Association between non-alcoholic fatty liver disease and risk of atrial fibrillation in adult individuals: an updated meta-analysis

Alessandro Mantovani, MD[1], Marco Dauriz, MD, PhD[1], Damiano Sandri, MD[1], Stefano Bonapace, MD[2], Giacomo Zoppini, MD[1], Herbert Tilg, MD[3], Christopher D. Byrne, MB BCh, PhD[4,5], Giovanni Targher, MD[1]

[1] Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy

[2] Division of Cardiology, ''IRCCS Sacro Cuore - Don Calabria'' Hospital, Negrar (VR), Italy

[3] Department of Internal Medicine I, Gastroenterology, Hepatology & Metabolism, Medical University Innsbruck, Innsbruck, Austria

[4] Nutrition and Metabolism, Faculty of Medicine, University of Southampton, Southampton, UK

[5] Southampton National Institute for Health Research Biomedical Research Centre, University Hospital Southampton, Southampton General Hospital, Southampton SO16 6YD, UK

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Address for correspondence:
Prof. Giovanni Targher, MD
Section of Endocrinology, Diabetes and Metabolism
Department of Medicine
University and Azienda Ospedaliera Universitaria Integrata
Piazzale Stefani, 1
37126 Verona, Italy
Phone: +39-045-8123110
E-mail: giovanni.targher@univr.it

LIST OF ABBREVIATIONS

NAFLD, nonalcoholic fatty liver disease

AF, atrial fibrillation

CI, confidence interval

NASH, nonalcoholic steatohepatitis

FLI, fatty liver index

NOS, Newcastle-Ottawa Quality Assessment Scale

BMI, body mass index

OR, odds ratio

HR, hazard ratio

ICD, International Classification of Diseases

LAY SUMMARY

• Recent studies examined the association between NAFLD and risk of atrial fibrillation in adults but the findings have been inconsistent.

• This updated meta-analysis provides evidence of a significant association between NAFLD and risk of atrial fibrillation in middle-aged and elderly individuals.

• Further studies are needed to better understand the link between NAFLD and risk of atrial fibrillation.

ABSTRACT

Background & Aims: Recent studies examined the association between non-alcoholic fatty liver disease (NAFLD) and risk of atrial fibrillation (AF) in adults, but the findings have been inconsistent. We provided a quantitative estimate of the magnitude of the association between NAFLD and risk of AF.

Methods: We searched publication databases using predefined keywords to identify observational studies (published up to December 14, 2018), in which NAFLD was diagnosed by biopsy, imaging or biochemistry, and AF was diagnosed by medical history and electrocardiograms. Data from selected studies were extracted and meta-analysis was performed using random-effects modeling.

Results: Nine cross-sectional and longitudinal studies were included in final analysis (n=364,919 individuals). Meta-analysis of data from five cross-sectional studies showed that NAFLD was associated with an increased risk of prevalent AF (random-effects odds ratio 2.07, 95%CI 1.38-3.10; I^2=54.7%), independent of age, sex, body mass index, hypertension and other common AF risk factors. This risk was particularly high among patients with established diabetes (n=1 study; random-effects odds ratio 5.17, 95%CI 2.05-13.02). Meta-analysis of data from four longitudinal studies showed that NAFLD was independently associated with a 10-year increased risk of incident AF only in type 2 diabetic patients (n=1 study; random-effects hazard ratio 4.96, 95%CI 1.42-17.28). Sensitivity analyses did not modify these findings. Funnel plots did not reveal significant publication bias.

Conclusions: NAFLD is associated with an increased risk of AF in middle-aged and elderly individuals (especially with type 2 diabetes). However, the observational design of the eligible studies does not allow for proving causality.

Keywords: fatty liver; atrial fibrillation; arrhythmias; meta-analysis

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a global health problem, affecting up to one-quarter of the world's population. In parallel with the rise in obesity rates, the worldwide prevalence of NAFLD is expected to increase markedly.[1]

Over the past 10 years, it has become increasingly clear that NAFLD is just one facet of a multisystem disease that confers substantially increased morbidity and mortality to those patients who are affected and where the most common causes of death are cardiovascular disease (CVD) (~40–45% of the total deaths), followed by extra-hepatic malignancies and liver-related complications.[2-5] This concept implies that patients with NAFLD should undergo careful cardiovascular surveillance, as recommended by the most recent European, Italian and United States clinical practice guidelines for the management of NAFLD.[6-8]

Recently, it has also become increasingly clear that NAFLD adversely affects not only coronary arteries, but also all other anatomical structures of the heart, including the cardiac electrical system.[9] Atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice and is a major health problem worldwide, owing to its associated morbidity and increased mortality.[10] As it will be discussed in greater detail below, a number of observational studies have recently examined the association between NAFLD and risk of both prevalent and incident AF in middle-aged and elderly individuals, but these have produced conflicting results.[11-18]

We therefore carried out a comprehensive systematic review and meta-analysis of observational studies examining the association between NAFLD and risk of permanent (chronic) AF in middle-aged and elderly individuals. Our aim was to determine whether, and to what extent, NAFLD is associated with the risk of both prevalent and incident AF. Given the growing clinical burden of NAFLD worldwide, we believe that quantitative estimation of the magnitude of the association between NAFLD and risk of AF might also have relevant clinical implications for the prevention and treatment of this common and burdensome arrhythmic disorder.

