

Differences and similarities in early atherosclerosis between patients with non-alcoholic steatohepatitis and chronic hepatitis B and C

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Background/Aims: To compare carotid intima-media thickness (IMT) – an index of early atherosclerosis – among patients with non-alcoholic steatohepatitis (NASH), patients with chronic hepatitis B (HBV) or C (HCV) and control subjects.

Methods: We studied 60 consecutive patients with biopsy-proven NASH, 60 patients with HCV, 35 patients with HBV, and 60 healthy controls who were comparable for age and sex. Common carotid IMT was measured with ultrasonography in all participants by a single operator blinded to subjects’ characteristics.

Results: Carotid IMT measurements were markedly different among the groups; the lowest values were in controls, intermediate in patients with HBV or HCV, and highest in those with NASH (0.84 ± 0.1 vs. 0.97 ± 0.1 vs. 1.09 ± 0.2 vs. 1.23 ± 0.2 mm, respectively; $p < 0.001$). The marked differences in carotid IMT that were observed among the groups were little affected by adjustment for age, sex, body mass index, smoking, LDL cholesterol, insulin resistance (by homeostasis model assessment) and components of the Adult Treatment Panel III-defined metabolic syndrome. Concordantly, in logistic regression analysis, NASH, HBV and HCV predicted carotid IMT independent of potential confounders.

Conclusions: These data suggest that NASH, HCV and HBV are strongly associated with early atherosclerosis independent of classical risk factors, insulin resistance and metabolic syndrome components.

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) affects a substantial proportion of the general population and is commonly associated with many features of the metabolic syndrome (MetS) [1–4]. The importance of

NAFLD and its relationship to the MetS is now increasingly recognized, and this has stimulated an interest in the possible role of NAFLD in the development and progression of cardiovascular disease (CVD). Recent data suggest that NAFLD may also be linked to increased CVD prevalence and incidence [5–13], although this needs verifying in larger studies.

Currently, very few data are available on relationships between CVD and chronic hepatitis B (HBV) and C (HCV). Although the relationship between HBV and CVD remains controversial [14,15], a large cohort study recently found an increased prevalence of carotid atherosclerosis in HBV carriers [16]. Similarly,

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chronic HCV infection has been shown to be strongly associated with higher frequency of carotid atherosclerosis [17]. On the other hand, there are still controversial results as to whether HCV antibody seropositivity alone – regardless of HCV-RNA positivity – is associated with higher CVD risk [18–23].

To our knowledge, no studies have previously compared carotid intima-media thickness (IMT), a reliable index of early atherosclerosis [24], among patients with NAFLD and those with chronic HBV or HCV.

Clarification of this aspect may help to explain the underlying mechanisms, and may be of clinical importance in planning preventative and therapeutic strategies. Indeed, the impact of NAFLD and chronic viral hepatitis on CVD risk deserves particular attention in view of the implications for screening/surveillance strategies in the growing number of patients.

The purpose of this study was to assess carotid IMT in patients with NASH, and to compare them with those of patients with HBV or HCV, and control subjects.

2. Patients and methods

Sixty consecutive patients with diagnosis of NASH, 60 patients with diagnosis of chronic HCV infection and 35 patients with diagnosis of chronic HBV infection were recruited from our clinic. Sixty healthy volunteers were recruited from hospital staff members and relatives. The four groups of participants were selected for matching for age and sex. All patients had chronically elevated liver enzymes and most of them had hepatic steatosis on ultrasound. Control subjects had normal liver tests and negative ultrasonography. Most NASH patients have been included in a study assessing the association between carotid IMT and liver histopathology [9].

NASH diagnosis was based on liver biopsy and exclusion of other causes of chronic liver disease (alcohol intake >20 g/day, viral hepatitis, autoimmune hepatitis or medications). HCV diagnosis was based on seropositivity of anti-HCV and HCV-RNA and exclusion of other causes of chronic liver disease. HBV diagnosis was based on seropositivity of HBV antigens and antibodies and exclusion of other causes of chronic liver disease; all of our HBV-infected patients had seropositivity of HBsAg, anti-HBc and anti-HBe but seronegativity of HBeAg.

