Insulin resistance predicts rapid virological response in non-diabetic, non-cirrhotic genotype 1 HCV patients treated with peginterferon alpha-2b plus ribavirin

Alessandro Grasso1,*, Federica Malfatti1, Pasqualina De Leo2, Hugo Martines1, Paolo Fabris3, Federica Toscanini2, Marco Anselmo2, Giorgio Menardo1

1Internal Medicine and Gastroenterology Unit, San Paolo Hospital, Via Genova 30, 17100 Savona, Italy
2Infectious Diseases Unit, San Paolo Hospital, Savona, Italy
3Infectious Diseases and Tropical Medicine Unit, San Bortolo Hospital, Vicenza, Italy

Background/Aims: The rapid decline in hepatitis C virus RNA is crucial for determining the outcome of therapy in patients with genotype 1 chronic hepatitis C. However, the variables influencing the early phase of viral decay are still largely unexplored. We aimed to assess which pre-treatment variable may predict rapid virologic response (RVR) and sustained virologic response (SVR).

Methods: We evaluated 90 consecutive non-diabetic patients with genotype 1 chronic hepatitis C without cirrhosis, treated with peginterferon alpha-2b plus ribavirin. Viral load (COBAS Amplicore, Roche) was measured at 1, 4 and 12 weeks after starting treatment, and then 24 weeks after the end of treatment.

Results: The overall SVR was 47%. The SVR in patients with RVR was 100%. Age, GGT levels, viral load, steatosis, fibrosis and HOMA-IR were significantly associated with RVR in univariate analysis. After logistic regression, HOMA-IR proved to be the strongest independent predictor of RVR (OR 0.37, 95% CI: 0.16–0.89; \( p = 0.027 \)), whereas fibrosis had a weaker independent association with RVR (OR 0.32, 95% CI: 0.1–1.04; \( p = 0.057 \)). Among the eight pre-treatment variables, both BMI and steatosis were significantly associated with HOMA-IR, either in univariate or in multivariate analyses.

Conclusions: Our data suggest that insulin resistance is strongly associated with RVR, thus reflecting the important role played by metabolic factors in the early phase of viral kinetics. HOMA-IR would appear to be a useful tool in predicting RVR and should be evaluated at baseline in all chronic hepatitis C patients before initiating antiviral treatment.

Keywords: Insulin resistance; HOMA-IR; Rapid virologic response; Chronic hepatitis C; Genotype 1
1. Introduction

The introduction of pegylated interferons (PEG-IFN alpha) in combination with ribavirin in recent years has greatly improved the treatment outcome of hepatitis C virus (HCV) infection [1,2]. However, the sustained virologic response (SVR) rates are heterogeneous and vary significantly on the basis of HCV genotype, with the worst results being seen among subjects infected with genotype 1 and high viral load [1–4].

There is substantial evidence to prove that the probability of achieving SVR increases with the rapidity of HCV-RNA suppression. A retrospective analysis of data from patients treated with peginterferon alpha-2a (40 kd) plus ribavirin for 48 weeks in a randomized, international, phase III study demonstrated that 91% of genotype 1 patients who achieved rapid virologic response (RVR), i.e., defined as undetectable HCV-RNA at week 4 of treatment had a SVR, as compared with 48% or fewer of those who did not become HCV-RNA-negative until week 24 [5].

A high positive predictive value (PPV) of RVR for SVR (75–89%) was also reported in several retrospective analyses of large cohorts of genotype 1 HCV-infected patients treated with peginterferon alpha-2a and ribavirin [6–8]. Therefore, RVR has emerged as the single best predictor of SVR in patients with chronic hepatitis C and has become the most important on-treatment indicator when deciding the duration of treatment, thereby allowing us to limit costs and side effects [9,10].

