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Florence Legrand-Abrevanel, Philippe Colson, Helene Le Guillou-Guillemette, Laurent Alric, Isabelle Ravaux, Françoise Lunel-Fabiani, Magali Bouvier-Alias, Pascale Trimoulet, Marie-Laure Chaix, Christophe Hezode, et al.

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INFLUENCE OF THE HCV SUBTYPE ON THE VIROLOGICAL RESPONSE TO PEGYLATED INTERFERON AND RIBAVIRIN THERAPY

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Complete List of Authors:	<p>Abravanel, Florence; Toulouse University hospital, Virology; Inserm U563 Colson, Philippe; Marseille university hospital, virology Le Guillou-Guillemette, Helene; Angers university hospital, virology Alric, Laurent; Toulouse University Hospital, Medecine Interne Ravaux, Isabelle; infectious diseases; Marseille university hospital, virology Lunel-Fabiani, F; chu angers, maine et loire Bouvier-Alias, Magali; CHU Mondor, virology Trimoulet, Pascale; CHU de Bordeaux, Virologie; Université Victor Ségalen, Virologie EA2968 Chaix, Marie-Laure; CHU Necker, virology Fontaine, Helene; CHU Cochin, Gastroenterology Hezode, Christophe; CHU Mondor, gastroenterology Foucher, Juliette; CHU Bordeaux, Gastroenterology Roque-Afonso, Anne-Marie; CHU Paul Brousse, virology Gassin, Michele; CHU Nantes, Virology Schvoerer, Evelyne; CHU Stasbourg, virology Gaudy, Catherine; CHU Tours, virology Roche, Bruno; CHU Paul Brousse, Gastroenterology Doffoel, Michel; CHU Strasbourg, Gastroenterology Dalteroche, Louis; CHU Tours, gastroenterology Vallet, Sophie; CHU Brest, virology Baazia, Yazid; CHU Avicenne, virology Pozzetto, Bruno; CHU Saint Etienne, Virology Thibault, Vincent; CHU Pitié salpêtrière, virology Nousbaum, Jean-baptiste; CHU Brest, Gastroenterology Roulot, Dominique; Hôpital Jean Verdier, gastroenterology Coppere, Henry; CHU Saint Etienne, gastroenterology Poynard, Thierry; CHU pitie salpetriere, gastroenterology Payan, Christopher; CHU Brest, virology Izopet, Jacques; Institut Fédératif de Biologie de Purpan, Laboratoire de Virologie</p>

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Keywords:	HCV subtype, interferon, ribavirin



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INFLUENCE OF THE HCV SUBTYPE ON THE VIROLOGICAL RESPONSE TO PEGYLATED INTERFERON AND RIBAVIRIN THERAPY

5 Florence Legrand-Abravanel¹, Philippe Colson², H el ene Leguillou-Guillemette³,
15 Laurent Alric⁴, Isabelle Ravaux⁵, Fran oise Lunel-Fabiani³, Magali Bouviers-Alias⁷,
16 Pascale Trimoulet⁸, Marie Laure Chaix⁹, Christophe H ezode¹⁰, Juliette Foucher¹¹,
17 H el ene Fontaine¹², Anne-Marie Roque-Afonso¹³, Mich ele Gassin¹⁴, Evelyne
18 Schvoerer¹⁵, Catherine Gaudy¹⁶, Bruno Roche¹⁷, Michel Doffo el¹⁸, Louis
24 D'Alteroche¹⁹, Sophie Vallet²⁰, Yazid Baazia²¹, Bruno Pozzetto²², Vincent Thibault²³,
25 Jean-Baptiste Nousbaum²⁴, Dominique Roulot²⁵, Henry Coppere²⁶, Thierry
26 Poinard²⁷, Christopher Payan²⁰ and Jacques Izopet¹

