Effect of Hepatitis B virus on Steatosis in Hepatitis C virus co-infected subjects: a multicenter study and systematic review

Running title: BOSTIC: Steatosis in HBV-HCV coinfection

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Abstract:

It remains unclear whether hepatitis B virus (HBV) infection may modify the severity of viral steatosis in patients coinfected with chronic hepatitis C virus (HCV). We examined the influence of coinfection with HBV on prevalence of steatosis in chronic hepatitis C in a multi-center cohort of HBV-HCV subjects, and by performing a systematic review and meta-analysis of the literature. We centrally and blindly assessed steatosis prevalence and severity in a cohort of HBV-HCV coinfected subjects compared to HCV and HBV monoinfected controls and we performed a systematic review of studies addressing the prevalence of steatosis in HBV-HCV subjects compared to HCV controls. In the clinical cohort, we included 85 HBV-HCV, 69 HBV and 112 HCV subjects from 16 international centers. There was no significant difference in steatosis prevalence between the HBV-HCV and the HCV groups (33% vs 45%, p=0.11). In subgroup analysis, lean HBV-HCV subjects with detectable HBV DNA had less steatosis than lean HCV subjects matched for HCV viremia (15% vs 45%, p=0.02). Our literature search identified 5 additional studies included in a systematic review. Overall, prevalence of steatosis > 5% was similar in HBV-HCV infection compared to HCV (pooled odds ratio [OR] 0.91, 95% CI 0.53-1.6) although there was significant heterogeneity (I² 69%, p = 0.007). In conclusion, although the prevalence of steatosis is similar in HBV-HCV compared to HCV subjects, our analysis suggests that there may be an inhibitory effect of HCV-induced steatogenesis by HBV in certain subgroups of patients.
Key words: Steatosis, hepatitis C virus, hepatitis C-hepatitis B coinfection, metabolic syndrome.

The clinical association between steatosis and hepatitis C virus (HCV) infection was described even prior to the discovery of HCV (1). The prevalence of fatty liver in chronic HCV patients varies from 40 to 80% depending on cofactors (2). The association between steatosis and HCV infection is especially strong for genotype 3 infections where steatosis is more severe and frequent; its severity correlates with the level of viral replication and it disappears after successful HCV therapy (2-4). In non-3 genotypes, the association is less strong and steatosis correlates mostly with metabolic risk factors and largely persists despite successful antiviral therapy (2, 3). Mechanisms leading to fat accumulation in HCV infection are complex and have been reviewed elsewhere (2, 5). Thus, in HCV-infected individuals, two major types of steatosis are found, i.e. virally-induced and metabolic steatosis.

The association between steatosis and chronic hepatitis B virus (HBV) infection is less clear. Up to one third of chronic hepatitis B patients may present with steatosis. This is essentially due to the overlap of HBV infection with the metabolic syndrome (6). Its overall prevalence seems comparable to that of the general population (6), and may even be lower than in controls, after adjusting for demographic and metabolic factors (7). In a meta-analysis of 17 studies, chronic HBV infection was associated with a reduced risk of hepatic steatosis when compared to uninfected controls (6). In that study, steatosis in HBV subjects was positively associated with male gender, body mass index (BMI), obesity, diabetes, blood glucose, cholesterol and triglyceride levels, and moderate alcohol consumption and a strong negative association between steatosis and HBV DNA levels was reported, suggesting a direct viral effect on lipid metabolism (6) while other studies reported an association between HBV infection and a lower prevalence of fatty liver (7-9). The mechanism(s) responsible for this apparent, partial protection are unknown.

HBV-HCV coinfection occurs in 3 to 18% of hepatitis B surface antigen (HBsAg) positive patients and 2 to 10% of HCV subjects (10, 11). HBV-HCV coinfection is an appealing model to study the interaction of HBV and HCV and, in particular, the effect on steatosis as it remains unclear whether
the putative protective effect of HBV on triglyceride accumulation may also occur on viral steatosis. *In vitro* experiments have shown that the expression of HBV proteins does not lead to interference with HCV replication (12, 13), although this is a well-known phenomenon occurring in dually infected patients (14-16). Similarly, previous clinical studies have addressed the effect of HBV coinfection in HCV-induced steatosis, although none of these studies assessed the subgroup of genotype 3a HCV infected subjects and the findings were contradictory (17-21). For instance, one recent clinical study including 66 HBV-HCV co-infected subjects found that prevalence of steatosis was not significantly different between HBV-HCV subjects and matched HCV subjects, however the study was a single-centre cohort, and focused on the role of *PNPLA3* polymorphisms in this patient population (21). The question is clinically important as steatosis is reported to be an independent risk factor for cardiovascular outcomes and may favour the development of hepatocellular carcinoma in HCV-infected subjects (22-26).