MATERIALS AND METHODS

Registration of review protocol

The protocol for this systematic review and meta-analysis was registered in advance with PROSPERO (International Prospective Register of Systematic Reviews, no. CRD42018119762).

Data sources and searches

We conducted a systematic literature search in PubMed, Scopus and Web of Science databases for identifying all observational studies, published through December 14, 2018, which examined the association between NAFLD and risk of AF. The search free text terms were “fatty liver” (OR “non-alcoholic fatty liver disease” OR “NAFLD” OR “non-alcoholic steatohepatitis” OR “NASH”) AND "atrial fibrillation" OR "arrhythmias". We also searched for MeSH (Medical Subject Headings) terms. Searches were restricted to human studies. No language restrictions were applied. Additionally, we reviewed references from relevant original papers and review articles for identifying further eligible studies not covered by the original database searches. We performed a systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Because the included studies were observational in design, we followed the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines for the meta-analysis of these studies.[19]

Study selection

Original studies were included if they met the following inclusion criteria: 1) observational cross-sectional and longitudinal studies examining the association between NAFLD and risk of AF; 2) studies that reported either odds ratios (OR) or hazard ratios (HR) with 95% confidence intervals (95% CI) values for the outcome of interest; and 3) studies in which the diagnosis of NAFLD was based on biopsy, imaging techniques (mostly ultrasonography) or surrogate markers of NAFLD, such as the fatty liver index (FLI), which includes in its equation anthropometric variables, serum triglyceride and gamma-glutamyltransferase concentrations, in the absence of significant alcohol consumption and other competing causes of chronic liver disease. Study participants included in the meta-analysis were adult individuals (aged ≥18 years) of either sex without any restriction in terms of age, race or ethnicity.

Exclusion criteria were as follows: 1) case reports, reviews, practice guidelines, commentaries and editorials; 2) studies where NAFLD diagnosis was based exclusively on serum liver enzyme levels; 3) studies which did not exclude individuals with significant alcohol consumption and other known causes of chronic liver diseases; 4) studies performed in patients with established cirrhosis of any etiology or in patients with end-stage liver disease awaiting liver transplantation; and 5) studies which did not specifically report any OR or HR values for the outcome measure of interest.

Two investigators (AM and GT) 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. Discrepancies were resolved by consensus, referring back to the original article, in consultation with a third author.

Data extraction and quality assessment

For all eligible studies, we extracted information on study design, study size, publication year, study country, participants characteristics, methods used for diagnosing both NAFLD and AF, follow-up duration and list of covariates adjusted in multivariable regression analyses. In the case of multiple publications, the most up-to-date or comprehensive information was included.

Two investigators assessed the risk of bias independently. Any discrepancies were addressed by a re-evaluation of original articles by a third author. Quality assessment was performed according to the Newcastle-Ottawa Quality Assessment Scale (NOS), which is a validated scale for non-randomized studies in meta-analyses.[20] A NOS scale adapted for the cross-sectional studies was used.[21] This NOS 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 (or five stars in the case of cross-sectional studies), two stars for comparability, and three stars for outcome/exposure. We judged studies that received a score of at least eight stars to be at low risk of bias (*i.e.*, thus reflecting the highest quality).

Data synthesis and analysis

The primary outcome measure was either the presence or the occurrence of AF in individuals with and without NAFLD. The ORs (for cross-sectional studies) or HRs (for longitudinal studies) and 95% confidence intervals were considered as the effect size for all the eligible studies. When studies had several adjustment models, we extracted those that reflected the maximum extent of adjustment for potentially confounding variables. The adjusted OR/HRs of all eligible studies were pooled, and an overall estimate of effect size was calculated using a random-effects model, as this methodology considers any differences between studies even if there is no statistically significant heterogeneity.[20]

Visual inspection of the forest plots was used to evaluate the possibility of statistical heterogeneity. The statistical heterogeneity among studies was assessed by the I^2 statistic, which provides an estimate of percentage of variability across studies that is due to heterogeneity rather than chance alone. According to Higgins and Thompson,[22] a rough guide to interpretation is as follows: I^2 values of approximately 25% represent low heterogeneity; approximately 50% represent medium heterogeneity; and approximately 75% represent high heterogeneity.

The possibility of publication bias was evaluated using the funnel plot and the Egger's regression asymmetry test.[23]

To explore the possible sources of (expected) heterogeneity among the eligible studies and to test the robustness of associations, we conducted sensitivity/subgroup analyses and meta-regression analyses. In particular, based on data from eligible studies, the effect of NAFLD on risk of AF was assessed by stratifying the studies according to study design (cross-sectional *vs*. longitudinal), study country, methods used for diagnosing NAFLD, type of cohorts (cohorts with type 2 diabetes only *vs*. population-based/community-based/hospital-based cohorts), or whether the studies had at least eight stars on the NOS scale (*i.e*., the "high-quality" studies). We also performed univariable linear meta-regression analyses in order to examine the association of NAFLD with age, sex and body mass index (BMI).

