The impact of light to moderate alcohol consumption on progressive non-alcoholic fatty liver disease: A systematic review and meta-analysis

Short title

Alcohol and progressive NAFLD

Authors

Louise Magherman[1]*, Robbe Van Parys[1]*, Nele S. Pauwels[2], Xavier Verhelst[1], Lindsey Devisscher[3], Hans Van Vlierberghe[1], Anja Geerts[1], Sander Lefere[1,3]

* These authors contributed equally

Affiliations

[1] Hepatology Research Unit, Dpt. Internal Medicine and Pediatrics; Liver Research Center Ghent, Ghent University, Ghent, Belgium

[2] Knowledge Center for Health Ghent, Ghent University and Ghent University Hospital, Ghent, Belgium

[3] Gut-Liver Immunopharmacology Unit, Dpt. Basic and Applied Medical Sciences; Liver Research Center Ghent, Ghent University, Ghent, Belgium

Contact information

Sander Lefere, MD, PhD
Hepatology Research Unit, Dpt. Internal Medicine and Pediatrics; Liver Research Center Ghent; Ghent University, Building B, Entrance 36, Corneel Heymanslaan 10, B-9000 Ghent, Belgium
sander.lefere@ugent.be
Tel. +32 9 332 2361

Disclosures

The authors do not report any conflicts of interest.

Grant support

SL is supported by a grant from the Research Foundation – Flanders (FWO) (12R0321N). AG and HVV are senior clinical investigators of the FWO (1805718N and 1801721N). The funding agency was not involved in study design, analysis or reporting.

Data sharing statement

The data that underlie the results reported in this article, together with the template data collection form, will be shared with researchers upon reasonable request. To gain access, proposals should be sent to sander.lefere@ugent.be.

Abbreviations

aHR: adjusted hazard ratio; ALD: alcoholic liver disease; aOR: adjusted odds ratio; aRR: adjusted relative risk; AUDIT: Alcohol Use Disorders Identification Test; BMI: body mass index; CAC: coronary artery calcification; CI: confidence interval; CT: computed tomography; CVD: cardiovascular disease; F: fibrosis stage; FIB-4: fibrosis 4 calculator; FLI: fatty liver index; HCC: hepatocellular carcinoma; H-MRS: proton magnetic resonance spectroscopy; HR: hazard ratio; HSI: hepatic steatosis index; ICD, International Statistical Classification of Diseases and Related Health Problems; LACU: lifetime alcoholic cumulative units; MRI: magnetic resonance imaging; N/A: not applicable; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; NASH CRN: clinical research network in non-alcoholic steatohepatitis; NFS: NAFLD fibrosis score; OATD: Open Access Theses and Dissertations; OR: odds ratio; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-

Analyses; RR: relative risk; SE: standard error; SLDH: Skinner Lifetime Drinking History; T2DM: type 2 diabetes mellitus.

Word count

Abstract: 235
Revised manuscript (including references and figure legends): 6245
Figures: 3
Tables: 3

Summary

Background & Aims

Non-alcoholic fatty liver disease (NAFLD) is defined as fatty liver disease in the absence of heavy alcohol consumption. However, the impact of light to moderate alcohol consumption on progressive NAFLD and on mortality is presently unclear.

Methods

Medline, Embase, OATD and OpenGrey were systematically searched up to November 2022 for relevant cross-sectional, case-control, and cohort studies. The study outcomes were progressive NAFLD – steatohepatitis (NASH), fibrosis, cirrhosis, hepatocellular carcinoma (HCC) and mortality. The entire review process was performed by two independent reviewers. A narrative synthesis was performed for all outcomes, while meta-analyses, subgroup analyses and publication bias assessment were performed depending on the number of articles.

Results

After study selection, 32 articles were included. Cohort studies reported that moderate alcohol intake increased the risk for advanced fibrosis (pooled OR 1.56; 95% CI 1.08-2.26 and HR 1.39; 95% CI 1.22-1.57), which was not observed in cross-sectional studies. Alcohol use also increased the risk of developing liver cirrhosis and HCC, but seemed to lower the risk of steatohepatitis. Light alcohol consumption protected against all-cause mortality, an effect not observed in NAFLD patients with moderate intake.

Conclusions

There is wide heterogeneity in studies on the impact of alcohol on progressive NAFLD. Nevertheless, cohort studies reported a significant harmful effect of moderate alcohol consumption on the occurrence of advanced fibrosis. Further research is needed to make valid recommendations with regard to alcohol consumption in patients with NAFLD.

Keywords

Advanced fibrosis, liver fibrosis, HCC, mortality, ethanol

Introduction

Non-alcoholic fatty liver disease (NAFLD) is currently the most common liver disease worldwide with an estimated prevalence of approximately 25%[1,2]. It is defined by the presence of steatosis in more than 5% of hepatocytes, and can progress to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and hepatocellular carcinoma (HCC)[2]. In addition, NAFLD is associated with insulin resistance, metabolic syndrome and a higher cardiovascular risk[2,3].

The diagnosis of NAFLD is based on the presence of steatosis and the exclusion of both secondary causes of liver disease and a daily alcohol consumption of more than 30 g for men and 20 g for women[2]. However, many patients diagnosed with NAFLD consume light, moderate or even excessive amounts of alcohol. While alcohol abstinence has been proven to prevent progression and complications in patients with alcoholic liver disease (ALD), there is discussion in the literature on the impact of light to moderate alcohol use on the progression of NAFLD to NASH, fibrosis, cirrhosis and HCC[4,5]. While several studies argue that there is no safe limit of alcohol use to avoid liver injury, others report that light to moderate alcohol use may have a protective effect against disease progression[5].

Recent meta-analyses by Wijarnpreecha et al. and by Wongtrakul et al. have documented a significant association between modest alcohol consumption and decreased liver fibrosis in patients with NAFLD. However, the inclusion criteria of these studies were very selective, resulting in an incomplete overview of the present sample of studies. For instance, the study by Wijarnpreecha et al. only incorporated cross-sectional studies for the outcome of fibrosis, while Wongtrakul et al. only included studies in which the diagnosis of NAFLD was based on liver histology, potentially introducing selection bias[6,7]. In contrast, another recent systematic review, by Jarvis et al., which only incorporated a handful

of longitudinal observational cohort studies, reported that any level of alcohol consumption, even light to moderate alcohol consumption, is associated with worsening of liver outcomes in NAFLD[8].

Therefore, this systematic review and meta-analysis was performed to give an inclusive overview of the impact of light to moderate alcohol consumption in patients with NAFLD, on the development or occurrence of NASH, fibrosis, cirrhosis and HCC and on overall mortality, compared to abstinence. Our objective was to provide guidance on whether patients with NAFLD can safely consume light to moderate amounts of alcohol without further disease progression or not.

Methods

This systematic review and meta-analysis was reported following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) Statement[9]. The protocol was registered on Prospero (CRD42021246943).

Search strategy

We searched the databases MEDLINE (via the PubMed interface), EMBASE (via the embase.com interface), OATD (Open Access Theses and Dissertations) and Open Grey for studies published through February 2021. Updates were performed for studies through November 2022. The search strategy was designed and conducted by the study's investigators (L.M., R.V.P. and S.L.) with input from an experienced information specialist (N.S.P.). Medical Subject Headings and free-text terms were combined in the search strategy, which consisted of the following concepts: NAFLD, alcohol consumption, NASH, fibrosis and cirrhosis, HCC and overall mortality (the full search strategy is detailed in Supplementary Tables 1-4). A time filter from 1990 onwards was applied, as before this time the entity of NAFLD was not clearly recognized; no language restriction was imposed during the search. The reference lists of relevant articles were searched to identify additional studies of interest.

Eligibility criteria and study selection

We included published epidemiological studies of any quantitative design investigating the impact of light to moderate alcohol consumption on the development or prevalence of progressive NAFLD and/or mortality or cardiovascular disease in patients with NAFLD. Light to moderate alcohol use was defined as a daily alcohol consumption of no more than 30g. If a study measured the quantity of alcohol consumption using units or standard drinks, the maximum amount of daily alcohol consumption was 3 units/drinks. Studies with alcohol exposure above the previous limits were excluded. Studies had to report on adults with NAFLD, based on the presence of liver steatosis in the absence of other causes of liver disease. NAFLD could be diagnosed by liver biopsy, imaging (ultrasound, magnetic resonance imaging (MRI) or computed tomography (CT)), serum markers or risk scores. If a full-text paper was not available but the abstract presented sufficient data, this abstract was included. Case reports, editorials, reviews, or letters were excluded. In case of multiple reports from the same study or database, all were included if these studies could offer data on the different outcomes of interest.