Although it is known that some pre-treatment variables such as age, viral load, level of fibrosis and insulin resistance do affect SVR in genotype 1 HCV-infected patients, little information is available concerning which factors may interfere with the early phase of viral dynamics in these patients. The aim of this study was to determine which variables may influence early viral kinetics and which may predict RVR and SVR.

2. Patients and methods

The current study was designed to analyze data from 90 consecutive, non-diabetic patients with genotype 1 chronic HCV without cirrhosis who had not previously been treated with interferon alpha and/or ribavirin and who were recruited from a single referral liver unit. The current study was designed to analyze data from 90 consecutive, non-diabetic patients with genotype 1 chronic HCV without cirrhosis who had not previously been treated with interferon alpha and/or ribavirin and who were recruited from a single referral liver unit.

3. Study design

Patients were treated with peginterferon alpha-2b (Pegintron, Schering-Plough Corp.) at a dose of 1.5 µg/kg/week and ribavirin (Rebetol, Schering-Plough Corp.) either 1000 mg/day (body weight <75 kg) or 1200 mg/day (body weight >75 kg). During treatment, patients were evaluated at monthly intervals to monitor compliance and side effects (i.e., blood cell count, hemoglobin levels, platelets, thy-roid function and serum liver tests).

Patients who showed a $2\log_{10}$ drop in viral load at week 12 as compared to baseline, or those whose HCV-RNA level was positive at week 24 discontinued treatment on the basis of the international guidelines. Conversely, patients who had a $>2\log_{10}$ drop in viral load at week 12 as compared to baseline and whose HCV-RNA level was negative at week 24 continued the treatment schedule for a total of 48 weeks.

3.1. Definition of response

Sustained virologic response (SVR) was defined as undetectable HCV-RNA levels in the serum at week 24 (i.e., 24 weeks after the end of treatment, which was also the end of follow-up). The definition of on-treatment response was as follows: rapid virologic response (RVR) was defined as undetectable HCV-RNA at week 4, “complete” early virologic response (cEVR) was defined as undetectable HCV-RNA at week 4 but undetectable at week 12, and “partial” early virologic response (pEVR) was defined as positive HCV-RNA at weeks 4 and 12 but with $>2\log_{10}$ drop in viral load at week 12 as compared to baseline.

Patients whose viral load declined more slowly ($<2\log_{10}$ drop at week 12 as compared to baseline), those whose viral load dropped $>2\log_{10}$ at week 12 but undetectable at week 12, and “partial” early virologic response (pEVR) was defined as positive HCV-RNA at weeks 4 and 12 but with $>2\log_{10}$ drop in viral load at week 12 as compared to baseline.

4. Assessment of HCV-RNA load, viral kinetics and genotypes

All subjects were reactive for anti-HCV antibodies using a third generation enzyme immunoassay EIA (Abbott HCV 3.0 Elisa). Serum HCV-RNA levels were quantified in all patients at the beginning of treatment and then at weeks 4, 12, 48 and 72 by using quantitative reverse-transcription polymerase chain reaction (PCR) (Amplicor Monitor HCV v. 2.0; Roche Molecular Systems, Mannheim, Germany). HCV-RNA viral load was also assessed at week 1 in 70 patients. For statistical purposes we chose 400,000 IU/mL as the cut-off value to separate low (<400,000 IU/mL) from high (>400,000 IU/mL) viral load. Viral load was also expressed as $\log_{10}$. HCV-RNA was quantitatively detected using the reverse-transcription PCR test Roche Cobas Amplicor assay (sensitivity 50 copies/ml). HCV genotype was determined using the INNO-LiPA HCV II kit (Bayer Diagnostics, Emeryville, CA). Eighty-one patients (90%) had subtype 1b, whereas 9 (10%) had subtype 1a.
5. Liver histology

All patients underwent liver biopsy within the 18 months prior to treatment. Paraffin embedded biopsies were analyzed by a single pathologist unaware of the clinical and biological data, except for the presence of chronic hepatitis C. This analysis was performed after hematoxyline–phloxin–safran, Perls’, and picrosirius red staining.