35 ¹ Laboratoire de virologie, Centre Hospitalier-Universitaire, Toulouse ; ² Laboratoire
36 de virologie, Centre Hospitalier-Universitaire, Marseille ; ³ Laboratoire de virologie,
37 Centre Hospitalier-Universitaire, Angers ; ⁴ M edecine interne, Centre Hospitalier-
38 Universitaire, Toulouse ; ⁵ Service de maladie infectieuse Centre Hospitalier-
39 Universitaire, Marseille; ⁶ Service de Gastroent erologie, Centre Hospitalier-
40 Universitaire, Angers; ⁷ Laboratoire de virologie, Centre Hospitalier-Universitaire
41 Mondor, Cr eteil ; ⁸ Laboratoire de virologie, Centre Hospitalier-Universitaire,
42 Bordeaux ; ⁹ Laboratoire de virologie, Centre Hospitalier-Universitaire Necker, Paris ;
43 ¹⁰ Service de Gastroent erologie, Centre Hospitalier-Universitaire Mondor, Cr eteil ; ¹¹
44 Service de Gastroent erologie, Centre Hospitalier-Universitaire Bordeaux, ¹² Service
45 d'h epato-logie H opital Cochin, Paris ; ¹³ Laboratoire de virologie, Centre Hospitalier-
46 Universitaire Paul Brousse, Paris ; ¹⁴ Laboratoire de virologie, Centre Hospitalier-

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3 Universitaire, Nantes; ¹⁵ Laboratoire de virologie, Centre Hospitalier-Universitaire,
4
5 Strasbourg ; ¹⁶ Laboratoire de virologie, Centre Hospitalier-Universitaire, Tours ; ¹⁷
6
7 Centre hépatobiliaire Centre Hospitalier-Universitaire, Paul-Brousse, Paris ¹⁸ Service
8
9 de Gastroentérologie Centre Hospitalier-Universitaire, Strasbourg, ¹⁹ Service de
10
11 Gastroentérologie Centre Hospitalier-Universitaire, Tours, ²⁰ Laboratoire de virologie,
12
13 Centre Hospitalier-Universitaire, Brest ; ²¹ Laboratoire de virologie, Centre
14
15 Hospitalier-Universitaire Avicenne, Bobigny ; ²² Laboratoire de virologie, Centre
16
17 Hospitalier-Universitaire, Saint-Etienne ; ²³ Laboratoire de virologie, Centre
18
19 Hospitalier-Universitaire Pitié-Salpêtrière, Paris ; ²⁴ Service de Gastroentérologie,
20
21 Centre Hospitalier-Universitaire, Brest, ²⁵ Service de Gastroentérologie, Centre
22
23 Hospitalier Jean Verdier, Bondy, ²⁶ Service de Gastroentérologie Centre Hospitalier-
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25 Universitaire Saint-Etienne, ²⁷ Service de Gastroentérologie Centre Hospitalier-
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27 Universitaire Pitié-Salpêtrière, Paris.
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ABSTRACT

The hepatitis C virus genotype is considered to be the most important baseline predictor of a sustained virological response in patients with chronic hepatitis C treated with pegylated interferon and ribavirin. The influence of the subtype on the sustained virological response was investigated in patients infected with genotypes 1, 4, 5 or 6. This study was done on 597 patients with chronic hepatitis C who were given pegylated interferon and ribavirin for 48 weeks. The overall rate of sustained virological response in the 597 patients was 37.8%. Univariate analysis indicated that the sustained virological response of patients infected with subtype 1b (39%) tended to be higher than that of patients infected with subtype 1a (30.6%; $p=0.06$) and it was similar to those patients infected with subtypes 4a (51.3%, $p=0.12$) or 4d (51.7%, $p=0.16$). Multivariate analysis indicated that five factors were independently associated with sustained virological response: the age (OR 0.97; 95% CI = 0.95-0.99), absence of cirrhosis (OR: 2.92; 95% CI = 1.7-5.0; $p<0.01$), absence of HIV coinfection (OR: 2.08; 95% CI = 1.2-3.5; $p<0.01$), low baseline plasma HCV RNA concentration (OR: 1.74; 95% CI = 1.2-2.6; $p<0.01$) and the subtype 1b (OR: 1.61; 95% CI = 1.0-2.5; $p=0.04$) or subtypes 4a and 4d (OR: 2.03; 95% CI = 1.1-3.8; $p=0.03$). In conclusion, among difficult-to-treat genotypes, the subtype 1a is associated with a lower response to anti-HCV therapy than subtypes 1b, 4a and 4d.

