REVIEW

# Critical review of the use of erythropoietin in the treatment of anaemia during therapy for chronic hepatitis C

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SUMMARY. Combined pegylated interferon (PegIFN) and ribavirin represents the standard therapy for patients with chronic hepatitis C (CHC), which allows for sustained viral response (SVR) in up to 90% of patients depending on certain viral and host factors. Clinical studies have demonstrated the importance of adherence to therapy, that is, the ability of patients to tolerate and sustain a fully dosed therapy regimen. Adherence is markedly impaired by treatment-related adverse effects. In particular, haemolytic anaemia often requires dose reduction or termination of ribavirin treatment, which compromises treatment efficacy. Recent evidence points to a beneficial role of recombinant erythropoietin (EPO) in alleviating ribavirin-induced anaemia thereby improving quality of life, enabling higher ribavirin dosage and consequently improving SVR. However, no general consensus exists regarding the use of EPO for specific indications: its optimal dosing, treatment ben-efits and potential risks or cost efficiency. The Swiss Association for the Study of the Liver (SASL) has therefore organized an expert meeting to critically review and discuss the current evidence and to phrase recommendations for clinical practice. A consensus was reached recommending the use of EPO for patients infected with viral genotype 1 developing significant anaemia below 100 g/L haemoglobin and a haematocrit of <30% during standard therapy to improve quality of life and sustain optimal ribavirin dose. However, the evidence supporting its use in patients with pre-existing anaemia, non-1 viral genotypes, a former relapse or nonresponse, liver transplant recipients and cardiovascular or pulmonary disease is considered insufficient.

Keywords: chronic hepatitis C, erythropoietin, growth factors, haemolysis, peginterferon, quality of life.

## INTRODUCTION

Chronic hepatitis C (CHC) affects approximately 170 million individuals worldwide and can – if left untreated – progress to chronic liver failure owing to cirrhosis, hepatocellular cancer and death [1,2]. Treatment for CHC with a combination of pegylated interferon-α (PegIFN) and ribavirin has significantly improved therapeutic outcomes with sustained viral response (SVR) rates between 45% and 90% depending on viral genotypes, viral load and certain host factors such as age, gender, body weight and alcohol and cannabis con-sumption [3] and, likely, genetic variants that allow for an optimal interferon-mediated immune response [4,5]. However, despite major advances in treatment efficacy, treatment-associated adverse effects occur frequently and compromise quality of life (QoL), physical and mental performance and the overall ability to tolerate fully dosed therapy [6–8]. In addition, combined antiviral therapy can induce significant haematological complications in more than 30% of patients such as neutropenia, thrombocytopenia and anaemia [9–12]. Current recommendations to manage these haematological effects consist of dose reduction or cessation of PegIFN, ribavirin or both [13,14]. However, PegIFN or ribavirin dose reductions increase the rates of primary nonresponse to treatment and posttreatment relapse [15,16]. Therefore, the intention to avoid dose reductions has stimulated the widespread 'off-label' use of

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recombinant erythropoietin (EPO). In addition, several clinical trials investigating the adjuvant administration of erythropoietic growth factors in patients undergoing combination antiviral treatment for CHC have suggested a significant benefit of such intervention to improve anaemia and QoL [17,18] and to maintain full-dose ribavirin and possibly SVR rates [19,20]. Although EPO is routinely used by many clinicians, no accepted standards exist regarding the use of EPO for specific indications: its dosing, type of EPO, treatment benefits, potential risks and finally, cost effectiveness [21].

In March 2010, the Swiss Association for the Study of the Liver (SASL) has organized an expert meeting of academic hepatologists representing the largest referral centers for hepatitis C virus (HCV) patients of Switzerland to critically review and discuss the current scientific evidence supporting the use of EPO in CHC.

The panel of experts consented to recommendations on the use of EPO because the body of corresponding scientific literature was considered sufficient to yield an evidence-based agreement, whereas reports on the use of darbepoietin, granulocyte colony-stimulating factor (G-CSF) and thrombopoietin (eltrombopag) were felt to be too preliminary.

Prior to the expert meeting, randomized trials, uncontrolled clinical studies and case series on the use of EPO to treat ribavirin-induced anaemia in patients with CHC published between 1990 and 2010 were searched in PubMed and EMBASE databases using the terms 'chronic hepatitis C', 'epo', 'epoetin' 'erythropoietin', 'growth factors', 'haemolysis', 'peginterferon' and 'quality of life'. No language restriction was employed. Retrieved publications were searched for yet unidentified publications, reviewed and discussed, and graded according to a system proposed by the Oxford Centre of Evidence-Based Medicine (http://www.cebm.net/index.aspx?o=1023). Therein, the grade of evidence is classified into five levels and several sublevels leading to three different strengths of recommendation (Table 1).

