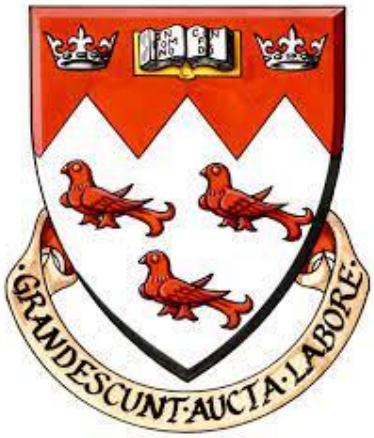

The Lasting Legacy of Metabolic Conditions in Hepatitis C:

Association Between Phenotypes of Metabolic Dysfunction-Associated Steatotic Liver Disease and Liver Fibrosis

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March 2025

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

This thesis includes a manuscript, titled **"Association of MASLD Phenotypes with Liver Fibrosis in Hepatitis C: The Role of Cardiometabolic Risk Factors,"** which has been formatted in accordance with the *Journal of Viral Hepatitis*'s submission guidelines. The manuscript was first published on January 27th, 2025. The references for this manuscript are included at the end of the chapter. References for the rest of the thesis are listed separately in the final bibliography.

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List of abbreviations

ANGPTL-3	Angiopoietin-like protein-3
COL1A1	Collagen type I alpha 1
DAAs	Direct acting antiviral agents
HCC	Hepatocellular carcinoma
HCV	Hepatitis C Virus
HDL	High density lipoprotein
HIV	Human Immunodeficiency virus
IL	Interleukin
IR	Insulin resistance
IRES	Internal ribosome entry site
LDL	Low density lipoproteins
LVPs	Lipoviroparticles
MASH	Metabolic dysfunction associated steatohepatitis
MASLD	Metabolic dysfunction associated steatotic liver disease
MASLD-HCC	MASLD-related hepatocellular carcinoma
METAVIR	Meta-analysis of Histological Data in Viral Hepatitis
MSM	Men who have sex with men
NAFLD	Nonalcoholic fatty liver disease
NPC1L1	Niemann–Pick C1-like protein 1
ORF	Open reading frame
RNA	Ribonucleic acid
SLD	Steatotic liver diseases
SVR	Sustained virologic response
Th17	T-helper 17
TNF-α	Tumor necrosis factor-alpha
UTRs	Untranslated region

Abstract

Hepatitis C virus (HCV) infection is a leading cause of chronic liver disease worldwide. While antiviral therapy achieves sustained virologic response (SVR) in over 95% of patients, metabolic dysfunction continues to influence liver fibrosis progression. Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) is increasingly recognized as a significant factor in liver health, yet its impact on fibrosis in individuals with a history of HCV infection remains underexplored. This thesis is centered on a cross-sectional study investigating the association between MASLD and significant liver fibrosis in a cohort of 590 individuals with history of chronic HCV infection from two Canadian academic centres. Liver fibrosis was assessed using innovative non-invasive imaging markers, and MASLD phenotypes were defined based on established metabolic risk factors. Unadjusted and adjusted logistic regression models were used to evaluate the relationship between MASLD phenotypes and significant fibrosis, controlling for potential confounders. The findings indicate that MASLD is strongly associated with significant liver fibrosis (adjusted odds ratio [aOR] 2.29, 95% confidence interval [CI] 1.07–4.87), with diabetic, hypertensive, and overweight MASLD phenotypes exhibiting the highest association in patients with HCV (aORs of 4.76 (95% CI 2.16–10.49), 3.44 (95% CI 1.77–6.68) and 2.54 (95% CI 1.27–5.07), respectively.) These results underscore the persistent role of metabolic dysfunction in liver disease progression, independent of viral eradication. The study highlights the necessity for an integrated, multidisciplinary approach in post-SVR management of individuals with history of chronic HCV infection that prioritizes metabolic health to mitigate fibrosis risk and optimize long-term liver outcomes.

Résumé

L'infection par le virus de l'hépatite C (VHC) est une cause majeure de maladie hépatique chronique. Bien que la thérapie antivirale permette d'atteindre une réponse virologique soutenue (RVS) chez plus de 95 % des patients, la dysfonction métabolique continue d'influencer la progression de la fibrose hépatique. La maladie du foie gras associée à une dysfonction métabolique (MASLD) est de plus en plus reconnue comme un facteur clé de la santé hépatique, mais son impact sur la fibrose chez les individus ayant des antécédents d'infection par le VHC reste peu exploré. Cette thèse porte sur une étude transversale évaluant l'association entre la MASLD et la fibrose hépatique significative dans une cohorte de 590 individus ayant des antécédents d'infection chronique par le VHC, recrutés dans deux centres académiques canadiens. La fibrose hépatique a été évaluée à l'aide de marqueurs d'imagerie non invasifs innovants, et les phénotypes MASLD ont été définis selon les facteurs de risque métaboliques établis. Des modèles de régression logistique, non ajustés et ajustés, ont été utilisés pour analyser la relation entre les phénotypes MASLD et la fibrose significative, en contrôlant les facteurs de confusion potentiels. Les résultats indiquent que le MASLD est fortement associé à une fibrose hépatique significative (rapport de cotes ajusté [RCA] de 2,29, intervalle de confiance [IC] à 95 % de 1,07 à 4,87), les phénotypes MASLD diabétique, hypertendu et en surpoids présentant la plus forte association chez les patients atteints du VHC (RCA de 4,76 (IC à 95 % de 2,16 à 10,49), 3,44 (IC à 95 % de 1,77 à 6,68) et 2,54 (IC à 95 % de 1,27 à 5,07), respectivement). Ces données soulignent le rôle persistant de la dysfonction métabolique dans la progression des maladies hépatiques, indépendamment de l'éradication virale. L'étude met en évidence la nécessité d'une approche intégrée et multidisciplinaire dans la prise en charge post-RVS des patients ayant des antécédents d'infection

chronique par le VHC, en mettant l'accent sur la santé métabolique afin de réduire le risque de fibrose et d'optimiser les résultats hépatiques à long terme.

Acknowledgement

First and foremost, I extend my deepest gratitude to Allah the Almighty, the Most Gracious, and the Most Merciful for granting me strength, patience, and wisdom throughout this journey. I am profoundly thankful for His countless blessings, guidance, and mercy, which have illuminated my path even during the most challenging times.

To my beloved family, I owe an immeasurable debt of gratitude for their unwavering love and support. To my parents, you have been my pillars of strength and my greatest role models. Your sacrifices, patience, and unconditional love have provided the solid foundation upon which I have built my dreams. The values of integrity, hard work, and compassion that you instilled in me continue to guide both my personal and academic life.

To my husband, Mohamed, I am deeply thankful for your boundless support, patience, and understanding. You have been by my side, offering reassurance and encouragement during the most intense periods of this journey. Your confidence in my abilities and your steadfast belief in my work have been a constant source of motivation, and I am incredibly blessed to share this journey with you. Your love, humor, and companionship have made this process more fulfilling and meaningful, and I am forever grateful.

A special note of thanks goes to my wonderful children, Mayar, Adam, and Ahmed. Your infectious joy, boundless energy, and unconditional love have inspired me in more ways than words can express. You remind me every day of the beauty of hope and the strength found in resilience. I cherish the happiness you bring into my life and am grateful for your presence on this journey.

I would also like to express my heartfelt appreciation to my supervisor, Dr. Giada Sebastiani, whose mentorship, wisdom, and guidance have been invaluable. Your confidence in my potential and your commitment to my success have profoundly impacted my academic journey. Thank you for challenging me, for your insightful feedback, and for encouraging me to strive for excellence.

I am also deeply grateful to the members of my thesis committee for their guidance, constructive feedback, and ongoing support throughout my research. Your insights and expertise have helped shape this work and have contributed significantly to my development as a researcher.

To my coauthors and collaborators, thank you for your critical contributions, shared knowledge, and valuable time. I am honored to have worked alongside such dedicated and brilliant colleagues. Your efforts have enriched this thesis and the research it presents.

Finally, to everyone who has supported, encouraged, or inspired me, whether in big or small ways, thank you. This thesis is not solely the product of my efforts but a testament to the love, guidance, and support I have received from so many remarkable people. I am deeply grateful to every one of you.

Contributions of authors

Thesis:

WE wrote, formatted, and revised the whole thesis. GS critically revised all thesis sections.

Manuscript: Association of MASLD Phenotypes with Liver Fibrosis in Hepatitis C: The Role of Cardiometabolic Risk Factors

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J Viral Hepat. 2025 Feb;32(2):e70004. doi: 10.1111/jvh.70004.

W. Elgretli contributed to study concept and design, acquisition of data, and interpretation of data and critical revision of the manuscript. M. Shengir, S. Sasson, L.R. Ballestreros, M. Deschenes, P. Wong, T. Chen, N. Kronfli, A. Keeshan, S. Tandon were involved in acquisition of data and critical revision of the manuscript. S. Saeed and A.V. Ramanakumar were involved in statistical analysis, interpretation of data and critical revision of the manuscript. C. Cooper was involved in acquisition and interpretation of data, critical revision of the manuscript and overall study supervision. G. Sebastiani was involved in study concept and design, acquisition and interpretation of data, analysis and drafting of manuscript, critical revision of the manuscript and overall study supervision. All the authors declare they have participated in the preparation of the manuscript and have seen and approved the final version.

Other contributions

First Author:

- **Hepatitis C Virus-Lipid Interplay: Pathogenesis and Clinical Impact**

Biomedicines. 2023 Jan 19;11(2):271. doi: 10.3390/biomedicines11020271.

Co-author:

- **Role of fatty liver in the epidemic of advanced chronic liver disease among people with HIV: protocol for the Canadian LIVEHIV multicentre prospective cohort**

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- **The effect of weight gain and metabolic dysfunction-associated steatotic liver disease on liver fibrosis progression and regression in people with HIV**

AIDS. 2024 Jul 15;38(9):1323-1332. doi: 10.1097/QAD.0000000000003903. Epub 2024 Apr 18.

- **Material deprivation is associated with liver stiffness and liver-related outcomes in people with HIV**

Liver Int. 2024 Oct;44(10):2615-2624. doi: 10.1111/liv.16022. Epub 2024 Jul 16.

- **Metabolic dysfunction-associated steatohepatitis exhibits sex differences in people with HIV**

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1. INTRODUCTION & LITERATURE REVIEW

In modern medicine, the boundaries between infectious and non-infectious diseases are becoming increasingly blurred. Historically, these conditions were treated as distinct domains; however, research now recognizes their complex interactions and mutual influence(1). Hepatitis C virus (HCV) infection is a clear example of this intersection, as it not only causes direct viral damage but also disrupts metabolic pathways, contributing to long-term health consequences. This interplay between viral infection and metabolic dysfunction is particularly relevant in the context of liver fibrosis, a key driver of liver-related morbidity and mortality worldwide(2).

The development of direct-acting antiviral agents (DAAs) has revolutionized HCV treatment, achieving cure rates exceeding 95%(3). Despite these therapeutic advancements, metabolic complications often persist after viral cure(4, 5). In particular, steatotic liver disease (SLD) and its newly defined subtype, metabolic dysfunction-associated steatotic liver disease (MASLD), are increasingly recognized as potential contributors to liver fibrosis progression even after viral eradication(4, 6, 7). While MASLD has been extensively studied in individuals without viral liver disease, its impact on liver fibrosis among individuals with a history of HCV infection remains underexplored. In addition, MASLD encompasses a heterogeneous spectrum of phenotypes defined by distinct cardiometabolic risk factors, such as diabetes, hypertension, dyslipidemia, and obesity(6). However, the relationship between these phenotypes and liver fibrosis in individuals with history of HCV infection has not been thoroughly investigated. Understanding these associations is critical, as liver fibrosis remains a primary determinant of long-term liver health. Identifying which MASLD phenotypes are most strongly associated with fibrosis could provide

valuable insights for clinical management, including the development of personalized, risk-based monitoring strategies for individuals with a history of HCV infection.

1.1. Background on hepatitis C and liver disease

Epidemiology of hepatitis C

HCV infection remains a global health concern, affecting approximately 50 million people worldwide and contributing to nearly 242,000 deaths annually due to complications like liver cirrhosis and hepatocellular carcinoma (HCC)(8). It is also a leading cause of liver transplantation(9). With significant prevalence across diverse populations, HCV is considered a major contributor to chronic liver disease, and it is especially concerning in areas where healthcare access, resources, and awareness are limited(10). As an RNA virus that infects liver cells and promotes chronic inflammation, HCV is a leading cause of advanced liver disease and associated metabolic complications(2), posing a significant challenge to healthcare systems worldwide.

Global and Regional Prevalence

Prevalence of HCV differs significantly by regions; the greatest burden is seen in countries in the Eastern Mediterranean, Sub-Saharan Africa and Central and East Asia, where prevalence rates often exceed 3% of the adult population. Egypt, for instance, historically had one of the highest prevalence rates, largely attributed to past medical practices, namely mass treatment campaigns for schistosomiasis, that inadvertently facilitated transmission. In high-income countries, prevalence rates are generally lower, yet HCV remains a considerable health burden due to its association with liver disease progression, cirrhosis, and HCC. Furthermore, the opioid epidemic in North America and parts of Europe has contributed to a resurgence in HCV transmission,

particularly through intravenous drug use, which accounts for a significant proportion of new infections in these regions(11-14).

Transmission and At-Risk Populations

HCV is primarily transmitted through blood-to-blood contact(14), making certain populations particularly vulnerable. The primary routes of transmission include:

- **Intravenous Drug Use:** Accounting for the majority of new infections in high-income countries, HCV transmission is highly prevalent among individuals who inject drugs, with an estimated 40–60% of people who inject drugs living with HCV.
- **Unsafe Medical Practices:** The reuse of needles and insufficient sterilization of medical equipment have historically played a role in HCV transmission. While blood supplies in high-income countries are highly secure, this route of transmission continues to be a major concern in low- and middle-income countries, although enhanced medical practices have helped mitigate this risk in numerous areas(14, 15).
- **Blood Transfusions and Organ Transplants:** Before the introduction of routine HCV screening of blood supplies, transfusion-related transmission was common. This route has since declined significantly due to widespread screening(14).
- **Vertical Transmission:** HCV can be transmitted from mother to child during childbirth, although this is a less common route compared to other modes of transmission. A meta-analysis by Benova *et al* estimated transmission rates of 5.8% in HCV-positive, HIV-negative women, and 10.8% in those co-infected with HIV(14, 16).

Other at-risk populations include incarcerated individuals, who face higher HCV prevalence due to intravenous drug use and unsafe tattooing practices within correctional facilities(17), and individuals undergoing hemodialysis, who may be exposed through blood-contaminated medical equipment(18). Men who have sex with men (MSM) represent a distinct population in the context of HCV transmission. While HCV is primarily transmitted through blood-to-blood contact, sexual transmission among MSM, particularly those who are HIV-positive, has been increasingly documented(19).

Disease Burden and Health Consequences

HCV contributes substantially to the global burden of liver disease. Acute HCV infection typically occurs within the first six months following exposure to the virus and is often asymptomatic. While approximately 20–30% of individuals spontaneously clear the infection, the majority of individuals with acute HCV infection progress to chronic infection, placing them at risk for long-term liver damage, including fibrosis, cirrhosis, and HCC. It is estimated that up to 15% of individuals with chronic HCV will develop cirrhosis within 20 years, with an additional 4–5% per year facing the risk of liver decompensation or liver cancer once cirrhosis has developed(20, 21). This progression underscores the importance of early diagnosis and treatment to prevent severe liver complications.

