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The impact of liver steatosis on early and sustained treatment response in chronic hepatitis C patients

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Abstract

Steatosis is a frequent feature of hepatitis C virus (HCV) infection. Steatosis may be an important cofactor in both accelerating fibrosis and increasing liver necroinflammatory activity in chronic hepatitis C. The main objective of this study was the evaluation of biological response rates, early viral response, sustained viral response in patients with chronic hepatitis C treated with Interferon-alpha (IFN-α), Pegylated (PEG)-IFN-α2a or -α2b plus Ribavirin and to relate it to the presence of hepatic steatosis. There were selected to take part to the research 210 patients with chronic hepatitis C who have fulfilled all inclusion and exclusion criteria and were treated with PEG-IFN plus Ribavirin. Patients' progress has been monitored by determining next parameters: age, gender; biochemical tests – alanine aminotransferase (ALT), aspartate aminotransferase (AST); serological assays – detect antibody to hepatitis C virus (anti-HCV); molecular assays – detect, quantify and/or characterize HCV-RNA; liver histopathological examination. Steatosis was graded using the Brunt system. These parameters were included in an area under curve (AUC) analysis. Purpose is to estimate their degree of influence on getting early viral response (EVR) and sustained viral response (SVR). Based on the obtained results, it appears that initial value of HCV-RNA, dVL parameter value (low relative percentage of viral load during the first 12 weeks of treatment), histological scores steatosis may be predictive in the viral response in chronic hepatitis C. Our research demonstrates that a high degree of liver steatosis impairs both EVR and SVR in chronic hepatitis C treated with standard PEG-IFN and Ribavirin for 48 weeks and that a steatosis score of ≤3 predicts EVR with a sensibility of 91.03% with specificity of 21.54%.

Keywords: steatosis, liver biopsy, chronic hepatitis C, ribavirin, sustained viral response.

Introduction

Viral hepatitis is the most difficult disease of infectious pathology due to the high incidence of acute infections, severity of chronic infections, risk of chronicity, and costs of treatment for each patient. Hepatitis is classified as acute or chronic liver inflammation and could be caused by drugs, alcohol, autoimmune diseases, viruses or metabolic diseases. Presence of viral or immunological markers in patients' serum is important to assess the etiology, level of activity, and treatment behavior [1–3].

Factors that may influence the rate of disease progression and response to therapy have been extensively investigated. These variables include age, gender, alcohol consumption, duration of infection, race, hepatitis C virus (HCV) genotype, viral burden and the stage of fibrosis. One other possible factor is steatosis, which is a common yet non-specific histological feature of chronic HCV infection [4–9].

Due to the increased recognition of the potential for some forms of fatty liver to result in progressive liver disease, the role of steatosis in HCV infection has become an area of interest. Liver steatosis is a frequent histological finding in patients with chronic hepatitis C, occurring in more than 50% of cases [10, 11].

Steatosis severity plays an important role in the liver fibrosis development and progression. Steatohepatitis is now regarded as an important cause of cirrhosis with unknown origin or as final stage for a part of liver lesions. Non-alcoholic steatohepatitis (NASH) pathogenesis involves insulin resistance oxidative stress, excess of fatty acids in hepatocytes, and lipid peroxidation. Lesion progression to cirrhosis is variable, but usually is slow. NASH is very often associated with central and visceral obesity, insulin resistance, hyperglycemia and hypertriglyceridemia. Difficulties encountered in diagnosis and treatment of this condition are linked to the lack of knowledge about pathogenic mechanism. Identification of specific factors responsible for hepatic steatosis to steatohepatitis transformation and cirrhosis are the final target of many studies [12–15].

Steatosis is a frequent feature of HCV infection. Steatosis may be an important cofactor in both accelerating

fibrosis and increasing liver necroinflammatory activity in chronic hepatitis C. Several studies suggested that steatosis induces resistance to interferon and Ribavirin combination treatment.

