Clinics and Research in Hepatology and Gastroenterology 49 (2025) 102639

Contents lists available at ScienceDirect

Clinics and Research in Hepatology and Gastroenterology

journal homepage: www.elsevier.com/locate/clinre

Original article

Metabolic factors drive early increase in hepatic steatosis despite improvement in non-invasive fibrosis markers after hepatitis C eradication with direct-acting antivirals

Mohamed Shengir [a], Wesal Elgretli [a], Felice Cinque [b,c], Agnihotram V. Ramanakumar [d], Rosa Lombardi [b,c], Annalisa Cespiati [b,c], Anna Ludovica Fracanzani [b,c], Luz Ballesteros [e], Marc Deschenes [e], Philip Wong [e], Tianyan Chen [e], Giada Sebastiani [a,e,*]

[a] Division of Experimental Medicine, Department of Medicine, McGill University, Montreal, Quebec, Canada
[b] Unit of Internal Medicine and Metabolic Disease, Fondazione Ca' Granda IRCCS Ospedale Maggiore Policlinico, Milan, Italy
[c] Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy
[d] Research Institute, McGill University Health Centre, Montreal, Quebec, Canada
[e] Division of Gastroenterology and Hepatology, Department of Medicine, McGill University Health Centre, Montreal, Quebec, Canada

ARTICLE INFO

Keywords:
Metabolic dysfunction-associated steatotic liver disease
Body mass index
Liver stiffness measurement
Controlled attenuation parameter
Sustained virological response

ABSTRACT

Background: While direct-acting antivirals (DAAs) achieve high sustained virologic response (SVR) rates in people with hepatitis C virus (HCV), their impact on hepatic steatosis (HS) remains unclear.
Methods: We conducted a retrospective cohort study of 108 HCV patients from McGill University and the University of Milan who achieved SVR following DAAs. Controlled attenuation parameter (CAP) and liver stiffness measurement (LSM) were used to assess HS and liver fibrosis at baseline and 24 weeks post-SVR. HS was defined as CAP ≥248 dB/m, significant liver fibrosis as LSM ≥8 kPa, and metabolic dysfunction-associated steatotic liver disease (MASLD) as HS plus ≥1 cardiometabolic risk factors. Changes were evaluated using Wilcoxon signed-rank test and standardized mean difference (SMD). Multivariable logistic regression identified predictors of post-SVR HS.
Results: HS prevalence increased from 47 % to 61 % post-SVR ($p = 0.0007$, SMD = 0.30). Among patients with baseline HS, 88 % had persistent steatosis. New-onset steatosis developed in 37 % of patients without baseline HS, with a significant CAP increase ($p < 0.0004$, SMD=0.48). In patients without baseline HS, total cholesterol and triglycerides increased ($p = 0.0084$, SDM = 0.43 and $p < 0.0001$, SDM = 0.71, respectively), whereas in those with baseline HS, only total cholesterol rose ($p = 0.0296$, SDM = 0.50). MASLD remained the leading etiology at both time points (94 % at baseline, 92 % post-SVR). Significant fibrosis declined markedly from 49 % to 17 % ($p < 0.0001$, SMD = –0.80). Higher BMI at 24 weeks was independently associated with HS (adjusted odds ratio 1.92, 95 %CI 1.22–3.03).
Conclusions: Despite improvement in liver fibrosis markers, HS often persists or emerges following DAAs therapy, particularly alongside metabolic dysfunctions marked by elevated cholesterol and triglycerides.

1. Introduction

Chronic hepatitis C virus (HCV) infection remains a significant global health concern, affecting over 58 million individuals worldwide and contributing to substantial liver-related morbidity and mortality, including cirrhosis and hepatocellular carcinoma (HCC) [1,2]. For nearly two decades, interferon-based regimens were the standard treatment for HCV infection, though they were limited by modest SVR rates, poor tolerability, and frequent adverse effects [3–5]. The introduction of safe and potent direct-acting antivirals (DAAs) has revolutionized HCV care. Currently, short-term treatment with pan-genotypic DAAs consistently achieves sustained virologic response (SVR) rates exceeding 95 % across HCV genotypes and diverse populations [6,7]. However, despite successful viral eradication, growing evidence

* Corresponding author at: Division of Gastroenterology and Hepatology, Chronic Viral Illness Service, Royal Victoria Hospital, McGill University Health Center, 1001 Décarie Blvd., Montreal, QC H4A 3J1, Canada.
E-mail address: giada.sebastiani@mcgill.ca (G. Sebastiani).

https://doi.org/10.1016/j.clinre.2025.102639

Available online 16 June 2025

suggests that people with HCV may continue to experience long-term hepatic and metabolic complications [8–10]. Among these, hepatic steatosis (HS) is particularly prevalent and clinically relevant, which may persist or even newly develop post-SVR [11–13].

HS is a common histological finding in people with HCV, with prevalence estimates ranging from 40 % to 86 % and an average of approximately 55 % in untreated patients [14]. These reported rates are influenced by population characteristics and HCV genotype distribution [15,16]. In genotype 3 infections, steatosis is typically driven by viral factors and is often referred to as "viral steatosis," closely correlating with HCV RNA levels [16,17]. This is largely due to HCV's ability to hijack host lipid metabolism to support its replication and assembly, with viral core proteins interacting with lipid droplets and modulating hepatic lipid transporters, ultimately impairing very low-density lipoprotein secretion and promoting intracellular fat accumulation [18,19]. In contrast, in non-genotype 3 infections, HS is primarily associated with host metabolic factors such as obesity, insulin resistance, and dyslipidemia, suggesting a phenotype more consistent with "metabolic steatosis" [15]. These distinctions raise important questions about the evolution of HS following SVR, particularly as viral steatosis may resolve, while metabolic dysfunction may persist or even emerge after viral clearance. However, data on the early trajectory of HS and metabolic dysfunction following SVR remain limited, as do insights into their contribution in sustaining the burden of liver disease.

In June 2023, a multisociety Delphi consensus introduced a revised nomenclature for fatty liver disease [20]. Under this new classification, the term metabolic dysfunction-associated steatotic liver disease (MASLD) replaced nonalcoholic fatty liver disease (NAFLD), emphasizing the role of cardiometabolic risk factors as central drivers of HS [21]. Importantly, unlike the previous NAFLD definition, MASLD framework permits the coexistence of other causes of HS, including viral hepatitis. MASLD is now recognized as a key coexisting liver condition that influences the post-SVR natural history of HCV [22,23]. Understanding how MASLD evolves following HCV cure is critical for guiding long-term risk stratification and liver disease surveillance in this population.

