Fatty liver is an independent predictor of early carotid atherosclerosis

Raluca Pais¹, Philippe Giral², Jean-François Khan², David Rosenbaum², Chantal Housset¹, Thierry Poynard¹, Vlad Ratziu¹*, for the LIDO Study Group

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.

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 [5] and with increased intima-media thickness (C-IMT) [6], 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 Prevention Center at Pitité-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 >130 or diastolic blood pressure >85 mmHg or treatment of previously diagnosed hypertension; fasting plasma glucose >5.6 mmol/L or previously diagnosed type 2 diabetes; triglycerides levels >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/m²); 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 characteristics are shown in Table 1. 50% of patients had more than 2 cardiovascular risk factors and 37% had the metabolic syndrome. Mean alcohol consumption was low, only 3.5 drinks per week. 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.

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:

\[
\text{FLI} = \frac{1093 \times \text{ALT level (IU/L)} + 718 \times \text{AST level (IU/L)}}{1 + 953 \times \text{GGT level (IU/L)} + 1.29 \times \text{BMI} + 0.139 \times \text{HbA1c (mmol/L)} + 0.053 \times \text{waist circumference - 15.745}}
\]

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

\[
\text{APRI} = \frac{\text{AST level (IU/L)}}{\text{Platelet counts (10^9/L)}} + 0.4375
\]

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 (Sequana 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 collinearity, 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 >60) were assessed using either Student’s t test, or χ² 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 >60 at follow-up) and steatosis regression (FLI >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 SPSS 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 characteristics are shown in Table 1. 50% of patients had more than 2 cardiovascular risk factors and 37% had the metabolic syndrome. Half of the entire cohort had a family history of cardiovascular disease. Mean alcohol consumption was low, only 3.5 drinks per week.
5% of patients consumed more than 30 g/day. Forty-six percent of patients were active or former smokers with a mean tobacco consumption of 18 ± 15 packs/year. The prevalence of TP was 35% and the mean FRS was 10 ± 8.

A third of patients (n = 1871, 33%) had a FLI >60 and were considered to have steatosis. Patients with steatosis were older, had higher BMI and waist circumference, higher prevalence of type 2 diabetes and high blood pressure, higher aminotransferase levels and high CRP than those without steatosis. They also had higher C-IMT and 10-year FRS (Table 1). Both C-IMT and FRS progressively increased across FLI quartiles (0.58 ± 0.12 mm, higher C-IMT and 10-year FRS (Table 1). Both C-IMT and FRS levels and hsCRP than those without steatosis. They also had had higher type 2 diabetes and dyslipidemia, those with steatosis had significantly higher C-IMT than those without steatosis (0.64 ± 0.14 mm vs. 0.61 ± 0.13 mm, respectively, p < 0.001) this difference disappeared when taking steatosis into consideration. Among patients with type 2 diabetes or dyslipidemia, regardless of steatosis.

Since ALT is a biochemical surrogate for hepatic necroinflammation and since some studies have shown that patients with non-alcoholic steatohepatitis (NASH) might be at higher cardiovascular risk than those with steatosis alone, we studied whether the impact of steatosis was independent from that of ALT. In this cohort, 45% of patients had normal-low ALT, 38% had normal-high ALT and 17% had high ALT. C-IMT values did not differ significantly according to ALT categories. Patients with steatosis had higher C-IMT values than patients without steatosis and this was true across all ALT categories (Fig. 3A). Patients with steatosis also had higher FRS than patients without steatosis, regardless of the ALT category (Fig. 3B).

In univariate analysis, age, sex, BMI, type 2 diabetes, high blood pressure and steatosis assessed by FLI were independent predictors of C-IMT (Supplementary Table 2). In multivariate analysis, FLI predicted C-IMT independent of the metabolic comorbidities, cardiovascular risk factors and markers of low grade inflammation, (β = 0.046, p = 0.002) (Table 2). When patients were further stratified according to the number of metabolic syndrome components, in the same model, FLI as continuous variable remained an independent predictor of C-IMT in patients with 2 or more MS components (β = 0.056, p = 0.001). However, when only patients with 4 or 5 MS components were considered, the impact of FLI on C-IMT was overpassed by classic CV risk factors. When FLI was replaced by its individual components, none of them, except for waist circumference, were independently associated with C-IMT (Table 2).