After removing duplicates using Endnote 20 and Rayyan, two reviewers (L.M. and R.V.P.) independently screened the titles and abstracts for initial eligibility criteria using the Rayyan software application[10], and read the full texts for final eligibility. Researchers were blinded to each other's decisions during the study selection process. Disagreements were resolved through a consensus meeting with a third reviewer (S.L.).

Outcome measures

Outcome measures were defined before performing the systematic review. The primary outcome was the occurrence of progressive NAFLD, e.g. NASH, liver fibrosis, cirrhosis, and/or HCC. Specifically, eligible studies diagnosed NASH by liver biopsy; liver fibrosis by biopsy, serum markers, fibrosis scores (FIB-4 and NFS) or transient elastography; liver cirrhosis by biopsy or transient elastography; and HCC by liver biopsy or imaging. Secondary outcome measures included cardiovascular events and overall mortality. Light alcohol consumption was defined as ≤ 10 g/day or ≤ 1 drink/day, moderate as ≤ 30 g/day or ≤ 3 drinks/day. Studies that did not differentiate regarding specific alcohol use were taken to

study moderate alcohol use as a whole. A separate sensitivity analysis excluding the latter articles (retaining only the papers defining moderate as 10-30 g/day or 1-3 drinks/day) was performed if enough studies were available.

Data extraction

Two reviewers (L.M. and R.V.P.) independently extracted data from eligible studies, using a piloted standardized data collection form. Discrepancies were resolved through consensus between the reviewers and consultation with a third reviewer (S.L.). The following information was abstracted from each study: study metadata, design, sample size, patient characteristics, details on the exposure and control conditions and how these were ascertained, method of NAFLD diagnosis and statistical methodology. Corresponding authors were contacted via email in case of missing data.

Quality assessment

To assess the risk of bias of both cohort and cross-sectional studies, the QUALSYST quality assessment tool[11] was used, with which each study was assessed on 14 criteria. Each criterion was scored, on the study and outcome levels, depending on the degree to which the specific criteria were met or reported ("yes" = 2, "partial" = 1, "no" = 0). Items not applicable to a particular study design were marked "N/A" and were excluded from the calculation of the summary score. The quality of the included articles was assessed by two independent reviewers (L.M. and R.V.P.). Disagreements between the two review authors were discussed with a third reviewer (S.L.). The completed checklist is presented in Supplementary Table 5.

Statistical analysis

A narrative synthesis of the data was performed, describing the type of outcome and exposure, the population characteristics, and the statistical results. A quantitative analysis was performed for outcomes for which enough studies were available, incorporating studies that reported odds ratios (OR), hazard ratios (HR) or relative risks (RR), and studies for which such an odds ratio could be calculated using the reported data. Adjusted ratios were used when possible, then reported adjusted

ratios and unadjusted ratios were pooled using the method of inverse variance. The meta-analysis was stratified according to light or moderate alcohol use. Subgroup analyses were carried out based on study design (cohort vs. cross-sectional study), geographic region, diagnostic tool, and the use of adjusted vs. unadjusted effect estimates. A random effect model was used to obtain a summary estimate of primary outcomes among patient groups as it takes into account the possibility of heterogeneity between studies. Heterogeneity was further assessed by the I^2 statistic, which was defined as low (I^2 <25%), moderate (I^2 25–50%) or high (I^2 >50%). A two-sided P-value < 0.05 was considered statistically significant for overall effect. Results were visually displayed by using forest plots, and publication bias was assessed using funnel plots when the meta-analysis consisted of more than ten articles (HR and OR were pooled together for publication bias assessment). All analyses were done in Review Manager 5.3 (Cochrane, UK).

Results

Study selection

Through systematic literature review, 12107 studies were identified. After deduplication, 9654 records were screened for eligibility based on title and abstract. A total of 177 records remained for further evaluation based on full text. Eventually, 32 articles met the inclusion criteria and were included in the systematic review. The other 145 studies were excluded (detailed in Supplementary Table 6). Of the 32 studies included in the systematic review, 17 studies were included in a meta-analysis (Figure 1).

Study characteristics

The characteristics of the included studies are presented in Tables 1-3; Table 1 summarizes studies on fibrosis and cirrhosis, Table 2 on NASH and HCC, and Table 3 on mortality and cardiovascular outcomes.

To assess the amount of alcohol consumption, 13 studies used an unspecified questionnaire[12-24], 8 studies the AUDIT or AUDIT-C[19,25-31], 5 studies the Skinner Lifetime Drinking History (SLDH) questionnaire[25,27,29,30,32], 13 studies an interview[14-16,18,26,28,33-39], and the method of assessing alcohol consumption was unknown in 4 studies[40-43] (some studies reported more than one method to assess

alcohol intake). In this study, light alcohol consumption was defined as ≤ 10 g/day or ≤ 1 drink/day and moderate alcohol consumption as ≤ 30 g/day or ≤ 3 drinks/day. The alcohol consumption could be categorized as light in 4 studies[21,32,34,42] and moderate in 18 studies[14,16,19,22,24-31,33,36-38,40,43]. A total of 10 studies investigated both the effect of light and moderate alcohol consumption[12,13,15,17,18,20,23,33,41].

Of the 32 included studies, 8 were performed in Europe (Italy, Finland, Sweden, United Kingdom, France, Spain)[17,26,28,29,33,38,39,43], 9 in Asia (China, Japan, South Korea)[12,13,15,16,20-22,37,42], 10 in the United States[23,25,27,30-32,34,35,40,41], 4 in Australia[14,18,19,24] and 1 study was international[36]. The diagnostic tools used for the diagnosis of NAFLD differed; 16 studies used liver biopsy[14,16,18,25-30,32,34,36,37,40,42], 10 studies used ultrasound[12,13,15,19,20,22,23], 2 studies the fatty liver index (FLI)[33], 1 study magnetic resonance imaging (MRI)[21], 1 study the hepatic steatosis index (HSI)[41], 1 study computed tomography (CT)[35], 1 study used ICD codes[17].

Quality assessment

The QualSyst tool[11] was used to assess the risk of bias; results are presented in Supplementary Table 5. The score of the included studies ranged from 11/22 to 21/22, with a mean score of 18.6/22. The most frequent limitations were (1) self-reported alcohol consumption, (2) cross-sectional design, and (3) small sample size.

Main outcomes

Liver fibrosis

Twenty-one papers investigated the effect of light to moderate alcohol consumption on the prevalence of (advanced) fibrosis in NAFLD patients (Table 1). Odds ratios were available in ten studies[15,18,19,26,28-30,34,39,43] and could be calculated from the available data or from data obtained after contact with the authors in eight[15,16,20-22,27,32,37]. The three remaining articles used HRs or RRs[12,13,17].

Advanced fibrosis

Advanced fibrosis was the main outcome in 15 of the 21 studies[12,13,15,16,18-22,26,27,30,32,37,43]. Six described the effect of light (≤ 1 drink/day or ≤ 10 g/day) and 13 the effect of moderate alcohol consumption (≤ 3 drinks/day or ≤ 30 g/day). Separate meta-analyses were performed for both categories. When no distinction was made in the original study, the study was included in the latter analysis.

Light alcohol consumption

Four cross-sectional studies investigated the odds of advanced fibrosis when consuming a light amount of alcohol compared to no drinking, using ORs[15,18,21,32], and two cohort studies investigated the development to advanced fibrosis using HRs[12,13].

Light alcohol consumption, compared to abstinence, was associated with a non-significantly decreased risk of advanced liver fibrosis in the cross-sectional studies (0.69 (95% CI 0.29-1.61)). The heterogeneity was high with an I^2 of 68%. Specifically, two studies based on liver biopsy reported a significantly lower prevalence of advanced fibrosis in those consuming low amounts of alcohol[18,32]. This association was not present in two larger studies employing non-invasive disease markers[15,21]. A meta-analysis of two nationwide Korean cohort studies reporting on the progression to advanced fibrosis, measured by the FIB-4 score, indicated a positive association between light alcohol consumption and advanced fibrosis, with a pooled HR of 1.11 (95% CI 1.05-1.18) (Figure 2A).

