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## **INFLUENCE OF HCV GENOTYPE 1 SUBTYPES ON THE VIRUS RESPONSE TO PEG INTERFERON ALPHA-2a PLUS RIBAVIRIN THERAPY**

Florence Nicot, Laurent Alric, Karl Barange, Sophie Métivier, Jean-Michel Dramard, Jean-Marc Combis, Bernard Castan, Jean-Jacques Meurisse, Jean-Louis Payen, Daniel Garipuy, et al.

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## INFLUENCE OF HCV GENOTYPE 1 SUBTYPES ON THE VIRUS RESPONSE TO PEG INTERFERON ALPHA-2a PLUS RIBAVIRIN THERAPY

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Keywords:	Hepatitis C, HCV subtype, RBV concentration

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1 Table I: Patient characteristics at inclusion

Parameter <sup>a</sup>	Result
Male sex, n (%)	67 (58)
Age, years, (range)	49 (31-76)
Weight, kilograms (range)	70 (44-113)
Hemoglobin at baseline, g/dL (range)	14.6 (8.5-17.9)
Neutrophil >1,500/ $\mu$ L at baseline, n (%)	100 (87)
Platelet > 90,000/ $\mu$ L at baseline, n (%)	103 (90)
Creatinine clearance at baseline, mL/min (range)	107 (35-138)
Fibrosis stage $\leq$ F2, n (%)	59 (57)
ALT > normal, n (%)	92 (80)
Risk factor for HCV, n (%)	
Injection drug use	34 (30)
Nosocomial	6 (5)
Transfusion	20 (17)
Unknown	55 (48)
HCV genotype, n (%)	
1a	37 (32)
1b	50 (44)
1 <sup>b</sup>	6 (5)
4a	9 (8)
4d	6 (5)
4	5 (4)
5	2 (2)
HCV viral load (log IU/mL)	6.27 $\pm$ 0.06
Genotype 1	6.31 $\pm$ 0.06
Genotype 4	6.13 $\pm$ 0.16

> 800,000 IU/mL, n (%)	87 (76)
HIV positive , n (%)	27 (23)
Patient treatment status at inclusion, n (%)	
Naïve	64 (56)
Non-responder	48 (42)
Unknown	3 (3)
Ribavirin dose, n (%)	
800 mg	14 (12)
1000 mg	74 (65)
1200 mg	27 (23)
Ribavirin weight-adjusted dose, mg/kg (range)	14.3 (9.2- 25)
Concomitant antiretroviral therapy, n	21 (18)

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1 <sup>a</sup> ALT, alanine aminotranferase

2 <sup>b</sup> HCV genotype 1: 1d (n=1), 1i (n=1), 1 with undetermined subtype (n=4)

1 Table II: Factors associated with an early virological response. Parameters with a p value  
 2 <0.10 by one-variable analysis are in bold.

Factor <sup>a</sup>	Univariate analysis		Multivariate analysis	
	OR (CI 95%) <sup>b</sup>	p	OR (CI 95%)	p
Sex (female)	1.30 [0.54;3.11]	0.55	—	
Age (< 40 years old)	1.20 [0.51;0.82]	0.65	—	
Weight (< 70 kg)	0.65 [0.27;1.59]	0.34	—	
HCV genotype (G4)	1.48 [0.45;4.92]	0.49	—	
HCV subtype (1b)	1.90 [0.72;4.99]	0.19	—	
HCV-RNA (< 800 000 log IU /mL)	<b>2.94 [1.19;7.26]</b>	<b>&lt;0.02</b>	—	
<b>Interferon naive patients</b>	<b>2.6 [1.06;6.17]</b>	<b>0.03</b>	<b>2.98 [1.15;7.72]</b>	<b>&lt;0.05</b>
Baseline ALT (elevated)	<b>2.7 [0.97;7.92]</b>	<b>0.06</b>	—	
ALT at week 12 (normal)	<b>3.13 [1.20;8.17]</b>	<b>0.02</b>	—	
Fibrosis score (< F3)	<b>2.3 [0.93;5.67]</b>	<b>0.07</b>	—	
Baseline CrCl (< 90 mL/min)	2.13 [0.68;6.62]	0.19	—	
Peg-IFN- $\alpha$ 2a at week 4 ( $\geq$ 67 ng/mL)	1.02 [0.41;2.55]	0.93	—	
Peg-IFN- $\alpha$ 2a at week 12 ( $\geq$ 67 ng/mL)	1.06 [0.42;2.66]	0.88	—	
Ribavirin at week 4 ( $\geq$ 2,200 ng/mL)	1.11 [0.46;2.67]	0.80	—	
Ribavirin at week 12 ( $\geq$ 2,200 ng/mL)	<b>2.6 [1.05;6.50]</b>	<b>&lt;0.05</b>	<b>3.41 [1.31; 8.90]</b>	<b>&lt;0.02</b>

