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Does Epoetin Beta Still Have a Place in Peginterferon Alpha-2a Plus Ribavirin Treatment Strategies for Chronic Hepatitis C?

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To investigate the impact of epoetin beta (EPO) on sustained virological response (SVR) in hepatitis C virus (HCV)-infected patients treated with peginterferon-ribavirin (RBV). Controlled, randomized, pragmatic multicenter study to assess 2 strategies, ie, the use (EPO group) or nonuse (control group) of EPO in terms of achieving SVR in treatment-naïve, genotype non-2/non-3 HCV-infected patients receiving a 48-week treatment regimen of pegylated interferon α -2a (peg-IFN) plus RBV (randomization 2:1). The single-nucleotide polymorphisms of interferon lambda 3 (*IFNL3*) (rs12979860 and rs8099917), interferon lambda 4 (*IFNL4*) (ss469415590), and inosine triphosphatase (*ITPA*) (rs1127354 and rs7270101) were determined retrospectively. Two hundred twenty-seven patients were included in the study. In the global population ($n=227$), the overall SVR rate was 52% (118/227). Non-response and relapse occurred in respectively 46/227 (20.3%) and 42/227 (18.5%) patients. In the intention-to-treat analysis, 55.5% of patients with anemia ($n=164$) had a SVR, specifically 57.4% in the EPO group versus 52.4% in the control group, but the difference was not statistically significant. In the anemic population, independent factors associated with SVR were *IFNL3* and *IFNL4* polymorphisms, pretreatment HCV RNA level, iron level, and aspartate aminotransferase/alanine aminotransferase (AST/ALT) ratio. EPO has little impact on SVR in patients treated with peg-IFN+RBV and should be recommended only for patients with severe anemia.

Introduction

HEPATITIS C VIRUS (HCV) infection is a major cause of morbidity and mortality, affecting 150 million people worldwide (Lavanchy 2009). In France, in 2004, there were 368,000 people affected by the disease, among these 221,000 were RNA positive (Meffre and others 2010). Extended studies of the natural history of HCV disease have shown that chronic infection is associated with an increased risk of complications, including cirrhosis, hepatic decompensation, and hepatocellular carcinoma. Starting in 1986, treatment consisted initially of interferon (IFN) alone (Hoofnagle and others 1986; Di Bisceglie and others 1990), which was later combined with ribavirin (RBV) (Hoofnagle

and others 1986; Di Bisceglie and others 1990). Pegylated interferon-alfa (peg-IFN) plus RBV was introduced in 2000 and this combination has been providing viral eradication in 40%–45% of patients infected with HCV genotype 1 and in 80% of patients infected with HCV genotypes 2 or 3 (Manns and others 2001; Reddy and others 2001; Fried and others 2002; Hadziyannis and others 2003).

In 2011, new direct-acting antiviral agents (DAAs), ie, protease inhibitors such as telaprevir and boceprevir, were approved in Europe and the United States for the treatment of patients infected with genotype 1. These first-generation DAAs provide sustained virological response (SVR) rates as high as 61%–69%, but at the cost of numerous adverse events (McHutchison and others 2009; Kwo and others 2010). New

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second-generation DAAs have been approved or are pending approval (ie, sofosbuvir, simeprevir, daclatasvir, ledipasvir, paritaprevir/ritonavir, ombitasvir, and dasabuvir) in the United States and Europe. However, these DAAs are very expensive, and thus, peg-IFN+RBV remains the standard of care (SOC) in many, particularly developing, countries around the world.

Under peg-IFN and RBV therapy, the SVR rate decreases dramatically when adherence to treatment is not optimal (McHutchison and others 2002). Adverse events are the main cause of poor compliance, and among them, dose-dependent hemolytic anemia induced by RBV stands out, as it causes dose reductions or early withdrawal in 10%–20% of patients (Dieterich and others 2003; Hadziyannis and others 2003; Afdhal and others 2004). Thus, to achieve a SVR and ultimately avoid long-term complications of HCV infection, improving therapeutic compliance is a priority.

Viral genotype and pretreatment HCV RNA viral load and its decline under treatment, age, ethnicity, and fibrosis stage are the main predicting factors of response to peg-IFN+RBV therapy. Recently, a genome-wide association study demonstrated an association between genetic variants and treatment response. Of the single-nucleotide polymorphisms (SNPs) studied, there were 2 (rs12979860 and rs8099917) located near the interferon lambda 3 (*IFNL3*) gene region (previously known as the IL28B gene region) that were strongly associated with treatment response in patients infected with genotype 1 (Ge and others 2009; Suppiah and others 2009; Tanaka and others 2009). This association was also observed later in patients infected with genotype 4 (Jimenez-Sousa and others 2013).

Evolving experience and recent clinical trials indicate that the use of hematopoietic growth factors, particularly epoetin beta (EPO), during HCV treatment with peg-IFN+RBV may permit the maintenance of optimal dose and duration and furthermore improve patient quality of life (Pockros and others 2004; Lebray and others 2005; Chapko and Dominitz 2006). The authors of a meta-analysis performed in 2011 concluded that EPO administration in patients who develop anemia can considerably enhance SVR (Alavian and others 2012). However, controlled studies comparing currently recommended standard treatment with or without EPO have not been performed (Talal and others 2001). This most likely explains why no official guidelines exist for treating anti-HCV therapy-associated anemia, and many issues remain unresolved.

