European Journal of Preventive Cardiology

Risk of Cardiovascular Events in Patients with Non-alcoholic Fatty Liver Disease: a Systematic Review and Meta-Analysis

--Manuscript Draft--

Manuscript Number:	EJPC-D-21-01272R2
Full Title:	Risk of Cardiovascular Events in Patients with Non-alcoholic Fatty Liver Disease: a Systematic Review and Meta-Analysis
Article Type:	Full Research Paper
Section/Category:	CVD Risk Factors
Keywords:	NAFLD, Myocardial Infarction, Ischemic Stroke, Atrial Fibrillation, Heart Failure
Corresponding Author:	Marco Proietti Universita degli Studi di Milano Milan, ITALY
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Universita degli Studi di Milano
Corresponding Author's Secondary Institution:	
First Author:	Livnat Alon, MD
First Author Secondary Information:	
Order of Authors:	Livnat Alon, MD
	Bernadette Corica, MD
	Valeria Raparelli, MD, PhD
	Roberto Cangemi, MD, PhD
	Stefania Basili, MD
	Marco Proietti, MD, PhD
	Giulio Francesco Romiti, MD
Order of Authors Secondary Information:	
Manuscript Region of Origin:	ITALY
Abstract:	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), Ischemic Stroke (IS), Atrial Fibrillation (AF) and Heart Failure (HF) in NAFLD patients. Methods: According to the PRISMA guidelines, we systematically searched PubMed and EMBASE, from inception to 6 th March 2021, and included all studies reporting the incidence of MI, IS, AF and HF in patients with and without NAFLD. Random-effect models 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. Results: Among 3,254 records retrieved from literature, 20 studies were included. NAFLD 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: NAFLD 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.

UNIVERSITÀ DEGLI STUDI DI MILANO

FONDAZIONE IRCCS CA' GRANDA
OSPEDALE MAGGIORE POLICLINICO

Milan, 24th November 2021

To Prof. Massimo Francesco Piepoli,

Editor-in-Chief of
European Journal of Preventive Cardiology,

Dear Editor,

Manuscript: **Risk of Cardiovascular Events in Patients with Non-alcoholic Fatty Liver Disease: a Systematic Review and Meta-Analysis**

We are pleased to submit the revised version of our paper, "*Risk of Cardiovascular Events in Patients with Non-alcoholic Fatty Liver Disease: a Systematic Review and Meta-Analysis"* for your consideration.

We want to thank again the editors for their useful comments and suggestions.
In this revised version of the manuscript, we have added a graphical abstract, and discussed adequately the reference suggested by the EiC.

We hope that our manuscript can now be acceptable for publication in the European Journal of Preventive Cardiology.

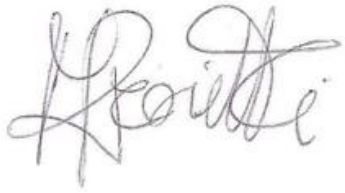
We confirm the following: 1) the paper is not under consideration elsewhere, 2) none of the paper's contents have been previously published, 3) all authors had access to all the study data, take responsibility for the accuracy of the analysis, had authority over manuscript preparation and the decision to submit the manuscript for publication and 4) have read and approved the manuscript; 4) the full disclosure of any potential conflict of interest has been made.

Yours sincerely,

Marco Proietti MD PhD FESC FEHRA

Assistant Professor in Geriatric Medicine
Department of Clinical Sciences and Community Health
University of Milan, Italy
Honorary Senior Research Fellow in Cardiology
Liverpool Centre for Cardiovascular Science,

University of Liverpool and Liverpool Heart & Chest Hospital, Liverpool



RESPONSE TO REVIEWERS

EiC Comments:

Reviewer #2: Thank you for your response for my comments.

EiC:
Consider adding a graphical Abstract (to enhance the visibility of the article).
Conisder also discussing your findings in the light of recent evidences [doi: 10.1093/eurjpc/zwab120].

Answer: *Thank you for your comment. We have added a graphical abstract to our submission, and we discussed the referenced suggested by the EiC (see page 13):*

"[…] Recently, simultaneous assessment of hepatic steatosis during coronary CT has showed improvement in the risk stratification of MACE in stable CAD patients, further underlining the tight relationship between NAFLD and ischemic heart disease.[42]"

Risk of Cardiovascular Events in Patients with Non-alcoholic Fatty Liver Disease: a Systematic Review and Meta-Analysis

Short Title: Cardiovascular Events in NAFLD

Livnat Alon[1*] MD, Bernadette Corica[1] MD*, Valeria Raparelli[2,3] MD, PhD, Roberto Cangemi[1] MD, PhD, Stefania Basili[1] MD, Marco Proietti[4,5,6] MD**, PhD, Giulio Francesco Romiti[1] MD**

[1]Department of Translational and Precision Medicine, Sapienza – University of Rome, Italy; [2]Department of Translational Medicine, University of Ferrara, Italy; [3]University of Alberta, Faculty of Nursing, Edmonton, Canada; [4]Department of Clinical Sciences and Community Health, University of Milan, Italy; [5]IRCCS Istituti Clinici Scientifici Maugeri, Milan, Italy; [6]Liverpool Centre for Cardiovascular Science, University of Liverpool and Liverpool Heart & Chest Hospital, Liverpool, United Kingdom

*These authors contributed equally.