FLI was also associated with 10-year FRS independent of C-IMT (β = 0.432, p < 0.001, R² = 0.257); similar results were obtained in diabetic patients only (N = 962, β = 0.269, p < 0.001).

Please cite this article in press as: Pais R et al. Fatty liver is an independent predictor of early carotid atherosclerosis. J Hepatol (2016), http://dx.doi.org/10.1016/j.jhep.2016.02.023
The prevalence of significant fibrosis defined as APRI >1.5 (according to the original publication and an upper limit of normal for aspartate aminotransferase (AST) of 26 IU/L) was rather low at 1.2%. The prevalence of patients without significant fibrosis (APRI <0.5) was 63%. When APRI was tested as a continuous variable alongside FLI (Model 3, Table 2) steatosis but not fibrosis was an independent predictor of C-IMT.

Impact of fatty liver on the progression of carotid atherosclerosis (longitudinal study)

Follow-up C-IMT measurements were available in 1872 patients (longitudinal cohort, mean time interval 8 ± 4 years) (Fig. 1). Patients with available follow-up C-IMT were younger, had lower BMI, waist circumference, and lower prevalence of steatosis but similar C-IMT and prevalence of CP. In patients with follow-up carotid ultrasound, changes in clinical and biological parameters during follow-up are shown in Supplementary Table 4. There was a significant tendency towards weight gain, increase in abdominal girth and a higher proportion of type 2 diabetes. At the same time these patients were more prevalently treated with lipid lowering agents (73% of them vs. 47% at beginning of follow-up), which resulted in a better control of lipid disorders, particularly, total cholesterol, low density lipoprotein (LDL)-cholesterol, HDL-cholesterol and triglycerides. The prevalence of steatosis increased significantly during follow-up (24% at baseline vs. 30% at follow-up, p <0.001). Steatosis occurred in 12% of patients and regressed in 6% at a crude rate of 0.75% per year. In parallel with the increasing proportion of steatosis, there was a degradation in liver enzymes as shown by increasing values of aminotransferases and GGT during follow-up. Interestingly, most of this increase was related to the occurrence of steatosis, since changes in liver enzymes were higher in patients that developed steatosis during follow-up. The prevalence of CP also increased during follow-up (39% vs. 57%, p <0.001), with 23% of patients developing CP vs. 5% no longer having them at the end of follow-up (Supplementary Table 4).

Overall, C-IMT increased from 0.61 ± 0.14 mm to 0.64 ± 0.14 mm, p <0.001. Patients with steatosis had higher C-IMT values at baseline than those without steatosis (0.60 ± 0.13 vs. 0.64 ± 0.14 mm, p <0.05). Patients with steatosis occurrence during follow-up had similar C-IMT at baseline as those that stayed free of steatosis (0.60 ± 0.13 vs. 0.61 ± 0.13 mm, p = 1);

---

**Table 2. Independent predictors of C-IMT (transversal cohort).**

<table>
<thead>
<tr>
<th>Variable</th>
<th>C-IMT (N = 5671)</th>
<th>Model 1</th>
<th>Model 2*</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p value</td>
<td>β</td>
<td>p value</td>
</tr>
<tr>
<td>Age</td>
<td>0.411</td>
<td>&lt;0.001</td>
<td>0.402</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>0.126</td>
<td>&lt;0.001</td>
<td>0.107</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tobacco</td>
<td>0.007</td>
<td>0.61</td>
<td>0.011</td>
<td>0.44</td>
</tr>
<tr>
<td>High blood pressure</td>
<td>0.063</td>
<td>&lt;0.001</td>
<td>0.061</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>0.012</td>
<td>0.39</td>
<td>0.008</td>
<td>0.56</td>
</tr>
<tr>
<td>hscCRP</td>
<td>0.035</td>
<td>0.012</td>
<td>0.023</td>
<td>0.11</td>
</tr>
<tr>
<td>Steatosis (FLI ≥60)</td>
<td>0.046</td>
<td>0.002</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>APRI**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BMI</td>
<td>0.018</td>
<td>0.53</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.080</td>
<td>0.009</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.004</td>
<td>0.80</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GGT</td>
<td>-0.007</td>
<td>0.64</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*In model 2 FLI was replaced with variables included in its calculation formula.
**APRI was tested as a continuous variable.
Discussion