Molecular Characteristics of Hepatitis C Virus and Its Role in Lipid Dysregulation

HCV is a small, enveloped virus classified under the Hepacivirus genus of the Flaviviridae family and classified into multiple different genotypes(2, 22). While genotypes 1 and 3 remain the most common globally, accounting for 49.1% and 17.9% of infections respectively, substantial regional

variation exists. Genotype 2 is predominantly found in West Africa and some parts of South America. Genotype 4 is highly prevalent in North Africa and the Middle East, especially Egypt, where it constitutes over 90% of infections. Genotype 5 is largely restricted to Southern Africa, whereas genotype 6 is most common in Southeast Asia. These genotype patterns are shaped by both historical factors such as the trans-Atlantic slave trade and medical practices like the use of unsterilized injections during past public health campaigns(23, 24). The HCV genome is a positive-sense, single-stranded RNA around 9600 nucleotides in length, consisting of a 5′ untranslated region (UTR), an open reading frame (ORF), and a 3′ UTR. The ORF is translated by the host cell's translation machinery into a polyprotein of 3000 amino acids, which is subsequently cleaved by viral proteases and cellular peptidases into 10 viral proteins, including three structural proteins and seven nonstructural (NS) proteins (Figure 1)(2). The structural proteins at the N-terminus of the glycoprotein comprise Core and two envelope glycoproteins (E1 and E2). The Core constructs the capsid shell that encases the viral genome, whereas the glycoproteins are situated within the lipid envelope that encircles the capsid(2). The NS proteins (P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) are located at the C-terminus of the polyprotein and are essential for viral RNA production, assembly, and various stages of the viral life cycle. P7 is a small protein located directly downstream of E2, which is essential for viral assembly and the release of viral particles(25-27). Studies has shown that, both in vitro and in vivo, the Core protein may significantly influence the transcriptional regulation of the lipid-regulating factor angiopoietin-like protein-3 (ANGPTL-3). ANGPTL-3 may serve as a persistent molecular fingerprint of HCV, potentially contributing to the ongoing dysregulation of lipid metabolism in individuals who have successfully eradicated the virus and may predispose some to the development of HCC through core-induced hepatocarcinogenic mechanisms(28, 29).

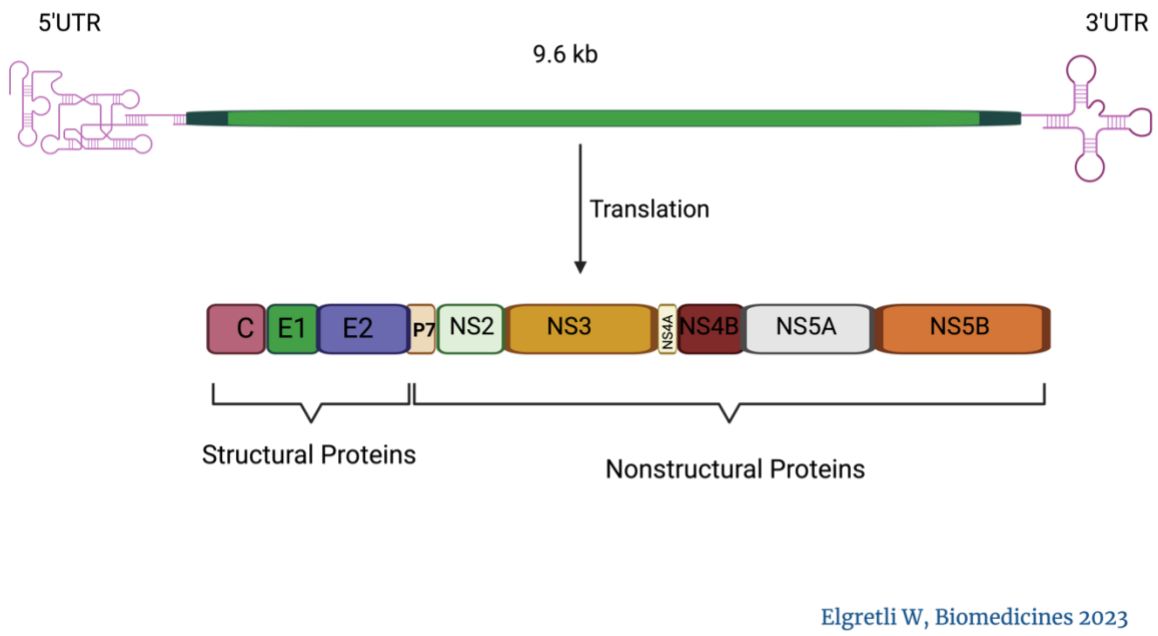

Figure 1. Schematic representation of the HCV genome and polyprotein precursor.

The HCV genome encodes a polyprotein of approximately 3000 amino acids and consists of an ORF bordered by 5′ and 3′ UTRs. After translation, the core, E1, and E2 proteins, along with p7, are cleaved from the polyprotein by cellular peptidases. The protease activity of NS2 cleaves NS2 from NS3, while the viral replication components (NS3-NS5B) are cleaved by the NS3-4A protease. C: core protein; E1 and E2: envelope glycoproteins E1 and E2; NS: nonstructural(2).

The Unique Biophysical and Lipid-Associated Properties of HCV Particles

The most notable feature of infectious HCV particles is their buoyant density, which is both remarkably low and heterogeneous for an enveloped RNA virus. HCV particles isolated from the patients' extracellular compartment are closely associated with lipoproteins, resulting in the formation of hybrid particles known as 'lipoviroparticles' (LVPs)(30, 31). The LVPs consist of viral components including a nucleocapsid containing the single-stranded RNA genome, which is associated with the viral core protein and an enveloping membrane containing surface

glycoproteins E1 and E2, alongside several apolipoproteins such as apoE, apoB, apoCI, apoCII, and apoCIII(32) (Figure 2). This leads to HCV exhibiting low-density properties distributed across a wide spectrum of density gradients ranging from 1.03 to 1.20 g/cm³(33). Additionally, HCV particles possess lipid and cholesteryl ester components analogous to those of very-low density lipoproteins (VLDL) and low-density lipoproteins (LDL)(34, 35). The presence of these apolipoproteins affects HCV stability and resistance to neutralizing antibodies, facilitating the virus's attachment to several lipoprotein receptors and its entry into hepatocytes(30, 36). Beyond structural and infectivity characteristics, LVPs have also been shown to induce robust inflammatory responses, particularly activating type 3 immunity. This includes the recruitment of T-helper 17 (Th17) cells and the interleukin IL-17/IL-23 axis, which promotes neutrophil infiltration and hepatic inflammation, key contributors to fibrosis and cirrhosis in chronic HCV infection(37).

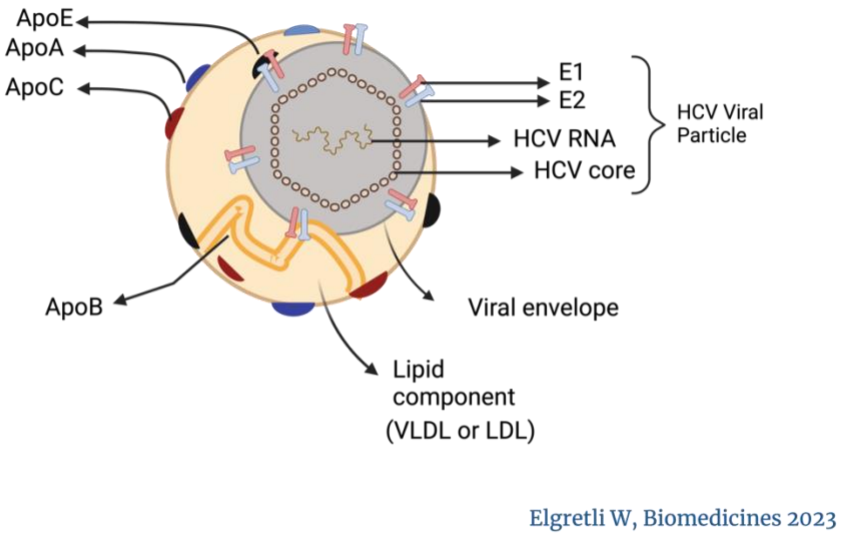

Figure 2. Hepatitis C Virus Lipoviroparticles (LVP).

The highly infectious HCV particle is a hybrid entity comprised of VLDL or LDL components and viral components, referred to as LVP. VLDL: very-low-density lipoproteins; LDL: low-density lipoproteins(2).

HCV Lifecycle and Dependence on Host Lipid Metabolism

The life cycle of HCV starts with its entry into cells. The procedure is a complex multistep mechanism involving the interaction between the virus and host cellular components(38, 39). The first stage of HCV entry from the bloodstream into target cells entails the interaction of apoE on the surface of HCV-LVPs with glycosaminoglycans and LDL receptors(40, 41). Under normal physiological environment, LDL receptors facilitate the intracellular transport of cholesterol-rich LDL via clathrin-mediated endocytosis. The competitive interaction between HCV and LDL for LDL receptors suggests that elevated levels of apoB-associated cholesterol, such as LDL, may predict HCV treatment response(42, 43). Upon the completion of the first phase, the viral envelope glycoprotein E2 engages with scavenger receptors class B type I, a high-density lipoprotein (HDL) receptor that additionally binds VLDL and LDL particles(44, 45). This interaction with scavenger receptors class B type I induces conformational changes in the E2 glycoprotein, facilitating HCV's association with tetraspanin CD81 to create the HCV-CD81 complex(46). Following its binding to HCV, CD81 translocates to the tight junctions and binds with claudin-1 and occludin. This interaction results in the cellular ingestion of the virus, facilitated by a clathrin-dependent endocytosis mechanism. This movement is dependent on various regulatory mechanisms, including receptor tyrosine kinases and Niemann–Pick C1-like protein 1 (NPC1L1)(47, 48). NPC1L1 is a transmembrane receptor for cholesterol absorption, predominantly found in intestinal enterocytes and hepatocytes(49). The NPC1L1 is essential for viral entrance through cholesterol

modulation, whereas receptor tyrosine kinases facilitate HCV entry by modulating CD81-claudin-1 co-receptor interactions and membrane fusion. Some studies suggest that receptor tyrosine kinases could serve as a potential target for the prevention and treatment of HCV infection(50). Another study demonstrated that the translocation of CD81 to the tight junction is dependent on the activation of the epidermal growth factor receptor, which initiates the actin-mediated lateral membrane diffusion of HCV-CD81 complexes. HCV internalization prompts the fusion of viral glycoproteins with early endosomes and the acidification of the vacuole(51). Subsequent to this pH-dependent mechanism, the HCV capsid is transported into the cytosol, degraded, and the resultant HCV genomic RNA is ready for translation to generate viral proteins and begin viral reproduction.

Following to the release of the HCV genome into the cytosol, viral replication is promoted by the interaction of HCV with several lipid-associated components. The positive-sense single-stranded RNA is made ready for translation by host ribosomal subunits located on the rough endoplasmic reticulum. Thereafter, the ribosome–RNA complex interacts with the endoplasmic reticulum membrane, starting HCV polyprotein translation. The translation of the HCV genome is predominantly regulated by a highly conserved structural area known as the internal ribosome entry site (IRES) and the microRNA-122 binding site, both situated in the 5′ UTR(52, 53). The 5′UTR IRES facilitates the binding of HCV viral RNA to the ribosomal subunit, thereby initiating translation. MicroRNA-122 is a liver-specific human microRNA that plays a crucial role in HCV virus replication within liver cells(54, 55). The binding of microRNA-122 to viral RNA leads to the overexpression of HCV RNA and genes associated with plasma cholesterol and hepatic fatty acid metabolism(56, 57). A solitary polyprotein of approximately 3000 amino acids is produced

by the translation process, thereafter, undergoing proteolytic processing within the rough endoplasmic reticulum utilizing both cellular and viral proteases. The final output consists of 10 mature HCV proteins, encompassing both structural and nonstructural proteins. Subsequent to the generation of viral proteins, the nonstructural proteins are securely incorporated into or linked with the endoplasmic reticulum membrane via a geranylgeranyl pyrophosphate-mediated mechanism(57-59). Geranylgeranyl pyrophosphate is a product of the cholesterol biosynthesis pathway, and its function in viral protein-membrane interaction is mostly influenced by the fatty acid composition of the cell. Inhibition of fatty acid production results in the suppression of HCV viral replication(57, 60). The viral proteins NS4B and NS5A activate cellular lipid lipase, resulting in modifications to the endoplasmic reticulum membrane structure that create clusters of cholesterol-rich double-membrane vesicles associated with intracellular lipid droplets(61-63). This constitutes the membranous web utilized for viral propagation. Various lipid transfer proteins, including Niemann–Pick C1 protein, which facilitates the transport of LDL-derived cholesterol, have been shown to be essential in the recruitment of cholesterol to the membranous web. Pharmacological suppression of Niemann–Pick C1 protein resulted in a reduction in cholesterol at replication sites, thereby diminishing HCV viral replication(64). Within the membrane web, nonstructural proteins function as a replication complex responsible for the replication of newly generated viral RNA. In the process facilitated by NS5B, the RNA-dependent RNA polymerase, the positive RNA genome acts as the template for the synthesis of negative HCV RNA strands. Consequently, the newly formed strands act as templates for the creation of positive HCV RNA strands. Simultaneously with the synthesis of new RNA strands, new viral proteins are produced during the translation process. Following the synthesis of positive HCV RNA strands and viral structural proteins, the assembly of new HCV particles can start(65).

To start virion assembly, it is believed that replicated genomes must be liberated from the membranous web to interact with the core protein that forms the virion capsid. The early phases of HCV assembly have been shown to require the interaction between the core protein and lipid droplets. Several studies have shown both the lipid droplets and VLDL formation pathways as significant contributors from the host cell to HCV assembly(66). lipid droplets are cytosolic storage organelles synthesized in the endoplasmic reticulum, consisting of triglycerides and cholesterol esters encased in phospholipid monolayers covered with various surface proteins(67). The core protein consists of two domains (D1 and D2), with the D2 domain facilitating the core–lipid droplets interaction. A mutation in D2 disrupts the core–lipid droplets association, resulting in considerably fewer infectious HCV production(68, 69). The core's ability to localize to lipid droplets is thought to be primarily dependent on host diacylglycerol acyltransferase 1, an enzyme necessary for triglyceride production in the endoplasmic reticulum, as well as lipid droplet and VLDL morphogenesis(70). Due to its localization to the lipid droplets, this facilitates the core protein's recruitment of newly synthesized HCV RNA from the membranous web and envelope E1 and E2 proteins from the endoplasmic reticulum(71). The NS5A protein is thought to assist in the transport of viral RNA to the lipid droplet for encapsulation by the core protein. The process necessitates the interaction of NS5A with the D1 domain of the core protein(72). Subsequently, the core–capsid complex translocates to the endoplasmic reticulum membrane, where it engages with viral E1/E2 proteins to generate the envelope, which is obtained through budding into the endoplasmic reticulum at lipoprotein sites, facilitating lipidation through the interaction between the virion and lipoproteins. The assembly of HCV has been associated with elements of the VLDL synthesis and secretion pathway, including microsomal triglyceride transfer protein (MTP)(73),

apoB(74), and apoE(75-77). The initial phase in VLDL synthesis involves the lipidation of apoB-100, facilitated by MTP, to produce a pre-VLDL particle(78). Pre-VLDL then merges with triglyceride-laden droplets to create VLDL(79). The incorporation of apo-E and apo-CIII on the surface of lipid droplets appears to be facilitated by MTP inhibitors(80). Studies showed that MTP inhibitors exert a more significant effect on HCV secretion compared to VLDL. HCV replication complexes derived from human hepatoma cells contain all proteins requisite for VLDL formation(73). The mature HCV associates with VLDL and is secreted via the VLDL secretion pathway as LVP.