3 <sup>a</sup> ALT, alanine aminotranferase; CrCl, creatinine clearance; peg-IFN- $\alpha$ 2a, pegylated  
 4 interferon  $\alpha$ -2a

5 <sup>b</sup> OR: odds ratio; CI: confidence interval.

1 Table III: Factors associated with sustained virological response. Parameters with a p value  
 2 <0.10 by univariate analysis are in bold.

Factor <sup>a</sup>	Univariate analysis		Multivariate analysis	
	OR (CI 95%) <sup>b</sup>	p	OR (CI 95%)	p
Sex (female)	1.19 [0.54;2.66]	0.66	—	
Age (< 40 years old)	0.82 [0.37;1.81]	0.59	—	
Weight (< 70 kg)	1.10 [0.48;2.50]	0.79	—	
HCV genotype (G4)	1.05 [0.37;2.99]	0.89	—	
HCV subtype (1b)	<b>3.15 [1.14;8.70]</b>	<b>&lt;0.05</b>	<b>6.82 [1.7;26.8]</b>	<b>&lt;0.01</b>
HCV-RNA (< 800 000 log IU /mL)	1.44 [0.65;3.19]	0.37	—	
Previous anti-HCV therapy (no)	1.56 [0.69;3.52]	0.28	—	
Baseline ALT (elevated)	1.41 [0.45;4.40]	0.52	—	
ALT at week 12 (normal)	<b>2.79 [1.14;6.86]</b>	<b>&lt;0.05</b>	—	
Fibrosis score (< F3)	1.15 [0.50;2.65]	0.72	—	
Baseline CrCl (< 90 mL/min)	1.17 [0.39;3.55]	0.75	—	
HCV RNA at week 4 (< 15 IU/mL)	<b>3.2 [0.96;10.66]</b>	<b>0.05</b>	—	
HCV RNA at week 12 (< 15 IU/mL)	<b>13 [4.86;34.95]</b>	<b>&lt;0.001</b>	<b>25 [6.4; 97.6]</b>	<b>&lt;0.001</b>
HCV RNA drop at week 12 (≥2 log)	<b>30 [3.9;234.6]</b>	<b>&lt;0.001</b>	—	
Peg-IFN-α2a at week 4 (≥ 67 ng/mL)	1.07 [0.45;2.5]	0.85	—	
Peg-IFN-α2a at week 12 (≥ 67 ng/mL)	1.33 [0.55;3.25]	0.50	—	
Ribavirin at week 4 (≥ 2,200 ng/mL)	0.78 [0.35;1.78]	0.54	—	
Ribavirin at week 12 (≥ 2,200 ng/mL)	<b>2.16 [0.93;5.05]</b>	<b>0.07</b>	—	

3 <sup>a</sup> ALT, alanine aminotranferase; CrCl, creatinine clearance; pegIFN-α2a, pegylated interferon