The main objective of this study was evaluation of biological response rates, early viral response, sustained viral response in patients with chronic hepatitis C treated with Interferon-alpha (IFN-α), Pegylated (PEG)-IFN-α2a or -α2b plus Ribavirin and to relate it to the presence of hepatic steatosis.

Patients, Materials and Methods

The research is performed within the Clinic of Internal Medicine, "Filantropia" University Hospital, Craiova, Romania, in 2011–2015. There were selected to take part to the research 210 patients who have fulfilled all inclusion and exclusion criteria. The inclusion criteria: biochemical: alanine aminotransferase (ALT) normal or elevated; virological: HCV-RNA detectable; histologically: liver biopsy consistent with chronic hepatitis; age ≤65 years. Patients older than 65 years will be evaluated based on comorbidities therapeutic risk. Exclusion criteria: we excluded from Interferon therapy patients with neurological diseases, psychiatric illnesses, decompensated diabetes, autoimmune diseases, ischemic heart disease or uncontrolled severe heart failure, uncontrolled severe respiratory disorders, hemoglobin (Hb) <11 g/dL, number of leukocytes <5000/mm^3, number of neutrophils <1500/mm^3. Patients treated with: PEG-IFN-α2a 180 μg/week + Ribavirin: 1000 mg/day body weight <75 kg; 1200 mg/day body weight >75 kg or PEG-IFN-α2b 1.5 mg/kg/week + Ribavirin: 1000 mg/day body weight <75 kg; 1200 mg/day for body weight >75 kg.

All subjects had given informed consent for the studies in which they participated and the Ethics Committee approved these studies.

The analyzed parameters were: demographic data (age, gender), laboratory determinations, diagnostic tests for the detection of HCV infection, followed by histological evaluation.

Laboratory determinations

We used standard procedures to ensure the reproducibility of the ALT and aspartate aminotransferase (AST) by spectrophotometric method.

Diagnostic tests for the detection of HCV infection

I. Serological assays – detect antibody to hepatitis C virus (anti-HCV) by enzyme-linked immunosorbent assay (ELISA).

II. Molecular assays – detection, quantification and/or characterization HCV-RNA. Detection of HCV-RNA using polymerase chain reaction (PCR) provides evidence of active HCV infection could be useful to confirm diagnosis and also for monitoring antiviral response to therapy. Optimal HCV–PCR assays, at present, have a sensitivity of less than 100 copies of HCV-RNA/mL of plasma or serum. Molecular tests have also been developed to classify HCV into distinct genotypes;

Histological evaluation

Liver histopathological examination (performed on liver fragment obtained from liver biopsy). A baseline biopsy was performed in all patients. Liver biopsy specimens were fixed in 10% buffered neutral formalin, processed by paraffin embedding and Hematoxylin–Eosin (HE) staining, as well as CD20 immunostaining. For visualization, it was used the LSAB2 (Labeled Streptavidin–Biotin) system (Dako). The presence of hepatocytes containing large-droplet fat was evaluated. Steatosis was scored using the Brunt grading system, in which steatosis is graded 0 to3.

Statistical analysis

For storing the information registered on the plug of study in a database, and also for statistical calculations, we used statistical software MedCalc® version 12.5.0.0 Medical (MedCalc® Software, Mariakerke, Belgium), Windows XP / Vista / 7 / 8.

Descriptive statistical analysis was used and distributions of categorical variables were compared using *chi*-square (χ^2) or Fisher's exact tests. Continuous data were expressed as means, medians and standard deviation (SD), predictive values. Comparisons between qualitative and quantitative variables were performed using the Student's *t*-test and ANOVA test. A value of $p<0.05$ was considered as statistically significant.

Results

We conducted a descriptive research of HCV patients group, which included 210 cases, of which 101 women with a mean age of 42.7 years and 109 men with a mean age of 43.4 years. Analyzing the ALT, we observed average values of 116.7 U/L for women and 124.5 U/L for men, and viral load mean of 3 578 702 UI/L for women and 4 020 733 UI/L for men (Table 1).

