miRNAs in patients with non-alcoholic fatty liver disease: A systematic review and meta-analysis

Chang-Hai Liu[1,3], Javier Ampuero[1,2,3], Antonio Gil-Gómez[1,3], Rocío Montero-Vallejo[1,3], Ángela Rojas[1], Rocío Muñoz-Hernández[1], Rocío Gallego-Durán[1], Manuel Romero-Gómez[1,2,3,⇑]

[1]*Institute of Biomedicine of Seville, Sevilla, Spain;* [2]*Unit of Digestive Diseases and Ciberehd, University Hospital Virgen del Rocío, Seville, Spain;* [3]*University of Seville, Seville, Spain*

Background & Aims: microRNAs (miRNAs) are deregulated in non-alcoholic fatty liver disease (NAFLD) and have been proposed as useful markers for the diagnosis and stratification of disease severity. We conducted a meta-analysis to identify the potential usefulness of miRNA biomarkers in the diagnosis and stratification of NAFLD severity.

Methods: After a systematic review, circulating miRNA expression consistency and mean fold-changes were analysed using a vote-counting strategy. The sensitivity, specificity, positive and negative likelihood ratios, diagnostic odds ratio and area under the curve (AUC) for the diagnosis of NAFLD or non-alcoholic steatohepatitis (NASH) were pooled using a bivariate meta-analysis. Deeks' funnel plot was used to assess the publication bias.

Results: Thirty-seven studies of miRNA expression profiles and six studies of diagnostic accuracy were ultimately included inthe quantitative analysis. miRNA-122 and miRNA-192 showed consistent upregulation. miRNA-122 was upregulated in every scenario used to distinguish NAFLD severity. The miRNA expression correlation between the serum and liver tissue was inconsistent across studies. miRNA-122 distinguished NAFLD from healthy controls with an AUC of 0.82 (95% CI 0.75–0.89), and miRNA-34a distinguished non-alcoholic steatohepatitis (NASH) from non-alcoholic fatty liver (NAFL) with an AUC of 0.78 (95% CI 0.67–0.88).

Conclusion: miRNA-34a, miRNA-122 and miRNA-192 were identified as potential diagnostic markers to segregate NAFL from NASH. Both miRNA-122, in distinguishing NAFLD from healthy controls, and miRNA-34a, in distinguishing NASH from NAFL, showed moderate diagnostic accuracy. miRNA-122 was upregulated in every scenario of NAFL, NASH and fibrosis.

Lay summary: microRNAs are deregulated in non-alcoholic fatty liver disease. The microRNAs, miRNA-34a, miRNA-122 and miRNA-192, were identified as potential biomarkers of non-alcoholic fatty liver and non-alcoholic steatohepatitis, at different stages of disease severity. The correlation between miRNA expression in the serum and in liver tissue was inconsis- tent, or even inverse.

Keywords: Non-alcoholic fatty liver disease; miRNA; Expression profile; Diagnostic accuracy.

* Corresponding author. Address: Digestive Diseases Unit, Virgen del Rocío University Hospital, Institute of Biomedicine of Seville, University of Seville, Avda Manuel Siurot s/n, Sevilla 41013, Spain. Tel.: +34 671535780; fax: +34 955 923 101.
E-mail address: mromerogomez@us.es (M. Romero-Gómez).

Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterised by excessive fat accumulation without a history of excessive alco-hol intake, and the absence of other known liver diseases, suchas hepatitis B and hepatitis C virus infection.[1–3] NAFLD has been described as the hepatic manifestation of metabolic syndrome, associated with insulin resistance and genetic susceptibility. NAFLD affects 30% to 40% of the United States population,[4] 2% to 44% of the European population,[5] and 15% to 45% of the Asian population,[6] while the Hispanic population is the most susceptible, as up to 45% of this population suffers from NAFLD.[7] Sedentary behaviour, low physical activity and poor diet have been defined as the ''triple-hit behavioural phenotype", whichis associated with cardio-metabolic health, NAFLD and overall mortality.[8] Moreover, the prevalence of NAFLD in children is increasing and was estimated to be approximately 10%.[9] NAFLD encompasses a wide spectrum of liver damage, ranging from non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH). NAFL is defined as the presence of hepatocyte steatosis without evidence of inflammation. It is usually non-progressive, while NASH (steatosis with the concomitant presence of inflammation and ballooning) is often progressive, eventually advancing to cirrhosis and hepatocellular carcinoma (HCC).[10] Approximately 2–3% of the general population is affected by NASH; this incidence is increased to 20%–30% among obese or diabetic individuals.[11,12] NASH is the second most common indication for liver transplantation in the United States[13] and is associated with an increased mortality with excess cardiovascular-, liver-, and cancer-related deaths.[14] Thus, improved detection of NASH is urgently needed. Moreover, distinguishing both NASH from NAFL and fibrosis from advanced fibrosis (>F3) are important goals.[1,15,16]

