell/mm ³	250 ± 19 x 10 ³
Hb level - g/dL	13.8 ± 0.9
Ferritin level - mg/dL	824 ± 20.3
Serum Albumin - mg/dL	4.4 ± 0.2
INR - %	0.9 ± 0.1
ISHAK score (overall)	10.1 ± 1.9
LASA energy score ^b	31.8 ± 16.2
LASA activity score ^b	40.2 ± 20.8

39 Abbreviations. ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; Hb: haemoglobin;
40 HCV: hepatitis C virus; HBV: hepatitis B virus; HIV: human immunodeficiency virus; PLTs: platelets;
41 INR: International Normalized Ratio; LASA: Linear Analog Self Assessment.

42 ^a Values are expressed as the mean ± standard deviation (SD). The body-mass index is the weight in
43 kilograms divided by the square of the height in meters. All liver specimens were assessed by a local
44 pathologist for histology status and reviewed by one expert pathologist, blinded about specimens group
45 origin.
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47 ^b The Linear Analog Self Assessment (LASA) scale, with both activity and energy score, was performed to
48 evaluate Quality Of Life (QOL) at the beginning of the study, and at weeks 4, 12, 24, 36 and 48.
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Table 2. Significant predictors of Sustained Virological Response in the Multiple Logistic Regression Model (n = 174) including baseline factors and Ribavirin exposure.

Factors (units)	Group 1 (67 pz)	Group 2 (67 pz)	Group 3 (40 pz)	p-value (Odds Ratio 95% CI)
Gender – n° (%)				
Male	33 (49.2)	32 (47.8)	21 (52.5)	ns
Female	34 (50.7)	35 (52.2)	19 (47.5)	ns
Age – years	47.3 ± 1.2	46.8 ± 1.1	47.2 ± 1.3	ns
Weight – Kg	67.8 ± 1.2	68.1 ± 1.0	66.7 ± 1.1	ns
Body-mass index	27.6 ± 1.2	26.3 ± 1.0	27.4 ± 1.0	ns
HOMA-IR	1.9 ± 0.1	1.8 ± 0.3	1.9 ± 0.2	ns
ALT serum level – UI/l	133 ± 2.2	135 ± 2.4	136 ± 2.0	ns
HCV-RNA– log ₁₀ IU/mL				
at week 0	6.32 ± 0.84 †	6.29 ± 0.78 ‡	6.30 ± 0.46 §	ns
at week 12 (EVR) ^a	2.09 ± 0.32 †	2.08 ± 0.25 ‡	2.12 ± 0.23 §	ns
RBV exposure – % of the optimal dose	97.1	80.2	96.5	< 0.001
Epoetin alpha (10000 UI/twice weekly) – n° (%)	67 (100)	0 (0)	0 (0)	< 0.001
ISHAK score (overall)	9.7 ± 1.8	9.5 ± 1.9	10.1 ± 1.3	ns

Abbreviations. HOMA-IR: homeostatic model assessment; ALT: Alanine Aminotransferase; HOMA-IR: Homeostatic Model Assessment index; Hb: haemoglobin; HCV: hepatitis C virus; EVR: early virological response; RBV: ribavirin.

Group 1: full RBV dose, with Epoetin alpha addition; Group 2: RBV dose reduction, as *Standard of Care*; Group 3: controls, full RBV dose.

Values are expressed as the mean ± standard deviation (SD). The body-mass index is the weight in kilograms divided by the square of the height in meters. All liver specimens were assessed by a local pathologist for histology status and reviewed by one expert pathologist, blinded about specimens group origin.

P was calculated by multiple logistic regression analysis. ANOVA was also performed inside each group.

^a Early Virological Response is defined as unquantifiable HCV RNA level or HCV RNA reduction > 2 log₁₀ IU/mL at week 12.

†, ‡, § HCV RNA level: week 0 vs week 12 (EVR): p < 0.001 in the three groups (ANOVA).

Table 3. ETR and SVR rates comparison between the three study groups.

	G1 (67 pz)	G2 (67 pz)	G3 (40 pz)
EVR	62.7%	52.2%	65.0%
SVR	59.7%	34.3%	60.0%

ETR: G1 vs G2 p= ns; G2 vs G3 p= ns. SVR: G1 vs G2 p<0.01; G2 vs G3 p<0.01

Abbreviations: ETR: End of Treatment Response; SVR: Sustained Virological Response; G1: group 1 (epoetin alpha); G2: group 2 (reduction in RBV dose); G3: controls.

P values were calculated using Fisher's exact test. Two-tailed p value <0.01 was considered statistically significant.

