IMPACT OF HEPATIC STEATOSIS ON DISEASE COURSE IN PATIENTS WITH COMPENSATED HEPATITIS C VIRUS-RELATED CIRRHOSIS RECEIVING INTERFERON-FREE THERAPY (PARITAPREVIR, RITONAVIR, OMBITASVIR DASABUVIR AND RIBAVIRINA)

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IMPACT OF HEPATIC STEATOSIS ON DISEASE COURSE IN PATIENTS WITH COMPENSATED HEPATITIS C VIRUS-RELATED CIRRHOSIS RECEIVING INTERFERON-FREE THERAPY (PARITAPREVIR, RITONAVIR, OMBITASVIR DASABUVIR AND RIBAVIRINA) (Abstract). Aim: In Romania, genotype 1 of hepatitis C virus (HCV) is the most common and the aim of this study was to assess comparatively the biochemical, hematological and virological parameters in patients with different degrees of steatosis before the initiation of interferon-free therapy (paritaprevir/ritonavir/ombitasvir/dasabuvir plus ribavirin) (PrOD-R) and at its completion. Material and methods: This retrospective study included 113 patients with compensated liver cirrhosis with C-genotype 1b, treated with PrOD-R evaluated and clinically-biologically monitored at the Iasi Hospital of Infectious Diseases between November 2015 and May 2017. Results: We found an advanced steatosis (grades ≥2) in 54% of patients. Associated cardiovascular and kidney diseases and diabetes mellitus were more frequent in the group of patients with advanced steatosis compared to the group with incipient steatosis (19.5% vs. 8% and 10.6% vs. 0.9%, respectively) (p = 0.02 and p = 0.003, respectively). In most patients the hepatic cytolysis syndrome was identified before the initiation of therapy (93%) with a significant difference between patients with advanced versus low grade steatosis (53% vs. 40%, respectively, p = 0.014). In patients with steatosis grade ≥2, GGT levels above the normal range were detected in 52.2% cases and in the group with steatosis grade <2 in 31% (p = 0.0003). At the time of treatment initiation, only 3.5% of patients presented various grades of anemia, but at the end of therapy 38% had different grades of anemia, this anomaly being more common in patients with advanced steatosis (23% vs. 15%, respectively, p = 0.27). At the end of therapy, only 14.2% still had higher-than-normal ALT levels, most of them in the group with advanced steatosis (11.5% vs. 2.6%, respectively, p = 0.018). Conclusions: We found a significant correlation between the
advanced grade of hepatic steatosis and the levels of hepatic cytolysis enzymes and GGT both before initiation of therapy and at its completion in patients with advanced steatosis. 

**Keywords:** STEATOSIS, HCV CIRRHOSIS, INTERFERON-FREE TREATMENT.

HCV infection has been reported to be strongly associated with hepatic steatosis. But there are other factors responsible for steatosis, including alcohol consumption, obesity and diabetes.

Experimental animal studies have shown that HCV core protein promotes liver steatosis. Also, when steatosis was studied in connection with HCV genotypes, it was found that although steatosis is induced by all HCV genotypes, it appears to be more evident and more frequent with genotype 3 infection, in these patients being a good correlation between the steatosis grade and HCV replication, the presence of HCV core protein in the liver, respectively (1). Moreover, it has been reported that in patients with genotype 3 infection steatosis regresses with successful antiviral therapy compared to those with other genotypes that remain steatosis (2).

Different mechanisms have been incriminated in the alteration of the lipid metabolism and steatosis progression in HCV-infected patients:

1. **Abnormal VLDL secretion**;
2. **Increased de novo free fatty acid synthesis**;
3. **Abnormal AGL degradation** (3,4,5).

Several viral proteins can occasionally cause cell lesions (by oxidative stress and steatosis), probably in a specific sequence and directly activating the hepatic stellate cells without participating in the inflammatory response (6). These findings may explain why some chronic hepatitis C patients may present significant fibrosis at the histological assessment, despite normal liver enzymes and minimal/ mild inflammation.

It is known that steatosis is a cofactor that influences the progression of fibrosis in chronic hepatitis C. Various studies have directly associated steatosis with HCV genotype 3. Thus, Kumar et al. identified steatosis reduction as a predictor of the virological response in the treatment of chronic hepatitis C in individuals infected with genotype 3 (2).

