Diagnostic efficacy of serum miRNA as a non-invasive method for nonalcoholic steatohepatitis: a systematic review and meta-analysis.

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Abstract

1. Background: Nonalcoholic steatohepatitis (NASH) is a key turning point in the progression of nonalcoholic fatty liver disease (NAFLD). As a non-invasive method , serum miRNA levels may provide an effective reference for the diagnosis of NASH. This article systematically reviewed related diagnostic trials to compare the difference in the efficacy of serum miRNAs in the diagnosis of NAFLD and its subtype, NASH, and identify the influencing factors.

2. Methods: We pooled the sensitivity (SEN), specificity (SPE), and area under receiver operating characteristics (AUROC) of each trail to determine the efficacy of serum miRNAs in the diagnosis of NAFLD and NASH; Clinical utility was evaluated by Fagan`s nomogram; Heterogeneity was evaluated by subgroup analysis and meta-regression. Publication bias was detected by Deek's funnel plot.

3. Results: We included 9 articles consisting of 27 trials and 2361 cases, 1775 NAFLD patients (not distinguishing between simple steatosis and NASH) and 586 NASH patients were collected. All cases were confirmed by biopsy. For NAFLD and NASH, the pooled values were SEN (0.71 vs. 0.74), SPE (0.76 vs. 0.85) and AUROC (0.80 vs. 0.86). miRNA had a high accuracy in distinction NASH from simple steatosis with AUROC at 0.91. Among the well-studied serum miRNAs, miRNA-34a showed a moderate accuracy with the lowest heterogeneity in diagnosing NAFLD (SPE I 2 : 5.73%, SPE I 2 : 33.16%, AUROC: 0.85). According to subgroup analysis and meta-regression, lower BMI (<30kg/m 2) may be a crucial source of heterogeneity and reduced the performance of serum miRNA in the diagnosis of NAFLD.

4. Conclusions: As a non-invasive method, serum miRNA should be considered a promising parameter in the diagnosis of NASH. Generally, NAFLD patients with higher BMI (≥ 30kg/m 2) are more likely to be diagnosed accurately by serum miRNA.

Background

Nonalcoholic fatty liver disease (NAFLD) has become a heavy burden in liver disease. The latest epidemiological data shows that the prevalence of NAFLD worldwide is approximately 25% [1]. In developed countries, such as the United States, the prevalence of NAFLD is 30% [1]. In developing countries, such as China, the prevalence also reaches 32.9% [2]. NAFLD comprises a spectrum of pathological conditions, including simple steatosis (NAFL), nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis and hepatocellular (HCC). NASH is the pivotal turning point in NAFLD progression. Studies have shown that approximately 1/6 of NAFL patients develop NASH [1]. Further, 20% of NASH patients will develop cirrhosis [3]. There are even studies that indicated patients with NASH are 60% more likely to develop HCC than that with simple steatosis [4]. In the past, NASH to HCC was considered to be a gradual development process, which must go through liver fibrosis and cirrhosis. Now It is found that NASH can jump directly to HCC [5]. Thus, early diagnosis of NAFLD, especially NASH, is quite important.

Liver biopsy is the gold standard in diagnosing NASH, but patients are usually reluctant. In addition, abnormal liver function can bring great risks to biopsy, such as bleeding. Therefore, non-invasive

approaches remain to be an interest. The most common serological biomarker for the diagnosis of NASH is cytokeratin 18 (CK-18). CK-18 is an indicator of liver cell apoptosis, suggesting damage to liver cells [6]. The specificity of CK-18 can reach 80%, and the area under the receiver operating characteristic curve (AUROC) can reach 0.83, but the sensitivity is low, only about 60% [7]. Other indexes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are also unsatisfactory [8]. Therefore, a search for better biomarkers to diagnose NASH is needed.

miRNA is a short, non-coding single-stranded RNA strand that is 20–25 nucleotides in length. miRNA plays a complicated and important role in regulating the expression of downstream genes [9]. At present, mountainous experiments and clinical trials confirm that miRNA is closely related to NAFLD [10–13]. miRNAs target a variety of lipid metabolism and pro-inflammatory related genes, and these genes are involved in the onset and progression of NAFLD [14]. A recent meta-analysis reported that circulating miRNA has a moderate diagnostic accuracy on NAFLD [15]. However, NAFLD is an expansive concept. NAFL, fibrosis and cirrhosis can be identified by B-scan ultrasonography, computed tomography (CT) or Fibroscan [16–18]. NASH is hidden but important, and cannot be diagnosed by such radiological methods. Hence, it is necessary to separately evaluate the efficacy of serum miRNA for NASH diagnosis. In this meta-analysis, we reorganized the existing studies and collected more related studies to analyze the value of serum miRNAs in diagnosis of NAFLD, especially NASH. Especially different, we would investigate whether body mass index (BMI) of NAFLD patient influent miRNA`s diagnostic efficacy.

Methods

1. Literature retrieval and selection.

We have submitted a review protocol on the website https://www.crd.york.ac.uk/prospero, (ID172385) and performed this study based on the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines (Additional file 1: PRISMA 2009 Checklist). We conducted literature retrieval in three databases: Pubmed, Science Direct, and Cochrane library. The retrieval strategy was (“NAFLD” OR “Non-alcoholic Fatty Liver Disease” OR “NASH” OR “Non-alcoholic Steatohepatitis”) AND (“microRNAs” OR “miRNA” OR “microRNA” OR “miR” OR “hsa-miR”). Retrieval time is up to Feb 1, 2020. Language is not limited. We totally collected 3956 records and selected according to selection strategy. The preliminary screening (title and abstract) were reviewed by two authors (SLX and QZ) independently and blindly. The repeated screening (full-text) were reviewed and discussed by all authors. Finally, 9 articles containing 27 trials were included according to eligibility criteria. Literatures were managed by Endnote X9.

Eligibility criteria. We included articles in the following conditions: (1) Control group and case group (NAFLD or NASH) were contained; (2) All NAFLD cases (involving NAFLD and NASH) were confirmed by liver biopsy; (3) serum miRNA level as a diagnostic tool; (4) Necessary statistical data (sensitivity, specificity and sample size) were provided. We excluded articles in the following conditions: (1) Other unrelated liver diseases (like alcoholic, viral and drug-induced liver damage); (2) miRNAs were tested from

non-blood sources; (3) Lack of necessary statistical data; (4) experimental researches; (5) duplicated records.

Characteristics of the included trials. (1) NAFLD was confirmed when more than 5% hepatocytes became steatosis; (2) NASH was confirmed by NAFLD score (NAS) (NAS ≥ 5); (3) Serum miRNA level was quantified by reverse transcription-polymerase chain reaction (RT-PCR); (3) In NAFLD trials, control group collected healthy individuals, case group collected NAFLD patients (not extinguishing between NAFL and NASH); In NASH trials, control group collected individuals with NAS < 5, case group collected NASH patients with NAS ≥ 5.