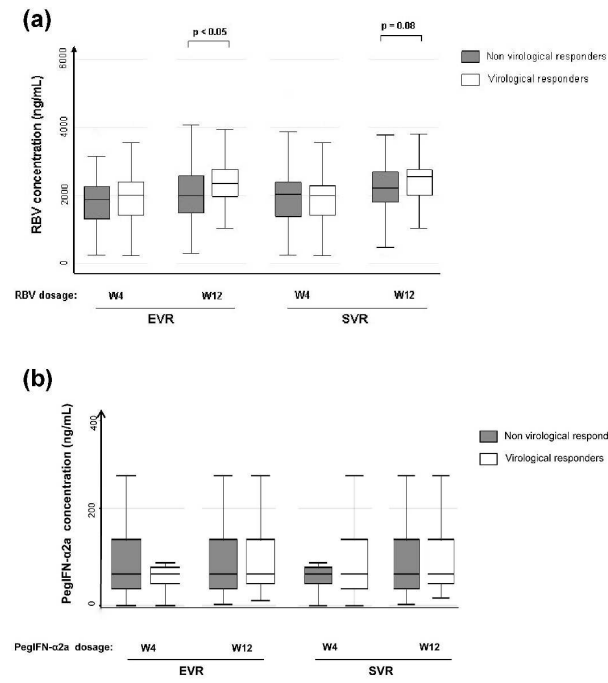
4 α-2a

5 <sup>b</sup> OR: odds ratio; CI: confidence interval.

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**Fig.1**



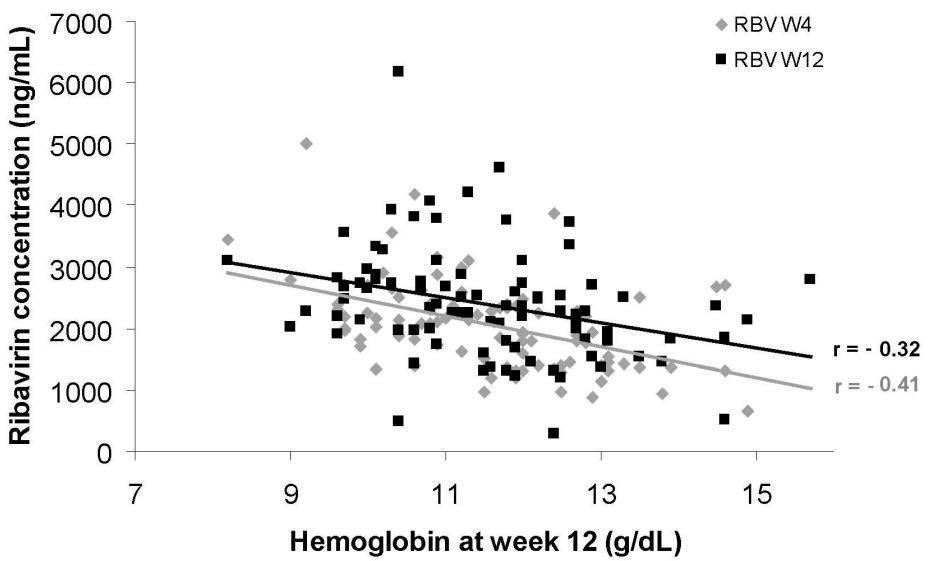
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Fig.2



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3 1 **INFLUENCE OF HCV GENOTYPE 1 SUBTYPES ON THE VIRUS RESPONSE TO PEG**  
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5 2 **INTERFERON ALPHA-2a PLUS RIBAVIRIN THERAPY**  
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10 4 **Running head: HCV genotype 1 subtypes and the response to anti-HCV therapy**  
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14 6 F. Nicot<sup>1</sup>, L. Alric<sup>2,3,4</sup>, K. Barange<sup>5</sup>, S. Métivier<sup>5</sup>, J.M. Dramard<sup>6</sup>, J.M. Combis<sup>7</sup>, B. Castan<sup>8</sup>, J.J  
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16 7 Meurisse<sup>9</sup>, J.L. Payen<sup>10</sup>, D.Garipuy<sup>11</sup>, H.Desmorat<sup>12</sup>, J.M Peron<sup>5</sup>, S. Thebault<sup>2</sup>, T.Morin<sup>13</sup>, C.  
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18 8 Renou<sup>14</sup>, P. Barel<sup>15</sup>, B. Guerin<sup>16</sup>, Y. Imbert<sup>17</sup>, S. Sire<sup>18</sup>, K. Sauné<sup>1,4,19</sup>, E. Chatelut<sup>4,20</sup>, J.  
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20 9 Izopet<sup>1,4,19\*</sup>  
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49 23 <sup>12</sup> Polyclinique du Parc, Toulouse, France;