P-values for chi-square tests are reported in all forest plots. A chi-square test *p*-value <0.10 was used to determine statistical significance considered for heterogeneity. The proportion of heterogeneity accounted for by between-study variability was also estimated using the I^2 index and adjudicated to be significant if I^2 was >50%. We used STATA® 14.2 (StataCorp, College Station, Texas) for all statistical analyses.

RESULTS

Literature search and study characteristics

Figure 1 shows the results of the literature research and study selection. After excluding duplicates, based on the titles and abstracts of 89 citations (in accordance with the aforementioned exclusion criteria of the meta-analysis), we initially identified 11 potentially relevant studies from PubMed, Web of Science and Scopus databases prior to December 14, 2018 (date last searched). After examining the full text of these eleven publications,[11-18,24,25] we further excluded two studies,[24,25] because of unsatisfactory inclusion criteria (*n*=1) or unsatisfactory outcome measures (*n*=1), as specified in the PRISMA flow diagram.

In total, nine observational studies were eligible for inclusion in the meta-analysis and were assessed for quality.[11-18] The main characteristics of the included studies are summarized in **Table 1**. The meta-analysis involved a total of 364,919 individuals (128,522 in cross-sectional studies and 236,397 in longitudinal studies, respectively), ~43% of whom had NAFLD and ~3% of whom had permanent AF (in cross-sectional studies). Most of these studies included middle-aged and elderly individuals (mean age: 60 years), predominantly women (57%) and overweight (mean BMI 27.8 kg/m^2). Five studies had a cross-sectional design, whereas four studies had a longitudinal design. Four studies were undertaken in Europe (Italy, Germany and Finland), two studies in the United States, and two studies in Asia (China and South Korea). Most of these studies were community-based or population-based cohorts, one study included a hospital-based cohort of middle-aged individuals in the United States and two studies included only Italian elderly individuals with established type 2 diabetes, attending a diabetes outpatient service. The diagnosis of NAFLD was based on International Classification of Diseases (ICD-9) code (*n*=1 hospital-based cohort study), ultrasonography (*n*=5 studies), computed tomography (*n*=1 study) or fatty liver index (*n*=1 study), in

the absence of significant alcohol consumption and other known causes of chronic liver disease. The diagnosis of AF was mostly based on medical history and standard resting electrocardiograms.

Of the nine observational studies included in the meta-analysis (**supplementary Tables 1-3**), six studies received at least eight stars on the NOS (indicating that those studies had a relatively low risk of bias), one study received seven stars, whilst the remaining two studies received five stars (*i.e.,* being at high risk of bias).

Effect of NAFLD on risk of prevalent AF

The distribution of the five cross-sectional studies (*n*=128,522 middle-aged and elderly individuals) by estimate of the association between NAFLD and risk of prevalent AF is plotted in **Figure 2**.

The presence of NAFLD was significantly associated with an increased risk of prevalent AF (*n*=5 studies; random-effects OR 2.07, 95% CI 1.38-3.10; I^2=54.7%). As we have always used the fully adjusted OR estimates for each eligible study (as specified in **Table 1**), in most of the included studies this random-effects OR was independent of age, sex, BMI, hypertension, dyslipidemia and other clinical AF risk factors.

As shown in the figure, stratifying by the type of cohorts (cohorts with type 2 diabetics only *vs*. community-based, population-based or hospital-based cohorts), the significant association between NAFLD and increased risk of AF was present in both types of cohort studies. However, this association appeared to be much stronger in the outpatient cohort of elderly patients with type 2 diabetes (*n*=1 cross-sectional study; random-effects OR 5.17, 95% CI 2.05-13.02) than in the population-based or community-based cohort studies (*n*=4 cross-sectional studies; random-effects OR 1.86, 95% CI 1.30-2.65, I^2=42.4%). That said, it is important to underline that this stratification abolished the significant (medium-high) heterogeneity observed in the pooled primary analysis.

Effect of NAFLD on risk of incident AF

Figure 3 shows the distribution of the four longitudinal studies (*n*=236,397 individuals) by effect size of the association between NAFLD and risk of developing AF.

The presence of NAFLD was not significantly associated with an increased risk of incident AF (*n*=4 studies; random-effects HR 1.34, 95% CI 0.92-1.95; I^2=65.4%). However, stratifying for the type of cohorts included (cohorts with type 2 diabetes only *vs*. community-based or population-based cohorts), the presence of NAFLD was significantly associated with a 10-year increased risk of incident AF only in the cohort of type 2 diabetic outpatients (*n*=1 prospective study; random-effects HR 4.96, 95% CI 1.42-17.28). This association was independent of age, sex, BMI, systolic blood pressure, hypertension treatment, electrocardiographic PR interval and left ventricular hypertrophy, and prior history of heart failure.

Subgroup/sensitivity analyses and meta-regressions

Table 2 summarizes the results of some sensitivity analyses of the eligible studies. Notably, the significant association we observed between NAFLD and risk of prevalent AF was consistent in all subgroups examined (*i.e.,* even after stratifying by study country and methods used for diagnosing NAFLD). With regards to the longitudinal studies, after stratifying by study country, we found a significant association between NAFLD and increased risk of incident AF in studies performed in the Europe and Asia, but not in the United States.