INTRODUCTION

Hepatitis C virus (HCV) infection is a major health care burden worldwide. HCV is a single-stranded, positive-polarity RNA virus belonging to the genus *Hepacivirus* of the family *Flaviviridae*. The HCV genome has the following gene order: 5'-C-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-3' and encodes a single long polyprotein [Lindenbach and Rice, 2005]. The sequences of the genomes of HCV variants differ considerably and have been divided into six genotypes. Each of these 6 genotypes can be further subdivided into a series of subtypes [Simmonds et al., 2005].

Anti-HCV therapy has progressed significantly in recent years. The standard and most effective initial treatment for chronic hepatitis C is a combination of pegylated interferon-alpha plus ribavirin [Fried et al., 2002; Manns et al., 2001]. The HCV genotype is considered to be the most important baseline predictor of a sustained virological response in patients with chronic hepatitis C. Treatment is effective in approximately 80% of patients infected with HCV genotype 2 or 3 but is less than 50% in those infected with HCV genotype 1 [Fried et al., 2002; Hadziyannis et al., 2004; Manns et al., 2001]. Genotype 4 is considered as a difficult-to-treat genotype [Legrand-Abravanel et al., 2005; Martin-Carbonero et al., 2008; Roulot et al., 2007; Zeuzem, 2004], even if high sustained virological response rates (67–70%) have been reported for patients in the Middle East infected with genotype 4 [Hasan et al., 2004; Kamal et al., 2005]. The sensitivity of genotypes 5 and 6 to interferon and ribavirin may be similar to that of genotypes 2 and 3 [Bonny et al., 2006; Fung et al., 2008; Hui et al., 2003; Legrand-Abravanel et al., 2004]. However, treatment for 48 weeks is still recommended for patients infected with genotypes 5 or 6.

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3 Most commercial assays determine the genotype by analysing the 5'UTR. But
4 the 5'UTR is highly conserved, which limits its use for discriminating genotype 6 from
5 genotype 1 and subtypes within genotypes 1, 2, 3, 4, and 6 [Cantaloube et al., 2006;
6 Chen and Weck, 2002; Sandres-Saune et al., 2003]. For example, the G residue at
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13 5 position 243 of the 5'UTR was originally considered to be representative of subtype
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25 10 making it impossible to study the influence of the subtype on the virological response,
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27 especially in difficult-to-treat patients infected with HCV genotypes 1 or 4.
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31 An observational study was therefore carried out on a large group of patients
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33 to evaluate the influence of HCV subtype on the response to pegylated interferon
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35 plus ribavirin.
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PATIENTS AND METHODS

Patient selection

A total of 14 centers in France took part in the study between January 2004 and June 2007. Patients were selected in the present study based on the following criteria: (i) patients were infected with HCV-1, 4, 5 and 6 only; (ii) subjects could be naïve or not for previous interferon-based therapy; (iii) patients could be or not coinfecting with human immunodeficiency virus (HIV) or the hepatitis B virus (HBV). The following data were collected: age, gender, geographical origin of the patients, source of transmission, and co-infection with HIV or HBV, and Metavir score of liver biopsies.

Patient description

Five hundred and ninety seven patients were included, 67.7% were men (Table 1). The mean age was 48.9 ± 11.1 years old. One hundred and ninety three (32.3%) had been previously treated with a standard interferon-based regimen. Fourteen patients were infected with HBV and 141 were also infected with HIV. Approximately one quarter of the patients (23.4%) who underwent a liver biopsy had cirrhosis.

