Predictive baseline criteria of primary therapeutic failure in chronic hepatitis C genotype 1

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ABSTRACT

Background and aims: more than half of patients with genotype 1 chronic hepatitis C (CHC) do not achieve a sustained viral response (SVR) to current antiviral therapy due to primary non-response, relapse or intolerance. Factors related to each of these unfavorable outcomes are different and the last two may be partially prevented. Our aim was to identify basal criteria to predict the risk of primary failure.

Patients and methods: we included 251 consecutive patients (152 males) from a single centre, infected with HCV genotype 1 and not previously treated. SVR was achieved in 141 patients and primary failure in 110.

Results: high vs. low viral load (> 400,000 IU/mL, OR = 6.17; 95% CI: 2.50-15.23), high serum GGT (>60 IU/mL, OR = 4.25; 95% CI: 2.49-7.24), low serum cholesterol (<178 mg/dL, OR = 2.93; 95% CI: 1.75-4.92) and older age (>47 yrs., OR = 1.79; 95% CI: 1.08-2.96) were associated to the risk of primary failure in the linear logistic regression analysis. From the 58 patients carrying all the first three negative criteria, 46 (79.3%) were primary non-responders.

Conclusions: the negative basal profile identified in this study is based on easily available data and provides information about the risk of primary therapeutic failure, and may help to decide whether antiviral therapy should be offered to a single patient.

Key words: Hepatitis C virus. Predictive factors. Pegylated interferon. Ribavirin. Thyroid hormones.

INTRODUCTION

Current therapy for genotype 1 chronic hepatitis C combines weekly doses of pegylated interferon (α2a or α2b) and daily oral ribavirin at weight-adjusted doses during 48 weeks. This therapy is considered successful if viral RNA is undetectable in peripheral blood after six months of the end of therapy with a high sensitivity assay. Less than 50% of patients obtain this “sustained viral response” (SVR) in most of the published pivotal clinical trials (1-3) and in many reports on real clinical practice (4-7). The remaining patients may experience: a) primary therapeutic failure, currently defined with the universally accepted “12 (2) and 24 (1) week stopping rules”; b) viral relapse after clearing viral RNA before the week 24 of therapy; and c) severe adverse events causing premature interruption of therapy (intolerance).

Although adverse events are frequent during treatment, most patients overcome them with specific supportive measures. Intolerance is largely unpredictable and causes premature interruption of therapy in less than 10% of treated patients. Viral relapse is related to a delayed response to therapy. Patients with a drop > 2 log 10 but still detectable viral load at week 12 are firm candidates to suffer from viral relapse if they achieve undetectable viral load at week 24 (8), and the current strategy is to prolong therapy until 72 weeks, in an attempt to improve the rate of SVR (9-11).

Most of published studies on this issue aimed at identifying the basal factors that influence the rate of therapeutic success compared patients reaching SVR with a group composed of primary non-responders, relapsers and non-tolerant patients. As these categories represent different types of failure and, in some cases (relapsers and patients with severe adverse events), strategies have been developed to improve the results of therapy, it seems that a change in perspective may be more informative about the risk of a patient to experience primary therapeutic failure,
the only scenario for which we do not have an alternative strategy.

Therefore, we designed the present study with the aim to elucidate whether there are clinical, biochemical and viral factors associated to the risk of primary therapeutic failure by comparing a group of patients with primary failure with a group of patients who reached SVR.

PATIENTS AND METHODS

This is a retrospective study based on the review of the clinical records of all the chronic hepatitis C patients treated at our Liver Unit with pegylated interferon and ribavirin since this combined therapy was available at our center (September 2001). All patients were treated in accordance with Spanish (11) and international (12,13) guidelines. A total of 633 patients had been treated and reached one of the four end-points previously defined. Five hundred and thirty of these patients had never been previously treated with interferon and/or ribavirin (i.e., they were naïve for therapy), and 376 were infected with genotype 1.

For this study, we selected for further analysis only the genotype 1 naïve patients, who obtained sustained viral response six months after the end of therapy or experienced primary failure at weeks 12 or 24 of therapy.

