

# Evaluation of direct-acting antiviral agents impact on liver biomarkers in patients with type-2 diabetes and hepatitis C virus infection

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## ABSTRACT

**Objectives.** Chronic hepatitis C (CHC) infection still affects a significant portion of the global population. Despite efforts to combat it, the global prevalence remains high. The use of direct antiviral therapy (DAA) has significantly improved cure rates. The study aimed to assess liver parameters and body mass index (BMI) dynamics in type 2 diabetes mellitus (T2DM) patients compared to non-diabetics, after obtaining sustained viral response (SVR) under DAA.

**Material and methods.** We conducted a retrospective study on 100 CHC infected patients treated with DAAs between September 2016 and August 2019 at “Prof. Dr. Matei Bals” National Institute for Infectious Diseases, Bucharest (INBI MB). The study group was divided into 2 subgroups according to the presence of diabetes. All patients were evaluated at initiation of treatment and one year after. FibroMax parameters and BMI were monitored.

**Outcomes.** The study found improvements in liver fibrosis, necroinflammatory activity, and steatosis in both groups after treatment. Notably, T2DM patients experienced a more significant decrease in necroinflammatory activity. Moreover, weight gain post-SVR was observed, potentially impacting liver health.

**Conclusions.** Post-SVR, CHC patients showed improvements in liver health markers, emphasizing the importance of early treatment to prevent severe liver complications. Future research should focus on long-term outcomes post-treatment.

**Keywords:** HCV infection, direct acting antivirals, sustained virological response, type 2 diabetes mellitus, FibroMax, BMI

## INTRODUCTION

CHC infection remains a global health issue, as it affects 1-2 % of the world population. [1] After the introduction of direct antiviral therapy (DAA), with a cure rate of over 95%, the World Health Organization (WHO) adopted a strategy to eliminate viral hepatitis by 2033, which includes a 65% reduction in mortality and 80% reduction in newly infected cases by the year 2030 [2]. However, despite all the efforts, the global prevalence of CHC infection re-

mains as high as 58 million, with approximately 1.5 million new infections per year [3]. WHO estimated that in 2019, 242,000 people died of hepatitis C. Mortality was mostly a result of complication of the chronic disease with cirrhosis and hepatocellular carcinoma (HCC) being the leading cause of mortality [1,3].

In addition to liver-related complications, CHC has been associated with multiple extrahepatic manifestations which contribute further to morbidity and mortality [4]. However, with the advent of

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DAAs, as more and more patients achieve SVR, the sequelae of hepatitis C (HCV) eradication are being extensively studied, primarily focusing on the extent of extrahepatic outcomes. The American Association of the Study of Liver Diseases (AASLD) recommends evaluation and management for modifiable risk factors for liver injury, such as fatty liver, and diabetes mellitus in order to optimize weight loss and glycemic control for these patients. [5]

It has been well documented that HCV infection induces insulin resistance (IR) which has been reported in up to 70% of CHC infected patients [6]. Moreover, CHC has been shown to be associated with an 11.5 times higher risk of developing T2DM. [7] The mechanism involved in these extrahepatic manifestations is related to chronic inflammation induced by a HCV infection that disturbs the central role of the liver in glucose homeostasis, insulin resistance (IR), and impaired glucose tolerance [8].

Over the years scientific data showed that IR and diabetes were considered negative predictors of a response to interferon-based HCV regimens [9,10]. Moreover, Interferon-based HCV treatment was associated with weight loss in up to 90% of patients, although most patients regained their initial weight after end of treatment. [11]

However, the burden of CHC could be greatly reduced, given the ongoing development of highly effective DAAs, but also with the availability of effective non-invasive biomarkers for identifying patients with severe liver disease, who should be given priority for the use of DAAs. Moreover, nowadays liver biopsy is no longer used as a primary method of assessing the stage of liver disease as it has been replaced with non-invasive methods which facilitate patient monitoring.