This study aimed to evaluate early changes in non-invasive markers of HS and liver fibrosis at 24 weeks post-SVR following DAA therapy. We focused on identifying metabolic factors associated with persistent or new-onset steatosis, with a particular emphasis on the emerging role of MASLD in the post-SVR context.

2. Methods

2.1. Study population

In this retrospective, bicenter cohort study, we included participants from McGill University Health Centre in Montreal, Canada, and the University of Milan in Milan, Italy, between 2016 and 2019. A total of 108 adult patients with chronic HCV infection were included. Eligible individuals were aged 18 years or older, had received DAA therapy, and achieved SVR, defined as undetectable HCV RNA 12 weeks after treatment completion. Exclusion criteria included a history of excessive alcohol intake, defined as consumption exceeding 140 g per week for females and 210 g per week for males, or other known causes of HS [24]. Patients with other known chronic liver disease, with or without clinical evidence of decompensated cirrhosis at baseline (e.g., ascites, bleeding esophageal varices, or hepatic encephalopathy), as well as those co-infected with hepatitis B virus or human immunodeficiency virus were excluded. We also excluded those who had missing controlled attenuation parameter (CAP) data at baseline. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, as outlined by the International Conference on Harmonization. The study protocol received approval (ID # 2022–8356) from the research ethics boards of both participating institutions, which waived the requirement for informed consent due to the retrospective design.

2.2. Study design and outcome measures

The overall study workflow is depicted in Fig. 1, which outlines the inclusion process, patient categorization, and follow-up assessments. This study was designed to evaluate longitudinal changes in HS and liver fibrosis in people with chronic HCV infection who achieved SVR following DAA therapy, using transient elastography (TE) with CAP. TE with CAP examinations were performed by an experienced operator using a commercially available device (FibroScan® 502, Echosens, Paris, France). At baseline, participants were stratified according to the presence or absence of HS, which was defined by a CAP value of ≥248 dB/m [25]. Individuals with HS were further assessed for cardiometabolic risk factors—including elevated body mass index (BMI) ≥25 kg/m^2, fasting plasma glucose ≥100 mg/dL or a diagnosis of type 2 diabetes, high blood pressure (≥130/85 mmHg), plasma triglycerides ≥150 mg/dL, and high-density lipoprotein cholesterol (HDL) ≤40 mg/dL in males or ≤50 mg/dL in females—to determine whether they met the criteria for MASLD. Furthermore, HS by CAP was categorized into three severity grades: mild (S1: 248 – 267 dB/m), moderate (S2: 268 – 279 dB/m), and severe (S3: >280 dB/m) [25–27]. Liver stiffness measurement (LSM) was used to assess liver fibrosis via TE. The M probe was used for participants with BMI <30 kg/m^2, and the XL probe for those with BMI ≥30 kg/m^2. Examinations were considered valid if they included at least 10 successful LSM readings with an interquartile range (IQR) <30 % of the median [27]. Significant liver fibrosis and cirrhosis were defined as LSM ≥8 kPa and ≥13 kPa, respectively [28]. Fibrosis assessment was supported by FIB-4 index, with a score of <1.45 indicating minimal fibrosis, 1.45–3.25 suggesting moderate fibrosis, and >3.25 indicating a high probability of advanced fibrosis [29,30]. All patients received standard-of-care DAA therapy and were reassessed 24 weeks post-SVR using TE/CAP.

The primary outcome was the change in HS status over time, categorized as resolution, persistence, or new onset. Secondary outcomes included change in MASLD status and change in the degree of liver fibrosis. This design allowed for comparison of steatosis and fibrosis trajectories within the cohort, stratified by baseline HS status.

2.3. Statistical analysis

We included all eligible patients with chronic HCV infection who achieved SVR following DAA therapy and had paired CAP and LSM measurements available at both baseline and 24 weeks post-SVR. No formal sample size calculation was performed; the study cohort size was determined by the availability of complete paired data within the study period. Paired comparisons of continuous variables between baseline and 24 weeks post-SVR were assessed using the Wilcoxon signed-rank test due to non-normal data distribution. Categorical variables were compared using the McNemar test for paired proportions or Chi-squared/Fisher's exact test for unpaired comparisons, as appropriate. To evaluate the magnitude of changes over time, standardized mean differences (SMDs) were calculated using rank-based methods. An SMD ≥0.20 was considered a small effect, ≥0.50 a moderate effect, and ≥0.80 a large effect. Lollipop charts were used to visually depict SMDs across key clinical and metabolic variables, enhancing the interpretability of effect sizes. Subgroup analyses were performed by stratifying participants according to the presence or absence of HS at baseline.

Multivariable logistic regression analysis was used to identify independent predictors of HS at 24 weeks. Covariates included in the model were selected based on clinical relevance and statistical significance in univariable analyses. Adjusted odds ratios (aORs) and 95 % confidence intervals (CIs) were reported. A two-sided p-value <0.05 was considered statistically significant. To assess multicollinearity, variance inflation factors were calculated for all covariates in the multivariable model. All variance inflation factors were below 2, indicating minimal collinearity

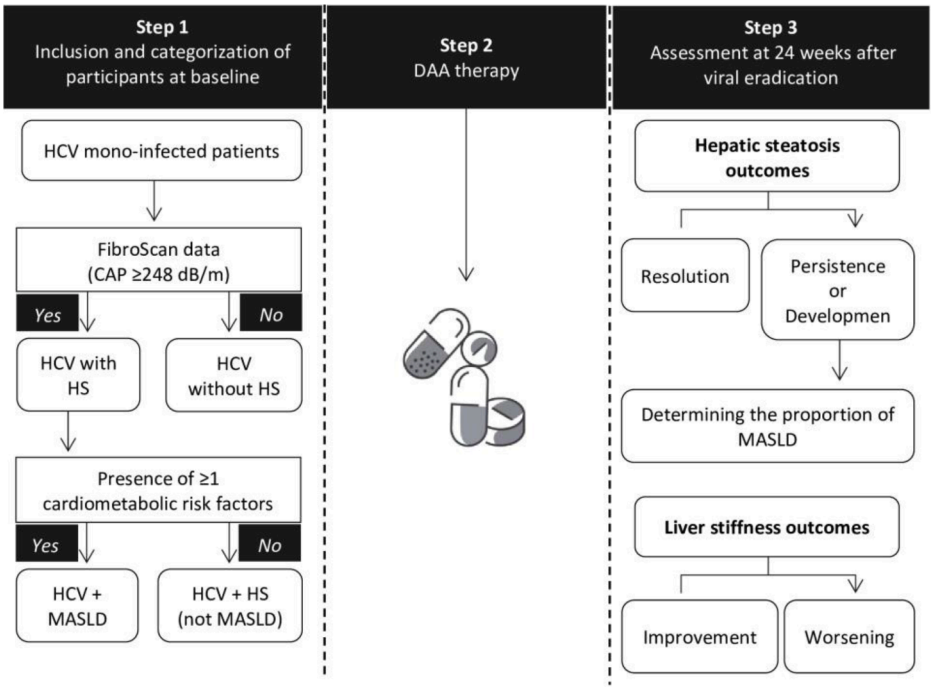

Fig. 1. Study flowchart illustrating patient selection, MASLD classification, and follow-up assessments at 24 weeks post-SVR.

and suggesting that individual predictors contributed distinct information. To minimize the risk of overfitting, we limited the number of covariates relative to the sample size and avoided unnecessary model complexity.