The diagnosis of chronic hepatitis C was based on clinical evaluation together with results of biochemical and virological tests. All patients were positive for anti-HCV since at least 6 months before the beginning of therapy, and HCV-RNA was detectable in blood samples of all individuals when treatment was started. Standard serological tests were used to exclude individuals with active infection with hepatitis B or human immunodeficiency viruses.

Quantitative analysis of HCV-RNA was performed with the Cobas Amplicor HCV Monitor version 2.0 (Roche Molecular Diagnostic). The detection range was 600 IU/mL to 8.5 x 10^5 IU/mL. Starting from July 2005, viral RNA was extracted automatically using Cobas Amplicore Prep, and the viral load was detected using Real-Time polymerase chain reaction (PCR) using Cobas TaqMan (Roche Diagnostics) which has a detection range of between 10 IU/mL and 2 x 10^6 IU/mL (14).

HCV genotypes were determined by a reverse hybridization assay (INNO-LiPA; Innogenetics). The genotypes are assigned on the basis of sequence variations in the 5’ untranslated region of HCV following gene amplification using reverse transcription polymerase chain reaction (RT-PCR).

Results of liver biopsy were also collected, when available; the stage of fibrosis was established according with Knodell et al. (15) and classified as null-low or advanced fibrosis (categories 0-1 and 3-4, respectively, of the item 4 of the Knodell scoring system).

### Statistical analysis

With the objective of establishing which parameters were significantly different between the two groups of patients, a comparison was made for age, gender, body mass index [body weight in kg/(height in m)^2], hemoglobin, leukocyte, neutrophil and platelet counts, AST, ALT, AST/ALT ratio, GGT, alkaline phosphatase, serum cholesterol, viral subtype (1b vs. 1 non-b), viral load (≤ 400,000 IU/mL vs. > 400,000 IU/mL), type of pegylated interferon (α2b vs. α2a), dose of ribavirin adjusted to BMI and, when available, histological stage of fibrosis. Continuous variables were compared with the Student’s t test or Mann-Whitney U test, each when adequate, depending on their Gaussian distribution. A p value < 0.05 was considered significant. Categorical variables were compared with the Chi² or the Fisher exact tests, each when appropriate, and the significance of differences was established by calculating the odds ratio with the 95% confidence interval.

The variables available in all patients and significantly different in the univariate analysis were included in a multivariate analysis based on a logistic regression model to identify if they were independently related to the risk of primary failure. To provide cut-off points useful in clinical practice to predict the risk of suffering primary failure, ROC curves were plotted and the odds ratios (with 95% confidence interval) for the median values of each significant independent variable in the multivariate analysis were calculated.

Data were analyzed using statistical software SPSS version 17.0 (SPSS, Chicago, IL, USA) and EPIDAT statistical package (Xunta de Galicia, Spain).

### RESULTS

Two hundred and fifty one patients (152 males) met the inclusion criteria. One hundred and forty one achieved a sustained viral response. In 110 patients the therapy was stopped due to primary viral failure, 58 in application of the 12 weeks stopping rule and 52 due to detectable viral load at week 24 of therapy. From the remaining 125 patients, 70 relapsed after transient viral response and 55 interrupted the therapy due to severe adverse events (intolerance).

Table I summarizes the demographic, analytical, virological and histological data of the two study groups. All patients were white (240 Spaniards), except one Chinese woman. In the univariate analysis, age, platelet count, AST/ALT ratio, GGT, serum cholesterol, viral subtype (1b vs. 1 non-b), low (≤ 400,000 IU/mL) vs. high (> 400,000 IU/mL) viral load, according to Witthöft et al. (5), and body mass index adjusted ribavirin dose were significantly different between SVR and therapy primary failure groups. From these, only age, GGT, serum cholesterol and low vs. high viral load were identified as inde-
pendent variables related to the risk of suffering from primary therapeutic failure. Table II summarizes the individual AUROC values for each continuous independent variable and the odds ratios for their respective median values (with the addition of viral load, the only significant categorical variable, which cut-off value is 400,000 IU/mL) to discriminate between SVR and primary failure groups.

