

Chronic Hepatitis C Treated with Peginterferon alfa plus Ribavirin in Clinical Practice

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ABBREVIATIONS:

Alanine Aminotransferase (ALT); Fibrosis Score (Fn); Hepatitis C Virus (HCV); Human Immunodeficiency Virus (HIV); Hepatitis B Virus (HBV); Ribonucleic Acid (RNA); Sustained Virological Response (SVR)

ABSTRACT

Background/Aims: The role of genotype and viremia were retrospectively evaluated on sustained virological response (SVR) rates in routine clinical practice.

Methodology: From 1907 patients with chronic hepatitis C proposed for treatment, we analysed 1380 (1124 naive and 256 treatment-experienced) with complete follow-up. Genotype and HCV RNA quantification were assayed by commercial tests. Viremia was considered high if >800,000IU/mL, and low if <400,000IU/mL. Liver fibrosis was staged in 614 patients.

Results: Genotype 1 was the most frequent (60%), followed by 3 (25%), 4 (9%) and 2 (2%); 3.2% had other or unclassified genotype. Genotype 1 was

more prevalent in central Portugal and genotype 4 in the south. Viremia was ≤800,000IU/mL in 54.6% and <400,000IU/mL in 34.6% of the patients, particularly in genotype 2 ($p<0.03$) and 4 ($p<0.001$). Genotype non-1 had a significantly lower viral load ($p=0.004$). Mild or moderate fibrosis was present in 71.7% and bridging fibrosis or cirrhosis in 28.3%, with no differences among genotypes. Treatment was discontinued in 19.8%. SVR was achieved in 55.3% of naive and 36.3% of re-treated patients.

Conclusions: Standard treatment of chronic hepatitis C in real-life achieves similar results obtained in clinical trials, despite differences of demographic and viral parameters.

INTRODUCTION

Hepatitis C is a worldwide disease with an estimated 170 million people chronically infected with the virus. Six genotypes and over 50 subtypes have been identified. Genotype 1 is predominant in the USA and Europe, while the genotypes 4 and 5 are found mainly in Africa and genotype 6 in Asia (1). The prevalence of genotypes varies according to the geography and changes in the prevalence within the same region or country may occur as a result of ethnic clusters, migratory flows or different contagiousness patterns of the populations studied (2,3).

In Portugal, the prevalence of people infected with the hepatitis C virus (HCV) is estimated at 1.5%. There is scarce and fragmented information on the prevalence of the different genotypes, although there appears to be an unequivocal prevalence of genotype 1 (4).

The genotype and baseline viremia are two essential factors in the clinical evaluation of patients with hepatitis C and are important pre-requisites for the establishment of the therapeutic strategy (5-8). Both factors are strong predictors of sustained viral response (SVR) as well as of viral kinetics on-treatment (9). The reason why the genotype influences the SVR to interferon is unknown, although it does not seem to result from any relation between the genotype and viremia, since no significant differences in the HCV RNA concentrations between the different genotypes of patients enrolled in clinical trials have been found (5,9,10). In fact, the discrepancy of nearly 30% in the SVR between genotypes 1 and 4 on the one hand, and genotypes 2 and 3 on the other, does not appear to result from the highest viral load, but rather from other viral factors which have not been determined (11).

The natural course of viremia in large populations of patients with hepatitis C and its relation with the natural history of the disease have not deserved special attention from researchers (12,13), although some works suggest that the severity of liver lesion and the clinical course of the disease do not depend on the HCV RNA serum levels (14).

In terms of antiviral therapy, a low baseline HCV RNA level is a strong predictor of rapid viral response (RVR) and SVR, so that the combination of those two parameters may allow shorter or extended treatment in order to optimise the response (15,16). The evolution of the quantification techniques for HCV RNA, detecting very low serum concentrations of the virus now permit a strict assessment of the viral kinetics during treatment. The threshold of 800,000IU/mL has been used to discriminate high and low viral loads (17), but this value is not consensual in the various therapeutic trials (6). With regard to genotype 1, some authors have considered lower levels, between 200,000 and 300,000IU/mL, as more reliable predictors of RVR and SVR (6,7). Ferenci *et al.* (18), in a recently published study, observed that SVR was approximately 15% higher in patients with baseline HCV RNA $\leq 400,000$ IU/mL when compared to HCV RNA $> 800,000$ IU/mL, but did not find a difference in the response above threshold of 400,000IU/mL (81.3% vs. 80.6%). Genotypes 2 and 3 have a similar behaviour as genotype 1 with identical baseline levels of viremia, although this is not so marked due to their high rates of SVR (8,19,20).