Our study aimed to evaluate the impact of advanced steatosis grade on virological, hematological and biochemical parameters in patients with HCV cirrhosis treated with Ombitasvir + Paritaprevir + Ritonavir + Dasabuvir and Ribavirin.

The need for such an assessment is justified by the existence of regional particularities regarding comorbidities, diet, age at the time of treatment, previous use of an antiviral drug and the response to it.

**MATERIAL AND METHODS**

In this retrospective study, we analyzed 113 patients with a diagnosis of compensated liver cirrhosis, with HCV genotype 1b, assessed, treated and clinically-biologically monitored at the Iasi “St. Parascheva” Hospital of Infectious Diseases (section 2) from November 2015 until May 2017.

Patient selection was strictly guided by the inclusion criteria set forth in the therapeutic protocol appropriate to DCI (Ombitasvirum + Paritaprevirum + Ritonavirum + Dasabuvirum, currently in force (7, 8).

Patient analysis required the collection and systematization of personal data of
Impact of hepatic steatosis on disease course in patients with compensated hepatitis C virus-related cirrhosis receiving interferon-free therapy (paritaprevir, ritonavir, ombitasvir dasabuvir and ribavirina)

Epidemiological, clinical and laboratory relevance from the medical records and the creation of databases (Excel, SPSS) for statistical evaluation and interpretation.

**Statistical analysis.** The chi-squared test was used for statistical analysis and a value of \( p < 0.05 \) was considered statistically significant.

**Ethical statement.** The objectives and procedures of the study were approved by the Ethics Committee of the Iasi Infectious Diseases Hospital. No written consent was given by the participants, as data were collected and analyzed anonymously.

**RESULTS**

In all patients, steatosis was graded using the baseline Fibromax test. We found an incipient steatosis (<2) in 52 (46%) patients and advanced steatosis (≥2) in 61 (54%) patients. Extreme steatosis grades, namely S0/S0-1 and S3, were recorded in 19 (18%) patients and 34 (30%) patients, respectively.

Seventy-five (66.37%) patients had been previously treated with peginterferon and ribavirin; of them 54 (47.8%) were considered non-responders to previous treatment and 21 (18.6%) patients relapsed. Thirty-eight (33.62%) patients did not receive previous interferon-based regimen.

Efficacy and safety of therapy were assessed in patients with advanced steatosis (≥2) and compared with data from patients with incipient steatosis (<2) (tab. I).

**TABLE I**

<table>
<thead>
<tr>
<th>Response to previous treatment with interferon + ribavirin and the presence of comorbidities by steatosis grade</th>
<th>Steatosis&lt;2 (no=52)</th>
<th>Steatosis≥2 (no=61)</th>
<th>Total (no=113)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Previous interferon-based regimen. (no=75)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.85</td>
</tr>
<tr>
<td>- Non-responder</td>
<td>27</td>
<td>36</td>
<td>27</td>
<td>36</td>
</tr>
<tr>
<td>- Relapse</td>
<td>11</td>
<td>14.7</td>
<td>10</td>
<td>13.3</td>
</tr>
<tr>
<td><strong>Treatment naïve (no=38)</strong></td>
<td>14</td>
<td>12.4</td>
<td>24</td>
<td>21.3</td>
</tr>
<tr>
<td><strong>With cardiovascular or kidney disease (no=31)</strong></td>
<td>9</td>
<td>8%</td>
<td>22</td>
<td>19.5</td>
</tr>
<tr>
<td><strong>With diabetes mellitus (no=13)</strong></td>
<td>1</td>
<td>0.89</td>
<td>12</td>
<td>10.6</td>
</tr>
</tbody>
</table>

A higher rate of the patients with cardiovascular, kidney and metabolic (diabetes mellitus) were in the advanced steatosis group compared with the early steatosis group (19.5% versus 8% and 10.6% vs. 0.9%, respectively) (\( p = 0.02 \) and \( p = 0.003 \), respectively).

Nearly half of the patients had thrombocytopenia (<150,000 / mm\(^3\)) at baseline and was identified in a slightly higher rate in the patients with steatosis ≥2 compared to those with steatosis <2 (28% vs. 22%, \( p = 0.64 \)).