Our aim was to assess, in a large multi-centre international cohort of HBV-HCV subjects (Effect of hepatitis B on steatosis in hepatitis C virus co-infected subjects study, BOSTIC), and by performing a systematic review and meta-analyses, whether coinfection with HBV may affect prevalence and severity of steatosis in chronic hepatitis C.

**Patients and Methods**

**BOSTIC Clinical cohort**

We retrospectively enrolled 266 patients with available liver biopsies with either HBV-HCV coinfection, HCV and HBV mono-infection from 13 international centers (the BOSTIC study, see analysis flowchart Supplementary Figure 1). HBV-HCV coinfection was defined as HBsAg and HCV RNA positive for more than 6 months and mono-infected controls were either HBsAg or HCV RNA positive for more than 6 months at time of liver biopsy. Patients were excluded if they had excessive alcohol consumption (defined as greater than 21 or 14 alcoholic drink units per week in men and women respectively), had type 2 diabetes or if they were undergoing active therapy for HBV.
or HCV less than 3 months prior to time of liver biopsy. Additional methods for the clinical cohort can be found in the Supplementary Methods.

Systematic review and meta-analysis

We followed the procedures for reporting systematic reviews and meta-analysis of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA, see Supplementary Table 1) (27). For detailed methods for the systematic review please see Supplementary Methods.

Results

BOSTIC Clinical cohort

Baseline characteristics of the BOSTIC subjects

A total of 85 HBV-HCV co-infected subjects, 112 HCV and 69 HBV mono-infected subjects with available histology for centralized histological assessment were included (see analysis flowchart Supplementary Figure 1). HCV genotype 3 subjects and HCV RNA levels were well-balanced between the HBV-HCV and HCV groups (p=0.40 and p=0.14 respectively) and BMI was similar in all 3 groups (HBV-HCV versus HCV p=0.56, HBV-HCV versus HBV p=0.14), suggesting that key cofactors affecting the presence of steatosis were homogeneously distributed (Table 1). As expected, HBV DNA levels were significantly lower in the HBV-HCV group compared to the HBV group (<5 IU/mL versus 1.5E5 IU/mL respectively, p<0.001).

The proportion of subjects with prediabetes, defined as fasting glucose greater than or equal to 5.6 mmol/L, was lower in the HBV-HCV group than in the HCV group (p=0.001) but there was no difference between the HBV-HCV group compared to the HBV group (p=1.0). Triglycerides were higher but total cholesterol was lower in the HBV-HCV group compared to the HBV subjects (p=0.04 and p<0.001 respectively), whereas the serum levels were similar in the HBV-HCV and HCV groups (p=0.10 and p=0.48 respectively). When comparing HBV to HCV groups, as expected prediabetes was more common in HCV (p=0.01), age was younger in the HBV group (p=0.001), total cholesterol levels were higher in the HCV group (p<0.001) although triglyceride serum levels were similar.
Comparison of histological parameters apart from steatosis (Table 2) among the groups showed less fibrosis in the HBV group compared to the HBV-HCV group (p=0.007) and to the HCV group (p=0.04).

Factors associated with steatosis

Interestingly, the only baseline factors associated with steatosis > 5% in the 85 HBV-HCV subjects were BMI above 25 kg/m$^2$ (odds ratio [OR] 3.8, 95% confidence interval [95% CI] 1.4-11.6, p=0.005) and fasting plasma glucose levels (p=0.02). HCV genotype 3 infection (OR 2.1, 95% CI 0.6-7.2, p=0.25), total cholesterol greater than 5.2 mmol/L (OR 5.1, 95% CI 0.6-245, p=0.14) and triglycerides over 1.7 mmol/L (OR 1.6, 95% CI 0.3-10.9, p=0.72) were not associated with presence of steatosis in HBV-HCV subjects. In HCV mono-infected subjects factors associated with significant steatosis included male sex (OR 3.5, 95% CI 1.4-9.7, p =0.004), genotype 3 HCV (OR 3.4, 95% CI 1.3-9.4, p=0.006), cirrhosis (OR 2.8, 95% CI 1.1-7.1, p=0.02), age more than 50 years (OR 2.1, 95% CI 1.2-3.7, p=0.006), BMI above 25 kg/m$^2$ (OR 2.6, 95% CI 1.1-6.1, p=0.02), serum glucose above 5.6 mmol/L (OR5.8, 95% CI 2.1-17, p<0.001) and triglycerides over 1.7 mmol/L (OR 4.0, 95% CI 1.03-19, p=0.04). In HBV mono-infected subjects only BMI above 25 kg/m$^2$ (OR 4.6, 95% CI 1.3-17, p =0.009) was associated with significant steatosis.