All statistical analyses were performed using R software (version 4.3.1; R Foundation for Statistical Computing, Vienna, Austria). To mitigate potential sources of bias, we employed a paired within-subject design, standardized non-invasive assessments (CAP and LSM), consistent diagnostic thresholds, and multivariable adjustment for key confounders. Inclusion of patients from two independent centers further enhanced generalizability. We used complete case analysis, including only patients with available paired CAP and LSM measurements at both baseline and 24 weeks post-SVR. Patients with missing data at either time point were excluded from the analysis**.** The manuscript was prepared according to the STROBE Statement-checklist of items [31].

3. Results

3.1. Baseline characteristics

A total of 108 participants were included, of whom 51 (47 %) had HS and 53 (49 %) had significant liver fibrosis or cirrhosis at baseline. Patients received DAA regimens as follows: 37 (34 %) received sofosbuvir+velpatasvir, 26 (24 %) glecaprevir+pibrentasvir, 19 (18 %) elbasvir+grazoprevir, and 26 (24 %) received other regimens. The overall median age was 62 years (IQR: 56 - 73) and 54 (50 %) were female. Compared to those without HS, patients with HS had higher BMI (27.2 vs. 23.9 kg/m^2, $p < 0.0001$) and higher triglyceride levels (90 vs. 78.5 mg/dL, $p = 0.0314$) (Table 1). No significant differences were observed in the prevalence of diabetes, hypertension, dyslipidemia, HCV genotype distribution, or viral load between groups. Among those with HS, 94 % met criteria for MASLD, with steatosis severity classified as mild in 43 %, moderate in 10 %, and severe in 47 %. In the subgroup of patients with HCV genotype 3 infection ($n = 8$), 4 (50 %) had hepatic steatosis at baseline, and 6 (75 %) had steatosis at 24 weeks post-SVR. Due to the small sample size, statistical comparisons were not performed.

3.2. Changes in liver and metabolic parameters

At 24 weeks post-SVR, the prevalence of HS increased from 47 % to 61 %, while MASLD remained highly prevalent, from 94 % to 92 %. In contrast the proportion of participants with significant liver fibrosis and cirrhosis decreased from 49 % to 17 %, and from 16 % to 5 %, respectively. Liver enzymes also improved, with elevated ALT and AST levels decreasing from 70 % and 66 %, respectively, to 5 % by the end of follow-up (Fig. 2). Among individuals with HS, the distribution of steatosis severity shifted over time. The proportion of mild steatosis decreased from 43 % to 30 %, moderate steatosis remained stable 10 % to 11 %, and severe steatosis increased from 47 % to 59 % at 24 weeks (Fig. 3a). When stratified by baseline HS status, 88 % of participants with baseline HS had persistent steatosis at follow-up, whereas 12 % showed resolution. In contrast, among those without HS at baseline, 37 % developed new-onset steatosis, while 63 % remained steatosis-free (Fig. 3b). Paired comparisons from baseline to end of follow-up showed modest worsening in CAP values rising from 245 to 260 dB/m ($p = 0.0007$), indicating a trend toward increased hepatic fat content despite viral eradication. In contrast, significant improvements were observed in liver fibrosis markers, with a notable reduction in median LSM from 7.9 to 5.6 kPa ($p < 0.0001$), and FIB-4 score from 2.13 to 1.58 ($p < 0.0001$). Liver enzymes, including ALT, AST, and GGT, also improved substantially (all $p < 0.0001$). Modest increases were observed in total cholesterol and triglycerides, while changes in HDL, LDL, BMI, and glucose were not significant (Table 2). These findings are further visualized in a lollipop plot of SMDs across key parameters (Fig. 4a).

3.3. Stratified analysis by baseline steatosis status

CAP values increased significantly in participants without HS at baseline, rising from 217 to 230 dB/m ($p = 0.0004$), while remaining relatively stable in those with HS (275 to 291 dB/m, $p = 0.2357$). Total cholesterol levels increased in both groups, with a more pronounced rise in patients without HS. Triglycerides increased significantly only in patients without HS. Platelet counts increased in both groups, with a greater rise observed among those with baseline HS. Importantly, both groups showed significant reductions in LSM, FIB-4 scores, and liver

Table 1
Baseline characteristics of the cohort stratified by HS status.