2. Data extraction and literature quality assessment

We built 2*2 contingency table for each trial and registered true positive (TP), false positive (FP), false negative (FN), true negative (TN). If original trial did not directly provide TP, FP, FN and TN values, we would calculate them based on simple size, sensitivity and specificity. In addition, other characteristics of trials were listed. Quality assessment of the included articles was conducted by two authors independently using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2). More details about QUADAS-2 could referred to the previous study. [18]

3. Statistical analysis

Stata SE 15 was used to perform this meta-analysis. The first step was to calculate pooled statistical values, including sensitivity (SEN), specificity (SPE), positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR). I^2 statistic was used to evaluate the heterogeneity among trails. If an $I^2 > 50\%$ was found, considerable heterogeneity was considered, thus the analysis should apply to random-effects model. The second step was to give a further analysis by SROC curve. If SROC plane appeared “shoulder-arm shape”, threshold effect should be considered. Area under receiver operating characteristics (AUROC) value of 0.5–0.7, 0.7–0.9 and 0.9–1.0 suggests low, moderate and high diagnostic accuracy, respectively. The third step was to detect the source of heterogeneity by subgroup analysis and meta-regression. For meta-regression, a covariate with $P < 0.05$ indicates statistically significant and this covariate should be considered as a crucial heterogeneity source. The fourth step was to test publication bias. Deek’s Funnel Plot was applied to examine the potential publication bias caused by asymmetry of the trials. $P < 0.05$ for the slope coefficient indicates test asymmetry and suggests a significant publication bias [19]. At last, we built Fagan`s nomogram and likelihood ratio scattergram to evaluate the clinical utility.

Results

1. Literature retrieval, literature characteristics and quality assessment

The literature retrieval generated 3956 records. After operated by Endnote X9, duplicate records (n = 295) and inappropriate article types (n = 636) were excluded. During preliminary screening (title and abstract),

we eliminated unrelated articles (n = 988) and experimental researches (n = 1967). During repeated screening (full-text), we included articles (n = 9) that met the eligibility criteria [20–28]. A flow diagram of the literature selection is presented in Fig. 1.

In total, 9 articles containing 27 trials were included in the present meta-analysis. We registered characteristics (trial number, first author, year, region, target miRNA, regulation mode, disease, sample size in control and case group, BMI, male proportion and diagnostic SEN, SPE) for each trial and showed in Additional file 1: Tab S1. The results are summarized as follows: Asian trials (n = 11), non-Asian trials (n = 16); Upregulation trials (n = 21), downregulation trials (n = 6); NASH trials (n = 13), NAFLD trials (not distinguishing between NAFL and NASH) (n = 14); BMI ≥ 30 kg/m^2 (n = 15), < 30 kg/m^2 (n = 9); male proportion ≥ 50% (n = 12), < 50% (n = 8).

The total sample size (including control and case) of the 27 trials was 4036, of which 2764 were in NAFLD trials and 1272 were in NASH trials Fig. 2-A. We summarized the target miRNAs involved in the 27 trials, the top 4 in total simple size were miRNA-122 (n = 1104), miRNA-99a (n = 792), miRNA-34a (n = 642) and miRNA panel (more than one miRNA) (n = 368), respectively Fig. 2-B. We built Cochrane bias graph to assess quality of each included article according to QUADAS-2 questionnaire Additional file 1: Fig S1.

2. Diagnostic Value Of Serum miRNAs In NAFLD

2.1 Pooling all trials (NO.1–27) to evaluate the efficacy of miRNA in diagnosis of the total NAFLD

Significant heterogeneity existed among the trials from the values of sensitivity and specificity

(pooled I^2 of SEN and SPE were 94.82% and 88.37%, respectively) Thus, we chose the random-effects model in our study. The pooled values were as follows: SEN 0.72 (95% CI: 0.64–0.79), SPE 0.81 (95% CI:0.75–0.86), PLR 3.77 (95% CI:2.86–4.96), NLR 0.34 (95% CI:0.27–0.44), DOR 10.95 (95% CI: 7.05–17.01), AUROC 0.84 (95% CI:0.80–0.87) Fig. 3. These results indicated that serum miRNA had moderate diagnostic accuracy in the total NAFLD.

Next, we evaluated the efficacy of the most studied serum miRNAs, miRNA-122, miRNA-99a and miRNA-34a, in diagnosis of the total NAFLD. (1) The pooled values of miRNA-122: SEN 0.84 (95% CI: 0.77–0.90) I^2 = 80.62%, SPE 0.72 (95% CI: 0.61–0.81) I^2 = 85.44%, PLR 3.01 (95% CI: 2.12–4.27), NLR 0.22 (95% CI: 0.14–0.33), DOR 13.79 (95% CI: 7.29–26.06), AUROC 0.86 (95% CI: 0.82–0.89) Additional file 1: Fig S2. (2) The pooled values of miRNA-99a: SEN 0.82 (95% CI: 0.71–0.89) I^2 = 93.46%, SPE 0.82 (95% CI: 0.53–0.95) I^2 = 96.90%, PLR 4.58 (95% CI:1.30-16.12), NLR 0.22 (95% CI: 0.11–0.47), DOR 20.42 (95% CI: 2.86–146.00), AUROC 0.87 (95% CI: 0.84–0.90) Additional file 1:Fig S3. (3) The pooled values of miRNA-34a: SEN 0.81 (95% CI: 0.76–0.85) I^2 = 5.73%, SPE 0.83 (95% CI: 0.77–0.87) I^2 = 33.16%, PLR 4.70 (95% CI: 3.51–6.30), NLR 0.23 (95% CI: 0.18–0.29), DOR 20.34 (95% CI: 13.08–31.60), AUROC 0.85 (95% CI: 0.82–0.88) Additional file 1: Fig S4. These results indicated all the three miRNAs had similar moderate diagnostic accuracy in the total NAFLD. Noteworthy, miRNA-34a showed the lowest heterogeneity, thus it might be more suitable to diagnose NAFLD.

2.2 Pooling trials (NO.1–13) and trials (NO.14–27) respectively to evaluate the efficacy of miRNA in the diagnosis of NASH and NAFLD.

(1) The pooled values of NASH trials: SEN 0.74 (95% CI: 0.66–0.81) I^2 = 74.97%, SPE 0.85 (95% CI: 0.77–0.91) I^2 = 79.60%, PLR 5.01 (95% CI: 3.11–8.05), NLR 0.31 (95% CI: 0.23–0.42), DOR 16.24 (95% CI: 8.17–32.28), AUROC 0.86 (95% CI: 0.83–0.89) Additional file 1: Fig S5.