Moderate alcohol consumption

Of the articles comparing no alcohol consumption with moderate alcohol consumption, four were cohort studies[12,13,16,22] and nine had a cross-sectional design[15,18-20,26,27,30,37,43]. In a random effects model meta-analysis of studies reporting an OR, moderate alcohol consumption was associated with a non-significantly increased risk for advanced fibrosis with a pooled OR of 1.28 (95% CI 0.81-2.03). The heterogeneity was high, with an I^2 of 78%. The meta-analysis of cohort studies using HR demonstrated a higher risk for developing advanced fibrosis in the moderate drinking group compared to the non-

drinking group, with a pooled HR of 1.39 (95% CI 1.22-1.57). The heterogeneity was high, with an I^2 of 73% (Figure 2B). To assess publication bias, a funnel plot was constructed for the studies included in the meta-analysis, which was symmetric, suggesting the absence of publication bias (Supplementary figure 1). A sensitivity analysis, excluding studies which grouped patients with light and moderate alcohol use together, yielded similar results, with a trend towards retention of studies showing more harmful effects (Supplementary Figure 2).

We performed a subgroup analysis according to study design, namely cross-sectional vs. cohort studies (Figure 2C), revealing that cross-sectional studies in aggregate did not find significantly different odds for advanced fibrosis in the moderate drinking group and the non-drinking group (pooled OR 1.20 (95% CI 0.65-2.20)); whereas cohort studies reported higher odds for advanced fibrosis in the moderate drinking group (pooled OR 1.56 (95% CI 1.08-2.26); I^2 0%). Indeed, the cohort studies by Chang et al. also demonstrated a statistically significant association between moderate alcohol consumption and worsening of non-invasive fibrosis markers over time. The results of the cross-sectional studies were inconsistent, on the other hand, with an I^2 of 81%. These are described in detail in Table 1. There was no clear association between the direction of the association and the use of specific diagnostic tools, e.g. liver biopsy versus non-invasive diagnosis by serum risk scores or imaging (Supplementary Figure 5). The paper by Dunn et al. was not included in the meta-analysis as the study population overlapped with that of Vilar-Gomez et al[27,30]. Both studies analyzed the NASH CRN database; the latter was included because of the larger sample size.

Other fibrosis outcomes

The remaining studies investigated either fibrosis stage as such or significant fibrosis (F≥2), reporting similar conflicting results as obtained in our meta-analysis (Table 1). A prospective cohort study reported that the worsening of fibrosis over time was similar among modest drinkers and non-drinkers (0.08 (95% CI -0.13-0.24) vs. 0.06 (95% CI -0.22-0.27); p = 0.85)[25]. Ekstedt et al. found that moderate alcohol use was not significantly associated with fibrosis (OR for weekly alcohol consumption 1.01 (95%

CI 1.00-1.03; p = 0.055))[28]. In contrast, Hägstrom et al. reported a significant association between low to moderate alcohol consumption and lower fibrosis stages (aOR for each unit increase 0.86 (95% CI 0.76-0.97; p = 0.017))[29].

Cirrhosis

Three papers studied the effect of light to moderate alcohol consumption on the progression of NAFLD to cirrhosis (Table 1). In a retrospective study, Kimura et al. reported an association between moderate alcohol consumption and cirrhosis (self-calculated OR 2.68 (95% CI 1.00-7.18; p = 0.05))[16]. A second study calculated the relative risk in healthy population groups with BMI 25-30 kg/m^2 and BMI $\geq$ 30 kg/m^2 compared to a reference group with a BMI 22.5-25 kg/m^2 and alcohol consumption of < 70 g/week). In subjects with a BMI 25-30 kg/m^2 and $\geq$ 30 kg/m^2, moderate alcohol consumption (70-150 g/week) was associated with a higher relative risk for cirrhosis (aRR 1.83 (95% CI 1.56-2.16) and 2.31 (95% CI 1.81-2.94), respectively), as there was an additive effect of both BMI and alcohol intake[17]. One cross-sectional study used LACU, an indicator for lifetime alcoholic cumulative units defined as drinking years multiplied by median alcohol units in a week divided by 7, to assess the impact of moderate alcohol consumption on the development of cirrhosis. Patients in the lower three quartiles of moderate alcohol use had a lower risk of cirrhosis compared to lifelong abstainers (OR 0.42 (95% CI 0.19-0.95; p = 0.037), an effect not observed for patients within the highest quartile (OR 1.35 (95% CI 0.54-3.36)[39].

Non-alcoholic steatohepatitis

Six papers studied the effect of light to moderate alcohol consumption on the prevalence of NASH in patients with biopsy-proven NAFLD (Table 2). Odds ratios were available in five studies[14,25,29,30,34] and could be calculated from the available data in the remaining study[27].

In a bariatric surgery cohort, weekly drinkers had similar odds of having steatohepatitis compared to less frequent drinkers (OR 1.14 (95% CI 0.50-2.60))[34]. In a similar setting, Dixon and colleagues reported that patients who consumed alcohol had a significantly lower prevalence of histological NASH (OR 0.35

(95% CI 0.12-1.0; p = 0.04)[14]. Two studies by the NASH CRN[27,30], with overlapping populations found that moderate drinkers had a lower risk of definite NASH compared to non-drinkers, with an OR of 0.70 (95% CI 0.54-0.91; p = 0.008) in the study of Vilar-Gomez et al, which included 1153 patients[30]. In accordance, Hägstrom et al. found that for each additional unit of alcohol, the odds of having NASH became lower, but the results were not significant (OR for each additional unit 0.98 (95% CI 0.86-1.11; p = 0.71)[29]. Challenging the beneficial impact of alcohol on NASH, a prospective cohort study by Ajmera et al. reported lower odds of NASH resolution in consistent modest drinkers compared to consistent non-drinkers (aOR 0.32 (95% CI 0.11-0.92; p = 0.04)[25].

Hepatocellular carcinoma

Six papers studied the effect of light to moderate alcohol consumption on development of HCC in patients with biopsy-proven NAFLD or biopsy-proven NAFLD with advanced fibrosis (Table 2). All three studies in patients with biopsy-proven NAFLD found that light or moderate alcohol consumption was associated with a higher risk for HCC. The first study reported an aHR of 3.8 (95% CI 1.6-8.9; p = 0.002) for social drinking (≤ 2 drinks/day or 3-6 drinks/day on weekends) compared to non-drinking[40]. In the second study, moderate consumption (< 20 g alcohol/day) in NAFLD patients was associated with a non-significantly increased aRR of 4.43 (95% CI 0.88-22.4; p = 0.07). However, in patients with advanced fibrosis, this association was statistically significant (aRR 4.83 (95% CI 1.01-23.00; p = 0.04))[16]. The third study reported that light alcohol consumption (< 30 g/week and < 20 g/week for men and women respectively) was a risk factor for HCC in non-cirrhotic NAFLD patients (OR 4.89 (95% CI 1.92-12.45; p < 0.01))[42]. The study by Vilar-Gomez et al. in patients with biopsy-proven NAFLD and advanced fibrosis found that moderate alcohol consumption increased the risk of HCC among cirrhotic patients (aHR 3.22 (95% CI 1.64-6.32; P <0.01)). However, no significant association was found among patients with bridging fibrosis[36]. In contrast, a large study in US veterans did not report an association between moderate alcohol use and HCC (HR 1.01 (95% CI 0.76-1.34))[31]. In the abovementioned study based on lifetime alcohol consumption, patients in the lower quartiles of alcohol use presented with a lower risk

of HCC compared to lifelong abstainers (OR 0.32 (95% CI 0.08-0.98; p = 0.043)), which was not observed in those within the highest quartile of moderate consumption (OR 1.79 (95% CI 0.54-5.92; p = 0.342))[39].

Secondary outcomes

Mortality

Six prospective cohort studies investigated the impact of light to moderate alcohol consumption on mortality in patients with NAFLD (Table 3). In a meta-analysis (of three of these studies) based on the reported hazard ratios, patients with light alcohol consumption had a significantly lower risk of all-cause death compared to patients consuming no alcohol (pooled HR 0.79; 95% CI 0.68-0.92; I^2 0%)[23,33,41] (Figure 3). However, patients with moderate alcohol consumption had a similar risk of death compared to patients with no alcohol consumption (pooled OR 1.07; 95% CI 0.73-1.56; I^2 61%)[23,33,41]. Decraecker et al. found that in NAFLD patients, drinking more than 7 units per week was associated with a significantly increased mortality (p = 0.001)[38]. A study performed in NAFLD patients with advanced fibrosis found that moderate alcohol consumption significantly increased the risk of death or transplantation in cirrhotic patients (aHR 2.30 (95% CI 1.32-4.02; p <0.01)), but not in patients with bridging fibrosis[36]. On the other hand, a study in US veterans with NAFLD cirrhosis reported a significantly lower overall mortality in patients with light-to-moderate alcohol use (aHR 0.86 (95% CI 0.79-0.95))[31].

