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Non-invasive quantification of liver fibrosis regression following successful treatment of chronic hepatitis C with direct acting antivirals

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Abstract

***Introduction**. The past years have revolutionized the treatment of hepatitis C virus (HCV) infection, with high rates of sustained virologic response (SVR). Furthermore, liver fibrosis has recently been redefined as a dynamic, reversible process. **Methods**. We performed a prospective cohort study to assess the role of laboratory evaluations and non-invasive measurement of liver stiffness in establishing the right time for starting treatment and in assessing the regression of liver fibrosis in Romanian patients treated with direct acting antivirals (DAA) for genotype 1b chronic hepatitis C. **Results**. We present the results for 102 patients, with a mean age of 58.5 years, and a rate of SVR of 100%. Our study has ruled out older age (p=0.628), IL28B non-CC genotype (p=0.693), baseline viral load above the cutoff of 600,000 IU/mL (p=0.353), and the presence of diabetes mellitus (p=0.272) or baseline steatosis (p=0.706) as factors potentially influencing the regression of liver fibrosis following DAA treatment of HCV infection with the 3D regimen. The quantitative regression of liver stiffness was inversely correlated with the duration of HCV infection (p=0.017), suggesting that timely treatment might associate better outcomes in terms of liver fibrosis. **Conclusion**. Our study's results point towards the need to start DAA treatment earlier in patients with HCV infection.*

***Keywords**: liver fibrosis, HCV, DAA, SVR, prognostic laboratory markers*
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Introduction

The past years have revolutionized the treatment of hepatitis C virus (HCV) infection, with direct acting antivirals (DAAs) now ensuring high rates of sustained virologic response (SVR), and leading to a change in the epidemiology of HCV infection in Romania (1, 2). Furthermore, liver fibrosis has recently been redefined as a dynamic, reversible process (3). A recent consensus statement published in Romania offers guidance on how to use two important types of tools for the non-invasive assessment of liver fibrosis, namely elastography and scores based on serum markers (4). However, to date, there is no specific data regarding the ideal tools for quantifying the regression of liver fibrosis following successful treatment of HCV infection (5). This study aimed to assess the role of laboratory evaluations and non-invasive measurement of liver stiffness in establishing the right time for starting DAA treatment and in assessing the regression of liver fibrosis in Romanian patients treated for genotype 1b (6) chronic hepatitis C and cirrhosis.

Material and methods

We performed a prospective cohort study to assess treatment response and non-invasive markers for regression of liver fibrosis in patients with chronic hepatitis C undergoing DAA treatment with ombitasvir/paritaprevir/ritonavir, dasabuvir with and without ribavirin for 12 weeks in a reference center in Romania. We present the results for the first 102 patients, included in the study from January 2015 to July 2016. All patients received nutritional and lifestyle counselling at baseline, with recommendations for hypocaloric balanced diets in those who were overweight, and normocaloric balanced diets in those within a healthy body mass index range. Personalized management of dyslipidemia was also ensured by the Institute's nutritionist at baseline.

Liver stiffness was assessed through shear-waves elastography on Aixplorer (SuperSonic Imagine, France) at baseline, at the end of treatment (EOT) and at a follow-up visit at 12 weeks post-treatment (PT).

We also assessed whether there is an association between the regression of liver fibrosis with classical prognostic factors previously used for calculating the chance of obtaining SVR following interferon-based treatment, such as: younger age (7, 8), female gender (9), IL28B CC *rs12979860* (10) or other genetic polymorphisms (11, 12), baseline viral load below 600,000 IU/mL (13, 14), absence of diabetes mellitus (15), shorter duration of HCV infection (16) calculated as the time (years) since the first positive anti-HCV test, and absence of surrogate markers for advanced liver disease at baseline.

Two different statistical models were applied to assess the performance of shear-waves elastography and to identify prognostic factors for the regression of liver fibrosis following SVR. In the first model we quantified the exact decrease in liver stiffness (in kPa) from baseline to EOT and from baseline to 12 weeks PT in patients with and without each prognostic factor, and in the second model we assessed the distribution of prognostic factors in two categories of patients: those who obtained a one stage decrease in liver fibrosis on the Metavir scale and those who failed to obtain this decrease by 12 weeks PT.

