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Improvement of carotid atherosclerosis and peripheral artery disease after hepatitis C virus eradication by direct-acting antivirals

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Abstract

Introduction: Recent research points to a link between chronic hepatitis C virus (HCV) infection and cardiovascular disease, especially carotid atherosclerosis, and suggests that HCV clearance may impact cardiovascular outcomes. *Aim*: To determine if viral eradication by the new oral direct-acting antiviral (DAA) agents has benefit regarding carotid atherosclerosis, peripheral artery disease (PAD), steatosis, and liver fibrosis. *Patients, Materials and Methods*: We conducted a prospective study on 168 patients diagnosed with chronic HCV infection or HCV-related cirrhosis. They were all treated with DAAs, with sustained virological response (SVR). Laboratory data, vibration-controlled transient elastography (VCTE), carotid *intima–media* thickness (IMT) measurement, and ankle–brachial index (ABI) were recorded in all patients. *Results*: We found an average IMT of 1.22±0.2 mm, with a variance range from 1.14±0.19 mm in the mild and moderate fibrosis (≤F2) group to 1.29±0.25 mm in the severe fibrosis (≥F3) group. Also, patients with severe fibrosis (≥F3) present a more critical decrease of IMT values, with the carotid thickness affecting only 18.2% of individuals in the follow-up period. At the baseline, the best values of ABI were recorded in patients having F1–F2 fibrosis stage (mean value 1.02±0.19). Instead, in the group with severe fibrosis, the average value of ABI was lower (0.91±0.16) at the baseline, with a significant increase at SVR evaluation (p<0.001). *Conclusions*: Our research highlights the beneficial effect of viral eradication on both carotid atherosclerosis and PAD, especially in those with advanced fibrosis and cirrhosis.

Keywords: chronic hepatitis C virus infection, sustained virological response, direct-acting antivirals therapy, atherosclerosis, *intima–media* thickness, ankle–brachial index.

Introduction

Hepatitis C virus (HCV) chronic infection continues to cast a shadow on global public health, affecting approximatively 71 million individuals across the planet, with million cases of new infections emerging annually, standing as a leading contributor to severe liver conditions – including cirrhosis and hepatocellular carcinoma, and to mortality [1]. Owing to its remarkable rate of persistence and chronicity, HCV contributes to various metabolic abnormalities, encompassing a wide spectrum of health issues, ranging from metabolic syndrome, obesity, and abnormal lipid levels to diabetes and an impaired response to insulin, which are major risk factors for atherosclerotic processes [2]. Slow progress and inapparent or discrete clinical features of chronic HCV infection may lead to a delayed diagnosis, in an advanced liver disease stage in many patients. For a long time, HCV infection has been associated with many extrahepatic manifestations including cardiovascular, renal, mixed cryoglobulinemia, diabetes mellitus [3, 4]. The supplementary burden caused by HCV multisystemic damage increased the need of an effective treatment. The revelation in antiviral treatment for HCV started with the new oral direct-acting antiviral (DAA) drugs, administered once daily, and very well tolerated, which managed to achieve >95% sustained virological response (SVR) rates after only eight to 12 weeks of treatment [5].

Additionally, new evidence points to a connection between cardiovascular changes and HCV infection. HCV infection has been linked to an increased incidence of myocardial damage [6], peripheral artery disease (PAD) [7], strokes and cardiovascular accidents [8, 9], and cerebro-/cardiovascular-associated mortality, as well as carotid and

coronary atherosclerosis [10, 11]. Contradictory findings, however, have also been recorded. This link is supported by associative data, theoretical conjectures, and equivocal experimental evidence [12]. Even in the face of significant variability, a recent meta-analysis found that HCV infection had a detrimental effect on cardiovascular changes and mortality [13]. Moreover, Mahale *et al.*, in a study which included 160 875 HCV-infected veterans, of whom 33.9% achieved SVR based on interferon (IFN) regimen, reported the impact of HCV clearance on extrahepatic symptoms. In their research, which is the largest study concerning the relationship between HCV eradication and IFN regimen, the authors found that patients who received IFN regimen (with or without SVR) had a lower risk of stroke compared with those untreated and the antiviral therapy had no effect for the risk of coronary artery disease [14]. The results that are currently available regarding the effect of SVR on cardiometabolic outcomes and the severity of liver damage are highly disputed. They range from the absence of any effect on the liver fibrosis to a beneficial effect on cardio-metabolic prognosis, as Mahale *et al.* reported [14], to a more noticeable effect in those patients having mild disease or in those being in a stage of with advanced fibrosis or even cirrhosis [15–17]. The discrepant findings could be explained by variations in baseline patient characteristics, individual metabolic and cardiac risk factors, follow-up period, and severity assessments of the liver condition [12]. These findings suggest that further research is still needed to fully understand this potential link.