To assess whether HBV-HCV was associated with different steatosis prevalence, we next assessed which factors were associated with steatosis > 5% in combined HBV-HCV co-infected subjects and HCV mono-infected subjects (Table 3). In univariable analysis, the presence of HBV-HCV coinfection was non-significantly associated with a slight reduction of steatosis (OR 0.6, 95% CI 0.3-1.1, p=0.10) and in multivariable analysis (Table 3), only male sex, BMI above 25 kg/m$^2$, HCV genotype 3, elevated AST and age above 50 years were associated with significant steatosis in combined HCV and HBV-HCV subjects.
Comparison of Steatosis prevalence

Prevalence of steatosis >5% in HBV-HCV subjects was comparable to that observed in HCV mono-infected subjects (33% versus 45% in HBV-HCV and HCV subjects, respectively, p=0.11, Figure 1A and Table 2). Similarly, there was no significant difference in steatosis prevalence between HBV-HCV co-infected and HBV mono-infected subjects (33% versus 28%, respectively, p=0.49, Figure 1A and Table 2). We found no significant difference in steatosis in genotype 3 HBV-HCV vs HCV subjects (47% and 67% respectively, p=0.23) although the number of subjects was limited (n=17, 20%).

Next, since 57% of HBV-HCV and 8% of HBV patients had undetectable HBV DNA, we assessed the role of HBV-HCV coinfection on steatosis considering only subjects with detectable serum HBV DNA levels (i.e. serum levels above 20 IU/mL). We found that HBV-HCV subjects with detectable HBV DNA had significantly less steatosis than HCV subjects (steatosis prevalence of 23% and 45% respectively, p=0.03) but very similar steatosis levels to HBV subjects (steatosis prevalence of 23% and 26% respectively, p=0.81) although we noted lower levels of serum HCV RNA in the HBV-HCV group compared to the HCV group (5.2E4 IU/mL versus 2.4E5 IU/mL respectively, p=0.04). To assess whether the reduced steatosis in the HBV-HCV group was due to reduced viral replication or a direct effect of HBV on lipid metabolism, we matched 20 lean HBV-HCV subjects with detectable HBV DNA with 40 lean HCV subjects based on serum HCV RNA levels (Figure 1B). Despite the well matched HCV viremia (1.1E5 vs 1.1E5, p=0.30, see also baseline characteristics of this subgroup in Supplementary Table 2), steatosis was significantly less frequent in the HBV-HCV group (15% vs 45% in the HBV-HCV and HCV groups respectively, p=0.03 [p=0.06 when adjusted for multiple testing]). Interestingly, similarly to the overall cohort, the proportion of HBV-HCV with impaired fasting glucose in this subgroup was significantly lower than HCV subjects (12% versus 48% respectively, p=0.02) leading to the question whether the effect on steatosis is a direct effect of HBV on lipid metabolic pathway (5) or an indirect effect acting via insulin resistance.
Systematic review and meta-analysis

Characteristics of included studies

After excluding duplicate studies, we screened 1,539 studies and assessed 19 studies for eligibility (Supplementary Figure 2). Of these, 16 studies were excluded (10 studies did not include HBV-HCV co-infected subjects, 2 studies with no liver biopsy, 3 studies assessed HIV co-infected subjects and 1 study, published as an abstract, had insufficient data for review or meta-analysis) and 5 studies were included for the final analysis (17-21). We also included the current international cohort of HBV-HCV subjects, the BOSTIC cohort, as a sixth study. All the studies were case-control studies published between 2004 and 2014, characteristics of included studies are summarized in Table 4. We assessed risk of bias and methodological quality of included studies using the NOS scale. 4/6 studies, including our current study (17, 18, 21), were found to have a low risk of bias, whereas 2/6 studies had a medium risk of bias (19, 20).

Findings of meta-analysis

All included studies reported sufficient data to assess the association of HBV-HCV co-infection with steatosis > 5%. When including all studies, the presence of HBV-HCV co-infection was not significantly associated with steatosis > 5% compared to HCV subjects (pooled OR 0.91, 95% CI 0.53-1.6, Figure 2A), although there was significant heterogeneity ($I^2$ 69%, $p = 0.007$). Similarly, there was no association between HBV-HCV co-infection and steatosis > 30% in the 4 studies (17, 18, 21) reporting this outcome (pooled OR 1.2, 95% CI 0.3-4.6, Figure 2B), although there was again significant heterogeneity ($I^2$ 88%, $p < 0.001$).

