Nutrition, Metabolism & Cardiovascular Diseases (2022) **32**, 2279–2288

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

SYSTEMATIC REVIEWS AND META-ANALYSES

Effect of pharmacological interventions and placebo on liver Histology in nonalcoholic steatohepatitis: A network meta-analysis

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Received 3 June 2022; received in revised form 1 July 2022; accepted 5 July 2022
Handling Editor: G. Targher
Available online 16 July 2022

KEYWORDS
NASH;
Meta-analysis;
Network meta-analysis

Abstract *Background:* The aims of this study were to quantify the histological improvement and its risk factors in patients with NASH enrolled in the placebo arms of randomized controlled trials (RCTs), and to indirectly compare the effect of several investigational drugs for NASH on validated histological outcomes.

Data synthesis: A comprehensive search was conducted to detect phase 2 and 3 RCTs comparing pharmacological interventions in patients with NASH. According to Food and Drug Administration (FDA) recommendations, primary outcomes included: 1) NASH resolution without worsening of fibrosis; 2) At least 1-point reduction in fibrosis without worsening of NASH. Meta-analysis and meta-regressions were conducted on placebo arms, while network meta-analysis was performed on intervention arms.

A total of 15 RCTs met the eligibility criteria. The meta-analysis on placebo arms showed a pooled estimate rate of 17% (95%C.I. 12%–23%;I^2 = 86%; p < 0.01) for NASH resolution without worsening of fibrosis and of 21% (95%C.I. 13%–31%;I^2 = 84%; p < 0.01) for ≥1stage improvement of fibrosis without worsening of NASH. Phase 3 (vs Phase 2)RCTs, older age and higher AST levels were significantly associated with progression of liver disease by univariate meta-regression. At network meta-analysis, Semaglutide (P-score 0.906), Pioglitazione alone (score 0.890) and plus Vitamin E (0.826) had the highest probability of being ranked the most effective intervention for NASH resolution without worsening of fibrosis, while Aldafermin (0.776), Lanifibranor (0.773) and Obeticholic acid (0.771) had the highest probability to achieve ≥1 stage of fibrosis improvement without worsening of NASH.

Abbreviations: NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; HCC, hepatocellular carcinoma; LD, liver decompensation; RCTs, randomized controlled trials; BMI, Body Mass Index; AST, aspartate aminotrasferase; ALT, alanine aminotrasferase; FDA, Food and Drug Administration; LRE, liver-related events; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

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https://doi.org/10.1016/j.numecd.2022.07.001

Conclusion: This study confirms the heterogeneity of histological progression of untreated patients with NASH and provides evidence to stratify patients according to identified risk factors in future RCTs of combination therapies. PROSPERO CRD42021287205.

1. Introduction

The prevalence of nonalcoholic fatty liver disease (NAFLD), because of the increasing spread of obesity and diabetes, is dramatically growing, and NAFLD has become the main cause of chronic liver disease worldwide in the last decade [1]. This picture is of great clinical and economic [2] impact because NAFLD patients, and especially those with nonalcoholic steatohepatitis (NASH) and liver fibrosis, are at high risk of developing liver-related events (LRE) – such as liver decompensation (LD) and hepatocellular carcinoma (HCC)- and extrahepatic complications – such as cardiovascular diseases and extrahepatic cancers [3–5].

Lifestyle modification, including dietary caloric restriction and increasing physical activity, with the goal of leading to weight loss of at least 5%–7% of baseline weight is the current backbone of NAFLD and NASH treatment because able to promote both NASH resolution and fibrosis improvement [6]. Unfortunately, structured and multidisciplinary programs are necessary to facilitate maintained weight loss achievement [7] and in real-life only a minority of patients can obtain weight loss. Therefore, the development of safe and effective pharmacological treatments for patients with NASH and fibrosis is a relevant unmet need.