Among the noninvasive alternatives to liver biopsy, it has been widely accepted the predictive value and a better benefit-to-risk ratio of five combinations of 10 serum biochemical markers in patients at risk of chronic liver diseases: FibroTest for the quantitative assessment of liver fibrosis; SteatoTest for the quantitative assessment of liver steatosis; ActiTest for the quantitative assessment of necroinflammatory activity in chronic viral hepatitis C and B; NashTest for the categorical diagnosis of nonalcoholic steatohepatitis; and AshTest for the quantitative assessment of alcoholic steatohepatitis.[12].

Using these methods, many studies concluded that all the above parameters seem to improve after antiviral treatment. [13]

Moreover, obtaining SVR after DAA treatment appears to be as effective in diabetic as well as in non-diabetic patients [14]. As they induce viral clearance, it seems that an improvement in the metabolism of glucose, but also in liver parameters has been observed even in patients with cirrhosis [15-

17]. However, a statistically significant improved outcome could not always be detected, and there is still a lack of prospective long-term studies which confirm a maintaining effect of SVR on IR over time [18-20].

In addition, studies conducted in patients who achieved SVR suggest substantial weight gain following successful DAA treatment. [11] Subsequent weight gain after DAA treatment may limit or even reverse the benefits gained from SVR achievement.

The mechanisms behind weight gain during DAA therapy are still being researched. However, possible mechanisms include modulating chronic inflammation, improvements in hepatic function, and improvements in quality of life after treatment [21-23]

Further research is necessary as weight gain in patients that attain SVR may predispose them to liver disease progression, including NAFLD, incident diabetes mellitus, and liver fibrosis evolution.

The aim of the present study was to assess the dynamics of hepatic parameters- fibrosis, necroinflammatory activity, steatosis and steatohepatitis evaluated by Fibromax and BMI kinetics in T2DM patients versus non diabetics one year after clearance of CHC infection achieved by treatment with DAAs.

## MATERIALS AND METHODS

We conducted a retrospective study on a cohort of 100 patients with CHC who underwent treatment with DAAs during September 2016-August 2019. The study took place at “Prof. Dr. Matei Bals” National Institute for Infectious Diseases, Bucharest, and was approved by the Local Ethical Committee, register no. 7440/19.09.2018. The patients included in the study met the following criteria: presence of HCV infection defined as positive HCV-RNA with various degrees of liver fibrosis and age over 18 years old. The exclusion criteria for the study were coinfection with other viruses - hepatitis B or HIV, chronic alcohol consumption and age under 18 years old. The therapeutic regimens administered were as follows: ombitasvir/paritaprevir/ritonavir (OBV/PTV/r) and dasabuvir (DSV), ledipasvir/ sofosbuvir (LDV/SOF) and sofosbuvir/velpatasvir, The antiviral treatment was administered as per protocol: OBV/PTV/r 12.5 mg/75 mg/50 mg, two pills once a day and DSV 250 mg twice a day; LDV/SOF 90 mg/400 mg once daily and SOF/VEL 400 mg/100 mg 1 pill daily. The duration of treatment was 12 weeks for all treatments. As part of the diagnostic protocol, the HCV genotype was determined in all patients as genotype 1b, the most common genotype in southeastern European countries. All patients were evaluated at initiation of treatment and 1 year after. Fibromax parameters and BMI were monitored.

Molecular determination of HCV-RNA was performed by real-time PCR using the automated instrument COBAS Amplicore (ROCHE diagnostics, Indianapolis, IN, USA). All patients achieved SVR defined as undetectable HCV-RNA 12 weeks after treatment completion.

The diagnosis of T2DM was made prior to the DAA treatment and was based on the glycated hemoglobin test (HbA1c) (above 6.5% for T2DM).

Fibromax is a non-invasive method of determining liver disease by evaluating 10 serum biomarkers. Five different sets of tests are included in Fibromax and are determined using Biopredictive tests: FibroTest for the quantitative assessment of fibrosis and it is staged F0-F4; SteatoTest measured the liver steatosis with a range of S0–3. The ActiTest was staged as grades A0–3 corresponding to the section of the scoring system assessing viral necroinflammatory activity in chronic viral hepatitis C and B; The NashTest evaluated the level of nonalcoholic steatohepatitis caused by the metabolic condition and was measured as N0–2. and AshTest for the quantitative assessment of alcoholic steatohepatitis with a scoring system H0-3. However we did not evaluate AshTest, as alcoholic patients were excluded from our study.

