

# Management of anemia induced by triple therapy in patients with chronic hepatitis C: Challenges, opportunities and recommendations

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## Summary

The addition of protease inhibitors, boceprevir or telaprevir, to peginterferon + ribavirin (PegIFN/RBV) increases the frequency as well as the severity, and hence, clinical relevance of anemia, which has now become one of the major complications associated with triple therapy. Most significant factors associated with anemia in patients receiving triple therapy include older age, lower body mass index (BMI), advanced fibrosis, and lower baseline hemoglobin. The variability in inosine triphosphate pyrophosphatase (*ITPA*) gene, which encodes a protein that hydrolyses inosine triphosphate (ITP), has been identified as an essential genetic factor for anemia both in dual and triple therapy. The correct management of anemia is based on anticipation, characterization and therapeutic management. Basically, anemia can be characterized in 3 types: ferropernic (mostly in fertile women), thalassemic type hemolytic anemia, and anemia from chronic processes. Functional deficit of iron should also be excluded in patients with normal ferritin and lower saturation of transferrin. Ribavirin dose reduction and epoetin, sequentially, are indicated in the management of anemia. Epoetin non-response can be caused by lack of time, type of anemia, functional iron deficit or erythropoietin resistance. In the transplantation setting, adding a protease inhibitor to PegIFN/RBV results in a significant increase in the incidence and severity of anemia and, as a consequence, a greater need for epoetin, transfusions, and ribavirin dose reductions. Packed red cell transfusions are utilized when hemoglobin decreases to less than 7.5 g/dl and/or there are clinical symptoms and/or there is no response to other therapeutic measures.

Keywords: Hepatitis C virus; Anemia; Boceprevir; Telaprevir; Pegylated interferon; Ribavirin; Epoetin; Protease inhibitor.

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Abbreviations: BMI, body mass index; *ITPA*, inosine triphosphate pyrophosphatase; HCV, hepatitis C virus; GWAS, genome-wide association study; SNP, single nucleotide polymorphisms; sRfT, transferrin soluble receptor; EPO, erythropoietin.

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## Introduction

Anemia is a major complication of antiviral therapy in chronic hepatitis C. With dual therapy, and despite its negative impact on quality of life, it was a desirable effect due to its association with higher sustained viral response rates. In patients treated with triple therapy, the impact of anemia on outcome is controversial; its incidence though is significantly higher and the management in this scenario is more complex, frequently requiring ribavirin dose reduction, epoetin and, in some cases, blood transfusions, jeopardizing the final efficacy of triple therapy.

In this review, we will try to answer the questions that physicians face regarding the management of anemia among patients treated with telaprevir or boceprevir triple therapy. We highlight the most relevant aspects with regards to the incidence of anemia, its clinical course, factors implicated in its development, characterization, and management.

## What are the definition, etiology (pathophysiological mechanism), incidence and natural course of anemia in patients receiving triple therapy?

Treatment of chronic hepatitis C depends on viral genotype. While patients with hepatitis C virus (HCV) genotype 1 are treated with triple therapy based on protease inhibitors (telaprevir or boceprevir) + peginterferon + ribavirin (PegIFN), those with HCV genotypes 2–6 are treated with dual therapy of PegIFN/RBV [1,2]. Anemia (defined in HCV studies as hemoglobin levels <10 g/dl) is one of the major adverse events in patients treated with PegIFN/RBV due to the hemolytic effect of ribavirin and, on rare occasions, the bone marrow depression induced by interferon. The addition of protease inhibitors, boceprevir, and telaprevir, increases the frequency as well as the severity, and hence, clinical relevance of anemia, which has now become one



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of the major complications associated with triple therapy [1]. In the pivotal studies of telaprevir and boceprevir, more than one in three patients developed anemia (hemoglobin <10 g/dl) with rates that varied between 29% and 49%, respectively [3–7]. More specifically, 34% and 8% of those treated with telaprevir-based therapy had a decrease in hemoglobin to levels <10 g/dl and 8 g/dl, respectively (compared to 14% and 2%, respectively, with dual therapy [5–7]). In the trials with boceprevir, the levels of hemoglobin were reduced to levels between 10 and 8.5 g/dl in 41–45% of patients (compared to 26% with dual therapy) and 5–9% reached levels of hemoglobin <8.5 g/dl (compared to 4% with dual therapy) [3,4]. In cirrhotic patients, the anemia is greater in severity and frequency than in non-cirrhotic patients [8]. In Phase II/III treatment-naïve patients receiving telaprevir, around 67% of cirrhotic patients developed significant anemia compared to 46% of non-cirrhotic individuals [6]. In treatment-experienced patients, these percentages were 42% vs. 27%, respectively [7]. In Phase III studies of treatment-naïve and non-responder patients receiving boceprevir, 54% of the cirrhotic patients vs. 46% of the F0–F2 patients developed anemia (defined as hemoglobin <11 g/dl) [4,5]. In clinical practice, studies including patients with cirrhosis or advanced fibrosis showed this percentage increased significantly. Nevertheless, less than 3% of patients discontinued therapy because of anemia attributable to protease inhibitors and/or ribavirin [8–10]. Observed rates of anemia in clinical practice and management outcomes are shown in Table 1.