**Joint senior authors

Manuscript Word Count: 3651

Corresponding Author
Marco Proietti MD PhD FESC FEHRA
Geriatric Unit, IRCCS Istituti Clinici Scientifici Maugeri
Via Camaldoli 64, 20138, Milan, Italy
ORCiD: 0000-0003-1452-2478
Twitter Handle: @MProiettiMD
e-mail: marco.proietti@unimi.it

ABSTRACT

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), Ischemic Stroke (IS), Atrial Fibrillation (AF) and Heart Failure (HF) in NAFLD patients.

Methods: According to the PRISMA guidelines, we systematically searched PubMed and EMBASE, from inception to 6th March 2021, and included all studies reporting the incidence of MI, IS, AF and HF in patients with and without NAFLD. Random-effect models 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.

Results: Among 3,254 records retrieved from literature, 20 studies were included. NAFLD 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: NAFLD 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.

KEYWORDS: NAFLD, Myocardial Infarction, Ischemic 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] NAFLD represent 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. CVDs 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, ischemic stroke, atrial fibrillation and heart failure.[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. [9] More recently, the role of gut microbioma

has received growing attention, according to its detrimental role in the development of cardiometabolic disease;[10] several studies have already depicted the contribution of dysbiosis in the progression and development of NAFLD and several CVDs.[10,11] 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[12], 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[13,14].

Our study aimed to provide a comprehensive systematic review and meta-analysis on the risk of myocardial infarction, ischemic stroke, atrial fibrillation, and heart failure 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.[15] 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 Materials.

Inclusion and Exclusion Criteria

Main inclusion criteria were: i) all studies reporting the number of patients, with and without NAFLD, who developed myocardial infarction, ischemic stroke, atrial fibrillation or heart failure, 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 myocardial infarction 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 3,254 studies were retrieved from the literature search (709 from PubMed and 2,545 from EMBASE). After duplicates removal, and sequential screening of title and abstract, we evaluated 94 full-texts, and eventually included 20 studies [16,17,26–35,18–25] (Figure S1 in Supplementary Materials). A summary of the main characteristics of the included studies is reported in Table 1. Briefly, 3 were case-control studies[18,23,27]; among the 17 cohort studies, 10 had a retrospective design[16,17,19,20,24,26,29,30,32,35] and 7 were prospective[21,22,25,28,31,33,34]. Overall, 5 studies were based on administrative databases[18,20,23,27,35]. 9 studies were held in Asia[16,19,20,22,25,28,29,33,35], 6 in Europe[17,18,21,24,27,31], 4 in North America[23,26,30,32] and 1 in Egypt.[34] 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 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; 2 studies enrolled only patients with type 2 diabetes mellitus[31,33], while 3 studies enrolled patients with suspected coronary artery disease[26,33] or referred for its evaluation[22]. Follow-up duration ranged from 2 years to over 17 years, with most studies reporting more than 4 years of observation.

13 studies reported data on myocardial infarction, 12 on ischemic stroke, 7 on atrial fibrillation, and 4 on heart failure. Overall, 9 studies were considered at high risk of bias[16,19,20,22,25,26,31,33,35]; 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 Table S4 in Supplementary Materials.

Across the studies included, Alexander et al.[27] pooled data of 4 different cohorts from Italy, Netherlands, Spain and United Kingdom; 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 NAFLD

Compared to patients without NAFLD, subjects with NAFLD showed significant increased risk of myocardial infarction (OR: 1.66, 95%CI 1.39-1.99, 95%PI 0.84-3.30, I^2=98%), ischemic stroke (OR: 1.41, 95%CI: 1.29-1.55, 95%PI: 1.03-1.93, I^2=93%), atrial fibrillation (OR: 1.27, 95%CI: 1.18-1.37, 95%CI: 1.07-1.52, I^2=65%) and heart failure (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 1, Panels A to D, respectively), compared to patients without NAFLD; 95%PI were significant for ischemic stroke, atrial fibrillation and heart failure, but not for the risk of myocardial infarction.

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 myocardial infarction, 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 myocardial infarction in NAFLD patients (Figure 2, panel A). 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 ischemic stroke ($p<0.01$ for all), with a trend similar to what observed for myocardial infarction; 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 to the primary analysis.

For atrial fibrillation, the only significant subgroup difference was found according to the NAFLD definition ($p=0.01$): higher risk of atrial fibrillation 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 heart failure.

Subgroup analyses for each outcome are reported in detail in supplementary materials, Figures S2 to S5.

Meta-Regression Analysis

Results of the univariable meta-regression analyses for each outcome are reported in Table S5-S7 in Supplementary Materials.