Vibration-controlled transient elastography (VCTE) with controlled attenuation parameter (CAP) is a non-invasive technique for assessing hepatic fibrosis and steatosis. When compared to liver biopsy, this method has the advantages of being quick, painless, and simple to use. Its high repeatability and reproducibility are also major benefits of this technique, which was implemented in clinical practice in the last two decades [18, 19]. Also, CAP uses the same ultrasonic probe and measures the ultrasound (US) attenuation at a frequency of, usually, 3.5 MHz, and it is performed at the same time with liver stiffness measurements (LSM) [20]. The research is lacking and contradictory when it comes to steatosis, despite some recent trials finding a significant decrease in liver fibrosis following DAA treatment [21–23].

The topic of whether HCV eradication can also improve cardiovascular outcomes was brought up by the availability of secure and efficient DAA-based regimens. Most of the data are drawn from populations of patients who were eligible for and treated with IFN-based therapies [11, 24–26]. The available evidence indicates HCV eradication lowers the risk for cardiovascular events; however, but the different studies methodology makes it impossible to determine if this decrease is caused by the virus eradication, by IFN, or by patient selection and other types of bias.

Aim

This study assessed the modifications of atherosclerosis using *intima–media* thickness (IMT) and ankle–brachial index (ABI) as surrogates' markers in individuals chronically HCV-infected who have obtained SVR, as assessed 12 weeks after the end of treatment.

Patients, Materials and Methods

Patients

One hundred sixty-eight consecutive patients with chronic HCV infection (hepatitis or compensated cirrhosis) were prospectively recruited at a tertiary center in Romania between January 2017 to March 2022. Patients were included if they met all the following criteria: (*1*) chronic hepatitis C/ HCV-related compensated cirrhosis; (*2*) failure to previous antiviral therapy or treatment-naïve; (*3*) detectable viral load. The criteria of exclusion were: (*1*) advanced stage of cirrhosis (defined as Child–Pugh class B or C); (*2*) other etiology of liver condition, than HCV infection; (*3*) alcohol intake >20 g/day in the last years; (*4*) previous history of heart or cerebral disease; (*5*) human immunodeficiency virus (HIV) infection; (*6*) hepatocarcinoma; (*7*) psychiatric disease; (*8*) contraindication to DAAs therapy.

Liver stiffness was measured by VCTE, and patients were stratified in two sub-groups: mild and moderate fibrosis (≤9.5 kPa) and advanced fibrosis and cirrhosis (>9.6 kPa and >12.5 kPa). All patients were evaluated at baseline, at SVR and during a follow-up period up to six months after SVR.

The study was performed according to the Declaration of Helsinki, and it was approved by the Ethics Committee. All patients signed an informed consent.

Clinical and laboratory assessment

All the patients had a comprehensive clinical exam, biological tests, as well as a VCTE evaluation during the same baseline visit. Data on gender, age, regular alcohol and smoking consumption, body mass index (BMI), diabetes type of treatment, and blood pressure were assessed. Blood indicators included hemoglobin, serum fibrinogen, the international normalized ratio (INR), serum glucose, glycosylated hemoglobin (HbA1c), alkaline phosphatase (ALP), serum albumin, serum bilirubin, total serum proteins and albumin, serum urea and creatinine, serum cholesterol and triglycerides levels, as well as low-density lipoprotein (LDL)-cholesterol. BMI was calculated by dividing the weight (expressed in kilos) by the square of height (expressed in meters). Overweight was defined as a BMI between 25–30 kg/m^2, and obesity as a BMI above. All patients underwent DAA therapy, being treated according to the national health protocol with the available therapeutic regimens (Sofosbuvir/Ledipasvir and Paritaprevir/Ritonavir/ Ombitasvir + Dasabuvir) for 12 weeks. SVR was evaluated 12 weeks after the end of the antiviral treatment.

VCTE examination

A trained, experienced physician, having operated more than 300 VCTE assessments evaluated patients included in our investigation for hepatic fibrosis and liver steatosis using the FibroScan® 520 (Echosens, Paris, France) supplied with two probes, M- (normal) and XL- (obesity). Patients were evaluated at least four hours after meal, with the right arm being positioned at maximum abduction. This increased the intercostal approach window for the right liver lobe. If between the liver capsule and the teguments the space was more than 25 mm, the XL- (obesity) probe

(corresponding to a frequency of 2.5 MHz) was suggested and utilized. The assessment was initially conducted with the standard M-probe (whose transducer has an intrinsic frequency of 3.5 MHz). A reliable measurement consisted of 10 acquisitions with an interquartile range divided by the median (IQR/M) of maximum 30% [19]. At baseline and SVR12, the LSM and CAP measurements were carried out. The steatosis grading, according to CAP measurements (having as global range the interval 100–400 dB-mW), had the following cut-offs: S1 (mild) >248 dB/m, S2 (moderate) ≥268 dB/m, and S3 (severe) ≥280 dB/m, respectively [24]. The LSM classification was made according to the following cut-off values: F1 (mild fibrosis) >5.6 kPa, F2 (significant fibrosis) >7.1 kPa, F3 (advanced fibrosis) >9.5 kPa, F4 (cirrhosis) >12.5 kPA [18].