Liver biopsy specimens not less than 15 mm in length, or the presence of at least 10 complete portal tracts were required. Necroinflammatory activity was graded and fibrosis was staged according to Ishak’s scoring system [11]. Steatosis was graded according to the percentage of hepatocytes containing cytoplasmic vacuoles. Since only 8 patients had steatosis >30% we chose to split them into 2 categories (≤5% and >5%), which reflects the absence (or minimal amount) versus the presence of larger amounts of steatosis.

6. Clinical and laboratory determinations

Age, sex and body mass index (BMI, calculated as weight in kilograms/height in square meters) were recorded for all patients at baseline, as were serum levels of alanine aminotransferase (ALT), aspartate aminotransferase, gamma-glutamyl transpeptidase (GGT), glucose (glucose HK UV enzymatic test [Olympus]) and insulin (measured by a two-site immunoenzymometric assay (ST-AIA-PACK IRI, Tosoh Corporation, Tokyo, Japan). Insulin resistance, a state in which a given concentration of insulin is associated with a sub-normal glucose response, was determined by the homeostasis model assessment (HOMA) method using the following equation: HOMA-IR = fasting insulin (mIU/L) × fasting glucose (mmol/L)/22.5) [12]. HOMA-IR has been validated in comparison with euglycemic/hyperinsulinemic clamp technique as the standard reference in both diabetic and non-diabetic patients [13].

7. Statistical analysis

Database management and statistical analyses were performed using a statistical software package (SPSS 10.0.5). Univariate analysis was carried out on thirteen pre-treatment variables that had already been evaluated in the literature for their possible association with on-treatment response. χ2-Test or the Pearson’s correlation test (for continuous variables) and t-test (for categorical variables) were used. Multivariate analysis was carried out by logistic regression. A p-value less than 0.05 was considered statistically significant.

For statistical purposes, HOMA-IR was expressed as a continuous variable or as a categorical variable (≤2 vs >2). A cut off value of 2 is reported in the literature as being clinically significant for its association with SVR [14]. A further HOMA-IR value of 3.23 was also identified on the basis of the 75th percentile of HOMA-IR values which represented the cutoff of insulin resistance in our population.

We also calculated the influence of HOMA-IR (<2 vs >2), steatosis (<5% vs >5%), fibrosis (<F2 vs >F2) and viral load (>400,000 IU/mL vs <400,000 IU/mL) on early viral dynamics as the decline in viral load (logs) between baseline and weeks 1, 4 and 12 (ΔHCV-RNA), respectively, by means of the Student’s t-test.

The calculated power of the F test (multiple regression) on the basis of alpha = 0.05, total number of patients (90), maximum number of predictors (6) and an effect size of at least 0.20, was 0.885.

8. Results

Clinical characteristics and on-treatment viral response rates for all 90 patients, as well as a break-down based on the presence or absence of SVR are shown in Table 1. Overall, 42 patients achieved SVR (47%). Patients with SVR were significantly younger, had less steatosis, fibrosis, viral load, and showed significantly higher RVR and cEVR rates compared with non-SVRs. HOMA-IR was not associated with SVR. The patients in the top quartile of HOMA-IR distribution values who were considered as having insulin resistance showed no significant differences in SVR rates (49%) compared with patients in the other three quartiles of HOMA-IR (39%).

However, when multivariate analysis was performed taking baseline variables into consideration, only viral load was independently associated with SVR (OR 4.75; 95% CI: 1.12-20.1; p = 0.0034).

Virological response according to viral on-treatment kinetics is shown in Fig. 1. Twenty-five patients (28%) whose HCV-RNA was undetectable at week 4 (RVR), all achieved SVR. On the other hand, SVR was achieved by 67% of the 21 cases whose HCV-RNA was still positive at week 4 but which became undetectable at week 12 (“non-RVR-cEVR”), while it was achieved by 23% of the 13 cases in whom a >2log viral decay was obtained (pEVR).

