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FULL RESEARCH PAPER

# Risk of cardiovascular events in patients with non-alcoholic fatty liver disease: a systematic review and meta-analysis

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**Aims** Non-alcoholic fatty liver disease (NAFLD) is a highly prevalent disease and has been repeatedly associated with an increased risk of cardiovascular disease. However, the extent of such association is unclear. We conducted a systematic review and meta-analysis of the literature to evaluate the risk of myocardial infarction (MI), ischaemic stroke (IS), atrial fibrillation (AF), and heart failure (HF) in NAFLD patients.

**Methods and results** According to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, we systematically searched PubMed and EMBASE, from inception to 6 March 2021, and included all studies reporting the incidence of MI, IS, AF, and HF in patients with and without NAFLD. Random-effect fmodels were used to estimate pooled odds ratio (OR), 95% confidence intervals (CI), and 95% prediction intervals (PI); subgroup analyses, meta-regressions, and sensitivity analyses were additionally performed. Among 3254 records retrieved from literature, 20 studies were included. Non-alcoholic fatty liver disease was associated with an increased risk of MI (OR: 1.66, 95% CI: 1.39–1.99, 95% PI: 0.84–3.30), IS (OR: 1.41, 95% CI: 1.29–1.55, 95% PI 1.03–1.93), AF (OR: 1.27, 95% CI: 1.18–1.37, 95% PI: 1.07–1.52), and HF (OR: 1.62, 95% CI: 1.43–1.84, 95% CI: 1.04–2.51). We identified significant subgroup differences according to geographical location, study design, NAFLD definition, and risk of bias; meta-regressions identified mean age, male sex, and study-level characteristics as potential moderators of the risk of MI and IS.

**Conclusions** Non-alcoholic fatty liver disease was associated with increased risk of MI, IS, AF, and HF. Age, sex, and study characteristics may moderate the strength of this association. Further studies are required to evaluate specific cardiovascular prevention strategies in patients with NAFLD.

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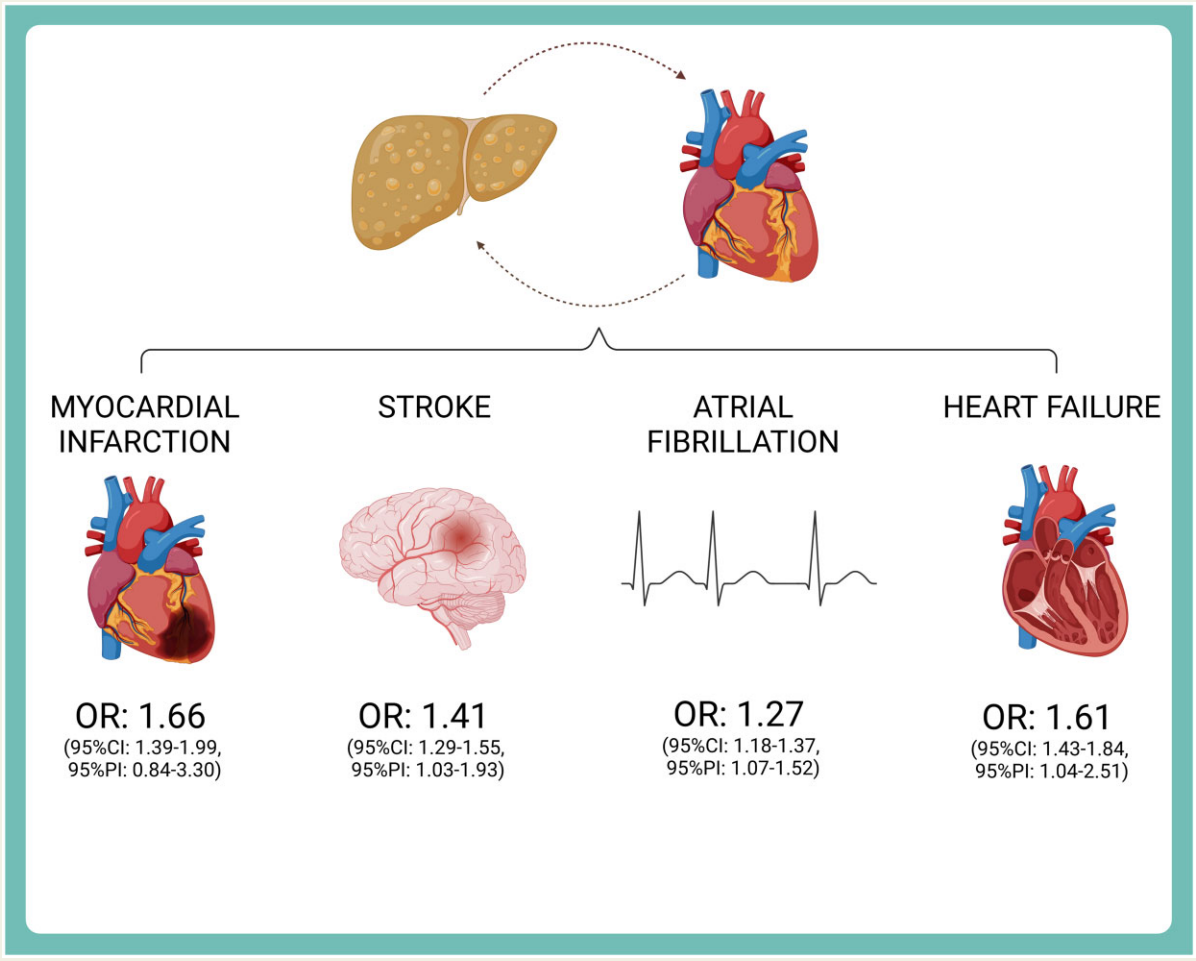