Cardiovascular outcomes

Seven papers reported on the effect of light to moderate alcohol consumption on cardiovascular outcomes in patients with NAFLD, based either on cardiovascular mortality, the incidence of cardiovascular events, or on measures of subclinical atherosclerosis (Table 3).

Moderate alcohol consumption, as compared to abstinence and light consumption in aggregate, did not affect cardiovascular mortality in an Australian population[24]. Aberg et al. investigated the impact of alcohol use on CVD events and concluded that both light and moderate alcohol consumption were associated with a reduced risk for CVD events. Nevertheless, this reached significance only for light

alcohol consumption (aHR 0.78 (95% CI 0.64-0.95) and 0.78 (95% CI 0.60-1.01) for light and moderate alcohol use, respectively)[33]. Conversely, moderate alcohol use slightly but significantly increased the risk of cardiovascular events in a French cohort (OR 1.04 (95% CI 1.01-1.07; p = 0.021))[38]. Vilar-Gomez et al. did not observe a significant association between moderate alcohol consumption and incident cardiovascular events in NAFLD patients with advanced fibrosis[36].

Two studies investigated the impact of alcohol on subclinical cardiovascular damage by using the coronary artery calcification (CAC) score as outcome measure (CAC score > 0). Both studies concluded that light and moderate alcohol use were not significantly associated with subclinical cardiovascular damage measured by CAC (Kashiwagi et al. OR 1.52 (95% CI 0.81-2.86; p = 0.19); VanWagner et al. OR 1.46 (95% CI 0.94-2.28; p = 0.09))[15,35]. In contrast, two studies estimating cardiovascular risk by carotid plaques on ultrasound reported a protective effect[15,20]. Kashiwagi et al. concluded that moderate, but not light drinking, tended to reduce subclinical cardiovascular damage.[15] Sinn et al. reported that both light and moderate drinking were associated with lower odds of having carotid plaques. Nevertheless, only the results for light drinking were statistically significant. Similarly, the authors found that light drinking was significantly associated with lower odds of carotid artery stenosis.[20]

Discussion

In this systematic review and meta-analysis, we report conflicting results regarding the impact of light to moderate alcohol consumption on the occurrence of progressive NAFLD. Fibrosis was the most intensively studied outcome, yet the results were heterogeneous. Alcohol use may increase the risk of developing cirrhosis and HCC in NAFLD patients. In contrast, light alcohol use could be a protective factor on all-cause mortality.

A closer examination reveals several explanatory factors for the conflicting results, which are most apparent in the analysis of liver fibrosis, given the amount of data available on the latter outcome. A subgroup analysis revealed that while several cohort studies found a significant dose-dependent association between alcohol use and fibrosis, the cross-sectional studies generally reported either no

such association, or the reverse. Cross-sectional studies, in which alcohol use and liver disease are assessed simultaneously, are more sensitive to bias and confounding. There is the potential for recall bias – patients might report different levels of alcohol intake depending on their knowledge of disease status, and reverse causation – patients with known liver disease might have lowered their intake. For the cross-sectional studies based on liver biopsy in particular, there is the additional issue of referral bias – patients referred for liver biopsy might not be representative of the NAFLD population at large. Although it is, in our view, important to include all available studies in a systematic review and meta-analysis, these issues illustrate that more weight should be given to the results of cohort studies, which suggest a harmful effect of moderate alcohol intake. Furthermore, one very large prospective cohort study which focused on cirrhosis (fibrosis stage 4) also reported a harmful association between moderate alcohol consumption and cirrhosis[17]. Similarly, none of the studies on fibrosis as such or on progression of fibrosis suggested a protective effect of alcohol consumption.

The results in this meta-analysis stand in contrast with previous meta-analyses that documented a significant association between modest alcohol consumption and decreased development of liver fibrosis[6,7]. However, this meta-analysis included four recent studies, not included in these other meta-analyses[15,22,26], all showing significant harmful effects of moderate alcohol use on fibrosis severity. Furthermore, the meta-analysis by Wijarnpreecha et al. only included studies with a cross-sectional design, without presenting a clear rationale for this choice. As discussed, these studies are more liable to bias. On the other hand, the meta-analysis by Wongtrakul et al. only included studies in which NAFLD was diagnosed by liver biopsy, thereby excluding several studies that were included in our systematic which demonstrated harmful effects of moderate alcohol consumption[15,19,22]. In addition, both meta-analyses did not investigate the impact of light and moderate alcohol consumption separately[6,7]. This distinction can be important, because light consumption might have a different impact, or conversely, could point towards a dose-dependent effect of alcohol, a strong biological argument for pathophysiological relevance. Lastly, Wongtrakul et al. used multiple hazard ratios of the same studies (Aberg et al. and Hajifathalian et al.) in the same meta-analysis about mortality. This could lead to bias

due to the higher weight of the results from that particular study[7]. In contrast, a systematic review by Jarvis et al., specifically including longitudinal observational cohort studies, found that any level of alcohol consumption, even light to moderate alcohol consumption, is associated with worsening of liver outcomes in NAFLD. Nevertheless, this review only included a handful of studies and could therefore not make a clear distinction between the different outcomes[8].

We found that moderate alcohol consumption may increase the chance of progressing to HCC in NAFLD patients. These results stand in contrast with our summary of studies investigating mortality, in which, mainly light, alcohol use seemed to be associated with a decreased risk of all-cause death. Although seemingly contradictory, these findings could also indicate that cardiovascular outcomes play a larger role than HCC in overall mortality in NAFLD patients. Coronary artery disease has the biggest impact on mortality of all cardiovascular disorders, and light to moderate drinkers in the general population, especially wine drinkers, seem to have a lower risk of coronary artery disease[44]. While alcohol is a known risk factor for several cancers, the positive features of moderate alcohol use on cardiovascular risk may compensate these negative effects and may be even beneficial overall[45]. However, most studies included in this review did not observe statistically significant effects, calling into question whether moderate alcohol consumption does reduce the risk of CVD in patients with NAFLD.

This systematic review and the included articles have some limitations. First, most studies used self-reported alcohol consumption, a rather subjective exposure measurement. Participants may have underreported their alcohol use due to social bias. The amount of alcohol use could also be affected by recall bias because of the use of retrospective questionnaires. On the other hand, nine studies employed validated questionnaires such as the AUDIT and SLDH [19,25-30-32], which are standard instruments in the assessment of alcohol intake. Second, in the meta-analysis, both unadjusted and adjusted ORs and HRs were included, resulting in a possible source of heterogeneity. Nevertheless, the trend towards increased risk of fibrosis for moderate alcohol intake remained in a subgroup analysis

based on the use of adjusted vs. unadjusted ORs/HRs (Supplementary Figure 3). Third, statistical heterogeneity was moderate to high in most of the meta-analyses and subgroup analyses. The main reasons for this heterogeneity between studies could be the differences in population characteristics, assessment methods of alcohol consumption, study design, adjustment for confounders and accuracy of diagnostic tools. Fourth, it is also important to consider sample size and geographic region, which is also a proxy of genetic background. The included studies by Chang et al.[12,13] for instance are large population-based Korean studies, which might introduce bias. However, the results of these studies are in line with other studies, and therefore a subgroup analysis based on geographic region did not show significant differences between Western and Asian countries (Supplementary Figure 4).

Despite these limitations, this systematic review has several strengths. First, this systematic review provides a very accurate representation of the literature available about this subject. Multiple electronic databases were thoroughly sought for relevant articles by two independent reviewers. Second, this review examined several aspects of NAFLD, including all-cause mortality and cardiovascular outcomes, which is of added value compared to the current literature. Lastly, the definition of light to moderate alcohol consumption varied between the included studies. However, a strength of our systematic review was reducing misclassification by categorizing the studies in subgroups with light alcohol consumption (≤ 10 g/day or ≤ 1 drink/day) and moderate alcohol consumption (≤ 30 g/day or ≤ 3 drinks/day), and excluding patients who consumed higher amounts, regardless of the definition used in the article.

In conclusion, this systematic review demonstrates the wide heterogeneity in studies. Whereas light to moderate alcohol consumption was associated with a reduced prevalence of NASH, our analysis suggests a harmful effect on disease progression to advanced fibrosis, cirrhosis and HCC. However, light, but not moderate, alcohol consumption was associated with a protective effect on mortality, possibly due to a decrease in cardiovascular events. Based on the results of this review, there is not enough evidence to recommend complete alcohol abstinence to prevent disease progression in NAFLD

patients. However, considering the harmful results, the intake of light and especially of moderate amounts of alcohol also cannot be recommended to patients with NAFLD. Future longitudinal prospective cohort studies with validated outcomes are needed to clarify clinical decision making. In addition, the impact of type of alcohol consumption and pattern of alcohol intake on NAFLD progression should be further investigated, as data on this are limited.