Carotid atherosclerosis evaluation

IMT was measured at the baseline and at 6–12 months after achieving SVR, with a high-resolution B-mode US equipment, using a linear multifrequency probe. The examination encompassed longitudinal views of the carotid arteries on both sides, precisely at the site of the common carotid artery, bulb and internal carotid (Figure 1). The technique involved three measurements in each patient. Carotid thickening was characterized as having an IMT equal to or greater than 1 mm (Figure 2). A carotid plaque was identified as a localized thickening measuring at least 1.5 mm at the common carotid artery level [25]. The IMT was evaluated in according with the clinical importance. An elevated IMT is a negative factor for subsequent coronary cardiac events and stroke. The IMT measurement can also provide additional prognostic information to the conventional risk factors for heart disease. The presence of atherosclerotic plaques and an IMT equal to or more than 1 mm has been linked to an increased risk for cardiovascular accidents [26] (Figure 3).

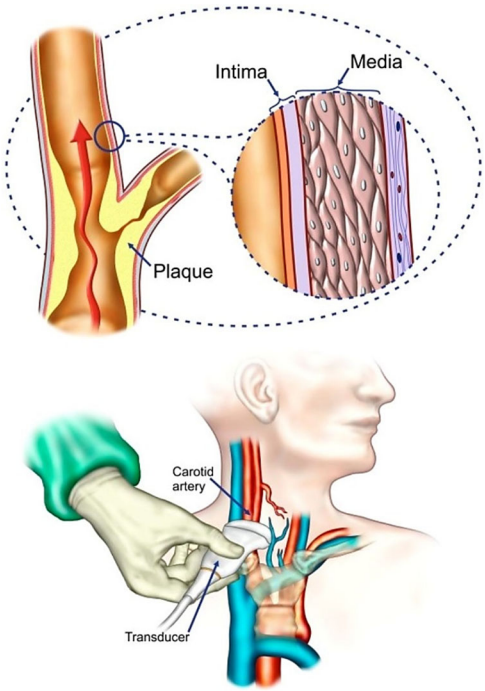

Figure 1 – ***The atherosclerotic plaque and ultrasound measurement.***

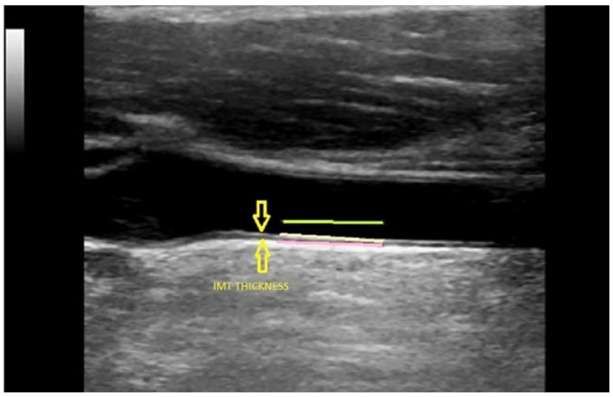

Figure 2 – ***Assessment of carotid IMT. Measurement of the IMT of the CCA by high-resolution B-mode ultrasonography. IMT was measured by an automatic function algorithm as represented by the yellow and pink lines (the green line in the lumen of the CCA represents the reference value for the arterial wall echo gradient calculations). The yellow arrows fence the IMT thickness. CCA: Common carotid artery; IMT: Intima–media thickness.***

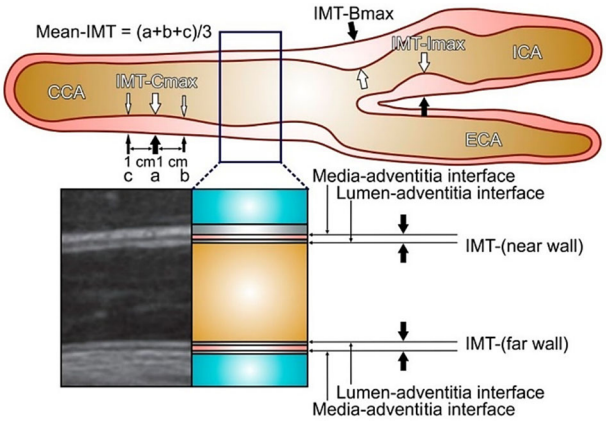

Figure 3 – ***The calculation of IMT. CCA: Common carotid artery; ECA: External carotid artery; ICA: Internal carotid artery; IMT: Intima–media thickness.***

Ankle–brachial index measurement

The patient is placed in the supine posture for 10 minutes before assessing the systolic blood pressure in both (right and left) brachial arteries, *dorsalis pedis*, and postero-tibial arteries. A portable 5- or 10-mHz Doppler device is used to record the systolic pressures. Typically, an ankle blood pressure cuff can be used. The blood pressure cuff should be the right size for the patient's lower calf to achieve the most reliable results, much like with arm pressures (immediately above the ankle). Due to the possibility that the blood pressure may fluctuate throughout the examination, we started at the level of the right arm, followed by the right leg, left leg, followed by the left arm. ABI was determined for each leg. The higher of the two artery pressures at the ankle was calculated as the ABI value and was divided by the systolic pressure of the brachial artery. For the ABI calculation, the most elevated between the two values of brachial systolic pressures was utilized [27].