Group distribution related with steatosis grade

In our group, the minimum value of the steatosis grade was 0 and the maximum 3. Most of the men had grade values between 1.74 and 2.11, the mean grade being 1.93. Regarding women, their grade values were between 2.06–2.43, with a mean of 2.25. There were no significant gender differences (Table 2).

Table 1 – ***Synoptic characterization of group***

Parameter	Women				Men			
	No. of patients	*Mean (95% CI)*	*Min.*	*Max.*	*No. of patients*	*Mean (95% CI)*	*Min.*	*Max.*
Age [years]	101	42.7 (40.4–45)	19	64	109	43.4 (41.2–45.6)	19	64
HCV-RNA [IU/mL]	101	3 578 702 (296 794–4 189 464)	130 000	15 600 000	109	4 020 733 (3 402 978–4 638 489)	99 000	13 600 000
ALT [U/L]	101	116.7 (104.4–129)	31	231	109	124.5 (113.7–135.3)	32	231

HCV-RNA: Hepatitis C virus-ribonucleic acid; ALT: Alanine aminotransferase; CI: Confidence interval.

Table 2 – ***Characterization of the group related with steatosis***

Parameter	Women				Men			
	Mean	*95% CI*	*Min.*	*Max.*	*Mean*	*95% CI*	*Min.*	*Max.*
Steatosis grade	2.25	2.06–2.43	0	3	1.93	1.74–2.11	0	3

CI: Confidence interval.

Histological examination

Upon microscopic examination of the cross-sections, we found: abundant lymphoplasmacytic infiltrate in the portal pace; hepatocytes with granular and vacuolar degeneration, dilated sinusoids with hematic content (Figure 1); severe steatosis with lymphoplasmacytic infiltrate in the portal space, hepatocytes with granular and vacuolar degeneration; dilated sinusoids with hematic content (Figure 2); mild steatosis with lymphoplasmacytic infiltrate in the portal space, with hepatocytes with granular and vacuolar degeneration, with fibrosis in the portal space and septa (Figure 3); moderate steatosis with lymphoplasmacytic infiltrate in the portal space and intralobular, hepatocytes with granular and vacuolar degeneration with the presence of binucleated hepatocytes (Figure 4); immunohistochemical staining for CD20 remarks the fibrocytes positivity in the portal space and septa with moderate steatosis (Figure 5); mild steatosis with abundant lymphoplasmacytic infiltrate in the portal space and septa, intense fibrosis with delimitation of pseudonodules (Figure 6).

Study on EVR (early viral response) – different degree of age, biochemical and histological parameters

Of all the subjects included in the study, 145 (69.04%) had therapeutic success (EVR = 1) and a mean age of 42.68 years. Regarding patients with treatment failure (EVR = 0), 65 (30.95%) had a mean age of 44.09 years.

These parameters were included in an area under curve analysis (AUC), in order to estimate their degree of influence on getting EVR.

The limit values from these parameters, could predict the therapeutic success in chronic hepatitis C, are shown in the tables below (Tables 3 and 4).

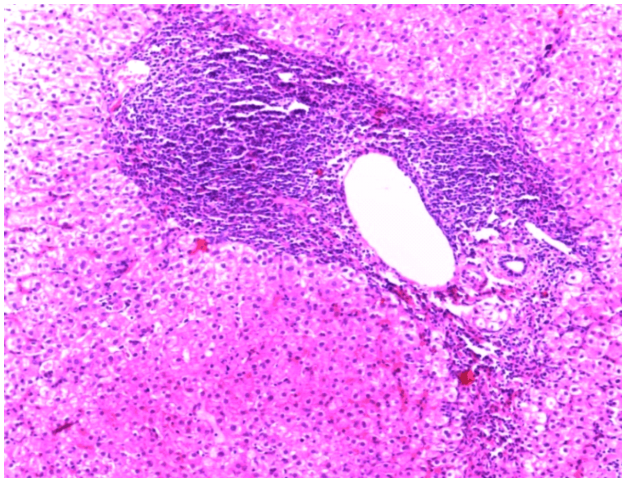

Figure 1 – ***Cross-section of the liver biopsy specimen of patient with chronic viral hepatitis C, severe activity (HE staining, ×100).***