Hepatitis C Virus and Lipid Metabolism: Clinical Implications

Circulating Hypocholesterolemia and Altered Lipid Profile

HCV infection, irrespective of its duration, has been shown to significantly alter plasma lipid levels. Both acute and chronic HCV infections are associated with reduced levels of circulating LDL, apolipoprotein B100, and total cholesterol when compared to healthy controls(81-83). This hypocholesterolemic state is particularly evident in individuals with non-genotype 1 infections, where apolipoprotein B100 levels are inversely correlated with viral load(83). HCV's impact on lipid metabolism stems from its interference with multiple pathways in hepatocytes, including enhanced lipid biosynthesis, impaired mitochondrial oxidation, reduced lipid degradation, and decreased secretion of apolipoproteins such as VLDL. These changes result in intracellular lipid accumulation and reduced circulating lipid levels(84). Interestingly, lipid levels, particularly LDL and HDL, have been linked to SVR rates. Higher levels of these lipids are associated with better treatment outcomes, possibly due to the dependence of HCV on LDL cholesterol and LDL receptors for cellular entry and replication. After successful antiviral treatment or spontaneous

clearance, cholesterol and apo-lipoprotein levels typically return to baseline, underscoring the role of HCV in driving these metabolic changes(43, 45). However, the normalization of lipid profiles post-SVR has been linked to adverse cardiovascular outcomes. The reversal of hypocholesterolemia often results in elevated levels of LDL, including the atherogenic small dense LDL, which increases the risk of atherosclerotic cardiovascular disease. Studies have reported a subset of patients requiring lipid-lowering therapy after achieving SVR due to significant hypercholesterolemia. These findings highlight the need for careful monitoring of lipid profiles and cardiovascular risk in patients with history of HCV infection after achieving SVR(85).

Hepatic Steatosis

Hepatic steatosis, characterized by the excessive accumulation of intrahepatic fat of at least 5% of liver volume, is a hallmark of HCV infection and has both clinical and prognostic implications(86). It can be detected in 40-85% of patients with chronic hepatitis C(2). This condition, first observed in liver biopsies of non-A, non-B hepatitis patients, is now recognized as a common histological finding in chronic HCV infection(87, 88). The prevalence and severity of hepatic steatosis vary by HCV genotype, with genotype 3 being most strongly associated with steatosis, often termed “viral steatosis”(89). This type of steatosis is directly linked to viral load and induced by HCV proteins, such as Core and NS5A, which disrupt lipid pathways, promote lipid droplet accumulation, and inhibit VLDL secretion(86, 89-92). In contrast, hepatic steatosis associated with non-genotype 3 HCV infections, referred to as “metabolic steatosis,” is frequently tied to host metabolic factors, including insulin resistance (IR), obesity, and elevated body mass index.

Although several studies addressed the potential processes involved, the mechanisms underpinning the development of hepatic steatosis in the context of HCV infection remain poorly understood. HCV leverages lipid pathways for its benefit. HCV may directly induce lipid accumulation in hepatocytes. It relies on intracellular lipid droplets for the buildup of viral proteins and the packaging of viral genomes. Viral proteins, including Core and NS5A, have been demonstrated to stimulate the synthesis and accumulation of lipid droplets. Moreover, viral proteins may influence the VLDL secretory pathway by inhibiting MTP, an enzyme essential for VLDL assembly, leading to triglyceride accumulation in liver cells and the subsequent onset of hepatic steatosis(93). Furthermore, the HCV core protein may induce mitochondrial malfunction, resulting in the accumulation of reactive oxygen species and the suppression of specific antioxidant mechanisms, thereby driving the onset of severe oxidative stress in HCV infection. Studies indicate that the HCV core protein may reduce the expression of peroxisome proliferator-activated receptor alpha, a crucial regulator of fatty acid breakdown in the liver, perhaps contributing to the onset of hepatic steatosis(94). IR may significantly contribute to the onset of hepatic steatosis in individuals infected with HCV. Numerous research has been conducted on the pathophysiology of the HCV-mediated pathways that induce IR. In HCV infection, IR may arise from elevated free fatty acids and increased levels of both suppressor of cytokine signaling 3 and tumor necrosis factor alpha (TNF-α). This may consequently lead to the downregulation of insulin receptor substrate signaling and, therefore, IR(95). Consequently, IR can result in elevated glucose levels, prompting heightened insulin secretion. The presence of abundant lipogenic substrates (glucose and free fatty acids) and elevated levels of lipogenic hormones (hyperinsulinemia) resulting in excessive activation of lipogenesis(96), ultimately leading to hepatic steatosis (Figure 3).

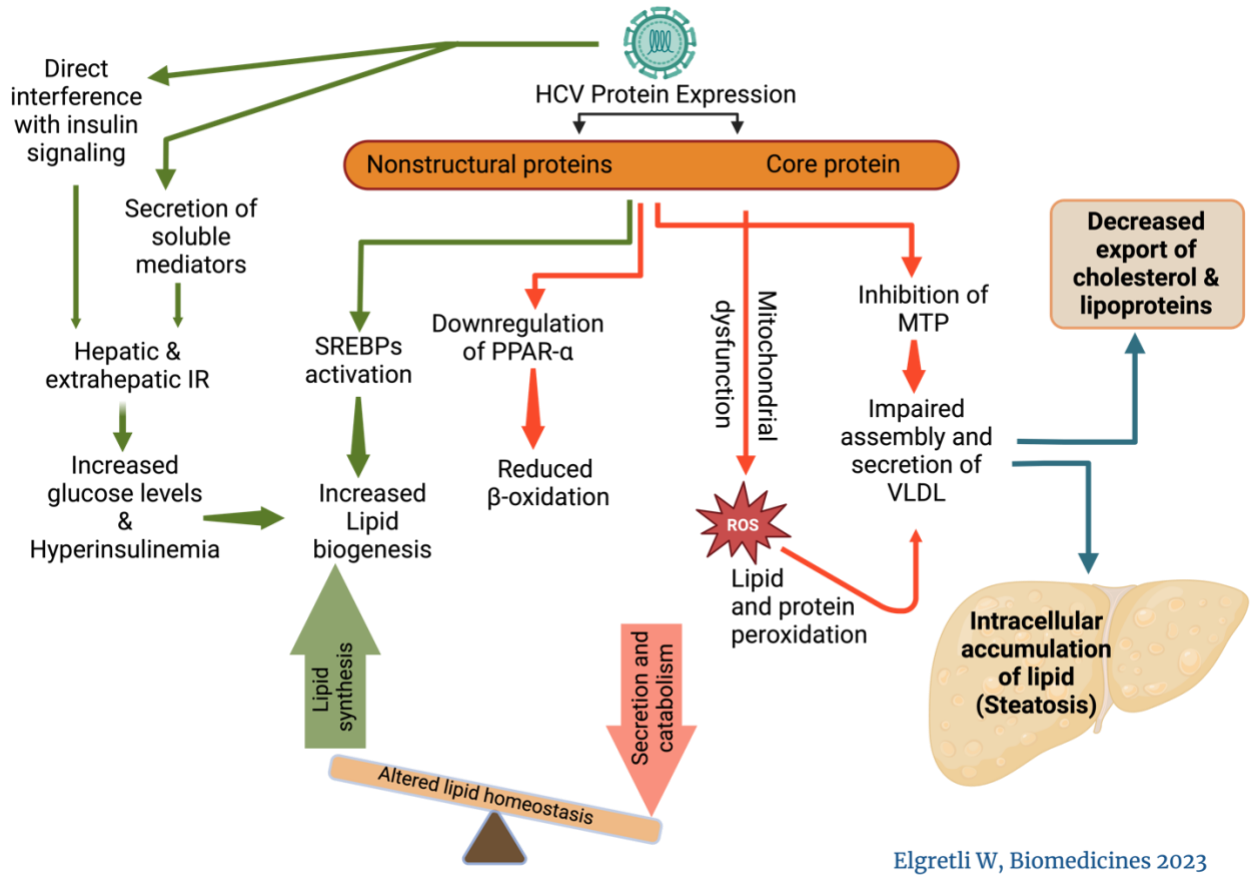

Figure 3. HCV-induced changes in lipid metabolism and steatosis.

IR: Insulin resistance; PPAR-γ: Peroxisome proliferator-activated receptor-γ; ROS: Reactive oxygen species; MTP: Microsomal triglyceride transfer protein; VLDL: very-low-density lipoproteins(2).

Treatment of Hepatitis C

The treatment landscape for HCV has evolved significantly over the past decade, transforming the prognosis for millions of patients worldwide. DAAs work by targeting and inhibiting key proteins in the HCV lifecycle, such as NS3/4A protease, NS5A, and NS5B polymerase, all of which are essential for viral replication and assembly. By disrupting these proteins, DAAs prevent the virus from replicating, allowing the immune system to clear the infection(97). Unlike older treatment

regimens that included interferon and ribavirin—which were associated with substantial side effects and lower success rates—DAAs are well-tolerated and achieve viral eradication in 8 to 12 weeks in most cases(98). The achievement of SVR, defined as undetectable HCV RNA 12 weeks after the end of treatment, is considered a functional cure and significantly reduces the risk of liver-related morbidity and mortality(99).

Residual Metabolic Challenges After HCV Cure

While DAAs effectively eliminate the virus, they do not directly address the metabolic disturbances induced by HCV, many of which can persist after achieving SVR(4, 5). For instance, HCV disrupts glucose metabolism, leading to IR, which may progress to type 2 diabetes. Viral clearance decreases the risk of developing glucose metabolic disturbances post-therapy; nonetheless, patients with pre-existing type 2 diabetes at the initiation of antiviral therapy continue to exhibit diabetes regardless of SVR, although they might require reduced dosages of antidiabetic medications. Consequently, type 2 diabetes may persist in influencing the clinical course of hepatitis C even post-cure(4, 100). Beyond glucose metabolism, achieving SVR also impacts lipid metabolism. In a recent meta-analysis, Mei *et al* found that patients with HCV infection who achieved SVR after treatment with DAAs experienced increased levels of TC and LDL from the end of treatment to one-year post-treatment. HDL levels also elevated significantly after treatment. However, triglycerides levels showed no significant change(101). This indicates a potential for dyslipidemia following SVR in these patients, necessitating monitoring and management. In parallel, hepatic steatosis emerges as another key metabolic concern post-SVR. Noureddin et al found a remarkable prevalence of steatosis at 47.5%, as assessed by controlled attenuation parameter, following SVR(102). Another study reported that, after achieving SVR in patients with

chronic hepatitis C, regression of hepatic steatosis was observed in only 31% of cases, while 23% experienced worsening results. Notably, 26% of these patients developed steatosis of 1–3 degrees despite having no steatosis prior to antiviral therapy(103). Several studies have shown that steatosis is responsible for discrepancy between biochemical and virological responses following antiviral treatment(4, 102, 104).

In summary, HCV uniquely manipulates host lipid metabolism as part of its lifecycle, setting it apart from other viral infections and positioning it as a direct driver of hepatic steatosis and lipid dysfunction. By hijacking lipid droplets, disrupting VLDL secretion, and fostering conditions favorable for lipid accumulation, HCV contributes to both immediate and long-term metabolic disturbances within the liver. These changes not only impair liver function during active infection but also set a metabolic foundation that can persist even after viral eradication, highlighting the importance of addressing these metabolic disruptions in HCV management.

1.2. Background on Metabolic Dysfunction-Associated Steatotic Liver Disease

With the growing understanding of metabolic influences on liver health, the term MASLD has been introduced to redefine liver disease associated with metabolic risk factors. MASLD represents a shift from the older term, “nonalcoholic fatty liver disease (NAFLD)”, to a diagnosis that emphasizes metabolic dysfunction as the primary driver, rather than merely defining the condition by absence of alcohol. MASLD is defined by the presence of hepatic steatosis in conjunction with at least one metabolic risk factor, such as obesity, diabetes, hypertension, or dyslipidemia. This new nomenclature better captures the metabolic origins of the condition and its close association with systemic metabolic dysfunctions that affect multiple organs, including the liver.

The diagnostic criteria for MASLD include hepatic steatosis confirmed by imaging or histology, along with one or more of the following metabolic risk factors:

- **Obesity or Overweight:** Body mass index (BMI) ≥ 25 kg/m;
- **Type 2 Diabetes or Prediabetes:** Fasting serum glucose ≥ 5.6 mmol/L (100 mg/dL) OR 2-hour post-load glucose levels ≥ 7.8 mmol/L (140 mg/dL) OR HBA1c ≥ 5.7% (39 mmol/L) OR type 2 diabetes OR treatment for type 2 diabetes;
- **Hypertension:** Blood pressure ≥ 130/85 mmHg OR specific antihypertensive drug treatment;
- **Dyslipidemia:** Plasma triglycerides ≥ 1.70 mmol/L. (≥150 mg/dL) OR Plasma HDL-cholesterol ≤ 1.0 mmol/L (40 mg/dL] (M) and ≤ 1.3 mmol/L (50 mg/dL) (F) OR lipid lowering treatment.

Unlike NAFLD, MASLD acknowledges that metabolic drivers can coexist with other liver disease, such as viral hepatitis. This shift in diagnostic criteria challenges the previously compartmentalized view of liver disease etiology, which often treated metabolic and viral -related liver diseases as mutually exclusive entities(6). This inclusion reflects a more holistic understanding of liver disease pathophysiology, where multiple contributing factors often overlap and compound liver injury.

Epidemiology of MASLD

MASLD has rapidly become the most prevalent chronic liver conditions globally, and its burden is increasing in parallel with the worldwide rise in obesity, type 2 diabetes, and metabolic syndrome. In high-income countries, where lifestyle-related metabolic conditions are common, MASLD is estimated to affect approximately 38.2% of the general population. Among individuals with obesity or diabetes, prevalence estimates are even higher, with up to 68% of patients with type 2 diabetes and nearly 90% of individuals with morbid obesity affected by MASLD(105-107). The burden of MASLD also varies by region, with higher prevalence rates observed in North America, Europe, the Middle East and parts of Asia, where lifestyle factors and urbanization have led to rising rates of metabolic syndrome. However, MASLD is increasingly common across all socioeconomic backgrounds and demographics, reflecting its close association with global trends in lifestyle and metabolic health(107). Lower socioeconomic status and material deprivation are increasingly recognized as contributors to MASLD progression and adverse liver-related outcomes. Individuals with lower socioeconomic status experience higher rates of food insecurity, poor diet quality, and limited healthcare access, which may exacerbate liver fibrosis and long-term hepatic complications(108, 109).

MASLD: A Spectrum of Disease and Its Impact on the Liver

MASLD represents a broad spectrum of liver conditions, ranging from simple hepatic steatosis to more severe forms such as metabolic dysfunction-associated steatohepatitis (MASH), which represents the inflammatory stage of MASLD, as well as fibrosis, cirrhosis, and MASLD-associated HCC (MASLD-HCC). This spectrum is influenced by a complex interplay of genetic, epigenetic, and environmental factors, including the gut microbiome and mitochondrial function(110-112). The underlying mechanisms involve lipotoxicity, oxidative stress, systemic inflammation, and hepatic stellate cell activation, all of which contribute to progressive liver injury. The presence of coexisting metabolic conditions, such as type 2 diabetes, hypertension, or obesity, amplifies these effects, increasing the risk of liver fibrosis and HCC(113).

Management of MASLD

The therapeutic management of MASLD involves a multifaceted approach that includes lifestyle modifications, pharmacological interventions, and potentially surgical options. Effective management strategies are crucial to prevent disease progression and associated complications (Figure 4).

Lifestyle modification

Lifestyle modification and weight management are fundamental in managing MASLD/MASH, as they help reduce metabolic stress, improve adipose tissue function, and support liver repair. Weight loss improves liver histology, whether achieved through lifestyle changes or bariatric surgery. A 3-5% weight loss can reduce steatosis, 7% can reverse MASH, and 10% may regress

fibrosis. Even lean MASLD patients benefit from subtle weight loss. Attention is needed to prevent sarcopenia, a condition linked to worsening liver disease, by ensuring sufficient protein intake and incorporating resistance exercises(114, 115).