Variable	Hepatic Steatosis: Absent ($n = 57$)	Hepatic Steatosis: Present ($n = 51$)	P-value
Age, years	62 (56 – 73)	62 (55 – 72)	0.7463
Male sex, n (%)	26 (46 %)	28 (55 %)	0.3526
Hypertension, n (%)	31 (54 %)	21 (41 %)	0.1790
Diabetes mellitus, n (%)	6 (11 %)	8 (16 %)	0.4479
Dyslipidemia, n (%)	9 (16 %)	9 (18 %)	0.7831
Body mass index, kg/m^2	23.9 (22.4 – 27)	27.2 (25.6 – 29.8)	0.0000**
HCV genotype, n (%)			
G1	32 (56 %)	31 (61 %)	0.6004
G2	16 (28 %)	10 (20 %)	0.3349
G3	4 (7 %)	4 (8 %)	0.8443
G4	5 (9 %)	6 (12 %)	0.6121
Viral load (log IU/mL)	6.02 (5.28 – 6.34)	5.92 (5.39 – 6.30)	0.7237
DAA regimen, n (%)			
Sofosbuvir/Velpatasvir (Epclusa)	20 (35 %)	16 (31 %)	0.6607
Glecaprevir/ Pibrentasvir (Maviret)	19 (33 %)	9 (18 %)	0.0771
Elbasvir/grazoprevir (Zepatier)	6 (11 %)	12 (24)	0.0748
Other regimens	12 (21 %)	14 (27 %)	0.4671
Total cholesterol, mg/dL	158 (139 – 189)	174 (140 – 191)	0.3144
LDL, mg/dL	88 (72 – 107)	97.5 (68 – 117)	0.3082
HDL, mg/dL	52.7 (41 – 65)	49.8 (38 – 58)	0.3377
Triglycerides, mg/dL	78.5 (53 – 100)	90 (69 – 127)	0.0314**
Serum glucose, mg/dL	94 (85 – 100)	97 (85 – 114)	0.2830
ALT, IU/L	61.5 (41 – 100)	56 (34 – 97)	0.6629
AST, IU/L	52 (36 – 69)	46 (32 –61)	0.4517
GGT, IU/L	46 (21 – 80)	42 (21 – 65)	0.5817
Platelets, $\times 10^9$/L	186 (149 – 242)	205 (172 – 250)	0.1159
Hepatic steatosis, n (%)			
S1 (CAP 248 – 267 dB/m)		22 (43 %)	–
S2 (CAP 268 – 279 dB/m)		5 (10 %)	–
S3 (CAP >280 dB/m)		24 (47 %)	–
MASLD, n (%)		48 (94 %)	–
LSM, kPa	7.7 (5.4 – 10.6)	8.6 (6.1 – 11.5)	0.2281
Liver fibrosis, n (%)			
F0 – F1 (LSM < 8 kPa)	34 (60 %)	21 (41 %)	0.0497
F2 – F3 (LSM ≥ 8 – < 13 kPa)	15 (26 %)	21 (41 %)	0.0997
F4 (LSM ≥ 13 kPa)	8 (14 %)	9 (18 %)	0.5720
FIB-4 score	2.22 (1.39 – 3.55)	1.91 (1.31 – 2.72)	0.1980
Fibrosis stage, n (%)			
0 – 1 (FIB-4 score < 1.45)	19 (33.5 %)	14 (27.5 %)	0.5016
2 – 3 (FIB-4 score 1.45 – 3.25)	23 (40.5 %)	25 (49 %)	0.3771
4 – 6 (FIB-4 score > 3.25)	15 (26 %)	12 (23.5 %)	0.7650

Statistical significances ($P < 0.05$**) were calculated using the Wilcoxon rank-sum test for continuous variables, reported as median and interquartile range (IQR), and the Chi-square test or Fisher's exact test, as appropriate, for categorical variables.
ALT: alanine aminotransferase; AST: aspartate aminotransferase; DAA: direct-acting antiviral; F: fibrosis; FIB-4: fibrosis-4 score; G: genotype; GGT: gamma-glutamyl transferase; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; LSM: liver stiffness measurement; MASLD: metabolic dysfunction-associated steatotic liver disease; S: steatosis.

enzymes, reflecting overall improvement in liver fibrosis markers and reduced hepatocellular injury (Table 3). These trends are further illustrated by stratified SMDs in the lollipop plot (Fig. 4b).

3.4. Predictors of HS at the end follow-up

In multivariable logistic regression model, BMI at 24 weeks was the only independent predictor of HS at the end of follow-up. Each unit increase in BMI was associated with a nearly two-fold increase in the odds of steatosis (aOR: 1.92; 95 % CI: 1.22 – 3.03; $p = 0.0049$). Other variables—including serum glucose, triglycerides, ALT, LSM, and total cholesterol, were not significantly associated with HS in the adjusted model (Table 4).

4. Discussion

In this retrospective cohort study of people with chronic HCV infection who achieved SVR following DAA therapy, we observed a significant shift in liver health indicators within 24 weeks post-SVR. Our findings confirm that SVR is associated with substantial improvements in non-invasive markers of liver fibrosis, consistent with the well-established benefits of viral eradication [32–34]. However, in parallel with this improvement, we identified a significant increase in HS prevalence, with nearly two-thirds of the cohort exhibiting HS at the end of follow-up. This trend was driven both by the persistence of steatosis in those with baseline involvement and the emergence of new-onset HS in individuals without prior steatosis. These findings underscore the growing importance of metabolic liver disease in the post-SVR era, suggesting that clearance of HCV does not fully eliminate the risk of progressive liver injury, particularly when metabolic risk factors are present [12,35,36].

A key finding of our study was the paradoxical pattern of improved non-invasive fibrosis markers alongside worsening steatosis. Liver stiffness and FIB-4 scores declined, and liver enzymes normalized, indicating a reduction in hepatic necroinflammation, hallmarks of early hepatic response following SVR. However, this was accompanied by a significant increase in CAP values and a rise in HS prevalence from 47 % to 61 %, despite successful viral eradication. This divergence reflects a shift in the underlying pathophysiology post-SVR, where viral-induced injury resolves, yet metabolic drivers of liver disease persist or worsen. These findings are consistent with those of Ogasawara et al., who similarly reported improvements in fibrosis coupled with worsening steatosis [37]. They also align with growing evidence that steatosis, particularly of metabolic origin, can progress independently of ongoing viral activity [38,39]. Interestingly, our findings suggest a reverse pattern of the so-called "burnout" phenomenon, where steatosis diminishes as fibrosis advances in later disease stages [40]. Instead, we observed increasing steatosis alongside improvements in non-invasive fibrosis markers, potentially reflecting an early or transitional phase of metabolic liver disease following SVR. However, it is important to acknowledge contrasting evidence. In a study by Shimizu et al., HS assessed by CAP was found to decrease after SVR, even in the context of rising LDL and HDL cholesterol levels—highlighting that post-SVR metabolic responses may be heterogeneous and influenced by population characteristics, baseline risk profiles, or duration of follow-up [41]. While the observed pattern in our cohort may initially suggest hepatic recovery, the persistence or emergence of steatosis raises concern that, if unaddressed, metabolic dysfunction could reinitiate fibrogenesis over time. These findings therefore highlight the importance of ongoing liver health surveillance and proactive metabolic risk management in the post-SVR population, even beyond virologic cure.