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51 24 <sup>13</sup> Gastroenterology, Bigorre Hospital, Tarbes, France;

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**ABSTRACT**

New factors that influence the viral response in HCV non-genotype 2/3 patients must be identified in order to optimize anti-HCV treatment. This multicenter prospective study evaluates the influence of HCV variability and pharmacological parameters on the virological response of these patients to pegylated interferon  $\alpha$ 2a (peg-IFN- $\alpha$ 2a: 180  $\mu$ g/week) and ribavirin (800-1200 mg/day) for 48 weeks. HCV subtypes were identified by sequencing the NS5B region. Serum ribavirin and peg-IFN- $\alpha$ 2a concentrations were measured at weeks 4 and 12. The 115 patients (67 men; median age=49, range [31-76]) included 64 who had never been treated and 27 co-infected with HIV. The mean baseline HCV RNA was  $6.30 \pm 0.06$  log IU/mL and the HCV genotypes were: G1 (n=93) with 1a (n=37) and 1b (n=50), G4 (n=20) and G5 (n=2). Most patients (79/108; 73%) had an early virological response. Independent predictors of an early virological response were interferon naive patients (OR=2.98 [1.15; 7.72]) and ribavirin of > 2,200 ng/mL at week 12 (OR=3.41 [1.31; 8.90]). Forty of 104 patients (38%) had a sustained virological response. The only independent predictors of a sustained virological response were subtype 1b (OR=6.82 [1.7; 26.8]), and HCV RNA <15 IU/mL at week 12 (OR=25 [6.4; 97.6]). Thus a serum ribavirin concentration of > 2,200 ng/mL was associated with an early virological response and patients infected with HCV subtype 1b had a better chance of a sustained virological response than did those infected with subtype 1a.

**KEY WORDS:** Hepatitis C, HCV subtype, ribavirin concentration

## 1 INTRODUCTION

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8 The hepatitis C virus (HCV) is very variable, with 6 genotypes and more than 70  
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10 subtypes [Simmonds et al., 2005; Simmonds et al., 1993]. Various factors (viral, host and  
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12 pharmacological one) can influence the overall rate of sustained virological response to  
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14 ribavirin (RBV) and pegylated interferon alpha (pegIFN $\alpha$ ) treatment that is only 50-60%  
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16 [Conjeevaram et al., 2006; Conjeevaram et al., 2007; Everson et al., 2006; Fried, 2002; Ge  
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18 et al., 2009; Manns et al., 2001; Zeuzem, 2004].

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20 Genotype is considered to be the most important factor predicting the virological  
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22 response. Patients infected with HCV genotype 1 or 4 are considered to be difficult-to-treat,  
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24 as only 40% achieve a sustained virological response [Fried et al., 2002; Hadziyannis et al.,  
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26 2004; Manns et al., 2001; Zeuzem, 2004]. Little is known at present about the influence of  
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28 HCV subtypes on the virological response. Most studies, including the pivotal studies, have  
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30 determined the genotype by analysing the 5'UTR region [Fried et al., 2002; Manns et al.,  
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32 2001]. This makes it difficult to discriminate accurately between HCV subtypes [Chen and  
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34 Weck, 2002; Halfon et al., 2001]. The NS5B region is considered to be the most appropriate  
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36 region of the HCV genome for accurately determining the HCV subtype [Cantaloube et al.,  
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38 2006; Nicot et al., 2005; Sandres-Saune et al., 2003] and for studying the virological  
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40 response of different subtypes.  
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45 There are few published data on the influence of pharmacological parameters on the  
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47 virological response and many of them are based on analyses of small numbers of patients.  
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49 Most studies have shown that a low ribavirin concentration is associated with a poor  
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51 virological response [Aguilar Marucco et al., 2008; Larrat et al., 2003; Lindahl et al., 2005;  
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53 Maynard et al., 2008; Nicot et al., 2008; Rendon et al., 2005] in patients infected with HCV  
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55 genotypes 1 or 4, but there are some conflicting data [Crespo et al., 2007; Dahari et al.,  
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57 2007; Lopez-Cortes et al., 2008]. Likewise, there are few data on the peg-IFN- $\alpha$   
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59 concentration and its influence on the virological response [Lopez-Cortes et al., 2008; Nicot  
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28 et al., 2008; Talal et al., 2006].