Public awareness campaigns are critical to addressing the effects of obesity and sedentary lifestyles. Smoking and alcohol should be avoided due to their role in MASLD progression and cancer risk, with strict alcohol abstinence advised for those with significant liver fibrosis or cirrhosis. Multidisciplinary care involving nutritionists, exercise therapists, psychological counselors, and digital therapies (e.g., smartphone apps) can provide tailored diet and exercise plans, enhancing patient engagement and adherence to lifestyle interventions(114, 115).

Pharmacological Treatment

Pharmacological treatments for MASLD are primarily used to complement lifestyle modifications, which remain the cornerstone of management. These treatments are particularly recommended when lifestyle changes alone fail to adequately slow disease progression(114). Several drugs that are typically used to manage cardiometabolic co-morbidities have shown potential efficacy in slowing the progression of MASLD. These medications can have beneficial effects on the liver by addressing underlying issues such as IR and adipose tissue dysfunction. Some of these pharmacological agents have demonstrated efficacy on histological endpoints, which are likely to translate into long-term clinical benefits for patients with MASLD. This suggests that these drugs not only manage symptoms but may also impact the disease at a cellular level(114).

- **Insulin Sensitizers:** Medications like metformin and thiazolidinediones (e.g., pioglitazone) are often used to improve insulin sensitivity, which can help reduce liver fat accumulation(114).

- **Lipid-Lowering Agents:** Statins and other lipid-lowering drugs may be used to manage dyslipidemia, a common co-morbidity in MASLD patients(114).
- **Antioxidants and Anti-inflammatory Agents:** Vitamin E and other antioxidants have been studied for their potential to reduce liver inflammation and oxidative stress(114).
- **Glucagon-Like Peptide-1 (GLP-1) Receptor Agonists:** These drugs, such as liraglutide, are used for their weight loss benefits and potential positive effects on liver histology(114).

There are several MASH-specific drugs currently in development that are expected to expand the therapeutic options available for MASLD, particularly in non-cirrhotic stages. These emerging treatments hold promise for more targeted and effective management of the disease.

- **Resmetirom**, a thyroid hormone receptor beta agonist. Resmetirom demonstrated significant benefits in fibrosis regression, MASH resolution, and other markers, with favorable safety and tolerability(114).

Other promising drugs in development include:

- **Lanifibranor**, a pan peroxisome proliferator-activated receptor agonist, and **semaglutide**, a GLP-1 receptor agonist, both in Phase 3 trials(114).
- **Pegozafermin**, a fibroblast growth factor 21 agonist, which has shown positive Phase 2 results and is entering Phase 3(114).
- **Dual and triple GLP-1/glucagon/glucose-dependent insulinotropic polypeptide agonists**, which induce significant weight loss and may have stronger intrahepatic effects on fibrosis compared to GLP-1 receptor agonists alone(114).

- Gene-targeting therapies, focusing on patatin-like phospholipase domain-containing protein 3 and hydroxysteroid 17-beta dehydrogenase 13, are being explored as potential avenues for treating MASH(116).

Bariatric surgery

Bariatric surgery is a highly effective intervention for individuals with MASLD, particularly those with severe obesity (BMI ≥35 kg/m²) and metabolic comorbidities. It is recognized not only for significant and sustained weight loss but also for its metabolic benefits, including improvements in insulin sensitivity, lipid profiles, and liver histology. Several studies have demonstrated that bariatric surgery can significantly reduce hepatic steatosis, inflammation, and fibrosis, even in patients with advanced liver disease(117, 118).

Liver transplantation

Liver transplantation is the definitive treatment for end-stage liver disease due to MASLD, particularly in cases of decompensated cirrhosis or HCC. MASLD is now a leading indication for liver transplantation, surpassing viral hepatitis in many regions due to the global rise in obesity and diabetes(119).

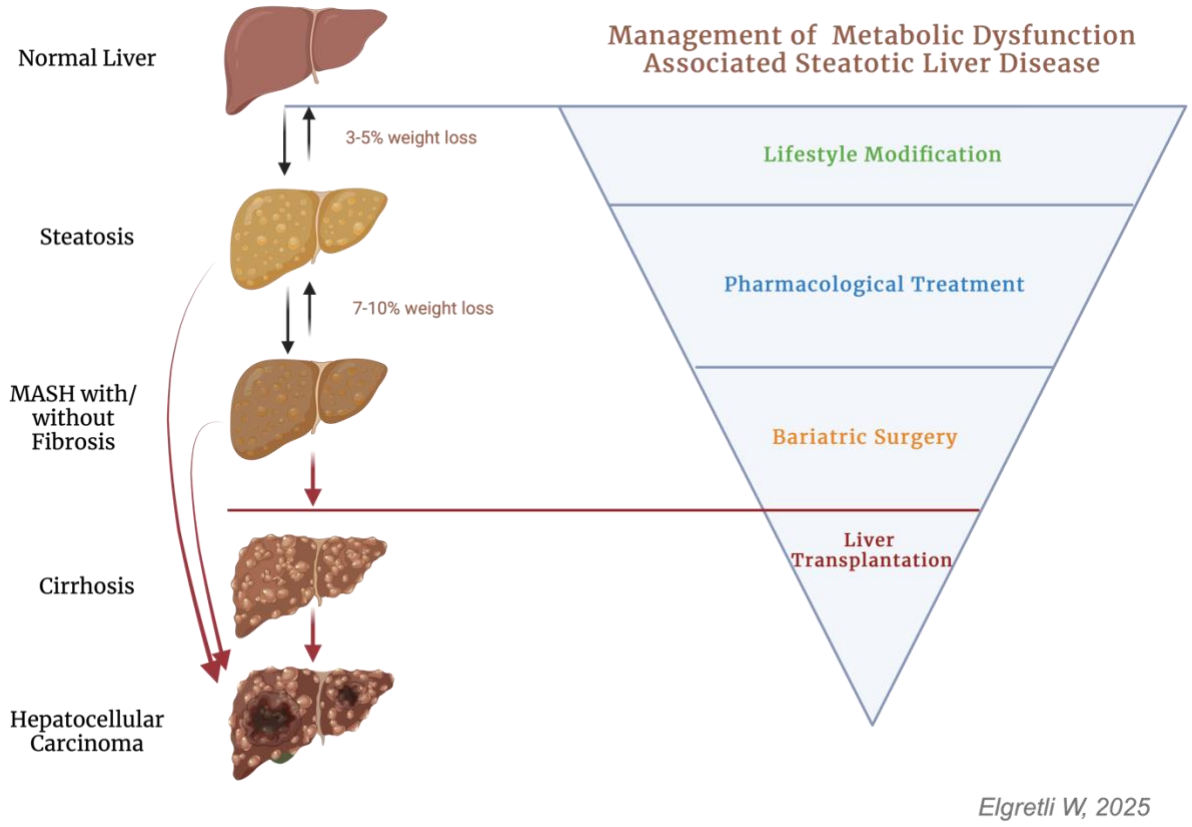

Figure 4. Management approach of metabolic dysfunction associated steatotic liver disease.

MASH: metabolic dysfunction-associated steatohepatitis

1.3. Hepatic fibrosis: Definition, stages, and consequences

Hepatic fibrosis is the excessive accumulation of extracellular matrix proteins, including collagen, in the liver as a response to chronic injury and inflammation. It is a wound-healing process that aims to contain liver damage, but over time, excessive fibrosis can disrupt normal liver structure and function(120). Unlike healthy liver tissue, which is soft and flexible, fibrotic tissue is dense and scar-like, progressively restricting blood flow and impairing liver function. Fibrosis itself is not considered liver failure; however, if left unchecked, it can progress to cirrhosis—a severe, irreversible condition associated with liver failure and high mortality risk(121, 122).

Stages of hepatic fibrosis

The progression of hepatic fibrosis is typically categorized into stages, with staging systems used to assess the degree of scarring. The most widely used staging system in viral hepatitis is the METAVIR score, which classifies fibrosis into five stages based on the extent of extracellular matrix deposition and architectural disruption(123):

- **Stage F0:** No fibrosis. The liver remains structurally intact with no significant scarring.
- **Stage F1:** Mild fibrosis. Fibrosis is limited to areas around the portal tracts (the entry points for blood vessels and bile ducts) without significant bridging between these areas.
- **Stage F2:** Moderate fibrosis. Fibrosis begins to bridge between portal tracts, forming small connections but without major architectural disruption.
- **Stage F3:** Severe fibrosis. Fibrotic tissue extensively bridges across the liver, linking portal tracts to each other and to central veins, creating structural distortions.
- **Stage F4:** Cirrhosis. Advanced fibrosis leads to the development of nodular architecture and liver stiffening, indicating severe scarring that impedes normal blood flow and liver

function. Cirrhosis greatly increases the risk of liver failure, complications from portal hypertension and HCC. Cirrhosis may be reversible in its early stages, but the precise threshold at which fibrosis becomes irreversible remains unknown(124).

Consequences of hepatic fibrosis

Hepatic fibrosis, particularly as it progresses to cirrhosis, has significant clinical implications. The consequences include:

- **Loss of liver function:** Fibrotic tissue reduces the liver's capacity to filter blood, produce essential proteins, and regulate metabolism, gradually leading to liver dysfunction(125).
- **Portal hypertension:** As fibrosis increases, blood flow through the liver is restricted, causing a rise in pressure within the portal vein system. Clinically significant portal hypertension is defined by a hepatic venous pressure gradient greater than 10 mmHg, and it is a critical factor in the progression from compensated to decompensated liver disease. The condition is associated with severe complications such as variceal hemorrhage, ascites, and hepatic encephalopathy, which significantly impact morbidity and mortality rates(126).
- **Increased risk of HCC:** The structural changes associated with fibrosis and cirrhosis predispose liver cells to malignant transformation, leading to a higher risk of liver cancer. Patients with cirrhosis are at significantly elevated risk, with HCC being one of the leading causes of death in patients with advanced liver disease(127).
- **Progressive risk of liver failure:** As fibrosis progresses, the liver's functional capacity declines, leading to liver failure in advanced cases. This often necessitates liver transplantation as the only viable treatment option in end-stage liver disease(128).

Fibrosis progression varies between individuals and depends on multiple factors, including the underlying cause, genetic predisposition, and coexisting conditions(129-131). Both HCV and MASLD can independently lead to fibrosis, each through distinct mechanisms.

Non-invasive Diagnosis of Hepatic Steatosis and Liver Fibrosis

The gold standard for diagnosing hepatic steatosis and fibrosis is liver biopsy, which provides direct histopathological assessment of liver tissue. Biopsy allows for steatosis grading based on the proportion of hepatocytes containing lipid droplets and fibrosis staging using scoring systems such as the METAVIR score in viral hepatitis or the NASH Clinical Research Network (NASH CRN) score in MASLD. Despite its diagnostic accuracy, liver biopsy has several limitations. It is an invasive procedure, associated with risks such as bleeding, pain, and infection, and it is prone to sampling error due to limited tissue collection. Additionally, interobserver variability in histological interpretation can affect diagnostic consistency. Due to these limitations, non-invasive methods, such as transient elastography (FibroScan®), are increasingly adopted in both clinical practice and research for assessing liver disease severity(132-134).

FibroScan is a widely used, non-invasive imaging technique for assessing hepatic steatosis and fibrosis, particularly in patients with chronic liver diseases, including MASLD and HCV-related liver disease. It is recognized for its reliability, reproducibility, and diagnostic accuracy, making it a valuable alternative to liver biopsy, the traditional gold standard for assessing liver pathology(132, 135).

FibroScan employs ultrasound-based elastography to measure liver stiffness, which correlates with fibrosis severity, and uses the controlled attenuation parameter (CAP) to estimate hepatic fat content, providing a comprehensive assessment of liver health. This method has gained widespread acceptance due to its non-invasiveness, quick procedure time, and low risk of complications, making it suitable for both clinical practice and large-scale epidemiological studies(132, 135, 136). During the procedure, patients lie in a supine position with their right arm raised to expose the liver area. The operator places the FibroScan probe between the rib spaces over the right liver lobe. The device then delivers multiple shear wave pulses to the liver, generating both liver stiffness measurements (LSM) for fibrosis assessment and CAP scores for steatosis quantification. To ensure accuracy, a minimum of 10 valid measurements are obtained, with results expressed in kilopascals (kPa) for stiffness and decibels per meter (dB/m) for steatosis. The procedure is painless, typically lasts 5–10 minutes, and requires no sedation or recovery time, making it highly suitable for clinical and research settings(132, 135).

Steatosis assessment using CAP

Hepatic steatosis is quantified using CAP, which measures the degree of ultrasound attenuation as the signal passes through the fatty liver tissue. Higher CAP values indicate greater steatosis severity. Steatosis is commonly defined using a diagnostic threshold of CAP ≥275 dB/m, a criterion aligned with the Clinical Practice Guidelines of the European Association for the Study of the Liver. CAP has been validated in large cohorts for its accuracy in detecting varying degrees of liver fat content, establishing it as an essential tool for diagnosing MASLD and monitoring liver health in individuals with HCV post-SVR(137, 138).

Fibrosis assessment using LSM

Liver fibrosis is assessed using LSM, a key diagnostic parameter in transient elastography. LSM reflects liver elasticity, which decreases as fibrosis progresses. Stiffness is measured in kPa, with higher values indicating more advanced fibrosis. Significant fibrosis is commonly defined as LSM ≥7.1 kPa, corresponding to METAVIR stage F2 or above, based on criteria from the European Association for the Study of the Liver and the American Association for the Study of Liver Diseases guidelines. LSM has demonstrated high diagnostic accuracy for staging liver fibrosis, with studies showing strong concordance with liver biopsy results, particularly in chronic hepatitis C and MASLD-related fibrosis(137, 139).

Role of Hepatitis C in Hepatic Fibrosis

HCV is a well-established cause of hepatic fibrosis. Chronic HCV infection leads to ongoing liver inflammation as the immune system attempts to eradicate the virus. Over time, this sustained inflammatory response promotes fibrogenesis within the liver(120, 140). Key mechanisms through which HCV drives fibrosis include:

- **Chronic Inflammation:** HCV infects hepatocytes, triggering a persistent inflammatory response. This inflammation recruits immune cells, which release pro-fibrotic cytokines and growth factors, leading to the activation of hepatic stellate cells. Once activated, hepatic stellate cells transform into myofibroblasts that produce and deposit collagen, resulting in scar tissue formation(140-142).
- **Oxidative Stress and Lipid Peroxidation:** HCV disrupts lipid metabolism, leading to the buildup of fatty acids in liver cells and increased oxidative stress. Oxidative stress further

stimulates hepatic stellate cells activation and collagen deposition, intensifying fibrotic processes(143).

- **Oxysterols and Interleukin-17 Pathway:** HCV-induced lipid metabolic dysregulation and oxidative stress contribute to the accumulation of oxysterols, oxygenated derivatives of cholesterol. Notably, Ikegami et al. reported significantly elevated levels of serum 7α-hydroxycholesterol, 25-hydroxycholesterol, and 4β-hydroxycholesterol in HCV-infected individuals, a pattern likely driven by non-enzymatic cholesterol autoxidation induced by persistent hepatic inflammation and ROS generation. These oxysterols are not only markers of oxidative stress but are also implicated in immunomodulatory effects, including the activation of IL-17–mediated inflammatory pathways. IL-17, predominantly secreted by Th17 cells, has been linked to hepatic injury and fibrogenesis, especially when metabolic dysfunction such as MASLD is present. The interplay between viral-induced oxidative lipid alterations and IL-17–driven immune responses may therefore contribute to liver disease progression in HCV-infected individuals(144). Furthermore, another study has identified oxysterols as potential immune-metabolic mediators that link lipid metabolism to chronic liver inflammation and fibrogenesis. Specifically, oxysterols such as 25-hydroxycholesterol and 7α-hydroxycholesterol can serve as ligands for the nuclear receptor retinoic acid-related orphan receptor gamma (RORγ), the master transcription factor regulating Th17 differentiation. Through this pathway, oxysterols indirectly promote IL-17 secretion, which is known to stimulate hepatic stellate cell activation and extracellular matrix deposition. Furthermore, IL-17 has been associated with insulin resistance, providing a plausible mechanism by which lipid-induced immune activation contributes to both metabolic dysfunction and hepatic fibrosis. This oxysterol–RORγ–IL-

17 axis may be particularly relevant in HCV-infected patients with coexisting MASLD, amplifying the fibrogenic milieu(144-146).