Patients with NAFLD die primarily of cardiovascular disease, and the extent to which the liver disease rather than associated comorbidities is responsible for excess cardiovascular death is still under debate [17]. Most studies have detailed the relationship between steatosis, associated cardiometabolic risk factors and cardiovascular events [18,19]. However, clinical events are by definition a late step in the atherogenic process, which makes it difficult to ascertain the contribution of steatosis per se. If steatosis were to play an independent role in the development of atherosclerosis, then it should promote the occurrence and progression of early, pre-atherosclerotic lesions. Such long-term, observational data are, however, limited.

In this study we demonstrated that steatosis, as assessed by FLI, a well validated biomarker panel, is associated with C-IMT, a pre-atherosclerotic lesion that predicts cardiovascular events. C-IMT increased proportionally with FLI, and this association was independent of traditional cardiometabolic risk factors. Steatosis identified patients with higher C-IMT better than type 2 diabetes or dyslipidemia. Importantly, patients that remained free of steatosis had lower baseline and follow-up C-IMTs, while those that had an occurrence of steatosis had the highest follow-up C-IMT values. Steatosis predicted the occurrence of CP during follow-up and was associated with higher cardiovascular risk at all times, both baseline and follow-up, as assessed by the FRS. Significant fibrosis at baseline did not predict the occurrence of CP during follow-up. This could be due to the low prevalence of significant fibrosis in our cohort. These results also suggest that while fibrosis is ultimately associated with increased CV mortality, steatosis and low grade inflammation plays a key role in occurrence and progression of early atherosclerotic lesions. Collectively, these data support a critical role for steatosis in the progression of atherosclerotic disease, a role that is largely independent of traditional risk factors. Since these data are derived from an at-risk primary prevention cohort rather than a highly selected population that had already experienced cardiovascular events, the conclusions of this study apply to the large majority of patients with steatosis from the general population. In the current series, the mean C-IMT was rather low (0.62 ± 0.13 mm, range from 0.28 to 1.6 mm). However, even lower levels of C-IMT have additive predictive value for

Table 3. Independent predictors of the occurrence of carotid plaques in Cox multivariate models.

<table>
<thead>
<tr>
<th></th>
<th>Occurrence of carotid plaques during follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1</td>
</tr>
<tr>
<td></td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Age</td>
<td>1.01 (0.98-1.03)</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.61 (0.43-0.88)</td>
</tr>
<tr>
<td>Tobacco</td>
<td>1.10 (0.78-1.55)</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>0.84 (0.54-1.33)</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>1.04 (0.73-1.47)</td>
</tr>
<tr>
<td>Baseline C-IMT</td>
<td>2.21 (0.67-7.25)</td>
</tr>
<tr>
<td>hsCRP</td>
<td>0.95 (0.89-1.01)</td>
</tr>
<tr>
<td>Steatosis at baseline</td>
<td>1.63 (1.10-2.41)</td>
</tr>
<tr>
<td>Lipid lowering treatment</td>
<td>-</td>
</tr>
<tr>
<td>Type 2 diabetes treatment</td>
<td>-</td>
</tr>
<tr>
<td>Cardiovascular treatment</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 4. Impact of baseline steatosis on the occurrence of CP during follow-up.
cardiovascular events, especially in asymptomatic individuals at intermediate cardiovascular risk [20–22].