Treatment

Patients were given pegylated interferon alpha-2a (Pegasys, Roche laboratories, Palo Alto, CA), 180 $\mu\text{g}/\text{week}$, or pegylated interferon alpha-2b (PEG-Intron, Schering Plough, Kenilworth, NJ), 1.5 $\mu\text{g}/\text{kg}/\text{week}$, at the discretion of the physician, combined with ribavirin (Copegus, Roche laboratories or Rebetol, Schering Plough), 1000 mg/day if the body weight was ≤ 75 kg, or 1200 mg/day if the body weight was > 75 kg, for 48 weeks. The doses of pegylated interferon and ribavirin were modified according to standard criteria and procedures [Dienstag and McHutchison, 2006].

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3 Patients with an undetectable HCV RNA or with a >2 log decline in HCV-RNA at
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5 week 12 were considered to be early virological responders and were treated for 48
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7 weeks. The treatment of patients with a <2 log decline at week 12 was stopped and
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9 they were considered to be non-responders. All patients were followed up for 24
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13 5 weeks after the end of treatment. The primary end point was undetectable serum
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15 HCV-RNA 24 weeks after treatment cessation.
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18 19 20 *HCV RNA quantitation*

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22 HCV-RNA was measured at baseline and 12 weeks after beginning the treatment
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25 10 with Versant HCV-RNA 3.0 bDNA (Siemens Healthcare Diagnostics, Tarrytown, NY)
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27 (limit of detection: 615 IU/ml), or Cobas Taqman Roche Diagnostics (Roche
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29 Molecular Systems, Pleasanton, CA) (limit of detection: 15 IU/ml), depending on the
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31 assay used routinely in each center. The plasma HCV RNA concentrations measured
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33 with the bDNA test were adjusted to take into account the mean difference between
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36 15 the two assays [Pittaluga et al., 2008]. Values of 0.49 log copies/ml for genotype 1a,
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38 0.45 log copies/ml for genotype 1b, and 0.26 log copies/ml for genotype 4 were
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40 added to plasma HCV RNA concentrations measured with the bDNA test (n=269).
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42 The plasma HCV RNA concentrations of patient infected with genotype 5 were not
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44 adjusted since the mean difference between the two assays is less than 0.05 log
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48 20 copies /ml [Sarrazin et al., 2006; Vermehren et al., 2008]. Similarly, the plasma RNA
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50 HCV concentration measured in patients infected with genotype 6 was not adjusted
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52 as no data are available on the quantitation for this genotype with the two assays.
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56 57 58 *HCV genotyping*

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3 The HCV genotype was determined by sequencing a 382 nt fragment within
4 the NS5B region of the HCV genome [Laperche et al., 2005; Sandres-Saune et al.,
5 2003].
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10 5 *Statistical analysis*

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12 Statistical analysis was performed with the software Stata 8.0 (Stata Corporation,
13 Grand Forks, ND). Non-parametric tests were used to compare the differences
14 between the groups (Mann-Whitney U test for continuous variable; X² test for
15 parametric variables). A p value of less than 0.05 was considered to be significant.
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10 Baseline predictors of the virological response were evaluated by univariate and
multivariate analyses. The following covariates were analysed: type of pegylated
interferon (pegylated interferon alpha-2a or pegylated interferon alpha-2b), previous
interferon-based therapy, sex, age as continuous variable, geographical origin of the
patients, liver cirrhosis (F4 score according to the METAVIR classification), HBV and
HIV co-infection, baseline plasma HCV RNA concentration, classified as $\leq 800\,000$
IU/ml (5.9 log IU/ml) or $> 800\,000$ IU/ml, HCV genotype and HCV subtypes 1a, 1b,
4a and 4d. Variables with a p value of 0.10 or less after univariate analysis were
entered into a multivariate, backward, stepwise logistic regression analysis to identify
significant variables associated independently with the virological response. Odds
ratios were estimated from the model and are given with their 95% confidence
intervals. Patients infected with non genotypes 1a, 1b, 4a and 4d were excluded from
the analyses due to the low number of patients within each subtype. Since they
presented the same early and sustained virological response, patients infected with
subtypes 4a and 4d were grouped to increase the number of subjects.