Supplementary Figure 1 shows the results of univariable meta-regression analyses showing the lack of any significant effect of age, sex or BMI on the association between NAFLD and risk of AF in the eligible cross-sectional studies.

Finally, as shown in **supplementary Figure 2**, the Egger's regression test did not show any statistically significant asymmetry of the funnel plots for both cross-sectional (*p*=0.93) and longitudinal studies (*p*=0.32, although this funnel plot appears to be asymmetric on visual inspection), thus suggesting that publication bias was unlikely, although it should be noted that the number of included studies was small.

DISCUSSION

In this updated systematic review and meta-analysis of nine observational studies (including nearly 365,000 middle-aged and elderly individuals), we found that NAFLD was significantly associated with a two-fold increased risk of prevalent AF (*n*=5 cross-sectional studies; random-effects OR 2.07, 95% CI 1.38-3.10; I^2=54.7%), independent of common risk factors for AF. Notably, this risk was much stronger in the cohort of outpatients with established type 2 diabetes (*n*=1 study; random-effects OR 5.17, 95% CI 2.05-13.02) compared with that observed in population-based or community-based cohort studies (*n*=4 studies; random-effects OR 1.86, 95% CI 1.30-2.65, I^2=42.4%). Conversely, meta-analysis of data from the four longitudinal studies showed that NAFLD was independently associated with a 10-year increased risk of incident AF only in the cohort of individuals with type 2 diabetes (*n*=1 prospective study; random-effects HR 4.96, 95% CI 1.42-17.28), whereas a significant association was not observed in population-based or community-based cohort studies (*n*=3 studies; random-effects HR 1.16, 95% CI 0.91-1.48, I^2=39.9%).

To our knowledge, our meta-analysis examining the association between NAFLD and risk of both prevalent and incident AF is the largest and most comprehensive assessment to date. In a previous meta-analysis of five observational studies (2 cross-sectional and 3 longitudinal), Wijarnpreecha *et al*.[26] reported that NAFLD was associated with a twofold increase in risk of AF (random-effects risk ratio 2.06, 95% CI 1.10-3.85, I^2=78%). In their analysis, the authors included five studies (published up to May 2017) that have also been incorporated into our meta-analysis.[11,12,16-18] However, we believe that the findings of this prior meta-analysis should be interpreted cautiously, especially because the authors have meta-analyzed data from cross-sectional and longitudinal studies all together and did not perform reliable analyses for excluding publication bias or sensitivity analyses in order to explore possible sources of significant heterogeneity observed in the primary pooled analysis. Almost identical considerations may also be applied to other two smaller meta-analyses, published in 2017, by Zhou *et al*.[27] (incorporating four studies[11,12,16,17]) and by Minhas *et al*.[28] (incorporating three studies[11,16,17]), respectively.

Compared to the meta-analysis by Wijarnpreecha *et al*.[26] and the other two previous smaller meta-analyses,[27,28] we have almost doubled both the number of studies (*n*=9 studies) and the total sample size (*n*=364,919 individuals included), especially by including newer observational studies

published in 2017 and 2018,[13-15] and conducted a more thorough statistical analysis (*e.g.*, performing separate analyses for cross-sectional and longitudinal studies as well as performing multiple sensitivity analyses and reliable analyses for excluding publication bias). As a result of this methodological effort, our meta-analysis provides strong evidence that NAFLD was significantly associated with an increased risk of prevalent AF. This risk was independent of age, sex, BMI, hypertension, dyslipidemia and other common risk factors for AF. Moreover, stratifying for the presence of diabetes, we also found that the risk of AF was much stronger in patients with type 2 diabetes and NAFLD. Notably, performing separate meta-analytic analyses for the four longitudinal studies, we have also observed that patients with type 2 diabetes and NAFLD were at higher risk for developing AF than those without NAFLD. In contrast, NAFLD was not significantly associated with increased AF incidence in the population-based or community-based cohort studies included in the meta-analysis. It is possible to hypothesize that the number of population-based/community-based cohort studies is too low or the mean duration of follow-up of these studies is relatively short for observing a significant association between NAFLD and risk of incident AF in nondiabetic populations. As discussed below, it is also possible to assume that people with type 2 diabetes and NAFLD are more likely to develop AF over time compared to persons without diabetes. It is known that diabetes is an established risk factor for AF (being associated with ~1.3 times greater risk of developing AF).[29,30] Compared to nondiabetic individuals, patients with type 2 diabetes show a greater propensity for ectopic and visceral fat deposition,[31] and have more severe forms of NAFLD (*i.e.,* NASH, advanced fibrosis or cirrhosis) that may promote the development and persistence of AF, possibly through the (direct) contribution of NAFLD to systemic low-grade, chronic inflammation, hypercoagulation and insulin resistance.[2,3,9] Previous population-based studies that have used mildly elevated levels of serum liver enzymes to diagnose NAFLD have shown that this liver disease is independently associated with increased long-term risk of developing AF;[32-34] however, increased concentrations of serum liver enzymes are a poor proxy for diagnosing NAFLD.[6-8] Thus, these data should be viewed with caution. Consequently, we consider that larger and longer prospective studies of well-characterized cohorts of people with NAFLD (involving both diabetic and nondiabetic individuals) are needed to better examine the impact of NAFLD and diabetes, singularly or in combination, on risk of incident AF.

