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Non-alcoholic fatty liver disease is strongly associated with carotid atherosclerosis: A systematic review☆

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***Background/Aims*: To perform a systematic review of the studies addressing the association between non-alcoholic fatty liver disease (NAFLD) and carotid intima-media thickness (IMT).**

***Methods*: Literature searches identified seven studies that met inclusion criteria: population-based or hospital-based case-control studies about the relation between NAFLD and carotid IMT, in which information on number of subjects in controls and NAFLD patients, and data to evaluate carotid IMT and carotid plaques (measured by carotid ultrasound) could be extracted.**

***Results*: From a total of 3497 subjects (1427 patients and 2070 controls), we found a significant association between NAFLD and carotid IMT either in fixed (*D*: 0.51, 95% CI: 0.44–0.58, $p < 1 \times 10^{-8}$) or random models (*D*: 1.44, CI: 95% 0.63–2.24, $p < 0.0006$). Meta-regression analysis showed that mean differences in alanine aminotransferase and γ-GT were strongly correlated with those in IMT ($p < 0.00006$ and 0.004, respectively). In addition, 5 reports including 3212 subjects showed that carotid plaques were more frequently observed in NAFLD patients in comparison with controls, fixed model ($p < 1 \times 10^{-10}$), OR: 1.97 95% CI: 1.67–2.32 and random model $p < 0.0002$, OR: 3.13, 95% CI: 1.75–5.58.**

***Conclusions*: Routine measurement of carotid IMT might be implemented in NAFLD patients, as they carry an increase of 13% of carotid IMT.**

***Keywords*: NASH; Carotid arteries; Carotid intima-media thickness; Carotid plaque; Cardiovascular risk**

1. Introduction

The prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing worldwide because of the rise of obesity and type 2 diabetes prevalence [1]. NAFLD is present in 10–24% of the general population in various countries [2] and is closely related to insulin resistance and markers of oxidative stress and endothelial

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dysfunction [3,4]. In fact, NAFLD is considered the hepatic manifestation of the metabolic syndrome (MS) [3,5].

People with metabolic syndrome are at risk for cardiovascular disease, including coronary heart disease and stroke [6]. The importance of NAFLD and its relationship with MS is now increasingly recognized as recent data suggest that NAFLD is linked to increased cardiovascular risk independently of the broad spectrum of risk factors of MS [7]. Indeed, it is hypothesized that NAFLD is not merely a marker of cardiovascular disease but may also be involved in its pathogenesis [8].

Various non-invasive markers of early arterial wall alteration are currently available, such as arterial wall thickening and stiffening, endothelial dysfunction and coronary artery calcification [9]. Of them, non-invasive assessment of carotid intima-media thickness (IMT) by high-resolution carotid B-mode ultrasonography (US) is widely used as a proxy end point for cardiovascular disease [10]. In addition, carotid US allows the evaluation – in a simple, safe and reproducible way – of lumen diameter, intima-media thickness, and the presence and extent of carotid plaques.

Recent studies have shown that NAFLD patients have significantly greater carotid IMT than age and sex-matched patients without NAFLD, independently of the classical risk factors of the metabolic syndrome. However, some degree of variability about the mean carotid IMT values' can be observed among all the published reports that result in a difficult evaluation of the magnitude of the observation. For instance, among the different studies, mean carotid IMT values in NAFLD patients range from 0.64 ± 0.10 mm to 1.24 ± 0.13 mm. The importance of this is to decide if further recommendations with regard to carotid atherosclerosis screening should be implemented in all NAFLD patients, as currently available epidemiological data indicate that a value of carotid IMT at or above 1 mm at any age is associated with a significantly increased risk of myocardial infarction and/or cerebrovascular disease [11].

In view of the evidence mentioned above, our primary purpose was to estimate from the available literature, the strength of the increased carotid IMT and carotid atherosclerosis observed in NAFLD patients. In addition, we systematically evaluated the study characteristics that could be responsible for the association.

2. Material and methods

2.1. Data sources and study selection

For the electronic searches, published studies were found through PubMed at the National Library of Medicine (http://ncbi.nlm.nih.gov/entrez/query) and in Medline databases for the query "(fatty liver or hepatic steatosis or non-alcoholic fatty liver disease) and (intima-media thickness, carotid artery, carotid plaque, cardiovascular disease and atherosclerosis)". In addition, the references of the articles were checked and the PubMed link 'related articles' was used to identify additional papers. This yielded 939 hits. The literature search was carried out on studies up to February 2008 and availability of an English-language abstract or paper for review. There were not country restrictions. All abstracts were reviewed for appropriateness on the research issue by the authors, and when so, the article was retrieved.