**Graphical Abstract:** Risk of Cardiovascular Events in patients with Non-Alcoholic Fatty Liver Disease (Created with Biorender.com). OR, odds ratio; 95% CI, 95% confidence intervals; 95% PI, 95% prediction intervals.

**Keywords** NAFLD • Myocardial infarction • Ischaemic stroke • Atrial fibrillation • Heart failure

## Introduction

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver condition, with an estimated prevalence that rose up to 25% of the adult population in the last decades.[1,2] Non-alcoholic fatty liver disease represents a spectrum of diseases, which includes non-alcoholic fatty liver (NAFL, characterized by steatosis, without inflammation or hepatocellular damage) and non-alcoholic steatohepatitis (NASH), characterized by hepatic steatosis, inflammation, and hepatocellular injury, with or without fibrosis.[3] Patients with NAFLD are often asymptomatic and can eventually progress to cirrhosis.[3] The contribution of NAFLD in the epidemiology of cirrhosis is expected to increase in the future.[4]

Beyond its liver-specific natural history, cardiovascular diseases (CVDs) have also been consistently associated with NAFLD. Cardiovascular diseases are among the main determinants of death and poor outcomes in NAFLD patients, being the second underlying cause of mortality in these patients after liver cirrhosis, and the largest contributory cause of death.[5] While these data underline the central role of CVDs in the prognosis and natural history of NAFLD patients, there is still great uncertainty and debate on the underlying mechanisms that link NAFLD and CVDs, and the strength of this relationship. From an epidemiological point of view, NAFLD and CVDs share several risk factors, including lifestyle habits and metabolic dysfunction[6]; consistently, previous studies suggested an association between NAFLD and the risk of several CVDs,[7] and particularly with myocardial infarction (MI), ischaemic stroke (IS), atrial fibrillation (AF), and heart failure (HF).[8] The pathophysiology of this relationship is only partially characterized, but it is likely complex and resulting from the interplay of different, bidirectional pathways, including endothelial dysfunction, vascular inflammation, and impaired glucose and lipid metabolism.[6] More recently, the role of gut microbiome has received growing attention, according to its detrimental role in the development of cardiometabolic disease[9]; several studies have already depicted the contribution of dysbiosis in the progression and development of NAFLD and several CVDs.[9,10] Further research on this

topic is ongoing, and will eventually explain the exact underlying mechanisms of this association.

Beyond that, clarification of the impact of NAFLD on the development of CVD is pivotal to design specific cardiovascular preventive and therapeutic strategies and to reduce the burden of CVDs on the prognosis of NAFLD patients. Although several systematic reviews and meta-analyses have already been performed to summarize findings from observational studies, most of them did not focus on specific CVDs[11] or did not include some of the most recent, large studies that have been published in recent years, and that provide new and valuable data on the causal effect of NAFLD on CVDs.[12,13]

Our study aimed to provide a comprehensive systematic review and meta-analysis on the risk of MI, IS, AF, and HF in patients with NAFLD.

# Methods

This systematic review has been conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and recommendations.[14] A protocol for this study was registered into the international prospective register of systematic reviews (PROSPERO), N. CRD42021241233.

Details on the search strategy, definition used, as well as studies selection, data extraction, and quality assessment processes and statistical analyses plan, are reported in Supplementary material online.

## Inclusion and exclusion criteria

Main inclusion criteria were: (i) all studies reporting the number of patients, with and without NAFLD, who developed MI, IS, AF, or HF and (ii) all studies with a minimum follow-up of 1 year. According to our aim, and to ensure that our estimates focus on the general population, we excluded those studies which enrolled only highly selected group of patients (i.e. cohorts composed only of patients with previous MI or previous stroke). Finally, we excluded cross-sectional studies, articles not in English, conference abstracts, comments, editorials, case reports, and systematic reviews, and studies that did not report the number of events according to NAFLD status. In the case of two or more studies based on the same cohort of patients, we selected the study with the highest number of patients included, or the most recently published one.