Based on the published studies, our meta-analysis did not permit us to systematically examine whether there was also a significant, graded relationship between the histological severity of NAFLD

and risk of AF. In line with previous studies showing that hepatic fibrosis stage is the strongest predictor of all-cause mortality and cardiovascular events in individuals with NAFLD,[4,6,7,35] it is reasonable to hypothesize that patients with NASH and hepatic fibrosis are at substantially higher risk of incident AF than those patients with simple steatosis alone, since it is likely that the mechanisms linking hepatic fibrosis and increased cardiovascular events are similar to those linking hepatic fibrosis and AF. However, in our meta-analysis there was only one hospital-based cohort study that had some information about liver biopsy for diagnosing NAFLD. Indeed, using the ICD-9 code, Whitsett *et al*.[15] reported that the prevalence of permanent AF was approximately twofold higher in a subgroup (*n*=215) of hospitalized patients with biopsy-proven NASH compared to a comparator cohort of patients without NAFLD/NASH evaluated at the same tertiary care center. However, no information was available in this cohort study about the existence of a significant, graded association between the severity of NAFLD histology and risk of AF.[15] Therefore, we believe that this important question remains currently unresolved. Larger studies of patients with biopsy-confirmed NAFLD are required in order to prove whether more severe liver disease within the spectrum that represents NAFLD, adversely affects risk of AF.

That said, we believe that the findings of our systematic review and meta-analysis are relevant and may have clinical practice implications for the potential screening and management of patients with NAFLD and AF. Coupled with the fact that the prevalence of NAFLD is increasing dramatically worldwide, it is reasonable to expect that the risk of AF in patients with NAFLD will increase in the near future with also a considerable increase in mortality rate, disability and healthcare expenditure. Thus, future development of effective pharmacological therapies for NAFLD is eagerly awaited that not only reduce the burden of hepatic disease but also decrease the burden of associated extra-hepatic (cardiovascular) complications that are linked to NAFLD. As highlighted in the study by Whitsett *et al*., it is also plausible to assume that AF is currently undertreated in patients with biopsy-proven NASH, given that nearly 40% of these patients did not receive guideline-recommended anticoagulation therapy for stroke risk reduction based on the CHA_2DS_2VASc score.[15] Despite clear clinical practice guidelines,[36] anticoagulation therapy for AF is challenging to implement, especially for patients with cirrhosis, due to a concern for increased risk of bleeding events. However, a recent systematic review showed that anticoagulation therapy (especially the use of the newer direct oral anticoagulants) is effective and safe for treatment of cirrhotic patients with AF, venous thromboembolism or splanchnic vein thrombosis.[37] In addition,

using the National Health Insurance Research Database in Taiwan (involving a total of ~290,000 patients with AF, ~5% of whom had cirrhosis), Kuo *et al*. recently reported that cirrhotic patients treated with warfarin had a substantially lower risk of ischemic stroke and a positive net clinical benefit compared with patients taking antiplatelet therapy or those without antithrombotic therapies, and thus, thromboprophylaxis should be considered for such patients.[38] However, future randomized controlled trials are needed to clarify whether guideline-based management of AF in patients with NASH reduces risk of thromboembolic events.

Although the pathophysiological inter-relationships between NAFLD and risk of AF are not well understood, there is now emerging evidence of biological plausibility that NAFLD may promote the development and persistence of AF. It remains debatable whether NAFLD is simply a marker of coexisting cardiometabolic risk factors or is an independent cardiovascular risk factor. In patients with NAFLD there is often ectopic fat accumulation in other organs (such as visceral adipose tissue, myocardium and pericardium) in people who may already be at increased risk for cardiac and arrhythmic complications. For example, in patients with NAFLD, the coexistence of myocardial steatosis and increased pericardial/epicardial fat accumulation might exert local adverse effects that result in functional and structural derangements of the myocardium as well as in pro-arrhythmogenic effects.[2,9] In an important study, Granér *et al*.[39] examined the effect of different ectopic fat depots (measured by both magnetic resonance imaging and magnetic resonance spectroscopy) on left ventricular function in men with NAFLD who did not have diabetes. The authors found that myocardial triglyceride, epicardial and pericardial fat increased with increasing amount of liver fat and visceral adipose tissue. Notably, in multivariable regression analyses, only hepatic triglyceride and visceral adipose tissue were independent predictors of left ventricular diastolic function, whereas myocardial triglyceride, epicardial and pericardial fat volumes were not associated with measures of diastolic function.[39] Therefore, the authors reasonably argued that the association between left ventricular diastolic function and both hepatic triglyceride and visceral adipose tissue might occur because of toxic systemic effects, rather than because of local effects in and around the heart.[39] Although these findings are convincing, a study by Lee *et al.* examined the longitudinal associations between pericardial, intra-thoracic, and visceral fat with incident AF in 2,135 adult participants from the Framingham Heart Study. These authors found that greater pericardial fat and intra-thoracic fat were significantly associated with an increased risk of incident AF (after adjusting for age and sex) over a median follow-up of 9.7 years. However,