In the last years, several drugs for the treatment of patients with NASH, tested against placebo and focused on different mechanisms of actions are under investigation in randomized controlled trials (RCTs) in various phases of development [8–10]. The Food and Drug Administration (FDA) defined the outcomes needed for drugs approval [11,12]. Specifically, in patients with non-cirrhotic NASH, $\geq$1 stage reduction in fibrosis stage without worsening of NASH and/or NASH resolution without worsening of fibrosis were the established surrogate histological endpoints of phase 3 RCTs necessary for the conditional approval of tested drugs, waiting for demonstrating the long-term effects on hard clinical outcomes like cirrhosis progression and development of LD and/or HCC [13–17]. However, the impact of FDA-defined histological outcomes on the natural history of NASH patients has been poorly studied to date.

Results from many RCTs in NASH patients reported a high variability in the response rate of placebo-treated arms, this relevant issue potentially affecting the overall meaning of the studies and sometimes leading to contrasting results from phase 2 to phase 3 trials [18–20]. Consistently, the identification of variables affecting the response rate in placebo-treated arms could lead to a better stratification of patients in placebo and active treatment arms. Furthermore, the lack of head-to-head comparison between the drugs in development, together with the before described high heterogeneity in placebo response, make it difficult to establish what drug could be preferred.

Therefore, by using FDA surrogate histological endpoints for clinical trials in non-cirrhotic NASH, the aims of the present study were 1) to provide an updated meta-analysis to quantify histological improvement in patients with NASH treated with placebo also identifying its predictors, and 2) to perform a comparative network meta-analysis to indirectly assess the benefit of drugs under investigation for NASH.

2. Methods

The reporting of this systematic review follows the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement [21]. Institutional review board approval and informed consent was waived as this was not an individual patient–data meta-analysis.

2.1. Search strategy and study selection

A systematic search of MEDLINE, EMBASE, and SCOPUS databases was performed including the following terms: “non-alcoholic steatohepatitis” OR “NASH” OR “non-alcoholic fatty liver” OR “NAFLD” combined with “randomized controlled trial”. The search included reports published until October 2021. The computer search was supplemented with manual searches of the reference lists of all review articles and primary studies retrieved to identify additional studies. When the results of a single study were reported in more than one publication, only the most recent and complete data were included in the meta-analysis. When the same intervention was evaluated in both phase 2 and 3 RCTs, only the phase 3 RCT was included for that intervention. Moreover, we performed a search for abstracts presented at main relevant hepatological conference proceedings (EASL, European Association for the Study of the Liver and AASLD, American Association for the Study of Liver Diseases) from 2017 to 2021. Abstracts that had been published subsequently as a full-text study were excluded if a full-text study already was included in the meta-analysis. Two independent investigators (GP, CiC) reviewed the titles and abstracts of all papers identified by the search. Full-text manuscripts were retrieved for the included abstracts and were subsequently

screened for eligibility by two independent investigators (GP, CiC). Discrepancies among reviewers were not frequent (interobserver variation <10%) and were resolved by discussion.

2.2. Eligibility criteria

Studies were included in the meta-analysis if they met the following criteria: 1) they were randomized phase 2 or phase 3 trials evaluating pharmacological agents for NASH; 2) they enrolled patients with biopsy-proven NASH; 3) they compared one or more pharmacological agent with placebo or with each other; 4) they reported at least one of the two FDA-based endpoints, [11] that are a) resolution of NASH without worsening of fibrosis or b) one or more stage reduction of fibrosis without worsening of NASH); 5) they had a minimum follow-up of 24 weeks.

Studies were excluded if: 1) they were observational studies; 2) they evaluated only lifestyle modifications; 3) they did not report histological results of follow-up liver biopsy after treatment; 4) they included less than 30 patients.

2.3. Data extraction

Study-level variables included the last name of the first author, the publication year, the trial phase, the number of centers (single versus multiple), the duration of follow-up, the names of intervention(s) and their dosing, the number of patients receiving intervention(s) and placebo and the definition of placebo. When different dosing of the same intervention or different combinations of interventions were evaluated in the same RCT, only data on the most effective arm were extracted.

Patient-level variables included age, gender, diabetes, body weight, body mass index (BMI), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and NAS score.

Risk of bias was assessed using the Cochrane risk of bias assessment tool in which studies were deemed to be at low, high, or unclear risk [22]. This tool includes the following domains: random sequence generation, allocation concealment, blinding, incomplete outcome data, and selective outcome reporting. Only full-text papers were evaluated for the risk of bias.