The aim of the present study was to evaluate prospectively the impact of EPO on SVR in anemic patients treated with peg-IFN+RBV.

Patients and Methods

Study design

We conducted a national, prospective, randomized, controlled phase 3b study in 31 French hospital centers between November 2007 and June 2009 (clinicaltrials.gov No. NCT00262379) to determine the impact of complementary EPO on SVR in treatment-naïve, genotype non-2/non-3 HCV-infected patients who experienced anemia under peg-IFN+RBV treatment. The determination of host IFN polymorphisms was performed retrospectively after the initial publication by Ge and others. A specific signed consent form approved by local and national ethical committees was obtained. Our aim in gathering this information was to exclude a disequilibrium of these genetic variants between the 2 groups,

which might have introduced a bias in the analysis (Ge and others 2009).

As EPO is efficient in preventing anemia during peg-IFN+RBV therapy, our trial was constructed as a pragmatic study with the goal of determining whether or not EPO should be used to increase SVR during peg-IFN and RBV therapy: α -risk and β -risk of respectively 100% and 0% were used (Schwartz and Lellouch 2009). We hypothesized a 15% difference in SVR between the experimental (with EPO) and control (without EPO) groups, ie, 65% for the former and 50% for the latter. For a γ -risk of 5% (error in the choice of the good strategy), the number of patients needed to conclude was 60 in each group. However, based on the article by Balan and others (2005), we expected that 50% of the patients would not meet the criteria to receive EPO. For this reason, we doubled the number of patients to be included in the EPO group (thus 60 patients in the control group and 120 patients in the EPO group). We also estimated first that 10% of the patients would not experience anemia during treatment and secondarily that 10% of the patients would have a major deviation from protocol and/or be lost to follow-up. Finally, the total number of patients included in the study was 222. Patients were 1:2 randomized to the control group or EPO group at inclusion.

Patients

Patients aged more than 18 years, infected with genotype 1, 4, 5, or 6 HCV, and presenting detectable HCV RNA were included. They had compensated liver disease (Child-Pugh ≤ 6) and were naïve of treatment. Patients with HIV or HBV coinfection or hepatocellular carcinoma were excluded from the study as were those who had received EPO in the 2 months before the start of HCV treatment. The peg-IFN α -2a and RBV therapy was conducted as per French recommendations (ANAES 2002). All patients provided signed informed consent.

Treatment regimen and follow-up

All patients were treated with SOC: peg-IFN α -2a 180 μ g weekly plus RBV 1,000–1,200 mg daily for 48 weeks. EPO (30,000 U weekly) was added to the SOC in the EPO group when the hemoglobin (Hb) level defined anemia, ie, below 12 g/dL in men and below 11 g/dL in women. EPO administration was 30,000 IU subcutaneously once per week for an initial period of 4 weeks. After this first period, if the Hb level increased more than 2 g/dL, the EPO dosage was decreased by at least 25%, if the Hb level increased between 1 and 2 g/dL, the EPO was maintained at 30,000 IU weekly, and if the Hb level did not increase to at least 1 g/dL, the EPO was increased to 60,000 IU weekly for another 4 weeks. The Hb level was reevaluated after this second 4-week period. If there was no response, the EPO was stopped. In all cases, the Hb level had to be maintained under 13 g/dL (French recommendation at the beginning of the trial, <http://ansm.sante.fr/>). The intention-to-treat (ITT) analysis considered patients who presented anemia (as defined above) during treatment. A *per protocol* analysis was also performed in patients who received 48 ± 4 weeks of the peg-IFN+RBV therapy.

Clinical endpoints

Our primary endpoint was SVR, defined as undetectable HCV RNA 24 weeks after the end of treatment (EOT).

Secondary endpoints were quality of life estimated by the Short Form Health Survey 36 Items (SF-36), Hepatitis Quality of Life Questionnaire (HQLQ) questionnaires, fatigue severity scale (FSS), a visual analog scale, and the cumulative dose of RBV between 0 and 24 weeks (Bayliss and others 1998; Dalgard and others 2004).

Clinical and laboratory assessments

Hematological parameters, platelet count, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were assessed at baseline, then at weeks 2 and 4, then every 4 weeks until EOT, and during the posttreatment period at weeks 52, 60, and 72. HCV RNA levels were determined at baseline and after 12, 24, and 48 weeks of treatment, and at week 72, 6 months after the EOT.