all these associations disappeared after additional adjustment for BMI and visceral fat.[40] Thus, to date, it remains unproven whether NAFLD is simply a marker of cardiac and arrhythmic complications or is causally implicated in the pathogenesis of these complications. Increasing evidence suggests that NAFLD (especially in its more severe histologic forms) exacerbates systemic/hepatic insulin resistance and causes the release of a variety of pro-inflammatory, pro-fibrogenic, pro-oxidant and vasoactive mediators that may promote the development and progression of AF, cardiomyopathy (mainly left ventricular diastolic dysfunction, left atrial enlargement and increased cardiac mass) and ischemic heart disease.[2,3,9,41-44] Further supporting a role for pro-inflammatory cytokines as potential triggers of AF, a meta-analysis has shown that increased levels of proinflammatory biomarkers (such as plasma interleukin-6 and C-reactive protein concentrations) are strongly associated with an increased risk of incident AF both in the general population and in patients undergoing coronary artery bypass grafting.[45] Therefore, lowering the chronic inflammatory burden in NAFLD might represent an effective intervention to reduce the risk of AF in patients with NAFLD.[9] However, in the light of all the evidence discussed above, we suggest further studies are needed to better clarify the differential role of NAFLD, abdominal visceral fat and cardiac ectopic fat depots in the development and persistence of AF.[46]

Our meta-analysis has some important limitations (strictly inherent to the nature of the eligible studies) that should be mentioned. First, the observational design of the studies does not allow establishing a causal association between NAFLD and increased risk of AF. Second, although most of the studies included in this meta-analysis have a relatively high quality (based on the NOS) and have adjusted their results for multiple clinical risk factors for AF, the possibility of residual confounding by unmeasured factors (*e.g*., waist circumference, pericardial/epicardial fat depots, left atrial dimensions and other echocardiographic measurements) cannot be ruled out. Third, although we used a random-effects model, the interpretation of some results of this meta-analysis requires some caution, given the medium-high heterogeneity (I^2=54.7%) observed in the pooled primary analysis of cross-sectional studies. However, when we systematically examined the possible sources of statistical heterogeneity using stratified analyses, we found that this medium-high heterogeneity most likely reflected differences in the cohort type of the eligible studies (cohorts with type 2 diabetes only *vs*. population-based/community-based cohorts). Fourth, the studies included in the meta-analysis used standard resting electrocardiograms for diagnosing permanent (chronic) AF, but did not perform 24-hour Holter monitoring. However, it is reasonable

to hypothesize that AF was likely to be under-diagnosed since a significant proportion of patients with NAFLD could have paroxysmal AF that has yet to be diagnosed. Finally, another potential limitation of the meta-analysis is that most of the eligible studies used liver ultrasonography, which is the recommended first-line imaging method for diagnosing NAFLD in clinical practice, whereas only a hospital-based cohort study used liver biopsy, which is the reference method for diagnosing and staging NAFLD.[6,8,47,48] That said, future studies in larger cohorts of well-characterized patients with NAFLD (as diagnosed by magnetic resonance-proton density fat fraction and magnetic resonance elastography, which are rapidly being recognized as being as good as liver biopsies)[48] are needed to clarify whether the severity of NAFLD may differentially affect risk of developing AF.[49]

Despite these limitations, our meta-analysis has also important strengths. We believe that the topic of our meta-analysis is clinically relevant, given the increasing prevalence and incidence of both NAFLD and AF globally and the emerging mechanistic data regarding the underlying mechanisms linking NAFLD to AF. Our systematic review and meta-analysis provides the most comprehensive and updated assessment on the association between NAFLD and risk of AF. Moreover, we have used standardized risk estimates from all eligible studies to allow consistent combination of estimates across studies. Finally, although a selective reporting bias of eligible studies could be not definitely excluded, we also searched for 'grey' literature in Scopus and Web of Science databases and made every effort to rule out very low-quality studies by using stringent inclusion criteria. We believe that our comprehensive search has made it unlikely that any published reports were missed and visual inspection of funnel plots and formal tests demonstrated no statistical evidence of publication bias (although the interpretation of the Egger's regression test should be viewed cautiously because the number of studies included was low).

In conclusion, the findings of this comprehensive and updated meta-analysis of observational studies show that NAFLD is significantly associated with an increased risk of AF in middle-aged and elderly individuals (especially in people with type 2 diabetes). However, since the observational design of the eligible studies does not allow for proving causality, further intervention studies are needed to test whether pharmacological/non-pharmacological treatments targeting NAFLD may reduce risk of AF. Additional larger prospective studies with longer follow-up durations and

mechanistic studies are also needed to better understand the link between NAFLD and long-term risk of AF.

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FIGURE LEGENDS

Figure 1. The PRISMA flow diagram for search and selection processes of the meta-analysis.

Figure 2. Forest plot and pooled estimates of the effect of NAFLD on the risk of prevalent atrial fibrillation in five cross-sectional studies, stratified by cohorts of patients with established type 2 diabetes.