Liver biopsy remains the gold standard for the diagnosis of NASH. A common scoring system to distinguish NAFL and NASH is the NAFLD activity score, which is defined by the sum score of steatosis, ballooning, and lobular inflammation.[17] Morerecently, a new scoring system based on the degree of steatosis (S), the grade of necro-inflammatory activity (A) and fibrosis (F), also known as the SAF score, appears to help distinguish NAFL from NASH.[18] However, the potential risks of liver biopsytogether with sampling and interpretation variability make it

unsuitable for screening in the populations at risk. Biomarker discovery remains a big challenge because no reliable non-invasive disease biomarker can accurately distinguish mild from severe histological disease stages.

MicroRNAs (miRNAs) are non-coding small RNAs capable of controlling translation and modulating gene expression at the post-transcriptional level. Their impact on gene expression profiles can modify a variety of biological functions, such as lipid and glucose metabolism and thyroid, adipose tissue, stomach,muscle and liver function.[19] The miRNAs are very stable because they are resistant to degradation by ribonucleases.[20] Thus, cir- culating miRNAs, *i.e.*, miRNAs detected in serum or plasma, have been proposed as attractive diagnostic tools.[21] The scope of applications associated with miRNAs is becoming broader because they are used in different clinical settings, such as early disease detection, disease prediction, monitoring of disease progression and response to treatment for a wide range of disorders.[22] miRNAs can also be found in extracellular vesicles(EVs), which are broadly divided into three types: microvesicles, apoptotic bodies and exosomes.[23,24]

To date, the miRNA expression profiling studies published in patients with NAFLD have reported inconsistent results. Potentially useful miRNA biomarkers need to be screened and identified. Moreover, no systematic review or meta-analysis has reported the diagnostic accuracy of miRNAs in distinguish- ing healthy people and individuals with NAFLD, NAFL, NASH or fibrosis. The present study aimed to identify potential biomarkers by analysing miRNA expression profiles and to demonstrate the diagnostic accuracy of miRNAs in patients with NAFLD.

Materials and methods

Study identification and selection

Seven investigators (CHL, JA, AGG, RMV, AR, RMH and RGD) worked as three teams and independently searched the MEDLINE (using PUBMED as the search engine), EMBASE and Cochrane databases. Databases were used to identify suitable studies that were published up to 2 February 2018. MeSH terms and keywords were used, and the search terms were as follows: miR, miRNA, microRNA, NAFLD, NASH, fatty liver, steatosis, and a combination of those MeSH terms. The searches were limited to English-language publications with human subjects. A manual search was conducted by using the references listed in the original articles and review articles retrieved. Oral presentations, abstracts and posters from liver disease confer- ences were also manually searched to collect all studies with both negative and positive results. The seven investigators collected all results separately. The inclusion criteria were as follows: a) studies that obtained miRNA expression profiles in patients with NAFLD or NASH; b) studies that reported miRNA diagnostic accuracy for NAFLD or NASH; and c) studies that used liver tissue, serum, plasma, blood, microvesicle, apoptotic body and/or exosome as the samples. The exclusion criteria were as follows: i) duplicate reports; ii) studies conducted in animals or cell lines; iii) case reports, comments and letters to the editors; and iv) systematic reviews or meta-analyses. This study was performed according to the PRISMA statement (supplementary information).[25]

Data extraction and quality assessment

From the full text and corresponding supplemental information, seven investigators (CHL, JA, AGG, RMV, AR, RMH and RGD) independently extracted miRNAs according to number, type of samples (liver tissue, serum, plasma, blood, microvesicle, apoptotic body and/or exosome), name of study and year, direction of expression difference (up- or downregulated), sample size, and fold-change from miRNA expression profile studies, as wellas data from two-by-two tables, cut-off value, sensitivity, speci- ficity, AUC, and methodological quality (e.g., patient selection, index test, reference standard, flow and timing, patient selection