The aim of this retrospective study was to assess the characteristics of Portuguese patients with chronic hepatitis C concerning prevalence of genotypes, viral load and histological lesions, and evaluate efficacy and safety of peginterferon alfa plus ribavirin therapy in routine clinical practice.

METHODOLOGY

We studied 1,907 adult patients with chronic hepatitis C referred to specialist outpatient hepatology clinics for treatment. From that group, 1,531 patients were consecutively treated with a combination of peginterferon alfa (peginterferon alfa-2a or 2b) and ribavirin therapy, between 2003 and 2008, *i.e.* immediately after the marketing of pegylated interferon in Portugal. The selection, doses and duration of treatment depended on medical criteria, in accordance with the good clinical practice and following the AASLD recommendations (21). Seven hospitals participated in the study, distributed among the three regions of the country: north (Hospital de S. João and Hospital de S. Marcos, with 249 patients), centre (Hospitais da Universidade de Coimbra, with 157 patients) and south (Hospital de Santa Maria, Hospital dos Capuchos, Hospital Egas Moniz and Hospital Fernando da Fonseca, with 1193 patients). One thousand three hundred and forty-three patients had never received treatment and 256 were non-responders or relapsers to the standard interferon combination with or with-

out ribavirin. Patients with HCV/HBV or HCV/HIV coinfection were excluded. The collection of data was obtained through a survey that included age, gender, alcohol consumption > 40 g/day, liver biopsy, stage of liver fibrosis, genotype, baseline viremia, rapid, early and sustained responses to treatment and re-treatment, and use of erythropoietin or granulocyte-stimulating agents.

Baseline HCV RNA was quantified with commercial assays, initially with Cobas Amplicor HCV Monitor V2.0 (Roche Molecular Systems, Indianapolis, USA) in 4 hospitals, Versant HCV Quant (Siemens Health Care Diagnostics, Tarrytown, NY, USA) in 2 hospitals; and, later by Abbott RealTime (Abbott Diagnostics) in 1 hospital and by Cobas Amplicor / Cobas Taqman HCV Test (Roche Molecular Systems, Indianapolis, USA) in the other hospitals. HCV genotype was determined with INNO-LiPa HCV II (Innogenetics, Ghent, Belgium), or Versant HCV Genotyping Assay 2.0 (Siemens Health Care Diagnostics, Tarrytown, NY, USA), except in 1 hospital where an in-house method was used. Viremia was considered high if HCV RNA $> 800,000$ IU/mL, intermediate 400,000-800,000IU/mL and low $< 400,000$ IU/mL (17). The stage of liver fibrosis was assessed by the METAVIR scoring system.

Regarding the treatment, only patients with complete follow-up were analysed, *i.e.* with HCV RNA determination six months after treatment completion. The SVR was defined, initially, as having undetectable HCV RNA at the end of follow-up by a qualitative PCR assay (COBAS Amplicor HCV Test, version 2.0, limit of detection, 50IU/mL), and at over the last 2 years, by PCR-real time. The study protocol was performed in accordance with guidelines of the 1975 Declaration of Helsinki.

Statistical analysis

All patients who received a single dose of medication were included in the efficacy analyses. Statistic analysis was descriptive. Chi-square was applied to compare different rates. Data analysis was made using the statistical package SPSS for Windows (version 14.0; SPSS Inc, Chicago, IL). A *p*-value below 5% was considered as significant.

RESULTS

Patient population

The study population consisted of 1907 patients, being 1274 (66.8%) male. The mean age of the patients was 41.1 years. Fourteen per cent had excessive alcohol consumption: 14% in the north and 17% in the south. Information from two hospitals, one of them from the centre, was not obtained.