We followed the appropriate methods for conducting a meta-analysis as stipulated in the Guidelines for Meta-Analyses of Observational Studies in Epidemiology (MOOSE) group [12].

2.2. Inclusion and exclusion selection criteria

2.2.1. Inclusion criteria

Observational or cohort (population-based or hospital-based) case-control studies that provided raw data dealing with non-alcoholic fatty liver disease and carotid IMT and that were conducted in adult populations, in which information about number of subjects in each category (control subjects and NAFLD patients evaluated at least by abdominal ultrasound), and data to evaluate carotid IMT and carotid plaques could be extracted (measured in all the cases by carotid ultrasound).

2.2.2. Exclusion criteria

Duplicate publications and unpublished papers. Secondary causes of steatosis, including alcohol abuse ($\geqslant 30$ g alcohol daily for men and $\geqslant 20$ g for women), total parenteral nutrition, hepatitis B and hepatitis C virus infection, and the use of drugs known to precipitate steatosis were also exclusion criteria.

2.3. Data collection

The primary outcome was carotid IMT in NAFLD patients compared with non-fatty liver controls. Secondary outcome was the presence of carotid plaques in the same groups.

For each study, information was collected concerning the demographic information of the subjects (age, sex, country of origin as a proxy of ethnicity), study design, and biochemical determinations, such as alanine aminotransferase (ALT) and γ-glutamyl transferase (γ-GT), were also analyzed. Besides, assessment of carotid atherosclerosis (evaluated either by the maximum carotid IMT-expressed in mm- or by the mean value of carotid IMT), and carotid plaque were included in the analysis (for each subject, three measurements of both sides were performed in all the reports, and all readings were averaged).

All quantitative variables had to be expressed as means ± SD; SE or 95% CI were converted to SD. Odd ratios (OR) for carotid plaques were obtained or calculated for NAFLD patients against healthy control subjects (absence of fatty liver disease).

2.4. Statistical analysis

For carotid IMT, effect stands for standardized difference (D) that is the mean difference (between cases and controls) divided by the common within-group standard deviation for IMT. The same calculations were also performed for other continuous variables such as ALT and γ-GT. For the dichotomic variable carotid plaque, effect stands for odd ratio (OR). Summary OR and corresponding 95% CI was estimated by both fixed and random effects meta-analysis. Fixed and random effect models using the Mantel-Haenszel method were used to summarize results, obtaining the corresponding pooled OR.

The DerSimonian and Laird method was used to combine the OR and D for the outcomes of interest using a random-effects meta-analytical technique. In this type of model, it is assumed that there is variation among studies and therefore the calculated ratios have a more conservative value [13]. This method is based on the inverse-variance approach, making an adjustment to the study weights according to the extent of variation, or heterogeneity, among the varying intervention effects. We assessed heterogeneity by using Q statistics.

For D, Cohen test (which is used for expressing the magnitude of differences between groups) was used to summarize the results, and heterogeneity was evaluated with Q statistic and the $I2$ statistic, a transformation of Q that estimates the percentage of the variation in effect sizes that is due to heterogeneity. An I_2 value of 0% indicates no observed heterogeneity, and larger values show increasing heterogeneity.

Meta-regression analysis (random effects model, within-study variance estimated with the unrestricted maximum-likelihood method) was also performed to assess the influence of study-related factors on outcomes and analysis of heterogeneity. Particularly, as a potential source of heterogeneity might be disease severity we assessed the correlation between D for carotid IMT and D for ALT and γ-GT when the information was available.

A sensitivity analysis was also conducted, whereby each study was omitted in turn.

To check for publication bias, we used the Begg and Mazumdar's rank correlation test (this test reports the rank correlation-also known as rank correlation coefficient or simply Kendall's tau – between the standardized effect size and the variances – or standard errors-of theses effects) [14].

All calculations were performed using the Comprehensive Meta-Analysis computer program (Biostat, Englewood, NJ, USA).

A p value equal or less than 0.05 was considered to be statistically significant.

