Role of interferon lambda 4 and ALT levels in optimising treatment of HCV for patients with low-stage fibrosis

F. Figorilli¹, S. Onali¹, S. Catone², C. Argentini², S. Casu¹, C. Balestrieri³, M. Conti³, G. Serra³, M. Casale¹, M.C. Pasetto¹, L. Matta¹, L. Barca¹, R. Scioscia¹, I. Canini⁴, M.G. Quaranta², D. Genovese², S. Vella², L. Chessa¹

¹Center for the Study of Liver Disease, Department of Medical Sciences “M. Aresu”, University of Cagliari, Monserrato (CA), Italy.
²Department of Therapeutic Research and Medicines Evaluation, Istituto Superiore di Sanità, Rome, Italy.
³Department of Internal Medicine, AOU Cagliari, Monserrato (CA), Italy.
⁴Department of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy.

ABSTRACT: The use of new anti-HCV drugs is currently limited by high costs and dual therapy; pegylated interferon and ribavirin (peg-IFN+RBV) still represents the only affordable treatment in patients with low-stage fibrosis. We evaluated the role of Interferon lambda4 (IFNL4) polymorphisms and its combination with on-treatment alanine transaminase (ALT) modification in predicting sustained virological response (SVR) in HCV genotype 1 and 4 patients with low-stage fibrosis. We retrospectively analysed 124 patients with Metavir ≤F2, who received dual therapy at our centre. Genotyping for IFNL4 polymorphisms was assessed at baseline, as well as ALT levels (baseline and week 2, 4, 12 and 24 of therapy). Thirty patients (24%) were TT/TT, 74 (60%) TT/DG and 20 (16%) DG/DG. The SVR rate was significantly higher in TT/TT genotype compared to TT/DG and DG/DG (97% vs. 53% and 50%, respectively, p=0.001). Patients that achieved a 60% reduction of ALT baseline value after 4 weeks of therapy had a significantly higher SVR rate (94% vs. 52%, p<0.001). Factors significantly associated with SVR were TT/TT genotype (p=0.029), RVR (p=0.019) and 60% ALT reduction at 4 week of therapy (p=0.005). The absence of both TT/TT genotype and 60% ALT reduction were negative predictors of SVR (p<0.001). In conclusion, the combined use of IFNL4 polymorphisms and ALT reduction at 4 week of treatment is able to optimize candidates’ selection for peg-IFN+RBV, discriminating those that could still benefit from dual therapy from the ones that need the new regimens.

Keywords: Chronic hepatitis C, Sustained virological response, Pegylated interferon, Ribavirin.

ABBREVIATIONS


INTRODUCTION

Hepatitis C virus (HCV) infection is a major global health issue with more than 170 millions infected people worldwide¹. Genotype 1 and 4 account for the majority of chronic HCV infections particularly in the Middle East, North Africa and sub-Saharan Africa². Until 2011, the dual therapy based on pegylated interferon and ribavirin (peg-IFN+RBV) for 48 weeks was the only ap-
The advent of the new direct antiviral agents (DAAs) has radically changed the prognosis of patients with HCV, achieving a SVR rate greater than 90%. For this reason, in current guidelines dual therapy with peg-IFN+RBV has been replaced by new regimens that contain usually one DAA. The recommended regimen to treat naïve patients with HCV genotype 1 and 4 and low-stage fibrosis is Sofosbuvir+peg-IFN+RBV for 12 weeks. Unfortunately, for most of the countries, especially the developing ones, the new guidelines are economically unsustainable due to the high costs of the new drugs. In this setting, DAAs availability has been limited to patients with advanced liver disease and peg-IFN+RBV is still the only treatment option for most of the patients. Several predictive factors of SVR have been identified in the last decade, such as early virological response (EVR). Genome-wide association studies have identified a single nucleotide polymorphism on chromosome 19q13 near the interferon lambda 3 gene (formerly known as IL28B) as variants positively associated with the response to peg-IFN+RBV treatment. More recently, a dinucleotide polymorphism (ss469415590) that creates or disrupts an open reading frame in a recently discovered gene, interferon lambda 4 (IFNL4), has been showed to be strongly involved in response to antiviral therapy. The IFNL4 gene (encoding IFNL4) is situated upstream of interferon lambda 3. The one-base deletion in the delta-G (DG) variant results in a frame shift, which in turn produces the full-length protein designated as IFNL4; the TT variant does not produce IFNL4. The TT/TT variant seems to be associated with impaired clearance of HCV RNA and response to peg-IFN therapy. In this study we explored the role of IFNL4 polymorphisms and on-treatment change of viral load and alanine transaminase (ALT) in predicting the response to peg-IFN+RBV therapy in a cohort of patients affected by chronic HCV genotype 1-4 infection and low-stage fibrosis.