- **IR and Steatosis:** HCV also promotes IR, contributing to hepatic steatosis, especially in patients with genotype 3. The combined effect of steatosis and viral infection can accelerate fibrosis progression, as the presence of steatosis has been shown to exacerbate liver inflammation and scar formation in HCV-infected individuals(147).

Even after achieving a virologic cure, fibrosis progression may continue in some patients due to the lasting impact of HCV-related metabolic disruptions, including steatosis and IR.

Role of MASLD in Hepatic Fibrosis

MASLD is another major driver of hepatic fibrosis, particularly in populations with high rates of obesity, diabetes, and metabolic syndrome. While the association between MASLD and liver fibrosis is well established, the underlying mechanisms remain incompletely understood. Several interrelated pathways have been proposed:

- **Hepatic Steatosis and Lipotoxicity:** Excess free fatty acids and their toxic metabolites (e.g., ceramides, diacylglycerols) accumulate in hepatocytes. These toxic lipids disrupt cellular membranes and organelles, particularly mitochondria and the endoplasmic reticulum, increases the production of reactive oxygen species within hepatocytes causing oxidative stress, and ultimately hepatocyte apoptosis (programmed cell death). Damaged hepatocytes release signals that activate hepatic stellate cells, initiating fibrogenesis(148).
- **Systemic and Hepatic Inflammation:** IR, a common feature of MASLD, contributes to a pro-inflammatory environment within the liver. Inflammatory cytokines, such as TNF-α

and interleukin-6, drive chronic inflammation, further activating hepatic stellate cells and promoting collagen deposition(149). Emerging evidence underscores the contribution of type 3 immunity in MASLD-related fibrosis. Oxysterols produced under oxidative stress act through RORγ to promote IL-17 expression, a cytokine known to directly activate hepatic stellate cells and upregulate fibrogenic genes such as collagen type I alpha 1 (COL1A1). IL-6 and transforming growth factor-β further amplify this fibrogenic response by enhancing Th17 differentiation and fibrotic signaling cascades(150, 151).

- **Adipokines and Hormonal Dysregulation:** MASLD is also associated with altered adipokine levels (e.g., leptin, adiponectin) that influence liver health. Leptin promotes liver fibrosis through multiple pathways. Traditionally, leptin was believed to exert its pro-fibrotic effects primarily through direct activation of hepatic stellate cells, promoting collagen production and extracellular matrix deposition. However, recent research has revealed a more complex immuno-metabolic role**.** Leptin stimulates differentiation of naïve CD4+ T cells into Th17 lymphocytes by upregulating the nuclear receptor RORγ, a key transcription factor in Th17 polarization. These Th17 cells secrete IL-17A, a cytokine that not only reinforces hepatic inflammation but also acts directly on hepatic stellate cells to upregulate fibrogenic genes, including *COL1A1*, further promoting fibrosis. This leptin–RORγ–Th17–IL-17 axis provides a mechanistic link between adipose tissue–derived signaling and liver fibrogenesis in MASLD. Reduced adiponectin levels further compound this process by limiting anti-inflammatory and insulin-sensitizing signals, creating a permissive environment for fibrosis progression(152-154).
- **Dysbiosis:** The gut microbiota plays a significant role in MASLD-induced fibrosis through multiple interconnected mechanisms. Dysbiosis, or microbial imbalance,

increases intestinal permeability, allowing lipopolysaccharide to enter the portal circulation, where it activates Toll-like receptor 4 on Kupffer cells and hepatic stellate cells, triggering inflammation and hepatic stellate cell activation, a key step in fibrosis. Additionally, microbial metabolites influence fibrogenesis: short-chain fatty acids from beneficial bacteria reduce inflammation and inhibit hepatic stellate cell activation, while harmful metabolites such as trimethylamine-N-oxide and ethanol promote oxidative stress and fibrosis. Dysbiosis also alters bile acid metabolism, reducing anti-fibrotic secondary bile acids. Furthermore, microbial imbalance exacerbates chronic inflammation by increasing pro-inflammatory cytokines and disrupting immune tolerance, which amplifies hepatic stellate cell activation. Lastly, dysbiosis contributes to IR, a key driver of MASLD, which promotes lipotoxicity, oxidative stress, and further fibrosis progression(149, 155, 156).

2. REASEARCH QUESTIONS & HYPOTHESES:

Even after achieving SVR with DAAs, individuals with a history of HCV infection remain at risk for liver fibrosis progression. Emerging evidence suggests that metabolic dysfunction, particularly MASLD, may play a critical role in fibrosis development, yet its impact in this population remains underexplored. In particular, different MASLD phenotypes—such as diabetes, hypertension, overweight, and dyslipidemia—may contribute uniquely to fibrosis risk. Understanding these relationships is essential for improving post-SVR management and guiding targeted clinical interventions.

This thesis seeks to answer the following key research questions:

1. Is MASLD independently associated with an increased prevalence of significant liver fibrosis in individuals with a history of HCV infection, regardless of viral clearance?
2. Do specific MASLD phenotypes (diabetic MASLD, hypertensive MASLD, overweight MASLD, and dyslipidemic MASLD) exhibit differential associations with significant liver fibrosis?
3. In hepatitis C, how does the prevalence of significant liver fibrosis compare between individuals with MASLD, those with HCV-related steatosis, and those without steatosis?

To systematically investigate these questions, we propose the following hypotheses:

Null Hypotheses (H_0)

- There is no association between MASLD and the prevalence of significant liver fibrosis in individuals with a history of HCV infection.

- MASLD phenotypes (obese MASLD, hypertensive MASLD, diabetic MASLD, and dyslipidemic MASLD) are not associated with differences in the prevalence of significant liver fibrosis in individuals with a history of HCV infection.
- In hepatitis C, the prevalence of significant liver fibrosis does not differ between MASLD patients and those with steatosis without cardiometabolic conditions (HCV-related steatosis) or those without steatosis.

Alternative Hypotheses (H_1)

- MASLD is associated with an increased prevalence of significant liver fibrosis in individuals with a history of HCV infection.
- MASLD phenotypes (obese MASLD, hypertensive MASLD, diabetic MASLD, and dyslipidemic MASLD) are associated with differences in the prevalence of significant liver fibrosis in individuals with a history of HCV infection.
- In hepatitis C, the prevalence of significant liver fibrosis differs between MASLD patients and those with SLD without cardiometabolic conditions (HCV-related steatosis) or those without steatosis.

To address these research questions, this study conducted a cross-sectional analysis of 590 individuals with a history of chronic HCV infection from two Canadian academic centers. Liver fibrosis was assessed using non-invasive imaging markers, and MASLD phenotypes were classified according to established metabolic risk factors.

3. MANUSCRIPT

Association of MASLD Phenotypes with Liver Fibrosis in Hepatitis C: The Role of Cardiometabolic Risk Factors

Running title: MASLD and liver fibrosis in hepatitis C

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First published: 27 January 2025

https://doi.org/10.1111/jvh.70004

Funding

The study was not funded. W. Elgretli and M. Shengir have received MSc and PhD fellowships, respectively, from the Canadian Network on Hepatitis C (CanHepC). CanHepC is funded by a joint initiative of the Canadian Institutes of Health Research (NPC-178912) and the Public Health Agency of Canada. G. Sebastiani is supported by a Senior Salary Award from *Fonds de Recherche du Quebec – Sante (FRQS)* (#296306).

3.1. Abstract

Steatotic liver disease is prevalent among people with hepatitis C virus (HCV). The new definition of metabolic dysfunction–associated steatotic liver disease (MASLD) emphasises the metabolic drivers of steatosis and recognises its frequent coexistence with other chronic liver diseases, including HCV. We aimed to evaluate the association of coexisting MASLD and HCV with liver fibrosis. Individuals with HCV who underwent transient elastography (TE) with associated controlled attenuation parameter (CAP) were included from two clinical centres. MASLD and significant liver fibrosis were defined as the presence of steatosis (CAP ≥ 275 dB/m) with at least one cardiometabolic risk factor, and liver stiffness measurement (LSM) ≥ 7.1 kPa measured by TE, respectively. Associated cofactors of significant liver fibrosis were determined using stepwise regression and cross-validation by LASSO models to select confounders. Among 590 participants, 31% were diagnosed with MASLD. The prevalence of significant liver fibrosis was the highest among people with MASLD (58%) followed by HCV-related steatosis (45%) and the non-steatosis group (39%). After adjusting for potential confounders, MASLD was associated with significant liver fibrosis (adjusted odds ratio [aOR] 2.29, 95% confidence interval [CI] 1.07–4.87). Furthermore, specific MASLD phenotypes including diabetes, hypertension and overweight were associated with significant liver fibrosis, with aORs of 4.76 (95% CI 2.16–10.49), 3.44 (95% CI 1.77–6.68) and 2.54 (95% CI 1.27–5.07), respectively. In conclusion, MASLD is associated with liver fibrosis in people with HCV, specifically the diabetes, hypertensive and overweight phenotypes. Beyond pursuing a virological cure, healthcare providers should prioritise managing metabolic conditions, particularly diabetes, hypertension and obesity.

Keywords: hepatic steatosis; transient elastography; controlled attenuation parameter; liver fibrosis; hepatitis C virus.

3.2. Introduction

Chronic hepatitis C remains a major public health concern, with 50 million people living with the hepatitis C virus (HCV) globally and approximately 1 million new infections annually [1]. HCV is a leading cause of liver cirrhosis and hepatocellular carcinoma, often requiring liver transplantation(2). The advent of direct-acting antiviral agents (DAAs) has revolutionized HCV treatment, achieving sustained virologic response (SVR) rates as high as 98%(3,4). However, despite these excellent cure rates, unresolved clinically significant issues persist. Metabolic complications associated with HCV, both during chronic infection and post-SVR, remain a particular concern. Hepatic steatosis is a frequent condition linked to HCV, with a prevalence ranging from 40% to 86%(5). The occurrence of steatosis in HCV patients can be attributed to either the direct viral effect on lipid metabolism, termed 'viral steatosis', or to the high incidence of metabolic syndrome features associated with HCV, known as 'metabolic steatosis'(6). Several studies have shown that steatosis often persists after achieving SVR and is linked to an increased risk of liver fibrosis, especially in patients with pre-treatment metabolic conditions such as obesity, diabetes and dyslipidemia(7,8). Thus, these findings highlight the critical role of metabolic factors in the development of liver fibrosis.

In June 2023, an international consensus panel introduced a revised nomenclature for fatty liver disease to more accurately reflect its underlying mechanisms. The term steatotic liver disease (SLD) was introduced as an umbrella category encompassing all causes of steatosis(9), while metabolic dysfunction–associated steatotic liver disease (MASLD) replaced nonalcoholic fatty liver disease (NAFLD). MASLD, which is now the second leading indication for liver transplantation after HCV(7), is defined as the presence of steatosis, either by histology or imaging,

along with at least one cardiometabolic risk factor among obesity, prediabetes or diabetes, hypertension or lipid disturbances. This new nomenclature emphasises the metabolic mechanisms underlying steatosis, shifting away from potentially stigmatising terms like ‘fatty’ and ‘alcoholic’ (9). Importantly, MASLD does not exclude the coexistence of other causes of steatosis, such as HCV.

The co-occurrence of MASLD and HCV-related metabolic complications, whether during chronic HCV infection or post-SVR, may contribute to a synergistic effect, exacerbating liver injury through steatosis, oxidative stress and cellular dysfunction. It remains uncertain whether steatosis alone is responsible for this effect or if other factors are involved. Therefore, we aimed to investigate whether the presence of MASLD was associated with a higher prevalence of significant liver fibrosis in individuals with a history of HCV infection (either active chronic infection or post-SVR). Additionally, we sought to identify which cardiometabolic risk factors within the MASLD phenotypes were associated with liver fibrosis.

3.3. Methods

Study Design and Population

This was a retrospective cross-sectional study conducted at the McGill University Health Centre (MUHC) and The Ottawa Hospital, Canada. A total of 2,401 individuals with a history of HCV infection were screened, including 1,557 participants from MUHC and 844 from the Ottawa Hospital. We included consecutive adults aged 18 years or older with a history of chronic hepatitis C (either with an active infection or who had achieved SVR following antiviral treatment) who underwent transient elastography (TE) with controlled attenuation parameter (CAP) between

October 2015 and December 2023. Exclusion criteria were as follows: (a) excessive alcohol intake, defined by an Alcohol Use Disorders Identification Test (AUDIT-C) score ≥ 5(10); (b) positivity for hepatitis B virus (HBV) surface antigen or HIV antibody; (c) history of pre-existing liver disease (autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, haemochromatosis, Wilson's disease, alpha-1 anti-trypsin); (d) failure to perform TE examination or acquisition of at least 10 valid measurements. The manuscript was prepared according to the STROBE Statement-checklist of items. The Research Ethics Board (REB) of the Research Institute of the MUHC (study code 14-026-GEN 2015-1134) and the Ottawa Hospital approved the study, which followed the principles of the Declaration of Helsinki. Given that the data were collected retrospectively, the REB waived the requirement for obtaining informed consent from patients.

Clinical and Biomedical Parameters

Patient's records were reviewed retrospectively to extract clinical and biomedical parameters within 3 months of the TE examination. Collected parameters included platelets, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total cholesterol, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), triglycerides and glycated haemoglobin (HbA1c). Additional information on age, sex, ethnicity, smoking habits (current, former, or never), alcohol consumption, presence of diabetes and/or hypertension and medication use (for type 2 diabetes, hypertension, dyslipidaemia and HCV infection) was also collected. Participants with AUDIT-C scores below 5 were considered to have non-hazardous alcohol consumption(10). SVR was defined as either an undetectable qualitative polymerase chain reaction (e.g., Amplicor HCV Test v2.0) or a quantitative HCV viral load below the detection limit (e.g., Abbott Real Time HCV).

We also collected data on liver-related events, defined as a history of any among classical hepatic decompensation, hepatocellular carcinoma and liver transplantation. Classical decompensation was defined as ascites, variceal bleeding or overt hepatic encephalopathy.

Non-Invasive Diagnosis of Hepatic Steatosis and Liver Fibrosis

Liver stiffness measurement (LSM) by TE examination was performed using FibroScan (Echosens, Paris, France) on patients who had fasted for at least a 3 h, by experienced operators (> 500 examinations prior to this study). The standard M probe was used initially in all patients. The XL probe was used if BMI was ≥ 30 kg/m^2 or if the M probe failed. Valid examinations required a minimum of 10 valid measurements with an interquartile range (IQR) < 30% of the median(11,12). Steatosis was defined as CAP ≥ 275 dB/m(13). Significant liver fibrosis (stage ≥ F2 out of 4) was defined as LSM > 7.1 kPa(14).