In patients receiving dual therapy, the levels of hemoglobin decrease by about 3 g/dl during the first 3 months of therapy. The addition of a protease inhibitor (telaprevir or boceprevir) represents an additional decrease of around 1 g/dl as well as a modification in the rapidity with which these changes occur. The changes in hemoglobin concentration with telaprevir are practically identical to those of boceprevir during the first 3 months of triple therapy. A sharp decrease in the levels of hemoglobin occurs in the first 4 months, with the most significant decrease occurring between weeks 2 and 6. The lowest value of hemoglobin is observed between weeks 12 and 14. After this, the changes in hemoglobin concentration diverge between patients treated with telaprevir and those treated with boceprevir. At the end of treatment with telaprevir, at week 12, the values of hemoglobin increase gradually and reach the values of the control arm of dual therapy. Conversely, with boceprevir, the levels follow a pattern in which the decrease in hemoglobin concentration is maintained up to the end of treatment at week 48. Based on the natural history of anemia, most experts recommend baseline complete blood count before starting therapy followed by monitoring levels at weeks 2, 4, 8, and 12 during the first 12 weeks of therapy. In patients at high risk of anemia (see later) or those with comorbidities that increase the risks associated with anemia, stricter monitoring is required, i.e., weekly testing (Figs. 1 and 2).

### What baseline and on-treatment factors predict more severe anemia with triple therapy? Is there a factor predictive of a sharp decrease in hemoglobin concentration during treatment?

Factors associated with anemia both with dual and triple therapy are shown in Table 2. During dual therapy, factors predictive of anemia include age above 50 years, female gender, low platelet

count (<150,000 platelets/mm<sup>3</sup>), baseline renal dysfunction (creatinine >1.5 g/dl), fast decrease of hemoglobin (>1.5–2 g/dl in 15 days), high ribavirin dose (>12 mg/kg), haptoglobin phenotype (Hp 1-1) and the presence of high function inosine triphosphatase (*ITPA*) genotype [9,10]. Baseline factors associated with anemia among naïve patients treated with telaprevir-based triple therapy include low baseline hemoglobin, high ribavirin doses, older age, and presence of cirrhosis. In turn, baseline factors associated with anemia in experienced patients receiving telaprevir-based triple therapy include older age (>50 years), lower body mass index (BMI <25 kg/m<sup>2</sup>), and lower baseline hemoglobin [11]. In addition, when pretreatment factors were taken into account, low haemoglobin levels (<13 g/dl) at week 2 was a significant prognostic indicator for the development of treatment-emergent anemia. Both in naïve and experienced patients, women developed more frequently anemia than men, but this was thought to be related to lower baseline haemoglobin values, lower BMI, and higher ribavirin dosing in females [11]. In turn, in patients receiving boceprevir, the development of anemia is influenced by baseline levels of hemoglobin, female gender, older age (>40 years), use of statins, a decrease in hemoglobin during prior therapy (or during the lead-in phase) with PegIFN/RBV, and/or the rate of creatinine clearance [12,13]. The mechanisms that explain these associations are not completely understood. Low body mass index patients can be overexposed to protease inhibitors and/or ribavirin. In turn, patients with low baseline hemoglobin levels and older patients are inherently expected to be at greater risk of developing anemia; patients with advanced fibrosis, particularly cirrhotic patients, may have a special sensitivity to the hemolytic effects of ribavirin, or perhaps also to the central anemia induced by protease inhibitors. Finally, in patients treated with a lead-in phase with PegIFN/RBV, the decrease in the levels of hemoglobin can help predict further development of anemia, so that in those with a decrease of more than 3 g by week 4, the risk of developing anemia in the following 4 weeks of triple therapy is greater than 80%. Finally, genetic factors influence anemia in both dual and triple therapy [15]. The variability in inosine triphosphate pyrophosphatase (*ITPA*) gene, which encodes a protein that hydrolyses inosine triphosphate (ITP), has been identified as an essential factor in a genome-wide association study (GWAS) [10] in patients receiving PegIFN/RBV. Single nucleotide polymorphisms (SNP) rs1127354 and rs7270101 affect the expression of *ITPA*. Individuals with *ITPA* deficiency gain protection from the hemolytic anemia induced by ribavirin, as the accumulation of ITP in erythrocytes increases the toxicity of purine analogue drugs. Thompson *et al.* confirmed this finding in 304 patients with HCV-1, as well as the decrease of ribavirin dose reduction in patients with polymorphisms that cause ITPase deficiency [16]. Subsequent studies have shown similar conclusions in patients on dual therapy [17]. There are a few studies about the role of *ITPA* on triple therapy. Suzuki *et al.* [18] assessed 61 HCV-1 patients treated with triple therapy with PegIFN, ribavirin, and telaprevir. Ribavirin dose reduction and nadir haemoglobin were compared between patients with favourable (CA/AA) and unfavourable (CC) genotypes in the *ITPA* gene (rs1127354). Decreases in haemoglobin levels were greater in patients with CC than CA/AA genotypes at week 2 and week 4, as well as at the end of treatment. The total ribavirin dose during the overall period of 24 weeks of therapy was comparable between patients with CC and CA/AA genotypes. However, ribavirin dose had to be reduced more often in patients with CC than CA/AA genotypes

