Facts and fictions of HCV and comorbidities: steatosis, diabetes mellitus, and cardiovascular diseases

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Abstract

The hepatitis C virus (HCV) is a major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma worldwide. A significant portion of the morbidity and mortality associated with HCV is a consequence of numerous HCV-associated comorbidities. Type 2 diabetes and atherosclerosis, two known complications of the metabolic syndrome, are noteworthy, because HCV has been suggested to play a role in their pathogenesis. In addition, HCV also causes steatosis, which may increase the risk of cardiovascular events. This review summarizes the evidence supporting the association between HCV and steatosis, insulin resistance/type 2 diabetes and cardiovascular morbidity and mortality. Their diagnostic, prognostic and management aspects are discussed.

Reference


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Facts and fictions of HCV and comorbidities: Steatosis, diabetes mellitus, and cardiovascular diseases

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Summary

The hepatitis C virus (HCV) is a major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma worldwide. A significant portion of the morbidity and mortality associated with HCV is a consequence of numerous HCV-associated comorbidities. Type 2 diabetes and atherosclerosis, two known complications of the metabolic syndrome, are noteworthy, because HCV has been suggested to play a role in their pathogenesis. In addition, HCV also causes steatosis, which may increase the risk of cardiovascular events. This review summarizes the evidence supporting the association between HCV and steatosis, insulin resistance/type 2 diabetes and cardiovascular morbidity and mortality. Their diagnostic, prognostic and management aspects are discussed.

Introduction

The hepatitis C virus (HCV) is a major cause of cirrhosis and hepatocellular carcinoma worldwide. In a recent systematic literature review, the global HCV prevalence was estimated to be ~2.8% of the world’s population, corresponding to ~185,000,000 persons infected [1]. The same study reported recently that in 2010 there were approximately 10 times more deaths attributable to viral hepatitis in the European Union than to human immunodeficiency virus (HIV), with two thirds of the viral hepatitis deaths associated with HCV [2]. Similar data have been reported in the USA, where HCV-associated mortalities surpassed those due to HIV in 2007 [3]. This already worrisome mortality rate is bound to increase at least until 2030 [4], as complications of end stage liver disease occur decades after infection, the vast majority of which occurred in the 1960's and 1970's. This will impose a significant burden on the health care systems worldwide [5]. Although cost-effectiveness studies on measures aimed at battling the HCV epidemic have essentially focused on liver disorder-related costs, a significant portion of the health burden associated with HCV is the consequence of a number of HCV-associated comorbidities [6] (Fig. 1). Among these, type 2 diabetes and atherosclerosis, two otherwise well-known, major complications of the metabolic syndrome, are noteworthy, because HCV infection has been linked to their pathogenesis [7]. In addition, HCV also causes steatosis [8], which has been suggested to increase the risk of cardiovascular morbidity [9]. This raises the legitimate question as to whether the successful management of HCV may also impact the future morbidity and mortality due to diabetes and atherosclerosis. The scope of this review is to discuss some aspects related to the epidemiology and pathogenesis of such manifestations, and to discuss their management.

Key Points

- Chronic hepatitis C patients often present with steatosis, which shows a strong genotype dependence, correlates with viral load, and disappears in case of successful therapy
- Viral steatosis is not associated with rapid fibrosis progression or poor response to IFN-α, but may be a risk factor for HCC
- HCV is associated with insulin resistance that may progress to type 2 diabetes in patients at risk, and leads to poor response to IFN-α, accelerated fibrosis progression and HCC
- HCV-infected persons are at higher risk of cardiovascular events, independently of other risk factors
- Successful antiviral therapy may reduce the risk of developing type 2 diabetes and ischaemic stroke

Keywords: Hepatitis C virus; Steatosis; Insulin resistance; Diabetes; Coronary artery disease; Atherosclerosis; Stroke.