**Table 1** Level of evidence / strength of recommendation

| Recommendation | Grade of evidence | Type of study |
|---|---|---|
| A | Ia | Meta-analysis of RCT |
| | Ib | At least one RCT |
| B | IIa | At least one controlled study |
| | IIb | Well-designed cohort study |
| C | IIIa | Systematic review of case–control studies |
| | IIIb | At least one case–control study |
| C | IV | Case series |
| C | V | Expert opinion |

RCT: randomized, controlled trial.

## DEFINITION AND INCIDENCE OF HAEMOLYTIC ANAEMIA DURING CHC THERAPY

Haemolytic anaemia is common during the treatment of HCV-infected patients with PegIFN and ribavirin. Registration trials with low ribavirin doses of 800 mg reported an incidence of anaemia in 9–13%, which rose to up to 37% in studies applying weight-based dosages of ribavirin [9–12]. In a retrospective analysis from Gaeta and co-workers, 108 (24.5%) of 441 patients treated for HCV infection with standard interferon and ribavirin discontinued therapy because of adverse effects, particularly during the first 6 months of treatment [22]. Haemolytic anaemia represented the most frequent cause with 36.1% of all discontinuations.

Clearly, the true incidence of significant anaemia requiring treatment modifications depends on its definition. The World Health Organization (WHO) defines anaemia as haemoglobin (Hb) levels <12 g/dL in women and <13 g/dL in men (http://whqlibdoc.who.int/publications/2008/9789241596657_eng.pdf). The National Anaemia Action Council (NAAC) defines anaemia as a 'reduction in the number of circulating red blood cells, Hb concentration or volume of packed red cells in the blood' (http://www.anemia.org/professionals/monograph) below the lower end of a normal range of values for age- and sex-matched subjects. However, neither the WHO nor the NAAC statements define thresholds at which the risks of treatment-related anaemia outweigh the potential benefits of an intervention. In the setting of CHC, NAAC recommends that 'patients with pre-existing haemolysis or anaemia (Hb <11 g/dL or haematocrit <33%) should not receive ribavirin'. The manufacturers of ribavirin advise a dose reduction at Hb levels below 10 g/dL and discontinuation of ribavirin at a level below 8.5 g/dL based on the two corresponding registration trials [23,24]. Both threshold values correspond to grades I (10 g/dL) and II (8.5 g/dL) of toxicity, respectively, as suggested by the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm) (*grade of evidence V, recommendation C*).

## PATHOPHYSIOLOGY AND RISK FACTORS OF ANAEMIA DURING HCV THERAPY

While much of the hardships of anti-HCV therapy relate to adverse reactions to PegIFN, anaemia is predominantly because of ribavirin. In a study with 133 patients treated for CHC with either interferon-α or PegIFN, with or without ribavirin, patients receiving combination therapy showed Hb decline of 2–3 g/dL within the first 4 weeks of therapy. Patients receiving interferon- or PegIFN monotherapy had only mild haemoglobin reductions [25], which are likely related to interferon-mediated myelosuppression with consecutive pancytopenia [26]. Ribavirin-induced haemolytic

anaemia is still incompletely understood but clearly dose-dependent and significantly increases at doses >800 mg/day. It is likely related to its marked accumulation in erythrocytes up to 60-fold compared with plasma levels with a half-life of 40 days [27]. Ribavirin is delivered into erythrocytes by a membrane transporter termed equilibrative nucleoside transporter 1 (ENT1), which facilitates the bidirectional diffusion of nucleosides across the cell membrane in a concentration-dependent manner [28] and is converted into ribavirin mono-, bi- and finally triphosphate in erythrocytes. Because of a lack of phosphatases, red blood cells cannot hydrolyse these triphosphates, which leads to ribavirin accumulation and depletion of adenosine triphosphate (ATP). ATP deficiency is believed to render erythrocytes more amenable to oxidative stress and subsequent haemolysis [29]. Support for this hypothesis derives from a study by Grattagliano and co-workers, who showed that low erythrocyte membrane protein sulfhydrils prior to therapy are predictive of ribavirin-induced major haemolysis [30]. *In vitro* incubation of red blood cells with glutathione ester and dipyridamol reduced ribavirin-associated oxidative stress.

Clinically, various risk factors have been identified rendering subjects more susceptible to ribavirin-induced anaemia (Table 2), and clinical studies have consistently demonstrated a higher risk for ribavirin-induced anaemia in women and patients with >50 years of age, pretreatment platelet counts <150 000/mm$^3$, elevated serum creatinine levels and a rapid Hb decline of >2 g/dL within the first 2 weeks of treatment ('2-by-2 rule').