The PPV for SVR of RVR, cEVR, “non-RVR”-cEVR and pEVR was 100%, 85%, 67%, 23%, while the NPV was 74%, 93%, 93% and 100%, respectively.

8.0.1. Influence of pre-treatment variables on early viral dynamics

Assuming that the degree of viral decay during antiviral therapy actually expresses the sensitivity of the virus–host system to that treatment, we assessed the influence of HOMA-IR, steatosis, fibrosis and pre-treatment viral load on early viral dynamics calculated as the decline in viral load (−logs) between baseline and weeks 1, 4 and 12 (ΔHCV-RNA) (Table 2). As shown in the
UNCORRECTED PROOF

243 Table, all the variables we evaluated do significantly affect viral HCV decay from the end of the first week of treatment up to 12 weeks, likely indicating that a relationship among them does exist.

9. Pre-treatment variables associated with RVR

To understand the relative weight of the pre-treatment variables influencing RVR, both univariate and multivariate analyses were performed. Univariate analysis showed that mean age, GGT, HOMA-IR, fibrosis and steatosis (<5%/>5%) at histology, as well as viral load (<400,000 IU/mL/>400,000 IU/mL) were significantly associated with RVR. However, multivariate analysis revealed that HOMA-IR was the main independent pre-treatment variable to be statistically associated with RVR, whereas fibrosis showed a weaker association (Table 3). Both HOMA-IR and fibrosis showed a stronger independent association with RVR when HOMA-IR was included in the multivariate model as a categorical variable (HOMA-IR <2/>2: OR 0.14; 95% CI: 0.03–0.65; p = 0.013; Fibrosis: OR 0.31; 95% CI: 0.1–0.13; p = 0.037).

10. Insulin resistance

In order to assess which variables were associated with insulin resistance, both univariate and multivariate analysis were applied. Among the 8 pre-treatment variable (age, sex, ALT, GGT, BMI, steatosis, fibrosis and viral load), only BMI and steatosis were found to be associated with HOMA-IR univariately. Both BMI and steatosis were also significantly and independently associated with HOMA-IR when linear regression analysis was performed (BMI: OR 0.37; 95% CI: 0.12–0.44; p = 0.001; steatosis: OR 0.32; 95% CI: 0.31–2.46; p = 0.013).

11. Discussion

Several studies have found that early viral kinetics is important for predicting outcome following peginterferon alpha plus ribavirin treatment in HCV patients [15–17]. In our study, all patients who became HCV-RNA negative within 4 weeks from baseline achieved SVR compared with 67% of patients in whom the negativity of viremia occurred between the 4th and the 12th weeks of treatment, and compared with only 23% of patients whose HCV-RNA was still positive at week 12 but who had a >2 log<sub>10</sub> drop in viral load from baseline. These data strengthen the hypothesis that the crucial point for achieving complete and sustained response after antiviral treatment is the speed in abating
viral load from baseline. In terms of predictive power for SVR, our results confirm the data reported in the literature (PPV of RVR = 100% and NPV of cEVR = 93%). Among baseline variables, viral load but not HOMA-IR, proved to be a predictor of SVR, suggesting that viral factors are more heavily involved than metabolic factors in achieving SVR.

Few studies have made any attempts to assess which factors may influence rapid virological response in HCV genotype 1 patients. Jensen and colleagues [6] found that in their cohort of patients who were treated for 24 weeks, those with low baseline HCV viremia (less than 600,000 IU/mL) and/or HCV infection with subtype 1b were more likely to achieve RVR than those with HCV viremia higher than 600,000 IU/mL or HCV infection with subtype 1a. In a recent prospective trial, Ferenci and colleagues. reported that younger age, lower body weight, genotype 4 and low baseline HCV-RNA (<400,000 IU/ml) and/or HCV infection treated with peginterferon alpha-2a plus ribavirin. J Hepatol (2009), doi: 10.1016/j.jhep.2009.07.008

The fact that HOMA-IR, rather than viral load, is able to predict RVR is surprising and reinforces the idea that insulin resistance is actually involved in the early phase of viral kinetics in some genotype 1 individuals.