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3 1 This prospective study on difficult-to-treat patients infected with HCV genotypes 1, 4  
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5 2 or 5 investigates the intrinsic sensitivity of HCV subtypes to peg-IFN- $\alpha$ 2a and ribavirin and  
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7 3 the influence of pharmacological parameters on the virological response (early virological  
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9 4 response and sustained virological response).  
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## PATIENTS AND METHODS

### Patients

A total of 115 patients infected chronically with HCV attending the Toulouse University Hospital and 13 General Hospitals in the Midi-Pyrénées area were enrolled in this prospective study between June 2005 and December 2007. The inclusion criteria were: age >18 years, HCV genotype 1, 4, 5 or 6 and treatment initiation with peg-IFN- $\alpha$ 2a and ribavirin. [Patients coinfecting with HCV and HIV were also included.](#)

This study was approved by the Toulouse University Hospital Ethic Committee. All the patients provided written informed consent before participating in the study.

### Treatment and monitoring

Patients were treated with 180  $\mu$ g peg-IFN- $\alpha$ 2a (Pegasys<sup>®</sup>, Roche), given subcutaneously once a week, in combination with ribavirin (Copegus<sup>®</sup>, Roche) adjusted for body weight, for 48 weeks. Treatment was stopped if the plasma HCV RNA concentration at week 12 had decreased by <2 log IU/mL. All patients were seen 4, 8, 12, 24, 48 weeks after the initial interview and 4 and 24 weeks after treatment withdrawal. Serum samples were collected at each time, centrifuged within 2 hours and stored at -80°C. Patients with an undetectable HCV RNA concentration at week 4 were considered to be rapid virological responders. Patients with a > 2 log decrease in plasma HCV RNA at week 12 were considered to be early virological responders. Patients with undetectable HCV RNA 24 weeks after treatment withdrawal were considered to be sustained virological responders.

## **Virological parameters**

Plasma HCV-RNA was measured at baseline, 4, 12, 48, 52 and 72 weeks by the real-time RT-PCR COBAS™ Ampliprep/COBAS™ Taqman HCV test (CAP/CTM; Roche Diagnostics, Meylan, France), according to the manufacturer's instructions. Plasma HIV-RNA was measured every 3 months by the real-time RT-PCR COBAS™ Ampliprep/COBAS™ Taqman HIV test (CAP/CTM; Roche Diagnostics, Meylan, France) according to the manufacturer's instructions. HCV genotypes and subtypes were determined by phylogenetic analysis of the NS5b region of the HCV genome as described previously [Sandres-Saune et al., 2003].

## **Determination of serum peg-IFN- $\alpha$ 2a concentrations**

The residual peg-IFN- $\alpha$ 2a concentrations in serum samples taken at 4 and 12 weeks were measured with a bioassay [Boulestin et al., 2004]. The assay evaluates the capacity of the peg-IFN- $\alpha$ 2a in serum samples to reduce the cytopathic effect of the vesicular stomatitis virus on MDBK cells in culture. The reference solutions contained 2.8 to 180 ng/mL of peg-IFN- $\alpha$ 2a (Roche, France).

## **Determination of serum ribavirin concentrations**

Residual serum ribavirin concentrations ( $C_{\text{residual}}$ ) were determined 4 and 12 weeks after treatment initiation by high-performance liquid chromatography (HPLC) [Kamar et al., 2004; Svensson et al., 2000]. Briefly, ribavirin was extracted with phenyl boronic acid Bond Elute 100 mg/PK cartridges (Varian SA) and analysed on a Bischoff Nucléosil<sup>R</sup> C18 7  $\mu$ m column with ultraviolet detection at 215 nm. The method was linear from 250 to 6,000 ng/mL. The average within-run and between-run precision was better than 15 % for high and medium concentration control samples, and better than 20 % for low concentrations.