IFNL3, interferon lambda 4 (*IFNL4*) and inosine triphosphatase (*ITPA*) polymorphisms were determined retrospectively using genomic DNA obtained from peripheral blood mononuclear cells or saliva after obtaining specific signed informed consent. Genomic DNA was extracted with the easyMAG automated system (BioMerieux) from peripheral blood mononuclear cells. Saliva was collected with the ORAGENE OG-500 DNA Collection Kit (DNA Genotek, Inc.), and genomic DNA extraction performed according to the manufacturer's precipitation protocol or with the MN plasma XS kit (Macherey Nagel) (Witt and others 2012). Genotyping of *IFNL3* SNPs rs12979860 and rs8099917 was performed by pyrosequencing using 2 specific primers for each SNP. This method is based on luminometric detection of pyrophosphate released upon nucleotide incorporation during DNA synthesis. The reaction generates a flash recorded as a peak in a program (Ronaghi 2001; Royo and others 2009). *IFNL4* and *ITPA* genotyping was performed using TaqMan real-time polymerase chain reaction (PCR) discrimination assays (Life Technologies) with the Type-it Fast Probe PCR Master Mix (Qiagen) and the Rotor-Gene Q PCR instrument (Qiagen). PCRs were performed in 100-well Rotor-Disc (Qiagen) rotors. The reaction mixture (10 μ L) contained 5 μ L of genomic DNA (2 ng/ μ L) and 5 μ L of master mix and the SNP assay. Each SNP assay contained 2 primers for amplifying the sequence of interest and 2 TaqMan probes for detecting alleles. *ITPA* SNP assays were ordered from Life Technologies as functionally tested assays (references C_27465000_10 and C_29168507_10 for rs1127354 and *ITPA*-rs7270101, respectively). *IFNL4*-ss469415590 assay was a custom TaqMan genotyping assay (Assay ID: AHRSOBV; forward primer: GCCTGCTGCAGAAGCAGAGAT, reverse primer: GCTC CAGCGAGCGGTAGTG, VIC-Reporter: ATCGCAGAAG GCC, FAM-reporter: ATCGCAGCGGCC).

Genotypes were defined as follows: *IFNL3*-rs12979860: CC, CT, or TT (minor allele=T); *IFNL3*-rs8099917: TT, TG, or GG (minor allele=G) and *IFNL4*-ss469415590: TT/TT or G carriers; *ITPA*-rs1127354: CC, CA, or AA (minor allele=A) and *ITPA*-rs7270101: AA, AC, or CC (minor allele=C). Genotype distributions conformed to the Hardy-Weinberg equilibrium (chi-square test $P>0.05$).

Liver fibrosis was assessed with liver biopsy, transient elastometry, fibrotest, or fibrometer when data were available in each investigation center and we calculated aspartate aminotransferase to platelet ratio index (APRI) and Fibrosis 4 score (FIB-4) scores retrospectively for all patients. For homogenization of the data, the severity of fibrosis was classified

as clinically significant fibrosis (CSF) and no CSF and cirrhosis or no cirrhosis. No CSF was defined by an APRI score ≤ 0.5 and CSF by an APRI ≥ 1.5 ; no cirrhosis was defined by an APRI ≤ 1.0 and cirrhosis by an APRI ≥ 2.0 . In parallel, FIB-4 was used to determine no severe fibrosis ($\text{FIB-4} \leq 1.5$) or severe fibrosis ($\text{FIB-4} \geq 3.25$) (Wai and others 2003; Sterling and others 2006). The severity of fibrosis was established, secondarily, according to available results from liver biopsy staging, transient elastometry, fibrometer, and fibrotest.

Statistical analysis

Quantitative data are described by means or medians with standard deviations or 95% confidence intervals. Group comparisons of categorical variables were performed using the Pearson chi-square test or Fisher's exact test. To compare numerical variables, the Student's *t*-test or Mann-Whitney U-test were used when appropriate. Multivariate logistic regressions by forward stepwise analysis were performed with SVR or anemia as the dependent variable and were evaluated using several statistical tests (mainly log-likelihood ratio test and the Hosmer-Lemeshow test). Multivariate analysis was split in 2 ways, with or without SNPs. To compare the performance of the logistic regression models, StAR software was used to plot receiver operator curves (ROCs) and perform statistical comparisons of the area under the curve (AUC) of each ROC (Vergara and others 2008). Statistical analysis was performed with SPSS software version 15 (SPSS, Inc.). A 2-sided *P* value of 0.05 was considered statistically significant.

Results

Population characteristics and virological response

Figure 1 provides a flowchart of the trial and Tables 1 and 2 the characteristics of the global and ITT (anemic patients) patient populations, respectively. No significant differences were observed between the EPO and control groups. Considering all patients ($n=227$), 17.2% had cirrhosis. The overall rate of SVR in all patients (52%) was similar to that found in previous studies on peg-IFN+RBV treatment. Interestingly, only 46 patients (20.3%) did not respond, 42 (18.5%) relapsed after treatment, and 5 (2.2%) had a breakthrough (Table 3). In the ITT population ($n=164$), SVR was obtained in 91 patients (55.5%), with a slightly higher rate in the EPO group (57.4%) than in the control group (52.4%), but the difference was not statistically significant (Table 3). A comparable difference was observed in the *per protocol* analysis (60.3% versus 55.8%, NS). The retrospectively calculated gamma-risks were 26.6% and 31.0% in the ITT and *per protocol* analyses, respectively. According to HCV genotype, we noticed a close rate of SVR for genotypes 1 and 4 (53.0% and 51.4%, respectively).