None of participants had any clinical evidence of cirrhosis, overt nephropathy, diabetes or CVD. Among HCV-infected patients, only eight of them had been treated previously for HCV (non-responders to therapy), but therapy was completed at least 6 months prior to study enrolment. The local Ethics Committee approved the study. All participants provided written informed consent.

Venous blood was drawn in the morning after an overnight fast. Plasma liver tests and other biochemical blood measurements were determined by standard laboratory procedures. Reference ranges for serum aminotransferase concentrations were 10–35 U/L for women and 10–50 U/L for men, respectively. Serum insulin was measured with a radio-immunoassay method. Hepatitis C virus serology was determined by qualitative detection of HCV antibody using an enzyme-linked immunosorbent assay (The Vitros anti-HCV assay, Ortho-Clinical Diagnostics, Amersham, UK), and HCV-RNA levels and its major genotypes using reverse-transcription polymerase chain reaction (Amplicor HCV test, v2.0, Roche Diagnostics, Milan, Italy). Hepatitis B virus serology was determined by qualitative detection of HBV antigens and antibodies using enzyme-linked immunosorbent assays (Ortho-Clinical Diagnostics). Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald's formula. An oral glucose-tolerance test was performed in all participants; four NASH patients had impaired glucose tolerance (2-h glucose >7.8 and <11.1 mmol/L), whereas the

remaining participants had normal glucose tolerance. Insulin resistance was calculated by the homeostasis model assessment (HOMA-IR score) [25].

Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared. Waist circumference was measured at the level of the umbilicus. Blood pressure was measured in triplicate with a standard mercury manometer. Information on alcohol consumption and smoking history was obtained from all participants by a questionnaire [9].

Metabolic syndrome was diagnosed by the Adult Treatment Panel (ATP)-III definition [26]. In accordance with this definition, a person was classified as having the syndrome if he/she had at least three of the following five components: (1) waist circumference >102 cm in males or >88 cm in females, (2) fasting glucose ≥ 6.1 mmol/L or on treatment, (3) triglycerides ≥ 1.70 mmol/L, (4) HDL <1.0 mmol/L in males and <1.29 mmol/L in females, or on treatment, and (5) blood pressure $\geq 130/85$ mmHg or on treatment. Only eight patients were treated with statins or ramipril.

A single operator blinded to subjects' characteristics measured common carotid IMT using ultrasonography. IMT measurements were made bilaterally in the 1-cm segment proximal to the dilatation of the carotid bulb, as previously described [5,7,9]. All ultrasonic examinations were stored on a super VHS video system for subsequent offline processing. Video images were captured in end-diastole of the cardiac cycle by triggering the electrocardiogram. The frozen video images were digitized and transferred for further analysis to a personal computer. IMT measurements were made by edge-detection techniques (and not by electronic calipers) by a single operator blinded to patients' details. For each subject, three maximum values of carotid IMT on both sides were measured, on the anterior, lateral and posterior projection of the near and far wall. All readings were then averaged. Repeated measurements on the same subjects gave coefficients of variation below 9% [9]. A carotid plaque was defined as a focal thickening of ≥ 1.2 mm at the level of common carotid artery [6,9,24]. No participants had clinically relevant carotid stenosis (>60%).

Liver biopsy specimens were available only in NASH patients. An experienced hepato-pathologist blinded to subjects' details scored liver biopsy specimens using the classification of Brunt [27]. NASH was defined as the presence of steatosis *plus* lobular inflammation *plus* hepatocellular ballooning or steatosis *plus* any stage of fibrosis. Liver histology results in NASH patients showed NASH with fibrosis stage of 0 in 21 subjects, NASH/fibrosis stage 1 in 23 subjects, NASH/fibrosis stage 2 in 11 subjects, and NASH/fibrosis stage 3 in 5 subjects; none had cirrhosis.