At univariable analysis, study design and NAFLD definition were significantly associated with the risk of myocardial infarction 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 ischemic stroke, 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 atrial fibrillation; finally, we were not able to perform meta-regression for the risk of heart failure, 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 (Figure S6 in Supplementary Materials).

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[31,33], or subjects referred for suspected CAD[22,26,33]. All the analyses showed consistency with main estimates (Figure S7, panel A to D); the exclusion of studies that used ICD codes lead to slightly higher pooled ORs for myocardial infarction and ischemic stroke (Figure S7, panel A 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, 6 studies reported adjusted HRs[18,22,27–30], and 2 studies reported adjusted OR[12,24]. No studies reported adjusted estimates for heart failure. Compared to the primary analysis, the use of adjusted effect size led to lower figures for the risk of both myocardial infarction and ischemic stroke. Significant subgroup differences were found for both outcomes, between studies analyzed according to adjusted effect sizes vs. those analyzed according to event counts ($p<0.01$ for both, Figure S8 Panel A and B respectively). Similar estimates compared to primary were found for atrial fibrillation (Figure S8 Panel C).

Publication Bias

Results of the publication bias analyses are reported in Figure S9. Visual inspection of the funnel plot for myocardial infarction 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 Figure S10. The imputation of 5 additional studies to reduce asymmetry of the funnel plot led to higher pooled estimates for the risk of myocardial infarction, compared to 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 ischemic stroke, atrial fibrillation and heart failure.

DISCUSSION

In this systematic review and meta-analysis, we found that patients with NAFLD are at a higher risk of myocardial infarction, ischemic stroke, atrial fibrillation and heart failure compared to 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 myocardial infarction, 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 influenced 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 myocardial infarction, while the type of study influenced both risks of myocardial infarction and ischemic stroke 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 atrial fibrillation represents a new finding, not found in a previous meta-analysis on the topic[14]; to our knowledge, our study is also the first to provide a meta-analysis on the risk of heart failure. Notably, we found comparable estimates for the risk of all outcomes investigated, although the 95% PIs confirmed the association for ischemic stroke, atrial fibrillation, and heart failure, but not for myocardial infarction. This suggest that while NAFLD may represent a common determinant of the risk of several CVDs (perhaps through different

pathophysiological pathways), differences in 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 myocardial infarction and cerebrovascular accident have been more extensively investigated[36]. In fact, NAFLD is part of a complex spectrum of metabolic dysfunctions, and can promote a pro-atherogenic lipid profile[37,38], endothelial dysfunction[39], and oxidative stress[39]. Interestingly, severity and stage of NAFLD seem to influence the extent of these processes[38,40]. Patient with NAFLD often show systemic inflammation[41], and are also frequently overweight or obese. All these factors can lead to a higher risk of CVDs, and specifically myocardial infarction and stroke. Recently, simultaneous assessment of hepatic steatosis during coronary CT has showed improvement in the risk stratification of MACE in stable CAD patients, further underlining the tight relationship between NAFLD and ischemic heart disease.[42]

On the other side, the mechanisms underlying the interplay between NAFLD, heart failure, and atrial fibrillation are less characterized. NAFLD has been associated itself with cardiac remodeling, including changes in left ventricular structure and increased left atrial size, which may promote the onset of heart failure and atrial fibrillation [31,43–46]. Moreover, oxidative stress, inflammation and insuline resistance promoted by NAFLD may contribute to the development of heart failure, and particularly to heart failure with preserved ejection fraction.[47] Finally, NAFLD may increase the risk of atrial fibrillation through the epicardial fat [48,49], which has been associated with incident atrial fibrillation.[50]

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[51]; currently, loss of weight and treatment of established concurrent risk factors, including diabetes, dyslipidemia and hypertension represent potential approaches to tackle CVDs risk.[51]

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 myocardial infarction and ischemic stroke, with lower figures found in European-based studies for both outcomes. Similarly, lower risk of myocardial infarction and ischemic stroke 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 atrial fibrillation.

Identification of NAFLD is pivotal to analyze 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[1,52]; 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 diagnosing 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 myocardial infarction and ischemic stroke. 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 myocardial infarction; on the other side, mean age was inversely associated with the OR for ischemic stroke 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[12], 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 included only 5 studies for the analysis on the risk of ischemic stroke[53]; similarly, Mantovani analyzed 4 studies for the risk of incident AF in patients with vs. without NAFLD[14], and did not found significant association; however, 4 newer studies were published thereafter[18,20,21,23], including 2 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, and 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 analyze. 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 HR 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 HR 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 HR included were close to 1, when the risk of observing significant difference with OR is reduced[54].

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

NAFLD is associated with increased risk of myocardial infarction, ischemic stroke, atrial fibrillation and heart failure; 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.

Funding:

GFR and BC were supported by grants (AR11916B84DD8DCE and AR120172B872270D) issued by Sapienza – University of Rome, Rome, Italy.

Conflict of Interest:

SB received research grant from MSD. Other authors have nothing to disclose.