For normally distributed continuous variables we report the mean and standard deviation (SD) and the results of the one-way analysis of covariance, while for non-normally distributed variables, we report the median and interquartile range (IQR), and the results of the Mann-Whitney U test along with Spearman's rank-order correlation. For categorical variables we present the frequency and percentage, along with the results of the Chi square test. Effect sizes are provided for all statistical comparisons. Data were analyzed using SPSS Statistics for Windows

(v22.0, IBM Corp, USA), with a p value <0.05 considered to be statistically significant.

Results

We present the results for 102 patients with genotype 1b chronic HCV infection, with a mean age and SD of 58.5±10.9 years, and a male-to-female ratio of 1.1:1. Most patients (n=69, 72.6%) presented comorbidities, namely arterial hypertension in 32 (36%) cases, diabetes mellitus in 18 (20.2%) cases, hypertriglyceridemia in 16 (15.7%) cases, hypercholesterolemia in 13 (12.7%) cases, thyroid dysfunction in 7 (7.9%) cases, and co-infection with HIV in 1 case (0.98%). Only 27.3% (12/44) of patients did not present steatosis at baseline, with 50% presenting minimal steatosis (22/44), 6.8% significant steatosis (3/44) and 6.9% severe steatosis (7/44) on FibroMax evaluation.

Most patients (n=68, 66.7%) had received at least one prior interferon-based anti-HCV treatment regimen, and their median (IQR) viral load at baseline was 899,500 IU/mL (376,111-2,613,916 IU/mL), with 51 patients (59.3%) presenting a baseline viral load above the classical cutoff of 600,000 IU/mL. Most patients (n=66, 83.5%) had a non-CC IL28B polymorphism. The median (IQR) duration of HCV infection was 10 (5-15) years, and 46 (59.7%) patients had cirrhosis as baseline, with a median (IQR) liver stiffness of 15.25 (10-21.8) kPa in the whole study group.

All patients received the full course of 12 weeks of DAA treatment, and 70 (72.2%) of them required the association of ribavirin in the treatment regimen. All patients achieved SVR, and the median (IQR) decrease in liver stiffness was 1.8 (0.7-3.7) kPa by EOT and 3.8 (1.9-5.3) kPa by 12 weeks PT. By EOT 20/53 (37.7%) patients had achieved a one stage decrease in liver fibrosis on the Metavir scale, and by 12 weeks PT another 12 patients, with an overall 59.3% of patients (32/54), had achieved a one stage regression in liver fibrosis (p=0.026, z-score=-2.2). Table 1 presents the baseline characteristics of patients who achieved and who failed to achieve this one stage regression in liver fibrosis by 12 weeks PT.

Age

Age was not significantly correlated with the quantitative decrease in liver stiffness at EOT (p=0.561, r_s=0.08, N=52) or 12 weeks PT (p=0.174, r_s=0.19, N=53) and neither with the qualitative one stage decrease in liver fibrosis, with the median (IQR) age lacking a statistically significant difference in patients who achieved regression of fibrosis (median: 61, IQR: 52-66.5 years) and those who failed to achieve regression (median: 59, IQR: 53-65 years), U=324.5, p=0.628, r=0.06.

Gender

The median (IQR) liver stiffness at baseline was 22.4 kPa in females and 21.4 kPa in males, U=627.5, p=0.245, r=0.1 (Figure 1A). Females presented a median decrease in liver stiffness of 2.4 kPa at EOT compared to 1.2 kPa in males (Figure 1B), and a median decrease of 4.3 kPa at 12 weeks PT compared to 2.3 in males (Figure 1C), but these changes failed to reach statistical significance at both EOT (U=257, p=0.158, r=0.2) and 12 weeks PT (U=262.5, p=0.126, r=0.2), even after adjusting for the baseline liver stiffness (F(1, 50)=0.133, p=0.717). The qualitative regression of liver fibrosis was also slightly more frequent in females (15/24, 62.5%) than in males (17/30, 56.7%), but without statistical significance (p=0.439, χ(1)=0.2, Phi=0.06).