Similar data were recently published by Nasta and colleagues in HIV/HCV co-infected patients. However, important and confounding interference of the protease inhibitor exposure on both viral load and insulin resistance has been hypothesized in these patients [18].

There is growing interest in the relationship between hepatitis C virus and host, especially in patients with steatosis and insulin resistance. Some experimental evidence suggests a direct effect of the HCV genotype 1 core protein in inducing steatosis [19]. Shintani and colleagues have shown that insulin resistance precedes the occurrence of steatosis in transgenic mice expressing the HCV core protein, thus suggesting that insulin resistance is not a consequence of hepatic steatosis in mice [20]. Similar evidence has been described in humans [21]. Moreover, insulin resistance is involved in fibrogenesis and is associated with more severe fibrosis in genotype 1 patients [22].

The relationship between antiviral treatment and insulin resistance in patients with chronic hepatitis C has not yet been completely elucidated. It is known that hyperinsulinemia is associated with increased HCV replication in genotype 1 patients [23,24] and that higher insulin resistance is associated with poor response to antiviral treatment [14]. However, the relationship between early viral kinetics during antiviral treatment in genotype 1 patients and insulin resistance are unknown.

### Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>RVR</th>
<th>Non-RVR</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, range)</td>
<td>41 (31–67)</td>
<td>57 (29–68)</td>
<td>0.94</td>
<td>0.90–0.98</td>
<td><strong>0.004</strong></td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>14/11</td>
<td>35/30</td>
<td>1.13</td>
<td>0.44–2.9</td>
<td>0.81</td>
</tr>
<tr>
<td>ALT (IU/L) (median, range)</td>
<td>142.5 (36–386)</td>
<td>77 (33–432)</td>
<td>1.00</td>
<td>0.99–1.01</td>
<td>0.11</td>
</tr>
<tr>
<td>GGT (IU/L) (median, range)</td>
<td>26.5 (8–127)</td>
<td>52 (10–759)</td>
<td>0.98</td>
<td>0.96–0.99</td>
<td><strong>0.01</strong></td>
</tr>
<tr>
<td>BMI (median, range)</td>
<td>23 (17.2–28.4)</td>
<td>24.1 (20.2–31.4)</td>
<td>0.88</td>
<td>0.74–1.05</td>
<td>0.16</td>
</tr>
<tr>
<td>Steatosis (&lt;5%/&gt;5%)</td>
<td>0.85</td>
<td>0.85</td>
<td>0.56</td>
<td>0.36–0.89</td>
<td><strong>0.013</strong></td>
</tr>
<tr>
<td>Fibrosis (median, range)</td>
<td>20/5</td>
<td>27/33</td>
<td>0.18</td>
<td>0.05–0.61</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td>Viral load (IU/ml) (&lt;400,000/&gt;400,000)</td>
<td>11/14</td>
<td>10/55</td>
<td>4.39</td>
<td>1.51–12.7</td>
<td><strong>0.006</strong></td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, range)</td>
<td>RVR</td>
<td>Non-RVR</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>14/11</td>
<td>35/30</td>
</tr>
<tr>
<td>ALT (IU/L) (median, range)</td>
<td>142.5 (36–386)</td>
<td>77 (33–432)</td>
</tr>
<tr>
<td>GGT (IU/L) (median, range)</td>
<td>26.5 (8–127)</td>
<td>52 (10–759)</td>
</tr>
<tr>
<td>BMI (median, range)</td>
<td>23 (17.2–28.4)</td>
<td>24.1 (20.2–31.4)</td>
</tr>
<tr>
<td>Steatosis (&lt;5%/&gt;5%)</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>Fibrosis (median, range)</td>
<td>20/5</td>
<td>27/33</td>
</tr>
<tr>
<td>Viral load (IU/ml) (&lt;400,000/&gt;400,000)</td>
<td>11/14</td>
<td>10/55</td>
</tr>
</tbody>
</table>
In this area, there is growing evidence of a complex interplay involving HCV genotype 1, cytokines, interferon and insulin signaling.