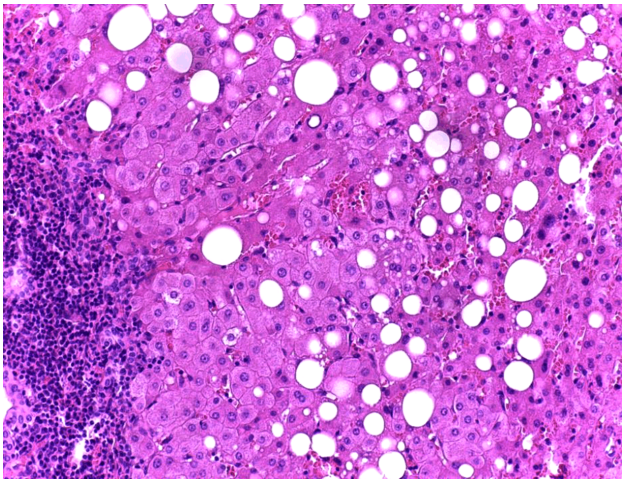

Figure 2 – ***Histological aspect of chronic viral hepatitis C with severe steatosis (HE staining, ×200).***

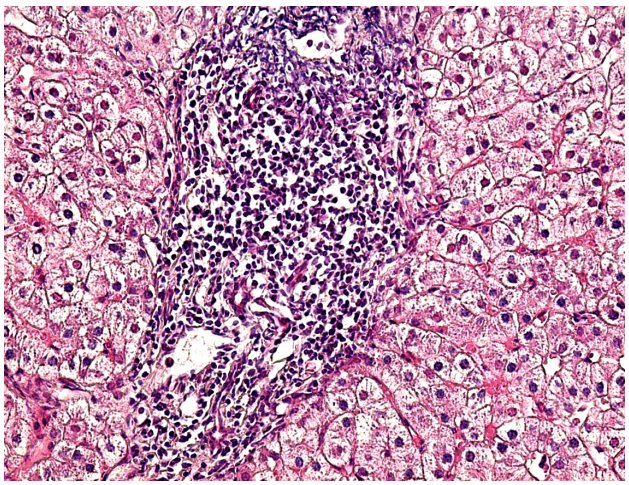

Figure 3 – ***Histological aspect of chronic viral hepatitis C with moderate activity (HE staining, ×200).***

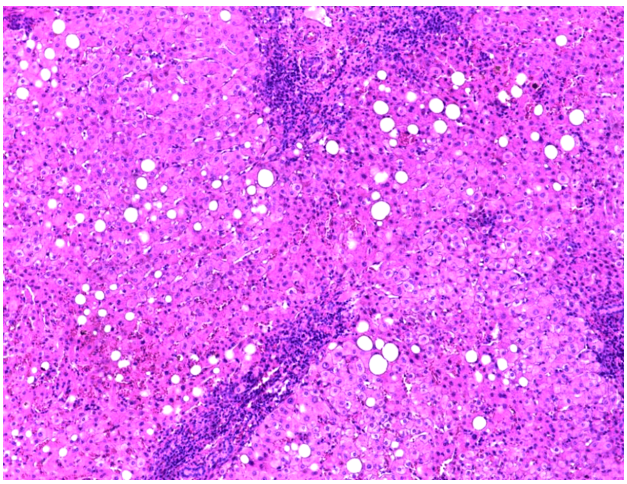

Figure 4 – ***Histological aspect of chronic viral hepatitis C with mild activity (HE staining, ×100).***