EPO prescription and RBV dose reduction

Overall, 82 of 149 (55.0%) patients received EPO, and 18 of 82 (22.0%) received a dose of 60,000 IU/week during antiviral treatment. For all patients, during the first 24 weeks of treatment, RBV dose reduction was observed in 24.7% of the patients in the control group (19/77) versus 9.5% of those in the EPO group (14/148) ($P=0.004$). RBV discontinuation was observed in 10.5% of the control patients (8/76) and in 14.2% of the EPO patients (21/148) ($P=0.53$). The

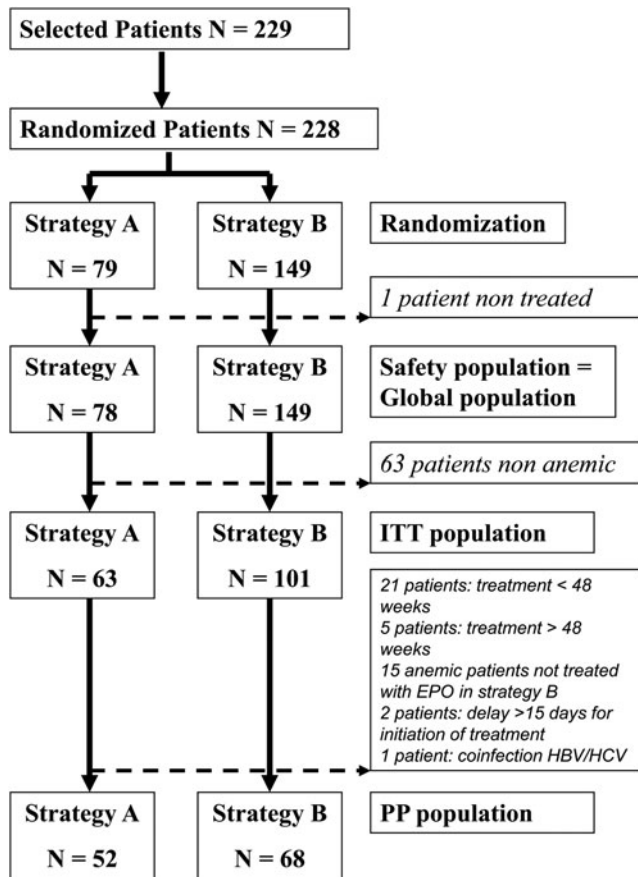


FIG. 1. Flow chart. Strategy A: no EPO treatment. Strategy B: EPO treatment. EPO, epoetin beta.

cumulative doses of RBV, at week 24, were not different between the control and EPO groups, respectively, $2,329.1 \pm 532.9$ and $2,337.3 \pm 588.1$ mg/kg ($P=0.97$, Table 4).

In the ITT population, RBV dose reduction was statistically higher in the control group [27.4% (17/61) versus 13% (13/100) in the EPO group, $P=0.036$] but not RBV discontinuation [11.5% (7/61) versus 10% (10/100), $P=0.79$]. The cumulative dose of RBV, at week 24, was higher in the EPO group, but the difference was not statistically significant (control group: $2,321.2 \pm 584.2$ mg/kg versus EPO group: $2,455.7 \pm 530.3$ mg/kg, $P=0.34$, Table 4).

We found no differences between the control and EPO groups regarding the cumulative doses of RBV at weeks 8, 12, and 48 (Table 4).

Adverse events

The rate of adverse clinical events was more than 95.6% for all patients, with asthenia (56.4%), insomnia (37.9%), and pruritus (30.8%) being the most frequent (Table 5). Asthenia was more frequent in the EPO group than in the control group (respectively, 64.6% versus 51.7%), as were insomnia and pruritus (35.4% versus 28.3% and 39.0% versus 37.2%). In contrast, dyspnea was significantly less frequent in patients who received EPO (11.0%) than in those who did not (22.0%) ($P<0.04$). Sixty-three of the 78 (80.8%) patients included in the control group presented an anemia as defined in our protocol (Hb <11 g/dL for women and <12 g/dL for men) and would have normally received EPO.

TABLE 1. BASELINE CHARACTERISTICS OF PATIENTS

	All patients (n = 227)	Control group (n = 78)	EPO group (n = 149)	P value
Age (years)	48.8 ± 12.4	49.7 ± 11.3	48.3 ± 13.0	0.45
Sex (male/ female)	138/89	49/29	89/60	0.67
Weight (kg)	72.4 ± 14.9	73.1 ± 12.4	72.0 ± 15.4	0.62
Height (cm)	170.2 ± 9.2	170.4 ± 8.5	170.1 ± 9.6	0.80
Hb (g/dL)	14.66 ± 1.31	14.70 ± 1.41	14.64 ± 1.25	0.92
Platelets (g/L)	226.7 ± 75.7	219.7 ± 75.4	228.8 ± 76.0	0.52
AST (U/L)	66.8 ± 52.8	64.8 ± 48.1	67.8 ± 55.2	0.53
ALT (U/L)	96.4 ± 76.8	95.4 ± 69.7	96.9 ± 80.4	0.25
Baseline HCV RNA ($\log_{10}$ IU/mL)	5.92 ± 0.73	5.98 ± 0.72	5.88 ± 0.73	0.38
Genotype				
1 (%)	185 (81.5)	66 (84.6)	119 (79.9)	0.65
4 (%)	35 (15.4)	11 (14.1)	24 (16.1)	
5 (%)	6 (2.6)	1 (1.3)	5 (3.4)	
3 (%)	1 (0.5)	—	1 (0.7)	
Cirrhosis, n (%)	39 (17.2)	16 (20.5)	23 (15.4)	0.36
rs12979860				>0.99
CC	30	12	18	
non-CC	70	27	43	
rs8099917				0.84
TT	51	21	30	
non-TT	48	18	30	
ss469415590				0.35
TT/TT	25	12	13	
Δ G carriers	73	27	46	
rs1127354				0.09
CC	83	36	47	
non-CC	16	3	13	
rs7270101				0.15
AA	75	33	42	
non-AA	24	6	18	

Results are shown as mean $\pm$ SD.

