DOUBLE THERAPY WITH PEGYLATED INTERFERON AND RIBAVIRIN FOR CHRONIC HEPATITIS C. A PHARMACOGENETIC GUIDE FOR PREDICTING ADVERSE EVENTS

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Abstract

Over 180 million people are infected with hepatitis C virus worldwide. Until 2016, the standard of care for patients with chronic hepatitis C was double therapy with PegInterferon and Ribavirin over a course of 48 weeks. Unfortunately, the treatment induces a wide variety of side effects. The aim of this study was to determine whether genetic variants can protect against or predict any of the adverse events. We included 267 patients, diagnosed with chronic HCV infection. Two genotypes were investigated: ITPA rs1127354 and C20orf194 rs6051702. Homozygous variants of the minor ITPA gene allele proved to be protective for anemia during therapy, whereas the “AA” allele of the c20orf194 gene is an important predictor for anemia (p < 0.01). The ITPA rs1127354 major C allele was found to be a positive predictor for a haemoglobin (Hb) drop over 2.5 g/dL at week 4 of treatment (p < 0.01). The minor AA allele of the c20orf194 gene proved to be an important protective factor for developing leucopenia (p = 0.03), neutropenia and thrombocytopenia (p < 0.01). We also discovered that the c20orf194 rs6051702 gene variants correlated to some extent to achieving sustained virological response, with 115 (43.07%) patients with SVR and AA allele compared to 30 (11.24%) with AC allele and 4 (1.5%) with CC allele (p < 0.01). These findings demonstrate that pharmacogenetic tools could play a very important role in individual designing every treatment.

Rezumat

Peste 180 milioane de persoane sunt infectate cu virusul hepatitis C la nivel mondial. Până în 2016, standardul în tratamentul pacienților cu hepatită cronică cu virus C era dubla terapie cu PegInterferon și Ribavirin timp de 48 de săptămâni. Însă acest tratament produce o varietate de efecte adverse. Scopul prezentului studiu este de a analiza dacă particulații genetice pot proteja sau prezice apariția efectelor adverse. În studiul au fost incluși 267 de pacienți diagnosticați cu infecție cronică cu virus hepatitis C. Au fost investigate 2 genotipuri: ITPA rs1127354 și C20orf194 rs6051702. Varianta homozigotă a alelei minore ITPA s-a dovedit protectoare împotriva apariției anemiei în timpul tratamentului, în timp ce alela AA a genei c20orf194 este un important factor predictiv pentru anemie (p < 0.01). Alela majoră C a genei ITPA rs1127354 este un factor predictiv pozitiv pentru scăderea hemoglobinei cu peste 2.5 g/dL în săptămâna 4 de tratament (p < 0.01). Alela minoră AA a genei c20orf194 rs6051702 s-a dovedit un factor predictiv pentru apariția leucopeniei (p = 0.03), neutropeniei și trombocitopeniei (p < 0.01). De asemenea, s-a observat că mutațiile genei c20orf194 rs6051702 sunt correlate cu apariția răspunsului virusologic susținut, cu 115 (43,07%) pacienți cu RVS și alela AA comparativ cu 30 (11,24%) pacienți cu alela AC și doar 4 (1,5%) pacienți cu alela CC (p < 0.01). Putem concluziona că farmacogenomica joacă un rol important în alegerea terapiei individualizate.

Keywords: pharmacogenetics, PegInterferon, ribavirin, side adverse events
Introduction