2.4. Outcomes

The number of patients who achieved NASH resolution without worsening of fibrosis and the number of patients who achieved the improvement of one or more stage of fibrosis without worsening of NASH were extracted as main histological outcome measures for intervention and placebo arms of each included trial. The proportion of patients achieving these endpoints was calculated after the exclusion of patients with no follow-up biopsy.

2.5. Statistical analysis

First, we performed a conventional meta-analysis of placebo arms of included RCTs. The pooled estimates of the achievement of the two main histological outcomes were obtained using a random-effects model with the generic inverse variance method. The method of moments estimator, proposed by DerSimonian and Laird, was used to assess between-study variance [23]. Heterogeneity was assessed with the Pearson chi-square test and the I^2 statistic. Univariate logistic meta-regression analysis was used to examine associations between patient- or study-level covariates and main histological outcomes and to explore and explain the heterogeneity in the placebo rate. Variables with a P value less than 0.10 was considered statistically significant. The amount of heterogeneity in the outcomes explained by risk factors was evaluated with the R2 index.

Next, we performed a frequentist network meta-analysis of intervention arms. For the two main histological outcomes, odds ratios (OR) and 95% confidence intervals (95% C.I.) were meta-analysed using the DerSimonian and Laird random-effects model [23]. The frequentist approach provides a point estimate from the network along with 95% Confidence Interval (95% C.I.) from the frequency distribution of the estimate. We calculated the relative ranking of the interventions for achieving the main histological outcomes as their P-scores [24]. P-score ranges between 0 when a treatment is certainly the worst, and 1 when a treatment is certainly the best. Univariate logistic meta-regression analysis was used to examine associations between patient- or study-level covariates and main histological outcomes. Finally, P-scores of treatments for the two main histological outcomes were added together to identify the most effective drugs overall. Sensitivity analyses included Bayesian network meta-analysis with relative ranking of interventions calculated as their surface under the cumulative ranking (SUCRA) [25] and Bayesian meta-regression analysis. The Egger regression test was performed to evaluate the asymmetry of the Begg funnel plot and potential publication bias. All analyses were performed with R (R Core Team, 2020).

2.6. Certainty of evidence

The GRADE approach was used to rate the certainty of evidence of estimates derived from direct meta-analysis [26,27]. According to the GRADE approach, direct evidence from RCTs starts at high certainty and can be rated down, based on risk of bias, indirectness, imprecision, inconsistency (or heterogeneity) and/or publication bias, to levels of moderate, low and very low.

The certainty of evidence (ie, certainty in the estimates) was evaluated using the Confidence in Network Meta-Analysis (CINeMA) approach [28]. CINeMA approach includes the following six domains: within-study bias (i.e. the impact of risk of bias in the included studies), reporting bias (i.e. publication and other reporting bias),

indirectness, imprecision, heterogeneity and incoherence. The reviewer's input is required at the study level for within-study bias and indirectness. After the application of the user-defined rules, judgments for each domain are made as: no concerns, some concerns, or major concerns. Judgments across domains are summarised to obtain four levels of certainty for each relative treatment effect: very low, low, moderate or high certainty of evidence [28].

3. Results

3.1. Literature search and study characteristics

The flow-chart of the study selection process is showed in Fig. S1. Our primary search identified 5108 titles and abstracts. After review of the studies, 15 RCTs (14 full-papers and 1 abstract) [29–43] enrolling 4.499 patients (2.714 patients receiving interventions and 1.785 receiving placebo) fulfilled the inclusion criteria and were selected for meta-analysis. Study-level characteristics of included RCTs are shown in Table S1. Eleven studies [29,31–33,35–37,39–42] were phase 2 RCTs and all but one [33] were multicenter RCTs. Only one RCT [30] had two active arms (Pioglitazone and Vitamin E). Follow-up duration ranged from 24 [32,42] to 96 weeks [29,30].