**Table 1. Observed anemia rate in clinical practice and its management.**

	CUPIC cohort [8]		EAP telaprevir cohort [33]	Veterans cohort [34]	
	TPV n = 205	BOC n = 292		TPV n = 198	BOC n = 661
Anemia (grade 2), Hb 8-9.9 g/dl	19%	23%	-	37%	43%
Anemia (grade 3-4), Hb <8 g/dl	12%	4%	29%	13%	7%
RBV dose reduction	13%	11%	34%	38%	44%
ESA use	54%	46%	24%	26%	25%
Blood transfusion	16%	6%	11%	8%	5%

TPV, telaprevir; BOC, boceprevir; RBV, ribavirin; ESA, erythropoietic stimulating agents.

while receiving telaprevir. The authors concluded that *ITPA* polymorphism influences haemoglobin levels during triple therapy, especially while telaprevir is given. Similar results were obtained by Chayama *et al.* [19]. They included 94 Japanese patients with HCV genotype 1 treated with PegIFN, ribavirin and telaprevir. Patients with unfavourable (CC) *ITPA* SNP rs1127354 genotype required RBV dose reduction earlier than did patients with other genotypes. Besides, sustained virological response was not associated with *ITPA* polymorphism. In summary, *ITPA* genotyping is able to detect HCV-infected patients, treated with dual or triple therapy, who have higher risk of developing anaemia. These patients should be monitored more closely with, perhaps, earlier reduction of ribavirin dose.

**Does the development of anemia impact sustained viral response?**

Interestingly, with dual therapy, the development of anemia, despite its association with poorer quality of life and potential development of severe complications, was considered a desirable effect since it was an indirect marker of higher efficacy, i.e., developing anemia was an indirect confirmation that the patient had adhered to the treatment, and that the ribavirin molecule had entered not only the hepatocyte but also the erythrocyte and, in doing so, had led to the adverse as well as desired effects at the same time.

In patients receiving telaprevir in Phase III trials (ADVANCE and ILLUMINATE), in which the percentage of cirrhotic patients was low, the efficacy was similar regardless of the development of anemia (74% in those with anemia vs. 73% in those without) or the need for ribavirin dose reduction (76% in those requiring ribavirin dose reductions vs. 72% in those without dose reductions) [20]. The results with boceprevir are different. In a *post-hoc* analysis of Phase III trials (SRINT-2 & RESPOND-2), SVR rates were greater in patients who developed anemia compared to those who did not develop this complication [13]. A sustained viral response was achieved in 58% (212/363) of treatment-naïve patients who did not develop anemia, 74% (95/129) of those who developed anemia and were treated with erythropoietin, 78% (29/37) of those who developed anemia and needed a dose reduction of ribavirin, 71% (109/153) of those who needed erythropoietin and ribavirin dose reductions and in 68% (30/44) of those who developed anemia and did not need specific treatment. In treatment-experienced patients with treatment failure (relapsers or partial responders), a sustained viral response was achieved in 50% of those who did not develop anemia compared to 76% of those who did.

**What is the appropriate dose of ribavirin in triple therapy? Is ribavirin necessary with the use of the new protease inhibitors?**