IL28B polymorphism

The median (IQR) liver stiffness decrease at EOT was 1.1 (0.7-2.4) kPa in patients with CC IL28B genotype and 1.8 (0.6-3.7) kPa in those with non-CC genotype, the differences lacking statistical significance (U=158.5, p=0.511,

Table 1. Characteristics of patients who achieved and who failed to achieve a one stage decrease in liver fibrosis following DAA treatment

Characteristic	Patients with regression of liver fibrosis	Patients without regression of liver fibrosis	Statistical analysis
Gender (female), n/N (%)	15/32 (46.9%)	9/22 (40.9%)	$\chi(1)=0.188$, p=0.665, Phi=0.06
IL28B (CC), n/N (%)	6/31 (19.4%)	2/19 (10.5%)	$\chi(1)=0.683$, p=0.409, Phi=-0.1
Steatosis at baseline, n/N (%)	15/19 (78.9%)	12/17 (70.6%)	$\chi(1)=0.334$, p=0.706, Phi=0.1
Baseline liver stiffness (kPa)	14.2 (9.8-21.9)	20.3 (15.7-30)	U=235.5, p=0.040, r=0.3
Baseline viral load (IU/mL)	1,134,683 (467,883-3,822,612)	1,130,459 (380,235-2,771,489)	U=276, p=0.716, r=0.1
Age (years)	61 (52-66.5)	59.5 (53-65)	U=324.5, p=0.628, r=0.06
Duration of HCV infection (years)	12 (7-16)	13.5 (9-16)	U=320.5, p=0.578, r=0.08
ALT (U/L)	84 (56-126)	119 (78-165)	U=243.5, p=0.093, r=0.2
AST (U/L)	60 (41-89)	94 (66-132)	U=142.5, p=0.036, r=0.4
Total bilirubin (mg/dL)	0.9 (0.7-1.05)	1.3 (1.05-1.5)	U=169, p=0.006, r=0.4
Thrombocyte count $x10^3/\mu L$	164 (135-212)	132 (63-174)	U=217.5, p=0.044, r=0.3
Baseline prothrombin concentration (%)	101 (91-112)	96 (74.25-105.5)	U=183.5, p=0.052, r=0.28
EOT prothrombin concentration (%)	103.5 (89.4-113.05)	92.5 (74-99)	U=121, p=0.005, r=0.4
On treatment decrease in liver stiffness (kPa)	2.4 (0.85-5.65)	1.15 (0.7-2.1)	U=153, p=0.109, r=0.247

r=0.09). The median (IQR) decrease at 12 weeks PT was 2 (0.9-4.4) kPa and 4.1 (2.3-5.5) kPa in the two groups, respectively (U=132, p=0.387, r=0.1). Qualitative regression of liver fibrosis was seen in 6/8 (75%) and 25/42 (59.5%) of those with CC and non-CC polymorphism, respectively (p=0.693, $\chi(1)=0.7$, Phi=-0.1).

Baseline viral load

A cutoff of 600,000 IU/mL of the baseline viral load has not been confirmed as a predictive factor for regression of liver fibrosis, with a median (IQR) stiffness decrease at EOT of 1.8 (0.6-3.5) kPa in patients with baseline viral load below the cutoff and 1.5 (0.7-3.6) kPa in those with baseline viral loads above the cutoff (U=297, p=0.802, r=0.04), and a median (IQR) liver stiffness decrease at 12 weeks PT of 3.8 (1.1-5.5) kPa and 3.4 (2.0-5.2) kPa in patients with baseline viral loads below and above the cutoff, respectively (U=268, p=0.727, r=0.05). Regression of liver fibrosis occurred in both groups of patients, regardless of the baseline viral load (r_s=-0.094, p=0.522, N=49). The quali-