Table S2 shows the patient-level characteristics of included RCTs. The mean age ranged from 46.6 [30] to 60 years [35] and from 45.4 [30] to 61 years [38] in the intervention group and placebo group, respectively. One study included only nondiabetic patients [30], while one study [35] included only diabetic patients; in the remaining studies, the percentage of patients with diabetes ranged from 28% [32] to 76% [38] for interventions and from 28% [32] to 78% [38] for placebo. Mean baseline BMI ranged from 29.5 [36] to 35.8 kg/m2 [34,38] for interventions and from 32.2 [36] to 36.8 kg/m2 [38] for placebo. Mean baseline ALT levels ranged from 40 [35] to 86 IU/mL [30] for interventions and from 44 [38] to 81 IU/mL [30] for placebo. Mean baseline AST levels ranged from 32 [35] to 59 IU/mL [31] for interventions and from 32 [32] to 59 IU/mL [34] for placebo. The mean baseline NAS ranged from 3.7 [35] to 5.7 [40] for interventions and from 4.2 [35] to 5.5 [37] for placebo.

Table S3 reports the risk of bias assessment for the included studies, showing an overall low risk of bias.

3.2. Meta-analysis of placebo arms, response rate in NASH patients

The pooled estimate for the rate of NASH resolution without worsening of fibrosis (that was available in 15 RCTs [29–43] including 1610 patients) was 17% (95% C.I. 12%–23%, I^2 = 86%, p < 0.01), ranging from 5% [41] to 36% [31] (Fig. 1A). Among the variables assessed by univariate meta-regression analysis, trial phase 3 and age were significantly associated with a lower placebo rate, accounting for 47% and 39% of the overall heterogeneity, respectively (Table 1).

The pooled estimate for the rate of ≥1 stage reduction of fibrosis without worsening of NASH (that was available in 7 RCTs [34,37–42] including 941 patients) was 21% (95% C.I. 13%–31%, I^2 = 84%, p < 0.01), ranging from 12% [34] to 39% [42] (Fig. 1B). Univariate logistic meta-regression analysis was performed to identify potential sources of heterogeneity among studies. Among the variables assessed, trial phase 3 and AST levels was significantly associated with a lower placebo rate, accounting for 58% and 59% of the overall heterogeneity, respectively (Table 1).

3.3. Network meta-analysis of NASH resolution without worsening of fibrosis

Sixteen interventions from 15 RCTs were evaluated for this outcome (Fig. 2). Compared to placebo, Semaglutide (OR 6.89 42%, 95% C.I. 2.90–16.35), Pioglitazone 45 mg (OR 6.44, 95% C.I. 2.59–16.06), Pioglitazone plus Vitamin E (OR 5.10, 95% C.I. 1.70–15.29), Vitamin E (OR 3.24, 95% C.I. 1.64–6.42), Lanifibranor (OR 3.11, 95% C.I. 1.58–6.10) and Pioglitazone 30 mg (OR 2.63, 95% C.I. 1.30-5-32) were significantly better than placebo (Fig. 3). Other interventions did not show superiority against placebo. According to P-score, Semaglutide (P-score 0.906), Pioglitazone 45 mg (P-score 0.890) and Pioglitazone plus Vitamin E (P-score 0.826) had the highest probability of being ranked the most effective intervention. Conversely, EPA-E (P-score 0.186), Emricasan (P-score 0.119) and Selonsertib (P-score 0.071) had the lowest probability of being ranked the most effective intervention. Heterogeneity evaluated by I^2 was 0% (p = 0.99). Univariate meta-regression analysis did not find any variables significantly associated with this outcome. In the sensitivity analysis, Bayesian network meta-analysis with SUCRA ranking and Bayesian meta-regression analysis showed similar results (Figs. S2 and S3).

3.4. Network meta-analysis of ≥1 stage reduction of fibrosis without worsening of NASH

Seven interventions were evaluated for this outcome (Fig. 4). Compared to placebo, Lanifibranor (OR 2.57, 95% C.I. 1.12–5.88) and Obeticholic Acid (OR 2.22, 95% C.I. 1.14–4.32) were significantly better than placebo (Fig. 5). Other interventions did not show superiority against placebo. According to P-score, Aldafermin (P-score 0.776), Lanifibranor (P-score 0.773) and Obeticholic Acid (P-score 0.711) had the highest probability of being ranked the most effective intervention, while Selonsertib (P-score 0.243) and Emricasan (P-score 0.066) had the lowest probability of being ranked the most effective intervention. Heterogeneity evaluated by I^2 was 49.3% (p = 0.16). Univariate meta-regression analysis did not find any variables significantly associated with this outcome. In the sensitivity analysis, Bayesian network meta-analysis with SUCRA ranking and Bayesian meta-regression analysis showed similar results (Figs. S4 and S5).