BMI was calculated taking into account sex, age, weight, and height of the subject. A BMI equal or higher than 30 kg/m<sup>2</sup> was considered obesity.

## RESULTS

A total of 100 patients were enrolled in the study, consisting of 43 males and 57 females. The study group was divided into 2 subgroups according to the presence of diabetes. Thus group 1 consisted of 42 non diabetic patients with CHC and group 2 was represented by 58 patients with CHC and T2DM.

The mean age at treatment initiation was 63 years, with a standard deviation of 9.1 years. Regardless of the presence or absence of T2DM all patients achieved SVR and no treatment side effects or drug interactions were reported.

Regardless of the presence or absence of T2DM at treatment initiation the study group had a median FibroTest score of 0,76 indicating an overall stage 4 liver fibrosis (e.g. cirrhosis). The staging of fibrosis at treatment initiation revealed the following distribution: 58.1% with F4 grade, 28% with F3 grade, 10,8% with F2 grade and 3,2% with F1 grade. One year after treatment initiation, the median FibroTest score had a statistically significant decrease, reaching a median score of 0,68, with the following distribution: 34.8% of patients with F4 grade, 34,8% with F3 grade, 21,7% with F2 grade, 7,6% with F1 grade, 1,1% with F0 grade,  $p < 0.001$  (Table 1).

The median ActiTest score at treatment initiation was 0,43 corresponding to A1-2 grade, and consisted

of 35,2% of patients with A3 grade, 6,8% with A2 grade, 33% with A1 grade, 25% with A0 grade, and one year after treatment initiation the median had a significant decrease, with a median score of 0.2 divided as it follows: 5,6% with A3 grade, 6,7% with A2 grade, 22,2% with A1 grade, 65,6% with A0 grade, with  $p < 0.001$ . (Table 1)

The median SteatoTest score was 0,48 corresponding to S1 grade at treatment initiation with the following distribution: 11,1% had S3 grade, 24,4% had S2 grade, 38,9% had S1 grade, 25,6% had S0 grade, whereas at one year after treatment we noticed a decrease in steatosis score, of 0.4 with the following distribution: 6,5% S3, 12% S2, 34,8% S1, 46,7% S0 ( $p < 0.001$ ). (Table 1)

At treatment initiation we obtained a NASHTest score of 0.5, corresponding to N1 grade with the following distribution: 39,4% of patients with N3 grade, 29,8% with N1 grade, 30,9% with N0 grade, whereas one year after treatment we can observe an overall decrease in NASHTest grade, although the overall median score was 0.5: 12,5% with N2 grade, 38,5% with N1 grade, 49% with N0 grade ( $p < 0.001$ ). (Table 1)

One year after study initiation all FibroMax tests showed a statistically significant decrease in all parameters, regardless of study group proving that achieving SVR after DAA improves significantly all liver tests. (Table 1)

**TABLE 1.** FibroMax variables at treatment initiation and one year after treatment initiation

	Treatment initiation	One year after treatment initiation	P value
FibroTest median score (IQR)	0.76 (0.64, 0.87)	0.68 (0.56, 0.78)	<0.001
ActiTest median score (IQR)	0.43 (0.28, 0.72)	0.2 (0.12, 0.37)	<0.001
SteatoTest median score (IQR)	0.48 (0.37, 0.67)	0.4 (0.27, 0.52)	<0.001
NASHTest median SCORE (IQR)	0.5 (0.25, 0.75)	0.5 (0.25, 0.5)	<0.001

Regarding BMI kinetics, if at treatment initiation 41% of patients had a BMI over 30 kg/m<sup>2</sup>, we noticed a decrease in the total number of obese patients to 31% at one year after treatment.

Out of the total of 58 patients with T2DM, 27 (46.6%) followed only a dietary regime, compared to 31 (53.4%) cases with antidiabetic treatment as follows: treatment with oral antidiabetics (OAD) - 13 cases (22.4% of all cases with diabetes, insulin treatment -16 (27.59% of all cases with diabetes) and OAD + insulin - 2 cases (3.45% of the total).