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Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; LDL, low density lipoprotein; HCC, hepatocellular carcinoma; PTEN, phosphatase and tensin homologue deleted on chromosome 10; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; HBV, hepatitis B virus; IR, insulin resistance; HOMA-IR, homeostasis model for assessment of insulin resistance; BMI, body mass index; SVR, sustained virological response; CCL2, chemokine [C-C motif] ligand 2; IFN, interferon; IMT, intima-media thickness.
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The various viral genotypes, in leading to the appearance of large involvement of additional mechanisms in case of an infection with frequent and severe in genotype 3 infection, suggesting the changes are shared by all viral genotypes while steatosis is more own replication and virion production, these pathophysiological when infection relapses [12]. The dependence of HCV replication at the time HCC occurs [32–34]. Thus, evidence supporting the association between steatosis and hepatocellular carcinoma (HCC). Several studies have associated steatosis and the risk for HCC in chronic hepatitis C [26–28]. Due to the paucity of patients with HCV genotype 3 included in these studies, making any inference about a causal link between viral steatosis and HCC is problematic. More likely, this association may have been accounted for by the known relationship between overweight (leading to steatosis) and HCC [29]. On the other hand, HCV genotype 3 infection is indeed associated with an increased risk for HCC [21,30,31], but whether this is due to viral steatosis is unknown. Association does not imply causation and, besides, steatosis is often reduced or absent in late stages of liver disease, i.e., at the time HCC occurs [32–34]. Thus, evidence supporting the argument that viral steatosis directly leads to HCC is lacking. Some patients with genotype 3 may present a deregulation of intracellular pathways leading to both steatosis and HCC: potentially culprits include the production of reactive oxygen species [35] or the downregulation of the tumour suppressor PTEN [36].

Clinical impact of steatosis in HCV infection

Whatever the mechanism, viral steatosis does not seem to impact liver fibrosis progression rate [20], although HCV genotype 3 is independently associated with increased fibrosis progression [20,21]. Viral steatosis does not impair response to interferon-α (IFN-α) [22,23]. Alternatively, steatosis due to the metabolic syndrome is associated with both accelerated fibrosis progression [24,25] and poor response to IFN-α-based therapy [22,23]. A distinct issue is the association between steatosis and hepatocellular carcinoma (HCC). Several studies have associated steatosis and the risk for HCC in chronic hepatitis C [26–28]. Due to the paucity of patients with HCV genotype 3 included in these studies, making any inference about a causal link between viral steatosis and HCC is problematic. More likely, this association may have been accounted for by the known relationship between overweight (leading to steatosis) and HCC [29]. On the other hand, HCV genotype 3 infection is indeed associated with an increased risk for HCC [21,30,31], but whether this is due to viral steatosis is unknown. Association does not imply causation and, besides, steatosis is often reduced or absent in late stages of liver disease, i.e., at the time HCC occurs [32–34]. Thus, evidence supporting the argument that viral steatosis directly leads to HCC is lacking. Some patients with genotype 3 may present a deregulation of intracellular pathways leading to both steatosis and HCC: potentially culprits include the production of reactive oxygen species [35] or the downregulation of the tumour suppressor PTEN [36].

Viral vs. metabolic steatosis

Based on the discussion above, it seems important, from the prognostic point of view, to distinguish viral steatosis from steatosis of a different origin, especially metabolic (Table 1). Unfortunately, viral steatosis does not present clear-cut histopathological features allowing to differentiate it from steatosis due to other causes. Thus, the differential diagnosis must rely on the history, the presence of risk factors, serum biochemistry assays and responsiveness to antivirals. HCV infected patients tend to have lower levels of circulating components of lipoproteins, such as cholesterol [25,37], especially in patients with genotype 3 [37]. This peculiar lipid profile is reverted after successful therapy [37,38], but a precise correlation between hypocholesterolemia and steatosis has rarely been reported [23].

Both HCV and the metabolic syndrome are frequent disorders, hence there is the probability of overlap. Non-alcoholic fatty liver disease (NAFLD) is the liver manifestation of the metabolic syndrome. The occurrence and significance of NAFLD and – more importantly – non-alcoholic steatohepatitis (NASH) in HCV infection have rarely been studied. In an important work, the features of patients with hepatitis C and NASH were analysed in detail [39]. Patients with chronic hepatitis C and NASH had higher