Additional analyses

When including the 4 studies with a low risk of bias (NOS score above 5), there was also no association between HBV-HCV coinfection and steatosis above 5% (pooled OR 1.2, 95% CI 0.61-2.6) or steatosis above 30% (pooled OR 1.2, 95% CI 0.29-4.6), although there remained significant heterogeneity ($I^2$ 74%, $p = 0.009$ and $I^2$ 88%, $p < 0.001$ respectively). When including the 3 studies with more than 50 HBV-HCV co-infected subjects, there remained no association between HBV-
HCV and steatosis above 5% (pooled OR 0.93, 95% CI 0.64-1.4) and heterogeneity was reduced ($I^2$ 19%, $p = 0.29$). Due to the lower number of studies reporting steatosis above 30% it was more challenging to assess heterogeneity although we note that the significant heterogeneity may be explained by the increased discrepancy among pathologists in the assessment of high (moderate and severe) grades of steatosis (i.e. ≥ 30%) (28).

Although we assessed a low number of studies, the assessment of funnel plots (Supplementary Figure 3) suggested potential reporting bias although, as recommended by the Cochrane group (29), we did not formally test significance for funnel plot asymmetry due to the low number of studies.

**Discussion**

In a large, retrospective cohort of HBV-HCV subjects from 13 international centers, we did not find a significant difference in the prevalence of steatosis between HBV-HCV co-infected subjects and HCV mono-infected subjects. However, the subgroup of HBV-HCV subjects with detectable and quantifiable serum HBV DNA had significantly less steatosis compared to HCV subjects and this was confirmed in lean subjects with detectable HBV DNA matched with HCV controls for serum HCV RNA levels.

These results seem to suggest that if HBV exerts any effect on viral steatosis, this may be a direct effect on lipid metabolism rather than an indirect one brought about by interference in HCV replication. Alternatively, the lower rate of impaired fasting glucose levels in the HBV-HCV group may suggest that this effect may be linked to HBV-mediated improved insulin resistance. However, this effect seems weak and should be confirmed in further studies with larger number of patients.

Next, in a systematic review and meta-analysis of the existing literature, we failed to identify any significant difference in steatosis prevalence between HBV-HCV and HCV subjects, although there was significant heterogeneity among studies. Heterogeneity was partially reduced when including only studies with more than 50 HBV-HCV subjects, suggesting that smaller studies may have introduced a significant bias.
Most studies investigating relationship between HBV and the host lipid metabolism show that HBV seroprevalence is associated with a reduced risk of metabolic syndrome, especially among men, and largely due to a favorable effect on dyslipidemia (7, 9, 30-34). Our recent observation that HCV may induce fatty liver via the downregulation of the PTEN tumor suppressor prompted us to study the effect of HBV on HCV-induced steatosis (23). Interestingly, in the present study, we observed that HCV genotype 3 infection among HBV-HCV subjects was not associated with steatosis, as opposed to the expected association seen among HCV mono-infected subjects, although this analysis may have been underpowered due to the small number of genotype 3 HBV-HCV subjects. In addition, lean patients with detectable HBV DNA had less steatosis than HCV mono-infected controls matched for serum HCV RNA levels. Thus, we wonder whether this effect of HBV on HCV-induced lipid accumulation observed in our study – albeit weak – may proceed via an interaction between HBV proteins and the tumor suppressor PTEN. This hypothesis warrants further testing in both experimental and clinical settings.

The interaction between HCV infection, steatosis and insulin resistance (IR) is complex (35). In particular, systematic reviews have shown that there is a significant clinical association between HCV and IR and/or type 2 diabetes, suggesting that HCV increases the risk of diabetes especially in predisposed individuals (36). To exclude confounding causes of steatosis, we excluded subjects with type 2 diabetes, although we still found that HBV-HCV subjects had a lower prevalence of prediabetes than HCV subjects but similar to HBV subjects suggesting that HBV may interact with HCV by reducing IR as opposed to what is seen in HBV mono-infection (37, 38), although a viral interference, i.e. suppression of HCV replication by HBV for example through secretion of HCV-induced cytokines (39), could also be involved. Further studies are warranted to assess this point.