References

1. Mitra S, De A, Chowdhury A. Epidemiology of non-alcoholic and alcoholic fatty liver diseases. Translational gastroenterology and hepatology. 2020;5:16.
2. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. Journal of hepatology. 2016;64(6):1388-402.
3. Targher G, Byrne CD, Lonardo A, Zoppini G, Barbui C. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis. J Hepatol. 2016;65(3):589-600.
4. Wandji LCN, Gnemmi V, Mathurin P, Louvet A. Combined alcoholic and non-alcoholic steatohepatitis. JHEP reports. 2020;2(3):100101.
5. Åberg F, Färkkilä M. Drinking and Obesity: Alcoholic Liver Disease/Nonalcoholic Fatty Liver Disease Interactions. Seminars in liver disease. 2020;40(2):154-62.
6. Wijarnpreecha K, Aby ES, Panjawatanan P, et al. Modest alcohol consumption and risk of advanced liver fibrosis in nonalcoholic fatty liver disease: a systematic review and meta-analysis. Annals of gastroenterology. 2021;34(4):568-74.
7. Wongtrakul W, Niltwat S, Charatcharoenwitthaya P. The Effects of Modest Alcohol Consumption on Non-alcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis. Frontiers in medicine. 2021;8:744713.
8. Jarvis H, O'Keefe H, Craig D, Stow D, Hanratty B, Anstee QM. Does moderate alcohol consumption accelerate the progression of liver disease in NAFLD? A systematic review and narrative synthesis. BMJ Open. 2022;12(1):e049767.
9. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. British Medical Journal. 2021;372:n71.
10. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. Systematic reviews. 2016;5(1):210.
11. Kmet LM, Lee RC, Cook LS. Standard Quality Assessment Criteria for Evaluating Primary Research Papers from a Variety of Fields. Edmonton: Alberta Heritage Foundation for Medical Research (AHFMR). 2004.
12. Chang Y, Cho YK, Kim Y, et al. Nonheavy Drinking and Worsening of Noninvasive Fibrosis Markers in Nonalcoholic Fatty Liver Disease: A Cohort Study. Hepatology. 2019;69(1):64-75.
13. Chang Y, Ryu S, Kim Y, et al. Low Levels of Alcohol Consumption, Obesity, and Development of Fatty Liver With and Without Evidence of Advanced Fibrosis. Hepatology. 2020;71(3):861-73.
14. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology. 2001;121(1):91-100.
15. Kashiwagi K, Yamaguchi A, Shiba S, et al. Moderate alcohol consumption is not associated with subclinical cardiovascular damage but with hepatic fibrosis in non-alcoholic fatty liver disease. Alcohol. 2020;89:1-7.
16. Kimura T, Tanaka N, Fujimori N, et al. Mild drinking habit is a risk factor for hepatocarcinogenesis in non-alcoholic fatty liver disease with advanced fibrosis. World journal of gastroenterology. 2018;24(13):1440-50.
17. Liu B, Balkwill A, Reeves G, Beral V. Body mass index and risk of liver cirrhosis in middle aged UK women: prospective study. BMJ (Clinical research ed). 2010;340:c912.
18. Mitchell T, Jeffrey GP, de Boer B, et al. Type and Pattern of Alcohol Consumption is Associated With Liver Fibrosis in Patients With Non-alcoholic Fatty Liver Disease. American Journal of Gastroenterology. 2018;113(10):1484-93.
19. Patel PJ, Smith D, Connor JP, et al. Alcohol consumption in diabetic patients with nonalcoholic fatty liver disease. Canadian Journal of Gastroenterology and Hepatology. 2017;2017.
20. Sinn DH, Gwak GY, Cho J, et al. Modest alcohol consumption and carotid plaques or carotid artery stenosis in men with non-alcoholic fatty liver disease. Atherosclerosis. 2014;234(2):270-5.

21. Wong VWS, Chu WCW, Wong GLH, et al. Prevalence of non-alcoholic fatty liver disease and advanced fibrosis in Hong Kong Chinese: A population study using proton-magnetic resonance spectroscopy and transient elastography. Gut. 2012;61(3):409-15.
22. Yamamura S, Eslam M, Kawaguchi T, et al. MAFLD identifies patients with significant hepatic fibrosis better than NAFLD. Liver Int. 2020;40(12):3018-30.
23. Younossi ZM, Stepanova M, Ong J, et al. Effects of Alcohol Consumption and Metabolic Syndrome on Mortality in Patients With Nonalcoholic and Alcohol-Related Fatty Liver Disease. Clinical Gastroenterology and Hepatology. 2019;17(8):1625-33.e1.
24. Janjua M, Knuiman M, Divitini M, et al. Alcohol Consumption and Cardiovascular Outcomes in Patients With Nonalcoholic Fatty Liver Disease: A Population-Based Cohort Study. Hepatology communications. 2022;6(3):526-34.
25. Ajmera V, Belt P, Wilson LA, et al. Among Patients With Nonalcoholic Fatty Liver Disease, Modest Alcohol Use Is Associated With Less Improvement in Histologic Steatosis and Steatohepatitis. Clinical Gastroenterology and Hepatology. 2018;16(9):1511-20.e5.
26. Blomdahl J, Nasr P, Ekstedt M, Kechagias S. Moderate alcohol consumption is associated with advanced fibrosis in non-alcoholic fatty liver disease and shows a synergistic effect with type 2 diabetes mellitus. Metabolism: clinical and experimental. 2021;115:154439.
27. Dunn W, Sanyal AJ, Brunt EM, et al. Modest alcohol consumption is associated with decreased prevalence of steatohepatitis in patients with non-alcoholic fatty liver disease (NAFLD). Journal of Hepatology. 2012;57(2):384-91.
28. Ekstedt M, Franzén LE, Holmqvist M, et al. Alcohol consumption is associated with progression of hepatic fibrosis in non-alcoholic fatty liver disease. Scandinavian Journal of Gastroenterology. 2009;44(3):366-74.
29. Hagström H, Nasr P, Ekstedt M, et al. Low to moderate lifetime alcohol consumption is associated with less advanced stages of fibrosis in non-alcoholic fatty liver disease. Scandinavian journal of gastroenterology. 2017;52(2):159-65.
30. Vilar-Gomez E, Sookoian S, Pirola CJ, et al. ADH1B∗2 Is Associated With Reduced Severity of Nonalcoholic Fatty Liver Disease in Adults, Independent of Alcohol Consumption. Gastroenterology. 2020;159(3):929-43.
31. Pearson MM, Kim NJ, Berry K, et al. Associations Between Alcohol Use and Liver-Related Outcomes in a Large National Cohort of Patients With Cirrhosis. Hepatology Communications. 2021;5(12):2080-95.
32. Kwon HK, Greenson JK, Conjeevaram HS. Effect of lifetime alcohol consumption on the histological severity of non-alcoholic fatty liver disease. Liver international : official journal of the International Association for the Study of the Liver. 2014;34(1):129-35.
33. Åberg F, Puukka P, Salomaa V, et al. Risks of Light and Moderate Alcohol Use in Fatty Liver Disease: Follow-Up of Population Cohorts. Hepatology. 2020;71(3):835-48.
34. Singla S, Charlton MR, Shah V, Dunn W. Influence of modest alcohol consumption on histologic severity of NAFLD in patients undergoing bariatric surgery. Gastroenterology. 2010;138(5):S806.
35. VanWagner LB, Ning H, Allen NB, et al. Alcohol Use and Cardiovascular Disease Risk in Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology. 2017;153(5):1260-72.e3.
36. Vilar-Gomez E, Calzadilla-Bertot L, Wai-Sun Wong V, et al. Fibrosis Severity as a Determinant of Cause-Specific Mortality in Patients With Advanced Nonalcoholic Fatty Liver Disease: A Multi-National Cohort Study. Gastroenterology. 2018;155(2):443-57 e17.
37. Yamada K, Mizukoshi E, Seike T, et al. Light alcohol consumption has the potential to suppress hepatocellular injury and liver fibrosis in non-alcoholic fatty liver disease. PloS one. 2018;13(1):e0191026.
38. Decraecker M, Dutartre D, Hiriart JB, et al. Long-term prognosis of patients with alcohol-related liver disease or non-alcoholic fatty liver disease according to metabolic syndrome or alcohol use. Liver Int. 2022;42(2):350-62.