2.1. Statistical analysis

Data are means \pm SD or proportions. Skewed variables (triglycerides, liver enzymes and HOMA-IR score) were logarithmically transformed to improve normality prior to analysis. Statistical analyses included one-way analysis of variance and chi-squared test (for categorical variables). Analysis of covariance was used to assess the significance of differences in carotid IMT among the groups after adjustment for potential confounders (sex, age, BMI, smoking, LDL cholesterol, HOMA-IR score and MetS components). After pooling subjects, the independence of associations of variables with carotid IMT was also assessed by multivariate linear (when the dependent variable, i.e., carotid IMT, was entered as a continuous variable) or logistic (when carotid IMT was modelled as categorical variable and subjects were stratified into those belonging to the top quartile vs. those belonging to the other three quartiles) regression analyses. In the fully adjusted regression models, together with the presence of NASH, HBV and HCV (included as categorical variables), sex, age, BMI, smoking, LDL cholesterol, HOMA-IR score and the individual components of the MetS (considered as continuous or categorical measures) were also included as covariates. *p*-values <0.05 were considered statistically significant.

3. Results

The baseline characteristics of the study participants are given in Table 1. Because of the study design, the

Table 1
Baseline characteristics of the study participants

	NASH patients	HCV patients	HBV patients	Control subjects
<i>n</i>	60	60	35	60
Sex (M/F)	34/16	34/16	23/12	34/16
Age (years)	46 ± 3	46 ± 3	47 ± 3	46 ± 3
BMI (kg/m ²)	28 ± 3 ^{a,b}	26 ± 3 ^a	24 ± 2	25 ± 2
Waist circumference (cm)	97 ± 6 ^a	93 ± 5 ^a	85 ± 3	86 ± 4
Systolic blood pressure (mmHg)	127 ± 7 ^{a,b}	121 ± 5	120 ± 4	123 ± 4
Diastolic blood pressure (mmHg)	81 ± 6	76 ± 4	77 ± 4	78 ± 4
Current smokers (%)	26.7%	25.0%	31.4%	33.3%
Fasting glucose (mmol/L)	5.6 ± 0.7	5.4 ± 0.6	5.3 ± 0.3	5.2 ± 0.3
Two-hour glucose (mmol/L)	6.9 ± 1.2	6.1 ± 1.0	6.0 ± 1.1	5.8 ± 1.1
Fasting insulin (mU/L)	15.0 ± 4 ^{a,b}	11.6 ± 4 ^a	5.9 ± 2	6.1 ± 2
HOMA-insulin resistance score	3.93 ± 2.1 ^{a,b}	2.91 ± 2.0 ^a	1.42 ± 1.9	1.45 ± 1.8
Triglycerides (mmol/L)	1.52 ± 1.0 ^{a,b}	1.17 ± 0.7 ^a	0.82 ± 0.4	0.90 ± 0.5
HDL cholesterol (mmol/L)	1.37 ± 0.6 ^a	1.46 ± 0.4	1.50 ± 0.5	1.51 ± 0.5
LDL cholesterol (mmol/L)	3.10 ± 0.5	2.80 ± 0.7	2.89 ± 0.3	3.04 ± 0.3
AST (U/L)	46 ± 51 ^a	58 ± 44 ^a	52 ± 41 ^a	18 ± 4
ALT (U/L)	106 ± 70 ^a	115 ± 83 ^a	102 ± 76 ^a	20 ± 5
GGT (U/L)	49 ± 36 ^a	41 ± 30 ^a	47 ± 27 ^a	17 ± 4
ATP III -metabolic syndrome (%)	52.5% ^{a,b}	25% ^a	0%	0%
Carotid IMT (mm)	1.23 ± 0.2 ^{a,b}	1.09 ± 0.2 ^a	0.97 ± 0.1 ^c	0.84 ± 0.1
Carotid plaques (%)	70.0% ^{a,b}	37.5% ^a	30.0% ^c	15.0%

Data are means ± SD unless otherwise indicated.

^a *p* < 0.001 vs. control subjects.

^b *p* < 0.05 or less vs. patients with HCV or HBV.