# Results

A total of 3254 studies were retrieved from the literature search (709 from PubMed and 2545 from EMBASE). After duplicates removal, and sequential screening of title and abstract, we evaluated 94 full texts, and eventually included 20 studies[15–34] (Supplementary material online, *Figure S1*). A summary of the main characteristics of the included studies is reported in *Table 1*. Briefly, 3 were case-control studies[17,22,26]; among the 17 cohort studies, 10 had a retrospective design[15,16,18,19,23,25,28,29,31,34]; and 7 were prospective.[20,21,24,27,30,32,33] Overall, five studies were based on administrative databases.[17,19,22,26,34] Nine studies were held in Asia,[15,18,19,21,24,27,28,32,34] six in Europe,[16,17,20,23,26,30] four in North America,[22,25,29,31] and one in Egypt.[33] Definition of NAFLD was different across studies; 10 (50%) of the studies used ultrasound (US) to diagnose NAFLD, 4 used computerized tomography (CT) scan assessment of liver steatosis, 3 diagnosed NAFLD according to international classification of disease (ICD) codes, and 3 defined NAFLD according to fatty liver index (FLI).

The mean age of the included studies ranged from 46.7 to 65 years old, with 14 (70%) studies reporting a mean age comprised between 50 and 60 years old. Males represented 39–94% of the patients enrolled in the original cohorts, with 14 studies (70%) that included at least 40% of female patients. Hypertension was among the most common comorbidities recorded; two studies enrolled only patients with type 2 diabetes mellitus,[30,32] while three studies enrolled patients with suspected coronary artery disease[25,32] or referred for its evaluation.[21] Follow-up duration ranged from 2 years to over 17 years, with most studies reporting more than 4 years of observation.

Thirteen studies reported data on MI, 12 on IS, 7 on AF, and 4 on HF. Overall, nine studies were considered at high risk of bias[15,18,19,21,24,25,30,32,34]; selection bias and comparability between NAFLD and non-NAFLD patients were among the most frequent concerns reported. Details on the bias assessment of the included studies are reported in Supplementary material online, *Table S4*.

Across the studies included, Alexander *et al.*[26] pooled data of four different cohorts from Italy, the Netherlands, Spain, and UK; for the purpose of our analyses and consistently with the original study's analysis design, we considered these cohorts separately.

## Risk of myocardial infarction, stroke, atrial fibrillation, and heart failure in patients with non-alcoholic fatty liver disease

Compared with patients without NAFLD, subjects with NAFLD showed significant increased risk of MI [odds ratio (OR): 1.66, 95% confidence intervals (CI) 1.39–1.99, 95% prediction intervals (PI) 0.84–3.30, $I^2$ = 98%], IS (OR: 1.41, 95% CI: 1.29–1.55, 95% PI: 1.03–1.93, $I^2$ = 93%), AF (OR: 1.27, 95% CI: 1.18–1.37, 95% CI: 1.07–1.52, $I^2$ = 65%), and HF (OR: 1.62, 95% CI: 1.43–1.84, 95% PI: 1.04–2.51, $I^2$ = 27%), with moderate to high heterogeneity found for all outcomes (*Figure 1A–D*, respectively); 95% PI were significant for IS, AF, and HF, but not for the risk of MI.

### Subgroup analysis

Subgroup analyses for each of the outcomes investigated are reported in *Figure 2*. Most of the subgroup analyses were consistent with the main estimates, particularly in terms of significance of the pooled estimates.

Among studies reporting data on MI, a significant interaction was found for geographical location, study design, and NAFLD definition ($P = 0.03$, $P < 0.01$, and $P < 0.01$, respectively). Specifically, European-based cohorts, case-control studies, and NAFLD cohorts defined by ICD codes showed lower figures for the risk of MI in NAFLD patients (*Figure 2A*). No heterogeneity was found among the subgroup of case-control and ICD codes-based studies.

Significant interaction was found across all the subgroups evaluated for the risk of IS ($P < 0.01$ for all), with a trend similar to what observed for MI; moreover, studies with low risk of bias showed lower estimates than those with a high risk of bias. Heterogeneity was found reduced in most of the subgroup investigated, compared with the primary analysis.