We also observed dynamic shifts in steatosis severity: while a subset of patients progressed from mild to more severe steatosis, resolution was uncommon. Notably, a significant proportion of participants without HS at baseline developed new-onset steatosis, further reinforcing the metabolic underpinning of liver disease in the post-SVR setting. Stratified analyses revealed that CAP progression was primarily driven by individuals who were initially steatosis-free, suggesting a delayed metabolic consequence of viral clearance. A plausible explanation lies in the rebound of lipid metabolism following SVR. With the cessation of

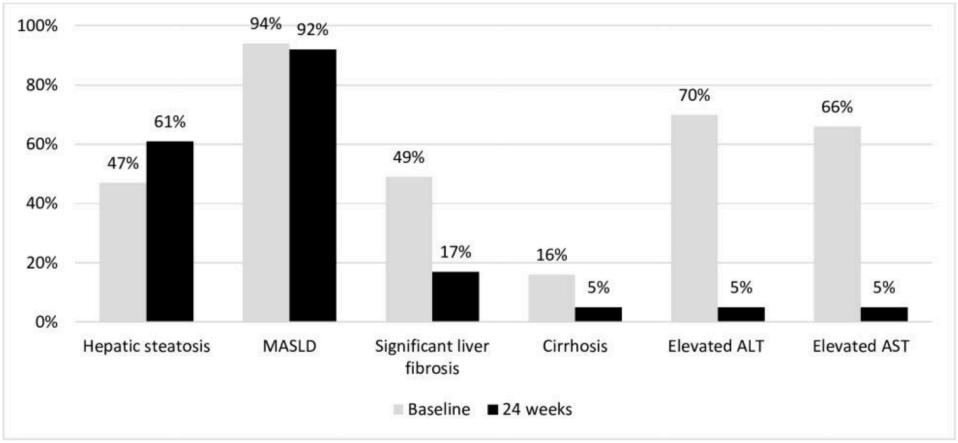

Fig. 2. Changes between baseline and 24 weeks post-SVR across liver parameters.

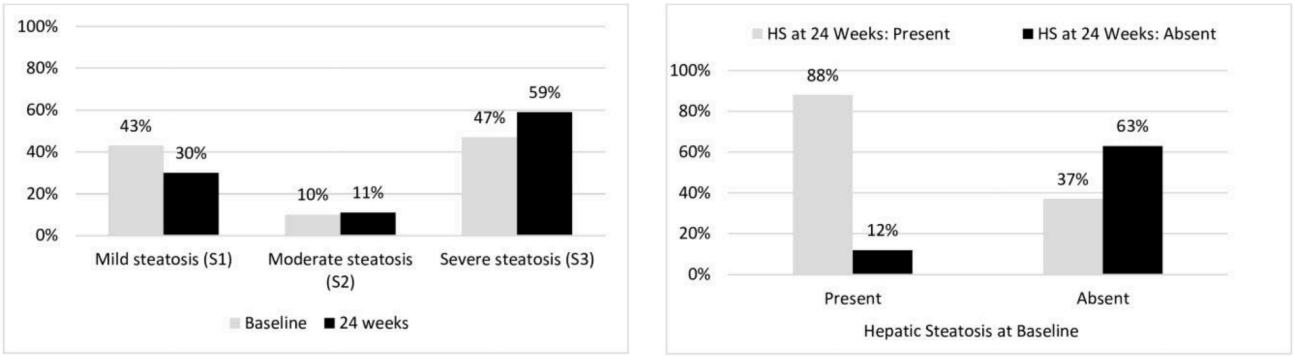

Fig. 3. a. Distribution of steatosis severity at baseline and 24 weeks post-SVR. b. Hepatic steatosis trajectories stratified by baseline status.

Table 2
Comparison of clinical and laboratory parameters at baseline and 24 weeks post-SVR.

Variable	Baseline (median [IQR]	24 weeks (median [IQR]	P-value
CAP (dB/m)	245 (214 – 272)	260 (220 – 305)	0.0007**
LSM (kPa)	7.9 (5.9 – 11)	5.6 (4.5 – 7.4)	0.0000**
FIB-4 score	2.13 (5.9 – 11)	1.58 (4.5 – 7.4)	0.0000**
Total cholesterol (mg/dL)	172 (139 – 196)	184.4 (161.6 – 199)	0.0008**
LDL (mg/dL)	98.5 (82 – 116.0)	106.5 (89 – 124.0)	0.0643
HDL (mg/dL)	52 (44.0 – 60)	51 (43 – 60)	0.6886
Triglycerides (mg/dL)	89 (67.6 – 112.2)	93.5 (69 – 137.5)	0.0123**
BMI (kg/m^2)	26 (23.9 – 29.1)	26.4 (24.6 – 29.4)	0.2329
Glucose (mg/dL)	95 (85 – 103)	93.6 (87 – 105)	0.9392
ALT (I/U)	62 (41 – 100)	18 (14 – 26)	0.0000**
AST (I/U)	49.5 (36 – 69)	23 (18 – 28)	0.0000**
GGT (I/U)	46 (21 – 80)	18 (13 – 27)	0.0000**
PLT ($\times 10^9$/L)	196 (149 – 242)	218 (172 – 250)	0.0002**

Data represented as median & interquartile range (IQR). ** $P < 0.05$, based on Wilcoxon signed-rank test.
ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; CAP: controlled attenuation parameter; FIB-4: fibrosis-4 score; GGT: gamma-glutamyl transferase; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; LSM: liver stiffness measurement; PLT: platelets.

viral replication, the HCV-induced disruption of lipid handling is expected to normalize. However, the restoration of hepatic lipoprotein synthesis can paradoxically elevate serum lipid levels. In individuals with pre-existing or emerging metabolic risk, this shift may lead to persistent or new-onset steatosis [42–45]. Therefore, SVR may act as a metabolic inflection point that shifts the pathogenesis of HS from a predominantly viral mechanism to one driven by metabolic dysfunction [46,47]. This hypothesis is supported by emerging evidence. A recent study linked post-SVR lipid shifts to new-onset or persistent steatosis, even in patients with minimal baseline metabolic risk before treatment [45]. Similarly, Tokuchi et al., reported an increased prevalence of fatty liver post-SVR accompanied by elevated LDL cholesterol, independent of changes in BMI [48]. In our cohort, increases in total cholesterol and triglyceride levels were more pronounced among participants without baseline steatosis, suggesting that restoration of lipid homeostasis may play a key role in driving hepatic fat accumulation. These findings highlight the need for closer monitoring of lipid profiles and steatosis development in the post-SVR period, particularly in individuals previously considered metabolically low risk. Understanding the early metabolic trajectory after viral cure is essential for identifying at-risk patients and implementing timely interventions to prevent long-term liver damage.

Multivariable analysis identified BMI at 24 weeks post-SVR as the only independent predictor of HS. This finding aligns with the pathophysiology of MASLD, in which adiposity is a key driver of hepatic fat accumulation [20]. Notably, traditional biochemical markers, including glucose, triglycerides, ALT, and cholesterol, were not independently associated with steatosis, underscoring the predominant role of body composition over isolated metabolic parameters.