Author Contributions:

LA, BC and GFR contributed to the conception and design of this study, acquired data and drafted the manuscript. GFR analysed data; LA, BC, MP and GFR interpreted the results of the analysis. VR, RC, SB and MP critically revised the manuscript and gave important intellectual contribution. All the authors gave final approval.

FIGURE LEGENDS

Graphical Abstract (created with Biorender.com)

Legend: OR= Odds Ratio; 95%CI=95% Confidence Intervals; 95%PI= 95% Prediction Intervals

Figure 1: Risk of Myocardial Infarction, Ischemic Stroke, Atrial Fibrillation and Heart Failure in patients with vs. without NAFLD

Legend: CI= Confidence Interval; MH= Mantel-Haenszel; NAFLD= Non Alcoholic Fatty Liver Disease; PI= Prediction Interval.

Figure 2: Subgroup Analysis for the risk of Myocardial Infarction, Ischemic Stroke, Atrial Fibrillation and Heart Failure in patients with vs. without NAFLD

Panel A) Myocardial Infarction; Panel B) Ischemic Stroke; Panel C) Atrial Fibrillation; Panel D) Heart Failure.

Legend: CI= Confidence Interval; CT= Computerized Tomography; ICD= International Classification of Diseases; FLI= Fatty Liver Index; I2=Inconsistency Index; OR= Odds Ratio; US= Ultrasound

REFERENCES

1. Powell EE, Wong VW-S, Rinella M. Non-alcoholic fatty liver disease. *Lancet* 2021;**0**, DOI: 10.1016/S0140-6736(20)32511-3.

2. Younossi ZM, Koenig AB, Abdelatif D *et al.* Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;**64**:73–84.

3. Chalasani N, Younossi Z, Lavine JE *et al.* The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018;**67**:328–57.

4. Sepanlou SG, Safiri S, Bisignano C *et al.* The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2020;**5**:245–66.

5. Paik JM, Henry L, De Avila L *et al.* Mortality Related to Nonalcoholic Fatty Liver Disease Is Increasing in the United States. *Hepatol Commun* 2019;**3**:1459–71.

6. Kasper P, Martin A, Lang S *et al.* NAFLD and cardiovascular diseases: a clinical review. *Clin Res Cardiol* 2020:1–17.

7. Lonardo A, Sookoian S, Pirola CJ *et al.* Non-alcoholic fatty liver disease and risk of cardiovascular disease. *Metabolism* 2016;**65**:1136–50.

8. Stahl EP, Dhindsa DS, Lee SK *et al.* Nonalcoholic Fatty Liver Disease and the Heart: JACC State-of-the-Art Review. *J Am Coll Cardiol* 2019;**73**:948–63.

9. Kasper P, Martin A, Lang S *et al.* NAFLD and cardiovascular diseases: a clinical review. *Clin Res Cardiol* 2021;**110**:921–37.

10. Tang WHW, Kitai T, Hazen SL. Gut microbiota in cardiovascular health and disease. *Circ Res* 2017;**120**:1183–96.

11. Tang WHW, Bäckhed F, Landmesser U *et al.* Intestinal Microbiota in Cardiovascular Health and Disease: JACC State-of-the-Art Review. *J Am Coll Cardiol* 2019;**73**:2089–105.

12. Targher G, Byrne CD, Lonardo A *et al.* Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis. *J Hepatol* 2016;**65**:589–600.

13. Hu J, Xu Y, He Z *et al.* Increased risk of cerebrovascular accident related to non-alcoholic fatty liver disease: A meta-analysis. *Oncotarget* 2018;**9**:2752–60.

14. Mantovani A, Dauriz M, Sandri D *et al.* Association between non-alcoholic fatty liver disease and risk of atrial fibrillation in adult individuals: An updated meta-analysis. *Liver Int* 2019;**39**:758–69.

15. Page MJ, McKenzie JE, Bossuyt PM *et al.* The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ* 2021;**372**, DOI: 10.1136/bmj.n71.

16. Moon SH, Hong SP, Cho YS *et al.* Hepatic FDG uptake is associated with future cardiovascular events in asymptomatic individuals with non-alcoholic fatty liver disease. *J Nucl Cardiol* 2017;**24**:892–9.

17. Pisto P, Santaniemi M, Bloigu R *et al.* Fatty liver predicts the risk for cardiovascular events in middle-aged population: a population-based cohort study. *BMJ Open* 2014;**4**:e004973.

18. Labenz C, Huber Y, Michel M *et al.* Impact of NAFLD on the Incidence of Cardiovascular Diseases in a Primary Care Population in Germany. *Dig Dis Sci* 2020;**65**:2112–9.

19. Yang Y-J, Jung M-H, Jeong S-H *et al.* The Association between Nonalcoholic Fatty Liver Disease and Stroke: Results from the Korean Genome and Epidemiology Study (KoGES). *Int J Environ Res Public Health* 2020;**17**:9568.

20. Lee SR, Han K Do, Choi EK *et al.* Nonalcoholic fatty liver disease and the risk of atrial fibrillation stratified by body mass index: a nationwide population-based study. *Sci Rep* 2021;**11**:3737.