Statistical analysis

Analysis was done utilizing Statistical Package for the Social Sciences (SPSS) version 24.0. Continuous variables

that followed a normal distribution were presented as the mean with standard deviation (SD), whereas categorical variables were presented as absolute values and percentages. To compare categorical data, the χ^2 (*chi*-squared) test was applied, and for quantitative variables with a normal distribution, the Student's *t*-test was utilized. To ascertain if the data distributions were normal, we used nonparametric procedures for normal data, as the Kolmogorov–Smirnov and the Mann–Whitney *U*-tests. To ascertain the statistical significance of the results, a *p*-value of 0.05 was utilized.

Results

The study cohort consisted of 168 patients, 54.7% of whom were females. The average BMI was 26.78±3.07 kg/m^2, and the average age was 58.14 years, with a SD of 8.8 years. Among all patients, more than half (55.3%) presented arterial hypertension, and approximately one out of five (22%) individuals had obesity according to BMI values. Furthermore, 26.1% of people had dyslipidemia, 25.6% of people had an IMT more than (≥1.5 mm), which is associated with the atherosclerotic plaques, and 31 (18.4%) of individuals had an ABI ≤0.90, a common marker for PAD. According to VCTE examinations, 86 (51.3%) of patients had lower stages of liver fibrosis (F0 and F1), and approximately one third (34.5%) of the individuals had at least advanced fibrosis (≥F3). Regarding CAP measurements, there were approximately two-thirds (63.1%) of the patients with liver steatosis, with a mean CAP value of 243.68±44.66 dB/m (Table 1).

Table 1 – *Baseline characteristics of studied population*

Baseline characteristics	Overall cohort (*n*=168)
Age [years]	58.14±8.8
Females, *n* (%)	92 (54.7)
Weight [kg]	76.23±15.26
Height [cm]	166.8±9.32
BMI [kg/m^2]	26.78±3.07
Underweight, *n* (%)	7 (4.2)
Lean subjects, *n* (%)	63 (37.5)
Overweight, *n* (%)	61 (36.3)
Obesity, *n* (%)	31 (22)
Hypertension, *n* (%)	93 (55.3)
Dyslipidemia, *n* (%)	44 (26.1)
IMT ≥1.5 mm, *n* (%)	43 (25.6)
ABI ≤0.90, *n* (%)	31 (18.4)
Fibrosis stage, *n* (%)	
LSM <5.6 kPa, *n* (%)	29 (17.3)
LSM ≥5.6 kPa, *n* (%)	57 (34)
LSM ≥7.1 kPa, *n* (%)	24 (14.2)
LSM ≥9.5 kPa, *n* (%)	36 (21.4)
LSM ≥12.5 kPa, *n* (%)	22 (13.1)
LSM [kPa]	8.68±5.02
Steatosis degree, *n* (%)	
CAP <248 dB/m	62 (36.9)
CAP ≥248 dB/m	46 (30.4)
CAP ≥268 dB/m	34 (20.2)
CAP ≥280 dB/m	21 (12.5)
CAP [dB/m]	243.68±44.66

ABI: Ankle–brachial index; BMI: Body mass index; CAP: Controlled attenuation parameter; IMT: *Intima–media* thickness; LSM: Liver stiffness measurement; *n*: No. of patients.

In terms of comparing the laboratory changes before treatment and, respectively, after the DAA treatment, a non-significant drop of fasting serum glucose (p=0.068), high-density lipoprotein (HDL)-cholesterol (p=0.186), and total bilirubin (p=0.548) values was noticed, with significant rise of triglycerides levels (p=0.046), and gamma-glutamyl transferase (GGT) (p=0.044). Overall, alanine transaminase (ALT) and aspartate transaminase (AST) levels decreased, but not by a statistically significant amount [for ALT, 40.8±23.61 IU/L *vs.* 36.84±23.18 IU/L (p=0.105), and for AST 34.83±20.28 IU/L *vs.* 30.11±21.43 IU/L (p=0.092)]. Moreover, we noticed a substantial decline in LSM values (p=0.018) and CAP score when assessing the parameters acquired by VCTE (p<0.001). In addition, regarding cardiovascular parameters, there was a significant decrease (p<0.001) of IMT values, and an important increase of the ABI levels (p<0.001) (Table 2).