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3 1 **Statistical analysis**  
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8 3 Statistical analyses were performed with STATA 8.0 software (Stata corporation, Grand  
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10 4 Forks, ND). Continuous variables were evaluated with the Mann-Whitney or Wilcoxon tests.  
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12 5 Categorical variables were tested by the Chi-squared test. Receiving operating  
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14 6 characteristics (ROC) curves were constructed with the ribavirin concentrations at week 12 to  
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16 7 determine the cut-off point that best discriminated between virological responders and non-  
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18 8 responders at week 12. A p value of less than 0.05 was considered to be significant.  
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20 9 Predictors of early virological response and sustained virological response were evaluated by  
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22 10 univariate and multivariate analyses. Variables with a p value of 0.10 or less after univariate  
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24 11 analysis were entered into a multivariate, backward, stepwise logistic regression analysis to  
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26 12 identify significant variables independently associated with the virological response.  
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## RESULTS

### Patient characteristics, treatment response and tolerance

The baseline patient characteristics are summarized in Table I. Most were infected with HCV genotype 1 (n=93, 81%) and HCV genotype 4 (n=20, 17%) and 27 of them (23%) were coinfecting with HIV.

Only 13/108 patients (12%) achieved a rapid virological response at week 4, while 79/108 patients (73%) achieved an early virological response at week 12, including 53 (49%) who had undetectable HCV RNA. At the end of follow-up, 40/104 patients (38%) had a sustained virological response, as did 35/88 patients (39.8%) with HCV-1 and 7/19 (36.8%) with HCV-4 (p=NS). One of the patients infected with HCV-5 discontinued treatment before week 12 and the other achieved a sustained virological response. Also 7/31 (22.6%) patients infected with HCV-1a achieved a sustained virological response, as did 23/48 (48%) of those infected with HCV-1b (p<0.02), and in 2/6 (33%) of those infected with HCV-1 other than 1a or 1b. A sustained virological response was observed in 8/25 patients (32%) coinfecting with HIV. This percentage was not significantly different from that of patients infected with HCV alone (40.5%) (p=NS). A sustained virological response was observed in 1/8 (12.5%) patients infected with HCV genotype 1a who were coinfecting with HIV and in 3/4 (75%) patients infected with HCV genotype 1b coinfecting with HIV (p=0.03).

Seven patients discontinued treatment before week 12 due to intolerance to treatment (severe anemia or neutropenia, n=6), or death unrelated to anti-HCV treatment (n=1). Four patients with an undetectable HCV RNA viral load at week 48 were lost to follow-up after week 48. Adverse haematological events led to the initial treatment of 32 patients (29.6%) being modified. Fourteen patients were given erythropoietin and 7 were given neutrophil growth factors.

### 1 Serum concentrations of ribavirin

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The mean serum ribavirin  $C_{\text{residual}}$  were  $1,991 \pm 76$  ng/mL at week 4 and  $2,355 \pm 88$  ng/mL at week 12. Figure 1a shows the serum ribavirin concentrations at weeks 4 and 12 in terms of the early virological response and sustained virological response. The serum ribavirin  $C_{\text{residual}}$  concentrations at week 12 were significantly higher in patients with an early virological response ( $2,424 \pm 94$  ng/mL) than in those with no early virological response ( $2,162 \pm 205$  ng/mL) ( $p < 0.05$ ). ROC curve analysis showed that the best cut-off value for discriminating between patients with an early virological response and those with no early virological response was 2,200 ng/mL (area under the curve = 0.66; 95% CI, 0.53-0.79; sensitivity: 62%, specificity: 69%). The serum ribavirin concentration at week 12 tended to be higher in patients with a sustained virological response ( $2,538 \pm 121$  ng/mL) than in those with no sustained virological response ( $2,296 \pm 123$  ng/mL) ( $p = 0.08$ ).

The ribavirin treatment led to a drop in circulating hemoglobin (Hb) of  $-3.1$  mg/dL ( $-6.30; 3.13$ ) between baseline and week 12; it reached a median value of 11.7 g/dL (8.2; 15.7) at week 12. The serum ribavirin concentrations at week 4 ( $r = -0.41$ ,  $p < 0.001$ ) and week 12 ( $r = -0.32$ ,  $p < 0.002$ ) were inversely correlated with the Hb concentration at week 12 (Figure 2).