The strengths of our approach include a centralized histological assessment, multi-center cohort and rigorous inclusion and exclusion criteria, and combination with a systematic review of the literature. Nevertheless, our study had some limitations, as our clinical cohort study was underpowered to assess the difference in steatosis in subjects with HCV genotype 3 and BMI below 25 kg/m², which would allow investigation of the difference in prevalence in steatosis in subjects without other major factors of steatosis. As for the systematic review, despite contacting the authors
we had incomplete data for steatosis over 30% for 2 studies (19, 20), potentially biasing our findings, although these were also the 2 studies at highest risk of bias.

In conclusion, we have shown that there is no major difference in steatosis distribution between HBV-HCV co-infected subjects and HCV mono-infected subjects, although our analysis of our clinical cohort also suggests that HBV may have a weak effect on lipid metabolic pathways affected by HCV, thus impacting viral steatosis. Further studies should clarify the effect of HBV-HCV coinfection in HCV genotype 3 subjects to further characterize the influence of HBV on pure viral-induced steatosis. However, if these lipid metabolic pathways are also affecting oncogenesis (e.g. PTEN downregulation), this effect (albeit weak) may become clinically significant. In the meantime, we believe that our findings have shed an unexpected new light on the complexity of the viral and clinical interactions between the HBV and HCV viruses.

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Declaration of personal interests
None

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References


Table 1: Baseline clinical, virological and biological characteristics of patients by study group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HBV-HCV co-infected</th>
<th>HCV</th>
<th>HBV</th>
<th>p-value (HBV-HCV vs HCV)</th>
<th>p-value (HBV-HCV vs HBV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>85</td>
<td>112</td>
<td>69</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Clinical**
- Male, n (%)  
  - HBV-HCV co-infected: 53 (77%)  
  - HCV: 74 (67%)  
  - HBV: 61 (72%)  
  - p-value (HBV-HCV vs HCV): 0.53  
  - p-value (HBV-HCV vs HBV): 0.58
- Age, median (IQR)  
  - HBV-HCV co-infected: 44 (36-55)  
  - HCV: 47 (37-54)  
  - HBV: 40 (30-48)  
  - p-value (HBV-HCV vs HCV): 0.63  
  - p-value (HBV-HCV vs HBV): 0.01
- BMI (kg/m²), median (IQR)  
  - HBV-HCV co-infected: 24 (23-27)  
  - HCV: 24 (22-27)  
  - HBV: 24 (22-26)  
  - p-value (HBV-HCV vs HCV): 0.56  
  - p-value (HBV-HCV vs HBV): 0.14
- BMI greater than 25kg/m², n (%)  
  - HBV-HCV co-infected: 35 (43%)  
  - HCV: 46 (41%)  
  - HBV: 22 (32%)  
  - p-value (HBV-HCV vs HCV): 0.88  
  - p-value (HBV-HCV vs HBV): 0.24

**Virological**
- HCV genotype 3, n (%)  
  - HBV-HCV co-infected: 17 (20%)  
  - HCV: 30 (27%)  
  - HBV: -  
  - p-value (HBV-HCV vs HCV): 0.40  
  - p-value (HBV-HCV vs HBV): -
- HCV RNA (IU/mL), median (IQR)  
  - HBV-HCV co-infected: 3.6E5 (2.2E4-8.6E5)  
  - HCV: 3.3E5 (9.3E4-1.6E6)  
  - HBV: -  
  - p-value (HBV-HCV vs HCV): 0.14  
  - p-value (HBV-HCV vs HBV): -
- HBV DNA (IU/mL), median (IQR)  
  - HBV-HCV co-infected: <5 (<5-3.2E3)  
  - HCV: -  
  - HBV: 1.5E5 (3.3E3-4.1E7)  
  - p-value (HBV-HCV vs HCV): -  
  - p-value (HBV-HCV vs HBV): <0.001

**Biological**
- Bilirubin (µmol/L), median (IQR)  
  - HBV-HCV co-infected: 10 (6-17)  
  - HCV: 11 (9-17)  
  - HBV: 15 (11-20)  
  - p-value (HBV-HCV vs HCV): 0.60  
  - p-value (HBV-HCV vs HBV): 0.06
- ALT (IU/L), median (IQR)  
  - HBV-HCV co-infected: 70 (46-117)  
  - HCV: 74 (45-131)  
  - HBV: 52 (30-102)  
  - p-value (HBV-HCV vs HCV): 0.43  
  - p-value (HBV-HCV vs HBV): 0.14
- AST (IU/L), median (IQR)  
  - HBV-HCV co-infected: 58 (35-81)  
  - HCV: 60 (39-100)  
  - HBV: 36 (30-59)  
  - p-value (HBV-HCV vs HCV): 0.35  
  - p-value (HBV-HCV vs HBV): 0.01
- GGT (IU/L), median (IQR)  
  - HBV-HCV co-infected: 49 (30-77)  
  - HCV: 64 (35-113)  
  - HBV: 32 (22-62)  
  - p-value (HBV-HCV vs HCV): 0.12  
  - p-value (HBV-HCV vs HBV): 0.02
- AP (IU/L), median (IQR)  
  - HBV-HCV co-infected: 99 (79-159)  
  - HCV: 75 (57-96)  
  - HBV: 75 (61-92)  
  - p-value (HBV-HCV vs HCV): <0.001  
  - p-value (HBV-HCV vs HBV): <0.001