When we compared Fibromax parameters between non diabetic patients and the T2DM group we observed the following:

## FibroTest

Both groups had a median F4 grade, with a higher score in nondiabetic patients (0.77 versus 0.76,  $p = 0.892$ ) at treatment initiation, as shown in table 2. No statistically significant differences were observed. One year after initiation of treatment FibroTest scores decreased in both groups with reaching a median value of 0.7 in group 1 and 0.65 in group 2 respectively, ( $p=0.277$ ) (table 3). Nondiabetic patients had a lower median difference in FibroTest score of 0.04 when compared to T2DM patients which had a median difference score of 0.08, although not statistically significant ( $p=0.192$ ). (Table 4)

## ActiTest

At treatment initiation the Actitest median score was significantly higher in the T2DM patients than nondiabetics (0.52 versus 0.31,  $p=0.003$ ) (Table 2). One year after treatment initiation both groups had similar scores, of 0.2 ( $p=0.997$ ) (Table 3). However, patients with T2DM showed a more significant decrease in necroinflammatory score than nondiabetics, with a median decrease of 0.22 compared to 0.11 in nondiabetic patients,  $p=0.002$  (Table 4).

## SteatoTest

Patients with T2DM had a higher median score compared to non-diabetics (0.54 versus 0.39,  $p=0.002$ ) at treatment initiation (Table 2). One year after treatment diabetic patients maintained a significantly higher SteatoTest score (0.44 versus 0.33,  $p=0.009$ ). (Table 3)

Patients with T2DM also had a higher median decrease, 0.1 vs 0.04 in non-diabetic patients ( $p=0.098$ ). (Table 4)

**TABLE 2.** Study parameters at treatment initiation

Study parameters	Non-diabetic patients N=42	T2DM patients N=58	P value
Male gender (%)	16 (38.09%)	27 (46.55%)	0.399
Mean age $\pm$ standard deviation	63.9 $\pm$ 8.6	62.72 $\pm$ 9.5	0.525
Median FibroTest score, (IQR)	0.77 (0.61, 0.88)	0.76 (0.67, 0.86)	0.892
Median ActiTest score (IQR)	0.31 (0.21, 0.6)	0.52 (0.34, 0.74)	0.003
Median SteatoTest score(IQR)	0.39 (0.25, 0.53)	0.54 (0.44, 0.7)	0.002
Median NASHTest score (IQR)	0.42 (0.25, 0.75)	0.64 (0.5, 0.75)	<0.001

## NASHTest

Nashtest score was also significantly higher in T2DM patients when compared to nondiabetic patients 0.64 and 0.42 respectively,  $p < 0.001$  at treat-

ment initiation (Table 2). One year after treatment T2DM group maintained a significantly higher score (0.5 versus 0.25,  $p=0.002$ ) (Table 3). We did not obtain a significant difference between median differences ( $p=0.179$ ) (Table 4).

**TABLE 3.** Study parameters one year after treatment initiation

Study parameters	Non-diabetic patients N=42	T2DM patients N=58	P value
Mediana Scor Fibrotest, (IQR)	0.7 (0.57, 0.82)	0.65 (0.54, 0.75)	0.277
Mediana Scor ActiTest (IQR)	0.2 (0.11, 0.38)	0.2 (0.12, 0.35)	0.997
Mediana Scor Steatostest (IQR)	0.33 (0.25, 0.43)	0.44 (0.3, 0.58)	0.009
Mediana Scor NASH (IQR)	0.25 (0.25, 0.5)	0.5 (0.25, 0.5)	0.002

**TABLE 4.** FibroMax kinetics one year after treatment initiation in non-diabetic and T2DM patients

	Non-diabetic patients	T2DM patients	P value
Median difference in FibroTest score one year after treatment - treatment initiation (IQR)	-0.04 (-0.15, -0.02)	-0.08 (-0.16, -0.02)	0.192
Median difference in ActiTest score one year after treatment - treatment initiation (IQR)	-0.11 (-0.21, -0.02)	-0.22 (-0.44, -0.10)	0.002
Median difference in SteatoTest score one year after treatment - treatment initiation (IQR)	-0.04 (-0.21, 0.05)	-0.1 (-0.22, -0.02)	0.098
Median difference in NASHTest score one year after treatment - treatment initiation (IQR)	0 (-0.25, 0)	0 (-0.25, 0)	0.179