Forty six (79.3%) of the 58 patients with all three unfavorable values for viral load, GGT and cholesterol were primary non-responders, whereas 11 (92%) of the 12 patients with the three favorable values obtained SVR. However, these 70 patients with extreme values only represent 29% of the whole series. Basal plasma uric acid levels were available in 185 patients (Table I), without significant differences between the 102 who achieved SVR and the 83 primary non-responders (Table I). The cut-off point for serum uric acid ≥ 5.8 proposed by Pellicano et al. (16) had no predictive role in our study, as SVR was obtained by 56.7% and 53.1% of patients under and over the cut off value, respectively.

Results of a liver biopsy were available in 176 patients. There was a significant excess of patients with advanced fibrosis in the group of primary therapeutic failure (56.6 vs. 38.0%, OR = 2.13, 95% CI 1.16-3.90). Cirrhosis was diagnosed in 16 cases, eight in each group of response.

**DISCUSSION**

Contrary to many previous studies, the main objective of this study was to elucidate which factors are related to the risk of primary therapeutic failure instead of which ones define the probability of reaching SVR. Our purpose was to eliminate the heterogeneity of a comparison group composed of three categories of disappointing end-points of therapy: primary failure, relapse and intolerance, each depending on different causes and mechanisms.

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### Table I. Baseline characteristics included in the search of predictive criteria of primary failure of therapy

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>SVR (141 cases)</th>
<th>Primary failure (110 cases)</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>87/54</td>
<td>65/45</td>
<td>OR = 0.89 (95% CI 0.54-1.49)</td>
</tr>
<tr>
<td>Age (yrs.)</td>
<td>45.5 (10.8)</td>
<td>50.4 (10.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI</td>
<td>25.32 (3.62)</td>
<td>25.74 (3.38)</td>
<td>0.334</td>
</tr>
<tr>
<td>PEG-α-IFN (2b/2a)</td>
<td>103/38</td>
<td>88/22</td>
<td>OR = 1.48 (95% CI 0.81-2.68)</td>
</tr>
<tr>
<td>RBV dose (mg/day)/BMI</td>
<td>40.5 (5.2)</td>
<td>38.5 (4.5)</td>
<td>0.024</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>15.0 (1.2)</td>
<td>15.2 (1.2)</td>
<td>0.281</td>
</tr>
<tr>
<td>Leucocytes (µL)</td>
<td>6496 (1726)</td>
<td>6527 (1999)</td>
<td>0.897</td>
</tr>
<tr>
<td>Neutrophils (µL)</td>
<td>3461 (1266)</td>
<td>3396 (15,41)</td>
<td>0.570</td>
</tr>
<tr>
<td>Platelets (10^9/L)</td>
<td>218 (63)</td>
<td>192 (58)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.86 (0.37)</td>
<td>0.95 (0.49)</td>
<td>0.059</td>
</tr>
<tr>
<td>AST (IU)</td>
<td>69 (52)</td>
<td>81 (69)</td>
<td>0.08</td>
</tr>
<tr>
<td>ALT (IU)</td>
<td>116 (89)</td>
<td>114 (93)</td>
<td>0.502</td>
</tr>
<tr>
<td>AST/ALT</td>
<td>0.65 (0.25)</td>
<td>0.76 (0.27)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>GGT (IU)</td>
<td>62 (64)</td>
<td>134 (172)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>189 (37)</td>
<td>165 (34)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Viral subtype (1b/1 non b)</td>
<td>97/44</td>
<td>99/12</td>
<td>0.491</td>
</tr>
<tr>
<td>Viral load (≤ 400,000 IU/mL ≥ 400,000 IU/mL)</td>
<td>37/104</td>
<td>6/104</td>
<td>OR = 1.92 (95% CI 1.06-3.48)</td>
</tr>
<tr>
<td>Uric acid (mg/dL) (185 patients)</td>
<td>5.41 (1.41)</td>
<td>5.59 (1.32)</td>
<td>0.383 Not included</td>
</tr>
<tr>
<td>Advanced fibrosis (no/yes) (176 patients)</td>
<td>62/38</td>
<td>33/43</td>
<td>OR = 2.13 (95% CI 1.16-3.90)</td>
</tr>
</tbody>
</table>

1Continuous variables are expressed as mean (SD); 2BMI: body mass index.