^c *p* < 0.05 vs. control subjects.

four groups were almost identical in terms of age and sex. Compared with controls, HBV-infected patients did not significantly differ in any of the study variables, except for liver enzymes. MetS and its individual components occurred more frequently in patients with NASH or HCV compared with HBV-infected patients and control subjects. Accordingly, NASH and HCV patients also had higher values of insulin and HOMA-IR score – that were remarkably higher in the former – than those in HBV-infected patients and control subjects. Smoking status, plasma LDL cholesterol and glucose concentrations did not differ among the groups.

Among HCV patients, forty-three (71.7%) patients were infected with HCV genotype 1, 15 (25%) with genotype 2 and remaining 2 (3.3%) patients with genotype 3.

Carotid IMT measurements were remarkably different among the study participants (Table 1). The lowest values were in controls, the intermediate in patients with HBV or HCV, and the highest levels in those with NASH. Patients with NASH or chronic viral hepatitis also had greater prevalence of carotid plaques (focal thickening ≥ 1.2 mm at the level of carotid artery) than controls. No differences in carotid IMTs were found in HCV-infected patients after stratification according to HCV genotype distribution (not shown).

As shown in Fig. 1, the differences in carotid IMT and plaques that were observed among the groups were little affected by adjustment for age, sex, BMI,

smoking, LDL cholesterol, insulin resistance and MetS components (included as continuous measures).

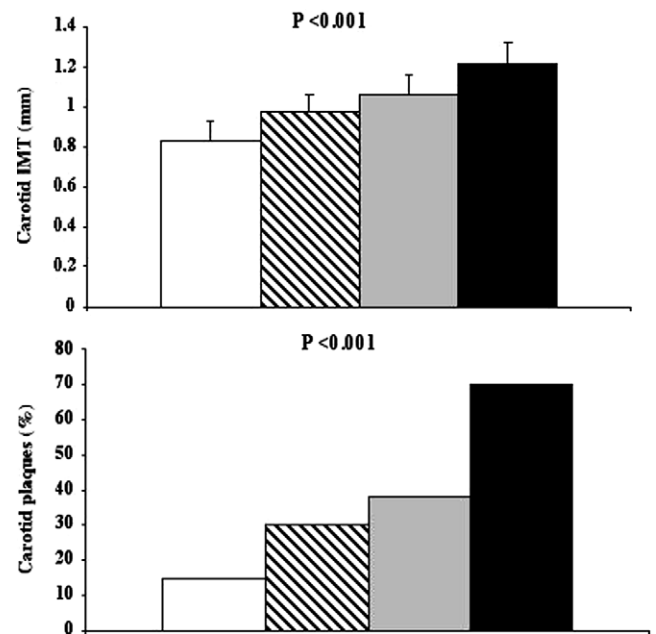


Fig. 1. Mean (±SD) values of carotid artery IMT and prevalence of carotid atherosclerotic plaques in controls (white bars, *n* = 60), HBV-infected patients (lined bars, *n* = 30), HCV-infected patients (grey bars, *n* = 60) and NASH patients (black bars, *n* = 60). All data are adjusted for age, sex, body mass index, cigarette smoking, LDL-cholesterol, HOMA-estimated insulin resistance and the individual components of the ATP III-defined metabolic syndrome.

Almost identical results were found when patients ($n = 8$) on statins or anti-hypertensive drugs have been excluded.

In univariate logistic regression analysis, together with NASH (OR 2.41, 95% CI 1.6–3.9; $p < 0.001$), HCV (OR 1.97, 95% CI 1.3–3.1; $p < 0.001$) and HBV (OR 1.45, 95% CI 1.1–1.9; $p < 0.05$), older age, male sex, BMI, waist circumference, blood pressure, smoking, HOMA-IR score and LDL cholesterol were significantly associated with carotid IMT. In the fully adjusted regression model, older age (OR 1.14, 95% CI 1.06–1.18; $p < 0.001$), raised blood pressure values (OR 2.01, 95% CI 1.5–2.7; $p < 0.001$), HOMA-IR score (OR 1.73, 95% CI 1.2–2.3; $p < 0.001$), and presence of NASH (OR 1.96, 95% CI 1.3–2.9; $p < 0.001$), HCV (OR 1.61, 95% CI 1.1–2.5; $p < 0.001$) or HBV (OR 1.40, 95% CI 1.1–1.7; $p < 0.05$) predicted carotid IMT (4th quartile vs. quartiles 1st–3rd) independent of other potential confounding factors (sex, smoking, BMI, waist circumference, LDL cholesterol and triglycerides). Almost identical results were found in models in which the MetS components were entered as categorical measures (not shown).