Definition of MASLD Phenotypes

MASLD phenotypes were determined by the presence of hepatic steatosis and at least one cardiometabolic risk factor, and were as follows:

– overweight MASLD: BMI ≥ 25 kg/m^2;

– hypertensive MASLD: blood pressure ≥ 130/85 mmHg or on antihypertensive medication;

– diabetic MASLD: prediabetes (fasting glucose levels 5.6–6.9 mmol/L, 2-h post-load glucose levels 7.8–11.0 mmol/L, or an HbA1c 5.7%–6.4%) or type 2 diabetes (fasting glucose levels > 6.9 mmol/L, 2-h post-load glucose levels > 11.0 mmol/L, or HbA1c > 6.4%) or receiving diabetes treatment;

– dyslipidaemic MASLD: triglycerides ≥ 1.70 mmol/L or HDL cholesterol < 1.0 mmol/L for men and < 1.3 mmol/L for women, or on lipid-lowering treatment(9).

These phenotypes were not mutually exclusive, and there could be overlap among individuals with multiple cardiometabolic risk factors.

Outcome Measures

The primary study outcome was the association between MASLD and its phenotypes with significant liver fibrosis in individuals with a history of HCV infection. Additionally, we compared the prevalence of significant liver fibrosis in MASLD patients to those without steatosis and those with SLD without cardiometabolic conditions (HCV-related steatosis).

Statistical Analysis

The chi-squared test or Fisher's exact test (for categorical variables), the unpaired Student's *t*-test (for normally distributed continuous variables) and the Mann–Whitney test (for non-normally distributed continuous variables) were used to compare study groups. Overlaps between MASLD phenotypes were visualised using an UpSet plot. Cofactors such as age, sex, ethnicity, smoking status, AST, ALT, platelets, total cholesterol, LDL cholesterol, genotype and detectable HCV viral load were examined using the change-in-estimate method, supported by stepwise regression. Only those cofactors that demonstrated significance through the change-in-estimate approach were included in the final models. All models were cross-validated by LASSO models (10-fold validation) to select confounders. Results were reported as adjusted odds ratios (aORs) with 95% confidence interval (CI). A complete case analysis was performed, as missing values for included

variables were < 10%. All tests were two-tailed, with a significance level of $\alpha = 0.05$. Statistical analyses were conducted using STATA 17.2 (STATA Corp. LP, College Station, Texas, USA).

3.4. Results

After applying the exclusion criteria, 590 patients met the selection criteria and were included in the final analysis (Figure 1). The TE failure rate was 15%, consistent with previous studies(15). The primary reason for TE failure was an unreliable result, including fewer than 10 valid measurements and/or an IQR > 30%. The XL probe was used in 36% of cases, while the standard M probe was applied for the remaining patients. The characteristics of the study population are summarised in Table 1. The overall median age was 53 years; 61% of the study population were male and 76% of White ethnicity. The mean duration of HCV infection was 10 years, and 74% of the study population achieved SVR. Significant liver fibrosis was present in 266 (45%) patients of the whole cohort. Patients with significant liver fibrosis were older, predominantly male, and had a higher body mass index (BMI). They also had lower platelets, higher liver transaminases and higher CAP. Hypertension and diabetes were significantly more prevalent among patients with significant liver fibrosis. We also reported on a history of liver-related events, acknowledging that the cross-sectional design of our study limited our ability to assess incidence rates or establish temporal relationships. A history of liver-related events, specifically hepatocellular carcinoma and variceal bleeding, was more frequent in patients with significant liver fibrosis compared to those without (Table 1).

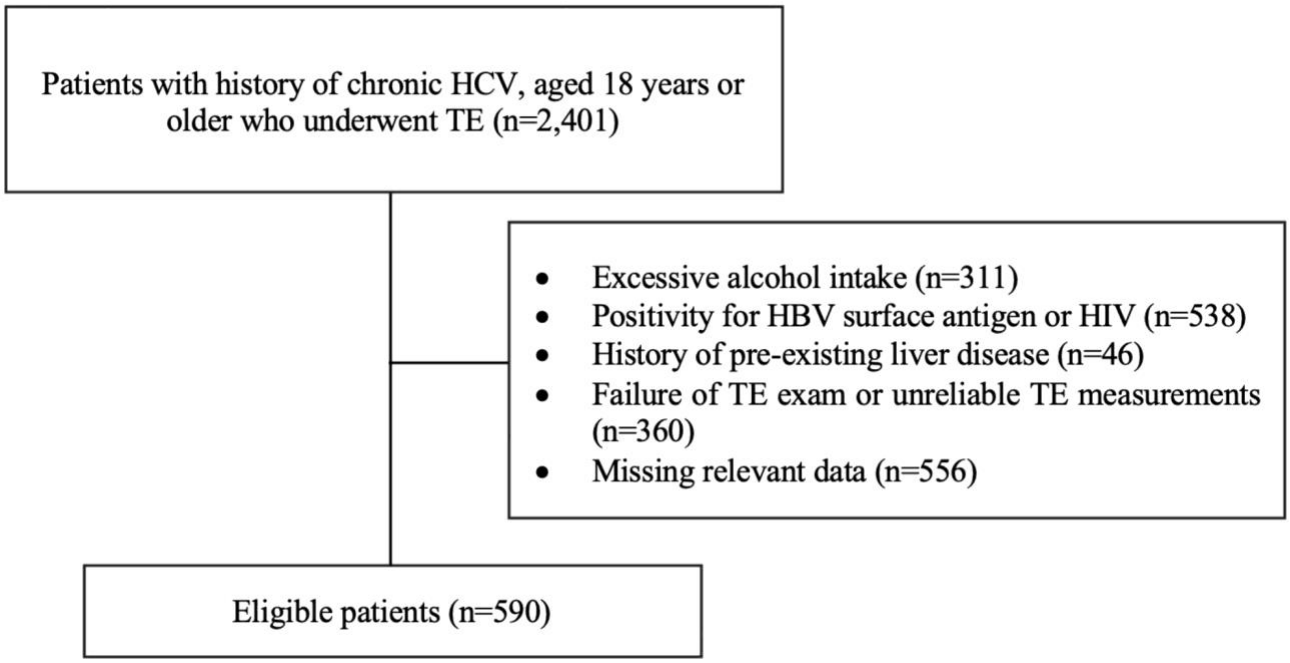

Figure 1. 1. Flow chart displaying the selection of the study participants.

Table 1. Demographic, clinical, biochemical and virological characteristics of the study population and univariate analysis by significant liver fibrosis status (n=590).

Variable	Total cohort (n=590)	Significant liver fibrosis (n=266)	No significant liver fibrosis (n=324)	p-value
Age (median years, IQR)	53 (43 – 60)	54 (46 – 61)	51(41 – 59)	**0.002**
Male sex (%)	359 (61)	181 (68)	178 (55)	**0.001**
Ethnicity (%)				
White	450 (76.2)	201 (76)	249 (77)	0.088
Black	32 (5.4)	11 (4)	21 (6)	
First Nation	20 (3.4)	14 (5)	6 (2)	
Others	88 (15)	40 (15)	58 (15)	
Smoking status (%)				
Never	242 (41)	100 (38)	142 (44)	**0.008**
Former	94 (16)	56 (21)	38 (12)	
Current	254 (43)	110 (41)	144 (44)	
Modality of HCV transmission (%)				
Intravenous drug use	105 (18)	56 (21)	49 (15)	0.061
Blood transfusion	36 (6)	14 (5)	22 (7)	0.066
Tattoos	19 (3.2)	7 (3)	12 (4)	0.493
Body piercing	8 (1.4)	5 (2)	3 (1)	0.319
Mixed	195 (33)	85 (32)	110 (34)	0.608
Unknown	227 (38.4)	99 (37)	128 (39)	0.570

ALT (median U/L, IQR)	49 (27 – 81)	64 (31 – 104)	41 (25 – 70)	**<0.001**
AST (median U/L, IQR)	40 (26 – 63)	48 (29 – 81)	33 (23 – 48)	**<0.001**
Platelet count (median 10^9/L, IQR)	215 (168 – 250)	193 (142 – 241)	228 (182 – 260)	**<0.001**
CAP (median dB/m, IQR)	248 (204 – 292)	266 (224 – 307)	231 (194 – 280)	**<0.001**
MASLD (%)	182 (31)	106 (40)	76 (23)	**<0.001**
BMI (median kg/m^2, IQR)	26 (23 – 32)	28 (24 – 34)	26 (22 – 30)	0.003
Diabetes mellitus or prediabetes (%)	145 (25)	83 (31)	62 (19)	0.001
Hypertension (%)	318 (54)	173 (65)	145 (45)	**<0.001**
Blood glucose (median mmol/L, IQR)	5.2 (4.8 – 6.2)	5.4 (4.9 – 6.9)	5 (4.6 – 5.6)	**<0.001**
HbA1c (median %, IQR)	5.6 (5.2 – 5.7)	5.7 (5.2 – 5.7)	5.5 (5.2 – 5.7)	0.139
Triglycerides (median mmol/L, IQR)	1.2 (0.8 – 1.5)	1.2 (0.8 – 1.5)	1.1 (0.8 – 1.6)	0.609
Total cholesterol (median mmol/L, IQR)	4.1 (3.6 – 4.9)	4.0 (3.4 – 4.6)	4.4 (3.7 – 5.3)	**<0.001**
HDL-cholesterol (median mmol/L, IQR)	1.2 (1.0 – 1.6)	1.2 (1.0 – 1.6)	1.3 (1.0 – 1.6)	0.550
LDL-cholesterol (median mmol/L, IQR)	2.1 (1.6 – 2.8)	1.9 (1.4 – 2.5)	2.3 (1.8 – 3.1)	**<0.001**
SVR (%)	379 (74)	155 (68)	224 (79)	**0.005**
Genotypes (%)				
Genotype-1	369 (63)	171 (64)	198 (61)	0.428
Genotype-2	31 (5)	17 (6)	14 (4)	0.272
Genotype-3	84 (14)	39 (15)	45 (14)	0.789
Others	41 (7)	14 (5)	27 (8)	0.193
Unknown	65 (11)	25 (9)	40 (12)	0.291
Time since HCV diagnosis (mean years, SD)	10.3 (9.1)	10.7 (9)	9.7 (9.4)	0.331

Legend: Continuous variables are expressed as median (IQR) and categorical variables as frequency and percentage (%). The p-values are based on the Student t-test, χ^2 test, or Fisher's exact test between groups with and without significant liver fibrosis. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; HbA1c, glycated hemoglobin; HCV, hepatitis C virus; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; MASLD, metabolic dysfunction-associated steatotic liver disease; SVR, sustained virologic response.

Prevalence of MASLD and its phenotypes, HCV-related steatosis and liver fibrosis

Among the 590 individuals with a history of HCV infection, 213 (36%) had SLD. Of these, 182 (31%) had steatosis with at least one cardiometabolic risk factor, meeting the criteria for MASLD, while 31 (5%) were classified as having HCV-related steatosis. To further categorise MASLD into its phenotypes, we used an UpSet plot to visualise overlapping MASLD phenotypes within the study population (Figure 2). The most frequent phenotypes were the intersection of overweight and hypertensive MASLD and hypertensive MASLD alone, both accounting for 30% of MASLD cases. The overweight MASLD alone was found in 12% of the cases. Other significant intersections include diabetic hypertensive MASLD, overweight diabetic hypertensive MASLD and overweight hypertensive dyslipidaemic MASLD, each present in 8% of the cases. More minor intersections represented fewer individuals, ranging from 0.5% to 6%. The prevalence of liver fibrosis was significantly higher in the MASLD group compared to the non-SLD group (58% vs. 39%, $p < 0.001$). In contrast, no difference in fibrosis prevalence was observed between HCV-related steatosis and the no-SLD or MASLD groups (Figure 3a). When examining the SVR status in relation to the presence of significant liver fibrosis within the MASLD group, individuals with significant liver fibrosis were more likely to have achieved SVR compared to those without

significant fibrosis (49.4% vs. 33.3%, $p = 0.047$). To explore the association between the number of cardiometabolic risk factors and liver fibrosis, MASLD patients were stratified into three groups according to the number of cardiometabolic risk factors: 1, 2 or ≥ 3. The prevalence of significant liver fibrosis increased proportionally with the number of cardiometabolic risk factors (Figure 3b).

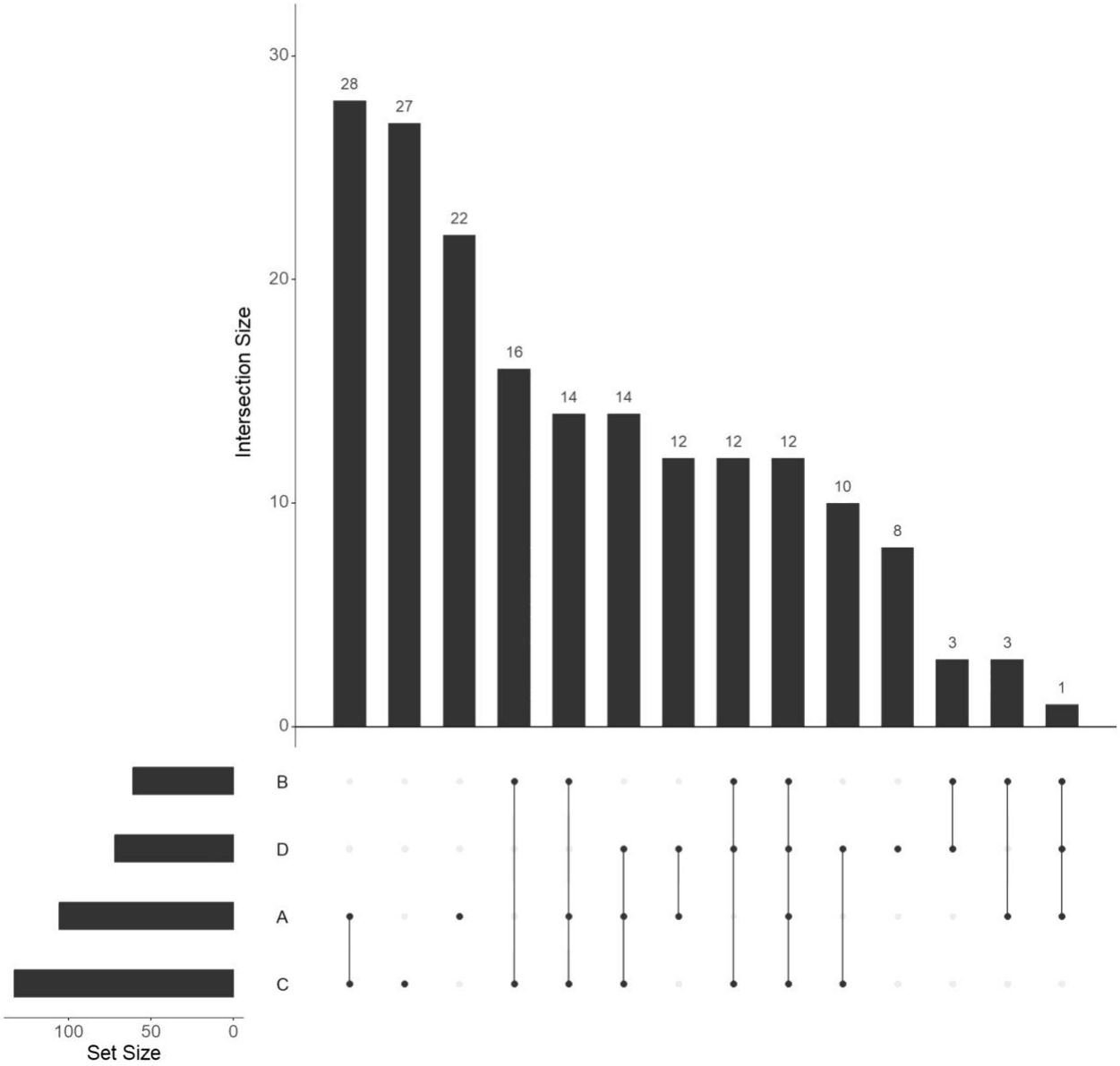

Figure 1. 2. UpSet plot showing the intersection of MASLD phenotypes.