21. Baratta F, Pastori D, Angelico F *et al.* Nonalcoholic Fatty Liver Disease and Fibrosis Associated With Increased Risk of Cardiovascular Events in a Prospective Study. *Clin Gastroenterol Hepatol* 2020;**18**:2324-2331.e4.

22. Wong VW, Wong GL, Yeung JC *et al.* Long-term clinical outcomes after fatty liver screening in patients undergoing coronary angiogram: A prospective cohort study. *Hepatology* 2016;**63**:754–63.

23. Allen AM, Therneau TM, Mara KC *et al.* Women With Nonalcoholic Fatty Liver Disease Lose Protection Against Cardiovascular Disease: A Longitudinal Cohort Study. *Am J Gastroenterol* 2019;**114**:1764–71.

24. Käräjämäki AJ, Pätsi OP, Savolainen M *et al.* Non-Alcoholic Fatty Liver Disease as a Predictor of Atrial Fibrillation in Middle-Aged Population (OPERA Study). *PLoS One* 2015;**10**:e0142937.

25. Hamaguchi M, Kojima T, Takeda N *et al.* Nonalcolholic fatty liver disease is a novel predictor cardiovascular disease. *World J Gastroenterol* 2007;**13**:1579–84.

26. Meyersohn NM, Mayrhofer T, Corey KE *et al.* Association of Hepatic Steatosis With Major Adverse Cardiovascular Events, Independent of Coronary Artery Disease. *Clin Gastroenterol Hepatol* 2020, DOI: 10.1016/j.cgh.2020.07.030.

27. Alexander M, Loomis AK, van der Lei J *et al.* Non-alcoholic fatty liver disease and risk of incident acute myocardial infarction and stroke: findings from matched cohort study of 18 million European adults. *BMJ* 2019;**367**:l5367.

28. Xu J, Dai L, Zhang Y *et al.* Severity of Nonalcoholic Fatty Liver Disease and Risk of Future Ischemic Stroke Events. *Stroke* 2021;**52**:103–10.

29. Sinn DH, Kang D, Chang Y *et al.* Non-alcoholic fatty liver disease and the incidence of myocardial infarction: A cohort study. *J Gastroenterol Hepatol* 2020;**35**:833–9.

30. Long MT, Yin X, Larson MG *et al.* Relations of Liver Fat With Prevalent and Incident Atrial Fibrillation in the Framingham Heart Study. *J Am Hear Assoc* 2017;**6**, DOI: 10.1161/jaha.116.005227.

31. Targher G, Valbusa F, Bonapace S *et al.* Non-alcoholic fatty liver disease is associated with an increased incidence of atrial fibrillation in patients with type 2 diabetes. *PLoS One* 2013;**8**:e57183.

32. VanWagner LB, Wilcox JE, Ning H *et al.* Longitudinal Association of Non-Alcoholic Fatty Liver Disease With Changes in Myocardial Structure and Function: The CARDIA Study. *J Am Hear Assoc* 2020;**9**:e014279.

33. Ichikawa K, Miyoshi T, Osawa K *et al.* Prognostic value of non-alcoholic fatty liver disease for predicting cardiovascular events in patients with diabetes mellitus with suspected coronary artery disease: a prospective cohort study. *Cardiovasc Diabetol* 2021;**20**:8.

34. El Azeem HA, Khalek E-SA, El-Akabawy H *et al.* Association between nonalcoholic fatty liver disease and the incidence of cardiovascular and renal events. *J Saudi Hear Assoc* 2013;**25**:239–46.

35. Lee H, Lee Y ho, Kim SU *et al.* Metabolic Dysfunction-Associated Fatty Liver Disease and Incident Cardiovascular Disease Risk: A Nationwide Cohort Study. *Clin Gastroenterol Hepatol* 2021, DOI: 10.1016/j.cgh.2020.12.022.

36. Targher G. Non-alcoholic fatty liver disease, the metabolic syndrome and the risk of cardiovascular disease: The plot thickens. *Diabet Med* 2007;**24**:1–6.

37. Sonmez A, Nikolic D, Dogru T *et al.* Low- and high-density lipoprotein subclasses

in subjects with nonalcoholic fatty liver disease. *J Clin Lipidol* 2015;**9**:576–82.

38. Francque SM, van der Graaff D, Kwanten WJ. Non-alcoholic fatty liver disease and cardiovascular risk: Pathophysiological mechanisms and implications. *J Hepatol* 2016;**65**:425–43.

39. Villanova N, Moscatiello S, Ramilli S *et al.* Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease. *Hepatology* 2005;**42**:473–80.

40. Musso G, Gambino R, De Michieli F *et al.* Association of liver disease with postprandial large intestinal triglyceride-rich lipoprotein accumulation and pro/antioxidant imbalance in normolipidemic non-alcoholic steatohepatitis. *Ann Med* 2008;**40**:383–94.