Similar results were found when we considered carotid IMT as a continuous measure and performed multivariate linear regression analyses. Also in this case, NASH (multiple-adjusted r -value = 0.63; $p < 0.001$), HCV (r -value = 0.44; $p < 0.001$) and HBV (r -value = 0.32; $p < 0.05$) were significantly associated with carotid IMT after adjusting for the above-reported covariates.

4. Discussion

The main findings of this study are as follows: (1) compared with controls, patients with NASH and those with HCV or HBV have increased carotid IMT and plaques, as early signs of atherosclerosis, (2) carotid IMT and plaques are higher for individuals with NASH than for those with HCV or HBV, and (3) the differences in early carotid atherosclerosis that are observed among the groups remained significant after adjustment for a broad spectrum of CVD risk factors, including insulin resistance and components of the MetS, a chronic inflammatory cardiovascular condition that is closely related to NASH and HCV [1–4,28].

As regards NASH, our findings complement recent observations that NAFLD is independently associated with greater carotid IMT and plaques [5–9], and lower endothelial flow-mediated vasodilation [29], and that NAFLD is associated with higher all-cause death, and predicts the risk of future CVD events [11–13]. The possible biological mechanisms linking NAFLD and CVD have been extensively reviewed elsewhere [30].

As regards HCV and HBV infections, few data are available on relationships between chronic viral hepatitis and CVD. In particular, our results extend those of the only one previously published study demonstrating that chronic HCV infection was associated with higher frequency of carotid atherosclerotic plaques independent of other prognostic factors [17]. On the contrary, there is uncertainty whether HCV antibody seropositivity alone is associated with higher CVD risk [18–23]. Similarly, although the data on relationships between HBV and CVD are still controversial [14–16,20], a recent cross-sectional study from a health-screening test cohort found a strong association between HBV carrier status and carotid atherosclerosis [16].

A significant strength of this study was that carotid IMT was measured in a large sample of consecutive patients with biopsy-proven NASH and, for comparison, in samples of HCV- and HBV-infected patients who were comparable for age and sex (i.e., the strongest CVD risk factors). Notably, our patients were free of diagnosed CVD, diabetes and cirrhosis, and had a well-preserved liver function; the evaluation of patients with such complications would have probably confounded the interpretation of the data.

The key finding of this study was that carotid IMT and plaques were greater for patients with NASH than for those with HBV or HCV (or controls). This finding partly supports the results of Sanyal [31], who compared the outcomes of cirrhotic patients with NASH or HCV. They found that compensated cirrhosis due to NASH is associated with a lower total mortality compared with that due to HCV, but that CVD mortality is greater in those with NASH. Moreover, this finding supports recent data suggesting an increased CVD incidence among cardiac or renal transplant recipients infected with HCV or HBV [32–34].

Clearly, we must be cautious in making any causal inference, given the cross-sectional design of the study. The biological mechanisms by which NASH, HBV and HCV might contribute to CVD are poorly understood. In particular, the most likely explanation for our findings could be that pre-clinical carotid atherosclerosis in NASH and HCV – but probably not in HBV which is a chronic liver disease not specifically associated with the MetS components – mainly reflects the presence of underlying metabolic abnormalities, principally visceral obesity and insulin resistance. However, NASH and HCV might also act as a stimulus for further increased whole-body insulin resistance and dyslipidaemia, leading to accelerated atherosclerosis. This hypothesis is partly supported by prospective studies demonstrating that NASH and HCV predict new-onset type 2 diabetes independent of obesity [35–39].