### 20 Serum concentrations of peg-IFN- $\alpha$ 2a

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The mean serum peg-IFN- $\alpha$ 2a  $C_{\text{residual}}$  was  $92 \pm 9$  ng/mL at week 4, and  $100 \pm 11$  ng/mL at week 12. No relationship was found between the serum peg-IFN- $\alpha$ 2a concentrations at weeks 4 and 12 and the early or sustained virological responses (Figure 1b).

## 1 Factors predicting the virological response

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One-variable analysis found that 6 factors were associated with an early virological response (Table II). The factors that remained independently associated with the early virological response in the logistic regression analysis were: interferon naive patients, and a serum ribavirin  $C_{\text{residual}} > 2,200$  ng/mL at week 12 (Table II). As all the patients with a rapid virological response (n=13) also had an early virological response, this factor was not included in the statistical analysis.

One-variable analysis revealed 6 factors that were associated with a sustained virological response (Table III). The factors that remained independently associated with a sustained virological response in the logistic regression model were subtype 1b and an undetectable HCV RNA concentration at week 12 (Table III).

## DISCUSSION

This is the first prospective study on HCV genotypes 1 or 4 that evaluates the influence of the virus subtype and the serum concentrations of peg-IFN $\alpha$ -2a and ribavirin on the virological response. A ribavirin concentration of > 2,200 ng/mL was identified as a factor associated with an early virological response. HCV subtype 1b was identified as a predictive factor of a sustained virological response. Unlike clinical trials, this cohort study included patients that were representative of the population followed by the clinicians.

Several studies have reported a correlation between an early virological response and/or a sustained virological response and the ribavirin C<sub>residual</sub> [Jen et al., 2000; Larrat et al., 2003; Maynard et al., 2008; Rendon et al., 2005; Tsubota et al., 2003]. A plasma ribavirin concentration threshold of 2,000 to 3,000 ng/mL at weeks 4 or 12 was proposed to maximize the rate of response [Larrat et al., 2003; Lindahl et al., 2005; Maynard et al., 2008; Nicot et al., 2008]. The patients in the present study with a serum ribavirin concentration of >2,200 ng/mL had more chance of achieving an early virological response. Although no association was found between the serum ribavirin concentration at week 12 and the sustained virological response (probably due to a lack of statistical power), this concentration tended to be higher in responders than in non-responders, as observed in a previous study on patients infected with HCV genotype 1 [Breilh et al., 2009]. The Hb concentration at week 12 was inversely correlated with the ribavirin concentrations, which is consistent with other studies [Arase et al., 2005; Jen et al., 2000; Loustaud-Ratti et al., 2008; Maeda et al., 2004]. Cut-off values of 2,300 or 2,800 ng/mL were proposed for predicting haematological toxicity in patients infected with both HIV and HCV [Aguilar Marucco et al., 2008; Rendon et al., 2005]. The optimal times for measuring the ribavirin concentration and the clinical ribavirin threshold in order to maximize the anti-HCV response and minimize the haematological effects have yet to be determined.

The data in the present study indicate that the serum peg-IFN- $\alpha$ 2a concentrations were not associated with an early virological response or a sustained virological response.

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3 1 This is in keeping with the findings for patients infected with HCV and HIV [Nicot et al., 2008;  
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5 2 Talal et al., 2006]. Another study on HIV-positive patients infected with HCV genotype 1 or 4  
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7 3 found that patients with a high plasma peg-IFN- $\alpha$ 2a concentration throughout treatment were  
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9 4 3 to 4 times more likely to achieve a sustained virological response than those with a low  
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11 5 plasma peg-IFN- $\alpha$ 2a concentration [Lopez-Cortes et al., 2008]. [These discordant results](#)  
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13 6 [between studies can be explained at least in part by differences in time of sampling.](#) Its  
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15 7 impact on the virological response remains to determine.