**Table 1** Main characteristics of the studies included in the systematic review

| Study | Year | Geog. location | Study type | Incl./excl. criteria | NAFLD definition | *N* | NAFLD | Age (mean) | *M* (%) | HTN (%) | DM (%) | FU (YRS) | Outcome reported |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alexander *et al.*[26] | 2019 | Europe | Case-Control | Pts. without history of MI or Stroke | ICD codes | 9 768 439[a] | 120 795[a] | 54.2[a] | 50[a] | 29[a] | 9[a] | 3.8[a] | MI, stroke |
| Allen *et al.*[22] | 2019 | North America | Case-Control | Unselected pts. with NAFLD | ICD codes | 19 078 | 3869 | 53[b] | 48 | 28 | 13 | 7 | AF, HF, MI, stroke |
| Baratta *et al.*[20] | 2020 | Europe | Cohort Study | Pts. with at least one comorbidity | US | 898 | 643 | 56.5 | 62 | 70 | 25 | 3.5 | AF, MI, stroke |
| El Azeem *et al.*[33] | 2013 | Other | Cohort Study | Pts. without history of CVD | US | 747 | 268 | 51.5 | 49 | 32 | 58 | 3 | MI, stroke |
| Hamaguchi *et al.*[24] | 2007 | Asia | Cohort Study | Pts. without history of MI or stroke | US | 1221 | 231 | 48 | NA | NA | NA | 5.8 | MI, stroke |
| Ichikawa *et al.*[32] | 2021 | Asia | Cohort Study | Pts. with DM and suspected CAD, without history of CVD | CT | 529 | 143 | 65 | 61 | 71 | 100 | 4.4 | HF, MI, stroke |
| Käräjämäki *et al.*[23] | 2015 | Europe | Cohort Study | Pts. 40–59 years with or without HTN | US | 958 | 249 | 51.3 | 47 | 51 | 10 | 16.3 | AF |
| Labenz *et al.*[17] | 2020 | Europe | Case-Control | Pts. without history of AF, MI, stroke | ICD Codes | 44 096 | 22 048 | 55.6 | 50 | 25 | 6 | 10 | AF, MI, stroke |
| Lee *et al.*[34] | 2020 | Asia | Cohort Study | Pts. 40–64 years without history of HF, MI, stroke | FLI | 8 962 813 | 2 461 072 | 50[b] | 48 | 23 | 9 | 10.1 | HF, MI, stroke |
| Lee *et al.*[19] | 2021 | Asia | Cohort Study | Pts. >20 years, without history of AF | FLI | 8 048 055 | 2 738 621 | 46.7 | 52 | 24 | 8 | 8.3 | AF |
| Long *et al.*[29] | 2017 | North America | Cohort Study | Pts. without history of AF | CT | 2060 | 406 | 59 | 47 | 26 | 7 | 9.3 | AF |
| Meyersohn *et al.*[25] | 2020 | North America | Cohort Study | Pts with suspected CAD, without previous MI | CT | 3756 | 959 | 60.6 | 48 | 64 | 20 | 2.1 | MI |
| Moon *et al.*[15] | 2017 | Asia | Cohort Study | Pts. screened for cancer | US | 815 | 394 | 51.8 | 94 | 21 | 9 | 4.2 | Stroke |
| Pisto *et al.*[16] | 2014 | Europe | Cohort Study | Pts. 40–59 years with or without HTN | US | 988 | 268 | 51.1 | 49 | 49 | 9 | 17.7 | MI, stroke |
| Sinn *et al.*[28] | 2020 | Asia | Cohort Study | Pts. without history of MI or CVD | US | 111 492 | 37 263 | 52 | 51 | 26 | 9 | 6.5 | MI |
| Targher *et al.*[30] | 2013 | Europe | Cohort Study | Pts. with DM, without previous AF | US | 400 | 281 | 63.3 | 59 | 71 | 100 | 10 | AF |
| VanWagner *et al.*[31] | 2021 | North America | Cohort Study | Unselected pts. that underwent CT | CT | 1827 | 159 | 50 | 39 | 31 | 11 | 5 | HF |
| Wong *et al.*[21] | 2016 | Asia | Cohort Study | Pts. referred for coronary CT angiogram | US | 612 | 356 | 63 | 71 | 66 | 31 | 6 | MI |
| Xu *et al.*[27] | 2021 | Asia | Cohort Study | Pts. without history of MI or stroke | US | 79 905 | 24 874 | 51.4[b] | 74 | 1 | 1 | 10.3 | MI, stroke |
| Yang *et al.*[18] | 2020 | Asia | Cohort Study | Pts. 40–69 years, without stroke | FLI | 7964 | 3414 | 52.5 | 42 | 39 | 9 | 12 | stroke |

AF, atrial fibrillation; CAD, coronary artery disease; CT, computerized tomography; CVD, cardiovascular disease; DM, type 2 diabetes mellitus; FLI, fatty liver index; FU, follow-up; HF, heart failure; HTN, hypertension; ICD, international classification of disease; M, males; MI, myocardial infarction; NA, not available; NAFLD, non-alcoholic fatty liver disease; US, ultrasound.

[a]The figures are for the whole pooled cohort of the study, although each cohort was analysed individually in the meta-analysis.

[b]Median values.