Table 2 – *Individuals' laboratory data pre- and post-DAAs regimens*

Variable	Pre-treatment	Post-treatment	*p*-value
HGB [g/dL]	13.08±1.54	13.11±1.41	0.682
Platelet count [G/L]	195.23±65.08	202.67±64.22	0.743
Total bilirubin [mg/dL]	1.16±0.59	1.11±0.52	0.548
AST [IU/L]	34.83±20.28	30.11±21.43	0.092
ALT [IU/L]	40.8±23.61	36.84±23.18	0.105
GGT [IU/L]	103.52±64.5	71.32±26.6	0.044
Albumin [mg/dL]	3.98±0.78	4.06±0.62	0.062
Glucose [mg/dL]	115.08±34.9	109.26±29.43	0.068
Total cholesterol [mg/dL]	183.24±33.78	187.28±39.46	0.054
Triglycerides [mg/dL]	143.7±43.54	152.61±51.28	0.046
LDL [mg/dL]	122.35±38.8	127.62±47.7	0.077
HDL [mg/dL]	42.6±12.4	41.58±12.7	0.186
IMT [mm]	1.22±0.2	1.13±0.17	<0.001
ABI	0.97±0.18	1.06±0.22	<0.001
LSM [kPa]	8.68±5.02	7.81±4.72	0.018
CAP [dB/m]	243.68±44.66	264±59.78	<0.001

ABI: Ankle–brachial index; ALT: Alanine transaminase; AST: Aspartate transaminase; CAP: Controlled attenuation parameter; DAA: Direct-acting antiviral; GGT: Gamma-glutamyl transferase; HDL: High-density lipoprotein cholesterol; HGB: Total hemoglobin; IMT: *Intima–media* thickness; LDL: Low-density lipoprotein cholesterol; LSM: Liver stiffness measurements.

Even though abnormal levels are not necessarily connected with the presence of this vascular degenerative process, IMT is a crucial marker for the evaluation of subclinical atherosclerosis. A higher IMT can also be observed in several non-atherosclerotic processes that affect the circumference of the vascular lumen, the tension in the arterial wall, or the adaptive response of the arteries to blood flow. It was deemed crucial to assess the study participants' cardiovascular risk factors as well as indirect indicators of atherosclerosis. The obtained results show an average IMT of 1.22±0.2 mm, with a variance range from 1.14±0.19 mm in mild and moderate fibrosis (≤F2) group to 1.29±0.25 mm in severe fibrosis (≥F3) group. Moreover, we identified a higher number of individuals (55.1%) with carotid thickness in the group with advanced fibrosis and cirrhosis before treatment, compared to those with F0–F2 fibrosis (41.8%). Also, patients with severe fibrosis (≥F3) present a more important decrease of IMT values, the carotid thickness affecting only 18.2%

of individuals in the follow-up period. Instead, in the group with F0–F2 fibrosis the decrease of IMT values was not so important, at the post-SVR evaluation 35.4% of the patients had carotid thickening. Regarding the number of carotid plaques, our results revealed that 74.4% of the investigated patients did not present atherosclerosis plaques in the examined carotid segment at the baseline. It is noteworthy to highlight that the degree of liver fibrosis had an impact on the quantity of atheroma plaques. In the F1–F2 fibrosis group, 23 (20.9%) patients were identified with atheroma plaques, in contrast to individuals with advanced fibrosis and cirrhosis, where the examination found that 20 (34.4%) individuals had atheroma plaques. Moreover, a significant decrease of the number of patients with carotid plaques appeared after DAAs regimen, only 10 (17.2%) individuals with severe fibrosis (≥F3) had carotid plaques at post-SVR evaluation (Table 3).

Table 3 – ***Modifications from baseline to follow-up in IMT and in the prevalence of carotid thickening (IMT≥1 mm) and carotid plaques according to each visit for evaluation***

		Patients, *n* (%)	IMT [mm]	IMT ≥1 mm, *n* (%)	Carotid plaques, *n* (%)
Baseline	Patients, *n* (%)	168	1.22±0.2	78 (46.4%)	43 (25.6%)
	≤F2	110 (65.5%)	1.14±0.19	46 (41.8%)	23 (20.9%)
	≥F3	58 (34.5%)	1.29±0.25	32 (55.1%)	20 (34.4%)
SVR	Patients, *n* (%)	168	1.13±0.17	67 (39.9%)	31 (18.4%)
	≤F2	110 (65.5%)	1.10±0.16	42 (38.2%)	18 (16.3%)
	≥F3	58 (34.5%)	1.16±0.18	25 (43.1%)	13 (22.4%)
Post-SVR evaluation (six months after SVR)	Patients, *n* (%)	168	1.11±0.16	59 (35.1%)	27 (16.1%)
	≤F2	110 (65.5%)	1.07±0.14	39 (35.4%)	17 (15.5%)
	≥F3	58 (34.5%)	1.13±0.18	20 (18.2%)	10 (17.2%)

F2, F3: Fibrosis stages; IMT: *Intima–media* thickness; *n*: No. of patients; SVR: Sustained virological response.