Phenotypes are represented by the sets A (overweight MASLD), B (diabetic MASLD), C (hypertensive MASLD) and D (dyslipidaemic MASLD).

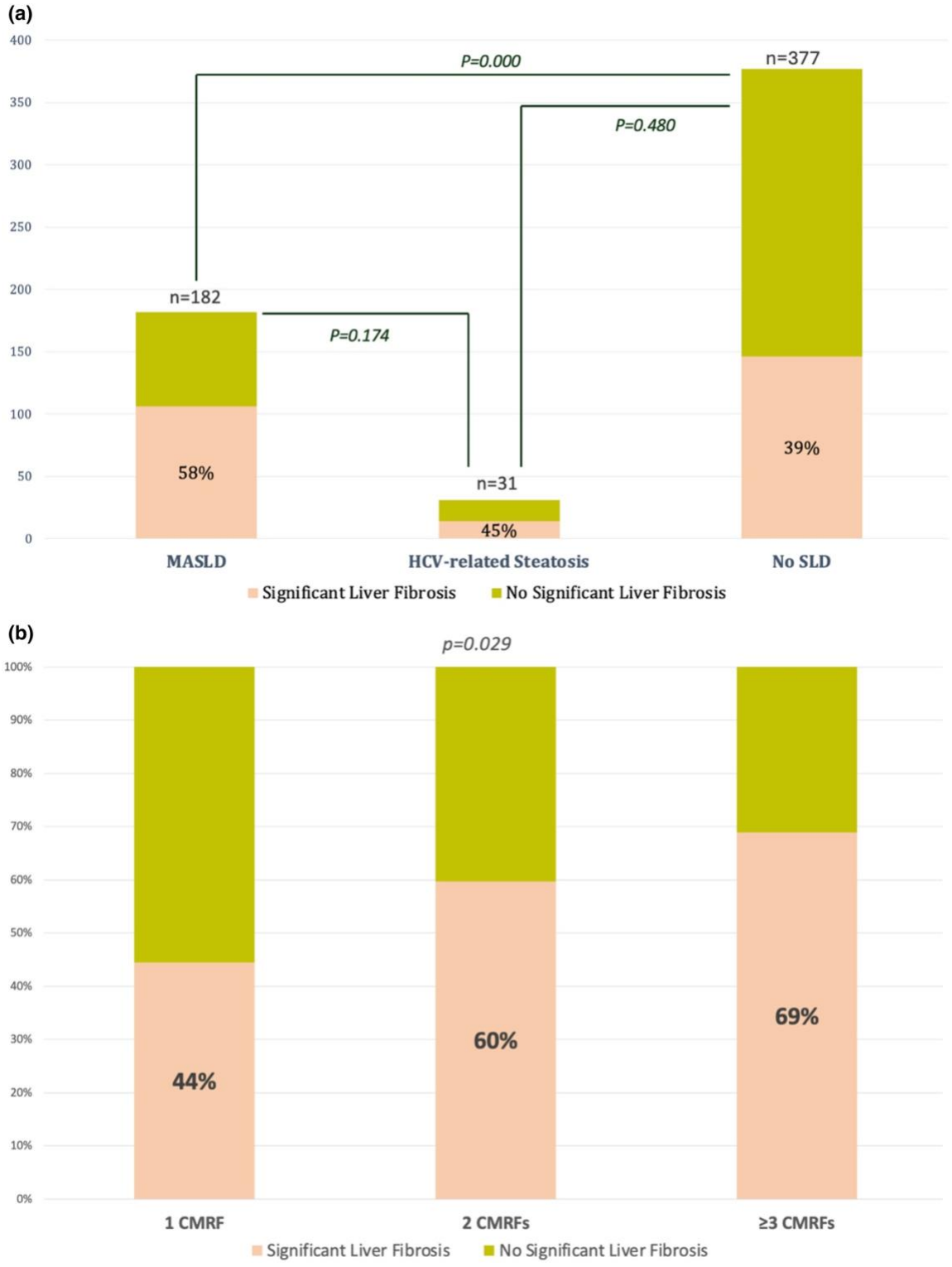

Figure 1. 3. Prevalence of significant liver fibrosis according to: (a) category of steatotic liver disease in the study population; (b) the number of cardiometabolic risk factors (CMRF).

Association of MASLD and its phenotypes with significant liver fibrosis

In the univariate analysis, patients with MASLD were more likely to have significant liver fibrosis. After adjusting for potential confounders, MASLD was associated with a more than 2-fold increase in the odds of significant liver fibrosis (aOR 2.29, 95% CI 1.07–4.87). Other factors associated with liver fibrosis included sex, AST and platelet count (Table 2). However, the presence of a detectable HCV viral load was not found to be associated with significant liver fibrosis. In a sensitivity analysis, the association of MASLD with significant liver fibrosis was confirmed in both patients with and without SVR (Table S1). The duration of SVR was available only in a subset of 283 patients. When incorporated into the multivariate analysis, it did not show a significant association with significant liver fibrosis (data not shown). When evaluating specific MASLD phenotypes associated with significant liver fibrosis, diabetic MASLD (OR 4.76, 95% CI 2.16–10.49), overweight MASLD (OR 2.54, 95% CI 1.27–5.07) and hypertensive MASLD (OR 3.44, 95% CI 1.77–6.68) demonstrated significant associations with significant liver fibrosis (Table 3). In contrast, dyslipidaemic MASLD did not show a significant association with liver fibrosis (OR 1.69, 95% CI 0.94–3.03). In an exploratory analysis of the association between MASLD and liver-related events, we found that patients with MASLD had a higher prevalence of a history of variceal bleeding compared to those without MASLD (3.3% vs. 0.7%, $p = 0.020$). No difference was observed for hepatocellular carcinoma, ascites or hepatic encephalopathy (data not shown).

Table 2. Univariate and multivariate analyses of cofactors associated with significant liver fibrosis.

	OR	95% CI	P-value	aOR	95% CI	P-value
MASLD	**2.16**	(1.51-3.08)	**0.000**	**2.29**	(1.07 – 4.87)	**0.031**
Female				0.42	(0.21 – 0.85)	**0.016**
AST				1.01	(1.00 – 1.02)	**<0.001**
Platelet				0.99	(0.99 – 1.00)	**0.032**

Legend: Odds ratios and 95% confidence intervals are shown for each variable analyzed in univariate and multivariate logistic regression analysis. Abbreviations: aOR, adjusted odds ratio; AST, aspartate aminotransferase; CI, confidence interval; MASLD, metabolic dysfunction-associated steatotic liver disease.

Table 3. Univariate and multivariate analyses exploring the association of each MASLD phenotype with significant liver fibrosis.

	MASLD Phenotypes							
	Overweight		**Diabetic**		**Hypertensive**		**Dyslipidemic**	
Univariate	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
	1.8 (1.18 – 2.77)	0.006	3.57 (1.99 – 6.42)	<0.001	2.48 (1.66 – 3.69)	<0.001	1.97 (1.18 – 3 .28)	0.010
Multivariate	**aOR (95% CI)**	**p-value**	**aOR (95% CI)**	**p-value**	**aOR (95% CI)**	**p-value**	**aOR (95% CI)**	**p-value**
	2.54 (1.27 – 5.07)	**0.008**	4.76 (2.16 – 10.49)	**<0.001**	3.44 (1.77 – 6.68)	**<0.001**	1.69 (0.94 – 3.03)	0.078

Age							1.02 (1.00 – 1.04)	**0.004**
Female					0.43 (0.24 – 0.76)	**0.004**	0.53 (0.36 – 0.80)	**0.002**
AST	1.01 (1.00 – 1.02)	**<0.001**	1.01 (1.00 – 1.02)	**0.001**	1.01 (1.00 – 1.02)	**<0.001**	1.01 (1.00 – 1.01)	**<0.001**
Platelets	0.99 (0.98 – 1.00)	**<0.001**	0.99 (0.98 – 0.99)	**<0.001**	0.99 (0.98 – 0.99)	**<0.001**		
Genotype-3	0.80 (0.29 – 2.20)	0.667						
Total Cholesterol			0.73 (0.61 – 0.87)	**<0.001**				
LDL Cholesterol	0.68 (0.45 – 1.00)	**0.009**			0.71 (0.53 – 0.95)	**0.024**		

Legend: Odds ratios and 95% confidence intervals are shown for each variable analyzed in univariate and multivariate logistic regression analysis. Abbreviations: aOR, adjusted odds ratio; AST, aspartate aminotransferase; CI, confidence interval; LDL, low-density lipoprotein; MASLD, metabolic dysfunction-associated steatotic liver disease.

3.5. Discussion

The present study demonstrates that MASLD is associated with significant liver fibrosis in individuals with a history of HCV infection. Interestingly, the prevalence of significant liver fibrosis varied across MASLD phenotypes, with individuals with diabetes, overweight and hypertensive MASLD showing higher figures. These findings suggest that specific metabolic abnormalities in these phenotypes may create a more pro-inflammatory environment, exacerbating hepatic fibrogenesis. In addition, the prevalence of significant liver fibrosis increased proportionally with the number of cardiometabolic risk factors.

MASLD is increasingly recognized as a key contributor to liver fibrosis progression. The meta-analysis by Singh et al.(16) highlighted that fibrosis in MASLD can advance rapidly, particularly in individuals with metabolic dysfunction-associated steatohepatitis (MASH) who progress by one stage every 7.1 years, compared to 14.3 years for those with less severe forms. The link between metabolic dysfunction, MASH and progression to advanced liver disease is well established(17). In our study, MASLD represented 85% of patients with SLD, while only a small proportion was attributable to HCV-related steatosis. This underlines the relevance of metabolic factors in driving SLD, even in the context of a history of HCV. Within the complex interplay of metabolic conditions contributing to MASLD, we identified a predominance of overweight and hypertensive MASLD phenotypes among individuals with a history of HCV. Additionally, there was a high prevalence of intersecting metabolic phenotypes, with hypertension and overweight being particularly common in HCV individuals with MASLD.

Our study reported that diabetic MASLD had the highest probability of significant liver fibrosis. There is a bidirectional relationship between MASLD and diabetes: MASLD can predict the onset of diabetes, and diabetes accelerates the progression of MASLD(18-26). A European MASLD registry corroborated this, showing that type 2 diabetes mellitus was associated with significant liver fibrosis (aOR 6.25, 95% CI 1.88–20)(27). Furthermore, these findings align with the outcomes of a biopsy-proven MASLD cohort, which demonstrated an elevated risk of liver-related mortality (adjusted hazard ratio [aHR] 2.19, 95% CI 1–4.81) and overall mortality (aHR 2.09, 95% CI 1.39–3.14) in individuals with diabetes(28). Insulin resistance (IR), a hallmark of diabetic MASLD, plays a critical role in the development and progression of liver fibrosis(29,30). It has also been identified as a pivotal factor in the intricate pathogenic mechanisms underlying

MASH(29,31). In addition, studies show that persistent hyperglycemia, arising from poorly controlled diabetes, promotes chronic glucotoxicity, thereby facilitating the progression of hepatic steatosis, necroinflammation and hepatocellular dysfunction(29, 32-40). Our study found a 25% prevalence of prediabetes and diabetes in the HCV population, higher than that reported in the general population(41). Numerous studies have shown that HCV infection increases the risk of IR and type 2 diabetes, which in turn exacerbate liver steatosis and fibrosis progression(42-44). One longitudinal study has demonstrated that individuals with HCV had an 11.5 times higher risk of incident type 2 diabetes compared to the general population(45). Several studies have consistently confirmed a positive association between HCV and IR(42,43,46,47). Moreover, IR can occur in non-obese, non-diabetic individuals with HCV, suggesting an independent role of HCV in inducing IR(48). A meta-analysis by Patel and colleagues further links the incidence of IR in HCV-infected patients with the degree of liver fibrosis induced by HCV(49). Patients with both HCV and type 2 diabetes are at a risk of developing steatosis and progressive liver disease, leading to severe clinical outcomes including hepatic decompensation, hepatocellular carcinoma and elevated risk of liver failure and mortality(38-40).

Our study also demonstrated that hypertensive MASLD was associated with significant liver fibrosis. Although the relationship between MASLD and hypertension is not fully understood, potential mechanisms include systemic IR, gut dysbiosis, fibrinolytic dysfunction via increased plasminogen activator inhibitor-1 levels, altered adipokine profile, chronic inflammation, oxidative stress and endothelial dysfunction(50-52). Previous studies have shown a direct link between hypertension and liver fibrosis, especially in MASLD(53-55). However, the relationship between hypertension and fibrosis seems to be complex in hepatitis C(56). A recent study has

shown that individuals with hepatitis C caused by HCV genotypes 1 and 4 have a higher prevalence of hypertension than those with HCV genotype 3(57). Chronic HCV infection induces a systemic inflammatory state that can lead to endothelial dysfunction, a precursor of hypertension(58,59). Several studies have shown a direct correlation between hypertension and the development of liver fibrosis(53,60). In a meta-analysis including 411 patients with biopsy-proven MASLD, hypertension was associated with fibrosis progression(16). However, while our study supports the association between hypertensive MASLD and significant liver fibrosis, conflicting data exist. In a cross-sectional analysis of the National Health and Nutrition Examination Survey data, Ciardullo et al.(61) found that, while obesity and diabetes were associated with both steatosis and fibrosis, there was no association between hypertension and liver fibrosis. This discrepancy may be due to differences in study populations, inclusion criteria and methodologies.

In our study, overweight MASLD was also associated with significant liver fibrosis. Obesity has long been recognised as an independent risk factor for fibrosis in MASLD(62,63). Furthermore, obesity seems to have a more detrimental effect than other metabolic abnormalities on the severity of advanced fibrosis(64). The mechanism underlying this relationship is multifaceted. Adipose tissue acts as hormonally active by releasing pro-inflammatory cytokines like tumour necrosis factor-alpha and interleukin-6 contributing to liver inflammation and fibrosis. Moreover, obesity alters adipokine profiles and gut microbiota, further promoting fibrogenesis(65). Similar mechanisms have been observed in patients with HCV infection(37,66,67). Indeed, elevated BMI is associated with steatosis progression in patients with chronic HCV infection(68). Notably, weight reduction in chronic HCV infection has been shown to improve not only steatosis and liver enzymes but also fibrosis, despite ongoing viral infection(69).

While previous studies have demonstrated a link between dyslipidaemia and liver fibrosis(70-72), our study found that dyslipidaemic MASLD does not exhibit an association with significant liver fibrosis in HCV infection. This may be partly due to the absence of data on lipid-lowering medications, such as statins, which have been shown to have anti-inflammatory and potentially anti-fibrotic effects(73).

In line with our findings, Yamamura et al. reported that the number of metabolic abnormalities is a key determinant of liver fibrosis progression in patients with SLD. We also observed a higher prevalence of significant liver fibrosis in MASLD patients compared to those with no SLD or HCV-related steatosis, suggesting that metabolic abnormalities, rather than steatosis per se, may drive fibrosis progression in HCV patients. This supports the superiority of the MASLD definition over NAFLD in identifying individuals at risk of fibrosis.

This study has several limitations. Its retrospective design restricts data availability, particularly for key metabolic parameters like homeostatic model assessment for insulin resistance, an important marker of IR and a major driver of fibrosis(74). Additionally, we lacked data on the use of lipid-lowering agents, which may have influenced the relationship between dyslipidaemia and fibrosis(73). Moreover, we were unable to account for the duration of MASLD and SVR, which may have influenced our findings. The cross-sectional nature of the study also limits our ability to establish causality and/or assess the temporal progression of fibrosis. Despite these limitations, our study has several strengths. It is one of the first to explore the association between MASLD phenotypes and liver fibrosis in individuals with a history of HCV infection. Additionally, the relatively large sample size and focus on HCV monoinfection enhance the robustness of our findings.