(2) The pooled values of NAFLD trials: SEN 0.71 (95% CI: 0.58–0.81) I^2 = 96.88%, SPE 0.76 (95% CI: 0.68–0.83) I^2 = 90.57%, PLR 2.99 (95% CI: 2.24–3.99), NLR 0.38 (95% CI: 0.26–0.55), DOR 7.93 (95% CI: 4.66–13.49), AUROC 0.80 (95% CI: 0.77–0.84) Additional file 1: Fig S6.

These results indicated that serum miRNA had moderate diagnostic accuracy either in NASH trials or in NAFLD trails. Very critically, serum miRNA showed better diagnostic efficacy in NASH than NAFLD for the higher DOR, AUROC and the lower heterogeneity.

2.3 Pooling trials (NO.9–13) to evaluate the diagnostic efficacy in distinguishing between NAFL and NASH

The pooled values were: SEN 0.83(95% CI: 0.70–0.91) I^2 = 86.22%, SPE 0.85 (95% CI: 0.74–0.92) I^2 = 85.11%, AUROC 0.91 (95% CI: 0.88–0.93). These results indicated that serum miRNA had a high accuracy in discriminating NASH from NAFL Additional file 1: Fig S7.

3. Subgroup Analysis

We divided trials (NO.1–27) into several subgroups according to different categories, and calculated pooled values of each subgroup. The categories were region, type of disease, regulation mode, male proportion and BMI Table 1. The results as follows:

(1) Region. Compared with Asian trials, Non-Asian trials had higher SEN (0.77 vs.0.64) and SPE (0.83 vs. 0.76) and significant lower heterogeneity (SEN I^2 81.33% vs. 96.08%, SPE I^2 76.03% vs. 92.08%). Non-Asian trials also had higher DOR (17 vs. 6) and AUROC (0.87 vs. 0.77);

(2) Type of disease. Results had already mentioned in Sect. 2.2;

(3) Regulation mode. Compared with upregulated-mode trials, downregulated-mode trials had lower SEN (0.66 vs.0.74) but higher SPE (0.89 vs. 0.78), and showed lower heterogeneity (SEN I^2 59.82% vs. 95.92%, SPE I^2 58.57% vs. 90.07%). downregulated-mode trials had higher DOR (16 vs. 10) but the same AUROC with upregulated-mode trials (0.83 vs. 0.83);

(4) Male proportion. Compared with male proportion ≥ 50%, trials with male proportion < 50% had higher SEN (0.77 vs.0.64) and SPE (0.87 vs. 0.74) and significant lower heterogeneity (SEN I^2 81.17% vs. 95.58%, SPE I^2 58.46% vs. 90.78%). Trials with male proportion < 50% also had higher DOR (21 vs. 5) and AUROC (0.89 vs. 0.75);

(5) BMI. Compared with BMI < 30 kg/m^2, trials with BMI ≥ 30 kg/m^2 had higher SEN (0.77 vs.0.63) and SPE (0.84 vs. 0.75) and significant lower heterogeneity (SEN I^2 82.34% vs. 96.69%, SPE I^2 76.67% vs. 93.11%). Trials with BMI ≥ 30 kg/m^2 also had higher DOR (17 vs. 5) and AUROC (0.87 vs. 0.76).

In summary, serum miRNA showed more accurate efficacy in the diagnosis of the total NAFLD in such trials: non-Asian, NASH related, woman predominated and BMI ≥ 30 kg/m^2. From heterogeneity prospective, all above five factors should be considered as sources of heterogeneity.

4. Meta-regression

To find out the significant source of heterogeneity, we preformed meta-regression. Region, disease type, miRNA regulation mode and miRNA profiling were set as covariates. Due to lack of some data, male proportion and BMI were not be included in meta-regression. We made the assignment as follows: region (Yes = Asian, No = Non-Asian), disease (Yes = NASH, No = NAFLD), regulation (Yes = upregulation, No = downregulation) and miRNA profiling (Yes = single miRNA, No = miRNA panel). As shown in Fig. 4, Only the region factor (Asian trials) caused statistical difference (SEN P < 0.01, SPE P < 0.001), suggesting that region factor (Asian trials) was the significant reason of heterogeneity.

In fact, the heterogeneity of region factor was likely to be mainly due to different BMI: In 16 Non-Asian trials, 15 trials with BMI ≥ 30 kg/m^2, 1 trials did not provide; In 11 Asian trials, 9 trials with BMI < 30 kg/m^2, 2 trials did not provide; Asian and non-Asian trials were basically the same in terms of diagnostic criteria, measurement methods, etc.; The statistical values of the subgroup categorized by region were very close to that of the subgroup categorized by BMI.

Therefore, although BMI was missing in some trials, we speculated that BMI (< 30 kg/m^2) was likely to be the true source of heterogeneity. When successively removed trials (BMI < 30 kg/m^2), trials (male proportion ≥ 50%) and trials (NAFLD + miRNA with upregulation mode), the heterogeneity of SEN and SPE showed a downward trend as compared to the original ones: SEN I^2 94.82% vs. 81.28% vs.80.39% vs. 0%, SPE I^2 88.37% vs. 72.92% vs. 76.92% vs. 25.99% Additional file 1: Tab S2.

5. Clinical Utility

We drew Fagan`s nomogram for NASH trials (NO.1–13) and NAFLD trials (NO.14–27), respectively, as shown in Fig. 5A-B. Pre-test probability was set at 50%. Results as follows: (1) In NASH trials, if a patient obtained a positive result from a serum miRNA test, the probability of suffering NASH was 83%. If the result was negative, the probability of not suffering NASH is 24%; (2) In NAFLD trials, if a patient obtained a positive result from a serum miRNA test, the probability of suffering NAFLD was 75%. If the result was negative, the probability of not suffering NAFLD is 27%. That indicated serum miRNA had higher positive diagnostic value for NASH than NAFLD. Furthermore, a likelihood ratio scattergram was constructed for NASH trials Fig. 5C. The result showed that 9/13 of the trials were located in the right lower quadrant,

which represented no exclusion or confirmation, indicating that the serum miRNA test had limited clinical utility for NASH. Thus, continual optimized methods about serum miRNA are needed in the future.

6. Publication Bias

Based on the Deeks' Funnel plot Fig. 6A-C, publication bias was not detected in the studies where serum miRNA was used to detect the total NAFLD (P = 0.77), NAFLD (P = 0.84) and NASH (P = 0.29). In addition, as shown in Fig. 6D, serum miRNA-34 did not show publication bias where used to detect the total NAFLD (P = 0.46).

Discussion

In this study, we systematically reviewed the diagnostic value of serum miRNA for NAFLD. Different from previous studies, we focused on NAFLD`s subtype, NASH. 27 trials were included in this meta-analysis with a total of 2361 NAFLD cases, which consist of 1775 NAFLD cases (not distinguishing between NAFL and NASH) and 586 NASH cases. In this study, we compare the differences in diagnostic efficacy between trials from two perspectives: the first is to compare the most studied serum miRNA-122, miRNA-99 and miRNA-34a in diagnosis of the total NAFLD, and the second is to compare serum miRNAs where used to diagnose the total NAFLD, NAFLD and NASH. In addition, we detected the sources of heterogeneity in pooled values from the aspects of region, disease type, miRNA regulation mode, male proportion, BMI and miRNA profiling.