2.5. Assessment of study quality

The quality of the studies was assessed using the "Quality Assessment of Diagnostic Accuracy Studies" (QUADAS) tool, an evidence-based instrument for the assessment of the quality of diagnostic accuracy in studies that was specifically developed for use in systematic reviews of diagnostic tests [15]. The final tool consists of a set of 14 items, phrased as questions, each of which should be scored as yes, no or unclear.

3. Results

We evaluated seven studies that met the selection criteria and that were identified using the search strategy described in Appendix Fig. A1. Studies characteristics are shown in Table 1. Data on one further study was unavailable because in the paper the authors did not disclose the raw data; the authors were contacted but they were unable to provide the data in the required format for evaluation [16]. In addition, the patients included in this study have fatty liver of unspecified aetiology.

Over 100% of the studies met the QUADAS quality-assessment criteria, which are summarized in Table 2. All the studies scored well in terms of adequate descriptions of selection criteria and reference test, blind assessment of the reference test and availability of clinical data.

3.1. Study characteristics

Five studies were hospital-based case-control [17–21], and other two were population-based case-control [22,23] studies. Study characteristics are shown in Table 1.

In all the cases, carotid IMT was measured by B-mode ultrasound by an operator who was blind to the clinical characteristics of the patients. Carotid IMT measurements were made bilaterally at the level of common carotid artery. Maximum value of carotid IMT was used in some of these studies [19,21,22], whereas mean value of carotid IMT readings was used in others [17,18,20,23].

Carotid plaque was defined as a focal thickening $\geqslant$1.2 [17–20] and $\geqslant$1.3 [23] at the level of carotid artery.

Diagnosis about fatty liver disease was performed in all the studies by abdominal ultrasound referred to as "bright" liver or diffuse increase of echogenicity of the liver compared to the of the kidney; in some studies fatty liver was confirmed by liver biopsy [17,19,22].

Control subjects were matched for age and sex in some studies [17,18,21], but for age, sex and BMI in others [19,20]. One study included only male but age matched subjects [22], and other study was a cross-sectional survey from a random sample of population that was selected using population registries [23].

3.2. IMT

Data regarding of carotid IMT extracted from the 7 studies included 3497 individuals, and showed a significant association of increased carotid IMT with NAFLD

Table 1
Characteristics of the studies about the relation between carotid IMT and nonalcoholic fatty liver disease (NAFLD) assessed by B-mode ultrasonography

First author, year	Ref.	Country	Study design and sample size (N)	Type of patients	Liver biopsy (n)	Carotid phenotype	Means ± SD of CIMT (mm) in controls	Means ± SD of CIMT (mm) in NADLD
Aygun, 2008	[17]	Turkey	Hospital-based $N = 80$	Hepatic steatosis	40	IMT, CP	0.54 ± 0.06	0.64 ± 0.10
Brea, 2005	[18]	Spain	Hospital-based $N = 80$	Hepatic steatosis	NA	IMT, CP	0.54 ± 0.13	0.70 ± 0.20
Fracanzani, 2008	[19]	Italy	Hospital-based $N = 375$	Hepatic steatosis	54	IMT, CP	0.64 ± 0.14	0.89 ± 0.26
Targher, 2006	[20]	Italy	Hospital-based $N = 245$	Hepatic steatosis	85	IMT, CP	0.82 ± 0.1	1.14 ± 0.2
Targher, 2006	[21]	Italy	Hospital-based $N = 200$	Diet-treated type 2 diabetic patients	NA	IMT	0.95 ± 0.11	1.24 ± 0.13
Targher, 2004	[22]	Italy	Population-based $N = 85$	Hepatic steatosis	NA	IMT, CP	0.94 ± 0.12	1.18 ± 0.14
Volzke, 2005	[23]	Germany	Population-based $N = 2432$	Hepatic steatosis	NA	IMT	0.78 ± 0.17	0.81 ± 0.17

Carotid intima-media thickness (CIMT), carotid plaque (CP), not available (NA).

either in the fixed ($p < 1 \times 10^{-8}$) or the random model ($p < 0.0006$) (Fig. 1).

We assessed between study heterogeneity by using the Q statistic and observed significant heterogeneity ($p < 0.0002$), I^2: 98.4%. Subjects were stratified by ethnicity, study design and associated disease condition but the heterogeneity remains significant.