Ribavirin-induced anaemia is also likely influenced by host genetic factors as demonstrated by the increased risk of anaemia in patients with Asian ethnicity [31–33]. A recent genome-wide association study showed that a single-nucleotide polymorphism (SNP) within the inosine triphosphatase gene (rs6051702 *ITPA*) confers protection against ribavirin-associated haemolytic anaemia with an impressive genome-wide significance level of $P = 1.1 \times 10^{-45}$ [34,35].

**Table 2** Risk factors for ribavirin-induced haemolytic anaemia

| Risk factor |
| --- |
| Age (>50 years) |
| Female gender |
| Low platelet levels (<150.000/mm$^3$) |
| Elevated serum creatinine concentration |
| Rapid haemoglobin decline after start of therapy (>1.5–2 g/dL within 2 weeks; '2-by-2 rule') |
| Ribavirin dose >12 mg/kg |
| Haptoglobin phenotype (Hp 1-1) ? |
| *ITPA* gene polymorphism |

## CLINICAL SIGNIFICANCE AND MANAGEMENT OF HAEMOLYTIC ANAEMIA

In many nonliver diseases, anaemia is associated with an increased morbidity and mortality, such as in haemodialysis patients who face a higher mortality with haematocrit levels <30% compared with those with levels >36% [36]. Similarly, anaemia increases mortality in patients after acute myocardial infarction, and in turn, blood transfusions may improve 30-day mortality in patients with haematocrit values lower than 33% [37]. Such associations have not been reported for patients developing anaemia during antiviral therapy for CHC, possibly because of their generally younger age and lack of co-existing diseases in which anaemia may precipitate major complications, e.g. cardiovascular and pulmonary events [38]. However, even without side effects from antiviral therapy, patients with CHC are burdened with a reduced QoL. Thus HCV-infected patients are afflicted by considerable fatigue, impaired ability to work and anxiety and often perceive HCV infection as a strong stigmatization. These consequences from infection with HCV are further worsened by combined antiviral treatment [7,8,39]. Importantly, evidence indicates that HCV-infected patients with low QoL scores are more likely to discontinue therapy prematurely [6].

Relevant issues of haemolytic anaemia are the extent and velocity of its evolution. Patients with a rapid decline are at risk of further progression of anaemia and are more likely to become intolerant against anaemia than those who present with a less acute drop of haemoglobin [33]. A decrease in Hb below 10 g/dL occurs in approximately 20% and below 8.5 g/dL in 5% of patients.

The magnitude of haemoglobin reduction was evaluated in a combined analysis of 594 patients from two studies with combination therapy. Hb declined at least 3 g/dL in 54% of patients and 5 g/dL or more in 10% of men and 7% of women [40], indicating a significant anaemia in a substantial proportion of patients.

Although anaemia adversely impacts patients' well-being, recent analyses of data from clinical trials indicate that those who experience a significant haemoglobin decline are more likely to eradicate the virus. Sulkowski *et al.* [41] retrospectively analysed 3023 patients with HCV genotype 1 included into a multicenter trial and found that subjects who experienced a drop in Hb concentrations of >3 g/dL had a significantly higher SVR rate of 43.7% *vs* 29.9% in those who had a less marked decrease. Interestingly, SVR was associated with early onset anaemia and the use of erythropoietic growth factors, but not with late onset anaemia. In a similar study from the United States, 871 therapy-naïve patients infected with HCV genotype 1 were studied retrospectively [42]. Again, a drop of haemoglobin of >3 g/dL and the development of anaemia were associated with a higher SVR rate. This association remained significant after exclusion of patients who received EPO. The precise

mechanism behind a better treatment response in anaemic patients is yet unknown, but may relate to a higher individual RBV exposure. As RBV clearance depends on body weight and kidney function and accumulates in red blood cells, a haemolysis-driven release of RBV into the extracellular (intravasal) space could lead to an increased exposure of HCV-infected cells (hepatocytes, immune cells) to RBV, thereby increasing its antiviral activity. Unfortunately, RBV plasma levels were not measured in the two large retrospective studies demonstrating anaemia as a predictor of SVR, so the hypothesis of 'Hb loss as a pharmacodynamic marker of RBV exposure' is not backed by data [42]. However, in a recent short report from the Netherlands, a median RBV concentration of 2.9 mg/L at week 24 of therapy was an independent predictor of SVR, whereas anaemia was not [43].

## CLINICAL STUDIES ON USE OF EPO IN CHC

The literature search retrieved nine published clinical trials on the use of adjuvant EPO administration for haemolytic anaemia during antiviral treatment of CHC with different study designs (Table 3). In the majority of studies (*n* = 7), EPOα was used [18–21,44–47] while the remaining two trials administered EPOβ [48,49]. Because of the restriction to a single uncontrolled trial, darbepoietin was not included into the present analysis [50].