Prevalence of genotypes

The genotype information was available in 1500 patients: genotype 1 was the most frequent (60.2%), followed by genotypes 3 (24.7%), 4 (9.4%) and 2 (2.0%). In 48 (3.2%) patients another genotype was found or it could not be classified (**Figure**

1). **Figure 2** shows the frequency of the different genotypes in the three regions of the country, showing that in the centre, compared with the other two regions, the prevalence of genotype 1 was lower ($p=0.005$), unlike what happened with genotype 3, although this difference was not statistically significant. In this region the largest number of non-1-4 genotypes were observed. Regarding genotype 4, the frequency increased from north to south (11%), and the difference between these two regions was significant ($p=0.013$).

Viremia

The baseline HCV RNA was quantified in 1213 patients: 551 (45.4%) had high viremia, 242 (19.9%) intermediate and 420 (34.6%) low. The viral distribution according to the genotype can be observed in **Figure 3**. With the exception of genotype 1, in which about half (49.6%) of the patients had a viral load higher than 800,000 IU/mL, in the other genotypes the HCV RNA serum levels were systematically below that level ($p=0.004$). Patients with genotypes 2 and 4 had a viral load lower than 400,000 IU/mL more frequently than the patients with genotype 1 ($p<0.03$ and $p<0.001$, respectively). **Table 1** shows the viral distribution according to genotype in the three regions of the country. There were no significant differences between regions, except with respect to genotypes 2/3, in which the high viral load was more frequent in the south when compared to the north ($p<0.03$), in opposition to what happened with the low viral load ($p<0.001$).

Evaluation of fibrosis

Six hundred and fourteen patients had a liver biopsy. Fibrosis was classified as F1 in 227 (38.7%), F2 in 192 (32.7%), F3 in 65 (11.1%) and F4 in 102 (17.4%). Thus, 440 (71.7%) patients had a mild fibrosis stage (F1/F2), while 174 (28.3%) had advanced fibrosis (F3/F4). There were no significant differences in the fibrosis stage between the different genotypes (**Figure 4**). For example, the comparison between genotypes 1 and 4 did not reveal significant differences between the stages F1/F2 (71.6% vs. 78.6%, respectively) and F3/F4 (28.4% vs. 21.4%, respectively).

Of the 28 patients with other/undetermined genotype, fibrosis score was F1 in 13 (46.4%), F2 in 8 (28.6%), F3 in 3 (10.7%) and F4 in 4 (14.3%).

Regarding the presence of cirrhosis, there was no significant difference between the different genotypes. A tendency towards a lower prevalence of cirrhosis on genotype 3 was observed in patients from the north and on genotype 1 in the patients from the centre, although without statistical significance.

Response to therapy

From the 1,599 patients treated, 1,380 had complete follow-up. In 274 (19.8%) patients the treatment was discontinued, in 127 (9.2%) due to adverse effects. From the 1124 patients that received treat-

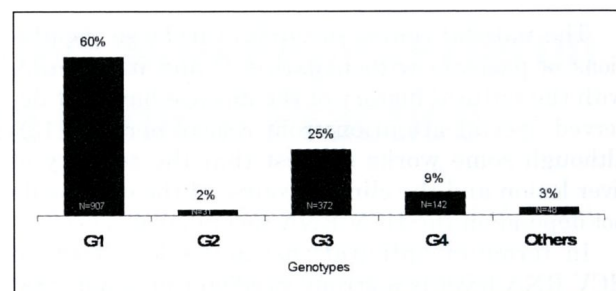


FIGURE 1 Frequency of genotypes in 1,500 Portuguese patients.