However, because in our study NASH, HBV and HCV were associated with carotid atherosclerosis independent of classical risk factors, insulin resistance and

MetS components, it might be also hypothesized that additional atherogenic mechanisms are involved, thus raising the possibility that liver may be not simply a marker of CVD but may also be involved in its pathogenesis [30].

A common, underlying, mechanism linking NASH, HCV, HBV and CVD might be represented by increased hepatic oxidative stress and chronic inflammation, which have been shown to be associated with CVD pathogenesis [40,41], and are thought to be key factors in the progression of these liver diseases [2,28,42]. Although this is still a controversial issue, similar to other infectious agents (cytomegalovirus, *Helicobacter pylori* and *Chlamydia pneumoniae*) possibly implicated in CVD pathogenesis [43], persistent HCV or HBV infections could induce hepatic/systemic chronic inflammation and thus might contribute to CVD pathogenesis. Consistent with the hypothesis that liver inflammation plays a role in CVD pathogenesis, we consider that the higher frequency of carotid plaques in NASH patients compared to HCV and HBV patients – which appears to be also independent of the higher MetS components in NASH – might be explained by a higher underlying liver inflammation in NASH. Indeed, we have previously found a strong, graded, relationship between the severity of NAFLD histology and carotid IMT and plaques in these patients [9]. However, the lack of liver biopsy specimens in our HCV- and HBV-infected patients does not allow us to definitively answer this question.

Decreased concentrations of adiponectin, an adipose-secreted protein with anti-inflammatory and anti-atherogenic properties [44], might represent another common, underlying, mechanism linking NASH, HCV and CVD. It has been demonstrated that hypoadiponectinemia correlates with the severity of NAFLD histology independent of insulin resistance and other MetS components [45–47]. Similar results were found in some, but not all, studies assessing plasma adiponectin concentrations among HCV-infected patients [48–51].

Interestingly, O’Leary reported that a carotid IMT value ≤ 0.86 mm carries a low risk of developing CVD, whereas an IMT value ≥ 1.10 carries a high risk of developing CVD [52]. It is worth emphasizing that the mean IMT values we found among controls, HBV, HCV and NASH patients were 0.84, 0.97, 1.09 and 1.23 mm, respectively. Thus, our findings might have important clinical implications. These data strongly emphasize the clinical importance of evaluating the global CVD risk in patients diagnosed with NASH or HBV or HCV; patients having increased carotid IMT could be candidates not only for aggressive treatment of their liver disease, but also for aggressive treatment of underlying CVD risk factors; this would help to modify and potentially decrease the global CVD risk of these patients.

This study has some limitations that should be noted. The cross-sectional design of our study precludes the establishment of causal or temporal relations among chronic liver diseases, carotid atherosclerosis and MetS. Prospective studies will be required to sort out the time sequence of events. Although our results have been adjusted for HOMA-IR score, a reliable method for estimating insulin resistance [25], we did not directly measure insulin sensitivity (by euglycemic clamp) in our population, so we cannot be certain that identical results could be obtained after adjustment for the clamp-measured insulin resistance (especially among patients with NASH or HCV who are hyperinsulinaemic and insulin resistant compared with controls). Another possible limitation of this study is that in the control subjects, the exclusion of NAFLD was based on medical history, blood testing, and ultrasound imaging, but was not confirmed by liver biopsy. However, although some non-differential misclassification of NAFLD on the basis of ultrasound is likely (i.e., some of the controls could have underlying NAFLD, despite normal liver enzymes and a negative ultrasound), this limitation would serve to attenuate the magnitude of our effect measures toward the null; thus, our results can probably be considered as conservative estimates of the relationship between NAFLD and carotid IMT.

In conclusion, our findings suggest that NASH, HCV and HBV (in patients without cirrhosis and with a well-preserved liver function) are associated with early signs of atherosclerosis, independent of classical risk factors, insulin resistance and components of the metabolic syndrome. Larger studies are needed to confirm these findings and to elucidate the underlying biologic mechanisms before causality can be firmly established.

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