Consistently, overall evidence indicates a benefit of EPO therapy in HCV-treated patients with regard to certain endpoints. These endpoints include the effect of EPO on improving *de novo* haemolytic anaemia, the possibility to maintain or even administer higher target ribavirin dosages and several composite scores assessing QoL. Data reflecting the impact of EPO administration on antiviral efficacy (SVR) are inconsistent, and if any benefit exists, it appears to be largely related to allowing for the maintenance of higher RBV doses and consecutive prevention of relapse.

### Anaemia and ribavirin dose maintenance

The most widely studied effect from EPO is that on the alleviation of RBV-induced haemolytic anaemia (Table 3). All published studies uniformly demonstrate a significant and marked improvement in ribavirin-induced anaemia following EPO administration (increase of 2–3 g/dL) or a significantly less pronounced drop of haemoglobin levels from baseline in those treated with EPO. Thus, EPO administration is effective in maintaining or recovering haemoglobin levels and thereby enabling standard of even higher ribavirin target doses. The latter seems noteworthy as strong scientific evidence indicates a pivotal role of higher ribavirin doses in achieving better SVR rates by the prevention of relapse [21,48,51]. This led the SASL expert panel to conclude that scientific evidence demonstrates that EPO treatment allows the maintenance of RBV dose in the majority of patients, however, at the expense of costs, frequent follow-up tests and the need for treatment modifications (*grade of evidence Ib; recommendation A*).

### Quality of life

Two randomized controlled studies have specifically investigated whether EPO would improve QoL in patients developing haemolytic anaemia during antiviral therapy [18–20]. Together, a significant improvement in QoL is a consistent finding of all studies addressing this issue either as a primary or as a secondary endpoint. The extent of improvement, indeed, is remarkable and applies to both of the used QoL scores, Medical Outcomes Survey Short Form-36 (SF-36) and Linear Analogue Scale Assessment (LASA). Interestingly, frequencies of adverse events such as fatigue, flu-like syndrome, myalgia, arthralgia and skin problems as well as adherence and discontinuation rates do not differ between patients receiving EPO and those who do not [18,21]. Altogether, the panel of experts felt that evidence from a well-performed, randomized, placebo-controlled crossover trial shows significant improvement in QoL across all parameters in patients treated with EPO for haemolytic anaemia supporting EPO use in patients with reduced QoL during antiviral combination therapy (*grade of evidence Ib; recommendation B*).

### SVR

An important issue in assessing the value of EPO as an addition in HCV therapy is its effect on treatment outcome, i.e. SVR. From the data available, EPO itself exerts no additional antiviral activity, but the higher ribavirin doses tolerated with EPO administration may favour elimination of HCV. Indeed, four trials specifically assessed the rates of SVR between patients receiving PegIFN and ribavirin with and without EPO [21,46,47,49]. Shiffman *et al.* [21] showed that PegIFN+higher ribavirin doses at 15.2 mg/kg/day were well tolerated with concomitant EPO and produced significantly higher SVR rates than PegIFN + standard ribavirin (13.3 mg/kg/day)+EPO or PegIFN+standard ribavirin without EPO (49% *vs* 29% and 19%, respectively). In a pilot trial from Sweden, extraordinary high levels of ribavirin (mean 2540 mg daily) resulted in SVR in 9 of 10 patients who were all treated with EPO between 9000 and 30 000 IU/week [48]. Two trials from Italy also provided evidence for a better SVR rate in patients receiving EPO together with PegIFN+ribavirin; however, ribavirin dose in the study from Falasca and co-workers was only 600 mg daily. In the remaining trial from Cash *et al.*, SVR was not improved by adjuvant EPO [46]. Overall, the expert panel consented that these trials lend support for increased SVR rates in patients receiving EPO, but these data are confined to higher than usual ribavirin doses (*grade of evidence Ib; recommendation B*).