EPO, epoetin beta; Hb, hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus; SD, standard deviation.

Few adverse events possibly attributable to EPO were observed in this trial. They were headache in 3 patients, hypertension in 2 patients, rash in 2 patients, malaise in 1 patient, and lithium overdose in 1 patient (the only serious adverse event due to EPO).

Predictive factors of SVR

In univariate analysis, age, pretreatment HCV RNA level, HCV RNA decline ≥ 2 log IU/mL at 12 weeks, fibrosis stage assessed by blood scores (fibrometer, FIB-4 score) or transient elastometry, γ -glutamyl transferase (GGT), platelets, iron, ferritin, vitamin B12, alkaline phosphatases, albumin, alpha-2 macroglobulin, alpha-fetoprotein levels, and the 2 polymorphisms ss469415590 and rs12979860 were all associated with SVR. In the global population, when SNP polymorphisms were not considered, multivariate analysis identified pretreatment HCV RNA level, GGT, and the AST/ALT ratio as being predictive for SVR. When host genetic factors were considered, pretreatment HCV RNA level, the dinucleotide ss469415590 (but not rs12979860), and the absence of clinically significant fibrosis were predictive for SVR (Table 6). In the ITT population, independent predictors of SVR were pretreatment HCV RNA,

TABLE 2. BASELINE CHARACTERISTICS OF THE INTENTION-TO-TREAT POPULATION

	All patients (n=164)	Control group (n=63)	EPO group (n=101)	P value
Age (years)	51.7 ± 11.6	52.3 ± 10.5	51.4 ± 12.2	0.61
Sex (male/female)	96/68	40/23	56/45	0.33
Weight (kg)	71.1 ± 14.8	72.4 ± 13.7	70.2 ± 15.5	0.37
Height (cm)	169.5 ± 9.4	171.0 ± 8.8	168.5 ± 9.6	0.11
Hb (g/dL)	14.52 ± 1.33	14.62 ± 1.40	14.45 ± 1.29	0.43
Platelets (g/L)	217.7 ± 66.4	211.5 ± 63.9	221.6 ± 67.9	0.34
AST (U/L)	67.1 ± 49.5	72.9 ± 51.4	63.5 ± 48.2	0.24
ALT (U/L)	94.9 ± 76.7	108.0 ± 91.4	86.8 ± 65.0	0.11
Baseline HCV RNA (log ₁₀ IU/mL)	5.93 ± 0.75	5.98 ± 0.73	5.90 ± 0.76	0.52
Genotype				0.98
1 (%)	135 (82.3)	52 (82.5)	83 (82.2)	
4 (%)	26 (15.9)	10 (15.9)	16 (15.8)	
5 (%)	3 (1.8)	1 (1.6)	2 (2.0)	
Cirrhosis, n (%)	31 (18.9)	14 (22.2)	17 (16.8)	0.42
rs12979860				>0.99
CC	25	11	14	
non-CC	52	23	29	
rs8099917				0.49
TT	41	20	21	
non-TT	35	14	21	
ss469415590				0.61
TT/TT	21	11	10	
ΔG carriers	54	23	31	
rs1127354				0.21
CC	64	31	33	
non-CC	12	3	9	
rs7270101				0.02
AA	60	31	29	
non-AA	16	3	13	

Results are shown as mean ± SD.

iron level, and AST/ALT ratio when host genetic markers were excluded and pretreatment HCV RNA, iron level, and the dinucleotide ss469415590 when host genetic markers were included (Table 6). The AUCs of the models in the global and ITT populations were higher with ss469415590

TABLE 3. VIROLOGICAL RESPONSE IN THE GLOBAL AND INTENTION-TO-TREAT POPULATIONS

Global population	NR	BT	RR	SVR	LC, AE
All patients n=227	46 20.3%	5 2.2%	42 18.5%	118 52.0%	16 7.0%
Control group n=78	18 23.1%	2 2.6%	14 17.9%	40 51.3%	4 5.1%
EPO group n=149	28 18.8%	3 2.0%	28 18.8%	78 52.3%	12 8.1%
ITT population	NR	BT	RR	SVR	LC, AE
All patients n=164	28 17.1%	3 1.8%	35 21.3%	91 55.5%	7 4.3%
Control group n=63	12 19.0%	2 3.2%	13 20.6%	33 52.4%	3 4.8%
EPO group n=101	16 15.8%	1 1.0%	22 21.8%	58 57.4%	7 4.0%

NR, nonresponders; BT, breakthrough; RR, responders-relapsers; SVR, sustained virological response; LC, lost to contact; AE, adverse event; ITT, intention-to-treat.