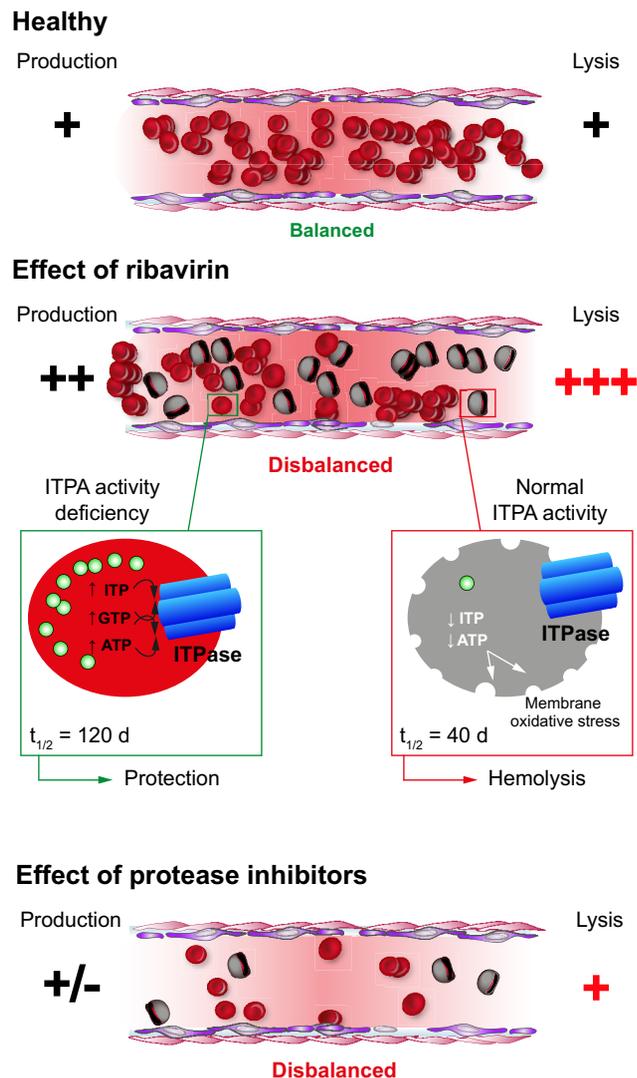
The optimum dose of ribavirin depends on body weight. In the PegIFN alfa-2b registration study, 10.6 mg/kg/d was established as the minimum effective dose. Subsequently, the results of treatment with PegIFN/RBV established the initial treatment dose of 15 mg/kg/d [1,3]. In clinical practice, when PegIFN alfa 2a is administered in patients of <75 kg body weight, a dose of 1000 mg/d is used while 1200 mg/d is recommended for body weight ≥75 kg. If PegIFN alfa 2b is used, the recommended dose is 800 mg/d for patients ≤65 kg, 1000 mg/d for patients between 65 and 85 kg, 1200 mg/d for those between 85 and 105 kg, and 1400 mg/d for ≥105 kg bodyweight. Currently, there is no evidence to justify the use of a lower initial dose. Besides, low-dose ribavirin was associated with a high rate of viral breakthrough and rate of relapse in a phase II study of naïve patients [14]. In this phase II study, a cohort of patients received BOC-based triple therapy with a low dose of ribavirin (ranging from 400 to 1000 mg/d). The sustained viral response rate was only 35% compared to 75% in the cohort receiving triple therapy and a high dose of ribavirin (800–1400 mg/d). In the near future, data from clinical trials that are being conducted in specific populations (co-infected or transplanted) may indicate that a lower, fixed dose of 800 mg can be adequate with triple therapy. Although ribavirin dose reductions (and perhaps lower starting doses) do not impair sustained viral response rates (see question 5), ribavirin is necessary when using protease inhibitors to achieve sustained viral response rates greater than those achieved with dual therapy. If ribavirin is discontinued, it should be restarted within 3 days of its discontinuation. If not possible, protease inhibitors should be also discontinued.

**What is the most effective strategy to manage anemia during triple therapy: to decrease ribavirin dose or to administer epoetin? Do the reductions of ribavirin dose compromise sustained viral response rates? Are transfusions a good solution in the presence of anemia, and when should they be administered?**

The correct management of anemia is based on **anticipation, characterization, and therapeutic management**. In order to **anticipate** the development of anemia, the dose of ribavirin should be adjusted to the patient's weight; baseline hemoglobin levels should be measured and if found to be low, strict

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**Fig. 1. Representation of the mechanism of anemia in patients receiving PegIFN/RBV or protease inhibitor-based triple therapy.** A balance between production and lysis is crucial. At the top, equilibrium between lysis and production in healthy people. In the middle, role of ITPA on ribavirin-induced hemolytic anemia, showing an overwhelming lysis. At the bottom, central anemia induced by protease inhibitors in which red cell production is compromised.

monitoring should be implemented. Both the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Disease (AASLD) recommend levels of haemoglobin  $>12$  g in females and  $>13$  g in males to start treatment [1,2]. Initiating therapy below these values is not contraindicated but each case needs to be assessed individually due to the potential for severe adverse events. Furthermore, in a patient with a history of clinically significant anemia in previous dual therapy, the patient should be informed that the anemia produced by dual therapy can be exacerbated by the addition of a protease inhibitor. These cases require a closer monitoring, mostly in the presence of comorbidities. Since both protease inhibitors are capable of producing clinically significant anemia, a history of anemia during a previous treatment should not influence the election of a specific protease inhibitor. In addition, the

doses of protease inhibitor should not be modified. If considered appropriate, in some cases, the dose of ribavirin can be reduced and/or epoetin administered preemptively [13].