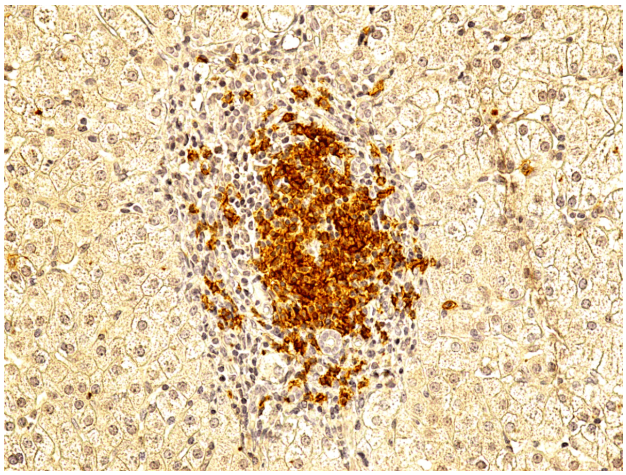

Figure 5 – ***Microscopic image of chronic viral hepatitis C with mild activity associated with CD20+ inflammatory cells in the portal space (Anti-CD20 antibody immunostaining, ×200).***

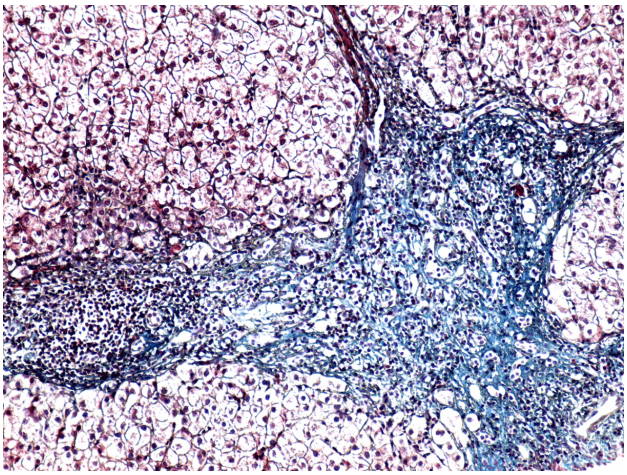

Figure 6 – ***Histological aspect of chronic viral hepatitis C with severe activity (Goldner–Szekely trichrome staining, ×100).***

Table 3 – ***Interpretation of the group through AUC analysis***

Parameter	AUC	Standard error	95% CI	Predictive value
Initial value of HCV-RNA	0.752	0.0326	0.688–0.809	Yes
ALT	0.563	0.0431	0.493–0.631	No
AST	0.557	0.0439	0.487–0.625	No
Age	0.536	0.0421	0.466–0.605	No
Steatosis score	0.682	0.0372	0.614–0.744	Yes

AUC: Area under curve; CI: Confidence interval; HCV-RNA: Hepatitis C virus-ribonucleic acid; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Table 4 – ***Values of predictive parameters in therapeutic success***

Parameter	Optimal value	95% CI	Sensitivity [%]	Specificity [%]
HCV-RNA value T_0	≤3 700 000	2 200 000–3 700 000	75.17	66.15
Steatosis score	≤3	2 to 2	91.03	21.54

CI: Confidence interval; HCV-RNA: Hepatitis C virus-ribonucleic acid.

Influence of steatosis score on EVR

The steatosis score mean 1.9 of patients with successful therapy (EVR = 1) is lower than of those without early viral response, showing that steatosis is a histological factor which could influence the antiviral therapy at 12 weeks (Table 5) (p<0.001). There is a significant correlation between the steatosis grade and early viral response.

Table 5 – ***Characterization of the group related with steatosis score***

Parameter	*n*	Mean steatosis score	*P*
EVR = 0	65	2.4	<0.001
EVR = 1	145	1.9	

n: No. of patients; EVR: Early viral response.