Our findings carry important clinical implications. First, they reinforce the role of TE with CAP as a non-invasive strategy for monitoring HS after SVR [49]. The significant increases in CAP observed in our cohort, despite improvement in non-invasive fibrosis markers and normalization of liver enzymes, highlight the ability of TE with CAP to detect early or subclinical metabolic liver changes that might otherwise

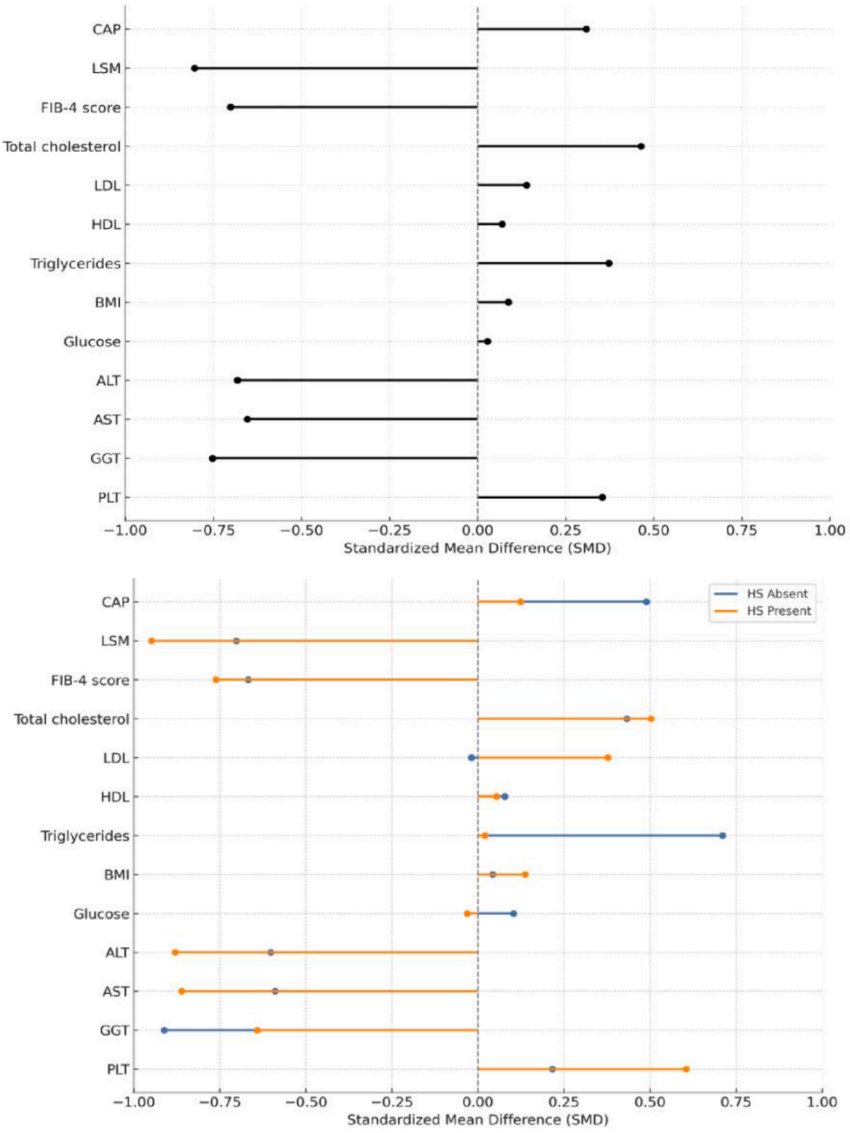

Fig. 4. a. Standardized mean differences of liver and metabolic parameters between baseline and 24 weeks post-SVR for the whole cohort. b. Standardized mean differences of liver and metabolic parameters between baseline and 24 weeks post-SVR stratified by HS status at baseline.
Legends: Effect sizes were interpreted as follows—≥0.20 as a small effect, ≥0.50 as a moderate effect, and ≥0.80 as a large effect.

Table 3
Changes in clinical and laboratory parameters from baseline to 24 weeks post-SVR stratified by HS status at baseline.

Variables	Group 1 HS at baseline: Absent			Group 2 HS at baseline: Present		
	Baseline (median)	24 weeks (median)	P-value	Baseline (median)	24 weeks (median)	P-value
CAP (dB/m)	217	230	0.0004**	275	291	0.2357
LSM (kPa)	7.7	5.3	0.0000**	8.6	6.2	0.0000**
FIB-4 score	2.22	1.58	0.0000**	1.92	1.64	0.0000**
Total Cholesterol (mg/dL)	162	184.4	0.0084**	177.5	184.5	0.0296**
LDL (mg/dL)	95	108	0.4270	100	104	0.0666
HDL (mg/dL)	51.8	52	0.6474	52.4	48.5	0.9888
Triglycerides (mg/dL)	69	92	0.0000**	108	95	0.7454
BMI (kg/m^2)	24.2	24.75	0.4422	27.5	27.8	0.3498
Glucose (mg/dL)	94	93.6	0.4923	97	94	0.5863
ALT (I/U)	61.5	18	0.0000**	62	18	0.0000**
AST (I/U)	52	24	0.0000**	49	23	0.0000**
GGT (I/U)	47	18	0.0000**	45	18	0.0000**
PLT ($\times 10^9$/L)	186	208	0.0605**	202	227.5	0.0001**

Data represented as median & interquartile range (IQR). ** $P < 0.05$, based on Wilcoxon signed-rank test.
ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; CAP: controlled attenuation parameter; FIB-4: fibrosis-4 score; GGT: gamma-glutamyl transferase; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; LSM: liver stiffness measurement; PLT: platelets.

Table 4
Multivariable logistic regression model to identify factors associated with HS at 24 weeks post-SVR.

Variable	Adjusted odds ratio (aOR)	95 % Confidence interval (CI)	P-value
BMI	1.923	1.22 – 3.03	0.0049
Glucose	0.987	0.94 – 1.03	0.5728
Triglycerides	1.017	0.99 – 1.04	0.1299
ALT	1.0	0.89 – 1.12	0.9945
LSM	0.997	0.71 – 1.39	0.9864
Total cholesterol	0.985	0.95 – 1.01	0.3391

remain unnoticed. Second, these results highlight the need for ongoing metabolic surveillance and targeted weight management strategies in the post-SVR population, particularly among individuals without baseline steatosis who may still be vulnerable to metabolic dysfunction over time.