### Table II. AUROC (for continuous variables) and odd ratios for the identified independent variables at specific cut-off values

<table>
<thead>
<tr>
<th>Variable</th>
<th>AUROC (95% CI)</th>
<th>Cut-off value</th>
<th>Odds ratio (95% CI)</th>
<th>p value (Chi² test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs.)</td>
<td>0.631 (0.561-0.700)</td>
<td>&gt; 47 yrs.</td>
<td>1.79 (1.08-2.96)</td>
<td>0.023</td>
</tr>
<tr>
<td>GGT (IU/mL)</td>
<td>0.749 (0.689-0.806)</td>
<td>&gt; 60 IU/mL</td>
<td>4.25 (2.49-7.24)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>1/Cholesterol (mg/dL)</td>
<td>0.678 (0.612-0.745)</td>
<td>&lt; 178 mg/dL</td>
<td>2.93 (1.75-4.92)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Low vs. high viral load</td>
<td>&gt; 400,000 IU/mL</td>
<td></td>
<td>6.17 (2.50-15.23)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Previous reports indicated that older age is a negative predictor of SVR in chronic hepatitis C genotype 1 (17-20), with only one contradictory study (21). From “the other side of the mirror”, our study confirmed that older age increases the risk of primary failure. Age directly correlates with the duration of the infection, although in many cases the date and mechanism of infection are unknown, a fact that justifies the relatively poor statistical power of this factor. We also confirmed previous reports signaling that a low viral load is the strongest predictor of SVR when the comparison is made with patients who suffer primary failure; unfortunately, only a minority of genotype 1 patients are included in this category (17,19,20,22,23). We used the limit of 400,000 IU/mL proposed by Witthöft et al. (5) as the best discriminant one between low and high viral rate, but results would not have changed if higher limits (600,000 or 800,000 IU/mL) had been used instead.

Craxi et al. (21) found that genotype 1a improves the chance of SVR, and our univariate analysis was in agreement with this finding, but its significance disappeared in the multivariate analysis, because this factor is closely related to age (data not shown). A low platelet count, a well known surrogate marker of advanced liver fibrosis (24) is another criterion that heralds primary failure, but in this study it did not reach statistical significance, probably due to the low proportion of patients with liver cirrhosis. Together with a high viral load, GGT and plasma cholesterol levels were the factors most strongly associated to the risk of primary failure in our study. GGT has been identified as a prognostic factor in other studies (17,23) and it is also a surrogate marker of liver fibrosis, as corroborates its inclusion in non-invasive scoring systems aimed to evaluate the stage of fibrosis (25). Moreover, GGT levels are related with an increased expression of TNFα in the liver that seems to reduce the efficacy of antiviral therapy (26). Low serum cholesterol levels have been unanimously identified as a negative predictor of therapeutic success (20,27,28), and they seem to be a consequence of specific defects in lipoprotein metabolism that cause low levels of LDL (28-31), but it is not clear how this may be related to the efficacy of antiviral therapy. Recently, Angelico et al. (32) have shown in a prospective analysis that included 65 patients (37 genotype 1) that low plasma cholesterol is associated with failure in achieving rapid virological response, defined as undetectable HCV RNA at week 4 of therapy.

This was a retrospective study. This drawback explains why we can not discuss the role of insulin resistance, which seems very important in determining the failure of antiviral therapy (22,33). However, our results did not confirm the suggested relation between the chance of SVR and basal values for serum uric acid (16) and neutrophil count (34,35).

We conclude that primary therapeutic failure may be predicted quite accurately in a low proportion of patients carrying an unfavorable combination of easily available basal criteria. Although many patients show an intermediate profile, our findings may help to establish the indication of antiviral therapy in patients with extreme values for these criteria.

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