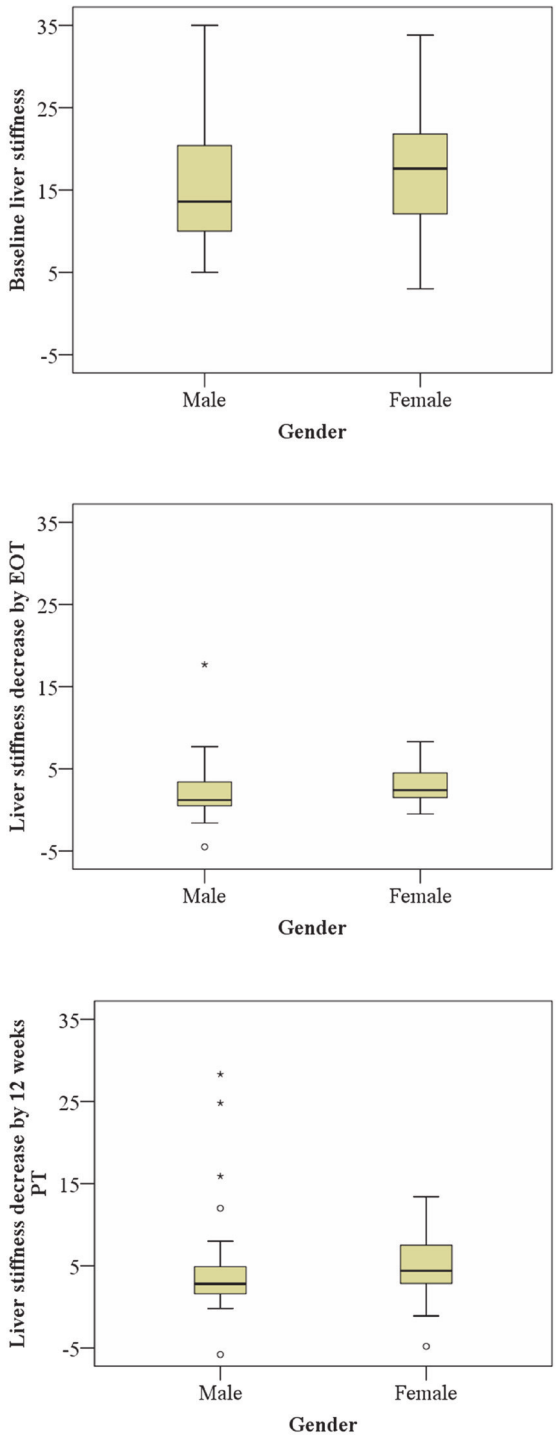

Figure 1. Differences in liver stiffness and fibrosis regression between genders

tative decrease was seen in 12/19 (63.2%) and 16/30 (53.3%) patients with baseline viral loads below and above the cutoff value, respectively (p=0.353, $\chi(1)=0.5$, Phi=-0.09).

Diabetes mellitus

At baseline, patients with diabetes mellitus had slightly higher liver stiffness, i.e., 20.0±12.7 kPa vs. 17.1±9.2 kPa (p=0.353, t(68)=-0.943, d=0.3). The median (IQR) decrease in liver stiffness at EOT was 0.3 (-2.5, 2.5) kPa and 1.8 (0.8-3.6) kPa in patients with and without diabetes (U=94, p=0.169, r=0.2). The median (IQR) decrease by 12 weeks PT was 2.1 (1.9-3.2) kPa and 4 (1.7-5.5) kPa in the two groups (U=110.5, p=0.128, r=0.21). Qualitative regression of liver fibrosis was seen in 6/8 (75%) and 24/43 (55.8%) patients with and without diabetes, respectively (p=0.272, $\chi(1)=1.0$, Phi=0.142).

Steatosis

Baseline liver stiffness was slightly higher in patients with any degree of steatosis, with a median (IQR) of 16.3 (9.8-23) kPa vs. 11.2 (8.5-19.7) kPa, but the differences failed to reach statistical significance (U=130, p=0.247, r=0.2). We also did not identify a significant difference in the quantitative decrease of liver stiffness in patients with and without steatosis at EOT (U=120, p=0.288, r=0.2) or 12 weeks PT (U=113.5, p=0.770, r=0.05). Qualitative regression of liver fibrosis was seen in 15/27 (55.6%) vs. 4/9 (44.4%) of patients with and without steatosis at baseline ($\chi(1)=0.334$, p=0.706, Phi=0.1).