Study on SVR (sustained viral response) – different degree of age, biochemical and histological parameters

The study of the factors involved in achieving SVR was conducted on the 145 patients who achieved early viral response, the other 65 with treatment failure were excluded from the statistics. Overall, SVR was identified in 106/145 (73.1%) patients of the total which was achieved EVR, namely 50.47% of all patients in the initial group (χ^2=30.04, p<0.001). These parameters were included in an AUC analysis in order to estimate their degree of influence on getting SVR.

Since we wanted to assess more precisely the dynamics of the viral load decrease in the first 12 weeks of treatment (EVR testing), it was introduced into the study a derivative measure (relative percentage decrease, nominated dVL), which represents the percentage fraction of the viral load decrease in relation to the initial value of HCV viremia (HCV-RNA T_0). It is calculated using the formula:

$$dVL = 100\times \frac{(\text{HCV-RNA } T_{12\text{ weeks}} - \text{HCV-RNA } T_0)}{\text{HCV-RNA } T_0}$$

where dVL – Low relative percentage of viral load during the first 12 weeks of treatment; HCV-RNA – Hepatitis C virus-ribonucleic acid.

The mean dVL of patients without sustained viral response is -98.43 and of those with SVR is -97.17. We can say that there are statistically significant differences, with p=0.001 (Table 6).

Table 6 – ***Characterization of the group related with dVL***

Parameter	*n*	Mean dVL	*P*
SVR = 0	39	-98.43	0.001
SVR = 1	106	-97.17	

n: No. of patients; dVL: Low relative percentage of viral load during the first 12 weeks of treatment; SVR: Sustained viral response.

Influence of steatosis score on SVR

In the table below, we observe that the mean steatosis score in patients with SVR is 1.89, much lower than in those without response (2.28); therefore, we can say that the presence of steatosis influences the sustained viral response (p<0.001) (Table 7).

We also performed a research regarding the influence of different clinical, biochemical or histological parameters.

These parameters were included in an AUC analysis in order to estimate their degree of influence on getting SVR. The results are shown in the table below (Table 8).

Table 7 – *Characterization of the group related with steatosis score*

Parameter	*n*	Mean steatosis score	*P*
SVR = 0	39	2.28	<0.001
SVR = 1	106	1.89	

n: No. of patients; SVR: Sustained viral response.

Table 8 – *Interpretation of the group through AUC analysis*

Parameter	AUC	Standard error	95% CI	Predictive value
Initial value of HCV-RNA	0.822	0.0342	0.755–0.889	Yes
dVL	0.686	0.0461	0.595–0.776	Yes
AST	0.564	0.0545	0.457–0.671	No
Age	0.517	0.0541	0.411–0.623	No
ALT	0.508	0.0551	0.400–0.616	No
Steatosis score	0.682	0.0532	0.577 to 0.786	Yes

AUC: Area under curve; CI: Confidence interval; HCV-RNA: Hepatitis C virus-ribonucleic acid; dVL: Low relative percentage of viral load during the first 12 weeks of treatment; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

Based on the obtained results, it appears that initial value of HCV-RNA, dVL parameter value (low relative percentage of viral load during the first 12 weeks of treatment), as well as values of steatosis histological scores may be predictive in the SVR in chronic hepatitis C. The limit values from which the set parameters could predict the SVR in chronic hepatitis C are shown in the table below (Table 9).

Table 9 – *Limit values of predictive parameters in SVR*

Parameter	Optimal value	95% CI	Sensitivity [%]	Specificity [%]
HCV-RNA value T_0	≤3 990 000	2 000 000–5 100 000	86.79	53.85
Steatosis score	≤3	3 to 3	99.06	30.77

SVR: Sustained viral response; CI: Confidence interval; HCV-RNA: Hepatitis C virus-ribonucleic acid.