Based on the ubiquity of the atherosclerotic process, in our study, ABI was calculated during each evaluation to identify the influence of SVR on peripheral arterial damage. The values obtained were expressed as mean ± SD. Later, correlations were made between IMT and ABI values, useful in highlighting the existence of subclinical atherosclerosis changes in the HCV population in the study. The results obtained showed an average value recorded at the level of the entire study group of 0.97±0.18. The mean values obtained from the study group indicate that the analyzed population falls within the normal range for ABI. However, when analyzing the results in relation to the extent of liver fibrosis, notable distinctions become apparent. At baseline, best values of ABI were recorded in individuals with F1–F2 stage of fibrosis, having a mean ABI value of 1.02±0.19. Instead, in the group with severe fibrosis, an average value of ABI was lower (0.91±0.16) at the baseline, with a significant increase at SVR evaluation (p<0.001). The results from the moment of SVR evaluation show us that, for the responders, the average value of IMT was 1.06±0.22, which suggests a significant increase compared to the previous evaluation (p<0.001). Moreover, this increase was observed at the follow-up period too, but without a significant change between SVR and post-SVR evaluation (p=0.139). When examining the outcomes in the context of fibrosis severity, it appears that individuals with severe fibrosis (>F3) show a marginal enhancement in their values from post-SVR evaluation, from 1.01±0.17 to 1.04±0.16, with a statistically significant p-value (p=0.038) (Table 4).

Table 4 – ***Changes from baseline to follow-up in ABI measurements to each point of evaluation***

		Patients, *n* (%)	ABI	*p*-value
Baseline	Patients, *n* (%)	168	0.97±0.18	
	≤F2	110 (65.5%)	1.02±0.19	
	≥F3	58 (34.5%)	0.91±0.16	
SVR	Patients, *n* (%)	168	1.06±0.22	<0.001
	≤F2	110 (65.5%)	1.10±0.2	<0.001
	≥F3	58 (34.5%)	1.01±0.17	<0.001
Post-SVR evaluation (six months after SVR)	Patients, *n* (%)	168	1.08±0.19	0.139
	≤F2	110 (65.5%)	1.13±0.17	0.234
	≥F3	58 (34.5%)	1.04±0.16	0.038

ABI: Ankle–brachial index; F2, F3: Fibrosis stages; *n*: No. of patients; SVR: Sustained virological response.

At the post-SVR evaluation, we discovered a strong association between the CAP score and clinical indicators of atherosclerosis, as IMT (r=0.061, p<0.001) or ABI (r=0.057, p<0.001) (Figure 4, A and B). Additionally, we found a strong association between the LSM at post-SVR evaluation and IMT values (r=0.84, p<0.001), and ABI levels, respectively (r=0.064, p<0.001) (Figure 5, A and B).

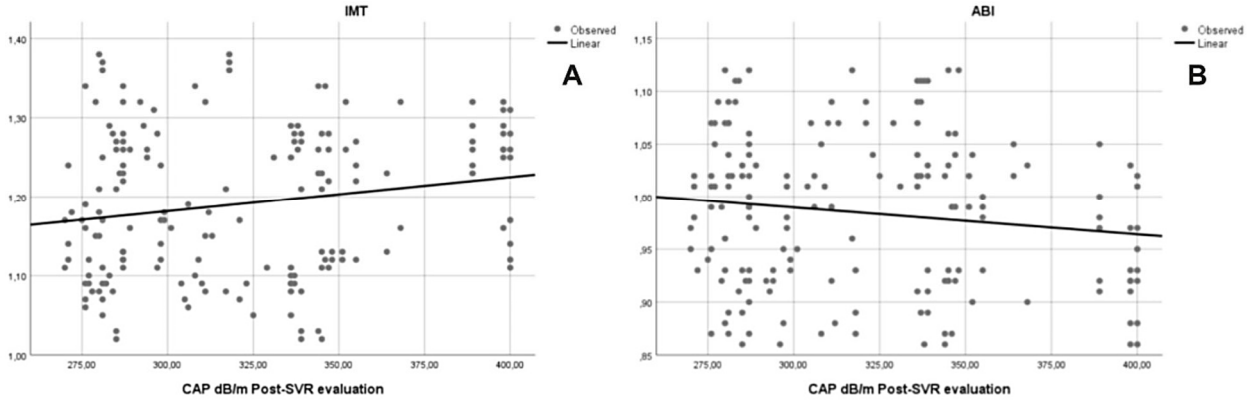

Figure 4 – ***Correlation between CAP score post-SVR evaluation and IMT (A), ABI (B). ABI: Ankle–brachial index; CAP: Controlled attenuation parameter; IMT: Intima–media thickness; SVR: Sustained virological response.***