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3 The statistical analysis was confirmed independently by Dr M Delobel-Ayoub (Inserm
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5 Unit 558).
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RESULTS

Genotype distribution

The demographic and clinical characteristics of the 597 patients are summarized in Table 1. The HCV genotypes were 1 (n=494), 4 (n=91), 5 (n=8), and 6 (n=4). The distribution of the subtypes is outlined in Table 2. Of the patients infected with genotype 1, 44.5% (n=220) were infected with the subtype 1a and 51.2% (n=253) with subtype 1b. The patients infected with genotype 4 showed a wider range of subtypes, with more than 9 subtypes identified, although subtypes 4a (40.7%) and 4d (31.8%) were the main ones.

Early virological response

Almost two-thirds of the patients (64.1%) had a virological response, defined as an undetectable HCV RNA or a 2-log decline from baseline HCV RNA, by week 12. The early virological responses for each subtype are shown in Table 2. Univariate analysis identified 6 factors that were associated with an early virological response: the type of pegylated interferon, previous interferon-based treatment, absence of cirrhosis, co-infection with HIV, low baseline HCV RNA and the genotype. Patients given pegylated interferon alpha 2a had a better early virological response (67.8%) than patients given pegylated interferon alpha 2b (54.3%, $p<0.01$). The early virological response of interferon-experienced patients was poorer (56%) than that of naive patients (69%, $p<0.01$). Similarly, the early virological response rate of cirrhotic patients was lower (48.4%) than that of non-cirrhotic patients (67.7%, $p<0.01$). Patients infected with both HIV and HCV had a lower early virological response rate (48.2%) than patients infected with HCV alone (69%, $p<0.01$). Patients with a low

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3 baseline HCV RNA ($\leq 800\,000$ IU/ml, or 5.9 log IU/ml) have a better early virological
4 response (73.6%) than patients with a high baseline HCV RNA ($> 800\,000$ IU/ml, or
5 5.9 log IU/ml) (58.4%). Patients infected with genotype 1 or 4 had similar early
6 virological responses (61.4 and 64.1%, $p=0.56$). Whereas, all the patients infected
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13 5 with genotypes 5 or 6 had undetectable HCV RNA by week 12. Patients infected with
14 genotype 1a or 1b had similar early virological responses (61.4% and 64.4%,
15 $p=0.52$), as did patients infected with genotype 4a or 4d (64.8% and 66.6%, $p=0.84$).
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25 10 independently associated with the early virological response: treatment with
26 pegylated interferon alpha-2a, interferon-naive patients, absence of cirrhosis,
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No difference according to gender, geographical origin of the patients or HBV co-
infection was observed. Multivariate analysis indicated that five factors were
independently associated with the early virological response: treatment with
pegylated interferon alpha-2a, interferon-naive patients, absence of cirrhosis,
absence of co-infection with HIV and low plasma HCV RNA concentration at baseline
(≤ 5.9 log UI/mL) (Table 3).

15 *Sustained virological response*

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The overall rate of sustained virological response was 37.8% for the 597
patients. The rates of sustained virological response according to the subtype are
shown in Table 2. Univariate analysis identified seven factors that were associated
with a sustained virological response: previous interferon-based treatment, age,
cirrhosis, co-infection with HIV, viral load before treatment, HCV genotype and HCV
subtype. Patients who had previously failed to respond interferon-based therapy
were less likely to have a sustained virological response (29%) than were naive
patients (43%, $p<0.01$). The responders were younger (47.3 years) than the non-
responders (49.8 years, $p<0.01$). The rate of sustained virological response of
patients without cirrhosis was 42.2%; it was only 19.1% in patients with a cirrhosis