The main three conclusions of this study were: (1) Compared with the total NAFLD and NAFLD, serum miRNA showed the highest diagnostic efficacy in NASH. Notably, serum miRNA also had high accuracy in discriminating NASH from NAFL; (2) Among the well-studied serum miRNAs, miRNA-34a showed the most stable efficacy with moderate accuracy in the diagnosis of the total NAFLD; (3) BMI < 30 kg/m^2 may be the critical reason of heterogeneity. Serum miRNA showed more accurate diagnostic efficacy in the total NAFLD patients with obesity (≥ 30 kg/m^2).

Firstly, we still need better non-invasive methods to diagnose NASH. NAFLD is the leading liver disease in the globe. It is estimated that by 2030, NAFLD patients in the United States will reach 100 million. Its subtype, NASH, covers approximately 7% -30% of NAFLD patients [1]. In Asia, the prevalence of NASH is even higher, reaching 63.5% in NAFLD liver biopsies [29]. NASH is an important turning point to develop to the severer liver disease, so early and accurate diagnosis of NASH is necessary. As we know, the gold standard for the diagnosis and classification of NASH is liver biopsy. However, biopsies cannot be widely used due to complications or patients` reluctance [30]. Therefore, non-invasive diagnosis is necessary. Liver function parameters, AST and ALT, can reflect the damage of hepatocytes, but depend on the severity of NASH. Even AST and ALT in some NASH patients are normal [31]; CK-18 reflects necrosis or apoptosis of hepatocytes. It is characterized by a good specificity but relatively low sensitivity. To increase the sensitivity, it needs to combine other indexes [7, 32–35]; Regarding inflammation indexes, they always show poor specificity [36]. At present, it is reported that the comprehensive score systems

(like NashTest and ActiTest) have low or moderate efficacy in diagnosis of NASH, but involving excessive indexes and costly [37]. Therefore, we still need better non-invasive methods to diagnose NASH.

Secondly, serum miRNA has more accurate efficacy in diagnosis of NASH. In recent years, more and more studies have shown the relationship between miRNA and NASH [38–40]. miRNA is a non-coding RNA with a length of about 20–25 nucleotides. Its regulatory mechanism is very complex. In general, it suppresses or promotes the expression of a target genes [41]. miRNAs widely participate in multiple pathological processes of NAFLD [14, 41], and miRNA levels are significantly different in the serum of healthy and NAFLD cases, so it becomes a new potential non-invasive biomarker for the diagnosis of NAFLD. (1) Our study found that serum miRNAs have a moderate accuracy to diagnose NAFLD, same conclusion obtained in the study of Cai C, et al [15]. The difference is that, our subgroup analysis showed that serum miRNA reaches more accurate efficacy in diagnosing NASH than NAFLD: the pooled values of NAFLD and NASH were SEN (0.71 vs. 0.74), SPE (0.76 vs. 0.85), DOR (7.93 vs. 16.24), AUROC (0.80 vs. 0.86), respectively. From healthy control, NAFL through NASH, the levels of serum miRNA gradually increase or decrease, accompanying by deterioration of disease [23, 42–43]. Therefore, the change of serum miRNA in NASH patients is more significant. We speculate that is one reason why serum miRNAs have more outstanding ability to diagnose NASH instead of NAFLD. Notably, in our study, a high accuracy of serum miRNA in discriminating NASH from NAFL was found with AUROC at 0.91. Similarly, a recent study reported that miRNA-34 had moderate accuracy to distinguish between NAFL and NASH [44]; (2) Our study involved a total of 14 miRNAs, which played roles in multiple pathological processes of NAFLD [14, 45]. For example, lipid synthesis (miRNA-122), fatty acid b-oxidation (miRNA-122, -34a), endoplasmic reticulum stress (miRNA-122, -34a, -30), inflammation (miRNA-146b, -99a), fibrosis (miRNA-122), tumorigenesis (miRNA-99a), cell autophagy and apoptosis (miRNA-34a). It can be seen that the relationships between miRNAs and NAFLD-associated processes can be both one-to-many and many-to-one. Therefore, compared with traditional non-invasive diagnostic methods, miRNA is the simple but comprehensive one that can efficiently diagnose NAFLD, especially NASH. Of course, as was shown in the likelihood ratio scattergram, we should optimize the strategy about serum miRNA to enhance its clinical utility.

Thirdly, serum miRNA-34a might be the suitable index in diagnosis of NAFLD. We summarized the most studied miRNAs (miRNA-122, -99a, -34a) and found similar moderate accuracy among them in diagnosing the total NAFLD, but miRNA-34a showed the lowest heterogeneity. The pooled values of miRNA-122, -99a, -34a were SEN I^2(80.62% vs. 93.46% vs.5.73%) SPE (85.44% vs. 96.90% vs.33.16%), DOR (13.79 vs. 20.42 vs.20.34) and AUROC (0.86vs. 0.87 vs.0.85), respectively. Thus, miRNA-34a might be the suitable index in diagnosis of NAFLD. miRNA-34a plays an important role in the regulation of NAFLD. Concerning lipid metabolism, miRNA-34a downregulates PPARα signal pathway. The main function of the target molecules of PPARα is to promote transport and β-oxidation of free fatty acid. Inhibition of PPARα signal pathway increases lipid accumulation in hepatocytes [46]; In addition, PPARα is involved in liver inflammation by regulating Kupffer cells [47]; Concerning cellular apoptosis, by repressing SIRT1, miRNA-34a increases p53 acetylation and transcription, leading to the induction of pro-

apoptotic genes such as PUMA and, finally, apoptosis [48]. To sum up, miRNA-34a participates in the “first hit” (abnormal lipid metabolism) and “more hits” (inflammation and apoptosis) of NAFLD. In other words, miRNA-34a involved in all the NAFLD process from onset to progression. Thus, it has more accurate and more stable efficacy in diagnosis of NAFLD than other common biomarkers and miRNAs. Due to limited researches, we did not compare the differences between miRNA-34a in the diagnosis of NAFLD and NASH. As a valuable reference, a study has shown that miRNA-34a can distinguish NASH from NAFL [45]