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**Steatosis**

*Evidence for a viral role in inducing fatty liver*

Chronic HCV infection is associated with steatosis [8,10]. This is suggested by the strong association of steatosis with HCV genotype 3: patients with this genotype have a ~5-fold probability of having moderate to severe steatosis, more than those with non-3 genotypes [11], hinting at viral sequences responsible for the fat accumulation. Occurrence and severity of steatosis in patients with HCV genotype 3 correlates with the viral load and the response to antivirals: fat accumulation disappears in patients who reach a sustained virological response (SVR), and reappears when infection relapses [12]. The dependence of HCV replication and spread on the host lipid metabolism partly explains this close association: (i) specific lipid species are essential for the HCV life cycle, as their depletion inhibits viral replication; (ii) virion assembly and egress depend on lipid droplets and exploit the hepatocyte lipoprotein secretion pathway; (iii) HCV circulates in blood associated with lipoproteins forming so-called lipoviroparticles; (iv) the latter ones bind to hepatocytes via interaction, among others, with the low-density lipoprotein (LDL) receptor [13].

Although HCV alters the host lipid metabolism to favour its own replication and virion production, these pathophysiological changes are shared by all viral genotypes while steatosis is more frequent and severe in genotype 3 infection, suggesting the involvement of additional mechanisms in case of an infection with this genotype. Unfortunately, the differential efficiency, shown by the various viral genotypes, in leading to the appearance of large fat droplets has been poorly studied [10]. In addition, although several mechanisms have been proposed to account for the viral steatosis (for a review, see [10]), no experimental model recapitulates the phenotype observed in humans. There are multiple reasons for the difference between in vivo and in vitro observations: (i) most models use hepatoma cells, (ii) the sequences used to induce metabolic alterations, supposed to lead to steatosis, are often derived from non-3 genotype isolates, and (iii) a direct comparison between different genotypes has been rarely performed, using the same model and experimental conditions.

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Both HCV and the metabolic syndrome are frequent disorders, hence there is the probability of overlap. Non-alcoholic fatty liver disease (NAFLD) is the liver manifestation of the metabolic syndrome. The occurrence and significance of NAFLD and – more importantly – non-alcoholic steatohepatitis (NASH) in HCV infection have rarely been studied. In an important work, the features of patients with hepatitis C and NASH were analysed in detail [39]. Patients with chronic hepatitis C and NASH had higher
steatosis and fibrosis scores, higher triglyceride levels and lower total and high density lipoprotein cholesterol levels than those with steatosis alone. Interestingly, they were more often infected with genotype 3. The authors concluded that patients with chronic hepatitis C and NASH are a distinct category of patients with more aggressive liver disease. Importantly, NASH seemed more common in genotype 3, where it lacked some of its typical features such as increased insulin resistance compared to patients with simple steatosis. The mechanisms underlying these peculiar patterns are unknown. However, clinicians and pathologists alike should be alerted by histopathological features suggesting NASH – typically the presence of hepatocyte ballooning – even when chronic hepatitis C patients present with genotype 3 and massive steatosis that may hastily be interpreted as purely viral.

### HCV and glucose metabolism disturbances

**HCV alters glucose homeostasis**

In a meta-analysis, White and collaborators showed that HCV infection is associated with an increased risk of type 2 diabetes in comparison to both uninfected and hepatitis B virus (HBV)-infected controls [40]. Among the numerous studies on this topic, the landmark cross-sectional, general population-based study by Mehta and collaborators, showed that HCV is associated with type 2 diabetes among persons aged 40 years or older [41]. Longitudinal studies in immunocompetent persons and in patients having undergone liver or kidney transplantation have further confirmed that HCV is a major risk factor for incident type 2 diabetes, especially in persons at risk due to obesity or age [42–47]. HCV infection precedes diabetes [48], rather than being an iatrogenic infection in highly medicalised diabetic patients. Overall, HCV infection seems to accelerate the occurrence of type 2 diabetes in predisposed individuals: in immunocompetent persons, this seems to occur at least a decade earlier compared to uninfected controls [41].