39. Ferri S, Stefanini B, Mulazzani L, et al. Very Low Alcohol Consumption Is Associated with Lower Prevalence of Cirrhosis and Hepatocellular Carcinoma in Patients with Non-Alcoholic Fatty Liver Disease. Nutrients. 2022;14(12).
40. Ascha MS, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. Hepatology (Baltimore, Md). 2010;51(6):1972-8.
41. Hajifathalian K, Sagv T, B, McCullough AJ. Effect of Alcohol Consumption on Survival in Nonalcoholic Fatty Liver Disease: A National Prospective Cohort Study. Hepatology. 2019;70(2):511-21.
42. Tobari M, Hashimoto E, Taniai M, et al. The characteristics and risk factors of hepatocellular carcinoma in nonalcoholic fatty liver disease without cirrhosis. Journal of Gastroenterology and Hepatology (Australia). 2020;35(5):862-9.
43. Vallejo-Senra N, Mauriz-Barreiro V, Molina-Perez E, Dominguez-Munoz J. Prevalence and risk factors associated with progression to advanced fibrosis and cirrhosis in non-alcoholic fatty liver disease: analysis of a prospective registry. UEGW Week; 9 October 2021: United European Gastroenterology Journal; 2021. p. 661.
44. Klatsky AL. Alcohol and cardiovascular diseases: where do we stand today? Journal of internal medicine. 2015;278(3):238-50.
45. EASL Clinical Practice Guidelines: Management of alcohol-related liver disease. Journal of hepatology. 2018;69(1):154-81.

Table 1. Baseline characteristics of included studies - Fibrosis, cirrhosis and other fibrosis outcomes

Author, Year, Study location	Study Design	Sample size (stratified by alcohol intake)	Mean age, years (SD)	Definition of alcohol intake categories	Diagnostic tool for outcome	Outcome	Findings	Confounders adjusted for
Advanced Fibrosis								
Blomdahl, J.[26] 2021 Sweden	Cross-sectional study	Non- or light drinkers: 70 Moderate drinkers: 16	59.8 (11.1)	Moderate consumption: 66-140g/week	Liver biopsy	Advanced fibrosis	OR 9.70 (95% CI 1.40-68.9) (reference: 0-2.99 g/week)	Age, sex, BMI, type 2 diabetes
Chang, Y.[12] 2019 South Korea	Prospective cohort study	Non-drinkers: 13032 Light drinkers: 23659 Moderate drinkers: 22236	37.7 (7.3)	Light consumption: 1-9.9 g/day Moderate consumption: 10-29.9 (men) or 10-19.9 (women) g/day	Liver ultrasound Fibrosis risk scores	Development of advanced fibrosis (FIB-4)	Light alcohol use: HR 1.09 (95% CI 1.00-1.19) * Moderate alcohol use: HR 1.32 (95% CI 1.21-1.44) *	Age, sex, center, year of screening, smoking status, exercise, BMI, education, hsCRP,, HOMA-IR, metabolic comorbidities
Chang, Y.[13] 2020 South Korea	Retrospective cohort study	Non-drinkers: 60443 Light drinkers: 84241 Moderate drinkers: 45364	35.5	Light consumption: 1-9.9 g/day Moderate consumption: 10-29.9 (men) or 10-19.9 (women) g/day	Liver ultrasound FIB-4 score	Development of NAFLD and advanced fibrosis	Light alcohol use: HR 1.15 (95% CI 1.04-1.27) * Moderate alcohol use: HR 1.49 (95% CI 1.33-1.66) *	Age, sex, center, year of screening, smoking status, exercise, BMI, education, metabolic comorbidities
Dunn, W.[27] 2012 USA	Cross-sectional study	Non-drinkers: 251 Moderate drinkers: 331	48.3	Moderate consumption: <20g/day	Liver biopsy	Advanced fibrosis	OR° 0.53 (95% CI 0.37-0.77; P = 0.001) *	N/A
Kashiwagi, K.[15] 2020 Japan	Cross-sectional study	Non-drinkers: 102 Light drinkers: 103 Moderate drinkers: 63	60.2	Light consumption: 0.1-6.9 drinks/week Moderate consumption: 7-20.9 (men) or 7-13.9 (women) drinks/week	Liver ultrasound Fibrosis risk scores	Advanced fibrosis (NFS)	Light alcohol use: OR° 1.19 (95% CI 0.69-2.06; P = 0.53) * Moderate alcohol use: OR 2.91 (95% CI 1.72-4.94; P < 0.01) *	Age, sex, BMI, visceral adipose tissue, hypertension, diabetes, hyperlipidemia, ever smoking, exercise, HOMA-IR, and hsCRP
Kimura, T.[16] 2018 Japan	Retrospective cohort study	Non-drinkers: 208 Moderate drinkers: 93	56	Moderate consumption: <20g/day	Liver biopsy	Advanced fibrosis	OR° 1.29 (95% CI 0.75-2.24; P = 0.36) *	N/A
Kwon, H.[32] 2013 USA	Cross-sectional study	Non-drinkers: 25 Light drinkers: 52	46.9	Light consumption: ≤40g/week	Liver biopsy	Advanced fibrosis	OR° 0.32 (95% CI 0.11-0.98; P = 0.046) *	N/A
Mitchell, T.[18] 2018 Australia	Cross-sectional study	Non-drinkers: 74 Light drinkers: 72 Moderate drinkers: 19	51.5 (12.1)	Light consumption: 1-69 g/week Moderate consumption: 70-210 (men) or 70-140 (women) g/week	Liver biopsy	Advanced fibrosis	Light alcohol use: OR 0.29 (95% CI 0.10-0.87; P = 0.027) * Moderate alcohol use: OR 0.23 (95% CI 0.02-2.55: P = 0.21) *	Age, sex, diabetes, BMI, HOMA, lifetime alcohol consumption
Patel, P.[19] 2017 Australia	Cross-sectional study	Non-drinkers: 46 Light drinkers: 70 Moderate drinkers: 35	61 (10.3)	Light consumption: always ≤ 20 g/day Moderate consumption: any period with intake > 20 g/day	Transient elastography with CAP	Advanced fibrosis	Light alcohol use: OR 1.79 (95% CI 0.67-4.82; P = 0.247) * Moderate alcohol use: OR 0.91 (95% CI 0.27-3.10; P = 0.88) *	Age, gender, BMI

Sinn, D.[20] 2014 South Korea	Cross-sectional study	Non-drinkers: 483 Moderate drinkers: 1797	51.8 (8.4)	Light consumption: < 10 g/day Moderate consumption: 10-20 g/day	Liver ultrasound NFS	Advanced fibrosis	OR° 0.27 (95% CI 0.07-1.07; P = 0.06) *	N/A
Vallejo-Senra, N.[43] 2021 Spain	Cross-sectional study	Non- or light drinkers: 308 Moderate drinkers: 187	58 (range 19-86)	Moderate consumption: 1-3 units (men) or 1-2 units (women) per day	Liver biopsy, imaging and biochemistry	Advanced fibrosis	OR 1.9 (95% CI 1.1-3.3)	Diabetes and obesity, others unknown
Vilar-Gomez, E.[30] 2020 USA	Cross-sectional study	Non-drinkers: 720 Moderate drinkers: 433	50.7 (11.5)	Moderate consumption: ≤ 14 (men) or ≤ 7 (women) drinks/week	Liver biopsy	Advanced fibrosis	OR 0.77 (95% CI 0.58-1.02; P = 0.073) *	Age, sex, BMI, T2DM, PNPLA3 rs738409
Wong, V.[21] 2011 China	Cross-sectional study	Non-drinkers: 190 Light drinkers: 56 Moderate drinkers: 18	51 (9)	Light consumption: < 70 g/week Moderate consumption: ≥ 70 g/week	Transient elastography	Advanced fibrosis	Light alcohol use: OR° 1.75 (95% CI 0.51-6.04: P = 0.38) * Data obtained through contact with author	N/A
Yamada, K.[37] 2018 Japan	Cross-sectional study	Non-drinkers: 101 Moderate drinkers: 77	Non-drinkers: 53.1 (17.2) Moderate drinkers: 46.1 (14,4)	Moderate consumption: ≤ 20 g/day	Liver biopsy	Advanced fibrosis	OR° 0.59 (95% CI 0.28-1.23; P = 0.16) *	N/A
Yamamura, S.[22] 2020 Japan	Prospective cohort study	Non-drinkers: 284 Moderate drinkers: 140	56	Moderate consumption: < 20 g/day	Shear wave elastography and fibrosis risk scores	Advanced fibrosis	OR° 1.82 (95% CI 1.10-3.00; P = 0.02) *	N/A
Cirrhosis								
Ferri, S.[39] 2022 Italy	Cross-sectional study	Non-drinkers: 92 Light drinkers: 117 Moderate drinkers: 67	59 (range 18-88)	Light consumption: < 70 g/week Moderate consumption: 70-210 (men) or 70-140 (women) g/week	Clinical and imaging diagnosis	Cirrhosis	OR 0.42 (95% CI 0.19-0.95; P = 0.037) for Q1-Q3 lifetime consumption OR 1.35 (95% CI 0.54-3.36) for Q4 lifetime consumption Both compared to lifelong abstinence	Sex, BMI, hypertension, diabetes
Kimura, T.[16] 2018 Japan	Retrospective cohort study	Non-drinkers: 208 Moderate drinkers: 93	56	Moderate consumption: <20g/day	Liver biopsy	Cirrhosis	OR° 2.68 (95% CI 1.00-7.18; P = 0.05) *	N/A
Liu, B.[17] 2010 UK	Prospective cohort study	BMI < 22.5: 237619 BMI 22.5 - 25: 331480 BMI 25 - 27.5: 266795 BMI 27.5 - 30: 173498 BMI 30 - 35: 156733 BMI ≥ 35: 64537	56 (4.7)	Light consumption: < 70 g/week Moderate consumption: 70 - 150 g/week	N/A	Cirrhosis	BMI 25-30: 70-150 g/week RR 1.83 (95% CI 1.56-2.16) (reference: BMI 22.5-25 and < 70 g/week) BMI ≥ 30: 70-150 g/week RR 2.31 (95% CI 1.81-2.94) (reference: BMI 22.5-25 and < 70 g/week)	Age, region, socioeconomic status, physical activity, smoking