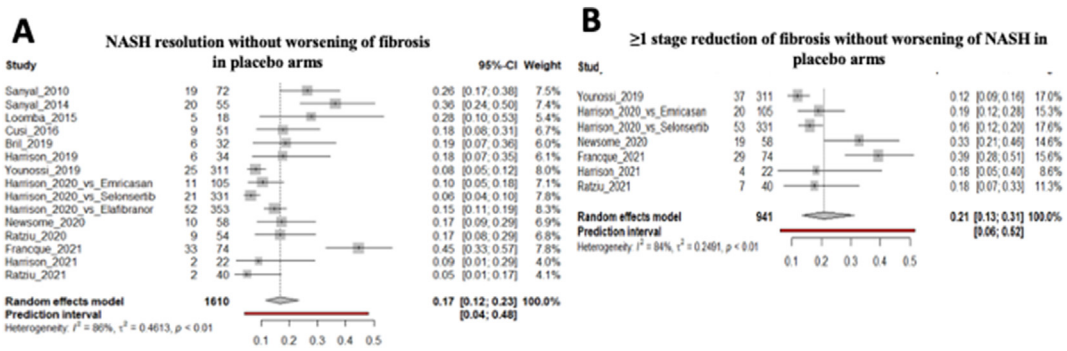

Figure 1 (A) Pooled estimate of the rate of NASH resolution without worsening of fibrosis. (B) Pooled estimate of the rate of ≥1 stage reduction of fibrosis without worsening of NASH.

3.5. Publication bias and certainty of evidence

Publication bias was not evaluable because only a single study was included for each drug comparison.

For the outcome of NASH resolution without worsening of fibrosis, Pioglitazone (both 30 and 45 mg), Vitamin E, Pioglitazone plus Vitamin E, Semaglutide and Lanifibranor had a better outcome when compared to Placebo, Cenicriviroc, Emricasan, Selonsertib, which was supported by high certainty of evidence. Pioglitazone 45 mg, Vitamin E, Pioglitazone plus Vitamin E, Semaglutide and Lanifibranor had a better outcome when compared to Elafibranor, with high certainty of evidence. Pioglitazone 45 mg and Semaglutide had a better outcome when compared with Obeticholic acid, Ezetimibe and Resmetiron, with high certainty of evidence. Pioglitazone 30 mg and Vitamin E had a better outcome when compared to EPA-E, with high certainty of evidence. Aramchol had a better outcome when compared with Emricasan and Selonsertib, with high certainty of evidence. Aldafermin, Elafibranor and Obeticholic acid had a better outcome when compared with Selonsertib, with high certainty of evidence. Other comparisons were supported by moderate to very low certainty of evidence, because of major concerns for within-study bias and imprecision (Supplementary Table S4).

For the outcome of ≥1 stage reduction of fibrosis without worsening of NASH, Obeticholic acid and Lanifibranor had a better outcome when compared to Placebo and Selonsertib, which was supported by high certainty of evidence. Obeticholic acid, Lanifibranor and Aldafermin had a better outcome when compared to Emricasan, with high certainty of evidence. Other comparisons were supported by low to very low certainty of evidence because of major concerns for imprecision (Supplementary Table S5).

4. Discussion

In this meta-analysis, collecting data of about 4500 patients enrolled in 15 placebo-RCTs on NASH drug treatment evaluating surrogate histological outcomes established by FDA for phase 3 trials, we found that the pooled rate of spontaneous NASH resolution without fibrosis worsening was 17% -ranging from 5% to 36%-, and the pooled rate of spontaneous ≥1 stage reduction of fibrosis without worsening of NASH was 21% -ranging from 12% to 39%. Meta-regression analysis showed older age and higher baseline AST levels were risk factors for more rapid histological progression, and that phase 2 vs phase 3 trials was an at lower risk setting. Moreover, network meta-analysis showed that Semaglutide and pioglitazone with/without Vitamin E had the highest probability of being the most effective therapeutic regimens for achieving NASH resolution without worsening of fibrosis, while aldafermin, lanifibranor and obethicolic acid had the highest probability of achieving ≥1 stage reduction of fibrosis without worsening of NASH.