**RESULTS**

**Baseline characteristics**

One hundred twenty-four outpatients followed between January 2003 and January 2012 at the Liver Unit of University of Cagliari were included in the study. Eighty-one (65.3%) were infected by HCV genotype 1 and 43 (34.7%) by genotype 4. The mean age was 43.4 years (SD 10.3) and 89 (71.8%) were males. All patients received antiviral treatment based with peg-IFN+RBV for a median period of 47 weeks. In the 24-week follow-up after therapy, 78 patients (62.9%) achieved SVR, 21 patients (16.9 %) were REL and 25 (20.2%) were NR.

**IFNL4 Genotyping**

The main characteristics of the patients stratified according the ss469415590 polymorphism (TT/TT, TT/DG, DG/DG) are shown in Table 1. Seventy-four (59.7%) patients were TT/DG, 30 (24.2%) TT/DG and 20 (16.1%)
DG/DG. At baseline, only gamma-glutamyl transpeptidase level was significantly lower in TT/TT patients (TT/TT 25.5 IU/L, TT/DG 60 IU/L, DG/DG 53.5 IU/L, p<0.001). RVR, EVR and SVR rate were significantly higher in TT/TT patients. The SVR rate in TT/TT was 96.7% compared to 52.7% in DG/DG (p<0.001).

**ALT variation**

The percentage of ALT decrease during therapy was different between the three genotypes of IFNL4. As showed in Figure 1a, after 2 weeks of treatment TT/TT patients had a median ALT decrease of 35.5%, which was significantly higher compared to genotypes TT/DG and DG/DG (13.7% and 8.4% respectively, p=0.002). After 4 weeks, the difference was lower but still significant (p=0.006) and at 12 weeks the reduction was similar between the three genotypes (p=0.082). The median rate of decrease was significantly higher in SVR group compared to REL and NR after 2 weeks (24.6% vs. 4.7%; p=0.001), after 4 weeks (44.6% vs. 23.5%; p=0.001), after 12 weeks (59.6% vs. 45.5%; p=0.004) and after 24 weeks (66.8% vs. 41.8%; p=0.002) (Figure 1b). The best cut-off obtained by ROC analysis was 20% at 2 weeks of therapy (AUC 0.679, sensitivity 60.3%, specificity 76.1%; p<0.001) and 60% at 4 weeks (AUC 0.687, sensitivity 38.5%, specificity 95.7%, p<0.001). As reported in Table 2, a decrease of ALT baseline level greater than 20% after 2 weeks of treatment was significantly associated with a higher rate of SVR (60.3% vs. 39.7%; p<0.001). Moreover patients that achieved a 60% reduction at week 4 of therapy had an SVR rate of 93.8% vs. 52.2% in patients with a lower decrease (p<0.001). Less patients with genotype DG/DG had a 60% reduction at week 4 (10%) compared to the other genotypes (20% in TT/DG, 50% in TT/TT, p=0.002). However, patients without favorable genotype, but showing ALT decrease, still achieved a higher SVR rate compared to those without ALT reduction (88% vs. 44%, p<0.001).

**Predictors of response to treatment**

In multivariate logistic regression (Table 2), factors significantly associated with SVR were TT/TT genotype (p=0.029, OR=10.74, 95%CI 1.28-89.84), RVR (p=0.019,
Figure 1. A, Rate of ALT decrease at different time points of treatment according to IFNL4 polymorphisms. B, Rate of ALT decrease at different time points of treatment according to virological response.

OR=6.68, 95%CI 1.36-32.87) and ALT reduction at week 4 of therapy >60% (p=0.005, OR=9.46, 95%CI 1.99-45.08). On the other hand, the absence of RVR (p=0.018, OR 6.75, 95%CI 1.38-33) and the combination of an unfavorable IFNL4 genotype (TT/DG or DG/DG) with a reduction of ALT at week 4 <60% (p<0.001, OR=0.06, 95%CI 0.02-0.23) were negative predictors of SVR. In particular, only 44.2% of patients showing this combination achieved a SVR compared to those with the same unfavorable genotype, but greater ALT decrease (88.2%, p<0.001) (Figure 2b).