When we compared Fibromax categories across diabetic patients, we found no statistical significance between patients with and without antidiabetic treatment. However, patients with T2DM who were under antidiabetic treatment tend to have a more pronounced median decrease in all categories. (Table 5)

## BMI

We evaluated the kinetics of BMI between the 2 groups of patients and also the influence of obesity (defined as a BMI  $>30$  kg/m<sup>2</sup> according to international definitions) over FibroMax parameters in diabetic patients.

Mean BMI at study initiation was 28,78 kg/m<sup>2</sup> regardless of the presence or absence of T2DM. The mean BMI was higher in patients with T2DM (29,23 kg/m<sup>2</sup> versus 27.38kg/m<sup>2</sup>), with 51.7 % diabetic obese

**TABLE 5.** FibroMax parameters kinetics in non-diabetics and T2DM patients

	T2DM patients following diet	T2DM patients following antidiabetic treatment	P value
Difference in Median FibroTest score (IQR)	-0.06 (-0.16, -0.02)	-0.11 (-0.16, -0.04)	0.107
Difference in Median ActiTest score (IQR)	-0.18 (-0.42, -0.08)	-0.24 (-0.45, -0.1)	0.446
Difference in Median SteatoTest score (IQR)	-0.07 (-0.21, 0)	-0.13 (-0.23, -0.04)	0.250
Difference in Median NASHTest score (IQR)	0 (-0.25, 0)	-0.04 (-0.25, 0)	0.993

patients and 26.2% nondiabetic obese patients. We did not find a statistically significant variation between the two groups (p =0.224). The same has been observed at one year after treatment initiation, with the T2DM group having a higher mean BMI than nondiabetic patients (29 kg/m<sup>2</sup> vs 27.87kg/m<sup>2</sup>, p= 0.239), however in the nondiabetic group the number of obese patients increased, with 31% of patients having a BMI over 30 kg/m<sup>2</sup>, whereas in the diabetic group the percentage of obese patients decreased to 43.1% (Table 6).

Overall the mean BMI decreased in both groups with a higher decrease in patients with T2DM than nondiabetics (0,29 kg/ m<sup>2</sup> versus 0,22) but non statistically significant (p=0.411) (Table 6)

**TABLE 6.** BMI kinetics in non-diabetic patients and diabetic patients one year after treatment initiation

	Non-diabetic patients	T2DM patients	P value
Mean BMI (kg/m <sup>2</sup> )± standard deviation at treatment initiation	27.38 ± 2.66	29.23 ± 4.53	0.224
Obese patients (%) at treatment initiation	26.2 %	51.7%	
Mean BMI (kg/m <sup>2</sup> ) ± standard deviation one year after treatment	27.87 ± 4.52	29 ± 4.79	0.239
Obese patients (%) one year after treatment	31%	43.1%	
BMI mean difference (kg/m <sup>2</sup> )at treatment initiation – one year after treatment initiation	0.22	0.29	0.411

Moreover T2DM patients with obesity behave differently than nonobese patients with T2DM when we evaluated the kinetics of FibroMax parameters at one year after treatment initiation. In the obese patients group we found that the median difference

of FibroTest decreased less than in nonobese patients, but not statistically significant, however the median difference for Actitest, steatostest, nashtest was higher in obese patients than nonobese patients with T2DM.

Furthermore the difference in median Actitest score was significantly higher in T2DM patients with obesity that non obese patients (p=0.016). (Table 7)

**TABLE 7.** Median difference of FibroMax parameters in T2DM patients at treatment initiation versus one year after treatment initiation

	T2DM patients without obesity one year after treatment	T2DM patients with obesity one year after treatment	P value
Median difference in FibroTest score (IQR)	-0.05 (-0.15, -0.02)	-0.07 (-0.17, -0.03)	0.228
Median difference in ActiTest score (IQR)	-0.12 (-0.3,-0.05)	-0.33 (-0.44, -0.12)	0.016
Median difference in SteatoTest score (IQR)	-0.06 (-0.23, 0.02)	-0.13 (-0.19, -0.04)	0.182
Median difference in NASHTest score (IQR)	0 (-0.25, 0)	0-0.04 (-0.25, 0)	0.597