As we hypothesized that heterogeneity may be due to liver disease severity, where sufficient data were available, we used regression analysis to investigate whether additional variables such liver enzymes (ALT and γ-GT) likely to be associated with severity of fat cytotoxicity were associated with the studied outcome effect and hence whether differences in these laboratory variables between the studies accounted for some of the observed heterogeneity. ALT and γ-GT were included as putative surrogate indicators of severity of fat cytotoxicity because carotid IMT value according to liver biopsy results (when available) was not disclosure by the authors except for one study [20]. The following six reports included data about ALT and γ-GT value [17–21,23], which were included in the analysis. According to meta-regression analysis, carotid IMT mean differences showed strong correlation with ALT and γ-GT mean differences. This analysis revealed that ALT had a significant impact on the carotid IMT, as D of carotid IMT strongly correlates with ALT D values (slope = 0.86, $p < 0.00006$) (Fig. 2A) and carotid IMT D was also correlated with γ-GT D values (slope 0.66, $p < 0.004$) (Fig. 2B).

From the Begg and Mazumdar's rank correlation test (Kendall's tau: 0.048, 2-tailed-$p < 1.0$), it seems that the there was no publication bias.

We estimated the NAFLD effect on carotid IMT by calculating, from the included studies, the percentage of increase of carotid IMT in NAFLD patients in relation to controls as follows: the weighted mean of NAFLD patient carotid IMT minus the weighted mean of controls, divided by the weighted mean of controls multiplied by 100. This analysis showed that NAFLD patients carry an estimated increase of 13% in carotid IMT.

3.3. Carotid plaque

We found five heterogeneous reports ($p < 0.001$, I^2: 77.7) that evaluated carotid plaque [17–20,23]. The comparison between cases and controls, including 3212 subjects, showed that carotid plaque was more frequently observed in NAFLD patients than controls by fixed ($p < 1 \times 10^{-10}$) or random effect ($p < 0.0002$) models (Fig. 3). To investigate the source of heterogeneity, we analyzed the data by grouping the reports by study design and after removing one study [23], the heterogeneity was removed. By meta-regression analysis, we observed that MH ORs for carotid plaques was correlated with ALT and γ-GT mean differences (slope: 0.451, $p < 0.001$ and slope: 0.395, $p < 0.003$, respectively).

Table 2
"Quality Assessment of Diagnostic Accuracy Studies" (QUADAS) tool

Author	Questions													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Aygun, et al., 2008 [17]	Y	Y	NA	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	U
Brea, et al., 2005 [18]	Y	Y	NA	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	U
Fracanzani, et al., 2008 [19]	Y	Y	NA	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	U
Targher, et al., 2006 [20]	Y	Y	NA	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	U
Targher, et al., 2006 [21]	Y	Y	NA	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	U
Targher, et al., 2004 [22]	Y	Y	NA	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	U
Volzke, et al., 2005 [23]	Y	Y	NA	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y

Y = yes; N = no; U = unknown, NA = not-applicable.

QUADAS Questions

1. Was the spectrum of patients representative of the patients who will receive the test in practice?
2. Were selection criteria clearly described?
3. Is the reference standard likely to classify the target condition correctly?
4. Is the period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?
5. Did the whole sample or a random selection of the sample receive verification using a reference standard?
6. Did patients receive the same reference standard regardless of the index test result?
7. Was the reference standard independent of the index test *i.e.*,the index test did not form part of the reference standard?
8. Was the execution of the index test described in sufficient detail to permit replication of the test?
9. Was the execution of the reference standard described in sufficient detail to permit its replication?
10. Were the index test results interpreted without knowledge of the results of the reference standard?
11. Were the reference standard results interpreted without knowledge of the results of the index test?
12. Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?
13. Were uninterpretable/intermediate test results reported?
14. Were withdrawals from the study explained?