**Table 3** Clinical trials reporting on the effect of adjuvant EPO in patients receiving antiviral therapy for chronic hepatitis C (CHC)

| Study | Study design | Patients (n) | Viral genotypes | Intervention | Endpoints | Results |
|---|---|---|---|---|---|---|
| Talal et al.[44] | Open-label pilot study | 18 | Not reported | Up to 40 000 IU EPOα/week if Hb<10 g/dL or drop of 2 g/dL from baseline | Hb recovery | EPO significantly increased mean Hb levels |
| Gergely et al.[45] | Open-label case series | 13; 10 therapy-naïve | GT1: 9 GT3: 2 GT4: 2 | 8–30 000 IU EPOα/week when anaemia developed (<10 g/L) | Hb recovery RBV maintenance | EPO significantly increased mean Hb levels EPO allowed for RBV dose maintenance |
| Dieterich et al.[18] | Open-label, randomized, controlled trial | EPO: 36 SOC: 28 | Not reported | Exp: 40 000 IU EPOα/week q.w. or SOC: RBV dose reduction when Hb<12 g/dL | Hb level at wk. 16 RBV maintenance QoL | EPO significantly increased mean Hb levels EPO allowed for RBV dose maintenance |
| Pockros et al.[19] & Afdhal et al. 2004 [20] | Double-blind, randomized, placebo-controlled trial | EPO: 93 Placebo: 92 129 therapy-naïve | GT1: 74% GT2: 15.7% GT3: 8.1% Other: 0.5% | 40–60 000 IU EPOα/week or placebo when Hb<12 g/dL after 12–14 weeks of SOC conducted with DBP, crossover and OLP | QoL assessed by SF-36 and LASA Hb recovery after 8 weeks of EPO | EPO significantly increased QoL EPO significantly increased mean Hb levels EPO allowed for RBV dose maintenance |
| Lindahl et al. [48] | Open-label pilot study | 10 all therapy-naïve | All GT1, high viraemia (>800 kIU/mL) | High-dose RBV (mean 2540 mg/day) + 9–30 000 IU EPOβ/week | SVR at week 72 (24 weeks after EOT) | 9 of 10 patients had SVR All patients had EPOβ 2 patients received blood transfusions |
| Shiffman et al. [21] | Open-label, randomized, controlled trial | 150; all therapy-naïve | All GT1 | A: PegIFNα-2b+RBV 13.3 mg/kg/day B: PegIFNα-2b + RBV 13.3 mg/kg/day + 40 000 IU EPOα/week C: PegIFNα-2b + RBV 15.2 mg/kg/day + 40 000 IU EPOα/week | Primary: SVR at week 72 (24 weeks after EOT) Relapse rate Secondary: RBV dose reduction, anaemia incidence (<10 g/dL) | SVR (ITT): A (29%), B (19%), C (49%) *Relapse:* A (36%), B (40%), C (8%) *RBV dose reduction:* A (40%), B (10%), C (31%) *Anaemia:* A (36%), B (11%), C (6%) |
| Cash et al.[46] | Retrospective, open-label, uncontrolled cohort study | 132; 121 therapy-naïve | GT1/4/6: 54 GT2/3: 78 | PegIFNα-2a or b+RBV (24/48 weeks) ± 4–39 000 IU EPOα/week ± GCSF 30 million IU/week | SVR at week 72 (24 weeks after EOT) | No significant difference between SOC and adjuvant therapy |

**Table 3** (Continued)

| Study | Study design | Patients (n) | Viral genotypes | Intervention | Endpoints | Results |
|---|---|---|---|---|---|---|
| Falasca et al. [49] | Open-label, randomized, controlled trial | 42 | GT1: 30<br>GT non-1: 12 | A: SOC + 30 000 IU EPOβ/week<br>B: SOC with RBV 600 mg/daily | SVR at week 72 (24 weeks after EOT) | SVR: 81.8% (A) vs 45% (B), P = 0.03 |
| Bertino et al. [47] | Open-label, randomized, controlled trial | EPO: 67<br>SOC: 67 | All GT1 | 1) PegIFNα-2a + RBV + 20 000 IU EPOα/week if Hb drop of 2 g/dL from baseline vs 2) PegIFNα-2a | SVR at week 72 (24 weeks after EOT) Hb recovery | SVR: 59.7% (1) vs 34.4% (2), P < 0.01 group 1 had higher initial Hb levels |

DBP, double-blind phase; EOT, end of therapy; EPO, erythropoietin; GCSF, granulocyte colony-stimulating factor; GT, genotype; Hb, haemoglobin; ITT, intention-to-treat; LASA, linear analogue scale assessment; OLP, open-label phase; QoL, quality of life; RBV, ribavirin; SF-36, Short Form-36 survey; SOC, standard of care.