Figure 3. Forest plot and pooled estimates of the effect of NAFLD on the risk of prevalent atrial fibrillation in four longitudinal studies, stratified by cohorts of patients with established type 2 diabetes.

Supplementary Figure 1. Univariable linear meta-regression analyses. A meta-analysis of the association of age (A), sex (B) and body mass index (C) with the risk of prevalent AF. Only cross-sectional studies were included in these analyses.

Supplementary Figure 2. Funnel plot of standard error by log-odds ratio for the risk of AF (for cross-sectional [A] and longitudinal [B] studies, separately).

Table 1. Principal observational (cross-sectional and longitudinal) studies examining the association between NAFLD and risk of both prevalent and incident atrial fibrillation (ordered by publication year and study design).

Authors, Year (Ref.)	Study Design, Sample Size, and Population Characteristics	Diagnosis and Prevalence of NAFLD	Diagnosis and Prevalence of Atrial Fibrillation	Covariate Adjustment(s)	Main Findings
Cross-sectional studies **(n=5)**					
Targher G *et al.* 2013 (11)	Cross-sectional hospital-based cohort: 702 Italian patients with type 2 diabetes discharged from hospital without known causes of chronic liver diseases. 379 men and 323 women; mean age 66±13 years; mean BMI 30.7±7 kg/m^2; 100% diabetics	Ultrasonography; 73.2% (n=514) had NAFLD	Medical history and standard ECG; 85 patients (12.1%) had permanent AF	Age, sex, systolic blood pressure, hemoglobin A1c, eGFR, total cholesterol, serum GGT, electrocardiographic left ventricular hypertrophy, chronic obstructive pulmonary disease, prior history of heart failure, valvular heart disease, hyperthyroidism and use of antihypertensive drugs, insulin and other medications	NAFLD was independently associated with increased risk of prevalent AF (adjusted OR 5.17, 95%CI 2.05-13.0)
Markus MR *et al.* 2016 (12)	Cross-sectional population-based study (Study of Health in Pomerania): 3,090 German middle-aged individuals without known causes of chronic liver diseases. 1,498 men and 1,592 women; median age 52 years; median BMI 26.9 kg/m^2; 9.7% diabetics	Ultrasonography; 30.3% (n=937) had NAFLD	Medical history and standard ECG; 46 individuals (1.5%) had permanent AF	Age, sex, weight, height, alcohol intake, smoking, systolic blood pressure, hemoglobin A1c, total cholesterol/HDL-C ratio, eGFR, chronic bronchitis, hyperthyroidism, prior history of myocardial infarction, valvular heart disease, left atrial diameter, left ventricular mass and ejection fraction, use of antihypertensive, hypoglycemic and lipid-lowering drugs	NAFLD was not independently associated with increased risk of prevalent AF (adjusted OR 1.20, 95%CI 0.43-3.37)
Long MT *et al.* 2017 (13)	Cross-sectional analysis of population-based study (Framingham Heart Study): 2,122 United States middle-aged individuals without known causes of chronic liver diseases. 1,002 men and 1,120 women; mean age 59±9.6 years; mean BMI 29±5 kg/m^2; 7% diabetics	Multi-detector computed tomography; 20% (n=424) had NAFLD	Medical history and standard ECG; 62 individuals (2.9%) had permanent AF	Age, sex, BMI, systolic and diastolic blood pressure, smoking, use of antihypertensive medication, diabetes, and prior history of heart failure or myocardial infarction	NAFLD was not independently associated with increased risk of prevalent AF (adjusted OR 1.12, 95%CI 0.58-2.18)
Zhang Y *et al.* 2018 (14)	Cross-sectional study: 1,688 Chinese elderly individuals without known causes of	Ultrasonography; 30.9% (n=522) had NAFLD	Medical history and standard ECG; 39 individuals (2.3%) had permanent AF	Age, sex, systolic blood pressure, fasting glucose, plasma lipid profile, serum	NAFLD was independently associated with increased risk of prevalent AF (adjusted OR