Several other studies have documented the association between steatosis and pre-atherosclerotic lesions or CP. In a landmark paper, Marchesini et al. showed that patients with steatosis and steatohepatitis have altered flow-mediated vasodilation of the brachial artery and a higher cardiovascular risk as measured by the FRS [23]. Several reports have now shown that steatosis increases the risk of coronary artery calcium scores, a sensitive indicator of early atherosclerosis, independent of features of the metabolic syndrome or conventional cardiovascular risk factors [8, 24]. An association between steatosis and arterial stiffness has been documented in several studies [25–27], although in adolescents, steatosis was associated with increased arterial stiffness only in individuals with high-risk metabolic profiles [28].

Steatosis has also been associated with CP compared to an age-, sex- [29] and sometimes BMI-matched control groups [30]. Similar to the present report, a previous study has shown that NAFLD is associated with increased C-IMT vs. age-, sex- and BMI-matched controls [31] and that this correlation is independent of potential confounders. This case-control study was much smaller than the current series, but NAFLD was diagnosed histologically and the authors were able to document significant associations between C-IMT and the degree of the main histological lesions of NAFLD, mainly steatosis, liver inflammation and fibrosis [31]. C-IMT is a non-invasive ultrasound marker of early atherosclerosis [32] that predicts cardiovascular events in the general population, independent of all major risk factors [10]. C-IMT is also an accepted surrogate for cardiovascular events that is strongly predictive of cardiovascular morbidity and mortality events, and hence recommended as a surrogate end-point for outcome cardiovascular trials [33]. Our study confirms the independent relationship between steatosis and C-IMT and provides important longitudinal data supporting this association in a very large population seen in a primary prevention centre. The association between steatosis and early atherosclerosis is corroborated in the same population by the prediction of occurrence of CP and by higher cardiovascular risk scores. One other report confirmed in a large transversal study that FLI predicted CP independent of age and smoking [34]. However, that study involved a healthy population devoid of any metabolic comorbidities; it thus did not address the question of whether steatosis by itself, independent of metabolic comorbidities, increases the risk of early atherosclerosis in a typical population of individuals at risk for NAFLD with one or several features of the metabolic syndrome.

Numerous studies have documented the predictive value of altered liver enzymes over the development of cardiovascular complications, both for gamma glutamyl transferase and aminotransferases [35–38]. Most of these studies, however, dealt with clinical events and not pre-atherosclerotic lesions. Siddiqui et al. showed that serum levels of aminotransferase were associated with increased atherogenesis, however their analysis did not take steatosis into account [39]. Interestingly, in our series, when controlling for steatosis, neither aminotransferases nor GGT were associated with C-IMT or CP or with the occurrence of CP in the follow-up study.

This study contributes to the growing body of evidence that steatosis can predate the phenotypical complications associated with insulin resistance and the clinical features of the metabolic syndrome [40]. NAFLD predicts cardiovascular disease but also type 2 diabetes and arterial hypertension [41–43]. The pathogenesis of these clinical observations has been partly elucidated. The fatty and inflamed liver results in hyperglycemia [44, 45] and overproduces triglyceride-rich very low density lipoprotein particles, which in turn leads to low HDL-cholesterol and increased small-density LDL particles [46], a pro-atherogenic profile. It also overproduces coagulation factors, including fibrinogen, which increases the risk of thromboembolic events [47]. Regardless of the mechanisms involved, the clinical implications are of first-hand importance, since patients at cardiovascular risk carrying one or several features of the metabolic syndrome have further increased risk if they have steatosis. Also patients with steatosis but only limited overweight and no type 2 diabetes or arterial hypertension are at higher risk of developing these complications than those without steatosis, which makes NAFLD a precursor of the metabolic syndrome. It follows that the diagnosis of steatosis is critical, and therefore a thorough cardiovascular and metabolic work-up and a strict monitoring of cardiovascular disease or of metabolic complications are needed in the clinical management of NAFLD patients. Whether the reduction of steatosis or the reversal of NAFLD will help improve the cardiometabolic risk is unknown, but it will be of critical importance as NASH therapies become available [48]. It is worth noting that in our longitudinal study, steatosis predicted incident CP, even after adjustment for lipid lowering or diabetes therapy. In patients with steatosis, C-IMT progressed despite a massive increase in the proportion of patients on statins during follow-up. This would suggest that correcting for the metabolic conditions might not be enough to minimize the risk of carotid atherosclerosis as long as liver steatosis is still present.