The mechanism whereby HCV induces diabetes is insulin resistance (IR). Patients with hepatitis C have significantly higher levels of IR (measured by the homeostasis model for assessment of IR [HOMA-IR]) compared to uninfected controls [49] or patients with chronic hepatitis B matched for other risk factors of IR, such as BMI, waist circumference, age, and sex [50]. The methods to measure IR vary, and the observations made in HCV-infected persons are conflicting depending on the technique used. Measuring the HOMA-IR to assess IR (defined by an arbitrary threshold of 2) [51] is very convenient when large study populations are to be evaluated, but it seems to overestimate the proportion of patients with IR, as post-load IR and stimulation assays [52,53] have suggested a lower prevalence and level of IR. However, the occurrence of IR in chronic hepatitis C patients without stigmata of the metabolic syndrome has been reliably confirmed using the euglycemic hyperinsulinemic clamp method, an otherwise laborious technique not suitable for routine diagnostic use [54,55]. These studies have also shown that different viral genotypes have comparable levels of both hepatic [52,54] and extrahepatic [54] IR.

**Direct and indirect mechanisms of HCV-associated insulin resistance**

In experimental models, HCV was shown to impair the hepatocyte insulin signalling pathway by a variety of postreceptorial mechanisms [56]. The central target of HCV appears to be the protein kinase B (PKB) or Akt, whose phosphorylation is impaired upon insulin stimulation [57]. On the other hand, in vitro experiments have shown that basal Akt phosphorylation is already elevated following HCV protein expression [14,58], a possible mechanism for promoting cell survival and protection from apoptosis [59,60]. Should this be the case in vivo, the basal activation of Akt may lead to infected cells, refractory to further insulin-mediated stimulation: whether we should redefine the HCV-induced IR more appropriately as a state of insulin refractoriness warrants further study.

Although HCV infects essentially the liver, a significant proportion of whole body IR in chronic hepatitis C patients without features of the metabolic syndrome originates from extrahepatic sites. Using a combination of euglycemic hyperinsulinemic clamp technique and calorimetry, two teams independently observed a reduced oxidative glucose consumption, secondary to a reduced capacity to take up glucose upon insulin stimulation [54,55]. This suggests that HCV infected hepatocytes may secrete mediators capable of endocrine effects at extrahepatic sites, notably the skeletal muscle.

**Clinical impact of insulin resistance and diabetes in HCV infection**

In chronic hepatitis C patients, IR and type 2 diabetes are major disease modifiers. They are in fact associated with several poor outcomes, including accelerated hepatic fibrosis progression, reduced SVR rates, the development of HCC, and some cardiovascular events such as stroke [61]. As mentioned above, steatosis – notably when due to metabolic changes, rather than viral replication – is associated with fibrosis [22]. In a retrospective study on serial liver biopsies from 135 chronic hepatitis C patients followed for a median of 61 months, the severity of steatosis at baseline (mostly metabolic, as 84% of patients had HCV non-3 genotypes) predicted the fibrosis progression [25].

Since metabolic steatosis is largely due to IR, the same association exists for IR. Among 121 chronic hepatitis C patients with minimal or no fibrosis, the HOMA-IR score (but not steatosis) was independently associated with fibrosis stage and progression rate [49]. IR and diabetes were further confirmed as being associated with fibrosis stage and progression in a variety of studies [11,62–65]. The fibrous tissue deposition and fibrosis progression in insulin resistant patients may be mediated by insulin itself, which can directly stimulate hepatic stellate cells to produce and secrete connective tissue growth factor [66]. On the other hand, the chronic inflammatory state associated with the metabolic syndrome involves the upregulation of pro-inflammatory...
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orders and stroke, as shown by a large population-based study [86,87]. A large study on 2842 chronic hepatitis C patients treated with led the HCV RNA decline, and that baseline HOMA-IR levels diated or disappears in patients treated with regimens containing negative association between IR and virological response is atten-
ated Cox regression analysis, diabetes was independently associ-
ated with type 2 diabetes in patients at risk. Most reports, with some excep-
tions remains controversial. Since this association has been the focus of major interest but also of some contradictory findings, it will be discussed in further detail. Early studies evaluated surrogate markers of cardiovascular outcomes, such as arterial intima-media thickness (IMT) and the presence of atherosclerotic plaques. These studies were prompted by the frequent observations that several bacterial [95] and viral infections [96] were associated with an increased risk of cardiovascular and cerebrovascular diseases. Kiechl et al. [95] reported the results of a 5-year prospective study on 826 persons, divided into those with a chronic infection (respiratory, urinary and others) and controls. As many as 40% incident atherosclerotic plaques were attributed to chronic infections, especially by Chlamydia pneumoniae. These observations led to the hypothesis that chronic infections may favour the atherogenic process by inducing a systemic inflammatory state. The first case-control study focusing on HCV infection analyzed 4784 individuals, 104 (2.2%) of which were anti-HCV-positive. After adjustment for confounding risk factors, the presence of anti-HCV was associated with an increased risk of carotid artery plaque (OR 1.92 [95% CI 1.56–2.38]) and carotid IMT (OR 2.85 [2.28–3.57]) [97]. A second case-control study from the same authors evaluated 1992 patients, of whom 496 had carotid artery plaques. Although only a minority (25, i.e., 1.3%) had circulating HCV core protein, this marker was a strong independent predictor of carotid plaques [98]. Tomiyama et al. [99] confirmed these findings measuring the pulse-wave velocity in 7514 subjects, and comparing results among patients with HBV, those with anti-HCV and uninfected controls. The association between pulse-wave velocity and HCV (but not with HBV) was independent from atherosclerotic risk factors. Targher et al. [100] further extended these observations by studying the carotid IMT in 60 patients with NASH, 60 with HCV, 35 with HBV, and 60 healthy controls, comparable for age and sex. These authors observed a gradient of thickness increasing from controls to HBV-infected to HCV-infected and finally to NASH patients. Interestingly, adjustments for age, sex, body mass index, smoking, LDL.