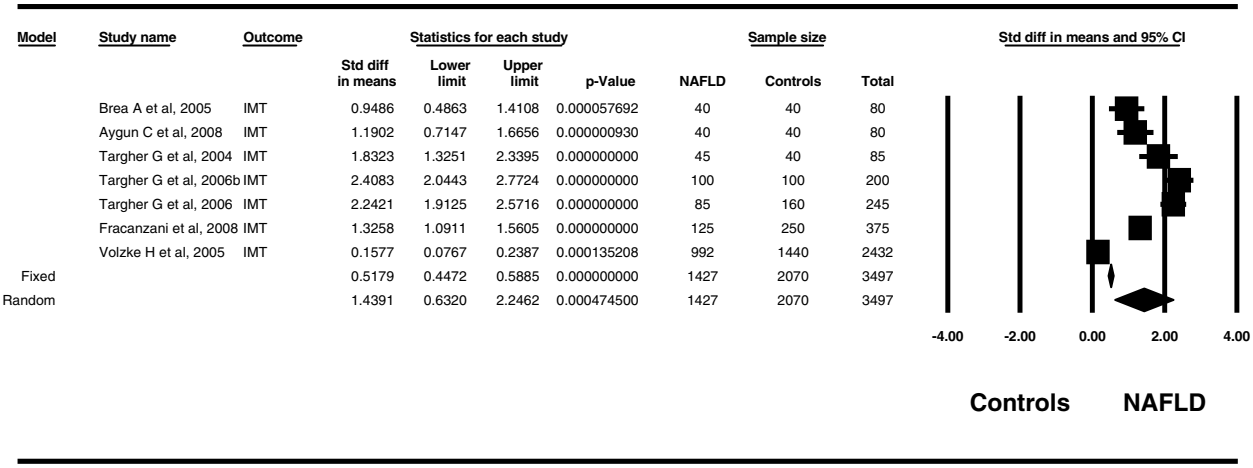

Fig. 1. Summary estimates for standardized difference (*D*) (effect), the corresponding 95% CI limits (lower and upper) and significance (*p* value) were estimated by fixed and random effects analysis for carotid intima-media thickness between NAFLD patients and controls. The first author of the study is indicated under citation. In the graph, numbers indicate *D* values, filled squares stand for the effect of individual studies and filled diamond express combined fixed and random effects.

Begg and Mazumdar's rank correlation test (Kendall's tau: 0.001, 2-tailed $p < 0.81$) showed that there seems not to be publication bias.

4. Discussion

Recent epidemiological studies suggested an increased incidence of major cardiovascular events in patients with ultrasound-diagnosed NAFLD independent of traditional risk factors and components of the metabolic syndrome [7,8,24]. Moreover, the 14-year risk of cardiovascular mortality was doubled in 129 patients with biopsy-proven NAFLD compared with that of the reference population [25].

A strong association between NAFLD and endothelial dysfunction as measured by brachial artery flow-mediated vasodilation – a reliable marker of early atherosclerosis, was recently described [26,27]. However, despite several previous studies demonstrated the association between NAFLD and carotid IMT and/or carotid plaque, no general consensus exists on the systematic screening of carotid atherosclerosis in patients with fatty liver disease. In fact, the only general recommendation for management of NAFLD patients to date is related to lifestyle changes and an attempt at gradual weight loss along with appropriate control of serum glucose and lipid levels [28,29].

To provide a more objective basis to clinical recommendations and to determine the impact of NAFLD on carotid atherosclerosis, we conducted a meta-analysis.

This study showed that carotid IMT is strongly associated with NAFLD, showing that patients with hepatic steatosis have an increase of 13% of IMT in comparison with individuals without fatty liver. This conclusion results from a total of 3497 individuals recruited from 7 heterogeneous studies. Besides, the presence of at least one carotid plaque was also strongly related with the