TABLE 4. RIBAVIRIN DOSE INTAKE BY PATIENTS AT DIFFERENT STEPS OF TREATMENT

Week	Population	Control group	EPO group	P value
8	GP	823.3 ± 128.4	831.9 ± 151.0	0.953
	ITT	829.6 ± 136.7	851.8 ± 160.7	0.591
12	GP	1,216.5 ± 201.9	1,221.0 ± 245.2	0.718
	ITT	1,221.2 ± 227.2	1,264.0 ± 248.2	0.533
24	GP	2,329.1 ± 532.9	2,337.3 ± 588.1	0.966
	ITT	2,321.2 ± 584.2	2,455.7 ± 530.3	0.344
48	GP	4,261.8 ± 1,498.2	4,233.9 ± 1,533.4	0.731
	ITT	4,260.2 ± 1,555.9	4,530.9 ± 1,414.0	0.522

GP, global population.

than with rs12979860 (0.825 versus 0.801 and 0.833 versus 0.820, respectively); no significant differences between the 2 AUCs obtained for each population were observed (global population, $P=0.15$, and ITT population, $P=0.42$).

Interestingly, in our study, 100% of our patients (11/11) with favorable genetic polymorphisms (*IFNL4* or *IFNL3*) and viral loads <800,000 IU/mL had SVRs.

Predictive factors of anemia during treatment and at week 4

We searched for predictive factors of anemia during treatment and at week 4 in the global and ITT populations. In univariate analysis, age, Hb level, red and white cell counts, neutrophil count, platelet count, creatinine, haptoglobin level, alpha-2 macroglobulin level, FIB-4 score, and *ITPA* rs1127354 were associated with anemia during treatment or at week 4. In multivariate analysis, the only independent factors associated with anemia during treatment were age and baseline Hb level in the global population, with no impact for *ITPA* rs1127354 (Table 7). Independent factors associated with onset of anemia at week 4 were red blood cell count, creatinine, haptoglobin, and the baseline Hb level; no impact for *ITPA* rs1127354 was observed (Table 7).

Quality of life

We did not observe marked differences between the control and EPO groups regarding quality of life during treatment (Fig. 2, no comparisons were significantly different). Surprisingly, in both the global and ITT populations during treatment (week 24), control patients felt better than EPO patients regarding bodily pain and mental health (SF-36 components), and in the global population, only they felt better for vitality and social functioning.

Discussion

DAA regimens free of IFN and RBV have transformed the therapeutic landscape for chronic hepatitis C, but their current cost makes them accessible only when significant financial means are available. Thus, in many countries, peg-IFN+RBV remains the SOC today. Previous clinical studies have pointed out that adherence to therapy, and particularly to RBV, was crucial for treatment efficacy, thus maintaining the initial RBV dosage may be useful for achieving viral clearance (Fried and others 2002; McHutchison and others 2002). Indeed, previous studies have shown that RBV is a key tool for hepatitis C treatment, working synergistically with peg-IFN to double the response obtained with this latter

TABLE 5. ADVERSE EVENTS ACCORDING TO EPOETIN BETA INTAKE

<i>Events</i>	<i>All patients</i>	<i>No EPO intake</i>	<i>EPO intake</i>	<i>P value</i>
All events/patients (%)	1,969/217 (95.6)	1,196/136 (93.8)	773/81 (98.8)	
Asthenia	170/128 (56.4)	102/75 (51.7)	68/53 (64.6)	<0.40
Influenza-like illness	47/46 (26.0)	37/33 (22.8)	27/26 (31.7)	<0.22
Irritability	47/46 (20.3)	32/31 (21.4)	15/15 (18.3)	<0.46
Fatigue	35/34 (15.0)	22/22 (15.2)	13/12 (14.6)	<0.80
Pruritus	86/70 (30.8)	48/41 (28.3)	38/29 (35.4)	0.39
Dry skin	44/40 (17.6)	30/28 (19.3)	14/12 (14.6)	<0.29
Alopecia	35/34 (15.0)	19/19 (13.1)	16/15 (18.3)	<0.38
Nausea	39/38 (16.7)	21/21 (14.5)	18/17 (20.7)	<0.30
Insomnia	95/86 (37.9)	61/54 (37.2)	34/32 (39.0)	>0.99
Cough	45/43 (18.9)	31/30 (20.7)	14/13 (15.9)	<0.70
Dyspnea	38/34 (15.0)	19/16 (11.0)	19/18 (22.0)	<0.05
Headache	59/55 (24.2)	32/29 (20.0)	27/26 (31.7)	<0.08
Myalgia	39/39 (17.2)	27/27 (18.6)	12/12 (14.6)	<0.36

alone (Hadziyannis and others 2004; Reddy and others 2007). The cumulative doses of RBV observed during the first 3 months have been shown to be predictive of SVR (Bain and others 2008), and very high doses of RBV have led to high SVR rates (Lindahl and others 2005; Bain and others 2008). An optimal early exposure to RBV, as measured by a week 4 trough concentration or better by a day 1 abbreviated area under the concentration curve, has been shown to be predictive of SVR (Loustaud-Ratti and others 2008; Maynard and others 2008). However, the main adverse effect of RBV is hemolytic anemia, which may require RBV dose reduction and thus compromise SVR. Anemia requiring EPO during the first 8 weeks of treatment has been shown to be predictive of SVR (McHutchison and others 2009). Studies have shown a beneficial role of recombinant EPO in alleviating RBV-induced anemia, thereby improving quality of life and furthermore permitting the maintenance of a high RBV dosage. However, few reports have studied the effect of EPO on SVR (Dieterich and others, 2003; Shiffman and others, 2007; Bertino and others, 2010; Falasca and others, 2010; Alavian and others, 2012).