Chronic hepatitis C virus (HCV) infection is one of the leading causes of chronic liver disease, including in its late stages, liver cirrhosis and hepatocellular carcinoma [4]. It is estimated that over 180 million people are infected with HCV worldwide. In Romania, the prevalence of HCV infection is 3.23%, according to a study conducted between 2006 and 2008 [10], and a little over 5% according to another cross-sectional epidemiological study between 2008 and 2009 [33]. Until 2011 worldwide and 2016 for Romania, the golden standard for the treatment of HCV infection was double antiviral therapy with Peg-Interferon (PegIfn), administered once a week subcutaneously and Ribavirin (RBV) given orally, for 48 weeks, which yielded sustained virological response (SVR) in up to 56% of patients [7, 21]. One of the main drawbacks of combination therapy is the development of side effects, which can result in suboptimal dosing or discontinuation of therapy [22]. This can limit the likelihood of SVR, since one of the determinants of SVR is the adequate dose and the duration of therapy. Among the haematologic abnormalities associated with combination therapy, anaemia is probably the most significant, as it can reduce patients’ health-related quality of life and may be the main determinant of fatigue [34]. Ribavirin-induced haemolytic anaemia is a common cause of dose reduction or discontinuation. Bone marrow suppression, the predominant mechanism for interferon-induced neutropenia and thrombocytopenia, also contributes to the anaemia by suppressing reticulocyte production. Although dose reduction or discontinuation of combination therapy can reverse these abnormalities, they may reduce the virological response [22].

Materials and Methods

Our study included 267 patients diagnosed with chronic HCV infection and treated with double antiviral therapy with PegIfn and RBV between October 2013 and November 2016 in the 2nd Medical Department of the Emergency County Hospital Craiova, Romania. All patients signed the informed consent prior to study initiation. The study protocol was designed according to the ethical guidelines of the Helsinki Declaration adopted at the 18th World Medical Assembly, Helsinki, 1964 and reviewed at the 29th World Medical Assembly, Tokyo, 1975, and the Patients’ Rights Act, 46/2003. The study was approved by the Ethics Review Committee of the University of Medicine and Pharmacy of Craiova. All included patients were tested positive for HCV-RNA (ribonucleic acid) (Quantiplex HCV RNA, Bayer Diagnostics, Puteaux, France and Cobas Amplicor HCV Monitor Test Cobas v2.0, Roche Diagnostics Systems, Meylan, France) and had compensated liver disease. All patients with HIV/HBV (human immunodeficiency virus/hepatitis B virus) co-infection, alcoholic liver disease, liver cirrhosis, pulmonary tuberculosis and any malignant tumours were excluded from enrolling in the study. The patients received a 48-weeks course of treatment with Pegylated Interferon and Ribavirin. Medical visits were performed regularly at 4, 12, 24, 48 weeks and another control visit at 24 weeks after completion of therapy. A complete blood count (CELL-DYN Ruby, Abbott Diagnostics, USA) was performed at every visit and every patient completed a questionnaire for subjective adverse events. Blood samples for genetic analysis were collected from every patient at the beginning of the study and were stored in an ultra-freezer at -86°C.

We analysed 2 single nucleotide polymorphisms (SNP) for this given population, ITTPA rs1127354 and C20orf194 rs6051702 in order to determine whether genetic variants influence or correlate with different treatment-related aspects. Genomic DNA (deoxyribonucleic acid) isolation was performed using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI). Regarding the single nucleotide polymorphisms (SNP) working protocol, we used TaqMan Pre-designed SNP Genotyping Assay, small scale, human, using the real-time polymerase chain reaction (RT-PCR) technique on the Corbett Rotor-Gene 6200 RPM platform with interchangeable blocks Viia™ 7 Applied Biosystems and automatic pipetting Thermocycler Eppendorf epMotion 5070 Mastercycler. Sequencing was performed using CEQ™ 80. TaqMan® Genotyping Assays genotypes single nucleotide polymorphism (SNP) was performed using 5’ nuclease activity to amplify and detect specific SNP alleles in genomic DNA samples. All data collected was processed using Data Analysis Toolpak add-in: Chi Test, Pivot Tables; fundamental statistical parameters, mean and standard deviation and coefficient of variation. The binary data were subjected to arcsin square root transform to adjust for ANOVA analysis of count data instead of continuous variables.