The overall novelty of our study lies in the evaluation of hard composite histological endpoints - NASH resolution without worsening of fibrosis or ≥1 stage reduction of

Table 1 Predictors of histological outcomes (NASH resolution without worsening of fibrosis and ≥1 stage reduction of fibrosis without worsening of NASH) in placebo arms by univariate meta-regression.

	Studies (n)	Patients (n)	β	95% CI	*p-value*	R^2
NASH resolution without worsening of fibrosis						
Study year (from oldest to most recent)	15	1610	−0.10	−0.20; 0.01	0.065	14.2%
Phase 3 (vs phase 2)	15	1610	−1.06	−1.81; −0.32	0.008	46.7%
Age	15	1610	−0.11	−0.19; −0.03	0.011	39.0%
≥1 stage reduction of fibrosis without worsening of NASH						
Phase 3 (vs phase 2)	7	941	−0.74	−1.47; −0.02	0.046	58.6%
AST levels	7	941	−0.07	−0.15; −0.01	0.044	59.0%

NASH, non-alcoholic steatohepatitis. 95% C.I., 95% Confidence interval.

Figure 2 Network meta-analysis of comparisons between interventions and placebo for NASH resolution without worsening of fibrosis. Width of the lines is proportional to the number of randomly assigned participants.

fibrosis without worsening of NASH - designed by FDA for the conditional approval of drugs tested in phase 3 RCTs for the treatment of patients with noncirrhotic NASH. Although previous published meta-analyses [44–46] and network meta-analysis [47–51] found a similar heterogeneity of the placebo arms response rate in NASH patients, and indirectly compared pharmacological agents in NASH, a concern of these studies was that they assessed only singular histological endpoints like NASH, NAS score, fibrosis, steatosis, etc., but not the histological surrogate endpoints established by FDA for conditional approval.

The association between phase 3 RCTs and lower placebo-response compared to phase 2 RCTs could result from the higher sample size of phase 3 RCTs leading to lower heterogeneity. A recent meta-analysis reported lower sample size as a factor significantly affecting mean change in magnetic resonance spectroscopy-intrahepatic triglyceride content in NAFLD patients receiving placebo [44]. Consistently, previous studies also reported that heterogeneity tends to be greater between small compared to larger studies [52]. We could speculate that

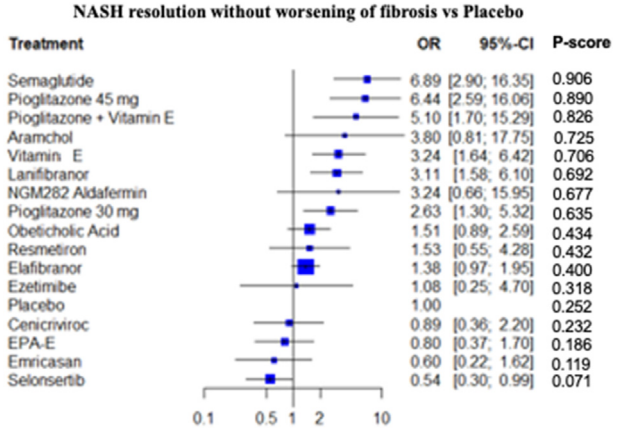

Figure 3 Forest plot of network meta-analysis of interventions compared to placebo ranking from best to worst based on P-score for NASH resolution without worsening of fibrosis.

ageing is a surrogate of the severity of liver disease and its risk factors, making more difficult an improvement in liver damage. When looking at AST, our data agree with a recent large study reporting that baseline AST levels and its changes can predict fibrosis regression/progression in NASH [44]. Although several previous studies downplayed the role of liver enzymes [53–57], showing that normal AST or ALT levels do not exclude the presence of significant histological abnormalities, it should be underlined that all these studies had a cross-sectional design. By contrast, our meta-regression analysis showed that high baseline AST levels were significantly associated with a lower probability of achieving a histological improvement at a second follow-up biopsy. Indeed, the proven efficacy of diet and physical activity in the improvement of NASH and fibrosis [6] may explain the response of placebo when lifestyle modifications were suggested. On the other side, the Hawthorne effect may also play a role to explain placebo rates in the absence of explicit lifestyle management in the trial design. The awareness of being observed may lead patients in placebo arms to modify their behavior with a progressive improvement of their histological features. From a clinical point of view, our meta-analysis can be helpful to better interpret the results of the effectiveness of pharmacological interventions and to properly design RCTs on NASH, better stratifying the baseline risk for liver disease progression.

