Research Article

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Fatty liver is an independent predictor of early carotid atherosclerosis

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Background & Aims: Whether steatosis is incidentally or causally associated with carotid atherosclerosis is debated, and long-term follow-up data are missing. This study aims to examine the impact of steatosis on the presence and progression of carotid intima-media thickness (C-IMT) and carotid plaques (CP) in a large cohort with longitudinal follow-up.

Methods: A retrospective single-center study between 1995 and 2012. Transversal cohort: patients with ⩾2 cardiovascular risk factors without previous cardiovascular events. Longitudinal cohort: patients with two consecutive C-IMT measurements more than 2 years apart. Steatosis was defined by a surrogate marker, the fatty liver index (FLI). CP and C-IMT were assessed by carotid ultrasound.

Results: In the transversal cohort (n = 5671) both C-IMT and the Framingham risk score (FRS) increased across FLI quartiles (0.58 ± 0.12, 0.61 ± 0.14, 0.63 ± 0.14, 0.64 ± 0.14 mm, and 5 ± 5%, 9 ± 7%, 12 ± 8%, 15 ± 9%, p <0.001 for both). Steatosis predicted C-IMT better than diabetes or dyslipidemia. Steatosis independently predicted C-IMT (p = 0.002) and FRS (p <0.001) after adjustment for metabolic syndrome and cardiovascular risk factors.

In the longitudinal cohort (n = 1872, mean follow-up 8 ± 4 years), steatosis occurred in 12% and CP in 23% of patients. C-IMT increased in patients with steatosis occurrence (from 0.60 ± 0.13 mm to 0.66 ± 0.14 mm, p = 0.001) whereas it did not change in those that stayed free of steatosis. Steatosis at baseline predicted CP occurrence (OR = 1.63, 95% CI 1.10–2.41, p = 0.014), independent of age, sex, type-2 diabetes, tobacco use, hsCRP, hypertension and C-IMT.

Conclusions: In patients with metabolic syndrome at risk for cardiovascular events, steatosis contributes to early atherosclerosis and progression thereof, independent of traditional cardiovascular risk factors.

Keywords: Fatty liver; Atherosclerosis; Carotid intima-media thickness; Carotid plaques; Cardiovascular risk; Metabolic syndrome; Type 2 diabetes.

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Abbreviations: C-IMT, carotid intima-media thickness; FLI, fatty liver index; NAFLD, non-alcoholic fatty liver disease; FRS, 10 year Framingham risk score.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is an increasingly common condition seen in patients with obesity, type 2 diabetes, atherogenic dyslipidemia and arterial hypertension. The leading cause of death in patients with NAFLD is cardiovascular mortality, which is not surprising given the high prevalence of the above-mentioned cardiometabolic risk factors [1,2]. However, a large body of data indicates that the fatty and inflamed liver expresses several pro-inflammatory and procoagulant factors, as well as genes involved in accelerated atherogenesis [3,4]. This raises the possibility that the link between NAFLD and cardiovascular mortality might not simply be mediated by shared, underlying, common risk factors but rather that NAFLD independently contributes to increasing this risk.

While an increased prevalence of cardiovascular disease in NAFLD is largely accepted, existing data also show an increased incidence [5–7]. This suggests that steatosis predates clinical cardiovascular disease, and that it may trigger or accelerate its occurrence. Providing support for this causal hypothesis, some reports have demonstrated an increased proportion of subclinical atherosclerosis or pre-atherosclerotic lesions in patients with NAFLD. For instance, ultrasound-diagnosed steatosis was associated with increased coronary calcium scores [8] and with increased intima-media thickness (C-IMT) [9], independent of conventional cardiovascular risk factors and insulin resistance. C-IMT is a marker of early atherosclerosis that predicts coronary and cerebrovascular events: a 0.1 mm increase in C-IMT increases the risk of myocardial infarction by 10–15% and the risk of stroke by 13–18% [10]. Taken together, these data suggest that steatosis actively contributes to atherogenesis. However, there are few, if any, longitudinal, long-term studies assessing the impact of steatosis on the progression of pre-atherosclerotic lesions. In this

study we hypothesized that steatosis is an independent predictor of C-IMT progression. Our objectives were: (1) to determine the relationship between steatosis, C-IMT and the 10-year Framingham risk score (FRS) in a population at risk for cardiovascular events; and (2) to determine in a longitudinal follow-up study if the occurrence or reversal of steatosis independently predicts the occurrence of carotid plaques (CP).