Our study is the first randomized, prospective controlled study testing the impact of EPO on SVR. We found that patients in the EPO group for whom the initial standard dose of RBV [adapted according to body weight (< or ≥75 kg)

1,000 or 1,200 mg daily] was maintained through the use of EPO after anemia onset had a rate of SVR slightly greater compared with the control patients, although the difference did not reach statistical significance. Some factors may have influenced our results.

- (1) We conducted a pragmatic, “real life” study. Experienced physicians involved in this study were aware of the importance of maintaining a RBV dose to obtain a high rate of SVR, and thus, they did not reduce the dose of RBV according to the drug registration recommendations in the control group. RBV dose reduction occurred less often in the EPO group, but this did not result in a significantly higher cumulative dose of RBV for those patients after 8, 12, and 24 weeks of treatment.
- (2) The global virological response for all treated patients was rather high (52%) for a real life study, including a high rate of patients with significant clinical fibrosis (46.3%), probably because the patients received thorough care. This relatively high rate of response may have masked the beneficial effect of EPO.
- (3) The limited improvement provided by the EPO strategy may also be explained by a disequilibrium of genetic polymorphisms that might have affected treatment re-

TABLE 6. PREDICTIVE FACTORS FOR SUSTAINED VIROLOGICAL RESPONSE IN MULTIVARIATE ANALYSIS

<i>Population</i>	<i>Factors</i>	<i>OR (95% CI)</i>	<i>P value</i>
Model 1: Global	HCV RNA level (log IU/mL)	0.579 (0.375–0.894)	0.014
	GGT (U/L)	0.993 (0.988–0.998)	0.005
	AST/ALT ratio	0.261 (0.082–0.832)	0.023
Model 2: Global with host genetic parameter	HCV RNA level (log IU/mL)	0.237 (0.097–0.574)	0.001
	ss469415590	9.575 (2.556–35.868)	0.001
	CSF	0.250 (0.094–0.665)	0.005
Model 3: ITT	HCV RNA level (log IU/mL)	0.339 (0.238–0.668)	<0.001
	Iron (μM)	0.947 (0.909–0.986)	0.008
	AST/ALT ratio	0.336 (0.123–0.986)	0.033
Model 4: ITT with host genetic parameter	HCV RNA level (log IU/mL)	0.183 (0.065–0.515)	0.001
	ss469415590	5.424 (1.362–21.596)	0.016
	Iron (μM)	0.916 (0.852–0.983)	0.016

OR, odds-ratio; 95% CI, 95% confidence interval; GGT, γ-glutamyl transferase; CSF, clinically significant fibrosis.

TABLE 7. PREDICTIVE FACTORS FOR ANEMIA AT WEEK 4 AND DURING TREATMENT

Anemia	Parameters	OR (95% CI)	P value
Model 1: GP—during treatment without including SNP	Age (years)	1.095 (1.048–1.143)	<0.001
	Baseline Hb (g/L)	0.591 (0.449–0.778)	0.005
Model 2: GP—during treatment including SNP	Age (years)	1.147 (1.072–1.228)	<0.001
	Baseline Hb (g/L)	0.611 (0.339–0.936)	0.024
Model 3: GP—at week 4 without including SNP	RBC count (g/L)	0.130 (0.049–0.350)	<0.001
	Creatinine (mM)	1.042 (1.013–1.072)	0.004
	Haptoglobin (g/L)	0.359 (0.162–0.798)	0.012
Model 4: GP—at week 4 including SNP	RBC count (g/L)	0.076 (0.017–0.346)	0.013
	Creatinine (mM)	1.052 (1.011–1.095)	<0.001
Model 5: ITT—at week 4 without including SNP	RBC count (g/L)	0.200 (0.074–0.539)	<0.001
	Creatinine (mM)	1.034 (1.005–1.064)	0.021
	Haptoglobin (g/L)	0.398 (0.168–0.943)	0.036
Model 6: ITT—at week 4 including SNP	Baseline Hb (g/L)	0.326 (0.194–0.677)	<0.001
	Creatinine (mM)	1.048 (1.004–1.094)	0.032

SNP, single-nucleotide polymorphism; RBC, red blood cell.