DISCUSSION

The new anti-HCV drugs are highly effective, being able to cure more than 90% of treated patients4. However, they are very expensive and their prescription to all infected subjects is economically unsustainable for most countries worldwide. Moreover, their availability is extremely limited in many low-income countries5. Similarly to anti-HIV treatment13, the possibility to identify those individuals that can be cured with the less expensive therapy (peg-IFN+RBV) would be very useful to focus the financial resources on patients that will require the new DAAs to achieve a sustained virological response. In this retrospective analysis we aimed at identifying factors that could predict the response to peg-IFN+RBV within 4 weeks of therapy. We focused on patients affected by HCV genotype 1 and 4 and low-stage fibrosis, because they represent the majority of HCV infected patients in the developing country7. First, we analysed the role of IFNL4 genes, a novel transcribed region recently identified by Prokunina-Olson et al9, and located close to the three interferon lambda genes: IFNL1 (IL29), IFNL2 (IL28A) and IFNL3 (IL28B). IL28B polymorphisms are the strongest predictors of response to therapy in HCV genotype 17,8,14-18. The region of IFNL4 harboured a dinucleotide variant (ss469415590) that has been found in two alternative forms, either DG or TT alleles. The one-base deletion in the DG variant results in a frameshift, which in turn produces the full-length protein, called IFNL4; on the opposite, the TT variant does not produce IFNL49,11. In our cohort, an important peculiarity was that IFNL4 and IL28B polymorphisms showed a perfect match: TT/TT with CC, TT/DG with CT and DG/DG with TT. Data from literature indicates that the correspondence between IL28B and IFNL4 genotypes is about 92% in the Caucasian ethnicity9, so it is not clear if our findings represented the real distribution of these alleles or if they were due to an artefact related to the small sample size. Therefore, from nowhere we refer only to IFNL4 genotype when commenting the results. It is noteworthy that all of patients with the TT/TT genotype cleared the virus after a peg-IFN+RBV course of treatment and only 1 of them relapsed. On the other hand, only half of the patients with the unfavourable genotype (DG/DG) or heterozygosis (TT/DG) achieved SVR. The pathophysiological mechanism that links IFNL4 polymorphisms and HCV clearance after treatment with peg-IFN+RBV has been investigated in several studies. In vitro, IFNL4 induces the expression of interferon-stimulated genes (ISG)
Table 2. Characteristics of patients stratified by treatment response and univariate and multivariate logistic analyses of predictors of sustained virological response (SVR).

<table>
<thead>
<tr>
<th></th>
<th>SVR</th>
<th>REL+NR</th>
<th>Univariate logistic regression</th>
<th>Multivariate logistic regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>78 (62.9%)</td>
<td>46 (37.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n. (%)</td>
<td>59 (66.3)</td>
<td>30 (33.7)</td>
<td>1.66</td>
<td>0.75-3.66 NS</td>
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<tr>
<td>Age, years (median)</td>
<td>43</td>
<td>42.5</td>
<td>0.99</td>
<td>0.96-1.03 NS</td>
</tr>
<tr>
<td>Previous therapy, n. (%)</td>
<td>17 (60.7)</td>
<td>11 (39.3)</td>
<td>0.89</td>
<td>0.37-2.11 NS</td>
</tr>
<tr>
<td>BMI, kg/m² (mean)</td>
<td>24.7</td>
<td>25.1</td>
<td>0.99</td>
<td>0.84-1.08 NS</td>
</tr>
<tr>
<td>HCV genotypes n. (%)</td>
<td>1</td>
<td>51 (63)</td>
<td>1.01</td>
<td>0.47-2.17 NS</td>
</tr>
<tr>
<td>INFL4 genotypes (%)</td>
<td>27 (62.8)</td>
<td>16 (37.2)</td>
<td>0.99</td>
<td>0.46-2.13 NS</td>
</tr>
<tr>
<td>TT/TT genotype</td>
<td>29 (96.7)</td>
<td>1 (3.3)</td>
<td>26.63</td>
<td>3.48-203.6 0.002</td>
</tr>
<tr>
<td>TT/DG genotype</td>
<td>39 (52.7)</td>
<td>35 (47.3)</td>
<td>0.314</td>
<td>0.14-0.71 0.005</td>
</tr>
<tr>
<td>Platelets, cells/mm³ (mean)</td>
<td>216413</td>
<td>223346</td>
<td>1.00</td>
<td>1.00-1.00 NS</td>
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<tr>
<td>AST, IU/L (median)</td>
<td>39.5</td>
<td>37.5</td>
<td>1.01</td>
<td>0.99-1.02 NS</td>
</tr>
<tr>
<td>ALT, IU/L (median)</td>
<td>67</td>
<td>62</td>
<td>1.01</td>
<td>1.00-1.02 NS</td>
</tr>
<tr>
<td>Decrease ALT after 2 weeks &gt;20%</td>
<td>30 (93.8)</td>
<td>2 (6.2)</td>
<td>13.75</td>
<td>3.10-60.93 0.001</td>
</tr>
<tr>
<td>Decrease ALT after 4 weeks &gt;60%</td>
<td>24.6%</td>
<td>4.7%</td>
<td>0.99</td>
<td>0.98-1.00 0.040</td>
</tr>
<tr>
<td>Decrease ALT after 4 weeks (median)</td>
<td>44.6%</td>
<td>23.5%</td>
<td>0.99</td>
<td>0.98-1.00 0.005</td>
</tr>
<tr>
<td>RVR (%)</td>
<td>25 (92.6)</td>
<td>2 (7.4)</td>
<td>10.38</td>
<td>2.33-46.26 0.002</td>
</tr>
</tbody>
</table>