Overall, results from our network meta-analysis and the complex pathophysiology of NASH [58,59] may suggest that NASH treatment could require targeting multiple pathways with different mechanisms of action, these considerations shedding light on the potential utility of multitargeted combination drugs. In fact, single drug therapy, even if targeting multiple pathogenic pathways, seems not enough effective on clinical relevant improvement of histological liver injury. Consistently, preliminary small studies searched for comparison of combination versus monotherapy in patients with NASH and advanced liver fibrosis [60]. All in all, we suggest that the design of future RCTs should explore the use of combination therapies for a successful treatment of NASH, for example a combination of GLP-1 agonists plus FXR/FGF19 agonists, or GLP-1 agonists in combination with panPPAR agonists. Semaglutide is a promising pharmacological intervention acting as GLP-1 receptor agonists given beneficial indirect effects on insulin resistance and body weight and given direct effects on lipotoxicity and liver inflammation [39,61]. Although our analysis showed that semaglutide was the most effective drug to achieve NASH resolution, we did not confirm a similar results for fibrosis improvement. This could be related to the short follow-up of RCT that evaluated semaglutide. Further, larger scale RCTs with a longer follow-up are needed to substantiate the long-term efficacy of semaglutide on fibrosis improvement. Pioglitazone regulates insulin sensitivity by the activation of peroxisome proliferator-activated receptor (PPAR)-γ, that modulates the transcription of genes involved in lipid metabolism and inducing mitochondrial beta-oxidation [33,62]. The addition of Vitamin E enhances antioxidant

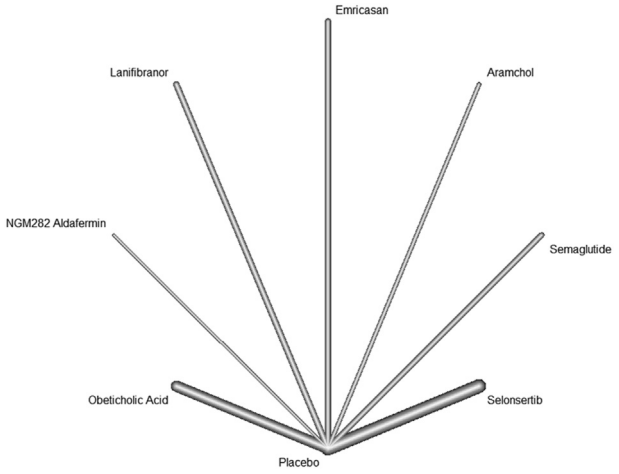

Figure 4 Network meta-analysis of comparisons between interventions and placebo for ≥1 stage reduction of fibrosis without worsening of NASH. Width of the lines is proportional to the number of randomly assigned participants.

effect from lipid peroxidation and oxygen-free radicals to prevent the cellular structure integrity [30,33]. Aldafermin is an engineered, non-tumorigenic analog of the human fibroblast growth factor 19 (FGF19) that leads to reduction in liver steatosis and improvement in insulin sensitivity [40]. Furthermore, Aldafermin reduces bile acid overload potentially accounting for an anti-fibrotic effect [63]. Lanifibranor acts as agonist in a well-balanced way on the three PPAR isoforms, better than single or dual agonism, resulting in simultaneous improvement of insulin sensitivity and inflammation; on the other hand, the multi-targerted mechanism of action likely gives a protective effect on fibrogenesis [42,62]. Obeticholic acid is a selective and potent agonist of farnesoid X receptor agonist, resulting in improvement of insulin sensitivity and decrease of hepatic lipid synthesis, as well as able it is able to modulate gut barrier integrity and to prompt an anti-inflammatory and antifibrotic effect [34].