Fourthly, the performance of serum miRNA in the diagnosis of NAFLD may depends on BMI. In our subgroup analysis, miRNA showed more accurate efficacy in this NAFLD cases with BMI ≥ 30 kg/m^2. The pooled values of BMI ≥ 30 kg/m^2 and BMI < 30 kg/m^2 were: SEN (0.77 vs.0.63) SPE (0.84 vs. 0.75) DOR (17 vs. 5) AUROC (0.87 vs. 0.76), respectively. In terms of meta-regression, only the difference in region was statistically significant, thus, region factor was a crucial reason of the heterogeneity. In fact, the major difference between Asian and non-Asian cases in our study was in BMI. We observed a clear reduction in heterogeneity when removing such trials with BMI < 30 kg/m^2: SEN I^2 94.82% vs. 81.28%, SPE I^2 88.37% vs. 72.92%. Accordingly, we could speculate that lower BMI might be the “real” source of the heterogeneity. In our study, the BMIs of Asian NAFLD patients ranged from 24 to 28 kg/m^2. Actually, according to a recent epidemiological investigation, in Asia, “lean” or “non-obese NAFLD” becomes a new trend. The prevalence of NAFLD in population with BMI < 25 kg/m^2 is 8–20% (China), 7% (India), 15% (Korea) and 13% (Japan) [49]. Distinguished from the classical pathogenesis in “obese” NAFLD, PNPLA3 polymorphism appears to be more important in the development of NAFLD in the non-obese population [50, 51]. As such, a caution should be exercised when using serum miRNA to diagnose Asian NAFLD (or NASH) cases.

Finally, there are still some defects in this study. (1) The cutoff value is an important cause of the heterogeneity, however, most trials in this study did not provide it; (2) We extracted multiple trials from one article, which may increase statistical deviation; (3) Due to limited trials, we did not evaluate the efficacy of miRNA-34a in the diagnosis of NASH; (4) Due to lack of some data, we did not directly analysis the BMI factor by meta-regression; (5) we may have ignored some relevant literature or part of the data.

Conclusion

In summary, this meta-analysis showed that serum miRNA is a promising valuable biomarker for diagnosing NASH. Among the well-studied serum miRNAs, miRNA-34a is the most suitable index for diagnosing NAFLD. What needs to be noticed is that BMI may influent the diagnostic performance, and more researches on this aspect are necessary in the future.

Abbreviations

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; AUROC: Area under the receiver operating characteristic curve; BMI: Body mass index; CI: Confidence interval; CK-18: Cytokeratin 18; DOR: Diagnostic odds ratio; FN: False negative; FP: False positive; PLR: Positive likelihood ratio; NAFL: Nonalcoholic fatty liver (simple steatosis); NAFLD: Nonalcoholic fatty liver disease; NAS: NAFLD activity score; NASH: Nonalcoholic steatohepatitis; NLR: Negative likelihood ratio; PPARα: Peroxisome proliferator-activated receptor α; PNPLA3: Patatin like phospholipase domain containing 3; PUMA: p53 up-regulated modulator of apoptosis; QUADAS: Quality assessment of diagnostic accuracy studies; RT-PCR: Reverse transcription-polymerase chain reaction; SE: Sensitivity; SIRT1: Sirtuin 1; SP: Specificity; TN: True negative; TP: True positive

Additional Files

Additional file 1

Figure S1: Quality assessment of the included trials. **Figure S2:** Assessment for the efficacy of serum miRNA-122 in the diagnosis of the total NAFLD (case vs. control). **Figure S3:** Assessment for the efficacy of serum miRNA-99a in the diagnosis of the total NAFLD (case vs. control). **Figure S4:** Assessment for the efficacy of serum miRNA-34a in the diagnosis of the total NAFLD (case vs. control). **Figure S5:** Assessment for the efficacy of serum miRNA in the diagnosis of NASH (NAS≥5 vs. NAS<5). **Figure S6:** Assessment for the efficacy of serum miRNA in the diagnosis of NAFLD (NAFLD vs. healthy control). **Figure S7:** Assessment for the efficacy of serum miRNA in the distinguishing between NASH and NAFL. **Table S1:** Basic characteristics of the included trials. **Table S2:** The heterogeneity of serum miRNA of diagnosing the total NAFLD after trial removal. **PRISMA 2009 Checklist.**

Declarations

(1) Ethics approval and consent to participate

Not applicable

(2) Consent for publication

Not applicable

(3) Availability of data and materials

Additional data not presented in the manuscript can be obtained by contacting the authors

(4) Competing interests

The authors declare that they have no competing interests

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(6) Authors' contributions

SLX: study concept and design, title, abstract, full-text screening, data abstraction, statistical analysis, data interpretation, and drafting the article. QZ, XFC: study design, data interpretation, critical revision of the manuscript. JHX and YYY: data interpretation, revision of the manuscript. All authors read and approved the final manuscript.