This study has several limitations, including its retrospective design, small sample size and relatively short follow-up period. However, as a real-world cohort analysis, it offers valuable insights into early post-SVR liver dynamics that may be overlooked in tightly controlled clinical trials. The use of routinely collected clinical data reflects real-life practice, enhancing the practical relevance of our findings for routine clinical decision-making. Moreover, this study is well-suited for hypothesis generation and can inform future prospective investigations. In addition, the number of patients with genotype 3 infection was small, limiting our ability to perform genotype-specific comparisons regarding HS dynamics. This is particularly relevant given the known association between genotype 3 and "viral steatosis." With respect to the regression analysis, although multicollinearity among predictors was assessed and found to be minimal, the sample size may still have limited power to detect smaller associations or interactions. The number of covariates was intentionally restricted to avoid overfitting, but residual confounding cannot be excluded. Despite its limitations, the inclusion of patients from two international centers strengthens the external validity of our results and supports their generalizability across diverse healthcare settings.

In conclusion, while non-invasive liver fibrosis markers show clear improvement following HCV cure with DAA, HS may persist or newly develop, particularly among individuals with elevated BMI. This paradoxical trajectory highlights a shift in the post-SVR landscape from viral to metabolically driven liver disease. Our findings emphasize the importance of integrated metabolic care and continued liver health monitoring in this population. BMI emerged as a strong independent predictor of steatosis at follow-up, suggesting it may serve as a practical target for clinical risk stratification and early intervention aimed at preventing progression to MASLD and its associated complications.

Financial support

MS received a PhD fellowship from Fonds de Recherche Québec - Santé (FRQ-S) and the Canadian Network on Hepatitis C (CanHepC). WE has received a MSc fellowship from CanHepC. CanHepC is funded by a joint initiative of the Canadian Institute of Health Research (NPC-178912) and the Public Health Agency of Canada. GS is supported by a Merite Salary Award from FRQS (#366391).

CRediT authorship contribution statement

Mohamed Shengir: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Wesal Elgretli:** Writing – review & editing, Validation, Data curation. **Felice Cinque:** Writing – review & editing, Investigation, Data curation. **Agnihotram V. Ramanakumar:** Writing – review & editing, Methodology, Formal analysis. **Rosa Lombardi:** Writing – review & editing, Resources, Investigation, Data curation. **Annalisa Cespiati:** Writing – review & editing, Data curation. **Anna Ludovica Fracanzani:** Writing – review & editing, Resources, Data curation. **Luz Ballesteros:** Writing – review & editing, Project administration, Data curation. **Marc Deschenes:** Writing – review & editing, Methodology, Investigation, Data curation. **Philip Wong:** Writing – review & editing, Data curation. **Tianyan Chen:** Writing – review & editing, Validation, Data curation. **Giada Sebastiani:** Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

GS has acted as speaker for Merck, Gilead, Abbvie, Novo Nordisk, Eli Lilly, Pfizer, served as an advisory board member for Gilead, GlaxoSmithKline, Merck, Novo Nordisk, Gilead and has received unrestricted research funding from Novo Nordisk.

FC, RL, AC, ALF, MD, PW, TC, MS, WE, AVR, and LB have nothing to disclose.

References

[1] (WHO) WHO. Hepatitis C [Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-c; 2023.
[2] Global change in hepatitis C virus prevalence and cascade of care between 2015 and 2020: a modelling study. Lancet Gastroenterol Hepatol 2022;7(5):396–415.
[3] Bota S, Sporea I, Sirli R, Popescu A, Neghină AM, Dănilă M, et al. Severe adverse events during antiviral therapy in hepatitis C virus cirrhotic patients: a systematic review. World J Hepatol 2013;5(3):120–6.
[4] Shehab TM, Fontana RJ, Oberhelman K, Marrero JA, Su GL, Lok AS. Effectiveness of interferon alpha-2b and ribavirin combination therapy in the treatment of naive chronic hepatitis C patients in clinical practice. Clin Gastroenterol Hepatol 2004;2(5):425–31.
[5] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales Jr FL, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347(13):975–82.
[6] Liu CH, Chang YP, Fang YJ, Cheng PN, Chen CY, Kao WY, et al. Dynamic change of metabolic dysfunction-associated steatotic liver disease in patients with hepatitis C virus infection after achieving sustained virologic response with direct-acting antivirals. J Gastroenterol 2024;59(7):609–20.
[7] Bhattacharya D, Aronsohn A, Price J, Lo Re V. Hepatitis C Guidance 2023 update: AASLD-IDSA recommendations for testing, managing, and treating Hepatitis C Virus infection. Clin Infect Dis 2023.
[8] Younossi ZM, Stepanova M, Henry L, Han KH, Ahn SH, Lim YS, et al. Sofosbuvir and ledipasvir are associated with high sustained virologic response and improvement of health-related quality of life in East Asian patients with hepatitis C virus infection. J Viral Hepat 2018;25(12):1429–37.
[9] Cuesta-Sancho S, Márquez-Coello M, Illanes-Álvarez F, Márquez-Ruiz D, Arizcorreta A, Galán-Sánchez F, et al. Hepatitis C: problems to extinction and residual hepatic and extrahepatic lesions after sustained virological response. World J Hepatol 2022;14(1):62–79.
[10] Negro F. Residual risk of liver disease after hepatitis C virus eradication. J Hepatol 2021;74(4):952–63.
[11] Terrault NA, Hassanein TI. Management of the patient with SVR. J Hepatol 2016;65(1 Suppl):S120–1s9.
[12] Noureddin M, Wong MM, Todo T, Lu SC, Sanyal AJ, Mena EA. Fatty liver in hepatitis C patients post-sustained virological response with direct-acting antivirals. World J Gastroenterol 2018;24(11):1269–77.
[13] Rout G, Nayak B, Patel AH, Gunjan D, Singh V, Kedia S, et al. Therapy with oral directly acting agents in Hepatitis C infection is associated with reduction in fibrosis and increase in hepatic steatosis on transient elastography. J Clin Exp Hepatol 2019;9(2):207–14.
[14] Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016;64(1):73–84.
[15] Sheridan DA, Shawa IT, Thomas EL, Felmlee DJ, Bridge SH, Neely D, et al. Infection with the hepatitis C virus causes viral genotype-specific differences in cholesterol metabolism and hepatic steatosis. Sci Rep 2022;12(1):5562.
[16] Adinolfi LE, Rinaldi L, Guerrera B, Restivo L, Marrone A, Giordano M, et al. NAFLD and NASH in HCV infection: prevalence and significance in hepatic and extrahepatic manifestations. Int J Mol Sci 2016;17(6).
[17] Abenavoli L, Masarone M, Peta V, Milic N, Kobyliak N, Rouabhia S, et al. Insulin resistance and liver steatosis in chronic hepatitis C infection genotype 3. World J Gastroenterol 2014;20(41):15233–40.
[18] Bartenschlager R, Penin F, Lohmann V, André P. Assembly of infectious hepatitis C virus particles. Trends Microbiol 2011;19(2):95–103.
[19] Herker E, Ott M. Unique ties between hepatitis C virus replication and intracellular lipids. Trends Endocrinol Metab 2011;22(6):241–8.