Duration of HCV infection

We identified a significant inverse correlation between the duration of HCV infection and the quantitative decrease in liver stiffness, r_s=-0.326, p=0.017, N=53, but this association was not statistically significant when comparing the qualitative regression in liver fibrosis, despite the fact that patients who achieved a one stage

decrease in fibrosis had a slightly lower median (IQR) duration of HCV infection, 12 (7-16) years, compared with those who did not achieve regression of liver fibrosis, 13.5 (9-16) years (U=320.5, p=0.578, r=0.08).

Surrogate markers for advanced liver disease

We have also identified a set of other predictive factors for qualitative regression of liver fibrosis, namely: lower baseline liver stiffness (U=235.5, p=0.040, r=0.3), lower EOT liver stiffness (U=114, p=0.003, r=0.4), lower transaminases at baseline (ALT: U=243.5, p=0.093, r=0.2; AST: U=142.5, p=0.036, r=0.4), lower total bilirubin values at baseline (U=169, p=0.006, r=0.4), higher thrombocyte count at baseline (U=217.5, p=0.044, r=0.3), EOT (U=156.6, p=0.012, r=0.4) and 12 weeks PT (U=145.5, p=0.03, r=0.3), higher prothrombin concentration at EOT (U=121, p=0.005, r=0.4).

We also identified that a higher on-treatment decrease in liver stiffness was positively correlated with a higher overall on- and off-treatment decrease (p=0.013, r_s=0.38, N=52).

Cholesterol levels were not correlated with the quantitative regression in liver stiffness at EOT (r_s=0.143, p=0.393, N=38), or at 12 weeks PT (r_s=0.116, p=0.471, N=41), and the same was true for triglyceride levels and liver stiffness at EOT (r_s=0.036, p=0.833, N=36), and at 12 weeks PT (r_s=0.088, p=0.594, N=39).

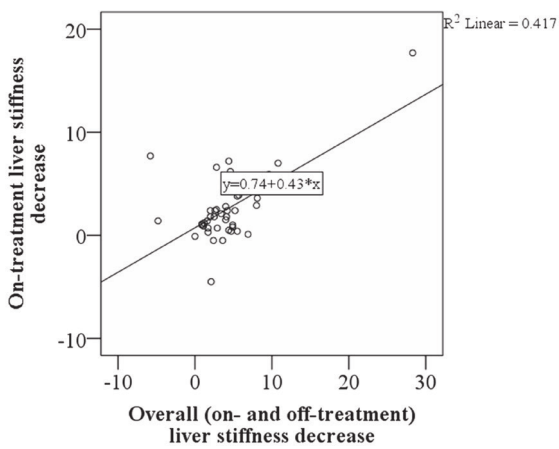

Figure 2. Dynamics and temporal trends in liver stiffness decrease

Discussion

With the improved treatment response following DAA administration, the landscape of HCV infection has dramatically changed (17, 18). In this context it becomes increasingly important to know when to start DAA treatment, and to establish which of the classical factors associated with good prognosis of hepatitis C may still play a role in HCV infection, particularly in the resolution or regression of liver fibrosis following SVR. We designed a study to assess the role of laboratory evaluations and non-invasive measurement of liver stiffness in establishing the right time for starting DAA treatment to achieve the best reduction in liver fibrosis.

Our study has ruled out older age (p=0.628), IL28B non-CC genotype (p=0.693), baseline viral load above the cutoff of 600,000 IU/mL (p=0.353), and the presence of diabetes mellitus (p=0.272) or baseline steatosis (p=0.706) as factors potentially influencing the regression of liver fibrosis following DAA treatment of HCV infection with the 3D regimen.

In terms of gender impact, we saw a tendency for higher regression of liver fibrosis in females compared to males (62.5% vs. 56.7%), but it failed to reach statistical significance (p=0.439) – Figure 1.

Diabetes mellitus and other types of glucose abnormalities have long been considered risk factors for faster progression of liver fibrosis in patients with hepatitis C (15). In our study patients with diabetes mellitus had slightly higher baseline liver stiffness, and we found that we can expect to see increasing liver stiffness during the course of the 12 weeks DAA treatment, but this

does not hinder the eventual regression in liver fibrosis by 12 weeks post-treatment (p=0.272), which occurs at a similar rate as that seen in patients without diabetes mellitus.