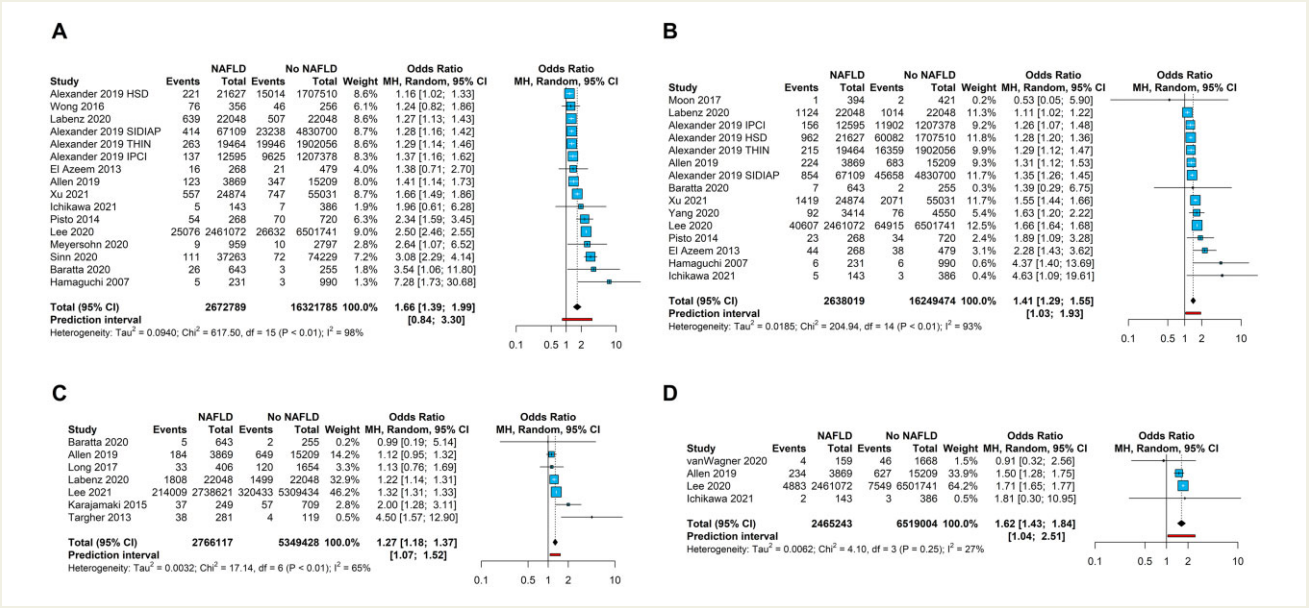


**Figure 1** Risk of myocardial infarction, ischaemic stroke, atrial fibrillation, and heart failure in patients with vs. without non-alcoholic fatty liver disease. *(A)* Myocardial infarction; *(B)* ischaemic stroke; *(C)* atrial fibrillation; and *(D)* heart failure. CI, confidence interval; MH, Mantel-Haenszel; NAFLD, non-alcoholic fatty liver disease; PI, prediction interval.

For AF, the only significant subgroup difference was found according to the NAFLD definition ($P = 0.01$): higher risk of AF was found among studies that used US, although this analysis was limited by the low number of cohorts included in each subgroup.

No significant subgroup difference was found for the risk of HF.

Subgroup analyses for each outcome are reported in detail in Supplementary material online, *Figures S2–S5*.

### Meta-regression analysis

Results of the univariable meta-regression analyses for each outcome are reported in Supplementary material online, *Tables S5–S7*.

At univariable analysis, study design and NAFLD definition were significantly associated with the risk of MI in patients with NAFLD. A multivariable model comprising study-level mean age, the proportion of males enrolled, and type of study explained the between-study variability found in the primary analysis ($R^2 = 100\%$), with proportion of male patients inversely associated with the risk of outcome, which was higher in cohort studies.

For the risk of IS, mean age, type of study, type of diagnosis, risk of bias, and geographical location were all associated with the outcome, with mean age being able to explain almost all of the between-study variability ($R^2 = 99.9\%$). Multivariable analysis was therefore not performed for this outcome.

None of the study-level characteristics was associated with the risk of AF; finally, we were not able to perform meta-regression for the risk of HF, according to the number of studies available for the analysis ($n = 4$).

### Sensitivity analysis

The first sensitivity analysis according to the 'leave-one-out' approach showed overall stability of both pooled estimates and heterogeneity for all outcomes, with little influence of individual studies (Supplementary material online, *Figure S6*).

We therefore excluded studies that defined NAFLD according to CT scan, ICD codes, or FLI, or those studies ($n = 4$) that enrolled only diabetic patients,[30,32] or subjects referred for suspected CAD.[21,25,32] All the analyses showed consistency with main estimates (Supplementary material online, *Figure S7A–D*); the exclusion of studies that used ICD codes lead to slightly higher pooled ORs for MI and IS (Supplementary material online, *Figure S7A and B*, respectively).

In the last sensitivity analysis, we replaced event counts with adjusted HRs or ORs for those studies that reported adjusted effect sizes. Overall, six studies reported adjusted HRs,[17,21,26–29] and two studies reported adjusted OR.[11,23] No studies reported adjusted estimates for HF. Compared with the primary analysis, the use of adjusted effect size led to lower figures for the risk of both MI and IS. Significant subgroup differences were found for both outcomes, between studies analysed according to adjusted effect sizes vs. those analysed according to event counts ($P < 0.01$ for both, Supplementary material online, *Figure S8A and B*, respectively). Similar estimates compared with primary were found for AF (Supplementary material online, Figure *S8C*).