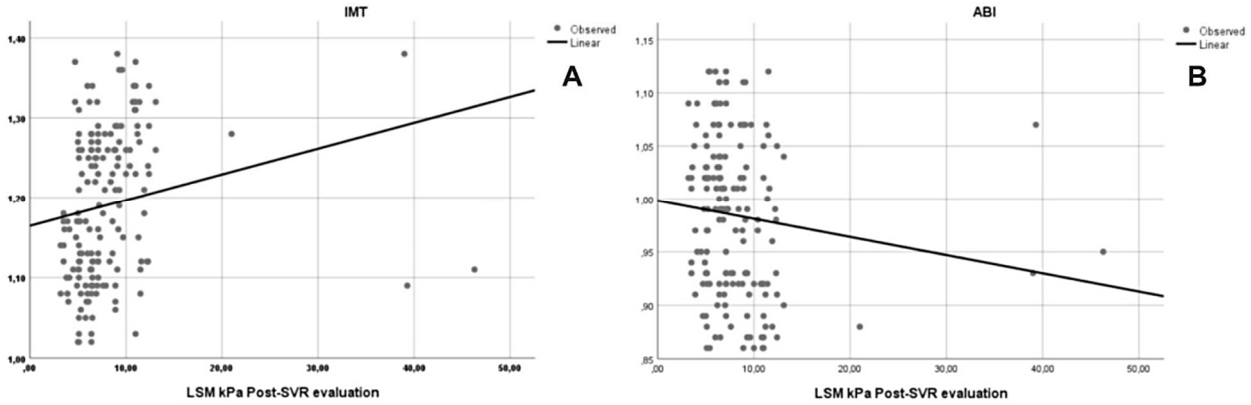

Figure 5 – ***Correlation between LSM score post-SVR evaluation and IMT (A), ABI (B). ABI: Ankle–brachial index; LSM: Liver stiffness measurements; IMT: Intima–media thickness; SVR: Sustained virological response.***

Discussions

During the evolution of chronic HCV infection, important changes occur in the lipid metabolism, these being influenced by the viral activity, the pharmacokinetics of the antivirals therapy, as well as the patient's diet and physical exercises regimen. Cardiovascular risk factors are variable and can be modified based on beneficial changes in metabolic syndrome, weight reduction, abdominal obesity decrease, effective blood pressure management, and lowered levels of serum triglycerides and cholesterol. IMT is being considered a surrogate marker for subclinical atherosclerosis, a major risk factor for cardiovascular events. The improvement in the IMT measurements cand bring new hope regarding the impact of DAA on the atherosclerotic process [28].

Our results obtained from the IMT measurement showed a decrease in the risk of the evolution of the atherosclerosis process after obtaining SVR (1.22±0.20 mm for patients with F0–F2 *vs.* 1.13±0.17 mm for patients with F3 and F4). IMT regression was noticed and differentiated, both in the case of patients with chronic hepatitis, but also in those with HCV-related cirrhosis. Improvement in IMT after SVR represents a decrease in the risk of cardiovascular events. This close association between a pathological IMT and possible future acute cardiovascular events has been highlighted in numerous studies in the literature. We recall the research carried out by Pignoli *et al.*, which stated that the cardiovascular risk increases by approximately 11% for every 0.1 mm above the acceptable limits of the IMT. This result has been replicated in recent years by five important studies that highlighted a possible correlation between IMT and increased risk of cardiovascular events [29–33]. The utility of using this carotid measurement along with classical cardiovascular risk factors, like those found in the Framingham score, for estimating a global cardiac risk, has been proven with minimal predictive power for the occurrence of new events [34–36]. This fact may be due to a lack of standardization of measurements according to age groups, patient gender and ethnicity. The results obtained by us are comparable with the data from the literature showing similar determinations of IMT in the population chronically infected with HCV.

Petta *et al.*, in a study conducted on 182 patients with chronic HCV infection, staged as hepatitis or compensated cirrhosis stages, demonstrated the beneficial effects of SVR on the cardiovascular risk. In this study, patients were evaluated by measuring IMT and through the quantification of atheroma plaques at baseline, in the end and also 9–12 months after the completion of the DAA therapy. The authors showed that the mean IMT values lowered from start until the therapy ended, but also during follow-up (0.94±0.29 mm at baseline, compared to 0.81±0.27 after SVR, respectively, $p<0.001$) [28]. Our study presents comparable results, in the post-SVR period an important decrease in IMT compared to the initial values was noted. The number of atherosclerotic plaques found at the carotid level was quantified in addition to the IMT assessment. As a result, a statistically significant link between their prevalence and the degree of liver fibrosis was discovered. In comparison to individuals with F1–F2 fibrosis, the group of patients with advanced fibrosis and cirrhosis displayed more atherosclerotic plaques. In their research, Petta *et al.* found a similar relationship between the quantity of carotid atherosclerotic plaques and the severity of liver disease. Their evolution following SVR was stationary, and the antiviral therapy had no regressive effect on these lesions that were already present [28].