Other fibrosis outcomes								
Ajmera, V.[25] 2018 USA	Prospective cohort study	Non-drinkers: 117 Moderate drinkers: 168	47 (11)	Moderate consumption: 1-2 drinks/day	Liver biopsy	Change in fibrosis	Change in fibrosis stage among modest drinkers vs. non-drinkers: +0.08 (95% CI -0.13-+0.24) vs. +0.06 (95% CI -0.22-+0.27); P = 0.85	N/A
Ekstedt, M.[28] 2009 Sweden	Prospective cohort study	Total: 71 No data on categories	61.2 (11.2)	Moderate consumption: ≤140g/week	Liver biopsy	Progression of fibrosis	Weekly alcohol use: OR 1.01 (95% CI 1.00-1.03; P = 0.055)	N/A
Hagström, H.[29] 2016 Sweden	Prospective cohort study	Total: 120 No data on categories The median lifetime consumption was 1.1 drinks/week	55.9	Moderate consumption: <14 drinks/week	Liver biopsy	Fibrosis	Per unit of alcohol/week: OR for a higher fibrosis stage 0.86 (95% CI 0.76-0.97; P = 0.017)	BMI, diabetes, arterial hypertension, smoking status, and age at biopsy
Singla, S.[34] 2010 USA	Cross-sectional study	Non-drinkers: 57 Yearly drinkers: 36 Monthly drinkers: 31 Weekly drinkers: 41	N/A	Yearly: < 1 drink/month Monthly: < 1 drink/week Weekly: ≤ 1 drink/day	Liver biopsy	Fibrosis	Weekly drinkers: OR 1.11 (95% CI 0.41-1.75) (reference: infrequent and non-drinkers)	Age, gender, BMI, diabetes

*: reference: non-drinkers; °: OR calculated by the authors

Table 2. Baseline characteristics of included studies - Steatohepatitis (NASH) and hepatocellular carcinoma (HCC)

Author, Year, Study location	Study Design	Sample size (stratified by alcohol intake)	Mean age, years (SD)	Definition of alcohol intake categories	Diagnostic tool for outcome	Outcome	Findings	Confounders adjusted for
Steatohepatitis								
Ajmera, V.[25] 2018 USA	Prospective cohort study	Non-drinkers: 117 Moderate drinkers: 168	47 (11)	Moderate consumption: 1-2 drinks/day	Liver biopsy	Resolution of NASH	OR 0.32 (95% CI 0.11-0.92; P = 0.04) *	Age at biopsy, sex, race, and ever smoker
Dixon, J.[14] 2001 Australia	Cross-sectional study	Non-drinkers: 48 Light to moderate drinkers: 57	41 (11)	Moderate consumption: ≤200g/week	Liver biopsy	NASH	OR 0.35 (95% CI 0.12-1.00; P = 0.04) *	N/A Not significant after controlling for diabetes or IR index
Dunn, W.[27] 2012 USA	Cross-sectional study	Non-drinkers: 251 Moderate drinkers: 331	48.3	Moderate consumption: <20g/day	Liver biopsy	NASH	OR° 0.67 (95% CI 0.44-1.02; P = 0.06) *	N/A
Hagström, H.[29] 2016 Sweden	Prospective cohort study	Total: 120 No data on categories	55.9	Moderate consumption: ≤13 drinks/week	Liver biopsy	NASH	Units of alcohol/week: OR for NASH 0.98 (95% CI 0.86-1.11; P = 0.71)	N/A
Singla, S.[34] 2010 USA	Cross-sectional study	Non-drinkers: 57 Yearly drinkers: 36 Monthly drinkers: 31 Weekly drinkers: 41	N/A	Yearly: < 1 drink/month Monthly: < 1 drink/week Weekly: ≤ 1 drink/day	Liver biopsy	NASH	OR 1.14 (95% CI 0.50-2.60) (reference: infrequent and non-drinkers)	Age, gender, BMI, and diabetes
Vilar-Gomez, E.[30] 2020 USA	Cross-sectional study	Non-drinkers: 720 Moderate drinkers: 433	50.7 (11.5)	Moderate consumption: ≤14 (men) or ≤7 (women) drinks/week	Liver biopsy	NASH	OR 0.70 (95% CI 0.54-0.91; P = 0.01) *	Age, sex, BMI, T2DM, and PNPLA3 rs738409
Hepatocellular carcinoma								
Ascha, M.[40] 2010 USA	Retrospective cohort study	Non-drinkers: 120 Moderate drinkers: 58	56.6	Moderate consumption: ≤2 drinks/day and ≤6 drinks/day on weekends	Imaging	HCC	HR 3.80 (95% CI 1.60-8.90; P = 0.002) *	Age at time of cirrhosis diagnosis, male sex, non-Caucasian, BMI, ever smoked, and diabetes
Ferri, S.[39] 2022 Italy	Cross-sectional study	Non-drinkers: 92 Light drinkers: 117 Moderate drinkers: 67	59 (range 18-88)	Light consumption: < 70 g/week Moderate consumption: 70-210 (men) or 70-140 (women) g/week	Clinical diagnosis	HCC	OR 0.32 (95% CI 0.08-0.98; P = 0.043) for Q1-Q3 lifetime consumption OR 1.79 (95% CI 0.54-5.92; P = 0.342) for Q4 lifetime consumption Both compared to lifelong abstinence *	Sex, BMI, hypertension, diabetes, cirrhosis
Kimura, T.[16] 2018 Japan	Retrospective cohort study	Non-drinkers: 208 Moderate drinkers: 93	56	Moderate consumption: <20g/day	Imaging	HCC	RR 4.43 (95% CI 0.88-22.40; P = 0.07) *	Fibrosis stage at baseline, T2DM and serum triglycerides
Pearson, M.[31] 2021 USA	Retrospective cohort study	Non-drinkers: 5094 Moderate drinkers: 1321	62.1 (8.0)	Moderate consumption: AUDIT-C score of 1-3 in men and 1-2 in women	Clinical diagnosis	HCC	HR 1.01 (95% CI 0.76-1.34) *	Age, sex, ethnicity, BMI, HIV/HBV infection, diabetes, platelet count, bilirubin, creatinine, albumin, INR, hemoglobin, AST/ALT and history of decompensation
Tobari, M.[42] 2019 Japan	Prospective cohort study	Non-drinkers: 456 Light drinkers: 204	Non-cirrhotic NAFLD: 49 (25.0),	Light consumption: <30 (men) or <20 (women) g/week	Liver biopsy or imaging	HCC	Non-cirrhotic NAFLD-HCC: OR 4.89 (95% CI 1.92-12.45; P < 0.01) *	Sex and FIB4-index

			non-cirrhotic HCC: 73 (10.8)					
Vilar-Gomez, E.[36] 2018 International	Prospective cohort study	Non-drinkers: 392 Moderate drinkers: 66	55.9 (11.2)	Moderate consumption: ≤20 (men) or ≤10 (women) g/day	Liver biopsy or imaging	HCC	HR 3.22 (95% CI 1.64-6.32; P <0.01) for patients with cirrhosis * No significant effect in patients with bridging fibrosis (P = 0.15) *	Age, sex, ethnicity, current smoking, T2DM, Child-Pugh score, BMI, hypertension, medication, and history of malignant neoplasm