## INDICATIONS FOR EPO USE

### HCV genotype 1

Genotype 1-infected patients face a less favourable treatment prognosis than those infected by non-1 genotypes and require longer treatment associated with a higher likelihood of adverse reactions. Very convincing data derives from the study by Shiffman et al. [21], who randomized 150 patients with genotype 1 HCV infection to standard of care with and without EPO and to a third group with higher daily ribavirin doses (Table 3). Patients receiving a full 48-week course of PegIFN+higher ribavirin doses at 15.2 mg/kg/day and EPO had the highest SVR rate, mostly because of a significantly lower relapse rate compared with the two other groups receiving PegIFN+standard ribavirin with or without EPO (8% vs 40% and 36%, respectively). In addition, rates of anaemia <10 g/dL were significantly lower (6% vs 9% and 34%, respectively). Bertino et al. showed a better SVR in genotype 1 HCV–infected patients when treated with EPO [47]. The potential of EPO to increase the patients' tolerance towards high ribavirin levels has been further demonstrated by Lindahl et al. [48], who had deliberately included patients with high viraemia and advanced fibrosis. Therefore, the panel of SASL experts agreed that sufficient evidence is available to conclude that there is an indication for EPO use in HCV genotype 1–infected patients who develop signs of anaemia to prevent unfavourable RBV dose reduction. The evidence seems to be even more convincing if higher ribavirin target doses are desired, e.g. in 'difficult-to-treat' patients (high viraemia and advanced fibrosis) (grade of evidence Ib; recommendation A).

### HCV genotype non-1

In contrast to genotype 1-infected patients, other genotypes, particular 2 and 3, face a significantly better treatment outcome with SVR rates as high as 90% in genotype 2 infections and only moderately lower in genotypes 3 and 4. Standard treatment of genotype 2 and 3 infection consists of shorter duration (24 vs 48 weeks) and applies lower ribavirin doses (800 mg vs 1000/1200 mg), which less likely produce significant anaemia. While effects of EPO administration on QoL, anaemia and the possibility to maintain RBV dose seem to be independent from viral genotypes, none of the published trials have specifically investigated the impact of EPO administration on SVR in HCV genotype non-1 patients (Table 3). Hence, the current evidence supporting EPO use in this subgroup of patients was estimated weaker than in genotype 1 patients. Thus, the SASL expert concluded that there may be an indication for EPO use in HCV genotype non-1–infected patients who develop significant anaemia to prevent unwanted RBV dose reduction, but unequivocal scientific evidence supporting its use is lacking (grade of evidence Ib; recommendation A).

## Retreatment of relapsers and nonresponders

So far, only few data are available demonstrating that the addition of EPO to standard therapy in patients who were nonresponders or relapsers to prior antiviral therapies results in better SVR rates than retreatment with standard protocols. Although clinical studies included therapy-experienced patients, subgroup analyses on SVR rates separating therapy-naïve patients from those with prior antiviral therapies were not performed. However, it seems plausible that EPO exerts similar effects on improving *de novo* haemolytic anaemia, the possibility to maintain or even administer higher target ribavirin dosages and QoL also in relapsers and responders. The experts therefore decided that there could be an indication for using EPO in anaemic patients retreated after prior treatment failure, particularly, if treatment was terminated for intractable haemolytic anaemia and if patients were treated with low RBV (or had RBV dose reductions) or were relapsers. However, direct scientific evidence supporting this notion is limited (*grade of evidence IV; recommendation C*).

## Cardiovascular and pulmonary comorbidities

Anaemia is associated with increased cardiovascular morbidity [52], mortality from cancer [53] and surgical mortality [54], and administering blood transfusions to elderly anaemic patients with myocardial infarction reduced mortality [37]. Also, several studies have reported an association between HCV and coronary artery diseases or carotid atherosclerosis [55–57], while others have not [58,59]. However, until now, no trial has demonstrated a significantly increased cardiovascular or pulmonary morbidity and mortality during antiviral treatment of CHC, and consequently, no study has provided evidence for a beneficial impact of EPO therapy for secondary vascular or pulmonary diseases except for patients with terminal kidney failure undergoing dialysis in whom EPO is regularly provided. In this particular subgroup, however, ribavirin is contraindicated outside clinical trials.

The panel of experts consented that the pre-emptive use of EPO in patients with pre-existing cardiovascular or pulmonary disease is not supported by scientific evidence and cannot be recommended routinely (*grade of evidence V; recommendation A*).

## Liver transplant recipients

The progression of fibrosis because of HCV graft reinfection after liver transplantation is faster than in the pretransplant setting, leading to histologically documented cirrhosis within 5 years in 25–30% of cases, and recurrent HCV is the main cause of graft failure and death after OLT [60]. Given this risk, it seems reasonable to consider antiviral therapy in liver transplant recipients. Many reports have shown rates of SVR

ranging from only 10% to 30% in liver transplant recipients with recurrent HCV treated for 48 weeks [61,62]. SVR rates were higher and comparable to patients without liver grafts in a retrospective analysis in 172 liver-transplanted patients from Canada receiving combination antiviral therapy demonstrating an overall SVR of 50% (genotype 1/4: 40%; genotype 2/3: 76%) [63]. However, a recent analysis concluded that considering the lack of clinical benefit and the frequent adverse events, there is currently no evidence to recommend antiviral treatment for recurrent liver graft infection with HCV and advocated further randomized clinical trials with adequate trial methodology and adequate duration of follow-up [64]. Hence, optimizing antiviral therapies for liver transplant recipients is an urgent task to improve graft and patient survival. However, no trial study has been carried out with EPO in liver transplant recipients demonstrating its benefit on tolerability and antiviral efficacy. So, the panel of experts inferred that there could be an indication for EPO in liver transplant recipients receiving combination antiviral treatment should anaemia emerge during treatment, but the evidence was found to be insufficient to advocate its routine use outside clinical trials (*grade of evidence V; recommendation A*).