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3 (p<0.01). The rate of sustained virological response for HIV-HCV co-infected patients
4 was lower (28.3%) than that of patients infected with HCV alone (40.7%; p<0.01).
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6 Patients with a low baseline HCV RNA ($\leq 800\,000$ IU/ml) have a better sustained
7 virological response (48.6%) than patients with an higher baseline HCV RNA (32.1%;
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13 5 p<0.01). The sustained virological response of patients infected with genotype 4 was
14 significantly higher (49.4%) than that of patients infected with genotype 1 (35.4%)
15 (p=0.01). Genotype 5 infected patients had a low sustained virological response rate
16 (37.5%) similar to that of patients infected with genotype 1. Those patients infected
17 with subtype 1a tended to have a lower sustained virological response rate (30.6%)
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25 10 than those infected with subtype 1b (39%; p=0.06), and it was lower than patients
26 infected with genotype 4a (51.3%, p<0.01) or 4d (51.7%, p< 0.01). Patients infected
27 with genotype 1b, 4a, and 4d presented the same rate of sustained virological
28 response (p>0.10). Neither gender, geographical origin, nor HBV co-infection
29 influenced the rate of sustained virological response. Multivariate analysis revealed
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36 15 five factors that were independently associated with a sustained virological response:
37 the age, absence of cirrhosis, absence of HIV coinfection, low baseline plasma HCV
38 RNA concentration (≤ 5.9 log UI/mL) and the subtypes 1b, 4a or 4d (Table 3).
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DISCUSSION