Discussion

In brief, our study demonstrates that factors connected to treatment failure in chronic hepatitis C are linked to liver steatosis, older age and high viral load.

Initial viral load is a strongly negative predictor for therapeutic success, older age has a negative correlation with viral response, but we noticed no influence related to the degree of cytolysis. In a study carried by García-Samaniego *et al.* [16] in patients treated with PEG-IFN-α2a/Ribavirin older age, genotype 1/4, and high gamma-glutamyl transferase (GGT) were associated with lack of EVR. The bivariate analysis showed that patients who failed to achieve EVR were older, had higher ALT, AST, GGT and ferritin levels, and a higher baseline viral load than patients reaching an EVR. Age <40 years, GGT <85 IU/mL, low ferritin levels and genotype other than 1/4 were identified as independent predictors for EVR in the multivariate analysis. In another study by Vanegas *et al.* [17] from of 272 naïve patients treated with PEG-IFN-α2a and Ribavirin, 243 completed the entire treatment. The overall SVR rate in intent-to-treat analysis was 66.5% and in treated patients was 74.5%. In a univariate analysis, the SVR was associated with age <40 years), pre-treatment viral load, non-genotype 1 HCV, non-cirrhosis or pre-cirrhosis rapid virological response (RVR) (91.4%) and EVR (83.8%). In the multivariate logistic regression analysis, the presence of an infection caused by a non-genotype 1 HCV and to achieve ERV were independent predictors of SVR. The RVR and histological stage of liver disease were not included in the multivariate analysis because these data were not available in most of the patients. Moreover, a meta-analysis by Rodriguez-Torres *et al.* [18] that comprised 1550 genotype 1 HCV patients from five clinical trials, randomized to PEG-IFN-α2a 180 μg/week plus Ribavirin 1000–1200 mg/day showed that overall, 15.6% of patients achieved RVR and 54% achieved complete EVR. Baseline factors predictive for RVR were serum HCV-RNA, ALT, non-cirrhotic status, age, and white ethnicity.

Our study observed that steatosis negatively influences the rate of response to antiviral treatment, as confirmed by large clinical trials. Poynard *et al.* [19], in a randomized trial, assessed the effect of PEG-IFN or IFN-α2b and Ribavirin on steatosis in 1428 naïve patients included. The variables associated with steatosis in logistic regression were genotype 3, triglycerides greater than 1.7 mmol/L, body mass index (BMI) >27 kg/m^2, age >40 years, and septal fibrosis. In genotype 3-infected patients, steatosis was associated with high viral load and with lower serum cholesterol. Steatosis was associated with lower sustained response rate, even after taking into account other factors. Among virological responders, steatosis was much improved in genotype 3, improvement of at least one grade in 77%, and disappearance in 46% compared with other genotypes, 46% and 29%, respectively. Steatosis was associated with genotype 3 HCV, triglycerides, high BMI, age, fibrosis stage, and lower virological response to treatment.

Bjøro *et al.* [20], in their study, also noticed that patients infected with genotypes 1a/1b, absence of steatosis was an independent predictor of SVR. In this study were enrolled 256 treatment-naïve HCV-RNA-positive patients with biopsy-confirmed chronic hepatitis in a randomized multicenter study. Also, the patients received standard combination therapy with 3 MIU IFN-α2b thrice-weekly for 26 weeks or 6 MIU IFN-α2b daily for four weeks and 3 MIU IFN-α2b three/seven days for 22 weeks. All patients received Ribavirin 1000 mg or 1200 mg (weight dependent) daily during the 26-week treatment period. During the study, patients were monitored for HCV-RNA treatment. For patients receiving IFN induction/Ribavirin and standard IFN/Ribavirin, respectively, SVR rates were 54% and 47%. Among patients infected with genotype 1a/1b, SVR rates were 32% and 35%. In patients infected with genotype 2b/3a HCV, IFN induction/Ribavirin led to a SVR rate of 80% as compared to 65% in the standard combination therapy group. Steatosis was frequently observed in liver biopsies from patients with genotype 3a compared to genotypes 1a/1b HCV. Among genotype