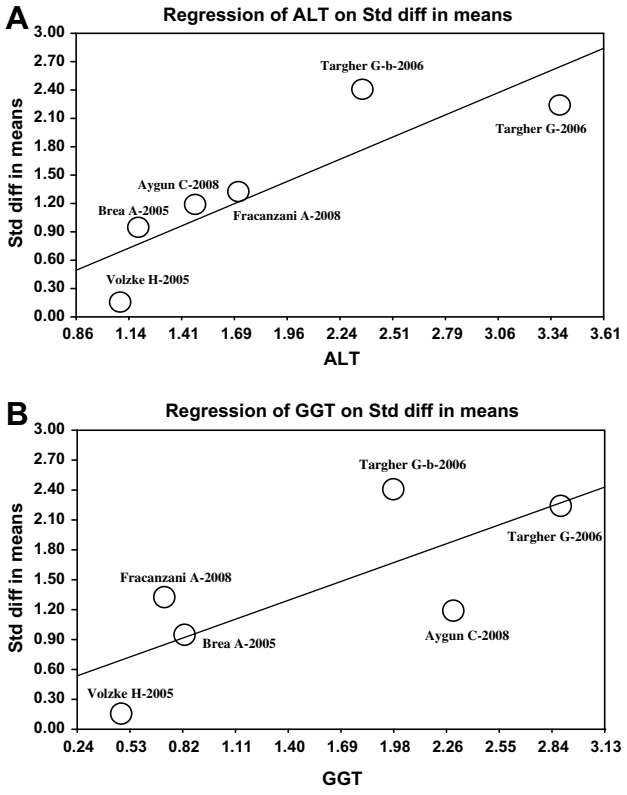

Fig. 2. (A) Meta-regression analysis of standardized difference (Std diff) in ALT means (*X* axis: ALT) on standardized difference (Std diff) in carotid intima-media thickness means (*Y* axis: Std diff in means). Each circle represents a study identified by the label. (B) Meta-regression analysis of standardized difference (Std diff) in γ-GT means (*X* axis: γ-GT) on standardized difference (Std diff) in carotid intima-media thickness means (*Y* axis: Std diff in means). Each circle represents a study identified by the label.

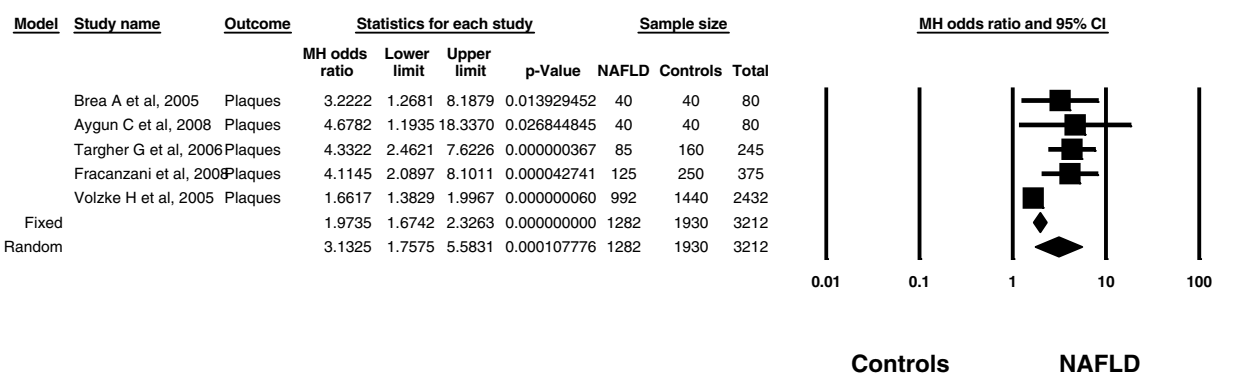

Fig. 3. Summary estimates for Mantel Haenszel Odds Ratios (OR, effect), the corresponding 95% CI limits (lower and upper) and significance (*p* value) were estimated by fixed and random effects meta-regression analysis for carotid plaque between the two groups (NAFLD patients and controls). The first author of the study is indicated under citation. In the graph, numbers indicate OR values, filled squares stand for the effect of individual studies and filled diamond express combined fixed and random effects.

presence of fatty liver disease in 3212 assessed individuals. Notably, meta-regression showed that liver function tests were strongly associated with carotid IMT and the presence of carotid plaques.

The main strength of this study is the large sample of individuals screened for both fatty liver disease and carotid IMT. In addition, this is the first study showing evidence-based data that may result in the formulation of management guidelines for NAFLD elsewhere in relation with prevention of cardiovascular events in individuals who until now are not currently included as carriers of potential risk factors for carotid atherosclerosis.

A note of caution should be added as the presence of heterogeneity may potentially restrict the interpretation of the pooled risk estimates. Heterogeneity in a meta-analysis is mostly produced by differences in study design and background characteristics of the subjects and the extent of heterogeneity might influence the conclusions. However, the random effect model, where heterogeneity is no longer a main issue, provided a significant result about both carotid features.