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8 A large observational study of chronic hepatitis C-infected patients was
9 conducted to assess the influence of virus subtype on the virological response to
10 pegylated interferon and ribavirin therapy. The subtype was accurately determined by
11 phylogenetic analyses of the NS5B region of the HCV genome. Multivariate analysis
12 revealed that the subtypes 1b, 4a, and 4d were independent variable associated with
13 the sustained virological response.
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22 Several factors influence the rate of sustained virological response to
23 pegylated interferon and ribavirin therapy: pharmacological, host and virological
24 factors [Everson et al., 2006; Fried et al., 2002; Manns et al., 2001; Nicot et al., 2008;
25 Zeuzem, 2004]. In the present study, patients treated with pegylated interferon alpha-
26 2a had a better early virological response than patient treated with pegylated
27 interferon alpha 2b. However, the type of pegylated interferon did not influence the
28 sustained virological response. This is in keeping with the results of the IDEAL trial
29 where patients treated with pegylated interferon alpha 2a and ribavirin had a better
30 end-of-treatment response than patients treated with pegylated interferon alpha 2b,
31 but the sustained virological responses of the two groups were similar [Sulkowski et
32 al., 2008]. HIV-HCV patients show the same difference in the early virological
33 responses of the patients treated with pegylated interferon alpha-2a and patients
34 treated with pegylated interferon alpha-2b [Vispo et al., 2008].
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53 Patients who had failed a previous interferon-based therapy had a lower
54 sustained virological response. Several trials on HCV-infected and HIV-HCV
55 coinfecting patients have shown that retreatment of (pegylated) interferon-
56 experienced patients with pegylated interferon and ribavirin combination therapy
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3 results in a much lower sustained virological response rate than treatment of
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5 (pegylated) interferon-naive patients [Camma et al., 2002; Krawitt et al., 2005;
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7 Sherman et al., 2006].
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11 In the present work, older age and cirrhosis and HIV coinfection were
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13 5 associated with a poor sustained virological response. It is well established that these
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15 host factors are independent predictors of nonresponse to interferon-based treatment
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17 of patients with chronic hepatitis C [Everson et al., 2006; Fried et al., 2002; Martin-
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19 Carbonero et al., 2008; Zeuzem, 2004].
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23 Baseline plasma HCV RNA concentration is a viral factor influencing the
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25 10 treatment in chronic hepatitis C in most studies [Fried et al., 2002; Manns et al.,
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27 2001; McHutchison et al., 1998; Poynard et al., 1998]. Baseline plasma HCV RNA
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29 concentration and virological response at week 4 may now influence the treatment
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31 duration in genotype 1 infected patients [Ferenci et al., 2008; Yu et al., 2008; Zeuzem
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33 et al., 2006]. In the present work, the statistical analyses found a higher early and
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35 sustained virological response rate in patients with low baseline plasma HCV RNA
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37 15 concentration. The same result was obtained when the analyses were conducted
38
39 without adjusting the HCV RNA concentrations for genotype 1 and 4.
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44 The HCV genotype is considered to be the most important baseline predictor
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46 of a sustained virological response in patients with chronic hepatitis C. A low
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48 20 sustained virological response rate was found for patients infected with genotype 5
49
50 (37.5%), unlike previous findings for this genotype [Bonny et al., 2006; Legrand-
51
52 Abravanel et al., 2004]. The findings of these two studies were supported by
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54 comparison with two control groups of patients infected with genotypes 1 or 2/3. The
55
56 discrepancy with previous work may be because genotype 5-infected patients in the
57
58 present work were more likely to suffer from cirrhosis and were older than the other
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3 patients in the present study. Only 4 patients were infected with genotype 6, but the
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5 rates of early or sustained virological response in this group of patients were very
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7 good, as previously described [Fung et al., 2008; Hui et al., 2003].
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11 Several clinical trials have found that patients infected with genotypes 1 or 4
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13 5 are less likely to achieve a sustained virological response [Fried et al., 2002;
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15 Legrand-Abravanel et al., 2005; Manns et al., 2001; Martin-Carbonero et al., 2008;
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17 Roulot et al., 2007]. A previous retrospective study found that French patients
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19 infected with subtype 4a had a higher rate of sustained virological response (58%)
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21 than those infected with subtype 4d (43%, $p=0.035$) [Roulot et al., 2007]. A study has
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23 reported a group of ten HIV-positive patients who were acutely infected with HCV
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25 10 genotype 4d. None of the 10 patients treated early with antiviral therapy had a
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27 sustained virological response, suggesting that this subtype is less sensitive to
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29 interferon-based therapy [Serpaggi et al., 2006]. However, no difference in the
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31 virological responses of patients infected with subtypes 4a or 4d was found at week
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36 15 12 or 6 months after completion of the therapy. By contrast, among difficult-to-treat
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38 genotypes, patients infected with subtype 1b, 4a and 4d had a higher sustained
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40 virological response than those infected with subtype 1a. This was not reported
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42 before because most studies, including pivotal studies on pegylated interferon-alpha
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44 2a and pegylated interferon-alpha 2b registration, determined the HCV genotype
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48 20 using a method based on the 5'UTR region that does not discriminate accurately the
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50 subtypes [Fried et al., 2002; Manns et al., 2001]. Therefore, the influence of the
51
52 subtype could not be determined.
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56 In conclusion, in patients infected with difficult-to-treat genotypes, the subtype
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58 1a may be considered as a pejorative factor of response to antiviral therapy. Further
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3 studies are needed to identify the molecular basis of this difference and to assess the
4
5 possibility of using the genotype 1 subtype to optimize anti-HCV therapy.
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For Peer Review

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Table 1. Baseline characteristics of the 597 treated patients