Results and Discussion

Demographics and SVR

The cohort characteristics regarding gender was as follows: 183 (68.54%) were females and 84 (31.46%) were males. The majority of patients, 153 (57.3%), were aged between 40 and 60 years old and most of them were women. The smallest incidence was among people under 30 years old (1.5%). From 267 patients included, 149 (55.81%) achieved SVR and 118 (44.19%) had detectable viremia at the end of treatment or 24 weeks after.
Adverse events recorded

Of the total of patients enrolled in the study, 100 (37.45%) patients experienced weight loss, 78 (28.08%) patients had headache, 81 (30.33%) patients had myalgia, 100 (37.45%) of patients experienced fever or flu-like symptoms, 85 (31.83%) patients had arthralgia, 79 (29.58%) patients experienced local reactions at the injection site, 110 (41.19%) patients experienced nausea, 126 (47.19%) of patients experienced loss of appetite, 29 patients (10.86%) experienced transit disorders, 12 (4.49%) patients had post-impeditive pruritus, 16 (5.99%) patients had insomnia and irritability, 6 (2.24%) patients had depression, 4 (1.49%) patients experienced shortness of breath, 15 (5.62%) patients had rebellious cough and 3 (1.12%) patients developed hyperthyroidism, hypothyroidism and dermatitis. Although not included in the graphical representation, there was one case of onset sarcoidosis secondary to Interferon therapy that had as presenting symptom – iridocyclitis.

Figure 1.

Adverse events during the 48 weeks course of treatment

Treatment with Peg-Interferon and Ribavirin is accompanied by multiple side effects as described earlier, but the most important of these are haematological ones. Of the total patients included in the study, 139 (52.06%) of patients developed anaemia, 123 (46.07%) patients had leucopenia, 95 (35.58%) patients developed neutropenia and 119 (44.56%) of patients experienced varying degrees of severity of thrombocytopenia (Figure 2).

The multitude of adverse events has been the subject of many research papers over the years. In our study, most of the adverse effects met with the data reported in previous studies. However, there have been some differences from the literature in this field. The most common adverse effects in therapy are asthenia, flu-like symptoms, adverse reactions in the gastrointestinal sphere, neuropsychiatric symptoms, haematological and endocrinological disorders [8].

The results of our study have shown a sizeable proportion of gastrointestinal side effects. According to a 2002 study [8], nausea was found in 43% of cases, and weight loss and weight loss accounted for 32%, which is broadly in line with our findings. According to previous reports, the percentage of typhoid dysfunctions or autoimmune side effects is low, with an approximate frequency of 5% endocrine disorders [14, 18]. In our study, only 3 (1.12%) patients had hypothyroidism and hyperthyroidism, the results being inferior to those from other researches [6].
Haematologic side effects are not limited to anaemia. Numerous studies have been published on the multitude of haematological effects [2, 25, 26, 35]. If anaemia is attributed to Ribavirin, the suite of haematological effects such as neutropenia and thrombocytopenia are attributed to the effect of interferon hematopoietic inhibition or autoimmune effects [11, 27].

### Single nucleotide polymorphism study

Baseline characteristics for our population are detailed in Table I.

<table>
<thead>
<tr>
<th>Rel. frequency per category (%)</th>
<th>AC Genotype</th>
<th>CC Genotype</th>
<th>AA Genotype</th>
<th>AC Genotype</th>
<th>CC Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M: 7 (34.87%)</td>
<td>57.05 ± 11.7</td>
<td>53.03 ± 10.77</td>
<td>52.00 ± 5.35</td>
<td>52.38 ± 10.54</td>
<td>55.86 ± 11.09</td>
</tr>
<tr>
<td>F: 14 (65.12%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M: 11 (4.76%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F: 227 (95.21%)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Anaemia patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (23.80%)</td>
<td>130 (54.62%)</td>
<td>4 (50.00%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No. of patients</strong></td>
<td>21 (7.87%)</td>
<td>238 (89.14%)</td>
<td>8 (3.00%)</td>
<td>182 (68.16%)</td>
<td>79 (29.59%)</td>
</tr>
</tbody>
</table>

One of the most important side effects of dual therapy was indisputably, anaemia. We tried to investigate whether the polymorphism of the ITPA or c20orf194 gene correlated with the occurrence of anaemia in PegInterferon and Ribavirin treated patients.