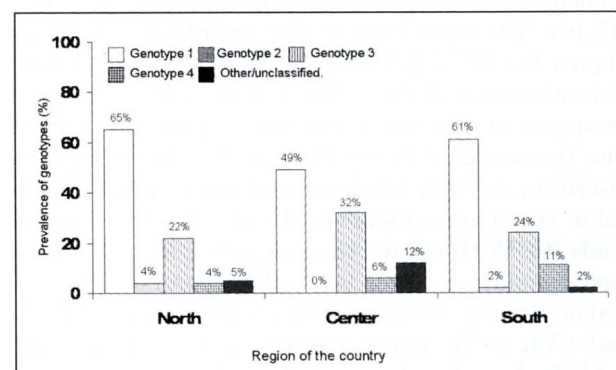


FIGURE 2 Distribution of HCV genotypes by region of the country ($n=1500$). Prevalence of genotype 4 is higher in south region when compared to the north ($p=0.013$).

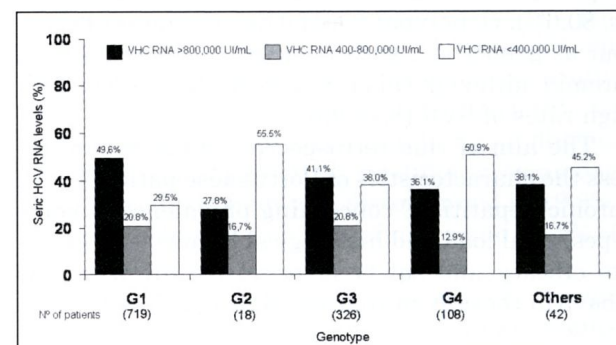


FIGURE 3 Distribution of HCV RNA levels categories (IU/mL) by genotype. Patients with non-1 genotype had significantly higher HCV RNA levels <800,000 IU/mL ($p=0.004$); patients with genotypes 2 and 4 had more frequently viral load <400,000 IU/mL than genotype 1 ($p<0.03$ and $p<0.001$, respectively).

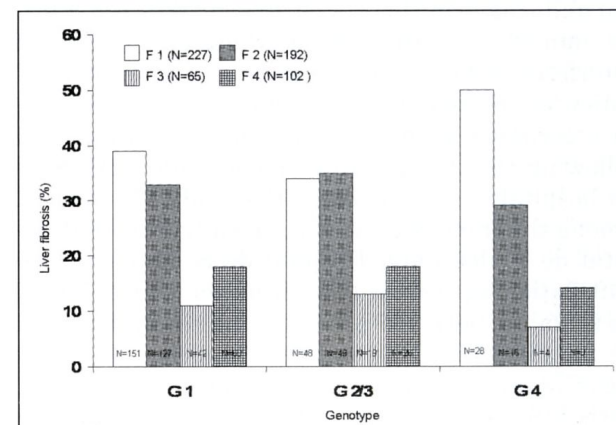


FIGURE 4 Classification of liver fibrosis with METAVIR score according to the genotype.

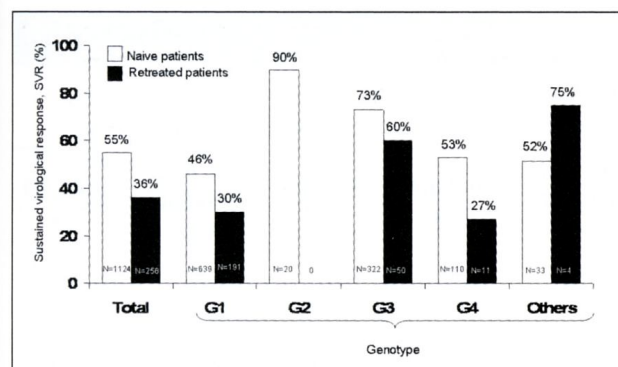


FIGURE 5 The overall and by genotype sustained virological response (SVR) rates to combination of peginterferon plus ribavirin in re-treated patients.

ment for the first time, 622 (55.3%) obtained SVR: 46.0% with genotype 1, 90% with genotype 2, 72.9% with genotype 3, 52.7% with genotype 4 and 51.5% with other or undetermined genotype (**Figure 5**). The SVR rate in the 256 re-treated patients was of 36.3%: 29.8% for genotype 1, 60.0% for genotype 3 and 27.3% for genotype 4. There was a wide variation among the hospitals in the SVR rate in naive and re-treated patients. In naive patients the highest SVR rates were 68% and 67% in two hospitals, and the lowest 48%. In re-treated patients, the highest SVR was 70% observed in one hospital and the lowest was 23%.