The cumulated risk for advanced fibrosis is even higher in patients who, on top of glucose abnormalities, also associate fatty liver disease, a condition which has been reported to have prevalences as high as 55% in patients with HCV infection (19), and in our study was present in 72.7% of patients, albeit only 13.7% presented advanced steatosis. In this context, a personalized medical nutrition regimen becomes a powerful instrument in preventing and/or treating fatty liver disease in patients with HCV infection (20). In our study, 27.3% of patients did not present steatosis, and 50% of them presented minimal steatosis. These findings suggest that in our cohort of patients, timely lifestyle and nutritional intervention may be used to try and prevent the progression of steatosis (21, 22).

The quantitative regression of liver stiffness was inversely correlated with the duration of HCV infection (p=0.017), suggesting that timely treatment might associate better outcomes in terms of liver fibrosis, as more mechanisms may be available earlier during the course of disease to provide regeneration of the extracellular matrix. This was also confirmed by a set of other baseline predictive factors for qualitative regression of liver fibrosis identified in our study, such as: lower liver stiffness (p=0.040), lower total bilirubin values (p=0.006), higher thrombocyte count (p=0.044), and higher prothrombin concentration at EOT (p=0.005). Therefore, it becomes apparent that treatment should be started as early as possible in the course of disease, preferably before developing significant liver fibrosis, and most importantly, before any signs of advanced liver disease appear. The absence of cirrhosis at baseline was a good prognostic factor for treatment response in the interferon era (14), and it appears to remain an important predictor for the regression of liver stiffness following SVR in the DAA era. This may be due to the fact that a good hepatic blood flow can allow natural killer and dendritic cells to accumulate in the liver, thereafter eliminating myofibroblasts originating from hepatic stellate cells, and using matrix metalloproteinases to degrade the fibrotic extracellular matrix (3).

Regression of liver fibrosis appears to be a dynamic process, starting early during the treatment phase and continuing into the off-treatment phase, the total regression in liver fibrosis being a factor of the fast initial on-treatment decrease in liver stiffness (p=0.013) – Figure 2. Shear-waves elastography can be used as a non-invasive instrument to measure liver stiffness at baseline and then quantify the regression of liver fibrosis during and after treatment.

Potential limitations of our study include the exploratory nature of the analysis, and the relatively low number of patients included in each sub-category. For example, when studying the IL28B polymorphism, only 13 (16.5%) of the patients presented CC genotype, and when analyzing the impact of comorbidities, only 18 (20.2%) of the patients presented diabetes. Also, FibroMax was not available in all patients to evaluate baseline steatosis. Therefore, the statistical significance of some of the results could have been potentially masked by the unequal distribution of patients within subgroups. To explore this issue, we propose that future studies be performed, adequately powered to assess the exact impact of each of these patient subcategories. Another limitation of the study resides in the fact that nutritional counseling for lifestyle and dyslipidemia management was only provided at baseline, and that the patients' compliance to the nutritional recommendations was not quantified during the course of the 12 weeks of treatment. Therefore, we are unable to provide close follow-up data regarding the nutritional and lifestyle management and its potential long term impact on the regression of

liver steatosis and dyslipidemia, an aspect which should be further explored in future studies.

In conclusion, most of the predictive factors for hepatitis C from the interferon era no longer play an important role in the DAA age, but we have identified certain laboratory markers suggestive for advanced liver disease which, along with the non-invasive quantification of liver fibrosis and the duration of HCV infection, can significantly impact the regression of liver fibrosis following sustained virological response. Our study's results consistently point towards the need to start DAA treatment earlier in patients with HCV infection.

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Conflicts of interest

OS, ASC, LLP, and ASC have been investigators in HCV clinical trials by AbbVie, Boehringer Ingelheim, Merck. MN and CV – no conflicts of interest.

Abbreviations

direct acting antivirals (DAAs)
end of treatment (EOT)
hepatitis C virus (HCV)
interquartile range (IQR)
post-treatment (PT)
standard deviation (SD)
sustained virologic response (SVR)

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