### Table II

Relationship between anaemia development at the end of the treatment and SNP c20orf194

<table>
<thead>
<tr>
<th>SNP c20orf194</th>
<th>Anaemia</th>
<th>No Anaemia</th>
<th>Grand Total</th>
<th>( \chi^2 )</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>110 (76.19%)</td>
<td>72 (23.81%)</td>
<td>182 (100.00%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC/CC</td>
<td>29 (34.12%)</td>
<td>56 (65.88%)</td>
<td>85 (100.00%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>139 (52.06%)</td>
<td>128 (47.94%)</td>
<td>267 (100.00%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p Anova</td>
<td>&lt; 0.0001</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Regarding the c20orf194 gene, 110 (60.43%) patients with AA genotype, 29 (34.11%) patients with AC/CC genotype developed anaemia during treatment, while 72 (39.56%) patients with AA genotype, 56 (65.88%) patients with AC/CC genotype did not develop anaemia \( (\chi^2 < 0.01) \). The data are presented in Table II and Figure 3. The results obtained were statistically significant, demonstrating that patients carrying the c20orf194 C allele are protected against anaemia and the patients carrying the A allele were more prone to develop anaemia during antiviral therapy.

![Figure 2. Haematological side effects during double antiviral therapy](image)

**Figure 2.**

Haematological side effects during double antiviral therapy

![Figure 3. Relationship between anaemia and SNP c20orf194](image)

**Figure 3.**

Relationship between anaemia and SNP c20orf194
We further investigated whether single nucleotide polymorphisms in the gene C20orf194 rs6051702 were associated with the decrease in haemoglobin at week 4 greater than 2.5 g/dL (Table III). The data obtained were as follows: 65 (35.71%) patients with AA genotype, 22 (27.85%) patients with AC genotype and no patient with CC genotype showed a decrease of > 2.5 g/dL of Hb, while 117 (35.71%) patients with AA genotype, 57 (72.15%) patients with AC genotype and 6 (100%) patients with CC genotype did not decrease. There was no association between AA, AC, CC and haemoglobin at week 4 with a p calculated by the Chi square test of 0.263, but the CC genotype proved to be a protective factor.

<table>
<thead>
<tr>
<th>SNP ITPA rs1127354</th>
<th>SNP C20orf194 rs6051702</th>
<th>(\chi^2) p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>AC</td>
<td>CC</td>
</tr>
<tr>
<td>4 (50%)</td>
<td>1 (4.76%)</td>
<td>82 (34.45%)</td>
</tr>
<tr>
<td>4 (50.00%)</td>
<td>20 (95.24%)</td>
<td>156 (65.55%)</td>
</tr>
<tr>
<td>8 (3.00%)</td>
<td>21 (7.87%)</td>
<td>238 (89.14%)</td>
</tr>
<tr>
<td>(\chi^2) p</td>
<td>0.0619</td>
<td>0.2845</td>
</tr>
</tbody>
</table>

Variations within the ITPA gene were responsible for the protection against haemolytic anaemia in HCV infected patients in the first Wide Genomic Association Study [9]. The ITPA gene encodes inositol triphosphatase (ITPase), a protein that hydrolyses inositol triphosphate (ITP). ITPase deficiency results in ITP accumulation in erythrocytes and increased toxicity of analogue purine drugs [3, 28]. Reduced ITP activity can be caused by a missense variant in exon 2 (rs1127354, resulting in a treolin proline substitution called P32T) and a polymorphism that creates splice changes in second intron 7270101 [1, 5, 29, 30]. Variations within the ITPA gene, lead to ITPase deficiency and protect against haemolytic anaemia in HCV infected patients during the first weeks of treatment; however, the effect of these ITPA gene variants on the outcome of therapy was inconclusive [16, 23]. During treatment based on Interferon and Ribavirin, anaemia is an important and frequent adverse effect, and therefore leads to the difficulty of patients to tolerate treatment [7, 13, 17, 19-21]. In particular, the host-genic variants of the inositol triphosphatase gene (ITPA) located on chromosome 20, the 20p13 region, leading to deficiency or decreased ITPase activity and having an overwhelming impact on protection against Ribavirin-induced haemolytic anaemia, and reducing the need for dose modification RBV at week 4 of treatment and throughout treatment [9, 23, 31]. In our study, it was demonstrated that the AC genotype was the strongest protector against the occurrence of anaemia. Of the total 21 patients with AC genotype of rs1127354, 76.19% of them did not have anaemia, totalling 16 patients, compared to 5 (23.81%) patients who experienced decreases in Hb during therapy. As far as CC genotype was concerned, of the 237 patients with this genetic variant, 54.62% had anaemia, demonstrating to a small extent that homozygous C allele patients are at a higher risk of developing anaemia during double therapies than patients carrying allele A. We also obtained suggestive data on the protective role of these genetic variants in relation to a decrease in haemoglobin of over 2.5 g/dL at week 4 of treatment. It was shown that patients with the AC genotype of the ITPA gene rs1127354, without a haemoglobin drop off over 2.5 g/dL at week 4 of treatment, accounted for 95.24% (20 patients) of all patients with this genotype, while only one patient (4.76%) showed a decrease in Hb at week 4 of treatment of over 2.5 g/dL. The study continued with the investigation of the same parameters in relation to the occurrence of leucopenia and neutropenia. The results were not conclusive; therefore, it was not possible to demonstrate a link between the occurrence of these haematological adverse effects and the ITPA gene rs1127354 polymorphisms. We further investigated whether the genetic polymorphism C20orf194 rs6051702 influenced leucopenia during treatment. The results obtained were significant with a p value of 0.03, but strong assumptions for this association cannot be supported. The data is presented in Table IV.
Neutropenia occurred in 95 patients; 59 (22.1%) patients tested positive for AA genotype, 36 (13.48%) patients tested positive for AC genotype, and no patients were positive for CC genotype (Figure 4).