### Publication bias

Results of the publication bias analyses are reported in Supplementary material online, *Figure S9*. Visual inspection of the funnel plot for MI revealed potential asymmetry in the right side of the forest plot for the studies with low standard error, and in the left bottom side of the plot for the studies with higher standard error.

The result of the analysis according to the 'trim-and-fill' approach is reported in Supplementary material online, *Figure S10*. The imputation of five additional studies to reduce asymmetry of the funnel plot

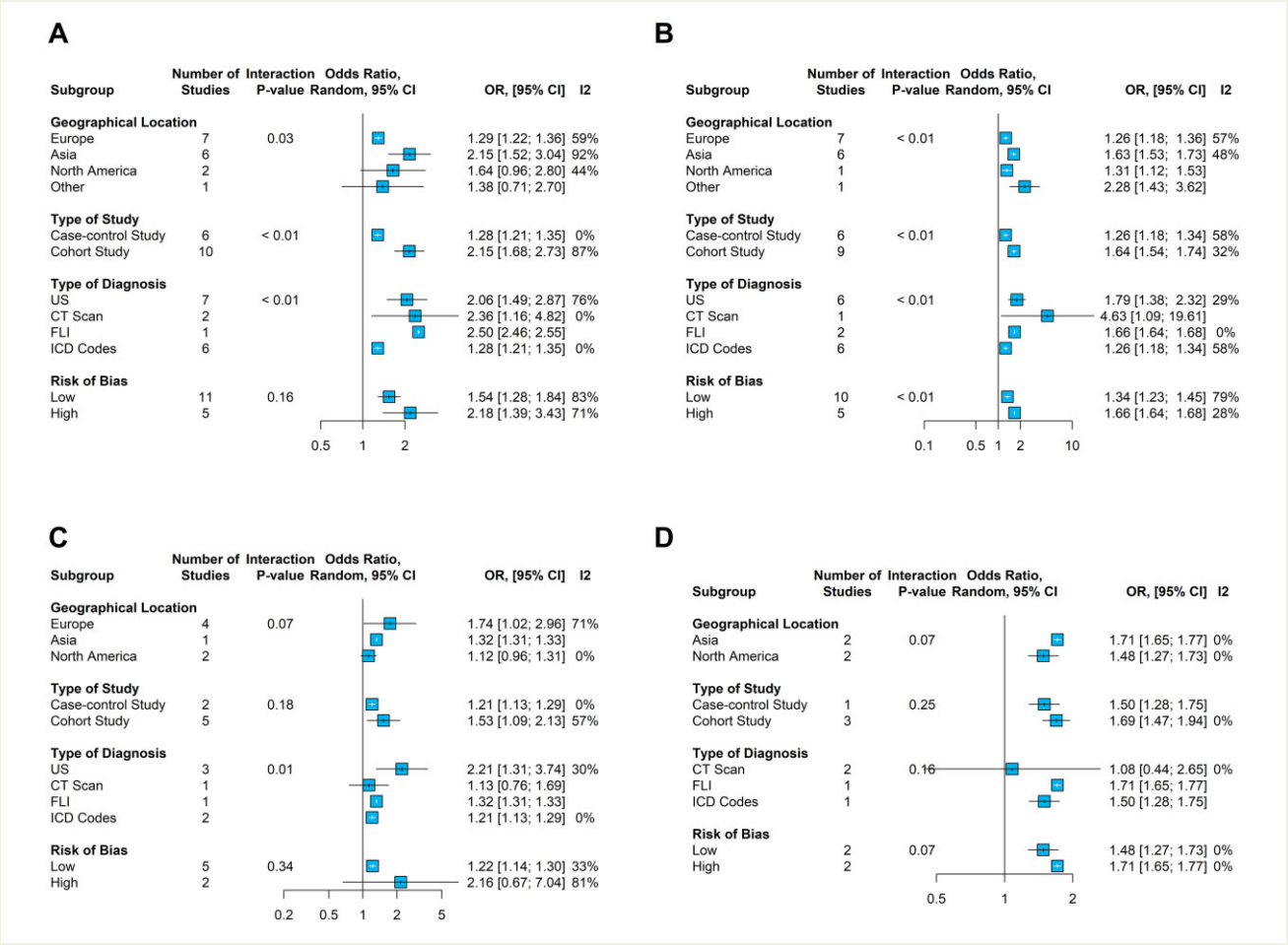


**Figure 2** Subgroup analysis for the risk of myocardial infarction, ischaemic stroke, atrial fibrillation, and heart failure in patients with vs. without non-alcoholic fatty liver disease. (*A*) Myocardial infarction; (*B*) ischaemic stroke; (*C*) atrial fibrillation; and (*D*) heart failure. CI, confidence interval; CT, computerized tomography; ICD, international classification of diseases; FLI, fatty liver index; $I^2$, inconsistency index; OR, odds ratio; US, ultrasound.

led to higher pooled estimates for the risk of MI, compared with the primary analysis (OR: 2.30, 95% CI: 1.78–2.97). Overall, these findings suggest that publication bias is unlikely to contribute to the significance of our results.