This study has several limitations. It is a retrospective study but the data was systematically collected in a well-defined cohort, and the risk of selection bias appears minimal. Not all patients had a follow-up carotid ultrasound. However, in those who did, the carotid ultrasound was centralized and performed by only two, experienced, operators. Importantly, there was no protocol-defined time interval for the follow-up carotid ultrasound which was performed on an individual basis. This could potentially induce a bias if patients considered at lower risk of progression had longer time intervals between the follow-up exams. However, in the clinical setting and at the time when these patients were managed, steatosis was not known to be a risk factor for pre-athertherosclerotic lesions. In addition, steatosis was not detected directly but calculated a posteriori through a composite surrogate. Therefore the timing of the follow-up ultrasound was not modified by the presence of steatosis at baseline. While this only reduces and does not exclude the risk for a lead-time bias, we acknowledge that only well-designed, prospective studies can definitively address this limitation. A second limitation is that steatosis was assessed by a surrogate biochemical marker and not histologically or by magnetic resonance spectroscopy. In large cohorts however, an invasive procedure or a complex, costly imaging method would not be feasible. Instead, FLI is a well-accepted surrogate of steatosis and has been validated in the general population, with an estimated accuracy of 84% [13]. When compared to liver biopsy, FLI discriminated between the presence or absence of >5% steatosis with good accuracy [49]. In addition many studies have shown that FLI also predicts overall and hepatic mortality, accelerated atherosclerosis, cardiovascular risk, insulin resistance and incident diabetes [50–52]. Interestingly, in the current study, FLI performed better than its individual components for both the association and the
prediction of early atherosclerosis. This further strengthens its validity as a surrogate for steatosis and not merely a biochemical panel that would simply represent the sum of its parts. Unfortunately, because histological data were not available, this study cannot provide evidence as to whether steatohepatitis, which combines steatosis, hepatic inflammation and liver cell injury, has a stronger association with early atherosclerosis than steatosis alone.

In conclusion, in this large cohort of patients at cardiovascular risk from a primary prevention program, steatosis was associated with lesions of early atherosclerosis, independent of traditional cardiovascular risk factors, both in transversal and longitudinal studies. This confirms the deleterious role of hepatic fat accumulation in the occurrence and worsening of features of the metabolic syndrome, which places patients at high cardiometabolic risk. From a metabolic point of view, steatosis is not an innocent bystander but rather a driving force, and hence an exhaustive work-up of the liver, cardiovascular and metabolic complications, strict monitoring and possibly reinforced therapies should be implemented.

Financial support
The research leading to these results has received funding from the European Community’s Seventh Framework Programme FP7/2007-2013 under grant agreement n° HEALTH-F2-2009-241762 for the project FLIP; PN-II-ID-PCE-2011-3-0917, no. 297/2011 of the Romanian Ministry of Education represented by UEFISCDI. The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Conflict of interest
The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Authors’ contributions
Study design: Raluca Pais, Philippe Giral, Vlad Ratziu; Acquisition of data: Philippe Giral, Jean Francois Khan and David Rosenbaum; Carotid Ultrasound: Jean Francois Khan and David Rosenbaum; Statistical analysis: Raluca Pais; Analysis and interpretation of data: Raluca Pais, Philippe Giral, Vlad Ratziu; Drafting of the manuscript: Raluca Pais, Philippe Giral, Vlad Ratziu; Critical revision of the manuscript for important intellectual content: Vlad Ratziu, Philippe Giral. Obtained funding: Vlad Ratziu; All authors approved the final document.

Acknowledgements

Supplementary data
Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2016.02.023.

References
Resarch article


[43] Sung KC, Wild SH, Byrne CD. Development of new fatty liver, or resolution of existing fatty liver, over five years of follow-up, and risk of incident hypertension. J Hepatol 2014;60:1040–1045.


Please cite this article in press as: Pais R et al. Fatty liver is an independent predictor of early carotid atherosclerosis. J Hepatol (2016), http://dx.doi.org/10.1016/j.jhep.2016.02.023