Materials and methods

Study population

This is a retrospective analysis of consecutive patients between 20 and 75 years of age referred to a Primary Cardiovascular Prévention Center at Pitié-Salpêtrière Hospital, Paris, France, between 1995 and 2012. Inclusion criteria were: (1) at least two cardiovascular risk factors among the following: age >60 years in women and >50 years in men; systolic blood pressure $\geqslant$130 or diastolic blood pressure $\geqslant$85 mmHg or treatment of previously diagnosed hypertension; fasting plasma glucose $\geqslant$5.6 mmol/L or previously diagnosed type 2 diabetes; triglycerides levels $\geqslant$1.7 mmol/L or high density lipoprotein (HDL) <1.03 mmol/L in males or <1.29 mmol/L in females or specific treatment for lipid disorders; overweight (BMI >25 kg/m^2); tobacco consumption; and (2) available carotid ultrasound with measurement of carotid intima-media thickness and of CP. Exclusion criteria were: patients with previous history of cardiovascular events (myocardial infarction, coronary by-pass surgery or coronary angioplasty, stroke); excessive alcohol consumption (>50 g/day in both men and women); any other identified causes of chronic liver disease including hepatitis B or C; positive test for human immunodeficiency virus; active malignancy; solid organ or bone marrow transplant recipients. Finally, 5671 patients met the inclusion and exclusion criteria (transversal cohort); among these, 1872 patients had a follow-up carotid ultrasound performed at least two years after the initial evaluation (longitudinal cohort).

Clinical and biological evaluation

Clinical data were recorded for each patient: age, gender, smoking status, alcohol consumption, (based on self-reported frequency and the amount of daily consumption), past medical history; systolic and diastolic blood pressure and anthropometric parameters were measured on the day of hospital visit. Fasting blood samples were collected the day of medical visit. Serum alanine aminotransferase (ALT) levels were classified as follows: (1) normal-low ALT: <19 IU/L in women and <30 IU/L in men; (2) normal-high ALT: between 19 IU/L and 40 IU/L in women and 30 IU/L and 40 IU/L in men; (3) high ALT: >40 IU/L both in men and in women [11]. Metabolic syndrome was defined according to the International Diabetes Federation (IDF) criteria [12].

Diagnosis of steatosis. A well validated, surrogate marker, the Fatty Liver Index (FLI) was used to identify patients with steatosis [13]. FLI was calculated as follows:

$$\mathrm{FLI} = \left(e^{0.953*\log_e(\text{triglycerides})+0.139*\mathrm{BMI}+0.718*\log_e(\mathrm{GGT})+0.053*\text{waist circumference}-15.745}\right) / \left(1+e^{0.953*\log_e(\text{triglycerides})+0.139*\mathrm{BMI}+0.718*\log_e(\mathrm{GGT})+0.053*\text{waist circumference}-15.745}\right)*100.$$

In accordance with the original report and subsequent validation studies, steatosis was defined as FLI $\geqslant$60. Steatosis occurrence during follow-up was defined as transition from a FLI <60 at baseline to a FLI $\geqslant$60 at the end of follow-up.