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<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/L), median (IQR)</td>
<td>144 (135-151)</td>
<td>144 (134-156)</td>
<td>148 (136-157)</td>
<td>0.96</td>
</tr>
<tr>
<td>WCC (x10^9/L), median (IQR)</td>
<td>6.8 (5.8-9.9)</td>
<td>6.0 (5.3-8.0)</td>
<td>5.2 (4.2-6.5)</td>
<td>0.22</td>
</tr>
<tr>
<td>Platelets (x10^9/L), median (IQR)</td>
<td>196 (154-249)</td>
<td>183 (143-220)</td>
<td>187 (145-234)</td>
<td>0.10</td>
</tr>
<tr>
<td>Fasting glucose &gt; 5.6 mmol/L (%)</td>
<td>10 (14%)</td>
<td>34 (37%)</td>
<td>7 (15%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/L), median (IQR)</td>
<td>1.2 (0.9-1.5)</td>
<td>1.0 (0.7-1.6)</td>
<td>1.0 (0.7-1.3)</td>
<td>0.10</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L), median (IQR)</td>
<td>4.5 (4.0-4.8)</td>
<td>4.3 (3.6-4.9)</td>
<td>5.2 (4.5-5.8)</td>
<td>0.48</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L), median (IQR)</td>
<td>1.1 (0.9-1.5)</td>
<td>1.2 (0.9-1.5)</td>
<td>1.3 (1.1-1.6)</td>
<td>0.73</td>
</tr>
<tr>
<td>Ferritin (µg/L), median (IQR)</td>
<td>198 (90-305)</td>
<td>167 (67-306)</td>
<td>91 (77-191)</td>
<td>0.62</td>
</tr>
</tbody>
</table>

AP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, Gamma-glutamyl transferase; HBV, hepatitis B virus; HCV, hepatitis C virus; HDL, high-density lipoprotein; HOMA, Homeostasis Model Assessment; IQR, interquartile range; WCC, white cell count
Table 2: Baseline histological characteristics by study group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HBV-HCV</th>
<th>HCV</th>
<th>HBV</th>
<th>p-value (HBV-HCV vs HCV)</th>
<th>p-value (HBV-HCV vs HBV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>METAVIR: A, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 0</td>
<td>13 (15%)</td>
<td>25 (22%)</td>
<td>15 (22%)</td>
<td>0.06</td>
<td>0.76</td>
</tr>
<tr>
<td>- 1</td>
<td>46 (55%)</td>
<td>70 (63%)</td>
<td>37 (54%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 2</td>
<td>24 (29%)</td>
<td>16 (14%)</td>
<td>16 (23%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 3</td>
<td>1 (1%)</td>
<td>1 (1%)</td>
<td>1 (1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>METAVIR: F, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 0</td>
<td>17 (20%)</td>
<td>23 (21%)</td>
<td>28 (41%)</td>
<td>0.11</td>
<td>0.007</td>
</tr>
<tr>
<td>- 1</td>
<td>29 (34%)</td>
<td>34 (30%)</td>
<td>12 (17%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 2</td>
<td>18 (21%)</td>
<td>12 (11%)</td>
<td>8 (12%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 3</td>
<td>8 (9%)</td>
<td>11 (10%)</td>
<td>4 (6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 4</td>
<td>13 (15%)</td>
<td>32 (29%)</td>
<td>17 (25%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steatosis, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- &lt;5%</td>
<td>57 (67%)</td>
<td>62 (55%)</td>
<td>50 (72%)</td>
<td>0.37</td>
<td>0.72</td>
</tr>
<tr>
<td>- 5-30%</td>
<td>21 (25%)</td>
<td>40 (36%)</td>
<td>16 (23%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 31-60%</td>
<td>6 (7%)</td>
<td>8 (7%)</td>
<td>2 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- &gt;60%</td>
<td>1 (1%)</td>
<td>2 (2%)</td>
<td>1 (1%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HBV, hepatitis B virus; HCV, hepatitis C virus; METAVIR: A, activity score; METAVIR: F, fibrosis score.