The hepatic cytolysis syndrome was identified before the initiation of treatment in most patients (93%) with a significant difference between those with advanced steatosis versus those with low-grade steatosis (53% vs. 40%, respectively, \( p = 0.014 \)).
TABLE II
Hematologic and biochemical parameters at the initiation of therapy PrOD- R by steatosis grade

<table>
<thead>
<tr>
<th>Parameters at the initiation of therapy</th>
<th>Steatosis &lt;2 (no=52)</th>
<th>Steatosis ≥2 (no=61)</th>
<th>Total (no=113)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Anemia (Hb&lt;11g/dL)</td>
<td>1</td>
<td>0.9</td>
<td>3</td>
<td>2.7</td>
</tr>
<tr>
<td>Thrombocytopenia (&lt;150,000/mmc)</td>
<td>25</td>
<td>22</td>
<td>32</td>
<td>28.3</td>
</tr>
<tr>
<td>Increased ALT level*</td>
<td>45</td>
<td>39.8</td>
<td>60</td>
<td>53.1</td>
</tr>
<tr>
<td>Increased bilirubin level (&gt;1.2mg/dL)</td>
<td>11</td>
<td>9.7</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Increased GGT level (&gt;65UI/L)</td>
<td>35</td>
<td>31</td>
<td>59</td>
<td>52.2</td>
</tr>
<tr>
<td>Increased AFP level &gt;20 UI</td>
<td>11</td>
<td>9.7</td>
<td>14</td>
<td>11.5</td>
</tr>
</tbody>
</table>

* > 30 UI in women and > 40 UI in men

In the study patients, not only the level of cytolytic enzymes was directly influenced by the steatosis grade but also the level of cholestasis enzymes. Thus, in patients with steatosis ≥2, elevated GGT levels were detected in 59 cases (52.2%), and in the group with steatosis<2, 35 patients (31%) showed levels above the normal (> 65UI /L) (p = 0.0003) (tab. II).

Approximately 18% of patients had initially mild hyperbilirubinemia, with no significant differences between the two study groups.

More clear-cut was the comparison of the extreme steatosis grades (S0 vs. S3) with respect to mean levels of viral load, ALT, GGT and AFP at baseline. Statistical analysis revealed significant differences between the obviously higher values in those with significantly advanced steatosis compared to those without steatosis.

Comparing the mean ALT levels in patients with steatosis S0 (71 IU) and steatosis S3 (172 IU), we noticed the significant influence of steatosis grade on the level of this enzyme (p = 0.002) (tab. III).

TABLE III
Mean levels of viral load, ALT, GGT and AFP at baseline by steatosis grade

<table>
<thead>
<tr>
<th>Mean levels</th>
<th>Steatosis: S0 (n=19)</th>
<th>Steatosis: S3 (n=34)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean viral load (UI/mL)</td>
<td>582,450</td>
<td>1,613,050</td>
<td>0.048</td>
</tr>
<tr>
<td>Mean ALT</td>
<td>71 UI</td>
<td>172 UI</td>
<td>0.002</td>
</tr>
<tr>
<td>Mean GGT</td>
<td>42.3 UI</td>
<td>162.4 UI</td>
<td>0.0003</td>
</tr>
<tr>
<td>Mean AFP</td>
<td>5.37 UI/mL</td>
<td>30.82 UI/mL</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Alpha-fetoprotein (AFP) is a very important parameter in the initial assessment being a predictor of hepatocellular carcinoma. In our study, we found a clear relationship between steatosis grade and AFP level. Thus, in S0 patients, mean AFP was 5.37 IU/mL compared to 30.82 IU/mL in S3 patients (p = 0.018).

If only 4 (3.5%) patients had anemia at baseline (Hb <11g/dL), at the end of therapy, 38% had different degrees of anemia, this hematological abnormality being more common in advanced steatosis cases (23% vs. 15%, respectively, p = 0.27) (tab. IV).

We also noticed a significant reduction in the number of thrombocytopenic patients

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Most of the treated patients responded by the return to normal of liver cytolytic enzymes, only 14.2% continuing to show ALT above the normal level at the end of therapy, their proportion being significantly higher in those with advanced steatosis (11.5% vs. 2.6%, respectively, p = 0.018).