Is HCV a cardiovascular risk factor?

Evidence that HCV infection is associated with atherosclerosis

Despite its association with IR, diabetes and steatosis, whether HCV is an independent risk factor also for cardiovascular disorders remains controversial. Since this association has been the focus of major interest but also of some contradictory findings, it will be discussed in further detail.

Management of glucose metabolism alterations in hepatitis C

If HCV alters glucose metabolism, curing HCV should result in the improvement of such disorders and into a decreased incidence of type 2 diabetes in patients at risk. Most reports, with some exceptions [84], have indeed shown that SVR is associated with an improved IR [51], and a reduced risk of incident IR [85] or other glucose metabolism disturbances, including type 2 diabetes [86,87]. A large study on 2842 chronic hepatitis C patients treated with various IFN-containing regimens showed that viral eradica-
coured a two-thirds reduction in the risk of incident type 2 diabetes, independently of age, presence of cirrhosis and of pre-
diabetes before therapy [87]. In addition, treatment of HCV may also reduce the risk of diabetes complications, such as renal disorders and stroke, as shown by a large population-based study from Taiwan [61]. Nevertheless, eradication of HCV in patients with the metabolic syndrome should not prevent proper management of IR and type 2 diabetes via life-style changes and specific drugs. Increased physical activity may reduce IR and other features of the metabolic syndrome [88]. Interestingly, in patients with NAFLD, even moderate- and low-intensity intervention programs improved their metabolic profile independently of body weight changes [89]. On the other hand, a more intensive program of body weight reduction and physical exercise was able to decrease liver steatosis and fibrosis scores in a small cohort of patients [90]. Finally, anti-diabetic drugs may reduce some liver-related outcomes. This beneficial effect has been reported for metformin, which has been shown to reduce significantly (by about 50%) the risk of developing HCC [91–94]. An optimal control of glycaemia is pivotal in reducing this risk, since the incidence of HCC was significantly higher in patients with glycosylated haemoglobin \( \geq 7\% \) in those with levels <7% [83].

Nevertheless, eradication of HCV in patients with the meta-

and downregulation of anti-inflammatory mediators [67]. Several substances secreted by adipocytes and macrophages infiltrating omental (but not subcutaneous) adipose tissue may induce inflammation, fibrosis deposition and, incidentally, also steatosis in hepatic tissue [68–70], contributing to the damage linked to hyperinsulinemia. These factors include interleukin-6, tumour necrosis factor-\( \alpha \), and the monocyte chemotactic protein-1 (also known as chemokine [C-C motif] ligand 2 or CCL2). The important role of CCL2 is suggested not only by experimental models [71,72], but also by the observation that increased CCL2 levels in chronic hepatitis C patients predict liver fibrosis progression [73]. Thus, hyperinsulinemia (caused by the metabolic syndrome and HCV) and the chronic inflammatory state (again, caused by the metabolic syndrome and very likely also HCV) contribute synergistically to liver damage severity and progression. However, it has to be mentioned that viral IR does not contribute to steatosis, as it is specifically associated with a derangement of glucose rather than lipid homeostasis [54].