P-values were computed using Fisher’s exact test.
Table 3: Factors associated with steatosis >5% in combined HBV-HCV co-infected and HCV mono-infected subjects by univariable and multivariable analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariable</th>
<th></th>
<th>Multivariable (selected by AIC)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>p-value</td>
<td>OR</td>
</tr>
<tr>
<td>Male sex</td>
<td>2.9</td>
<td>1.4-6.2</td>
<td>0.002</td>
<td>3.9</td>
</tr>
<tr>
<td>Age &gt; 50 years</td>
<td>2</td>
<td>1.1-3.8</td>
<td>0.02</td>
<td>2.9</td>
</tr>
<tr>
<td>HBV-HCV coinfection</td>
<td>0.6</td>
<td>0.3-1.1</td>
<td>0.10</td>
<td>-</td>
</tr>
<tr>
<td>• HBV-HCV co-infection with viremic HBV DNA</td>
<td>0.4</td>
<td>0.1-0.96</td>
<td>0.03</td>
<td>-</td>
</tr>
<tr>
<td>BMI &gt; 25kg/m²</td>
<td>2.7</td>
<td>1.4-5.2</td>
<td>0.001</td>
<td>8.5</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>2.1</td>
<td>1.0-4.3</td>
<td>0.04</td>
<td>-</td>
</tr>
<tr>
<td>HCV genotype 3</td>
<td>2.9</td>
<td>1.4-6.1</td>
<td>0.002</td>
<td>6</td>
</tr>
<tr>
<td>HCV viremia &gt; 8E5 IU/mL</td>
<td>1.5</td>
<td>0.8-3.0</td>
<td>0.20</td>
<td>-</td>
</tr>
<tr>
<td>Bilirubin &gt; 17 µmol/L</td>
<td>1.7</td>
<td>0.3-9.9</td>
<td>0.50</td>
<td>-</td>
</tr>
<tr>
<td>AST &gt; 50 IU/L</td>
<td>4</td>
<td>1.9-8.4</td>
<td>&lt;0.001</td>
<td>5</td>
</tr>
<tr>
<td>GGT &gt; 50 IU/L</td>
<td>3.7</td>
<td>1.7-8.2</td>
<td>&lt;0.001</td>
<td>-</td>
</tr>
<tr>
<td>Platelets &lt; 150 G/L</td>
<td>0.9</td>
<td>0.4-1.8</td>
<td>0.90</td>
<td>-</td>
</tr>
<tr>
<td>Fasting glucose &gt;5.6 mmol/L</td>
<td>2.8</td>
<td>1.4-5.8</td>
<td>0.001</td>
<td>2.3</td>
</tr>
<tr>
<td>Triglycerides &gt; 1.7 mmol/L</td>
<td>1.8</td>
<td>0.7-5.0</td>
<td>0.20</td>
<td>-</td>
</tr>
<tr>
<td>Total cholesterol &gt; 1.7 mmol/L</td>
<td>0.5</td>
<td>0.1-1.5</td>
<td>0.20</td>
<td>-</td>
</tr>
<tr>
<td>Ferritin &gt;300 µg/L</td>
<td>1.8</td>
<td>0.6-5.3</td>
<td>0.20</td>
<td>-</td>
</tr>
</tbody>
</table>

AIC, Akaike information criteria; AST, aspartate aminotransferase; BMI, body mass index; GGT, Gamma-glutamyl transferase; HBV, hepatitis B virus; HCV, hepatitis C virus; HOMA, Homeostasis Model Assessment

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Table 4: Characteristics of studies included for the systematic review and meta-analysis.