DISCUSSION

In this retrospective study we characterised the viral and histological aspects as well as the effectiveness of peginterferon and ribavirin combination therapy in clinical practice, in a wide group of patients with chronic hepatitis C from all regions of the country. The study reveals some facts that we believe are worthy of mention. Firstly, genotype 1 is the most frequent in Portugal; secondly, the distribution of genotypes is not uniform in the country, in particular genotype 4 with a prevalence revealing a north-south gradient; thirdly, viremia is relatively low in all genotypes except for genotype 1; fourthly, one third of the patients have advanced histological lesion (F3/F4); and finally, the overall SVR rate and per genotype with the peginterferon alfa-ribavirin combination is comparable to that described in randomised clinical trials.

The genotype distribution in Portugal follows, generically, the pattern observed in southern Europe (2,3,22), but is slightly different from that observed in the USA, where the prevalence of genotype 1 and, in particular, of genotype 2 are higher (23-25). The higher prevalence of genotype 4 in the south of Portugal may be explained, in our point of view, due to its proximity to north Africa, where genotype 4 is widely predominant, and due to the high expression of the African immigrant community in this area of the country. The migratory flows from North Africa to Europe are, according to some authors, the source of the increasing spread of genotype 4 among intravenous drug users of several European countries,

thus contributing to the alteration of the previous patterns of genotypic prevalence (22,26). In 3.2% of the patients it was not possible to establish a genotype classification, probably for technical reasons, with the exception in a few cases when the genotype 5 was found. We did not find mixed infections in any case, but on genotype 4 the association of subtypes c and d was frequent.

We did not find any relation between genotype and the severity of hepatic lesion. The histological classification of our patients was very similar to that described by Payan *et al.* (2) which, in the multivariable analysis of a series of 1,292 patients, did not find a relation between the genotype and the histological lesion, particularly with cirrhosis. On the contrary, cirrhosis was associated with age at exposure, alcohol consumption, high ALT level, and HIV and HBV coinfections.

Most of our patients (55%) had low viral load, *i.e.* less or equal to 800,000IU/mL, being 35% less than 400,000IU/mL, particularly in patients with genotypes 2 and 4. These viremias are lower than those recently described by Nainan *et al.* (25) in American patients, which can be explained by the influence of the greater prevalence of genotype 1 in that study comparatively to our (75.3% *vs.* 49.6%). In fact, in our patients infected with genotype 1, the HCV RNA serum levels were significantly higher than those of the other genotypes, but this correlation was not observed by Nainan *et al.* (25). However, our data are consistent with those of other authors (23,27) relating to genotypes 1, 2 and 3, and with those of Lau *et al.* (28) with regard to the genotype 4. It can be questioned if the viremia classification based on only one determination is reproducible. The available data are scarce for a definite response, although it can be possible to speculate that the viral load may remain stable over time (13).

As has been reported by other authors (13,14,28) we too did not find any relation between the viral load and the hepatic fibrosis stage. As reported by Romeo *et al.* (14), we also observed that the frequency of the various fibrosis stages, and in particular with respect to cirrhosis, were equally represented in the different genotypes.

The treatment of hepatitis C in the field of routine medical practice may result in a significant variation in the SVR rate when compared to randomised controlled clinical trials. In these trials, patients are chosen according to more restrictive inclusion and exclusion criteria, while in routine medical practice patient's selection is more permissive, no stratification is carried out and the intensive treatment of the adverse effects may reduce the discontinuation rate. Viral factors such as genotype (5) and viremia (6,7,9) may determine the response, but other factors related to the host, such as old age, race, overweight, alcohol abuse, cirrhosis and other co-morbidities may also influence the response (29).