**The characterization of anemia** is the second useful tool in the management of this complication. In general, there are 3 types of anemia: ferropenic anemia (mostly in fertile women), thalassemic type hemolytic anemia, and anemia from chronic processes. In the latter, a functional deficit of iron, a clinical situation that occurs in patients with ferritin levels higher than 100 mg/ml despite the presence of ferropenia, might be involved. In these cases, ferritin acts as an acute phase reactant generating false negative results. Transferrin saturation index, levels of transferrin soluble receptor (sRfT) and the reticulocytic hemoglobin concentration should be measured to make a definitive diagnosis. A functional iron deficit is characterized by a transferrin saturation index  $<20\%$ , a concentration of sRfT  $>5$  mg/dl or an sRfT/log quotient of ferritin  $<1.5$ . In patients treated with PegIFN, ribavirin and protease inhibitors, 3 types of anemia have been described: (i) hemolytic anemia characterized by degradation of haptoglobin; (ii) central anemia characterized by the absence of a reticulocyte response; and (iii) mixed anemia in which hemolysis and central anemia occur simultaneously. Both, **reduction of ribavirin** dose and administration of **epoetin** are used in the **management of anemia**. In the telaprevir trials registered to date, the use of epoetin was not allowed and the management of anemia was solely based on decreasing the ribavirin dose. Conversely, in the registered trials of boceprevir, dose reductions of ribavirin as well as epoetin administration were allowed at the discretion of the investigator. Retrospective analyses of these registered trials have shown that with both protease inhibitors, the decrease of ribavirin dose does not compromise sustained viral response rates. Interestingly, in patients treated with dual therapy, a decrease in the ribavirin dose was shown to compromise the success of therapy, mostly if the reduction was performed in the first weeks of therapy when the patient was still viremic [21]. However, the results of this study were not confirmed in the *post-hoc* analysis of the IDEAL study [22]. Moreover, sub-analyses of the studies using triple therapy advocate that the dose of ribavirin can be safely reduced without compromising the efficacy, irrespectively of the timing of dose reduction or the detectability of HCV RNA in serum [23]. Indeed, in subanalysis of the registered trials of triple therapy with telaprevir in treatment-naïve patients (ADVANCE and ILLUMINATE), no significant differences in sustained viral response rates were observed between patients in whom the dose of ribavirin was reduced vs. those in whom it was not (79% vs. 75%, respectively) [13]. The same results were obtained when analyzing previously-treated patients (REALIZE). In this setting, the outcomes were independent of the type of previous response: relapsers (82% vs. 83%), partial responders (66% vs. 50%) or non-responders (31% vs. 45%) [13]. Similar results were obtained in the subanalysis of registered trials of triple therapy with boceprevir in treatment-naïve patients (SPRINT-2) and previously-treated patients (RESPOND-2) in which there were no differences in the rates of sustained viral response between patient in whom the dose of ribavirin was reduced vs. those in whom it was not [24]. In a recent randomized study, ribavirin dose reduction was compared to epoetin use in the management of anemia induced by triple therapy with boceprevir in treatment-naïve patients [12]. Patients were randomized when they became anemic (hemoglobin  $<10$  g/dl, or if the rate of hemoglobin decline suggested that the value would

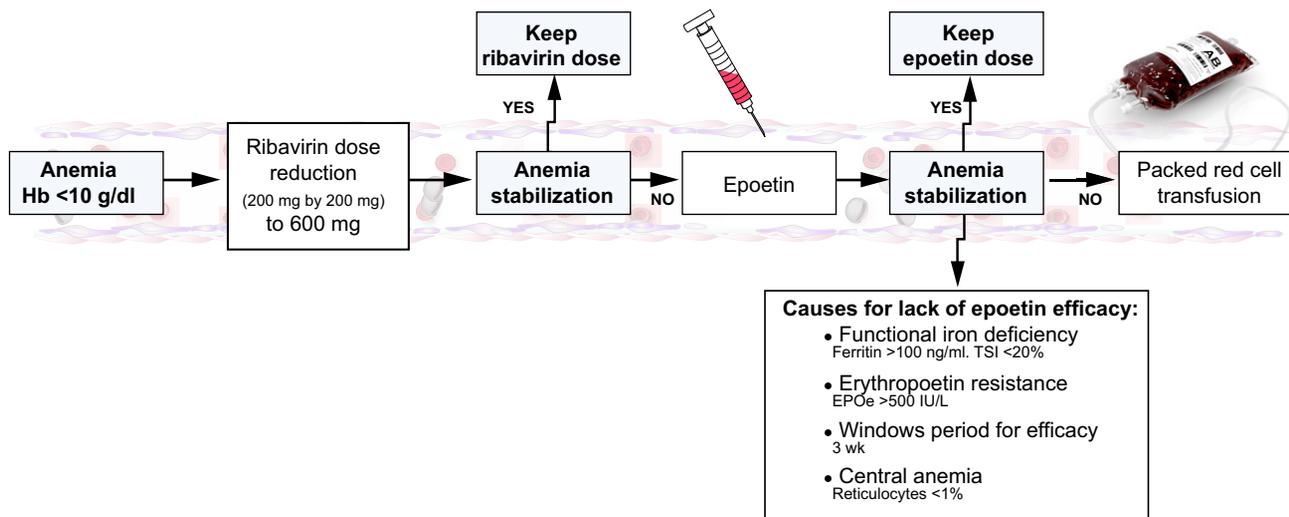


Fig. 2. Management of anemia in patients with chronic hepatitis C genotype 1 receiving protease inhibitor-based triple therapy with telaprevir or boceprevir.