Higher HOMA-IR scores are associated with lower SVR rates in patients treated with IFN-\( \alpha \)-based regimens, independently of HCV genotypes [51,73–76]. However, a direct causal link between IR and IFN-\( \alpha \) resistance seems improbable, since correction of IR with pioglitazone does not influence HCV RNA levels or virological response to therapy [77]. Treatment with metformin seems to affect early virological response to IFN-\( \alpha \) [78] but these promising results have still to be independently replicated. The negative association between IR and virological response is attenu-
ated or disappears in patients treated with regimens containing direct acting antivirals, such as telaprevir [79,80]. Danoprevir monotherapy showed that the HOMA-IR decrease mostly paralleled the HCV RNA decline, and that baseline HOMA-IR levels did not affect response [81]. It is likely that IR will not influence the virological response to the upcoming IFN-free regimens.

Type 2 diabetes is definitely an important risk factor for HCC development. In a prospective series on 541 chronic hepatitis C patients [82], with Ishak fibrosis scores of 4–6 (16% with diabetes at baseline), the 5-year incidence of HCC was 5.0% in patients without diabetes and 11.4% in those with diabetes. By multivari-
ate Cox regression analysis, diabetes was independently associ-
ated with HCC in patients with an Ishak fibrosis score of 6 (HR 3.28, 95% CI 1.35–7.97). A subsequent large retrospective study [83] showed that in chronic hepatitis C, the presence of type 2 diabetes increases the incidence of HCC nearly two-fold compared with non-diabetic patients.

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The role of inflammation was further suggested by a subsequent study [103], where IMT and carotid plaques were assessed in 174 chronic hepatitis C patients and 174 controls, attending an outpatient cardiology unit. Here, the independent factors associated with carotid plaques were older age and severe liver fibrosis, leading the authors to conclude that patients with chronic hepatitis C and severe fibrosis should be screened for early atherosclerosis independently of their age.

Finally, Adinolfi et al. [9] not only confirmed the atherosclerotic risk in young HCV-infected persons but also noted an association between carotid atherosclerosis and both steatosis and viral load, hinting to a dose-effect mechanism. In the setting of coinfection with HIV, the independent, pro-atherogenic role of HCV seems diluted by the presence of several confounders, and therefore the results are not univocal [104–106].

HCV and cardiac disorders

Subsequent work assessed the relationship between HCV infection and some specific cardiovascular outcomes associated with atherosclerosis. Coronary artery disease was evaluated in a case-control study on 491 patients and 195 controls hospitalized for other cardiac abnormalities but with normal coronary arteries. The HCV prevalence was significantly higher among patients than among controls (6.3% vs. 2%), and increased with the number of arteries affected [107]. By multivariate analysis, HCV independently increased the risk of coronary artery disease by a factor of four. Similar results were reported by measuring the Reardon score, which assesses the extent of stenosis in the proximal coronary circulation [108] or in patients having undergone solid organ transplantation [109–111]. HCV increased the risk of coronary artery disease also in the observational cohort of all HCV-infected veterans [112], including 82,083 HCV-infected and 89,582 uninfected subjects. By multivariate analysis, and despite their favourable lipid profile, HCV-infected veterans had a higher risk of coronary artery disease (HR, 1.25; 95% CI, 1.20–1.30) on top of classical risk factors such as age, arterial hypertension, chronic obstructive pulmonary disease, diabetes, and hyperlipidemia. A similar study, using a prospective cohort design on 8579 veterans infected by HCV, coinfected by HIV, or uninfected [113], and followed for a median of 7.3 years, concluded that HIV+ HCV+ veterans have an increased risk of coronary heart disease compared with HIV+ HCV− and HIV− HCV− veterans, and similar conclusions were reached by Bedimo et al. [114]. Myocardial ischemia was associated with arterial hypertension, metabolic syndrome and also HCV infection in a study conducted on 5015 persons from Taiwan evaluated by ECG for a general check-up [115].