If we take into account all patients from the study, we observe that one year after treatment initiation SteatoTest and NashTest positively correlate with BMI (p <0.001 in both cases), however FibroTest and ActiTest do not. (Table 8)

**TABLE 8.** Correlations between BMI and FibroMax tests one year after treatment initiation

	BMI one year after treatment initiation	
	Pearson’s correlation	P value
FibroTest score one year after treatment initiation	-0.070	0.508
ActiTest score one year after treatment initiation	-0.008	0.937
SteatoTest score one year after treatment initiation	0.579	<0.001
NASHTest score one year after treatment initiation	0.439	<0.001

## DISCUSSIONS

It has been well documented that HCV plays an important role in the evolution of metabolic dysregulations, and is particularly associated with insulin resistance. CHC infection represents a risk factor for the onset of T2DM and patients with insulin resistance (IR) and HCV tend to have significantly worse clinical outcomes. Moreover, T2DM was associated with increased liver fibrosis, higher rates of HCC,

and a reduced response to antiviral therapy especially in the era of interferon based treatment [24]. These data underlie the two-way association between insulin resistance and CHC infection. Moreover obtaining SVR by DAAs led to an important reduction in the risk of HCC. [25]

## BMI

Weight gain after SVR can negatively impact liver fibrosis. DAAs have been associated with an increase in BMI among patients who achieved SVR [26,27]. The pathophysiologic causes are not yet fully explained. It has been proposed that it may be the consequence of metabolic disorders during treatment or that a better quality of life following viral eradication may imply a less restrictive diet and, implicitly leading to weight gain. In their study, Shousha et al. evaluated the correlation between DAA treatment and an increase in BMI after achieving SVR. The results revealed an increase in BMI in more than a half of patients who obtained SVR [26].

As reported in this study, the lowest BMI was found in patients with low degree of hepatic steatosis, whereas patients with more profound steatosis had a higher BMI. These results are consistent with other studies that report BMI as a factor closely associated with hepatic steatosis [28,29,30]. Moreover, in terms of hepatic fibrosis, Ortiz et al. highlighted that a BMI over 30 kg/m<sup>2</sup> was a predictive parameter for fibrosis progression.[31].

Contrary to other studies our data showed that one year after treatment initiation the percentage of T2DM obese patients decreased from 51.7% to 43.1%. However, in the nondiabetic group the number of obese patients increased from 26.2% to 31% of patients having a BMI over 30, which is similar to other studies.

Schlevogt et al. evaluated the kinetics of BMI in 264 patients with HCV cirrhosis who received DAAs and concluded that 6 months after initiation of therapy there was an increase in BMI in 35% of the cases, moreover, at one year BMI increased in 41% of the patients enrolled in the study when compared to baseline. [37].

The study by Do et al. evaluated 11,469 patients in which weight gain occurred in 52.6% of patients and excessive weight gain occurred in 19.8% of patients. Other positive predictors of weight gain included advanced fibrosis, advanced hepatic steatosis and whether patients were obese at treatment initiation. [38]

## FIBROTEST

One year after treatment initiation both in patients with T2DM and in those without T2DM, significant improvements were observed in the degree of liver fibrosis. Comparing the 2 groups of patients, we note that the degree of liver fibrosis decreased

significantly in the case of both categories of patients; if at the beginning of the study more than 50% of both groups of patients had liver fibrosis grade 4, at one year the percentage of patients with F4 reached around 30%. Moreover, if at the beginning of the study less than 20% of patients without DM and a little over 10% of patients with T2DM fell into the F0-F2 range, one year after treatment initiation approximately 30% of patients without DM and 30% of those with DM fell into this range. Although in both groups of patients a significant decrease in the degree of liver fibrosis is observed at one year after treatment initiation, the study data show that in the case of patients with T2DM, the improvement of fibrosis is observed relatively frequently in the case of patients with a higher degree of fibrosis (F3-F4) while in the case of patients without T2DM, the improvement was uniform regardless of the degree of fibrosis in which the patients were initially classified.