41. Hamirani YS, Katz R, Nasir K *et al.* Association between Inflammatory Markers and Liver Fat: The Multi-Ethnic Study of Atherosclerosis. 2014, DOI: 10.4172/2155-9880.1000344.

42. Ichikawa K, Miyoshi T, Osawa K *et al.* Incremental prognostic value of non-alcoholic fatty liver disease over coronary computed tomography angiography findings in patients with suspected coronary artery disease. *Eur J Prev Cardiol* 2021, DOI: 10.1093/eurjpc/zwab120.

43. Trovato FM, Martines GF, Catalano D *et al.* Echocardiography and NAFLD (non-alcoholic fatty liver disease). *Int J Cardiol* 2016;**221**:275–9.

44. Hallsworth K, Hollingsworth KG, Thoma C *et al.* Cardiac structure and function are altered in adults with non-alcoholic fatty liver disease. *J Hepatol* 2013;**58**:757–62.

45. Simon TG, Bamira DG, Chung RT *et al.* Nonalcoholic Steatohepatitis is Associated with Cardiac Remodeling and Dysfunction. *Obesity (Silver Spring)* 2017;**25**:1313–6.

46. VanWagner LB, Wilcox JE, Colangelo LA *et al.* Association of nonalcoholic fatty liver disease with subclinical myocardial remodeling and dysfunction: A population-based study. *Hepatology* 2015;**62**:773–83.

47. Itier R, Guillaume M, Ricci J-E *et al.* Non-alcoholic fatty liver disease and heart failure with preserved ejection fraction: from pathophysiology to practical issues. *ESC Hear Fail* 2021;**8**:789–98.

48. Fracanzani AL, Pisano G, Consonni D *et al.* Epicardial Adipose Tissue (EAT) thickness is associated with cardiovascular and liver damage in nonalcoholic fatty liver disease. *PLoS One* 2016;**11**, DOI: 10.1371/journal.pone.0162473.

49. Liu B, Li Y, Li Y *et al.* Association of epicardial adipose tissue with non-alcoholic fatty liver disease: a meta-analysis. *Hepatol Int* 2019;**13**:757–65.

50. Wong CX, Ganesan AN, Selvanayagam JB. Epicardial fat and atrial fibrillation: current evidence, potential mechanisms, clinical implications, and future directions. *Eur Heart J* 2017;**38**:1294–302.

51. Stahl EP, Dhindsa DS, Lee SK *et al.* Nonalcoholic Fatty Liver Disease and the Heart: JACC State-of-the-Art Review. *J Am Coll Cardiol* 2019;**73**:948–63.

52. Marchesini G, Day CP, Dufour JF *et al.* EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016;**64**:1388–402.

53. Hu J, Xu Y, He Z *et al.* Increased risk of cerebrovascular accident related to non-alcoholic fatty liver disease: A meta-analysis. *Oncotarget* 2018;**9**:2752–60.

54. Zhang J, Yu KF. What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. *J Am Med Assoc* 1998;**280**:1690–1.

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[27]	2019	Europe	Case-Control	Pts. without history of MI or Stroke	ICD Codes	9768439*	120795*	54.2*	50*	29*	9*	3.8*	MI, Stroke
Allen[23]	2019	North America	Case-Control	Unselected pts. with NAFLD	ICD Codes	19078	3869	53†	48	28	13	7	AF, HF, MI, Stroke
Baratta[21]	2020	Europe	Cohort Study	Pts. with at least 1 comorbidity	US	898	643	56.5	62	70	25	3.5	AF, MI, Stroke
El Azeem[34]	2013	Other	Cohort Study	Pts. without history of CVD	US	747	268	51.5	49	32	58	3	MI, Stroke
Hamaguchi[25]	2007	Asia	Cohort Study	Pts. without history of MI or Stroke	US	1221	231	48	NA	NA	NA	5.8	MI, Stroke
Ichikawa[33]	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[24]	2015	Europe	Cohort Study	Pts. 40-59 years with or without HTN	US	958	249	51.3	47	51	10	16.3	AF
Labenz[18]	2020	Europe	Case-Control	Pts. without history of AF, MI, Stroke	ICD Codes	44096	22048	55.6	50	25	6	10	AF, MI, Stroke