Although heterogeneity was addressed statistically by applying a random effects model, we aimed to further investigate potential sources of it where possible. Thus, the full dataset was utilized for investigation of heterogeneity by meta-regression. When main outcomes were analyzed according to liver enzymes (ALT and γ-GT values) and entered into a meta-regression, they proved to be a statistically significant source of heterogeneity. The main hypothesis forwarded to explain these results is the observation that in NAFLD group were included patients with different stages of disease severity, and consequently patients with more severe fatty liver disease are those who show elevated liver function test results. These data are in agreement with previously published epidemiological studies that have related serum liver enzymes with atherothrombotic risk profile and elevated risk of cardiovascular disease [30–33].

A critique concerns the observation that few studies showed data about liver disease severity. The limited data on this issue may to some extent be attributable to the difficulties in performing liver biopsy as it is an invasive and costly procedure, and even in the most experienced hands it is prone to complications. Only one study disclosed the data of carotid IMT according to liver histology, and showed that carotid IMT was significantly different between patients with nonalcoholic steatohepatitis, patients with simple steatosis and controls subjects [20]. Therefore, this lack of information remains to be addressed.

The results of this meta-analysis have important clinical implications. First, this study shows that carotid atherosclerosis disease should be suspected when there are at least characteristic changes on hepatic ultrasonography that show fatty liver disease. Besides, recent data suggest that NAFLD is strongly associated with carotid atherosclerosis even in childhood [34]. Carotid plaques were more common in NAFLD patients. These data reinforce the importance of the described association as there is strong relation between plaque prevalence and stroke suggesting that the presence of any plaque on ultrasound increases the likelihood of clinical disease in the future [35].

Second, our analysis supports the association between liver enzymes and carotid atherosclerosis, and the potentially strong relation between ALT and γ-GT with carotid IMT. Finally, although this issue cannot be solved from the current study, our observation supports the notion that subjects with carotid

atherosclerosis should be assessed for fatty liver disease.

In summary, because carotid IMT is a marker of early arterial wall change, including atherosclerosis and/or vascular hypertrophy, and patients with NAFLD are at increased risk of higher carotid IMT, routine detection of carotid IMT by B-mode ultrasonography is strongly recommended in patients with fatty liver disease and vice versa. This proposal may provide benefits on primary prevention and in the decision to treat the existing but not diagnosed cardiovascular disease in patients with fatty liver disease.

Appendix

Search methods and process of study selection (see Fig. A1).

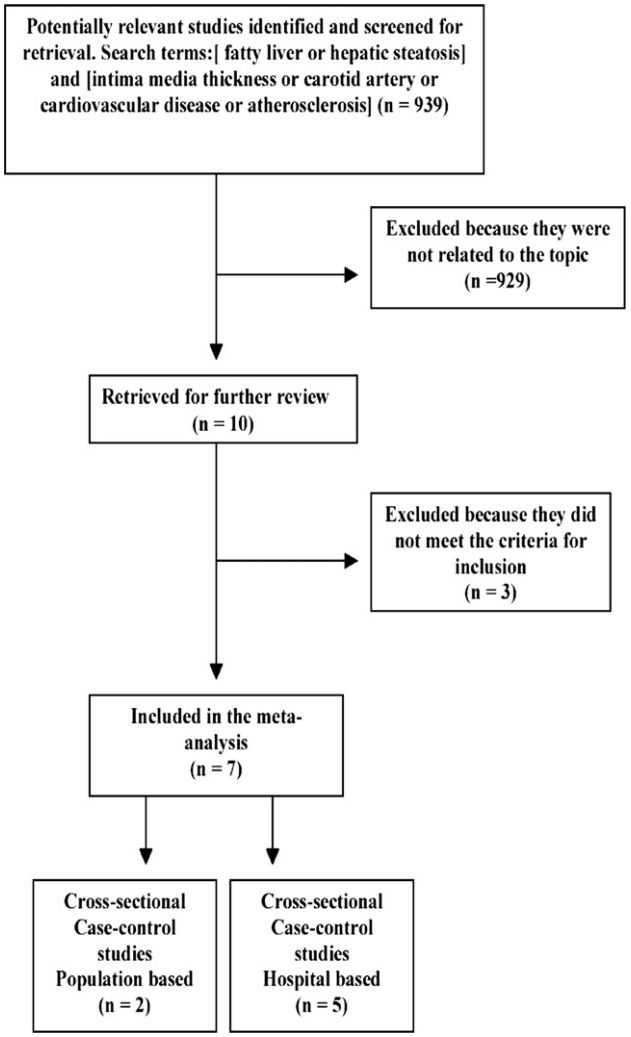

Fig. A1.

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