As for thrombocytopenia, we didn’t find any statistical links for the ITPA gene variants. We have conducted correlation studies between mild, moderate and severe thrombocytopenia and its development as a whole with the ITPA genotypes. We have not been able to demonstrate a statistical link between the parameters studied. However, in regard to the c20orf194 genotypes, we demonstrated a statistical significance for the correlation between the AA genotype and the protection against thrombocytopenia. From the total patients included in the study that presented with thrombocytopenia, 77 (28.84%) patients had AA genotype, 46 (17.23%) patients had AC genotype and 2 (0.75%) patients, CC genotype. The distribution of patients without thrombocytopenia was as follows: 105 (39.33%) patients with AA genotype, 33 (12.36%) patients with AC genotype and 4 (1.5%) patients with CC genotype (Figure 5).

The C20orf194 gene, rs6051702, located on the 20p13 chromosome in a 1.8 Mb region, encodes an uncharacterized protein and is associated with two genetic variants of the ITPA gene (rs1127354 and rs7270101). As regards to the single nucleotide polymorphisms of the C20orf194 gene, the data demonstrated that patients carrying AC/CC genotypes were protected against the development of anaemia. Genotype AA has been associated with higher rates of anaemia. Instead, there could be no correlation between these genotypes and a decrease in haemoglobin of over 2.5 g/dL at week 4. The data obtained corresponded to our previous findings in a smaller group of patients. Anaemia correlated with genotype AA rs6051702 and CC genotype rs1127354 [15]. Several studies have investigated the implication of these individual genetic variants in the prediction of other haematological side effects of dual antiviral therapy [12, 24, 32]. In our study we investigated the involvement of these polymorphisms in the occurrence of thrombocytopenia, neutropenia and leucopenia. As far as thrombocytopenia is concerned, we have noticed that the genotype AA of the C20orf194 gene has a protective role, but it has not been shown to link to the polymorphism of the ITPA gene. The occurrence of leucopenia could not
be correlated with any of the studied polymorphisms. The homozygous patients for allele A of the c20orf194 gene were protected against the development of neutropenia during antiviral treatment. Of the total AA patients, 53 (29.12%) patients experienced neutropenia, while 129 (70.88%) patients with AA genotype had no decrease in absolute Neutrophil count since initiation of therapy. A similar association could not be demonstrated for the genetic variants of the rs1127354 gene.

Our findings showed a powerful correlation between the genetic variants of the rs1127354 gene and anaemia or outcome after treatment with pegylated interferon and ribavirin. The homozygous patients for allele A and g. IVS2+2a or alfa-2b plus ribavirin in chronic hepatitis C virus-infected patients receiving combination therapy. Gut Liver, 2015; 9(2): 214.