[20] Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, et al. A multi-society Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol 2023.
[21] Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. J Hepatol 2020;73(1):202–9.
[22] Liu CH, Cheng PN, Fang YJ, Chen CY, Kao WY, Lin CL, et al. Risk of de novo HCC in patients with MASLD following direct-acting antiviral-induced cure of HCV infection. J Hepatol 2025;82(4):582–93.
[23] Elgretli W, Shengir M, Sasson S, Ramanakumar AV, Cinque F, Ballestreros LER, et al. Association of MASLD phenotypes with liver fibrosis in Hepatitis C: the role of cardiometabolic risk factors. J Viral Hepat 2025;32(2):e70004.
[24] Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2018;67(1):328–57.
[25] Sasso M, Tengher-Barna I, Ziol M, Miette V, Fournier C, Sandrin L, et al. Novel controlled attenuation parameter for non-invasive assessment of steatosis using Fibroscan (®): validation in chronic hepatitis C. J Viral Hepat 2012;19(4):244–53.
[26] Karlas T, Petroff D, Sasso M, Fan JG, Mi YQ, de Lédinghen V, et al. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. J Hepatol 2017;66(5):1022–30.
[27] EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis - 2021 update. J Hepatol 2021;75(3):659–89.
[28] EASL-ALEH Clinical Practice Guidelines: non-invasive tests for evaluation of liver disease severity and prognosis. J Hepatol 2015;63(1):237–64.
[29] Amer J, Alnees M, Salameh M, Daraghmeh A, Kabha A, AlHabil Y, et al. The diagnostic utility of FIB-4 as a non-invasive tool for liver fibrosis scoring among NAFLD patients: a retrospective cross-sectional study. Eur Rev Med Pharmacol Sci 2024;28(8):3104–11.
[30] Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, et al. Development of a simple non-invasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43(6):1317–25.
[31] von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The strengthening the reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol 2008;61(4):344–9.
[32] van der Meer AJ, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. JAMA 2012;308(24):2584–93.
[33] Pan JJ, Bao F, Du E, Skillin C, Frenette CT, Waalen J, et al. Morphometry confirms fibrosis regression from sustained virologic response to direct-acting antivirals for Hepatitis C. Hepatol Commun 2018;2(11):1320–30.
[34] Iwamoto T, Nozaki Y, Inoue T, Suda T, Mizumoto R, Arimoto Y, et al. Histological improvement of fibrosis in patients with hepatitis C who achieved a 5-year sustained virological response to treatment with direct-acting antivirals. J Gastroenterol 2025;60(2):197–209.
[35] Negro F. Hepatitis C virus-induced steatosis: an overview. Dig Dis 2010;28(1):294–9.
[36] Cespiati A, Petta S, Lombardi R, Di Marco V, Calvaruso V, Bertelli C, et al. Metabolic comorbidities and male sex influence steatosis in chronic hepatitis C after viral eradication by direct-acting antiviral therapy (DAAs): evaluation by the controlled attenuation parameter (CAP). Dig Liver Dis 2021;53(10):1301–7.
[37] Ogasawara N, Kobayashi M, Akuta N, Kominami Y, Fujiyama S, Kawamura Y, et al. Serial changes in liver stiffness and controlled attenuation parameter following direct-acting antiviral therapy against hepatitis C virus genotype 1b. J Med Virol 2018;90(2):313–9.
[38] Petta S, Maida M, Macaluso FS, Di Marco V, Cammà C, Cabibi D, et al. The severity of steatosis influences liver stiffness measurement in patients with nonalcoholic fatty liver disease. Hepatology 2015;62(4):1101–10.
[39] Pais R, Charlotte F, Fedchuk L, Bedossa P, Lebray P, Poynard T, et al. A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. J Hepatol 2013;59(3):550–6.
[40] Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, et al. Liver fibrosis, but No other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty Liver disease. Gastroenterology 2015;149(2):389–97. e10.
[41] Shimizu K, Soroida Y, Sato M, Hikita H, Kobayashi T, Endo M, et al. Eradication of hepatitis C virus is associated with the attenuation of steatosis as evaluated using a controlled attenuation parameter. Sci Rep 2018;8(1):7845.
[42] Hashimoto S, Yatsuhashi H, Abiru S, Yamasaki K, Komori A, Nagaoka S, et al. Rapid increase in serum low-density lipoprotein cholesterol concentration during Hepatitis C interferon-free treatment. PLoS One 2016;11(9):e0163644.
[43] Gitto S, Cicero AFG, Loggi E, Giovannini M, Conti F, Grandini E, et al. Worsening of serum lipid profile after direct acting antiviral treatment. Ann Hepatol 2018;17(1):64–75.
[44] Gualerzi A, Bellan M, Smirne C, Tran Minh M, Rigamonti C, Burlone ME, et al. Improvement of insulin sensitivity in diabetic and non diabetic patients with chronic hepatitis C treated with direct antiviral agents. PLoS One 2018;13(12):e0209216.
[45] Mei T, Huang X, Tang S, Liu M, Zhang W, Yu H. Effects of sustained viral response on lipid in Hepatitis C: a systematic review and meta-analysis. Lipids Health Dis 2024;23(1):74.
[46] Lonardo A, Adinolfi LE, Restivo L, Ballestri S, Romagnoli D, Baldelli E, et al. Pathogenesis and significance of hepatitis C virus steatosis: an update on survival strategy of a successful pathogen. World J Gastroenterol 2014;20(23):7089–103.
[47] Gonzalez-Aldaco K, Torres-Reyes LA, Ojeda-Granados C, Leal-Mercado L, Roman S, Panduro A. Metabolic dysfunction-associated steatotic liver disease in chronic Hepatitis C Virus infection: from basics to clinical and nutritional management. Clin Pr 2024;14(6):2542–58.
[48] Tokuchi Y, Suda G, Kawagishi N, Ohara M, Kohya R, Sasaki T, et al. Hepatitis C virus eradication by direct-acting antivirals causes a simultaneous increase in the prevalence of fatty liver and hyper low-density lipoprotein cholesterolemia without an increase in body weight. Hepatol Res 2023;53(7):595–606.
[49] Wong VW, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology 2010;51(2):454–62.