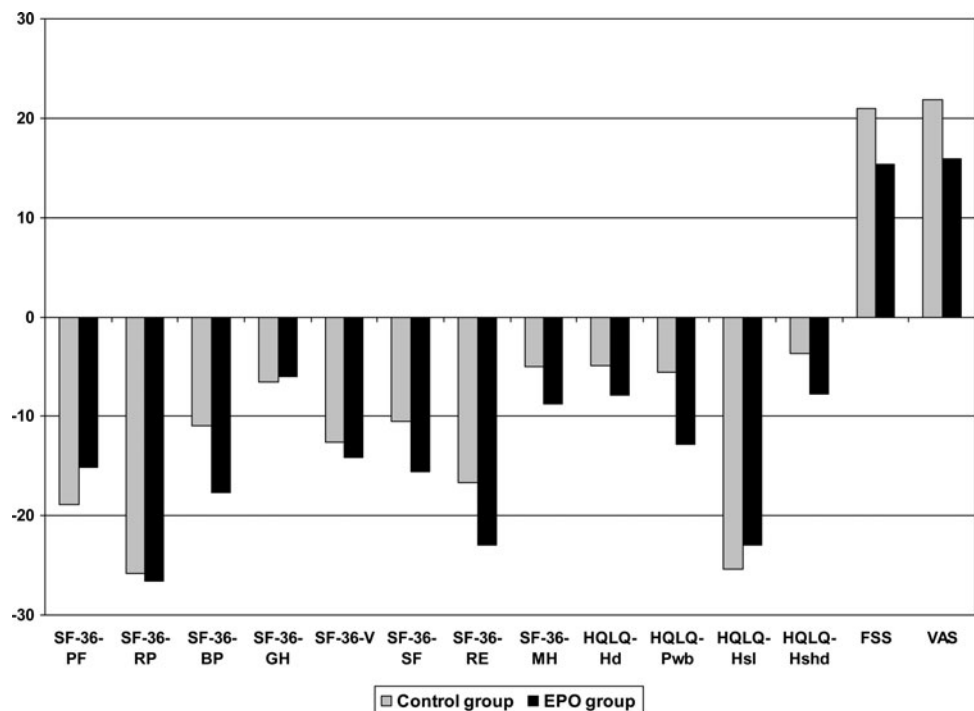
sponse (ie, *IFNL3* polymorphism) or anemia occurrence/severity (ie, *ITPA* polymorphism). However, the respective repartitions of these polymorphisms were not statistically different between the overall and ITT control and EPO groups (Tables 1 and 2).

The multivariate analysis of predictive factors of SVR (including genetic factors) was an important element of our study. Interestingly, as already reported, the 2 main factors associated with SVR were the *IFNL4* ss469415590 and the pretreatment viral load (Real and others 2014). Like Real and others, we too found that the AUC of the logistic regression model constructed with the *IFNL4* ss469415590 was higher than that constructed with *IFNL3* rs12979860, but with no significant differences between the AUCs in the 2 populations. We also found that the *ITPA* polymorphism was associated with neither virologi-

cal response nor anemia severity in multivariate analysis (Tables 5 and 6).

Our results are in accordance with previous non-randomized studies, except for improvement of quality of life, which was not observed in our EPO-treated group. In the study by Shiffman *et al.*, the virological response was higher in patients receiving high doses of RBV+EPO compared to those treated with a lower dose of RBV+/-EPO (Shiffman and others 2007). We obtained a SVR in our global population (52%) similar to the one reported by Cash and others (2010). However, SVR was higher in our ITT population (57.4% with EPO versus 52.4% without EPO) and in our *per protocol* population (60.3% with EPO versus 55.8% without EPO), illustrating an effect of EPO on SVR, even if this impact was lower than expected (+5%). There were high gamma risks in our ITT and *per protocol* populations (26.6% and 31.0%, respectively), suggesting a 25%

FIG. 2. Mean changes for SF-36, HQLQ, FSS, and VAS scores between day 0 and week 24 in the ITT population. ITT, intention-to-treat; PF, physical functioning; RP, role physical; BP, bodily pain; GH, general health; V, vitality; SF, social functioning; RE, role emotional; MH, mental health; Hd, health distress; Pwb, positive well-being; Hsl, hepatitis-specific limitations; Hshd, hepatitis-specific health distress; FSS, fatigue severity scale; VAS, visual analog scale; SF-36, Short Form Health Survey 36 Items; HQLQ, Hepatitis Quality of Life Questionnaire.



risk of concluding wrong. In a retrospective cohort study on 5,944 patients (of whom 915 received EPO), Backus and others (2007) also found that the use of EPO was a predictive factor of SVR in multivariate analysis.

Earlier studies have suggested that peg-IFN+RBV was responsible for the majority of adverse events during treatment, in contrast to EPO, which was linked with few (Afdhal and others 2004).

Although new highly active HCV treatments are available, their cost is so high that peg-IFN+RBV remains an option, even in developed countries, especially in patients with favorable predictive factors for SVR, ie, HCV genotype, low viral load, low fibrosis score, and favorable *IFNL3* and *IFNL4* polymorphisms.

In countries with no or low access to new DAAs, it may be thus well founded to systematically assess pretreatment predictive factors of response if available (baseline viral load, evaluation of liver fibrosis, viral genotype, genetic polymorphism, and so on) before deciding on a therapeutic strategy. Peg-IFN+RBV could then be proposed when patients have a high chance of response [ie, those with favorable *IFNL4* or *IFNL3* and low viral load (<800,000 IU/mL), who had a 100% chance of response to treatment in our study]. Moreover, close management of on-treatment patients to identify those who achieve a rapid virological response (ie, undetectable HCV RNA after 4 weeks of treatment) would extend limited budgets and permit the treatment of more patients with peg-IFN+RBV, with an ensuing increase in SVR (Heidrich and others 2014; Pearlman and Ehleben 2014).

Currently, in low-income countries, peg-IFN+RBV remains the SOC for the treatment of chronic hepatitis C. RBV may cause anemia, but reducing RBV dose decreases the response rate. In practice, EPO is frequently used to address anemia. However, our study suggests that when systematically prescribed once a definition of “anemia” has been reached, it only has a slightly favorable effect on SVR. Thus, we cannot recommend the systematic use of EPO in cases of RBV-induced anemia; instead, its use might be best reserved for patients who tolerate anemia poorly.

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All PEGEPO study collaborators for the clinical trial are listed in Appendix 1.

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Author Disclosure Statement

The authors declare that no competing financial interests exist.

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