*: reference: non-drinkers; °: OR calculated by the authors

Table 3. Baseline characteristics of included studies - Mortality and cardiovascular outcomes

Author, Year, Study location	Study Design	Sample size (stratified by alcohol intake)	Mean age, years (SD)	Definition of alcohol intake categories	Diagnostic tool for outcome	Outcome	Findings	Confounders adjusted for
Mortality								
Åberg, F.[33] 2020 Finland	Prospective cohort study	Non-drinkers: 993 Light drinkers: 4429 Moderate drinkers: 1448	53.7 (12.6)	Light consumption: 0-9g/day Moderate consumption: 10-19g/day	/	All-cause mortality	Light alcohol use: HR 0.79 (95% CI 0.66-0.95) * Moderate alcohol use: HR 0.87 (95% CI 0.68-1.11) *	Age, sex, ASA use, education, employment, smoking, exercise, WC, diabetes, systolic blood pressure, cholesterol, triglyceride level, GGT, and history of cancer
Decraecker, M.[38] 2021 France	Prospective cohort study	Non- or light drinkers: 1021 Moderate drinkers: 390	55.4 (IQR 46.8-63.1)	Moderate consumption: 8-21 units (men) or 8-14 units (women) per week	/	All-cause mortality	>7 vs. ≤7 units per week increased mortality (P = 0.001) >1 vs. ≤1 units per week increased mortality (P = 0.025)	/
Hajifathalian, K.[41] 2019 USA	Prospective cohort study	Non-drinkers: 3318 Light or moderate drinkers: 1250	Non-drinkers: 48.9 (16.2), drinkers: 47.8 (15.9)	Light consumption: 0.5-1.4 drinks/day Moderate consumption: 1.5-2 (women) or 3 (men)/day	/	All-cause mortality	Light alcohol use: HR 0.64 (95% CI 0.42-0.97; P = 0.035) * Moderate alcohol use: HR 1.45 (95% CI 1.01-2.10; P = 0.047) *	Age, gender, smoking, race, physical activity, diet, education, and diabetes status
Pearson, M.[31] 2021 USA	Retrospective cohort study	Non-drinkers: 5094 Moderate drinkers: 1321	62.1 (8.0)	Moderate consumption: AUDIT-C score of 1-3 in men and 1-2 in women	/	All-cause mortality	HR 0.86 (0.79-0.95) *	Age, sex, ethnicity, BMI, HIV/HBV infection, diabetes, platelet count, bilirubin, creatinine, albumin, INR, hemoglobin, AST/ALT and history of decompensation and HCC
Vilar-Gomez, E.[36] 2018 International	Prospective cohort study	Non-drinkers: 392 Moderate drinkers: 66	55.9 (11.2)	Moderate consumption: ≤20 (men) or ≤10 (women) g/day	/	Death or liver transplant	HR 2.30 (95% CI 1.32-4.02; P <0.01) for patients with cirrhosis * No significant effect in patients with bridging fibrosis (P = 0.48) *	Age, ethnicity, current smoking, T2DM, varices, Child-Pugh score, BMI, hypertension, medication, and history of malignant neoplasm
Younossi, Z.[23] 2019 USA	Prospective cohort study	Non-drinkers: 2369 Light drinkers: 790 Moderate drinkers: 717 Substantial drinkers: 158	45.9 (SE 0.42)	Light consumption: 0-3 drinks/week (men) Moderate consumption: 4 drinks/week – 2 drinks/ day (men) Substantial consumption: 2-3 drinks/day (men)	/	All-cause mortality	Light alcohol use: HR 0.94 (95% CI 0.70-1.27; P = 0.69) * Moderate alcohol use: HR 0.91 (95% CI 0.63-1.31; p = 0.60) * Substantial alcohol use: HR 0.95 (95% CI 0.43-2.11; P = 0.89) *	Age, sex, ethnicity, smoking status, education, income, metabolic syndrome
Cardiovascular outcomes								
Åberg, F.[33] 2020 Finland	Prospective cohort study	Non-drinkers: 993 Light drinkers: 4429 Moderate drinkers: 1448	53.7 (12.6)	Light consumption: 0-9g/day Moderate consumption: 10-19g/day	ICD codes	CV events	Light alcohol use: HR 0.78 (95% CI 0.64-0.95) * Moderate alcohol use: HR 0.78 (95% CI 0.60-1.01) *	Age, sex, ASA use, education, employment, smoking, exercise, WC, diabetes, systolic blood pressure, cholesterol, triglyceride level, GGT, and history of cancer

Decraecker, M.[38] 2021 France	Prospective cohort study	Non- or light drinkers: 1021 Moderate drinkers: 390	55.4 (IQR 46.8-63.1)	Moderate consumption: 8-21 units (men) or 8-14 units (women) per week	/	CV events	OR 1.04 (95% CI 1.01-1.07; P = 0.021) For >7 vs. ≤7 units per week	/
Janjua, M.[24] 2022 Australia	Prospective cohort study	Non-drinkers: 76 Light drinkers: 368 Moderate drinkers: 215	56.9 (14.0)	Light consumption: 1-7 drinks/week Moderate consumption: 7-21 drinks/week	ICD codes	CV mortality	HR 0.813 (95% CI 0.456-1.448) for moderate vs. no/light consumption	Age, sex, BMI, CVD history, cholesterol, HDL, smoking, diabetes, SBP, and hypertension treatment
Kashiwagi, K.[15] 2020 Japan	Cross-sectional study	Non-drinkers: 102 Light drinkers: 103 Moderate drinkers: 63	60.2	Light consumption: 0.1-6.9 drinks/week Moderate consumption: 7-20.9 (men) or 7-13.9 (women) drinks/week	CAC Carotid ultrasound	Subclinical athero-sclerosis	CAC > 0: Light alcohol use OR° 1.19 (95% CI 0.69-2.07; P = 0.53) Moderate alcohol use OR° 1.52 (95% CI 0.81-2.86; P = 0.19); Carotid plaque: Light alcohol use OR° 1.17 (95% CI 0.66-2.08; P = 0.59) * Moderate alcohol use OR° 0.74 (95% CI 0.37-1.48; P = 0.39) *	N/A
Sinn, D.[20] 2014 South Korea	Cross-sectional study	Non-drinkers: 483 Light or moderate drinkers: 1797	51.8 (8.4)	Light consumption: <10g/day Moderate consumption: 10-20g/day	Carotid ultrasound	Subclinical athero-sclerosis	Carotid plaques: Light alcohol use OR 0.71 (95% CI 0.56-0.90; P < 0.05) Moderate alcohol use OR 0.80 (95% CI 0.62-1.04) * Carotid artery stenosis: Light alcohol use OR 0.47 (95% CI 0.31-0.72; P < 0.05) Moderate alcohol use OR 0.94 (95% CI 0.61-1.46) *	Age, smoking, and metabolic syndrome
VanWagner, L.[35] 2017 USA	Prospective cohort study	Non-drinkers: 238 Moderate drinkers: 332	50.4 (3.6)	Moderate consumption: ≤21 (men) or ≤14 (women) drinks/week	CAC	Subclinical athero-sclerosis	CAC > 0: alcohol use OR 1.46 (95% CI 0.94-2.28; P = 0.09) *	Center, age, race, sex, education, income level, smoking, physical activity, systolic blood pressure, total and HDL cholesterol, BMI, and metabolic comorbidities
Vilar-Gomez, E.[36] 2018 International	Prospective cohort study	Non-drinkers: 392 Moderate drinkers: 66	55.9 (11.2)	Moderate consumption: ≤21 (men) or ≤14 (women) g/day	Follow-up visits	CV events	Not significant in univariate analysis P = 0.31 for bridging fibrosis P = 0.56 for patients with cirrhosis	N/A

*: reference: non-drinkers; °: OR calculated by the authors

Figure legends

Figure 1. PRISMA flowchart of the study selection process.

Figure 2. (A-B) Forest plots for the association between light (A) or moderate (B) alcohol consumption on advanced fibrosis in NAFLD patients, separated by use of OR or HR. (C) Subgroup analysis based on study design.

Figure 3. Forest plots for the association of alcohol intake and mortality, divided according to light (upper panel) or moderate (lower panel) alcohol consumption.