Hepatitis C virus has been reported to down-regulate the insulin receptor substrate 1 and 2 (IRS1/2) gene expression in liver tissue. Hepatic expression of the IRS1/2 central molecules that are involved in the insulin signaling pathway improves after HCV clearance in sustained responders to antiviral treatment [25]. On the other hand, recent studies reported that overexpression of the suppressor of cytokine signaling-3 (SOCS-3) gene in liver tissue, a gene involved in the interferon signaling pathway, is associated with poorer treatment outcome in patients with chronic hepatitis C viral genotype 1 [26]. In these patients, SOCS-3 gene overexpression is associated with obesity and metabolic syndrome [27].

Persico and colleagues suggested that HCV genotype 1b may directly induce up-regulation of SOCS-3 expression which is involved in triggering insulin resistance and metabolic syndrome onset, as well as in non-response to antiviral treatment [27]. On the basis of this model, higher insulin resistance seems to be a surrogate marker of genotype 1b-induced up-regulation of the SOCS3 gene in non-responders to antiviral treatment.

From this perspective, our data on the association between HOMA-IR and RVR suggest that HOMA-IR is a simple and useful pre-treatment tool that is able to predict RVR.

These data lead us to hypothesize a possible link between HCV infection, insulin resistance and the early phase of viral kinetics during antiviral treatment. This hypothesis leads us to a further consideration regarding the theoretical benefit of adjunctive therapies, such as weight loss or insulin sensitizers. Tarantino and colleagues demonstrated that a low-caloric diet for 3 months before initiating antiviral therapy in patients with genotype 1-chronic hepatitis C resulted in a significant improvement in insulin resistance as well as a 60% “end-of-treatment” response rate in the low-caloric diet group as compared to the control group (17.6%) [28]. Although these data do not provide information on long term outcome, they do suggest that the decrease in insulin resistance may induce a reduction in viral load.

The randomized, double-blind TRIC-1 trial by Romero-Gomez and colleagues [29] analyzed 125 naive genotype 1 patients treated with peginterferon alpha-2b and ribavirin plus metformin or placebo on an intention-to-treat basis. Their final results showed that there was a significant decrease in both HOMA index and viral load during the first 12 weeks, as well as an improvement in SVR rate in the metformin group as compared with the placebo group, but only in females.

On the other hand, the INSPIRED HCV study [30], in which previous non-responders to peginterferon/ribavirin combination were retreated with peginterferon alpha-2a plus ribavirin plus pioglitazone 15 mg QD for 12 weeks, failed to demonstrate an increase in early virological response. Therefore, HOMA values paradoxically increased in 2 of 5 patients. This finding is difficult to explain, and further studies, perhaps using an alternative schedule and/or different drugs, are needed to prospectively investigate the impact of adjunctive therapies on early viral kinetics and SVR.

On the other hand, a non-negligible number of patients without RVR (but with c-EVR or p-EVR) had SVR, suggesting that other factors besides rapid viral decay, such as baseline viral load, may play a role in inducing SVR. It would therefore be reasonable to propose alternative treatment schedules to these groups (for instance 18 months instead 12 months of combined therapy).

In conclusion, insulin resistance is strongly associated with RVR, thus confirming that metabolic factors play a key role in early viral kinetics. HOMA-IR emerges as a useful tool in predicting RVR in genotype 1-chronic hepatitis C patients treated with pegylated interferon alpha-2b plus ribavirin.

References