	chronic liver diseases. 930 men and 758 women; median age 72 years; mean BMI 23.8±3 kg/m^2			GGT and albumin levels	2.76, 95%CI 1.32-5.77)
Whitsett M *et al.* 2018 (15)	Cross-sectional hospital-based cohort: 9,108 United States hospitalized patients with NAFLD/NASH and 111,812 patients <65 years old without NAFLD/NASH. All these patients were retrospectively identified from a tertiary care center Electronic Database from 2002-2015. Among those with ICD-defined NAFLD, 215 patients had biopsy-proven NASH	ICD-9 code; 9,108 had NAFLD/NASH. Among these patients, 215 had biopsy-proven NASH (aged <65 years)	ICD-9 code; 3,571 patients had permanent AF. Among those with biopsy-proven NASH, 10 patients had AF	None	NAFLD was significantly associated with increased risk of prevalent AF (unadjusted OR 2.13, 95%CI 1.93-2.34). When the statistical analysis was restricted to patients with biopsy-proven NASH, NASH was not associated with risk of prevalent AF (unadjusted OR 1.74, 95%CI 0.92-3.28)
***Longitudinal studies* (n=4)**					
Targher G *et al.* 2013 (16)	Longitudinal cohort of individuals with type 2 diabetes: 400 Italian type 2 diabetic patients without previous history of AF, heart valve disease and known causes of chronic liver diseases, randomly selected among those attending a diabetes outpatient service. 235 men and 165 women; mean age 64±10 years; mean BMI 29±4 kg/m^2; 100% diabetics	Ultrasonography; 70.2% (n=522) had NAFLD	Standard ECG (annually); 42 individuals developed incident AF over a follow-up of 10 years	Age, sex, BMI, systolic blood pressure, hypertension treatment, history of heart failure, electrocardiographic PR interval and left ventricular hypertrophy	NAFLD was independently associated with increased risk of incident AF (adjusted HR 4.96, 95%CI 1.40-17.0)
Karajamaki AJ *et al.* 2015 (17)	Longitudinal cohort of middle-aged hypertensive subjects and age- and sex-adjusted normotensive subjects randomly selected from National Insurance registries (OPERA study): 958 Finnish individuals without previous history of AF and known causes of chronic liver diseases. 450 men and 508 women; mean age 51±6 years; mean BMI 28±5 kg/m^2; 10.1% diabetics	Ultrasonography; 26% (n=249) had NAFLD	AF diagnosis was made by ICD-10 code in the National Death Registry and/or hospital discharge registry; 94 individuals developed incident AF over a mean follow-up of 16.3 years	Age, sex, BMI, waist circumference, alcohol intake, smoking, systolic blood pressure, insulin-sensitivity check index, hypertension, diabetes, coronary heart disease, left ventricular mass index, left atrial diameter, serum ALT, atrial natriuretic peptide and C-reactive protein levels	NAFLD was independently associated with increased risk of incident AF (adjusted HR 1.88, 95%CI 1.03-3.45)

You SC *et al.* 2016 (18)	Longitudinal population-based study (Korean National Health Insurance Service): 232,979 South Korean adult individuals without previous history of AF, structural heart disease, and excess alcohol drinking. 84,571 men and 148,408 women; mean age 49±7 years	Fatty liver index (FLI); 15% (n=35,082) had NAFLD	Standard ECG; 2,261 individuals developed incident AF over a mean follow-up of 3.7 years	Age, sex, systolic blood pressure, serum creatinine, obesity, impaired fasting glucose, dyslipidemia and history of heart failure	NAFLD was independently associated with increased risk of incident AF (adjusted HR 1.13, 95%CI 1.03-1.24)
Long MT *et al.* 2017 (13)	Longitudinal analysis of population-based study (Framingham Heart Study): 2,060 United States middle-aged individuals without previous history of AF and known causes of chronic liver diseases. 53% women. Mean age 59±9.6 years; mean BMI 29±5 kg/m^2; 7% diabetics	Multi-detector computed tomography; 20% (n=424) had NAFLD	Standard ECG (at each visit); 153 individuals developed incident AF over a mean follow-up of 9.3 years	Age, sex, BMI, systolic and diastolic blood pressure, smoking status, use of antihypertensive medications, diabetes, and prior history of heart failure or myocardial infarction	NAFLD was not independently associated with increased risk of incident AF (adjusted HR 0.96, 95%CI 0.64-1.45)

Abbreviations: AF, atrial fibrillation; ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyltransferase; HR, hazard ratio; ICD, International Classification of Diseases; NASH, nonalcoholic steatohepatitis; OR, odds ratio.

Table 2. Sensitivity and subgroup analyses – Association between NAFLD and risk of AF in the eligible cross-sectional and longitudinal studies, stratified by diagnostic methods for NAFLD and study country.

	Cross-sectional studies (*n*=5)	**Longitudinal studies (*n*=4)**
Diagnostic methods for NAFLD		
Imaging techniques	Random-effects OR 2.07 (95% CI 1.03-4.16), I^2= 65.4% Number of studies: 4 *n*= 7,602	Random-effects HR 1.74 (95% CI 0.80-3.79), I^2= 74.9% Number of studies: 3 *n*= 3,418
International Classification Diseases (ICD-9) code	Random-effects OR 2.13 (95% CI 1.93-2.34) Number of studies: 1 *n*= 120,920	Not applicable
Fatty liver index (FLI)	Not applicable	Random-effects HR 1.13 (95% CI 1.03-1.24), Number of studies: 1 *n*= 232,979
Study country		
Europe	Random-effects OR 2.54 (95% CI 0.61-10.6), I^2= 76.7% Number of studies: 2 *n*= 3,792	Random-effects HR 2.60 (95% CI 1.06-6.38), I^2= 46.8% Number of studies: 2 *n*= 1,358
United States	Random-effects OR 1.69 (95% CI 0.92-3.09), I^2= 71.8% Number of studies: 2 *n*= 123,042	Random-effects HR 0.96 (95% CI 0.64-1.45), Number of studies: 1 *n*= 2,060
Asia	Random-effects OR 2.76 (95% CI 1.32-5.77) Number of studies: 1 *n*= 1,688	Random-effects HR 1.13 (95% CI 1.03-1.24), Number of studies: 1 *n*= 232,979