The discrepancies in SVR observed between the different hospital centres could neither be explained on basis of significant differences on geno-

TABLE 1 Viremia According to Genotype and Region of the Country

Region	Genotype 1			Genotype 2/3			Genotype 4		
	HCV RNA (IU/mL)			HCV RNA (IU/mL)			HCV RNA (IU/mL)		
	>800,000 (%)	400,000-800,000 (%)	<400,000 (%)	>800,000 (%)	400,000-800,000 (%)	<400,000 (%)	>800,000 (%)	400,000-800,000 (%)	<400,000 (%)
North	55 (47)	29 (24)	34 (29)	9 (23)	6 (15)	25 (63)	1 (13)	2 (25)	5 (63)
Center	35 (49)	13 (18)	23 (32)	24 (44)	6 (11)	24 (44)	3 (30)	2 (20)	5 (50)
South	267 (50)	108 (20)	155 (29)	106 (42)	59 (24)	85 (34)	35 (39)	10 (11)	45 (50)
Total	357 (50)	150 (21)	212 (29)	139 (40)	71 (21)	134 (39)	39 (36)	14 (13)	55 (51)

type distribution, particularly genotypes 2 and 3, which have higher response rates or from conventional therapeutic protocol adaptations. The overall SVR rate from our study, 55% for naïve patients, is consistent with the rate from other similar studies (30) and may compare with the rate of the clinical trials (9,10). However, our overall and by genotype SVR rate was clearly higher than that found by Backus *et al.* (29), perhaps due to the population characteristics of this study and to the much higher treatment discontinuation rate observed. Our patients were Caucasian, were not excluded from the clinical trials and decompensated cirrhotic patients were not included.

The SVR varied widely among the different hospitals, with 20% of difference for non-experienced patients and 47% for re-treated patients. These differences are not explained by the prevalence of genotypes nor by viremias since, for example, two hospitals from the same region presented differences of 19% in the SVR, while in the centre, despite the lower prevalence of genotype 1, the SVR rate was close to the overall average.

While in clinical trials the discontinuation rate ranged from 14%-32% and 7%-8% in the 48 and 24-week treatment regimens, respectively (6,9,10), in the routine daily treatment it ranged higher, between 30% and 68% (29,31). The lower discontinuation rate of our study may be due to the greater flexibility in the management of adverse effects and to the use of hematological growth factors out of the protocol, particularly the erythropoietin. Although some authors (32) highlight the importance of maintaining the cumulative dose of ribavirin above 60% in order not to affect SVR, the use of erythropoietin with the purpose of avoiding the reduction of ribavirin dose and increasing hemoglobin levels is not consensual (33,34).

The SVR rate to re-treatment (36%) with peginterferon alfa plus ribavirin was surprisingly high, particularly in some hospitals, but is in accordance with the results of other works (35-37). Our non-responders group was very heterogeneous, including null-responders, partial responders and

relapsers. On other hand, failure to respond could be to monotherapy with standard or pegylated interferon either with or without ribavirin. As we included patients just at the time when combination peginterferon plus ribavirin became available, re-treated patients had received in the past any of the schedules except for those containing pegylated interferon.

This study has some limitations in order to be considered a real epidemiological study. Firstly, it evaluates only patients with hepatitis C referred to tertiary centres for antiviral therapy. Nevertheless, we believe that this fact does not significantly compromise the prevalence of genotypes and viremia results, since none of these factors neither contribute to the enrolment or exclusion from treatment or for the severity of the liver lesion and thus in the selection of patients for therapy. Secondly, the hospitals were randomly chosen and their distribution in the territory is not homogeneous. We are convinced that the selected sample reproduces our national reality because patients living in areas beyond their area of influence were referred to the hospitals who took part in this study. However, we admit that the lack of hospitals from the extreme south of the country may have created a light distortion on the real prevalence of genotype 4 in our country.

In conclusion, in the largest study carried out in Portugal, a country in southern Europe, the prevalence of HCV genotypes is similar to that observed in other European countries, with higher prevalence of genotype 1, although it is interesting to note the meridional increase of genotype 4 frequency. The occurrence of low viremias in all genotypes seems to be a characteristic of our patients, which does not seem to significantly increase the SVR rate to the standard combination peginterferon plus ribavirin therapy for chronic hepatitis C within the scope of routine clinical practice.

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