A favorable evolution was also recorded for GGT; thus, only 16.8% of the treated patients had higher-than-normal levels, with significant differences between those with steatosis ≥2 vs. those with S <2 (13.3% vs. 3.5 %, respectively, p = 0.016).

A sustained virological response was recorded in all cases (at 12 weeks after completion of therapy) except for one person who discontinued medication for 4 days because of a death in the family.

**DISCUSSION**

The severity of hepatic steatosis in chronic HCV infection is due to the combination of several host factors (obesity, medication, alcoholism) and viral factors (genotype, viral load, mutations) that interfere with the hepatocyte lipid metabolism. Thus, steatosis occurs in chronic HCV infection more frequently than in any other chronic hepatitis and, more importantly, in patients with no other causes of fatty liver, indicating that hepatic steatosis is a direct cytopathic effect of the virus. That is why we considered extremely important to evaluate several aspects related to the presence and severity of steatosis in the study patients (2).

Of the total of 113 patients, 91.2% (103 patients) had various grades of steatosis, with a moderate-severe steatosis (grade ≥2) in over half of them. These results reveal a higher prevalence of steatosis in the study group compared to literature data reporting a prevalence of steatosis in patients with chronic HCV infection of 40-86% (9) or, in another case, of only 55% (10).

Another important aspect is the relationship between the viral genotype and the prevalence of steatosis. As already men-

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**TABLE IV**

**Hematological and biochemical parameters at the end of PrOD- R therapy by steatosis grade**

<table>
<thead>
<tr>
<th>Parameters at the end of therapy</th>
<th>Steatosis &lt;2 (no=52)</th>
<th>Steatosis ≥2 (no=61)</th>
<th>Total(no=113)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Anemia (&lt;12 g%)</td>
<td>17</td>
<td>26</td>
<td>43</td>
<td>38</td>
</tr>
<tr>
<td>Thrombocytopenia (&lt;150,000/mm³)</td>
<td>19</td>
<td>19</td>
<td>38</td>
<td>33.6</td>
</tr>
<tr>
<td>Increased ALT level</td>
<td>3</td>
<td>13</td>
<td>16</td>
<td>14.2</td>
</tr>
<tr>
<td>Increased bilirubin level (&gt;1.2mg/dl)</td>
<td>20</td>
<td>15</td>
<td>35</td>
<td>31</td>
</tr>
<tr>
<td>Increased GGT level (&gt;65UI/l)</td>
<td>4</td>
<td>15</td>
<td>19</td>
<td>16.8</td>
</tr>
<tr>
<td>Increased INR ↑ level (&gt;1.2)</td>
<td>44</td>
<td>53</td>
<td>97</td>
<td>85.8</td>
</tr>
</tbody>
</table>
tioned, all patients included in the study had HCV genotype 1b infection. It was determined (by Fibromax) that 90% of patients had various grades of steatosis, value significantly higher than those reported by other studies which showed a prevalence of steatosis in patients with HCV genotype 1 infection of about 50% (10). Also, most studies suggest that genotype 3 is the most steatogenic, with a prevalence of steatosis of >70%, associating it with the lowest rate of response to classical interferon-based therapies (10).

The introduction of new antiviral agents with direct mechanism has greatly improved the response rate of the patients with steatosis included, this parameter not being so important in predicting the response to treatment, but rather a prognostic factor for other comorbidities: accelerated atherosclerosis, cirrhosis or hepatocellular carcinoma (11, 12).

Thus, referring to the long-term negative potential of steatosis, we considered it necessary to evaluate its severity in our study patients. Over half of the patients enrolled in the study had moderate-severe steatosis grade S ≥2 (54% of the total).

The group of patients with steatosis grade S3 was the most numerous (25.7%), followed by grade S2 (23.9%), the smallest number of patients presenting steatosis grade S2-3 (4.4%). Only 10 of the total 113 patients showed no evidence of steatosis by Fibromax (S0), while 9 had steatosis grade S0-1, suggesting an incipient steatosis. Thus, there is an increased prevalence of moderate-severe steatosis in HCV genotype 1b patients, as opposed to other studies that rather highlight the involvement of genotype 3 in these advanced steatosis forms (moderate-severe in 40-50% of cases), and less of genotypes 1 or 4, with the presence of moderate-severe steatosis in only 10-15% of cases (11).

