Research Article

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Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis

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Background & Aims: There have been many studies of the effects of non-alcoholic fatty liver disease (NAFLD) and the risk of cardiovascular disease (CVD), but these have produced conflicting results. We performed a meta-analysis of these studies to quantify the magnitude of the association between NAFLD (and NAFLD severity) and risk of CVD events.

Methods: We searched PubMed, Google scholar, and Web of Science databases using terms "NAFLD", "cardiovascular events", "cardiovascular mortality", "prognosis" and their combinations to identify observational studies published through January 2016. We included only observational studies conducted in adults >18 years and in which NAFLD was diagnosed on imaging or histology. Data from selected studies were extracted and meta-analysis was then performed using random effects modelling.

Results: A total of 16 unique, observational prospective and retrospective studies with 34,043 adult individuals (36.3% with NAFLD) and approximately 2,600 CVD outcomes (>70% CVD deaths) over a median period of 6.9 years were included in the final analysis. Patients with NAFLD had a higher risk of fatal and/or non-fatal CVD events than those without NAFLD (random effect odds ratio [OR] 1.64, 95% CI 1.26–2.13). Patients with more 'severe' NAFLD were also more likely to develop fatal and non-fatal CVD events (OR 2.58; 1.78–3.75). Sensitivity analyses did not alter these findings. Funnel plot and Egger's test did not reveal significant publication bias.

Conclusions: NAFLD is associated with an increased risk of fatal and non-fatal CVD events. However, the observational design of the studies included does not allow to draw definitive causal inferences.

Lay summary: The data on whether NAFLD by itself is associated with increased cardiovascular events and death remains an issue of debate. The findings of this updated and large meta-analysis of observational studies indicate that NAFLD is significantly associated with an increased risk of fatal and non-fatal cardiovascular events. However, the observational design of the studies included does not allow us to prove that NAFLD causes cardiovascular disease. Clinicians who manage patients with NAFLD should not focus only on liver disease but should also consider the increased risk of cardiovascular disease and undertake early, aggressive risk factor modification.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a clinico-pathological syndrome that ranges from simple steatosis to non-alcoholic steatohepatitis (NASH) with varying amounts of fibrosis, and cirrhosis [1]. NAFLD is becoming the most common cause of chronic liver disease worldwide, affecting up to 30% of the adult population in the United States and Europe [1–3]. Over the past decade, it has become increasingly clear that NAFLD is not only associated with an increased risk of liver-related morbidity or mortality, but also it is a multisystem disease that affects a variety of extra-hepatic organ systems, including the cardiovascular system [3–7].

A recent comprehensive meta-analysis involving 27 cross-sectional studies has shown that NAFLD was associated with various markers of subclinical atherosclerosis, such as increased carotid artery intimal-medial thickness, impaired flow-mediated vasodilation, increased arterial stiffness or increased coronary artery calcification [8]. All these associations were independent of multiple cardio-metabolic risk factors across a wide range of patient populations [8].

Several studies have also demonstrated that the prevalence of clinically manifest cardiovascular disease (CVD) was also significantly increased among patients with NAFLD (as reviewed elsewhere) [5,6]. Worryingly, NAFLD was also associated with a higher prevalence of high risk and vulnerable coronary artery

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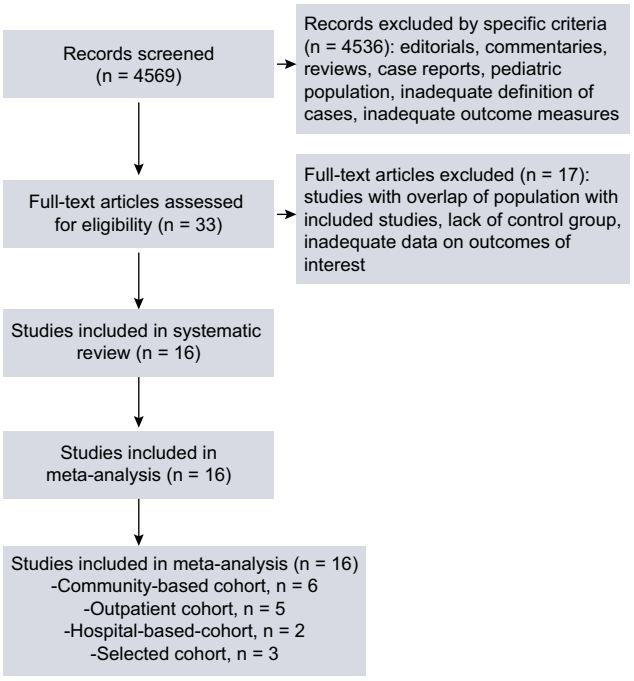

Fig. 1. Included and excluded studies: the MOOSE flow diagram.

plaques, independently of traditional CVD risk factors and the extent and severity of coronary atherosclerosis [9].