No significant publication bias was found for IS, AF, and HF.

## Discussion

In this systematic review and meta-analysis, we found that patients with NAFLD are at a higher risk of MI, IS, AF, and HF compared with patients without NAFLD. While moderate to high heterogeneity was found for all analyses, our results were supported by 95% PIs, which showed significance for all outcomes except MI and were further reinforced by the sensitivity analyses, which showed overall consistency of the significant associations, regardless of potentially biased definition of NAFLD, or the use of adjusted effect sizes. The subgroup analyses identified several study-level characteristics that may influence the extent of the associations observed. Finally, meta-regressions revealed that mean age and proportion of male sex might be relevant moderators of the association between NAFLD and MI, while the type of study influenced both risks of MI and IS in patients with NAFLD.

The association between NAFLD and CVDs represented one of the most vibrant and evolving topics in the last decades. In our study, we found that NAFLD is associated with several types of cardiovascular events, suggesting that the effects of NAFLD on the cardiovascular system are multifaceted. Moreover, the significant association between NAFLD and AF represents a new finding, not found in a previous meta-analysis on the topic[13]; to our knowledge, our study is also the first to provide a meta-analysis on the risk of HF. Notably, we found comparable estimates for the risk of all outcomes investigated, although the 95% PIs confirmed the association for IS, AF, and HF, but not for MI. This suggests that while NAFLD may represent a common determinant of the risk of several CVDs (perhaps through different pathophysiological pathways), differences in the extent of the association between different clinical scenarios may exist, and further research are needed to investigate the strength of the association between NAFLD and specific CVDs.

Overall, several hypotheses may explain the increased risk of CVD in NAFLD patients, although research on this topic is still ongoing.

From a pathophysiological point of view, the effects of NAFLD on the incidence of MI and cerebrovascular accident have been more extensively investigated.[35] In fact, NAFLD is part of a complex spectrum of metabolic dysfunctions and can promote a pro-atherogenic lipid profile,[36,37] endothelial dysfunction,[38] and oxidative stress.[38] Interestingly, severity and stage of NAFLD seem to influence the extent of these processes.[37,39] Patients with NAFLD often show systemic inflammation[40] and are also frequently overweight or obese. All these factors can lead to a higher risk of CVDs, and specifically MI and stroke. Recently, simultaneous assessment of hepatic steatosis during coronary CT has showed improvement in the risk stratification of major adverse cardiovascular events in stable CAD patients, further underlining the tight relationship between NAFLD and ischaemic heart disease.[41]

On the other side, the mechanisms underlying the interplay between NAFLD, HF, and AF are less characterized. Non-alcoholic fatty liver disease has been associated itself with cardiac remodelling, including changes in left ventricular structure and increased left atrial size, which may promote the onset of HF and AF.[30,42–45] Moreover, oxidative stress, inflammation, and insulin resistance promoted by NAFLD may contribute to the development of HF, and particularly to HF with preserved ejection fraction.[46] Finally, NAFLD may increase the risk of AF through the epicardial fat,[47,48] which has been associated with incident AF.[49]

Beyond speculations, a better understanding of the pathophysiology underlying these relationships is urgently needed to design specific therapeutic and preventive strategies, which are still undefined[8]; currently, loss of weight and treatment of established concurrent risk factors, including diabetes, dyslipidaemia, and hypertension represent potential approaches to tackle CVDs risk.[8]

We also found that several study-related characteristics, including geographical locations, NAFLD definition, and study design may influence cardiovascular risk in NAFLD patients. Geographical differences were observed for the risk of MI and IS, with lower figures found in European-based studies for both outcomes. Similarly, lower risk of MI and IS was also observed among case-control studies, and consistently in those cohorts in which NAFLD was defined according to ICD codes, this being significant also for AF.

Identification of NAFLD is pivotal to analyse the effect of the disease on the onset of CVD, and our results suggest that the criteria used to define NAFLD may influence the strength of the association with cardiovascular outcomes. Currently, the diagnosis of NAFLD is often made through imaging tests, although biopsy is required to differentiate reliably between NASH and NAFL[1,50]; moreover, surrogate marker, such as FLI, may be helpful to identify NAFLD in administrative databases. Different strengths of the association may reflect the unequal sensitivity between methods for the diagnosis of NAFLD. Similarly, case-control studies, in which NAFLD patients are matched with controls based on comorbidities and risk factors, may have provided a more reliable estimate of the true extent of the association between NAFLD and CVDs.