Some studies have analysed the potential effect of HCV infection on heart function, rather than on the coronary artery integrity. Maruyama and co-workers studied 217 HCV-infected patients by myocardial scintigraphy and found that 87% had myocardial perfusion defects, and that the severity of the defects was associated with the degree of liver necroinflammatory activity [116]. The interesting aspect of this study is that the response to antiviral therapy was accompanied by the improvement of anomalies, in contrast with the lack of response. However, using data of 19,741 persons (173 or 0.88% with detectable HCV RNA) from a general population survey [117], Younossi and co-workers found that HCV was independently associated with IR, diabetes, hypertension and congestive heart failure, but not with ischemic heart disease.

HCV and stroke

Regarding cerebrovascular outcomes, a cohort study from Taiwan found that chronic hepatitis C is an independent predictor of stroke [118]. As many as 4094 adults with HCV infection were compared to 16,376 adults without HCV and matched by age and sex. Compared with uninfected persons, the adjusted HR of stroke was 1.27 (95% CI 1.14 to 1.41) for people with HCV. Another community-based, prospective study found that HCV infection is an independent predictor of cerebrovascular death, and a significant dose-effect association was observed between serum HCV RNA levels and cerebrovascular mortality, suggestive of a causal link [119]. Importantly, IFN-based therapy reduced by ~60% the incidence of stroke, compared to no treatment, in another large, retrospective cohort study [120], further adding evidence for a causal association between HCV infection and cerebrovascular damage. Similar results were reported more recently in a prospective cohort study where 1411 diabetic HCV patients treated with IFN-α-based regimens were matched with 1411 diabetic, HCV-infected but untreated controls [61]. The treated cohort was further matched with 5644 diabetic uninfected controls. After 8 years of follow-up, the difference in the cumulative incidence of end-stage renal disease and stroke among treated, untreated, and uninfected cohorts was significant. In multivariate analysis, antiviral treatment was associated with a significantly reduced risk of end-stage renal disease (HR = 0.16; 95% CI, 0.07–0.33%) and of ischemic stroke (HR = 0.53; 95% CI, 0.30–0.93), although no data were available on the post-treatment HCV RNA status. These impressive results need confirmation in different areas of the world, but will likely influence the future paradigm of therapy for chronic hepatitis C by extending it to patients at risk of extrahepatic complications, irrespective of the severity of the underlying liver damage.

HCV and cardiovascular mortality

If HCV is implicated in the pathogenesis of cardiovascular lesions, this should result into an increased cardiovascular mortality, as shown by studies linking large databases with death registries. The first of such studies appeared in 2006 [121] and was conducted on 75,834 patients with hepatitis C. In this population, cardiovascular diseases were the third most common cause of death, with a standardized mortality ratio of 1.3 (95% CI 1.2–1.5) after adjustment for age, sex, and calendar year. However, no data were available on possible confounders such as smoking. In a following retrospective cohort study, Guerrini and collaborators studied 10,259 anti-HCV-positive blood donors and 10,259 HCV-seronegative donors matched for age, gender and other parameters [122]. Anti-HCV-positive donors presented an increased cardiovascular mortality (HR = 2.21, 95% CI: 1.41–3.46). Unfortunately, data for
socioeconomic status, smoking, cholesterol, diet, or other confounders were again missing; ethnicity data was incomplete, and chronic cocaine use data, linked to an increased risk of coronary artery calcification [123] was also missing. Finally, according to a vast study issued by the REVEAL cohort, 23,820 adults in Taiwan — 1095 of which were anti-HCV-positive and 760 had detectable HCV RNA — were prospectively followed for an average period of 16.2 years [124]. This important study showed that persistent HCV infection increased both hepatic and extrahepatic mortality compared to uninfected controls. In particular, anti-HCV-positive participants had a higher risk of dying from circulatory diseases and renal diseases, with a multivariate-adjusted HR of 1.50 (95% CI 1.10–2.03) and 2.77 (1.49–5.15), respectively, compared with seronegative persons. The association between HCV RNA and cardiovascular death showed that it is the replicating HCV rather than prior infection (i.e. seropositive for anti-HCV but negative for HCV RNA) that matters in predicting the risk of mortality. Furthermore, this study collected a vast array of data from enrolled persons, allowing adjustment of hazard ratios for age, sex, cigarette smoking, alcohol drinking, and central obesity; hazard ratios for circulatory diseases were additionally adjusted for personal history of diseases and baseline serum levels of cholesterol and triglycerides. Thus, based on these two studies from Taiwan, it seems as if treating HCV not only reduces the liver-related outcomes, but also the extrahepatic ones.