Although the cross-sectional association between NAFLD and increased CVD prevalence is strong and consistent, it remains uncertain whether the presence of NAFLD predicts incident CVD events or whether the more severe forms of NAFLD are associated with an even higher risk of future CVD events. Moreover, the mechanisms linking NAFLD to CVD are controversial and several putative mechanisms have been proposed which, however, are to be traced back to liver histologic changes, insulin resistance and oxidative stress [10].

In this context, we have carried out a comprehensive systematic review and meta-analysis of published observational studies to gauge precisely the nature and magnitude of the association between NAFLD and the risk of incident CVD events. We have also investigated whether the severity of NAFLD is associated with a higher risk of CVD events. Clarification of these issues may have important clinical implications for management of patients with NAFLD.

Materials and methods

Registration of review protocol

The protocol for this review was registered in advance with PROSPERO (International Prospective Register of Systematic Reviews, #CRD42016033481).

Type of studies, inclusion and exclusion criteria and definition of severe NAFLD

Studies were included if they were observational, prospective or retrospective studies that reported fatal and/or non-fatal CVD events in adult patients (>18 years old) with NAFLD as compared with adult individuals without NAFLD. Study participants were of either sex with no restrictions in terms of comorbid conditions. We included only studies in which the diagnosis of NAFLD was based on either radiological imaging or histology in the absence of competing causes of hepatic steatosis. Exclusion criteria were as follows: 1) studies that used only serum liver enzyme levels to diagnose NAFLD; 2) studies conducted in paediatric population (<18 years old); 3) studies performed in patients with NAFLD who received liver transplants; and 4) studies that compared long-term adverse outcomes of fibrosing NASH and NASH-cirrhosis with patients with chronic liver diseases of other aetiology.

Based on data from the eligible studies, 'severe' NAFLD was defined either by presence of steatosis on radiological imaging, plus either elevated serum gamma-glutamyltransferase (GGT) concentrations or high NAFLD fibrosis score or high hepatic ^{18}F-fluoro-2-deoxyglucose (FDG) uptake on positron emission tomography or by increasing fibrosis stage on liver histology. All these histological and non-histological/imaging criteria can identify more 'severe' NAFLD, e.g., NASH with varying amounts of fibrosis [1,2,11–14].

Included and excluded studies were collected following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram. Additionally, because included studies were observational in design, we followed the meta-analysis of observational studies in epidemiology (MOOSE) guidelines for the meta-analysis of observational studies.

Search strategy and data extraction

Relevant studies were identified by systematically searching PubMed, Google scholar and Web of Science up to January 2016 using the terms "fatty liver" (OR "NAFLD" OR "NASH" OR "non-alcoholic fatty liver disease" OR "non-alcoholic steatohepatitis") AND cardiovascular events, prognosis, cardiovascular mortality, mortality, CVD, myocardial infarction or stroke. No language restriction was applied. Reference lists of relevant papers and previous review articles were hand searched for other relevant studies. Two investigators independently examined all titles and abstracts, and obtained full texts of potentially relevant papers. Working independently and in duplicate, we read the papers and determined whether they met inclusion criteria. We resolved disagreement by consensus, and extracted data independently using an electronic spreadsheet. For all studies, we extracted information on study design, source of data, population characteristics, outcomes of interests, matching and confounding factors.

Assessment of risk of bias

Two authors assessed the risk of bias independently. Since all the included studies were nonrandomised and had a cohort design, the Newcastle–Ottawa scale (NOS) was used to judge study quality, as recommended by the Cochrane collaboration [15]. This scale uses a star system to assess the quality of a study in three domains: selection, comparability, and outcome/exposure. The NOS assigns a maximum of four stars for selection, two stars for comparability, and three stars for outcome/exposure. Therefore, nine stars reflect the highest quality. Any discrepancies were addressed by a joint revaluation of the original article with a third author. We recorded the review authors' judgments about the three NOS domains (selection, comparability and outcome) into the risk of bias tool of the Review Manager software of the Cochrane collaboration. This tool allowed us to provide a graphical representation of quality ratings similar to that produced by Cochrane reviews for randomized studies, as suggested by Wells *et al.* [16].

Data synthesis

The outcome measure of this meta-analysis was the incidence of fatal and/or non-fatal CVD events in individuals with NAFLD in comparison with individuals without NAFLD. When possible, we pooled adjusted odds ratios (ORs) or relative risks or hazard ratios, with their 95% confidence intervals, with the assumption that these are comparable measures of association given that CVD events are relatively rare [17]. Visual inspection of graphs was used to investigate the possibility of statistical heterogeneity. This was supplemented using, primarily, the I-squared statistic. This provides an estimate of the percentage of variability due to heterogeneity rather than chance alone. According to Higgins *et al.*, a rough guide to interpretation is as follows: I-squared values of approximately 25% represent low heterogeneity; approximately 50% represent medium heterogeneity; and approximately 75% represent high heterogeneity [18].

The results of studies were pooled and an overall estimate of odds ratio (OR) was obtained from a random effects model, as this methodology takes into account any differences between studies even if there is no statistically significant heterogeneity [19]. Publication bias was evaluated using the funnel plot and Egger's regression test [20].