Figure 2. A. Percentage of SVR according to the presence of TT/TT IFNL4 genotype, decrease greater than 60% after 4 weeks of treatment and Rapid Virological Response (RVR). B. Percentage of SVR according to the combination of TT/TT genotype and ALT decrease greater than 60% after 4 weeks of treatment: presence of both of them (+TT/+DEC), TT/TT genotype without decrease (+TT/-DEC), decrease >60% at 4 wk in unfavourable IFNL4 genotype (-TT/+DEC) and the absence of both of them (-TT/-DEC).
in a similar pattern to those induced by peg-IFN alpha and IL28B (9). A high baseline hepatic ISG expression correlates with poor response to peg-IFN therapy\(^{19,20}\) and it is associated with the unfavourable IL28B genotype\(^{21}\). Similarly, HCV infected patients carrying the unfavourable IFNL4 genotype DG/DG, and to a lesser extent ΔG/TT heterozygosis, showed a persistent and dose-dependent expression of IFNL4 ISG in the hepatocytes\(^{9}\). Although moderate ISG expression can partially impair viral replication, it also stimulates negative regulatory pathways that ultimately reduce hepatocellular sensitivity to exogenous IFN. Therefore the IFN-alpha administered during therapy might fail to induce an effective inflammatory response able to eradicate the infection\(^{22,23}\). On the contrary, patients with the favourable IFNL4 allele TT/TT show lower level of hepatic ISG expression at baseline\(^{9}\). Although this phenomenon might result in a higher baseline viral load, hepatocytes can be more sensitive to IFN stimulation compared to the unfavourable genotype\(^{22,23}\). We have also studied the potential role of the baseline level of alanine aminotransferase (ALT) and the decrease of this enzyme during therapy as predictors of SVR. Controversial results have been reported regarding the predictive value of ALT level at baseline\(^{24-28}\), while so far few studies have described ALT changes during therapy\(^{29}\). In our study, the absolute values of ALT at baseline and during treatment were not significantly associated with SVR. However, when considering the degree of variation at predefined time points during treatment, we found that patients achieving SVR had a higher decrease compared to nonresponders and relapers, especially after 2 weeks and 4 weeks of therapy. A significant difference has also been found among the IFNL4 genotypes, because TT/TT patients presented the highest decrease of ALT at each time point of therapy. Similarly to the differences among the classes of response, the gap with the non-favourable genotypes was greater after 2 weeks and 4 weeks. However the 60% cut-off of the decrease at week 4 showed a higher specificity than the 20% cut-off at 2 weeks of treatment (95.7% vs. 76.1%). These results were confirmed in the multivariate analysis, where a decrease greater than 60% after 4 weeks of therapy was a predictor of SVR together with TT/TT genotype and RVR. A significant difference has also been found among the IFNL4 genotypes, because TT/TT patients presented the highest decrease of ALT at each time point of therapy.

CONCLUSIONS

We suggest that dual therapy could still be an effective treatment in selected subsets of patients with HCV genotype 1 or 4 infection and low-stage fibrosis. Genotyping of IFNL4 polymorphisms and the variation of ALT serum level and viral load during treatment represent affordable tools to identify ideal candidates for peg-IFN+RBV therapy. The use of our algorithm may help to reduce the number of HCV-infected patients requiring the new DAAs and therefore contribute to optimize the healthcare-related costs.

CONFLICT OF INTERESTS:

The Authors declare that they have no conflict of interests.

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