## Haemodialysis patients

The prevalence of CHC in patients receiving long-term maintenance haemodialysis ranges between 7% and 30% in Western countries, and the natural course of CHC is worse than in patients without end-stage kidney diseases (ESKD). Several studies have clearly demonstrated a higher mortality risk of HCV-infected patients with ESKD [65–67] and a negative impact of HCV infection on QoL in dialysis patients [68]. Antiviral therapy in patients with ESKD using standard IFN and PegIFN with and without RBV has been amply described in a large number of small clinical trials and several meta-analyses [69]. Virological response rates between studies differed markedly between 0% and 100%, as did rates of therapy withdrawals (0–56%). A specific problem among patients with ESKD is a high prevalence of pretherapeutic cytopenias including low platelet numbers and anaemia, which can be further aggravated by interferons and RBV. The latter is officially contraindicated in patients with ESKD as it may elicit life-threatening haemolytic anaemia. However, several trials tested the antiviral efficacy of low-dose RBV (between 200 and 400 mg daily) in combination with low-dose PegIFN (135 μg/week), which achieved SVR rates between 7% and 97% [69] at an acceptable tolerability. However, a uniform observation in these studies was a high proportion of patients developing haemolytic anaemia in response to RBV even at such low dosages requiring adjuvant therapy with EPO in almost all patients. The panel of experts therefore concluded that there are sufficient data to recommend the use of EPO in patients with ESKD (*grade of evidence Ia; recommendation A*).

## GENERAL CONSIDERATIONS ON THE USE OF EPO

### Tolerability and toxicity of EPO treatment

The favourable impact of EPO on the antiviral treatment of CHC must be weighed against its potential side effects, which, however, are infrequently observed by the panel of experts. According to the manufacturers' sheets, both EPO-$\alpha$ and -$\beta$ can produce various adverse events including elevation of arterial pressure and headache. Recently, a revised black box warning from the FDA has been added to the package inserts associating EPO with increased mortality, serious cardiovascular and thromboembolic events and even tumour progression (http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm109375.htm). However, data documenting such risks also for patients treated for CHC and receiving EPO are lacking. A recent cohort study from Canada, including 174 patients treated with PegIFN/ribavirin, found no increased risk of cardiovascular events, malignancy, thrombosis or death in 34 HCV-infected patients during the HCV therapy and EPO treatment or in the period after therapy completion [38]. Similarly, among 282 of 403 patients treated for CHC with adjuvant EPO up to 40 000 IU/week, only 3 patients (1%) with cirrhosis developed hepatocellular carcinoma compared with 1 patient with cirrhosis of 123 (0.8%) who were not treated with EPO. None developed thromboembolic events [70]. Apart from these studies, several case reports have shown the potential development of neutralizing antibodies against EPO, which may also antagonize the effects of endogenous EPO and other recombinant preparations including darbepoietin [71,72]. Clinically, a sudden decrease in Hb levels despite EPO therapy should therefore prompt the discontinuation of EPO and antiviral therapy.

### Selection of type and dosage of erythropoietic growth factor

Three different erythropoietic compounds are currently approved to treat anaemia: erythropoietin-alpha (Eprex®, Janssen-Cilag, Beersen, Belgium), -beta (Recormon®, Roche, Basel, Switzerland) and darbepoietin (Aranesp®, Amgen, Thousand Oaks, CA, USA). All of them have been applied for treating ribavirin-induced haemolytic anaemia. Among the three products, darbepoietin has the advantage of being administered weekly or biweekly instead of thrice weekly as with erythropoietin-alpha and -beta. However, the effect of darbepoietin has so far been tested only in a single trial [50]. Younossi *et al.* analysed 101 patients treated with PegIFN-$\alpha$ 2b and ribavirin who were administered darbepoietin at 3 $\mu$g/kg biweekly when their haemoglobin levels were 10.5 g/dL or below and titrated to achieve a level of 12 g/dL in an uncontrolled, open-label phase II dose-finding study. Of the entire cohort, 41% of patients required darbepoietin, mostly within the first 12 weeks of treatment, which lead to an increase in haemoglobin by 1.9±1 g/dL with a dose of 2.9 ± 1.11 mg/kg

every other week. Treatment was well tolerated and allowed for ribavirin dose maintenance in 85% of patients.