18 8 The HCV genotype is a major determinant of the response to treatment for chronic  
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20 9 hepatitis C. Several clinical trials have found that patients infected with genotypes 1 or 4 are  
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22 10 less likely to achieve a sustained virological response than others [Fried et al., 2002;  
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24 11 Legrand-Abbravanel et al., 2005; Manns et al., 2001; Martin-Carbonero et al., 2004; Roulot et  
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26 12 al., 2007]. Many of them were performed with the first version of the INNO-LIPA HCV assay  
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28 13 that misclassifies a significant percentage of genotype 1a isolates as genotype 1b [Chen and  
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30 14 Weck, 2002]. HCV subtypes in the present study were identified by sequencing the NS5b  
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32 15 region, which accurately discriminates between subtypes [Cantaloube et al., 2006; Nicot et  
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34 16 al., 2005; Sandres-Saune et al., 2003; Thomas et al., 2007]. Those patients infected with  
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36 17 subtype 1a had less chance of clearing their HCV infection than did patients infected with  
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38 18 subtype 1b, which agrees well with a previous study [Legrand-Abbravanel et al., 2009]. [The](#)  
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40 19 [subtype did not influence the early virological response: the viral responses at week 12 in](#)  
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42 20 [patients infected with subtype 1a \(62.5%\) and 1b \(76%\) were similar.](#) Recent data suggest  
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44 21 that the treatment duration of patients infected with HCV genotype 1 could be adapted  
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46 22 according to the HCV RNA concentrations. Evidence from several clinical trials supports the  
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48 23 case for extending the treatment time for patients with HCV genotype 1 and a slow virological  
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50 24 response to 72 weeks [Berg et al., 2006; Pearlman et al., 2007]. Knowing the HCV subtype  
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52 25 of patients infected with genotype 1 could also help to optimize anti-HCV treatment. Several  
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54 26 new drugs that target the virus, including protease and polymerase inhibitors, have also  
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56 27 focused on HCV genotype 1 infections. Studies investigating telaprevir in combination with  
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58 28 peg-IFN- $\alpha$  and ribavirin found that a virological breakthrough due to resistance emerged

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3 1 more frequently in patients harboring subtype 1a [McHutchison et al., 2009; Sarrazin and  
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5 2 Zeuzem, 2010].  
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7 3 Recent independent genome-wide association studies have identified single  
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9 4 nucleotide polymorphisms near the IL28B region as being associated with the response to  
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11 5 peg-IFN- $\alpha$  and ribavirin treatment [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009].  
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13 6 The association was observed in individuals infected with HCV alone and in those coinfecting  
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15 7 with HCV and HIV. The association was strongest in patients with HCV genotypes 1 or 4  
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17 8 [Rauch et al., 2010]. These combined virological, pharmacological and host factors will be  
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19 9 analysed in future studies.  
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22 10 To conclude, a high ribavirin concentration is associated with a higher rate of early  
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24 11 virological response. An important finding is that the HCV subtype 1a responds less well to  
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26 12 treatment than does HCV subtype 1b. The HCV subtype could therefore be relevant for  
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28 13 managing the anti-HCV therapy of individual patients and for assessing the efficacy of new  
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30 14 combined treatments with drugs that target specifically the virus.  
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14 **6 CONFLICT OF INTEREST**  
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For Peer Review



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3 1 Figure 1:  
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5 2 (a) Serum ribavirin concentrations and (b) serum pegylated interferon alpha-2a (peg-IFN-  
6  $\alpha$ 2a) concentrations at week 4 (W4) and 12 (W12), according to the early virological  
7 response and the sustained virological response. Data are presented as box plots in which  
8 4 response and the sustained virological response. Data are presented as box plots in which  
9 5 50% of values lie within the box. The horizontal lines through the middle of the boxes  
10 6 represent the median values. The top and the bottom of each box are the 10<sup>th</sup> and the 90<sup>th</sup>  
11 7 percentiles of all values.  
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20 9 Figure 2:  
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22 10 Relationship between the plasma ribavirin concentrations at weeks 4 or 12 and the  
23 11 hemoglobin concentration at week 12. [The Spearman correlation rate is indicated for data at](#)  
24 12 [weeks 4 and 12.](#)  
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