**HCV and cardiovascular lesions: in search of a mechanism**

The fact that HCV seems to increase the risk of morbidity and mortality only for some specific cardiovascular events, and the observation that the atherogenic role of HCV appears independent of classical risk factors, including smoking and the metabolic syndrome, raise the issue of the pathogenesis of cardiovascular damage in HCV infection. Although metabolic factors, such as steatosis [9], may play a role, systemic chronic inflammation seems more likely to be a central factor [100,103,115], together with possible endothelial dysfunction [100] and the contribution of genetic and environmental factors. The proposal of a direct viral infection of the arterial wall [125] has not been independently confirmed, although HCV has been shown to infect the brain endothelial cells [126]. Finally, it is interesting to note that according to a very recent Danish study, some biomarkers predictive of coronary artery disease were more elevated in 60 HCV-infected patients than among 60 controls: these markers included high-sensitivity C-reactive protein, soluble intercellular adhesion molecule-1, soluble vascular cell adhesion molecule-1 and soluble E-selectin [127]. These concepts are summarized in Fig. 2. Further research is warranted in this fascinating field.

**HCV and cardiovascular risk: a note of caution**

This discussion would not be complete without mentioning the studies that have failed to identify an association between HCV infection and cardiovascular morbidity. A first case-control study from the northeast of Germany (Pomerania) [128] enrolled 233 cases with antibody to HCV or HBV surface antigen (anti-HBs), and 4033 seronegative controls. By multivariate analysis, there was no association between serostatus and myocardial infarction, stroke, carotid IMT, and carotid plaques. However, this study pooled patients with HCV infection together with those cured from HBV infection, and this may have diluted any effect due to HCV. On the other hand, a mere 21 patients were anti-HCV-positive. Similarly, a case-control study of 292 active-duty US military personnel hospitalized for acute myocardial infarction and 290 controls failed to find an association between HCV and acute myocardial infarction [129]. Although only 52 cases were anti-HCV-positive, the results were clearly arguing against a putative role of HCV in inducing myocardial infarction.

A subsequent 5-year longitudinal case-control study (40 HCV-infected patients and 40 uninfected controls, matched for classical atherosclerotic risk factors) analysed the progression of atherosclerotic plaques in carotid and femoral arteries [130]. Not only patients showed no changes in plaque and IMT during follow-up, but a significant increase in carotid IMT was observed only among controls, prompting the authors to suggest that HCV may even delay the atherosclerotic process, possibly via its favourable lipid profile. Another large prospective cohort study from Norway on 1010 HCV-positive patients followed for a median of 7 years [131] failed to show a statistically significant increase in the standardized mortality ratio due to cardiovascular diseases. However, data on smoking and other cardiovascular risk factors were missing. In addition, the diagnostic assays for HCV were performed by occasional blood testing, and therefore the number of HCV-related deaths may have been underestimated. Finally, a large retrospective cohort study conducted in the UK enrolled 4809 HCV-infected individuals matched for age, sex and medical practice with up to 15 randomly selected patients without HCV, leading to a staggering number of 71,668 controls [132]. Rates of incident MI during a median follow-up of 3.2 years were comparable between HCV-infected and uninfected cases.

The discrepancies between the numerous studies reported above have enlivened the debate regarding the association between HCV and cardiovascular outcomes. Large studies conducted with rigour and a wealth of data have produced compelling but conflicting results. It seems very likely that the increase in risk for selected outcomes may be small, to the point of being heavily conditioned by unknown confounders, including genetic variants. The latter may explain the striking differences among studies conducted in different ethnic populations. Only carefully conducted, prospective cohort studies, using appropriate stratifications and exploiting the high efficacy of novel antivirals may discriminate the effect of HCV on each single cardiovascular disorder.
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Conflict of interest
F. Negro is consultant for Roche and MSD, is advising Gilead, Janssen, Novartis, Bristol-Myers Squibb and Boehringer Ingelheim, and has received unrestricted research grants from Roche, Gilead and Novartis.

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