<table>
<thead>
<tr>
<th>Author (year) country</th>
<th>Study type</th>
<th>HBV-HCV (n)</th>
<th>HCV control (n)</th>
<th>HBV-HCV genotype 3 n (%)</th>
<th>Control HCV genotype 3 n (%)</th>
<th>HBV-HCV BMI (kg/m²)</th>
<th>HCV BMI (kg/m²)</th>
<th>NOS score</th>
<th>Centralised histology review</th>
<th>Matched groups (which variables)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sagnelli (2004) Italy</td>
<td>Case-control</td>
<td>27</td>
<td>58</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>5</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Bini (2010) USA</td>
<td>Case-control</td>
<td>26</td>
<td>658</td>
<td>5/73 (6.8%)**</td>
<td>109/1,257 (8.7%)**</td>
<td>NA</td>
<td>NA</td>
<td>6</td>
<td>Unclear</td>
<td>No</td>
</tr>
<tr>
<td>Hung (2010) Taiwan</td>
<td>Case-control</td>
<td>100</td>
<td>100</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>7</td>
<td>Unclear</td>
<td>Yes (age, sex, BMI)</td>
</tr>
<tr>
<td>Zampino (2014) Italy</td>
<td>Case-control</td>
<td>66</td>
<td>198</td>
<td>5/66 (7%)</td>
<td>17/198 (8.7%)</td>
<td>25.7</td>
<td>26.7</td>
<td>7</td>
<td>Unclear</td>
<td>Yes (age, sex, HCV genotype)</td>
</tr>
<tr>
<td>Morosan (2014) Romania</td>
<td>Case-control</td>
<td>39</td>
<td>1021</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>5</td>
<td>Unclear</td>
<td>No</td>
</tr>
<tr>
<td>BOSTIC (2016) International</td>
<td>Case-control</td>
<td>72*</td>
<td>112</td>
<td>17/85 (20%)</td>
<td>30/112 (27%)</td>
<td>24</td>
<td>24</td>
<td>6</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

* Excluding 13 subjects included in the Zampino 2010 study
** Numbers and proportion from entire cohort reported, not available for subset which had liver biopsy

The NOS score represents a scale for the assessment of the methodological quality of each study. The NOS is based on a cumulative score (total of 9) in each of three categories: selection of study groups, comparability of the cases and controls, and the ascertainment of exposure. Similar to previous reviews, we classified studies as high risk of bias (1–3 points), medium risk of bias (4–5 points), or low risk of bias (6–9 points).

BMI, body mass index; HBV, hepatitis B virus; HCV, hepatitis C virus; NOS, Newcastle-Ottawa Quality Assessment Scale

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Figure legends

**Figure 1:** Distribution of steatosis across all subjects and selected subgroups of subjects with HBV, HCV mono-infection and HBV-HCV co-infection. p-values denote comparison of steatosis distribution in the HCV or HBV mono-infected group to the HBV-HCV co-infected group.

A. Distribution of steatosis across all subjects.
B. Distribution of steatosis in lean subjects matched for HCV viral load after exclusion of HBV-infected subjects with undetectable HBV viral load.

BMI, body mass index; HBV, hepatitis B virus; HCV, hepatitis C virus

**Figure 2:** Forest plot for association of HBV-HCV co-infection with steatosis when compared to HCV mono-infection. The meta-analyses were performed using the random-effects model due to significant heterogeneity.

A. Forest plot for association of HBV-HCV with steatosis greater than 5%
B. Forest plot for association of HBV-HCV with steatosis greater than 30%.

Horizontal lines represent 95% CIs of each study. Squares, represent odds ratios of each individual study (the size represents the weight that the study was given in the meta-analysis). The diamond represents the pooled summary estimate. Odds ratio above one indicates more steatosis in HBV-HCV compared to HCV.

95% CI, 95% confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus; OR, odds ratio
### Table A

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sagnelli, 2004</td>
<td>27</td>
<td>0.4</td>
<td>0.2, 1.1</td>
</tr>
<tr>
<td>Bini, 2010</td>
<td>26</td>
<td>2.1</td>
<td>2.9, 158</td>
</tr>
<tr>
<td>Hung, 2010</td>
<td>100</td>
<td>1.3</td>
<td>0.7, 2.3</td>
</tr>
<tr>
<td>Zampino, 2014</td>
<td>66</td>
<td>0.9</td>
<td>0.5, 1.6</td>
</tr>
<tr>
<td>Moreau, 2014</td>
<td>39</td>
<td>0.6</td>
<td>0.3, 1.2</td>
</tr>
<tr>
<td>BOBSTIC</td>
<td>72</td>
<td>0.7</td>
<td>0.4, 1.2</td>
</tr>
</tbody>
</table>

**Summary**: 330 | 0.9 | 0.5, 1.6

---

### Table B

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bini, 2010</td>
<td>26</td>
<td>5.6</td>
<td>2.5, 13</td>
</tr>
<tr>
<td>Hung, 2010</td>
<td>100</td>
<td>2.2</td>
<td>1.1, 4.4</td>
</tr>
<tr>
<td>Zampino, 2014</td>
<td>66</td>
<td>0.1</td>
<td>0.02, 0.4</td>
</tr>
<tr>
<td>BOBSTIC</td>
<td>72</td>
<td>1.1</td>
<td>0.4, 3.0</td>
</tr>
</tbody>
</table>

**Summary**: 264 | 1.2 | 0.3, 4.6

---

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