The study by Ichikawa *et al.*, which examined a group of 48 patients who received treatment with DAAs, offers another viewpoint on IMT evaluated post-SVR. The patient group was assessed by IMT measurements, lipid profile, insulin resistance using the homeostatic model assessment (HOMA) technique, and BMI around one year after obtaining SVR. Moreover, Ichikawa *et al.* indicate significant variations in the reduction of cardiovascular risk following therapy with IFN or DAAs in their study. It's probable that the various antiviral mechanisms, the numerous negative effects of IFN, as well as the lengthy duration of this therapeutic regimen, led to a reduction in total cholesterol during antiviral therapy. The relationship between cholesterol fractions, especially LDL, and an elevated IMT at the start of treatment must be assessed both during treatment and after it is over. The study by Ichikawa *et al.* suggests that when the clinical situation incorporates persistent HCV infection, men are substantially more likely than women to have a greater IMT. This study has shown that a condition that predisposes to carotid atherosclerosis can be present in patients regardless of their gender [37]. Petta *et al.*, who performed a comparison between two groups of HCV patients who received antiviral treatment and achieved SVR and a control group without antiviral treatment, found that patients without DAAs regimen had a higher risk for

atherosclerosis progression. No significant changes in IMT were observed in the control group (0.88±0.30 mm) compared to the responders' group (0.94±0.40 mm) (p=0.29) [28].

HCV causes multisystemic damage, and the atherosclerotic process can also be found in the peripheral arterial areas; in this regard, the documentation of ABI offered us a new perspective on the distal arteriopathy seen in patients enrolled. However, demonstrating the impact of the SVR on PAD during the DAA era still remains an unsolved topic [38]. In a recent study published by Hsu *et al.*, patients with HCV who were over 65 years old had a 11.7 times greater incidence of PAD. It seems that even though antiviral medication can decrease the risk of heart attack and stroke, there were no changes in peripheral arteriopathy after SVR. According to Hsu *et al.*, this occurrence may be explained by the irreversibility of peripheral arteriopathy following antiviral therapy, a finding we underlined in our research [39]. It is therefore assumed that HCV acts on the atherosclerotic process in the early stages, which can explain why antiviral treatment is ineffective in cases of established lesions. This assumption is based on the idea that diffuse atherosclerotic process extension in the peripheral vessels can indicate an advanced stage of this vascular condition. According to our study results, ABI was improved at the time of the SVR assessment (0.95±0.20 *vs.* 0.96±0.17). Although the results fell short of statistical significance, it is important to emphasize that SVR can improve or slow down the atherosclerotic process.

Moritani *et al.* conducted a study on 1806 individuals and attempted to evaluate the risk of peripheral arteriopathy in this population segment to emphasize the significance of examining peripheral arteriopathy in patients with chronic HCV infection. The entire patient population had chronic hepatitis B virus or HCV infection. ABI index and the propagation speed of the pulse wave both in the designated group and in the comparison group, represented the chosen method to investigate peripheral arteriopathy. Results showed that patients with HCV had lower ABI values compared to patients in the control group. It is important to remember that chronic HCV infection tends to progress to liver cirrhosis, a condition that usually involves, due to hepatic insufficiency, a lower level of cholesterol, a fact that still requires studies on the atherosclerotic risk among these patients [40].

Study limitations

This study presents several limitations. The agreement between observers in assessing carotid atherosclerosis was not assessed, which may have impacted how our results should be interpreted. Nevertheless, all procedures were carried out by skilled personnel who adhered to the same protocol; furthermore, several relevant studies that investigated carotid atherosclerosis relied on data from multicenter cohorts and/or involved multiple operators; in the same time, some studies documented favorable agreement between different observers when it came to assessing carotid atherosclerosis using ultrasonography [41, 42]; and also, as we highlighted in our recent report, there is typically a strong level of consensus between different observers when evaluating carotid atherosclerosis in HCV patients [28]. The fact that the median follow-up length in our analysis was just 48 weeks after the end of the treatment course may be another drawback. Longer studies are necessary to track the progression of hepatic steatosis and fibrosis after DAA therapy in chronically HCV-infected patients. The absence of a histological examination is another issue to mention. Finally, the therapeutic application of our findings is further constrained by the lack of information regarding the primary cardiovascular outcomes and long-term follow-up [43].

Conclusions

Our research highlights the beneficial effect of HCV eradication by the new DAA regimens on both carotid atherosclerosis and PAD. All treated HCV-infected patients experienced significant improvement in IMT and ABI measurements. According to the fibrosis stage as recorded by the liver stiffness evaluation, the maximum benefit was obtained by patients with advanced fibrosis and cirrhosis.

Institutional Review Board Statement

The Ethics Committee of Grigore T. Popa University of Medicine and Pharmacy, Iaşi, Romania, approved the study (Protocol code 15046, date of approval July 2017) and it was carried out in accordance with the principles of the Declaration of Helsinki.

Informed Consent Statement

All participants in the study provided their written informed consent.

Data Availability Statement

The corresponding author can provide the data described in this study upon request. Since the data are the property of the Institute of Gastroenterology and Hepatology, St. Spiridon University Hospital, Iaşi, Romania, they are not accessible to the general public.

Conflict of interests

No conflict of interests is disclosed by the authors.

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