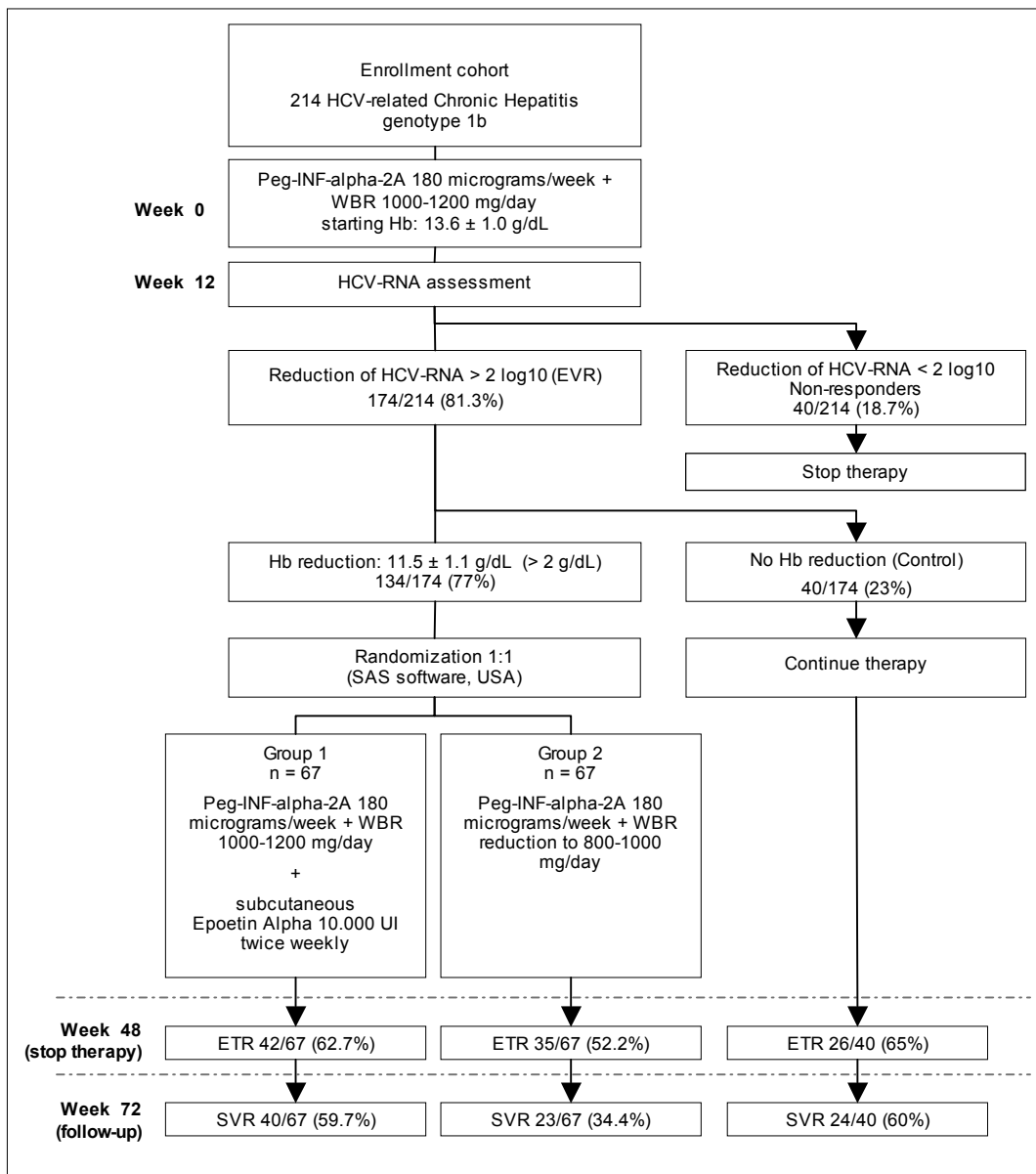


Fig. 1 – Flowchart of the study

Abbreviations: HCV: hepatitis C virus; PEG-IFN-alpha-2A: pegylated interferon alpha-2A; EVR: Early Virological Response; WBR: weight based ribavirin; Hb: haemoglobin; ETR: End of Treatment Response; SVR: Sustained Virological Response.

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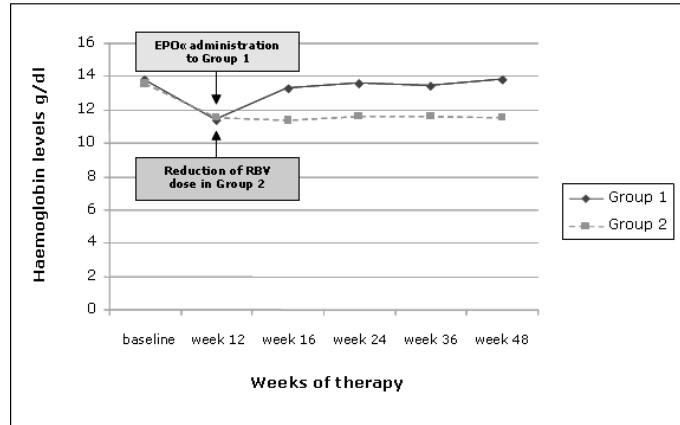


Fig. 2 - Comparison of haemoglobin levels between patients in group 1 treated with PEG-INF-alpha-2A and RBV at standard dose plus the addition of epoetin alpha and patients in group 2, undergone a reduction of RBV dose at week 12.
Abbreviations: RBV: ribavirin; Hb: haemoglobin.

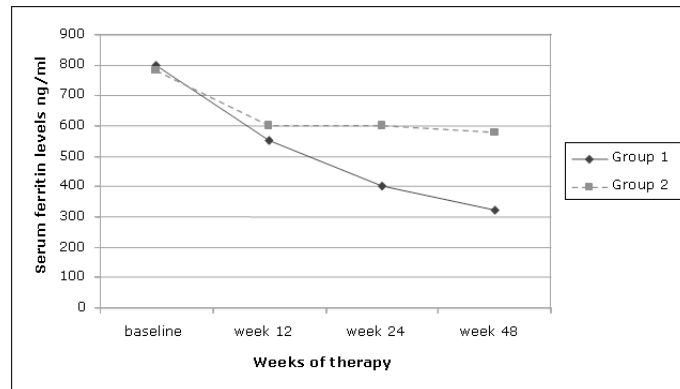


Fig. 3 - Comparison of serum ferritin levels between patients in group 1 undergone standard PEG-INF-alpha-2A and RBV dose with the addition of epoetin alpha and patients in group 2, undergone RBV dose reduction only.
Abbreviations: RBV: ribavirin.