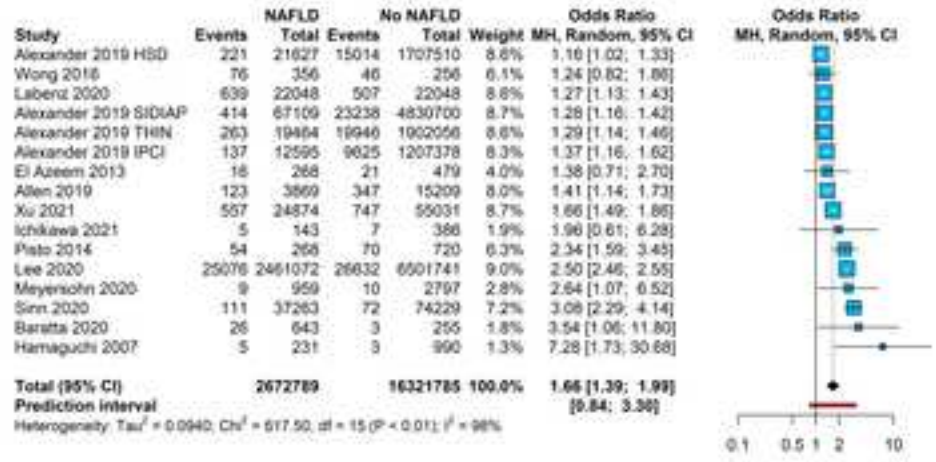
Lee[35]	2020	Asia	Cohort Study	Pts. 40-64 years without history of HF, MI, Stroke	FLI	8962813	2461072	50†	48	23	9	10.1	HF, MI, Stroke
Lee[20]	2021	Asia	Cohort Study	Pts. >20 years, without history of AF	FLI	8048055	2738621	46.7	52	24	8	8.3	AF
Long[30]	2017	North America	Cohort Study	Pts. without history of AF	CT	2060	406	59	47	26	7	9.3	AF
Meyersohn[26]	2020	North America	Cohort Study	Pts with suspected CAD, without previous MI	CT	3756	959	60.6	48	64	20	2.1	MI
Moon[16]	2017	Asia	Cohort Study	Pts. screened for cancer	US	815	394	51.8	94	21	9	4.2	Stroke
Pisto[17]	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[29]	2020	Asia	Cohort Study	Pts. without history of MI or CVD	US	111492	37263	52	51	26	9	6.5	MI
Targher[31]	2013	Europe	Cohort Study	Pts. with DM, without previous AF	US	400	281	63.3	59	71	100	10	AF
VanWagner[32]	2021	North America	Cohort Study	Unselected pts. that underwent CT	CT	1827	159	50	39	31	11	5	HF
Wong[22]	2016	Asia	Cohort Study	Pts. referred for coronary CT angiogram	US	612	356	63	71	66	31	6	MI

Xu[28]	2021	Asia	Cohort Study	Pts. without history of MI or Stroke	US	79905	24874	51.4†	74	1	1	10.3	MI, Stroke
Yang[19]	2020	Asia	Cohort Study	Pts. 40-69 years, without Stroke	FLI	7964	3414	52.5	42	39	9	12	Stroke

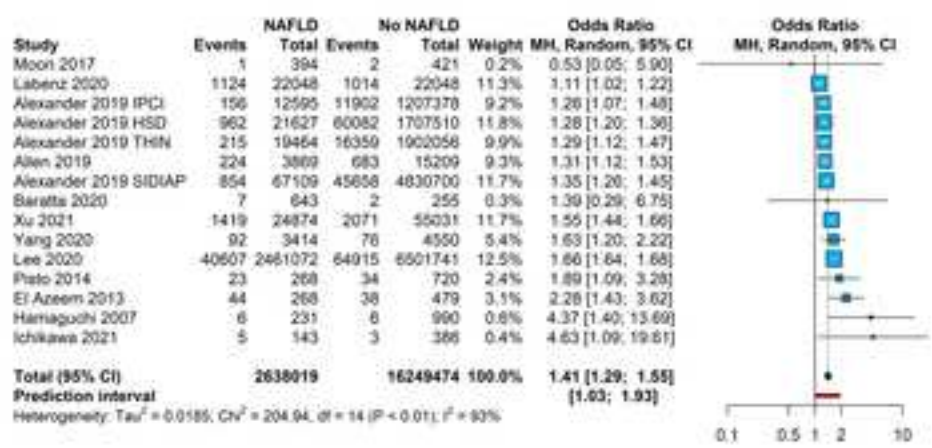
Legend: *The figures are for the whole pooled cohort of the study, although each cohort was analyzed individually in the meta-analysis; †median values; AF= Atrial Fibrillation; CAD= Coronary Artery Disease; CT= Computerized Tomography; CVD= Cardiovascular Disease; DM= Type 2 Diabetes Mellitus; FLI= Fatty Liver Index; FU= Follow-up; HF= Heart Failure; HTN= Hypertension ICD= International Classification of Disease; M= Males; MI= Myocardial Infarction; NA= Not Available; US= Ultrasound