This meta-analysis has some limitations. First, it was performed using aggregate data, therefore our summary results describe only between-study, not between-patient differences because they reflect group averages rather than individual data. Only an individual patient data meta-analysis can provide a more detailed treatment comparison in order to overcome the disease heterogeneity, finally implementing a personalized approach in NAFLD patients. Second, histological endpoints recommended by FDA for conditional approval were assessed as primary outcomes of our analysis. However, the surrogacy of these histological endpoints with clinical outcomes remains to be established. Third, limitation concerns about the intrinsic heterogeneity in meta-analysis and meta-regression. Differences in study design among trials (number of subjects and duration of RCTs, evolving definition of NASH resolution) and in outcome assessment, demographic and clinical features of patients enrolled, and the type of pharmacological interventions may limit the comparability of trials. Looking at the network meta-analysis, number of studies per comparisons and the network meta-analysis configuration may affect estimates of ranking probabilities [64]. However, our results were confirmed in a sensitivity analysis by using a Bayesian approach. Finally, small sample size of some of the evaluated trials like that on Aldafermin [40], and the lack of evaluation of the impact of experimental drugs on extra-hepatic outcomes, could further affect the interpretation of the results.

In conclusion, our meta-analyses performed on phase 2 and 3 RCTs assessing the efficacy of pharmacological treatments of NASH, have confirmed the highly heterogeneous spontaneous improvement rate in placebo-treated patients, affected by more advanced phase of study, older age and higher AST level at baseline. Semaglutide and pioglitazone appeared to be more effective on NASH activity while aldafermin, lanifibranor and obeticholic acid performed better on the evolution of fibrosis. In order to increase the efficacy of pharmacological treatments

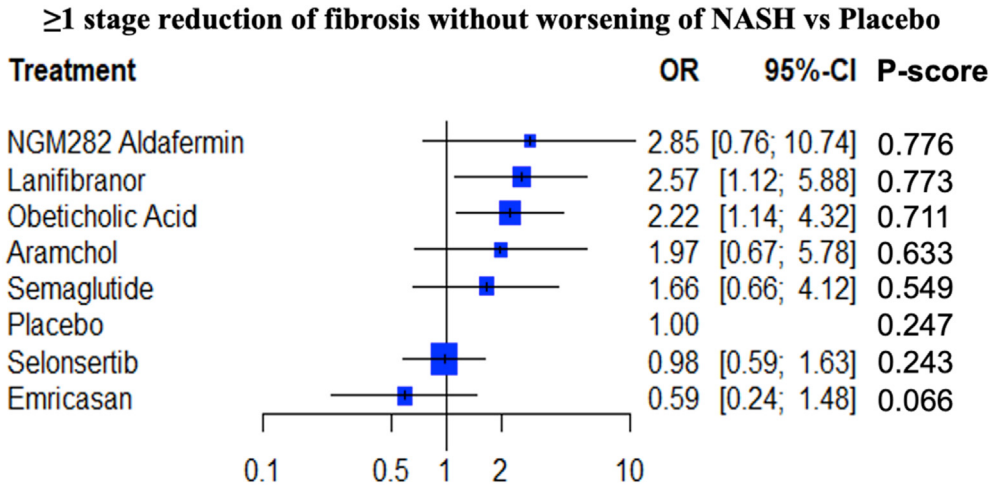

Figure 5 Forest plot of network meta-analysis of interventions compared to placebo ranking from best to worst based on P-score for ≥1 stage reduction of fibrosis without worsening of NASH.

reducing the time needed to reach clinically consistent histological improvements, combination of GLP-1 agonists plus FXR/FGF19 agonists, or GLP-1 agonists in combination with panPPAR agonists should be assessed prospectively.

Founding

None.

Author Contributions

Grazia Pennisi, Ciro Celsa, Marco Enea Marco Vaccaro, Vito Di Marco, Carlo Ciccioli, Giuseppe Infantino, Claudia La Mantia, Stefanie Parisi, Federica Vernuccio, Antonio Craxì, Calogero Cammà, Salvatore Petta had full control of the study design, data analysis and interpretation and preparation of article. All authors were involved in planning the analysis and drafting the article. The final draft article was approved by all the authors.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2022.07.001.

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