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Figures

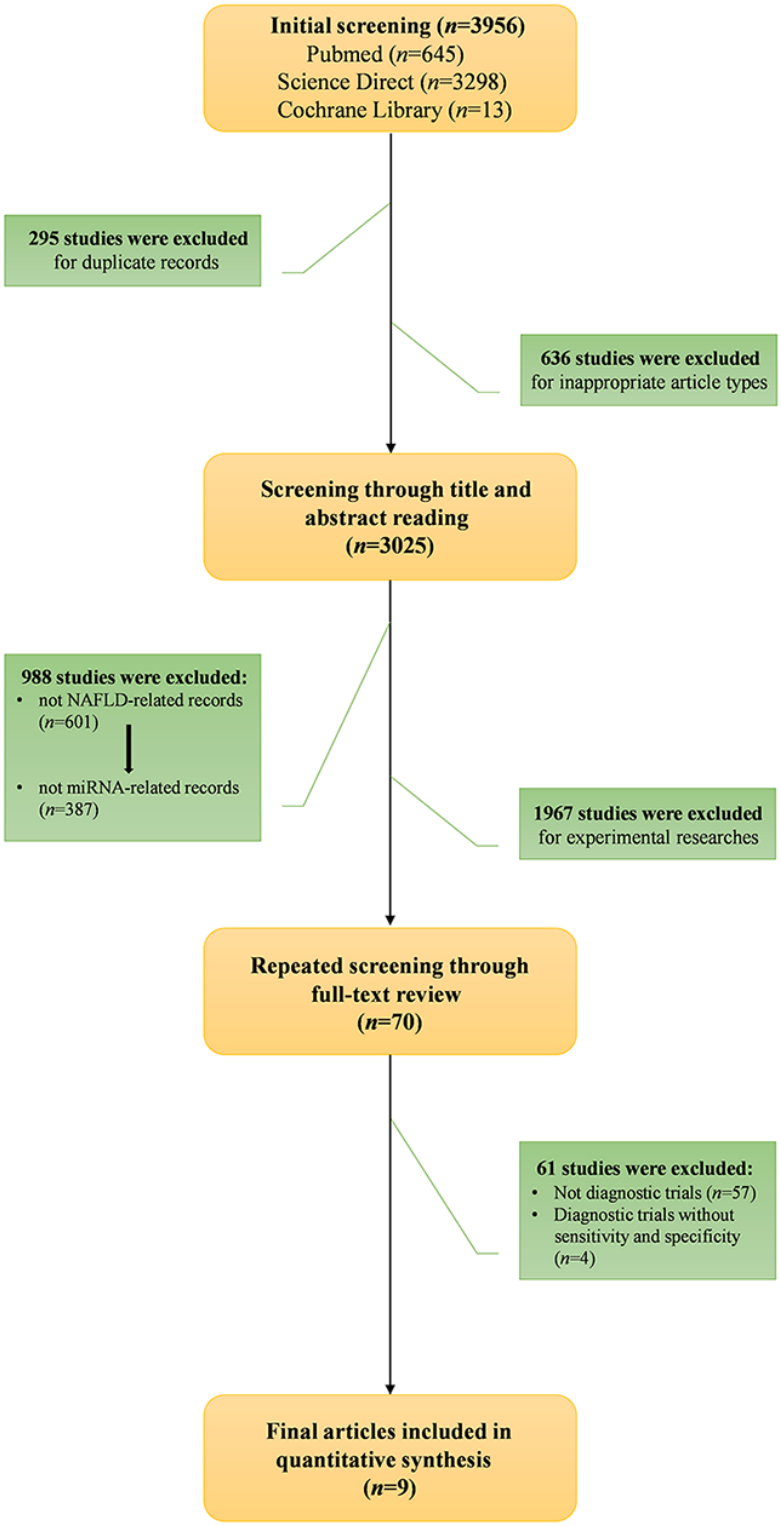

Figure 1

Article selection process.

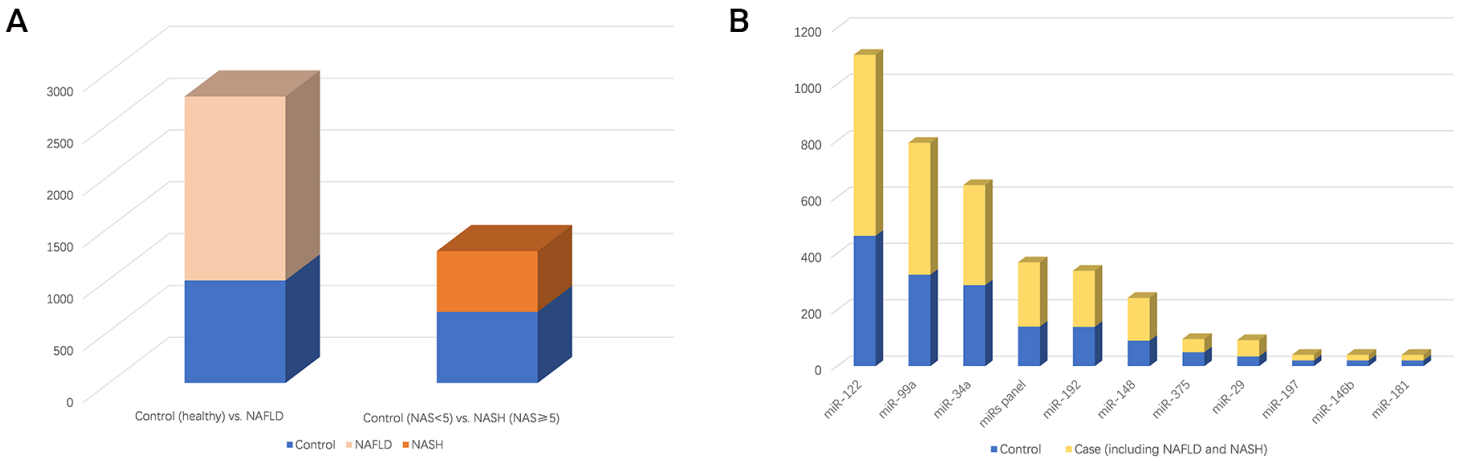

Figure 2

A: The total sample size of NAFLD trials and NASH trials. B: The total sample size of each involved serum miRNA in this study.