<i>Characteristic</i>	<i>Total (n=597)</i>	<i>Genotype 1 (n=494)</i>	<i>Genotype 4 (n=91)</i>	<i>Genotype 5 (n=8)</i>	<i>Genotype 6 (n=4)</i>
Gender, n (%)					
Female	193 (33)	159 (32.8)	30 (33)	4 (50)	0
Male	404 (67)	335 (67.8)	61 (67)	4 (50)	4 (100)
Age, mean years ± SD	48.9 ± 11.1	49.3 ± 11.2	45.2 ± 8.5	63.2 ± 8.9	59.8 ± 7.3
Source of infection, n (%)					
Blood transfusion	132 (22.1)	120 (24.3)	9 (9.9)	3 (37.5)	0
Drug abuse	194 (32.5)	159 (32.1)	35 (38.5)	0	0
Nosocomial	40 (6.7)	32 (6.5)	5 (5.5)	2 (25)	1 (25)
Sexual	11 (1.8)	8 (1.6)	3 (3.3)	0	0
Unknown	222 (37.3)	175 (35.5)	39 (42.8)	3 (37.5)	3 (75)
Liver Fibrosis, n (%)*					
F0-3	412 (76.6)	336 (75.2)	68 (86.1)	5 (62.5)	3 (75)
F4	126 (23.4)	111 (24.8)	11 (13.9)	3 (37.5)	1 (25)
Co-infection, n (%)					
HIV	141 (23.6)	112 (22.6)	29 (31.8)	0	0
HBV	14 (2.3)	12 (2.4)	2 (2.2)	0	0
Mean HCV RNA (log IU/mL) (± SD)	6.10 ± 0.6	6.15 ± 0.6	5.87 ± 0.6	5.80 ± 0.5	6.21 ± 0.8
Range HCV RNA (log IU/mL)	3.34 - 8.29	3.44 - 8.29	3.34 - 7.47	5.34 - 6.83	5.16 - 6.89
Previous interferon based therapy					
Yes	193 (32.3)	172 (34.8)	20 (22)	1 (12.)	0
No	404 (67.7)	322 (65.3)	71 (78)	7 (87.5)	4 (100)
Treatment					
Peg-IFN α -2a + Ribavirin	436 (73.1%)	361 (73.1%)	65 (74.1%)	4 (75%)	4 (100%)
Peg-IFN α -2b + Ribavirin	161 (26.9%)	133 (26.9%)	26 (28.6%)	2 (25%)	0

*Data were unavailable for 59 patients, including 47 in the genotype 1 group and 12 in the genotype 4 group.

Table 2. Virological responses according to the genotype and subtype

Genotype	Subtype	n	Early virological response, n (%)	Sustained virological response, n (%)
1	all subtypes	494	310 (62.7)	175 (35.4)
	1a	220	135 (61.4)	68 (30.6)
	1b	253	161 (64.1)	98 (39)
	other subtypes	21	14 (66.6)	9 (42.8)
4	all subtypes	91	60 (65.9)	48 (52.7)
	4a	37	24 (64.8)	19 (51.3)
	4d	29	22 (66.6)	15 (51.7)
	other subtypes	25	17 (68)	12 (48)
5	5a	8	8 (100)	3 (37.5)
6	all subtypes	4	4 (100)	3 (75)

Table 3: Multivariate analysis of factors associated with virological response.

	<i>n</i>	<i>Odds ratio</i>	<i>95% CI</i>	<i>P value</i>
Early virological response	538			
Type of pegylated interferon				
Peg-IFN α 2b	143	1		
Peg-IFN α 2a	395	2.57	1.7 - 3.9	<0.01
Previous interferon based therapy				
Yes	184	1		
No	354	1.50	1.0 - 2.2	0.04
Cirrhosis				
Yes	126	1		
No	412	2.02	1.3 - 3.1	<0.01
HIV coinfection				
Yes	124	1		
No	414	2.8	1.8 - 4.4	<0.01
Baseline HCV RNA				
> 5.9 log IU/ml	190	1		
\leq 5.9 log IU/ml	348	2.06	1.4 – 3.1	<0.01
Sustained virological response	484			
Age		0.97	0.95-0.99	<0.01
Cirrhosis				
Yes	117	1		
No	367	2.92	1.7 - 5.0	<0.01
HIV coinfection				
Yes	129	1		
No	355	2.08	1.2 – 3.5	<0.01
Baseline HCV RNA				
> 5.9 log IU/ml	323	1		
\leq 5.9 log IU/ml	164	1.74	1.2 – 2.6	< 0.01
Subtypes				0.03
1a	201	1		
1b	226	1.61	1.0 – 2.5	0.04
4a + 4d	57	2.03	1.1 - 3.8	0.03