be <10 g/dl before the next protocol specified visit and the value was <11 g/dl) to receive epoetin (40,000 IU s.c./week) or dose reduction of ribavirin (200 mg in 200 mg). There were 687 patients included. Anemia (hemoglobin <10 g/dl) developed in 500 patients (72%) who were then randomized to receive epoetin (n = 251) or ribavirin dose reduction (n = 249). The rates of sustained viral response were almost identical; 178/249 (71%) in the dose reduction arm and 178/251 (71%) in the epoetin arm. Indeed, sustained viral response rates were similar between both arms regardless of timing of the first ribavirin reduction and HCV-RNA detectability at the time of randomization. Furthermore, in the registered trials as well as in this prospective study, no significant differences were observed in sustained viral response rates as a function of ribavirin dose reduction up to 50%. The viral response was impaired if the ribavirin dose was lower than 50% (sustained viral response of only 18%). There is a paucity of data on the influence of ribavirin reduction in cirrhotic patients due to the low number of cirrhotic patients having been enrolled either in pivotal trials with telaprevir and boceprevir, or in randomized trials aimed at assessing the best strategy for anemia management. In the above trial, no major differences in sustained viral response were observed in cirrhotic patients (n = 60) who had ribavirin dose reduction vs. those who were treated with epoetin (57% vs. 64%). It is important to note that 38% of patients randomized in the EPO arm needed a secondary intervention to control the anemia while this second intervention was only needed in 18% of the patients in the ribavirin arm. This treatment failure rate occurred in 56% of cirrhotics. In this trial, the ribavirin dose reduction was performed by increments of 200 mg and of 400 mg if the initial dose of RBV was 1400 mg/day. In summary, ribavirin dose reduction, its magnitude and the timing when it occurs have no influence on viral clearance rates. Based on these results, ribavirin dose reduction has become the first measure in the management of anemia. Whether the reduction should be performed progressively (200 mg in 200 mg) or more abruptly (directly to 600 mg) requires further investigation. In addition, given that the data from clinical trials are very limited regarding subgroup analyses, there still remains a need to confirm whether the trials' findings remain true in standard clinical practice, particularly when treating cirrhotic patients.

In case of no response to ribavirin dose reduction, epoetin can be administered. However, ribavirin has an unusual pharmacokinetic profile that does not follow the standard models of absorption, distribution, and elimination. It is characterized by a very slow, and variable, eliminatory phase following rapid absorption and distribution phases. The half-life of elimination increases progressively from 79 h with a single dose up to 274–298 h (about 12 days) with multiple doses [24]. Based on these data, waiting 7–10 days to check whether ribavirin dose reductions have resulted in an effective response seems an appropriate time-frame. In addition, and based on available data, it seems appropriate to maintain, in case of anemia, a ribavirin dose >60% of the baseline recommended dose and, if possible, to complete at least 80% of the scheduled treatment duration [12,15,21]. Once the protease inhibitor is discontinued, evidence suggests that while there is no contraindication to increasing the dose of ribavirin, this strategy is not strictly required, i.e., the development of anemia probably reflects a high exposure and sensitivity to ribavirin. In cases in which the reductions of doses are drastic (with doses of 600 mg/d), the aim should be to achieve a total dose ≥60% of the dose prescribed [2].

**Erythropoietin (EPO)** is an essential factor in the production of red cells. Essentially, EPO is produced in adults in the kidneys (90–95%), especially in the interstitial fibroblastic cells in the internal cortex, and in smaller amounts in the liver, spleen, lungs, testicles, and brain. Treatment with EPO (or “epoetin”, as the pharmaceutical preparation is termed) is, in general, well tolerated and severe adverse events are rare. Pain at the injection site, headache and arterial hypertension are the most frequently reported. Isolated cases of convulsion, thrombotic phenomena, and red cell aplasia have also been described.

Epoetin is indicated in cases of ferropenic anemia, loss of red cells (bleeding), increases in red cells destruction (associated with anti-neoplastic, anti-viral or anti-inflammatory therapies) and anemia due to reduced/lack of endogenous erythropoietin typical of end-stage renal diseases. Different types of EPOs are available: alpha epoetin, beta epoetin, omega epoetin, theta epoetin, alfa darbepoetin, methoxy-polyethylene glycol beta epoetin, and activating agents of the endogenous erythropoietin receptor.