1a/1b HCV-infected patients, steatosis was a highly significant predictor of failure to achieve SVR. Logistic regression analysis reveals that independent predictors of SVR were low age, female gender, genotype 2b/3a and HCV-RNA negativity at two weeks. These observations are also sustained by Akuta *et al.* [21] that identified five independent factors associated with SVR; viral load <1.0 mEq/mL, total IFN dose ≥700 MIU, hepatocyte steatosis none or mild, albumin ≥3.9 g/dL, and ALT ≥75 IU/L. The kinetic study also showed that serum viral clearance at ≤1 week was the best predictor of SVR, and persistence at ≥4 weeks was a predictor of non-SVR.

The mechanism by which steatosis contributes to liver injury remain unclear [22, 23]. In Walsh *et al.* [24] study, were enrolled 125 patients with chronic hepatitis C. The main objective was to assess the effect of steatosis on liver cell apoptosis. Also, they assess the expression of Bcl-2, Bcl-x(L), Bax, tumor necrosis factor-alpha (TNF-α) and the relationship between liver cell apoptosis and disease severity. In liver sections with increasing grade of steatosis was seen a significant increase in liver cell apoptosis. We can see that increased liver cell apoptosis without steatosis was not associated with stellate cell activation or fibrosis. In contrast, in the presence of steatosis, increasing apoptosis was associated with activation of stellate cells and increased stage of fibrosis, supporting the premise that the steatotic liver is more vulnerable to liver injury. In patients with genotype 3 HCV [25], there was a significant correlation between TNF-α mRNA levels and active caspase-3 but further investigation will be required to determine the molecular pathways responsible for the proapoptotic effect of steatosis and whether this increase in apoptosis contributes directly to fibrogenesis [26].

Recent advances by Petta *et al.* [27] study show that the triglycerides×glucose (TyG) index can be proposed surrogate marker of insulin resistance (IR), calculated from fasting plasma triglyceride and glucose concentrations. The authors tested the host and viral factors associated with Tyg and homeostasis model assessment (HOMA) scores, comparing their associations with histological features and with SVR in patients with genotype 1 chronic hepatitis C (G1CHC). Three hundred and forty consecutive patients with G1CHC were considered. All had a liver biopsy scored by one pathologist for staging and grading and graded for steatosis (as we also did), which was considered moderate-severe if ≥30%. Anthropometric and metabolic measurements, including IR measured by both HOMA and TyG, were registered. By linear regression analysis, TyG was independently associated with waist circumference (WC), total cholesterol, presence of arterial hypertension, log_{10} HCV-RNA and steatosis. Similarly, WC and steatosis were significantly associated with HOMA. Older age, higher WC and higher TyG were linked to moderate-to-severe steatosis (≥30%) by multiple logistic regression analysis. When TyG was replaced by HOMA-IR in the model, the latter remained significantly associated with steatosis ≥30%. Receiver operating characteristic curves showed a similar performance of TyG (AUC 0.682) and HOMA-IR (AUC 0.699) in predicting moderate-severe steatosis. No independent associations were found between both TyG and HOMA and fibrosis or SVR. It can be possible that in the future, in patients with G1CHC, TyG, an easy-to-calculate and low-cost surrogate marker of IR, could be linked to liver steatosis showing an independent association with viral load.

Conclusions

Analyzing results of our study reveals that steatosis score may have a predictive value for obtaining an early and sustained viral response in chronic hepatitis C and the high viral load is a strongly negative predictor for therapeutic success. Low pre-treatment level of HCV-RNA was statistically significantly correlated with virological response in patients with chronic hepatitis C treated with IFN-α and Ribavirin.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

Alice Elena Găman and Anca Marilena Ungureanu equally contributed to the manuscript.

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