In conclusion, our findings underscore the importance of a comprehensive approach to managing patients with HCV, even after viral eradication. Early identification and management of metabolic risk factors, especially diabetes and hypertension, could potentially reduce the risk of fibrosis progression, improving long-term outcomes. Furthermore, the differential impact of MASLD phenotypes highlights the need for personalised care, where interventions are tailored based on individual risk profiles to prevent or slow the progression of liver disease. Further longitudinal studies are needed to evaluate the joint effects of HCV and MASLD on liver fibrosis and on the incidence of liver-related events.

Author Contributions

Wesal Elgretli contributed to the study concept and design, acquisition of data, interpretation of data and critical revision of the manuscript. Mohamed Shengir, Solomon Sasson, Luz Esther Ramos Ballestreros, Marc Deschenes, Phil Wong, Tianyan Chen, Nadine Kronfli, Alexa Keeshan and Saniya Tandon were involved in the acquisition of data and critical revision of the manuscript. Sahar Saeed and Agnihotram V. Ramanakumar were involved in statistical analysis, interpretation of data and critical revision of the manuscript. Curtis Cooper was involved in the acquisition and interpretation of data, critical revision of the manuscript and overall study supervision. Giada Sebastiani was involved in the study concept and design, acquisition and interpretation of data, analysis and drafting of the manuscript, critical revision of the manuscript and overall study supervision. All the authors declare they have participated in the preparation of the manuscript and have seen and approved the final version.

Conflicts of Interest

Marc Deschenes has served as an advisory board member for Merck, Janssen and Gilead. Phil Wong has acted as a consultant for BMS, Gilead, Merck and Novartis. Nadine Kronfli reports research funding from Gilead Sciences, advisory fees from Gilead Sciences, ViiV Healthcare, Merck and Abbvie, and speaker fees from Gilead Sciences, Abbvie and Merck. Sahar Saeed served as an advisory board member for Novo Nordisk. Curtis Cooper is a consultant and speaker for AbbVie and Gilead. Giada Sebastiani has acted as a speaker for Merck, Gilead, Abbvie, Eli Lilly and Novo Nordisk, and served as an advisory board member for Merck, Gilead, GlaxoSmithKline and Novo Nordisk. Wesal Elgretli, Mohamed Shengir, Solomon Sasson, Agnihotram V. Ramanakumar, Felice Cinque, Luz Esther Ramos Ballestreros, Tianyan Chen, Alexa Keeshan and Saniya Tandon declare no conflicts of interest.

Supplemental table S 1. Multivariate analysis of cofactors associated with significant liver fibrosis by SVR status (n=590).

	Patients with SVR (n=379)			**Patients without SVR** (n=211)		
	OR	**95% CI**	**P-value**	**aOR**	**95% CI**	**P-value**
MASLD	**1.83**	(1.05 – 3.19)	**0.032**	**2.51**	(1.07 – 5.90)	**0.034**
Female	0.66	(0.41 – 1.06)	0.086	0.69	(0.28 – 1.72)	0.425
AST	1.01	(1.01 – 1.02)	**<0.001**	1.03	(1.01 – 1.06)	**0.008**

Legend: Odds ratios and 95% confidence intervals are shown for each variable analyzed in univariate and multivariate logistic regression analysis. Abbreviations: aOR, adjusted odds ratio;

AST, aspartate aminotransferase; CI, confidence interval; MASLD, metabolic dysfunction-associated steatotic liver disease; SVR, sustained virological response.

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4. THESIS DISCUSSION

HCV is a hepatotropic virus that has developed sophisticated mechanisms to exploit the host's cellular machinery for survival and replication. A key pathway it manipulates is lipid metabolism, which is essential for both viral replication and liver injury. By altering lipid homeostasis, HCV facilitates its own lifecycle while contributing to hepatic steatosis and long-term metabolic dysfunction. Liver disease in individuals with a history of HCV infection has long been viewed primarily through the lens of viral pathogenesis. However, growing evidence suggests that metabolic dysfunction plays a crucial role in liver fibrosis, even after achieving SVR(5). The findings of this study reinforce this paradigm shift by demonstrating that MASLD is independently associated with significant fibrosis progression in individuals with prior HCV infection, with diabetic, hypertensive, and overweight MASLD phenotypes exhibiting the strongest associations. This aligns with recent shifts in hepatology, particularly the redefinition of NAFLD to MASLD. The revised terminology reflects a deeper understanding of the intricate relationship between metabolic dysfunction and liver health and acknowledges that metabolic dysfunction is not an isolated condition but instead coexists with and exacerbates other liver diseases, including HCV.

The strong association between MASLD and liver fibrosis observed in our study aligns with growing evidence that metabolic risk factors can independently drive fibrogenesis, especially in the presence of chronic liver insults such as HCV. Patients with HCV, even after achieving SVR, are known to remain vulnerable to liver-related complications, a vulnerability likely heightened by metabolic comorbidities(157). The persistence of fibrosis risk among patients with HCV and MASLD suggests that, while viral clearance halts the direct inflammatory impact of HCV, it does not reverse the pre-existing or emerging metabolic conditions that promote fibrosis(4, 5, 157, 158).

This finding supports the hypothesis that metabolic dysfunction may act as a secondary "hit" following HCV, independently perpetuating liver injury through chronic low-grade inflammation, altered lipid metabolism, and pro-fibrotic signaling.

The contribution of specific MASLD phenotypes, notably diabetic MASLD, to fibrosis is particularly informative. In recent study, diabetes significantly contributed to the development of advanced liver fibrosis, as evidenced by an odds ratio of 2.00 (95% CI 1.22–3.28; $p < 0.01$) in patients with MASLD(159). In other study, type 2 diabetes mellitus, was recognized as an independent risk factor for the progression of liver fibrosis(160). In this study, patients with diabetes and MASLD experienced faster fibrosis progression compared to non-diabetic individuals. IR associated with diabetes contributes to liver fat accumulation, inflammation, and oxidative stress, which promote liver injury and fibrosis. Additionally, metabolic endotoxemia and gut dysbiosis linked to diabetes further exacerbate liver disease progression, leading to advanced fibrosis and HCC(161). In patients with HCV, whose liver function is already compromised, these effects may be amplified, leading to more rapid fibrosis progression. In a study included 581 patients with chronic HCV infection, hyperglycemia was positively associated with the risk of persistent advanced fibrosis. While hyperglycemia did not show a substantial association with the progression of advanced fibrosis in the short term, it was identified as an independent risk factor for persistently advanced fibrosis(162). Thus, the intersection of diabetes and HCV infection appears to create a compounded environment for fibrogenesis, which may help explain the significantly elevated fibrosis risk observed in this subgroup.

The finding that hypertensive MASLD is associated with an increased risk of liver fibrosis in patients with history of HCV infection introduces important considerations regarding the mechanisms of vascular and hepatic interaction. Hypertension's role in fibrosis may be attributed to more than systemic vascular dysfunction; recent research suggests that hypertension contributes to hepatic endothelial dysfunction, which can disrupt sinusoidal blood flow and promote stellate cell activation within the liver. Moreover, hypertensive patients often exhibit increased levels of angiotensin II, a peptide hormone that promotes vasoconstriction and fibrogenesis by directly stimulating hepatic stellate cells(163-165). For patients with HCV, whose liver vasculature may already be compromised, hypertension's additive effect could represent a significant risk factor that accelerates the fibrotic process, even when viral activity is no longer a driving force. This points to a potential therapeutic role for anti-hypertensive treatments in mitigating fibrosis risk within this specific patient population. Bahde et al. found that hypertensive patients with HCV exhibited a higher stage of liver fibrosis compared to non-hypertensive patients. However, the use of angiotensin-converting enzyme inhibitors or angiotensin II receptor type 1 antagonists in these hypertensive patients was associated with a decrease in liver fibrosis compared to other antihypertensive treatments(166). Another study has shown that losartan, when administered to patients with chronic HCV and liver fibrosis, resulted in a significant decrease in fibrosis stage after one year of administration(167). Furthermore, a meta-analysis conducted by Kim et al. revealed that patients treated with renin–angiotensin system inhibitors exhibited significantly lower fibrosis scores, and smaller fibrosis areas compared to control groups, indicating a potential antifibrotic effect of these medications in patients with chronic liver disease(168).

Regarding overweight MASLD, the results of our study suggest that excess adiposity in MASLD patients with history of HCV infection is indeed associated with fibrogenic progression. A recent study examining abdominal obesity indices found that obesity was associated with an increased risk of fibrosis progression over time in individuals with MASLD(169). This may be partially explained by the concept of lipotoxicity, where excessive adipose tissue promotes the release of free fatty acids, which accumulate in the liver and cause cellular injury. Lipid peroxidation, often exacerbated in obesity, leads to oxidative stress and inflammatory cytokine release, both of which stimulate fibrotic pathways in hepatocytes and hepatic stellate cells(170, 171). Furthermore, obesity alters the secretion patterns of adipokines—hormones produced by adipose tissue—that regulate metabolic and inflammatory pathways. Leptin is typically elevated in obese individuals, and has been shown to promote fibrogenesis by directly activating hepatic stellate cells, thereby enhancing collagen production and deposition. Conversely, adiponectin levels are often reduced in obesity. This adipokine possesses anti-inflammatory and anti-fibrotic properties; its deficiency removes inhibitory signals on hepatic stellate cells activation, facilitating fibrosis progression(172-174). This imbalance may explain the observed association between overweight MASLD and fibrosis in patients with HCV, and suggests that weight management should be a critical component of post-SVR care.

The observed lack of a significant association between dyslipidemic MASLD and fibrosis in individuals with history of HCV infection is intriguing and may be influenced by several factors. While dyslipidemia is commonly linked to hepatic steatosis, its direct role in promoting liver fibrosis appears less pronounced compared to other metabolic conditions such as IR or chronic inflammation. The accumulation of lipids in the liver contributes to steatosis(2); however, the

progression to fibrosis often involves additional factors like oxidative stress and inflammatory pathways, which may not be as strongly activated by dyslipidemia alone. Additionally, many patients with dyslipidemia are prescribed statins, which have demonstrated anti-fibrotic properties. Statins not only lower lipid levels but also exert beneficial effects on liver inflammation and fibrosis. For instance, simvastatin has been shown to decrease hepatic inflammation and fibrosis through the inhibition of Ras and RhoA pathways, independent of changes in steatosis or intrahepatic cholesterol content(175). Another study demonstrated that combining simvastatin with a cyclooxygenase-2 inhibitor led to significant inhibition of hepatic stellate cell proliferation and attenuation of liver fibrosis. The combined treatment induced apoptosis in activated hepatic stellate cells through pathways involving extracellular signal-regulated kinase activation and the caspase cascade(176). Similarly, atorvastatin has been observed to attenuate hepatic fibrosis in animal models by reducing hepatic stellate cell turnover. These anti-fibrotic effects could mitigate the impact of dyslipidemia on fibrosis progression, potentially explaining the lack of a significant association observed in our study.

This study has several strengths. It is among the first to evaluate the association between MASLD phenotypes and liver fibrosis in a large, well-characterized cohort of individuals with a history of HCV infection. The use of non-invasive imaging markers provides a robust and clinically applicable assessment of liver fibrosis. Additionally, the inclusion of multiple MASLD phenotypes allows for a nuanced understanding of how different metabolic risk factors contribute to liver disease progression.

While this thesis provides valuable insights into the role of MASLD phenotypes in liver fibrosis among individuals with HCV, several limitations should be acknowledged. Addressing these limitations in future research will help refine our understanding and improve clinical strategies. One key limitation is the retrospective study design, which restricts causal inference and prevents confirmation that the observed associations between MASLD phenotypes and fibrosis are directly causative. Longitudinal, prospective studies are needed to track fibrosis progression in patients with HCV and MASLD over time, particularly after achieving SVR. Such studies would offer stronger evidence on how metabolic conditions impact fibrosis and help identify critical intervention points to prevent disease progression. Another constraint is the lack of medication and intervention data. Information on patient medications, such as anti-diabetic drugs, lipid-lowering agents (e.g., statins), or anti-hypertensive therapies, was unavailable, potentially confounding the observed associations. Given that statins, for example, have anti-fibrotic properties, their inclusion in future studies could clarify their role in fibrosis progression across different MASLD phenotypes. Future research should assess the impact of these pharmacological interventions and consider interventional studies evaluating the effectiveness of metabolic treatments in modifying fibrosis outcomes. Additionally, the study did not include biomarkers of IR (e.g., homeostatic model assessment for insulin resistance) or inflammatory markers, limiting its ability to explore mechanistic links between IR, systemic inflammation, and hepatic fibrosis. Future research should incorporate such biomarkers to better understand the biological pathways linking MASLD phenotypes with fibrosis and to develop targeted risk stratification models. The thesis also focuses exclusively on patients with a history of HCV infection, excluding those with viral coinfections (e.g., HIV/HCV or HBV/HCV). Since viral coinfections are known to exacerbate metabolic dysfunction, alter liver pathology, and influence fibrosis progression(177-

179), the findings may not be generalizable to individuals with history of coinfections. Future studies should explore how viral coinfections impact fibrosis risk in MASLD patients and whether MASLD phenotypes exert unique effects in these populations.

Beyond these limitations, several areas for further study warrant attention. One key question that remains unanswered is whether fibrosis progression differs between MASLD patients with prior HCV infection and those with primary MASLD (i.e., MASLD without a history of viral hepatitis). Given that HCV itself contributes to metabolic dysfunction and steatosis, it is possible that the fibrosis trajectory in post-HCV MASLD patients differs from that of individuals with primary MASLD. Longitudinal studies are needed to assess whether fibrosis progression and resolution follow distinct patterns in these two groups, which could inform more personalized monitoring and treatment strategies. Furthermore, given the increasing burden of MASLD among individuals with HCV history, interventional studies investigating metabolic and anti-fibrotic therapies in this population are crucial. Clinical trials evaluating the effects of glycemic control, lipid management, and weight reduction on fibrosis outcomes in HCV-related MASLD would provide actionable insights for personalized treatment. For instance, the anti-fibrotic potential of metformin in diabetic MASLD patients or the impact of ACE inhibitors in hypertensive MASLD patients should be explored. Additionally, studies assessing whether combining metabolic interventions (e.g., statins or GLP-1 receptor agonists) with emerging anti-fibrotic agents improves fibrosis outcomes could offer new therapeutic strategies. Moreover, genetic and molecular research could further refine our understanding of fibrosis mechanisms in MASLD. Fibrosis progression is influenced by individual variability, and identifying genetic susceptibility markers—such as patatin-like phospholipase domain-containing protein 3 and transmembrane 6 superfamily member 2

variants—could enhance risk stratification and lead to precision medicine approaches for managing fibrosis in MASLD patients with HCV. Research into gene-environment interactions may uncover novel targets for intervention, ultimately improving patient outcomes. Finally, health disparities and socioeconomic determinants of liver disease should be examined, as metabolic dysfunction often correlates with lifestyle factors that vary by socioeconomic status. Investigating how social determinants influence MASLD prevalence and fibrosis risk in HCV patients could inform public health initiatives aimed at reducing disparities in liver health.

In conclusion, this thesis makes a significant contribution to the understanding of liver fibrosis in patients with a history of HCV and metabolic dysfunction, highlighting the importance of metabolic factors as independent drivers of liver disease progression. By elucidating the unique contributions of MASLD phenotypes to fibrosis risk, it advocates for a shift in how liver disease is managed in patients with HCV, from a narrow focus on viral suppression to an integrated model that addresses both viral and metabolic health. These findings underscore the potential of multidisciplinary, personalized care in improving outcomes for patients with HCV and MASLD, while also laying the groundwork for future research and policy advancements in liver disease management. By addressing the dual burden of viral and metabolic liver disease, healthcare providers can move toward a more comprehensive, patient-centered approach to liver health, with the ultimate goal of reducing the burden of fibrosis and improving quality of life for affected individuals.

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