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**Table 2. Predictive factors associated with anemia in patients treated with dual or triple therapy.**

Type of treatment/Factor	Dual therapy	Triple therapy with telaprevir	Triple therapy with boceprevir
Age	>50 yr	>50 yr	>40 yr
Sex	Female	Female in univariate analysis	Female
Body mass index		<23 kg/m <sup>2</sup>	
Statin use			Statin use
Baseline hemoglobin levels	Lower baseline hemoglobin levels	Lower baseline hemoglobin levels	Lower baseline hemoglobin levels
Stage of disease	Cirrhosis	Advanced fibrosis	Advanced fibrosis
Renal function	Creatinine >1.5 mg/dl; creatinine clearance <80 ml/min		Creatinine clearance <80 ml/min
Ribavirin dose	>12 mg/kg		
<i>ITPA</i> polymorphism	<i>ITPA</i> polymorphism	<i>ITPA</i> polymorphism	<i>ITPA</i> polymorphism
On-treatment factors	Fast hemoglobin drop during the first weeks of treatment (>1.5-2 g/dl at week 2)	Low hemoglobin levels (<13 g/dl) at week 2	Degree of hemoglobin decrease during the lead-in phase

Those that have been most frequently used in the management of anemia associated with triple therapy are alfa epoetin (40,000 IU/week) and alfa darbepoetin (1.5 µg/kg/week) although, to date, there are no convincing scientific arguments in favour of either one. Although there is no fixed protocol as to how and when to initiate the administration of erythropoietin, most experts recommend its initiation if after three steps of 200 mg/d dose reductions over a period of 3 weeks, there is a failure to achieve recovery or stabilization of hemoglobin levels. The nadir at which to initiate erythropoietin is not fixed; generally experts recommend initiating EPO when the levels of hemoglobin remain lower than 10 g/dl despite ribavirin dose reductions. In patients with sharp decreases in hemoglobin (2 g/dl in 2 weeks, 3 g/dl in 4 weeks or 4 g/dl relative to baseline), but with levels between 10 and 12 g/dl, there needs to be a clinical assessment of the risk-benefit for the patient [25,26]. If there is resolution of symptoms and/or if the values of hemoglobin reach 10 to 12 g/dl, either the dose should be reduced or, alternatively, the interval extended until an optimal schedule is reached, which should then be maintained during the remaining duration of antiviral treatment. The doses of epoetin alfa and beta need to be decreased by 25% of the baseline dose in case of increases of >1 g/dl hemoglobin after 2 weeks of treatment, while a decrease of 49% is recommended when the drug used is darbepoetin. Discontinuation of erythropoietin should be considered in patients who do not respond after 8 weeks of therapy or if hemoglobin is >12 g/dl, as recommended by EMEA. There are no published data regarding predictive factors of response to epoetin during antiviral therapy. In other diseases, an increase in hemoglobin of at least 1 g/dl in the 4th week of EPO as well as low baseline levels of endogenous erythropoietin (in most studies the cut-off value is 100 mU/ml) are two parameters that have been significantly associated with response to EPO. Conversely, elevated baseline levels of erythropoietin (>500 mU/ml) and deficits of iron, vitamin B12 or folate have been associated with absence of response [24–26].

**A lack of response to epoetin** can be due to 3 main factors: (a) lack of time, i.e., the decrease in the levels of hemoglobin is very rapid and the EPO cannot promote a marrow response with enough celerity. It is estimated that erythropoietin requires about 3 weeks to generate an increase in the production of erythrocytes. Hence, in order to deal appropriately with this complication, anticipation is

crucial so that EPO therapy can be initiated before the first signs of progressive anemia develop; (b) the type of anemia, i.e., in patients with central type anemia with bone marrow suppression; (c) functional iron deficit. Indeed, absolute and functional iron deficit is the principal reason for the lack of response to epoetin in circumstances other than hematological neoplasm. If EPO is considered, levels of ferritin should not be <100 mg/dl and transferrin saturation not be <20%. In these cases, the administration of parenteral iron can increase the possibilities of response to EPO treatment. On rare occasions, there is B12 and, particularly, folate deficiency due to excessive consumption as a result of the expansion of the red cell population. Since these deficits occur infrequently, the systematic use of B12 and folate is not recommended, and should only be prescribed if a deficit has been detected by laboratory analysis; (d) erythropoietin resistance, which is observed in patients with plasma levels of erythropoietin >500 IU/L. Normal levels of endogenous erythropoietin vary between 10 IU/L and 30 IU/L. In patients with chronic hepatitis C treated with antivirals, a linear increment in the levels of endogenous erythropoietin occurs as anemia develops, with levels stabilizing around 200–400 IU/L. In patients with very high levels of erythropoietin, the use of exogenous EPO is not indicated due to the low probability of inducing a response.

**Packed red cell transfusions** are indicated when hemoglobin decreases to less than 7.5 g/dl and/or there are clinical symptoms and/or there is no response to other therapeutic measures. The transfusion of 400 ml packed cells should result in an increase in hemoglobin levels of 1.5 g/dl. Hence, the recommendation is to transfuse 2–3 units of packed cells to solve an acute episode while, at the same time, avoiding complications resulting from overload if larger quantities are administered.