Meta-regressions confirmed the importance of study-level characteristics, particularly for MI and IS. Moreover, a multivariable model comprising mean age, the proportion of male sex, and type of study was able to explain all the between-study variability for the risk of MI; on the other side, mean age was inversely associated with the OR for IS at the univariable level. These findings may suggest that other variables may be important in modulating the risk in NAFLD patients and that the effects of NAFLD on the incidence of cardiovascular events may be magnified in younger cohorts. Further studies are required to evaluate the effects of NAFLD on CVDs in different subgroup of patients, stratified according to age, sex, and overall cardiovascular risk.

Previous meta-analyses have summarized the findings of observational studies on the relationship between NAFLD and CVD. However, these meta-analyses did not provide specifications on the type of CVDs,[11] or were based on a limited number of studies and did not include many of the most recent, larger observational cohorts that were published thereafter. For example, Hu *et al.*[12] included only five studies for the analysis on the risk of IS; similarly, Mantovani *et al.*[13] analysed four studies for the risk of incident AF in patients with vs. without NAFLD, and did not found significant association; however, four newer studies were published thereafter,[17,19,20,22] including two based on large administrative cohorts, leading to significant results in our analysis.

Beyond the inclusion of newer cohorts, our study has several additional strengths. First, we performed a comprehensive analysis on the risk of four different CVD, thus providing an extensive outlook on the effect of NAFLD on cardiovascular system. Second, we performed exhaustive study of the heterogeneity, which help to identify potential moderators of the relationship investigated. We also provided 95% PIs, which are a more meaningful measure of uncertainty of the estimates reported, and performed several sensitivity analyses, which support the robustness of our results, even after the exclusion of studies that used different criteria for the diagnosis of NAFLD.

## Limitations

Our study has some limitations that should be noted. First, we included studies with different definitions of NAFLD to ensure comprehensiveness of our analysis. This may have introduced bias in the interpretation of the NAFLD-CVDs interplay, particularly due to the potential risk of incorrect classification of NAFLD (that was not histology-confirmed), and especially for those studies based on ICD codes or indirect assessment; this may have led to an incorrect estimate of the risk of CVDs in NAFLD patients. Although these limitations impose the need for a cautious interpretation of our findings, it should be noted that both subgroup and sensitivity analyses confirmed that, although diagnostic criteria may have influenced the extent of the association, they are unlikely to have contributed to the significance of the overall results. On the other side, the outcomes investigated were defined as per the original studies included; although this may have introduced heterogeneity in the assessment of CVD risk, the bias assessment revealed that concern on the quality of outcome detection was very low across the studies included, so that this factor is unlikely to have contributed to our results.

Second, we cannot exclude the contribution of unaccounted confounders on the strength of association between NAFLD and CVDs, including heterogeneity in baseline CVD risk due to other comorbidities and lifestyle habits, such as smoke, that we were unable to analyse. It is possible that all these factors contributed to the moderate to high heterogeneity observed for all the estimates,

which was partially expected due to the nature of our analysis. This issue is common to epidemiological meta-analysis, and we also performed an extensive study of the heterogeneity observed, and a sensitivity analysis with the inclusion of adjusted effect sizes rather than event counts, which broadly confirmed our results. Furthermore, we reported 95% PIs along with our estimates, which help to interpret our findings in view of the heterogeneity observed and provide a more reliable estimate of the true effect expected in a future similar study.

We had limited data on the severity and progression of NAFLD, as well as information on treatments (both for NAFLD and other comorbidities) and potential other confounders, including socio-demographical variables. We think that these variables may play a role in shaping the relationship between NAFLD and CVD, and further studies are required to clarify their impact on the natural history of NAFLD patients. Furthermore, the sensitivity analysis according to the adjusted effect sizes may have been biased by the fact that HR and OR are not easily interchangeable; however, we think that this limitation has reduced effect on the interpretation of our results, since the aim of the sensitivity analysis was to confirm the results of the main analysis, and according to the fact that most of the adjusted HRs included were close to 1, where the risk of observing significant difference with OR is reduced.[51]

Finally, despite our best efforts to include any relevant cohort in our systematic review, it is possible that some studies were not included (e.g. because not retrieved with our search strategy or excluded for irrelevance according to the title or abstract). However, we provided the most updated and large meta-analysis on the topic, which included roughly 2.5 million of NAFLD patients for each outcome investigated, and it is unlikely that any additional cohort would critically impact our pooled estimates.

# Conclusions

Non-alcoholic fatty liver disease is associated with increased risk of MI, IS, AF, and HF; the extent of the association was influenced by several study-related characteristics, including geographical locations and criteria used to define NAFLD. Age and sex may also represent other key moderators. Further studies are required to investigate the risk in specific subgroups of patients and define specific therapeutic and prevention strategies in NAFLD patients.

# Supplementary material

Supplementary material is available at *European Journal of Preventive Cardiology* online.

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**Conflict of interest:** S.B. received research grant from MSD. Other authors have nothing to disclose.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request, and after approval of all other co-investigators.

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