Clinical studies using EPO-alpha were exclusively carried out in the United States and all applied between 40 000 and 60 000 IU/weekly when defined thresholds of anaemia were undercut (Table 3). Similar results were achieved in two European trials on EPO-beta using 9000–30 000 IU/week according to their effect on haemoglobin levels. The panel of SASL experts concluded that there is currently no evidence-based algorithm for starting, escalating, reducing or stopping EPO in patients developing haemolytic anaemia during combined antiviral therapy for CHC. However, SASL currently investigates an algorithm using escalating doses of EPO in a randomized study (SASL 24 study; NCT00944684) in which EPO-beta is started at $3 \times 3000$ IU/week when haemoglobin levels fall below 10 g/dL. (*grade of evidence V; recommendation B*).

### Cost efficiency of EPO use in CHC

There is still a debate on whether the use of EPO in the given context is cost efficient. So far, four studies addressed the issue of cost efficiency and yielded controversial results. Two studies concluded that EPO administration seems to be cost efficient, both applied a Markov decision analytic model comparing two models of treatment: (i) EPO administration if haemoglobin levels fall by >3 g/dL, or instead (ii) ribavirin dose reduction [73,74]. Cost efficacy was found to be advantageous with darbepoietin compared with erythropoietin-alpha in one analysis [73], and the cost of darbepoietin per additional quality-adjusted life year (QALY) was $34 793 in genotype 1 and $33 832 in genotype 2 or 3 patients. Figures for EPO were $60 600 and $64 311, respectively. In the study by Spiegel, costs per QALY were calculated at $16 443 irrespective of the HCV genotype, which is only slightly higher than the costs for QALY in patients undergoing primary angioplasty *vs* thrombolysis in acute myocardial infarction [74,75]. Two further studies ascertained inconclusive or negative results with regard to cost effectiveness of EPO during antiviral therapy, at least for genotype 2/3 patients, and advocated the assessment of cost effectiveness during randomized trials [76,77]. Generally, cost effectiveness in the selected 'model' patients were found highly sensitive to variation in SVR, the definition of QoL, the age at which therapy was begun and the duration thereof rendering artificial cost analyses highly susceptible to an investigator-related bias.

In conclusion, cost efficiency remains an unresolved issue and is most probably restricted to patients with a high likelihood of developing anaemia (full-/high-dose ribavirin, established cirrhosis), a strong demand for fully dosed therapy (genotype 1 patients, high viraemia and advanced fibrosis) and a good response to EPO and antiviral treatment. The expert panel decided that cost efficiency is an open

question until further, prospective trials provide clear answers (*grade of evidence V; recommendation B*).

## CONCLUSION AND FUTURE ASPECTS

A consensus was reached among the experts that haemolytic anaemia requires intervention with Hb levels below 100 g/L and a haematocrit of <30%. The panel of experts recommends the use of EPO for patients infected with viral genotype 1 developing significant anaemia to improve QoL and sustain optimal ribavirin dose. Evidence supporting routine EPO use in patients with pre-existing anaemia, non-1 viral genotypes, a former relapse or nonresponse, liver transplant recipients and cardiovascular or pulmonary disease is not convincing and thus, further randomized clinical trials are advocated in this field. Whether the use of EPO will remain an adjuvant strategy to improve the tolerability of antiviral treatment in CHC patients with the expected introduction of novel and more effective antiviral therapies, must be judged as soon as sufficient clinical evidence has been generated. However, as ribavirin will continue to be an integral part of future combination therapies, the use of EPO will probably remain a valuable measure to improve therapy tolerability and potentially, to increase the antiviral effects of combination therapy by allowing for sustained ribavirin dosage.

Importantly, the market introduction of two novel direct antiviral agents (DAA), Boceprevir and Telaprevir, will not only improve SVR rates, but also impact the incidence of anaemia. Recently published phase III trials showed that compared with standard therapy, the addition of telaprevir increased the rate of anaemia from 17% to 27% (4% severe), while boceprevir, in particular, caused significant anaemia in almost half of all patients requiring the use of EPO in up to 46% [78]. Anaemia as a possible predictive marker of SVR, however, was not analysed. Overall, the new antiviral standard treatment for HCV will certainly improve the prognosis of patients with HCV genotype 1 infection, but the foreseeable rise in the incidence of treatment-associated anaemia will continue to call for the use of EPO to increase tolerability in an even larger proportion of patients than today.

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