Although our study did not show a statistically significant correlation between fibrosis decrease in T2DM patients and nonT2DM patients ( $p=0.192$ ), these findings coincide with other studies that demonstrated IR and /or T2DM to be an independent predictor of fibrosis in HCV infection [32]. The study of Hsu et al. found a strong association between the severity of fibrosis and diabetes [33]. Even if their findings are uniform, however, some studies failed to show this correlation. Overall, the majority of studies support the notion that diabetes and IR play an important role in liver fibrosis, but also, advanced liver fibrosis worsens the outcome of diabetic patients, thus proving the two-way interaction between HCV and T2DM.

## ACTITEST

The second monitored parameter and probably the most evocative was represented by liver necro-inflammatory activity. This parameter improved significantly both in the case of patients with T2DM and in the case of those without T2DM, one year after treatment initiation compared to the time of enrollment in the study. If at the beginning of the study the majority of patients in the group without diabetes presented A0 grade and the majority of those in the group with T2DM presented A3 grade, one year after treatment the distribution of the patients in the two groups became superimposed with over 75% of the patients with or without T2DM presenting A0 grade.

Our study shows a statistically significant median difference in actitest scores between diabetic and nondiabetic patients, with a higher decrease in actitest score in T2DM patients, proving that diabetes enhances the inflammation in HCV infection.

As emphasized by other studies conducted, the main reason the necroinflammatory activity score

was higher in the T2DM group seems to be the fact that the liver associated lesions generated by the CHC infection and diabetes are not only cumulative but also enhance each other [34]. This theory is further augmented by the fact that after HCV clearance the decrease in necroinflammatory activity was more prominent in the T2DM group. One of the arguments of this theory is the fact that patients with T2DM tend to have a higher prevalence of steatosis as studies showed that HCV replication is higher in patients with steatosis[35].

### STEATOTEST and NASHTEST

Both in the case of patients without T2DM and in the case of those who associated T2DM one year after treatment initiation, a significant decrease in the prevalence of hepatic steatosis was observed. At the time of initiation of the study, the prevalence of hepatic steatosis was clearly higher in the group of patients with T2DM, compared to patients without T2DM (86.3% vs. 50%). This ratio tends to be maintained even one year after initiation of DAAs, at which time 27.28% of the patients without T2DM had hepatic steatosis grade 1, 2 or 3 and 57.18% of the patients with T2DM still presented various degrees of hepatic steatosis.

It has been proven that HCV replication is higher in patients with hepatic steatosis. Concomitantly other studies showed that CHC infection favors the onset of liver steatosis, however the mechanisms behind this link are not yet fully elucidated but one thing is clear, patients with CHC infection have glucose and lipid metabolism alterations which influence liver parameters [36].

### CONCLUSIONS

HCV infection is now curable with DAAs, but it remains unclear whether hepatitis C extrahepatic sequelae are fully reversible after viral clearance.

The data from our study shows a significant decrease in liver parameters assessed through non-invasive measurements, after DAA induced SVR. Regarding liver fibrosis, an improvement is observed relatively frequently in the case of patients with a higher degree of fibrosis (F3-F4) while in the case of patients without DM, the improvement of fibrosis was uniform.

Moreover, there was a significant difference in ActiTest score between the diabetic and non-diabetic patients, with a higher decrease in T2DM patients, proving that diabetes enhances the inflammation in HCV infection. In terms of weight gain, our study showed that diabetic patients tend to lose weight, at least one year after treatment initiation.

In theory an early therapeutic approach not only allows for many of the extrahepatic manifestations that are still in a reversible stage of the disease to be cured, it can also prevent a significant portion of the extrahepatic diseases that develop due to delayed treatment.

However, whether these beneficial effects are long lasting or just temporary is still under investigation. Future studies should focus on longer follow-up to elucidate the long-term effects and metabolic outcomes in patients achieving viral clearance, in order to prevent them or tailor a personalized treatment.

*Conflict of interest:* the authors declare they do not have any financial or personal relationships that might bias the content of this work

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