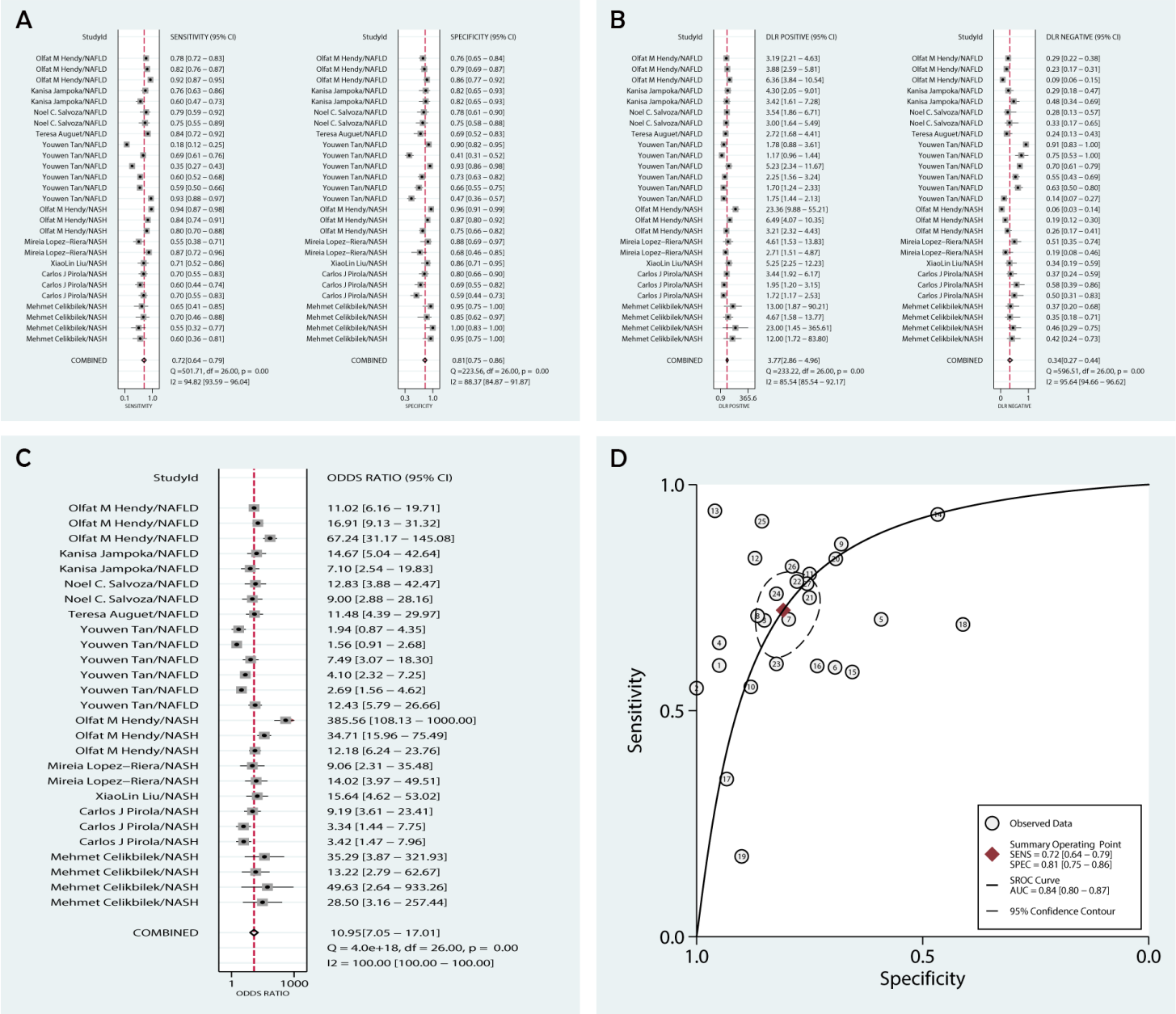

Figure 3

Forest plots and meta-analysis of trials showing pooled sensitivity and specificity (A), positive likelihood ratio and negative likelihood ratio (B) and diagnostic odds ratio (C) of serum miRNA for detection of the total NAFLD (case vs. control). D: Summary of AUROC of serum miRNA for detection of the total NAFLD (case vs. control).

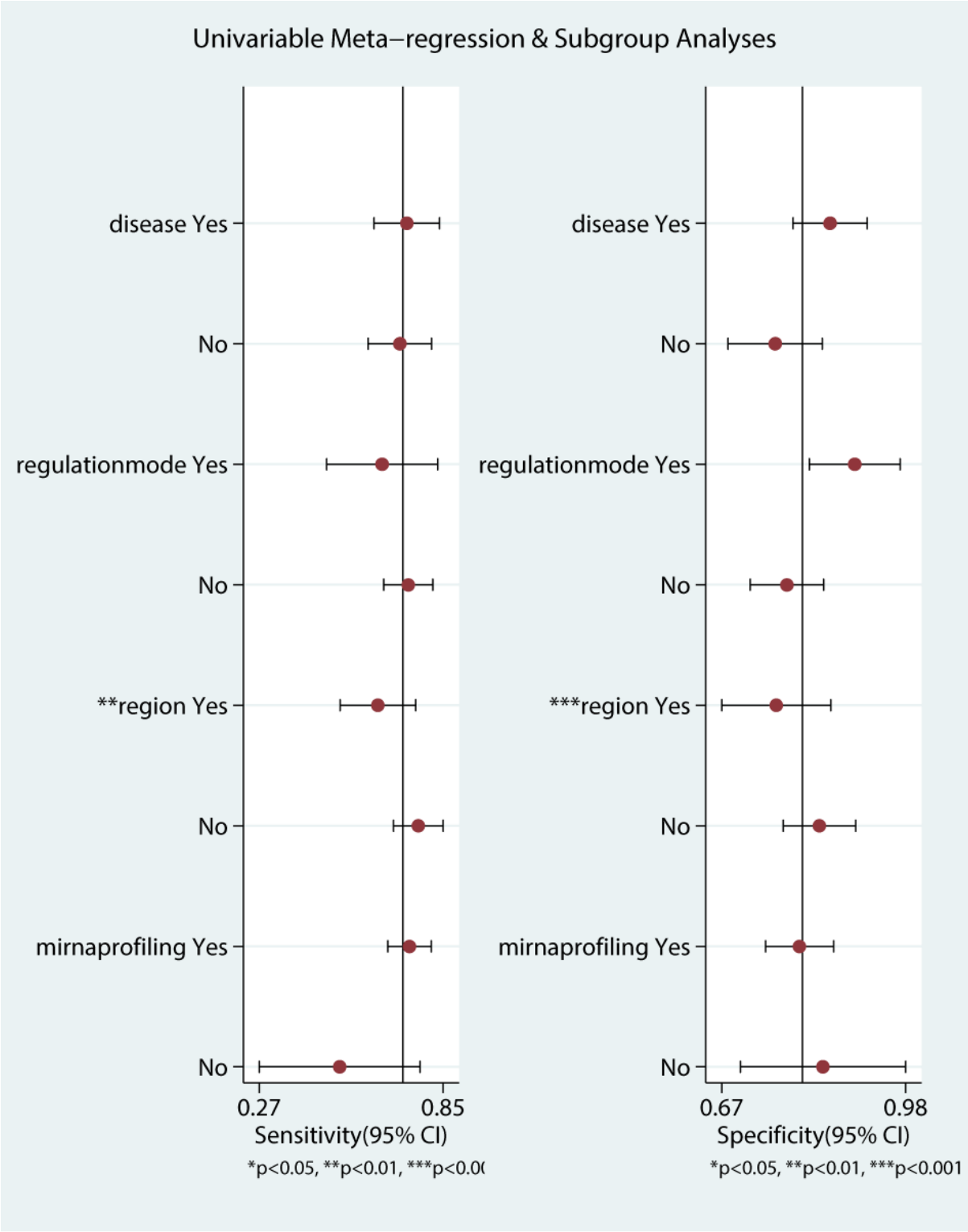

Figure 4

The meta-regression analysis of serum miRNA for detection of the total NAFLD (case vs. control). The assignment was made as follows: region (Yes=Asian, No=Non-Asian), disease (Yes=NASH, No=NAFLD), regulation (Yes=upregulation, No=downregulation) and miRNA profiling (Yes=single miRNA, No=miRNA panel).