Figure 1

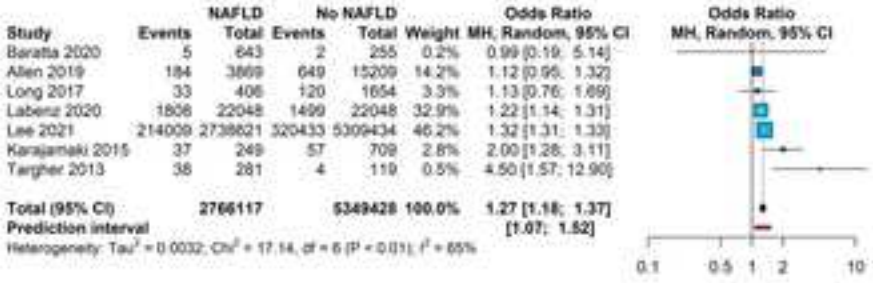
A



B



C



D

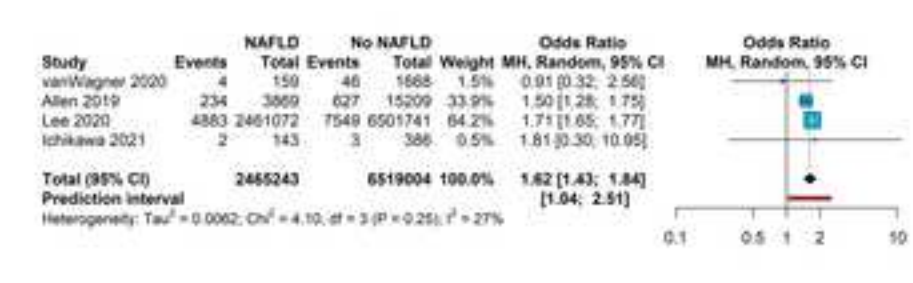
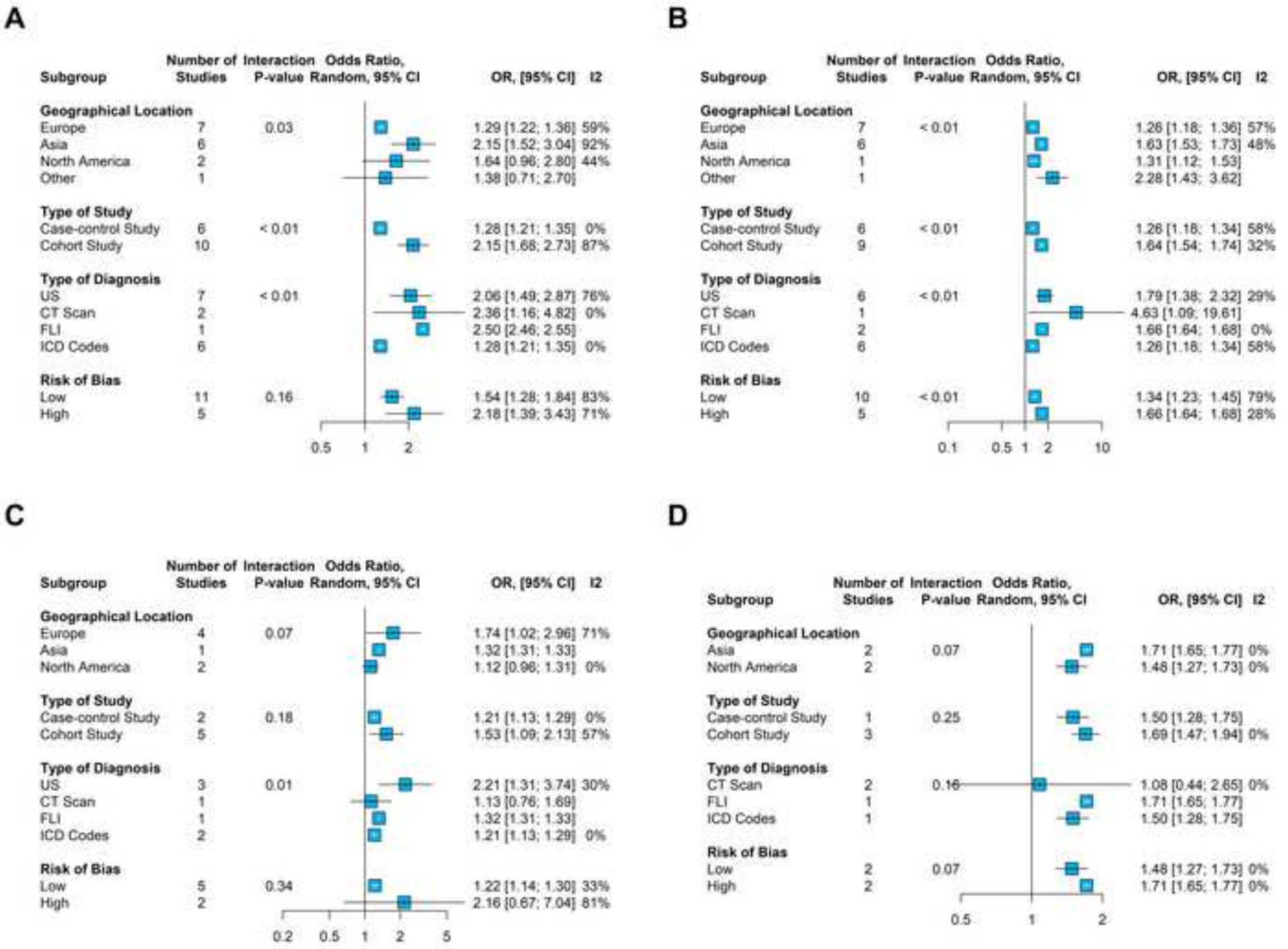


Figure 2



Graphical Abstract