Diagnosis of fibrosis. AST to Platelet Ratio Index (APRI) score was used to identify the presence/absence of significant fibrosis at two cut-off values: APRI >1.5 to rule in significant fibrosis and <0.5 to rule out significant fibrosis [14].

$$\mathrm{APRI} = (\text{AST level } (/\mathrm{ULN})/\text{Platelet counts } (10^9/\mathrm{L}))*100$$

Evaluation of pre-atherosclerotic lesions and of cardiovascular risk score

Carotid ultrasound was performed systematically in all patients as part of a primary prevention program. The C-IMT was measured on the far wall of the carotid artery as the distance between the lumen-intima interface and the media-adventitia using high resolution B-mode ultrasound (Sequoia 512, Acuson). All measurements of C-IMT were made at a site free of any plaque with the accuracy of the electronic caliper to the nearest 0.1 mm. The presence of plaques was defined as localized echo structures encroaching into the vessel lumen for which the distance between the media-adventitia interface and the internal side of the lesion was >1 mm. When a plaque was present, optimal frozen images (1 longitudinal and 1 transversal view), showing the plaque in its greatest thickness, were selected, measured and stored.

All measurements were done by two trained physicians who had more than 10 years of experience and 5000 examinations performed [15,16]. The interobserver coefficient of variation for C-IMT was <3%.

The 10-Year Framingham risk score (FRS) was calculated using gender specific score sheets.

Statistical methods

All quantitative data were expressed as mean ± standard deviation; categorical data were expressed as percentage. To avoid colinearity, we ensured that variables used in FLI calculation were not included in FRS formula.

Transversal study

The differences in patients' characteristics according to the presence of fatty liver (defined as FLI $\geqslant$60) were assessed using either Student's *t* test, or χ^2 as appropriate. ANOVA test with Bonferroni correction was used for multiple comparisons (significance level set for p <0.05). Multiple linear regression analysis was used to analyze the relationship between fatty liver, C-IMT and 10-year FRS. Variables in FRS or FLI were not included in multivariate models.

Longitudinal study

The evolution of clinical and biological variables between baseline and follow-up were compared using paired sample *t* test for continuous variables and McNemar test for categorical variables. Patients were divided according to the transition between steatosis categories during follow-up, i.e., patients without steatosis at baseline and follow-up, patients with steatosis at baseline and follow-up, steatosis occurrence (FLI <60 at baseline and $\geqslant$60 at follow-up) and steatosis regression (FLI $\geqslant$60 at baseline and <60 at follow-up). To determine the impact of steatosis on the occurrence of CP we used Kaplan-Meyer and Cox multivariate analysis models.

All statistical tests were two-sided and significance level was set at p <0.05. Statistical analyses were performed using SPPS v.21 MacOS statistical software (IBM, Chicago, IL).

Results

Relationship between steatosis, carotid atherosclerosis and 10-year FRS (transversal study)

5671 patients had available carotid ultrasound and met the inclusion and exclusion criteria (transversal cohort) (Fig. 1). Patient

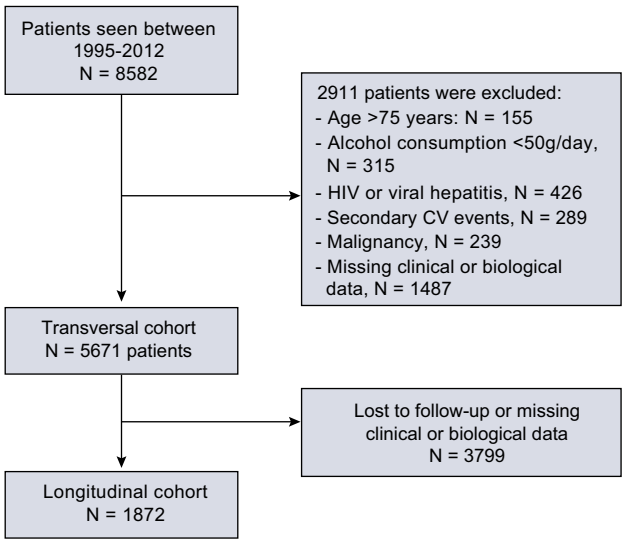

Fig. 1. Study flowchart.