**In conclusion**, the clinical status should guide our strategy to manage anemia. Usually, a decrease in the concentration of hemoglobin to less than 10 g/dl is associated with clinical symptoms requiring ribavirin dose reduction. If the levels of hemoglobin continue to decrease and/or symptoms do not improve, epoetin should be administered. In severe cases of anemia (hemoglobin ≤7.5 g/dl, hemodynamic instability), a transfusion may be required. This is especially relevant for patients with comorbidities, older age or ischemic heart disease. In these patients, the dose of PegIFN should be reduced at the same time

as ribavirin, when hemoglobin decreases to <10 g/dl. Triple therapy should be discontinued when there is a decrease in the hemoglobin level below 7 g/dl and/or when there is hemodynamic compromise (decrease of 2 g/dl in 2 weeks, 3 g/dl in 4 weeks, or 4 g/dl from baseline hemoglobin) and stabilization of hemoglobin >8.5 g/dl has not been achieved following the use of transfusion of packed red cells. If ribavirin is discontinued for a prolonged period of time due to lack of response, protease inhibitors should be discontinued as well.

**Do the incidence, natural course and management of anemia differ in the liver transplantation setting?**

In liver transplant recipients treated with PegIFN/RBV, treatment is associated with several adverse effects, particularly hematologic toxicity; the management has been treatment discontinuation and dose reductions in approximately 27% and 73% of patients, respectively [27–29]. Dose reduction or discontinuation of therapy due to anemia has been reported in 22% to 40% of treated recipients [30]. While the mechanisms involved in the development of anemia, such as interferon-related bone marrow suppression, myelosuppression due to concomitant therapies, renal insufficiency, HCV-interference with erythropoietic production, and, above all, ribavirin dose-dependent hemolysis are the same as those described in the immune competent population, their prevalence, particularly that of renal insufficiency and myelosuppression due to concomitant therapies, is higher in the liver transplant setting. In a recent study based on 164 liver transplant patients (75% men; mean age 55 years, range: 35–75) treated with PegIFN/RBV, 70% of patients developed anemia (hemoglobin <10 g/dl) and 40% developed significant anemia (hemoglobin drop >5 g/dl). Factors independently associated with anemia, and more specifically with the significant anemia, included renal insufficiency (RR: 0.97, 95%CI: 0.95–0.99; *p* = 0.03), longer time from transplantation to therapy (RR: 1.001, 95%CI: 1.000–1.001; *p* = 0.002), high baseline viremia (RR: 3.2, 95%CI: 1.3–8.1, *p* = 0.01), cyclosporine-based immunosuppression (RR: 0.4, 95%CI: 0.2–0.99; *p* = 0.049), and use of miconophenolate mofetil (RR: 3.4, 95%CI: 1.1–10.7; *p* = 0.03). Anemia was not associated with early viral response, end-of-treatment response, relapse or sustained viral response, PegIFN dose reduction or premature treatment discontinuation, but resulted in ribavirin dose reductions (*p* = 0.0001) [31]. Data with triple therapy in the liver transplant setting are still preliminary but, as with cirrhotic patients, it seems that adverse events, particularly anemia, are more frequent and severe compared to those seen with dual therapy. In a recent report on 18 boceprevir- and 19 telaprevir-treated liver transplant recipients, 100% and 84% developed anemia, which was severe (<8 g/dl) in 39% and 15%, respectively. Epoetin was used in >90% of cases, transfusions in 33% and 16%, respectively, and ribavirin dose reductions were required in more than two third of cases [32]. As with dual therapy, anemia did not seem to have an effect on on-treatment viral response. No data on sustained viral response have been provided, as yet. In conclusion, in liver transplant patients with recurrent hepatitis C treated with PegIFN/RBV, anemia is a very frequent complication, with almost 70% of patients developing a hemoglobin of <10 g/dl compared to 30% in immune competent patients, and a reduction of hemoglobin >5 g/dl in 41% of treated patients compared to 20–25% in non-transplanted patients.

Anemia results in ribavirin dose reductions and frequent use of EPO (60% compared to only 15% in immune competent patients treated with dual therapy). Adding a protease inhibitor to PegIFN/RBV results in increased incidence and severity of anemia and, as a consequence, a greater need for epoetin, transfusions and ribavirin dose reductions [32].

**Key Points 1**

In the registration trials, triple therapy with boceprevir or telaprevir was associated with an increase in the incidence and severity of anemia in comparisons with PegIFN plus ribavirin

**Key Points 2**

The incidence of anemia is even greater in real practice cohorts, especially in advanced fibrosis and in transplanted patients

**Key Points 3**

Age, female gender, low baseline hemoglobin and cirrhosis are the main factors associated with higher incidence of anemia during triple therapy

**Key Points 4**

Ribavirin dose reduction should be the preferred first strategy for anemia management. Epoetin belongs to the second line and pack red blood transfusion are eventually needed in a subgroup of patients

**Key Points 5**

The reduction of ribavirin does not adversely impact the ultimate SVR rate. This information is not very robust in difficult-to-cure patients, especially in patients with cirrhosis

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**Conflict of interest**

The declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Review

# Review

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