Another important aspect was the interpretation of the correlation between the initial viral load and steatosis grade. A significant correlation (p = 0.048) was found between steatosis grade and viral load, so that patients with steatosis S0 had a mean viral load of 582,450 IU/mL, significantly lower than that of patients with steatosis S3, with a viral load of 1,613,050 IU/mL. Considering that all our patients had HCV genotype 1b, these data are somewhat contradictory to those obtained by other international studies that have demonstrated the direct correlation between steatosis grade and baseline viral load in case of genotype 3 rather than in genotype 1 (13). Thus, Jackel-Cram investigated the effects of HCV 3a core protein compared to HCV 1b core on fatty acid synthase (involved in de novo synthesis of AG) and revealed a higher prevalence as well as an increased severity of steatosis in the case of genotype 3a compared to 1b (13).

However, a study by Kara (14) also highlights the direct relationship between HCV genotype 1 infection and the presence of steatosis in 82% of patients in the absence of any additional risk factors for this histopathological change. This study also demonstrated a significant link between initial viral load and steatosis grade, similar in relevance to the results of our study (p = 0.02 vs. p = 0.048).

Another interesting aspect in our study was the significant correlation between the high AFP level and grade of steatosis in patients with chronic HCV infection. Thus, in patients without steatosis, mean AFP level was 5.37 IU/mL, compared to 30.82 IU/mL in patients with steatosis S3 (p =0.018). Similar results have been reported.
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by many other studies, such as that conduct- ed by Mousa et al. (15), which compared two groups of patients (50 patients with steatosis, 50 without steatosis) and showed a significantly higher AFP level in patients with steatosis (p <0.01) In other studies are highlighted the clear association (p <0.05) of elevated AFP levels not only with hepatic steatosis, but also with elevated levels of uric acid, fasting blood glucose, vitamin D deficit and AST (16, 17, 18).

CONCLUSIONS

In North-eastern Romania, we found an increased prevalence of advanced steatosis in HCV genotype 1b infected cirrhotic patients, correlated with the presence of cardiovascular, renal and metabolic comorbidities in over 30% of cases. However, the sustained virologic response was very good (approximately 100%), with the normalization of cytolytic enzymes and platelets in significant percentages. We have found a significant correlation between the advanced grade of hepatic steatosis and the level of hepatic cytolytic enzymes and GGT both before the initiation of therapy and at its completion. This finding pleads for the need to reduce the steatosis grade prior to antiviral therapy to limit the level of hepatic cytolysis that would predispose to a more rapid progression to complications even in those who attained sustained virological response.

REFERENCES


**MAASTRICHT V: BEST EVIDENCE AND RELEVANCE TO THE MANAGEMENT OF HELICOBACTER PYLORI INFECTIONS**

The Maastricht conference first took the initiative in 1996 and since then has been repeated at intervals of 4–5 years. Experts invited were chosen for their expertise and contribution to *H pylori* research and/or guideline methodology to review and discuss all relevant clinical data to arrive at recommendations for the clinical management of *H pylori* infection. It has been made important progress in the management of *Helicobacter pylori* infection in this fifth edition of the Maastricht Consensus Report. Key aspects related to the clinical role of *H. pylori*, to the increasing *H. pylori* resistance to previously efficacious antibiotic regimens, to the feasibility and efficacy of gastric cancer prevention, to the recommendation of treatment of all *H. pylori* infected subjects, to the perspective of potential interactions with other microbiota were re-evaluated. The aim of this report is to serve as a state-of-the-art guide for the management of *H. pylori* infection (Malfertheiner P, Megraud F, O’Morain CA, Gisbert JP, Kuipers EJ, Axon AT, Bazzoli F, Gasbarrini A, Atherton J, Graham DY, Hunt R, Moya yedi P, Rokkas T, Rugge M, Selgrad M, Suerbaum S, Sugano K, El-Omar EM, European Helicobacter and Microbiota Study Group and Consensus panel. Management of *Helicobacter pylori* infection-the Maastricht V/Florence Consensus Report. *Gut* 2017; 66(1): 6-30).

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