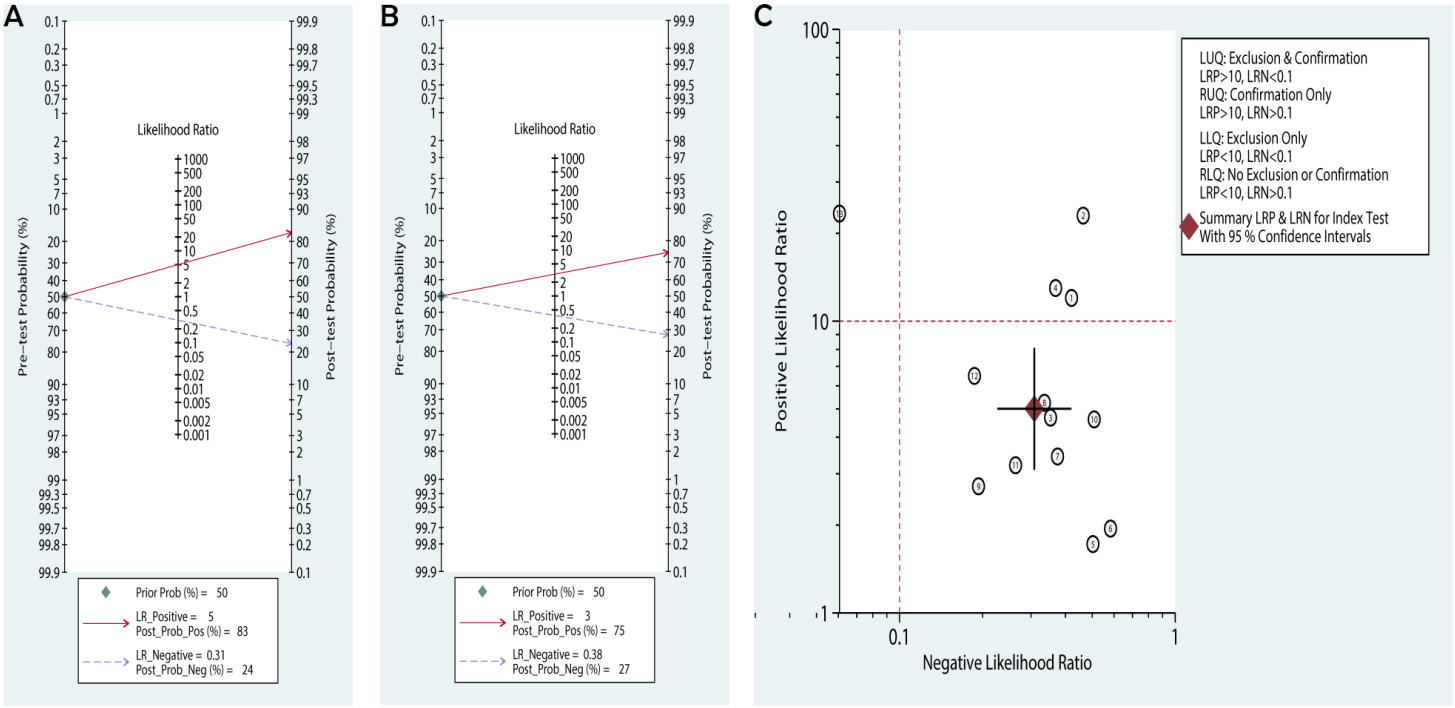

Figure 5

A: Fagan's nomogram of serum miRNA for detection of NASH (NAS≥5 vs. NAS<5). Pre-test probability=50% (patients with the moderate suspicion for NASH), the post-test positive and negative probability of NASH steatosis are 83% and 24%, respectively. B: Fagan's nomogram of serum miRNA for detection of NAFLD (NAFLD vs. healthy control). Pre-test probability=50% (patients with the moderate suspicion for NAFLD), the post-test positive and negative probability of NAFLD are 75% and 27%, respectively. C: The likelihood ratio scattergram of serum miRNA for detection of NASH (NAS≥5 vs. NAS<5). LRP: positive likelihood ratio; LRN: negative likelihood ratio; LUQ: left upper quadrant; RUQ: right upper quadrant; LLQ: left lower quadrant; RLQ: right lower quadrant.

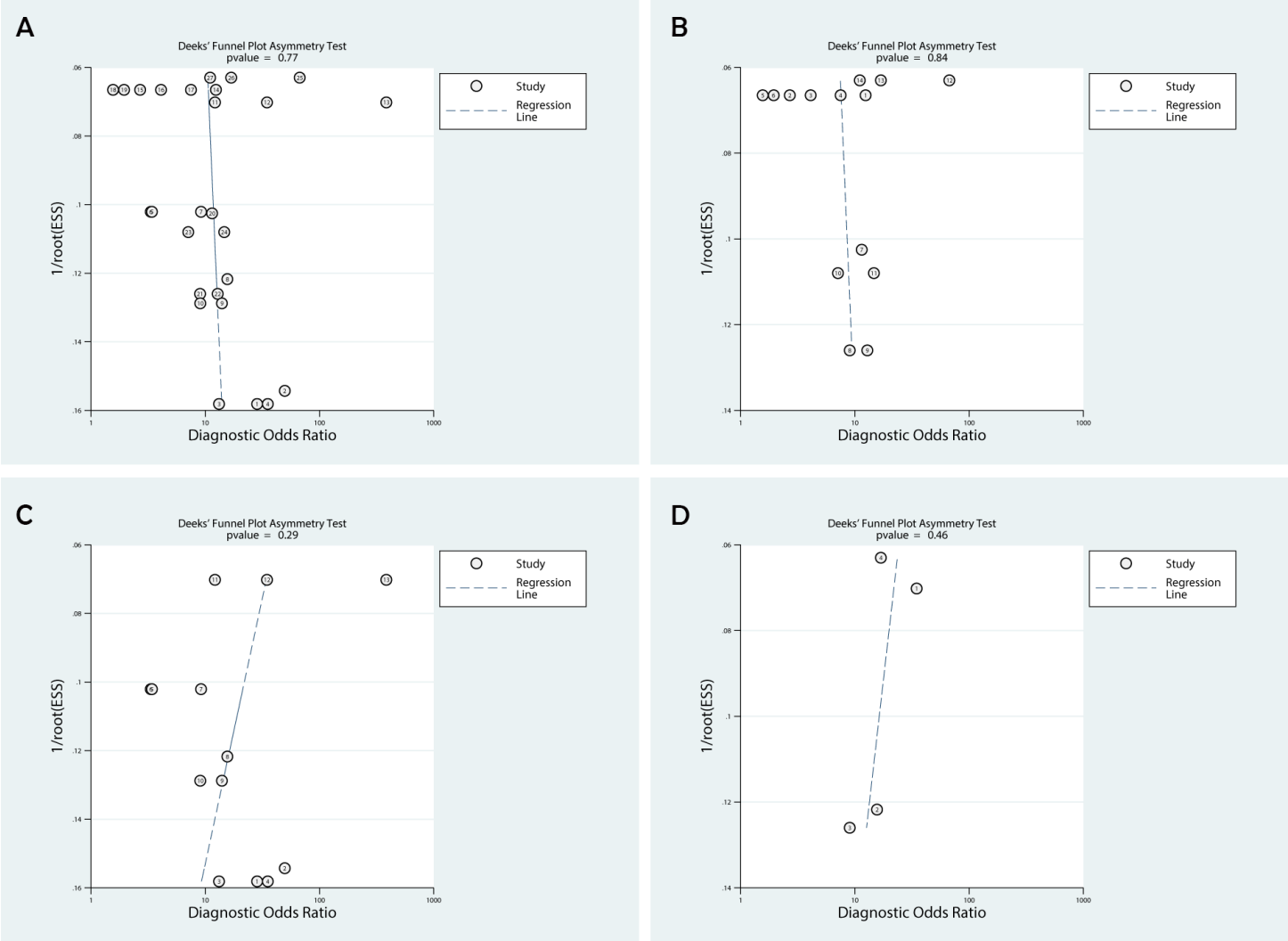

Figure 6

Estimation of the publication bias by Deek's Funnel plots of all involved trials (A), NAFLD trials (B), NASH trials (C) and miRNA-34a related trials (D).

Supplementary Files

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