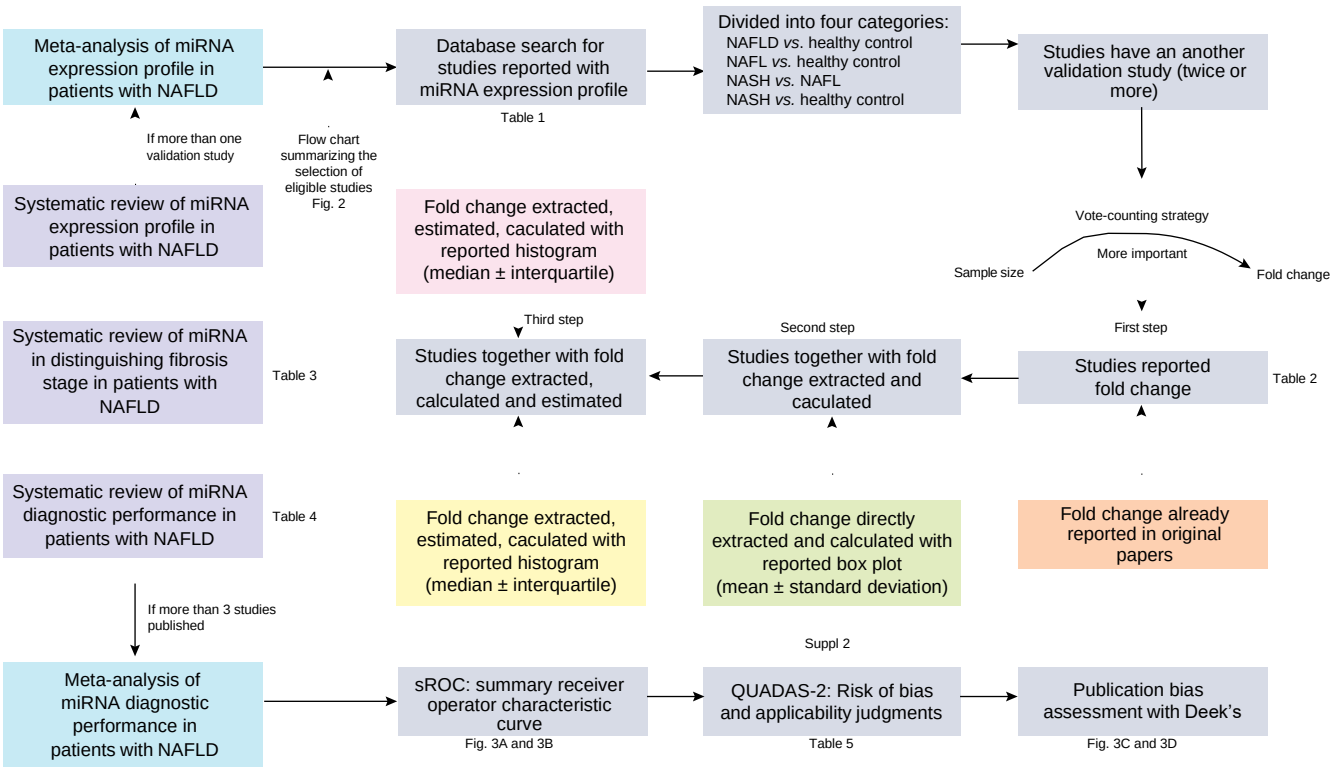

Fig. 1. Flow chart of the current study. NAFL, non-alcoholic fatty liver; NAFLD, NAFL disease; NASH, non-alcoholic steatohepatitis.

for the applicable concerns, index text for the applicable concerns and reference standards for the applicable concerns) from the diagnostic accuracy studies. If a certain study used both microarray platform, high-throughput sequencing (RNA- seq) and quantitative reverse transcription-PCR (RT-qPCR), the fold-changes were extrapolated from the RT-qPCR results because RT-qPCR is the most frequently used approach for validation of circulating miRNAs and has superior sensitivity and specificity. When the same population was published in several journals, we retained only the most informative article or complete study to avoid duplication. We extracted data from the graphical plots to calculate fold-changes, sensitivity and specificity by using WebPlotDigitizer (version 4.0.0). Accordingto the Cochrane book, the extracted median from box plots was directly used as the mean in our meta-analysis because the median is very similar to the mean when the distributionof the data is symmetric.[26] The extracted data, such as copy number, that could not be converted to fold-changes are reported as ‘‘not available”. If two investigators disagreed, a third author (MRG) was consulted. Two reviewers (CHL and RMV) independently assessed the qualities of eligible studies by using the Quality Assessment for Studies of DiagnosticAccuracy (QUADAS-2) tool.[27]

Data synthesis

The present study is reported separately in two main parts, namely, the expression profile and the diagnostic accuracy performance (Fig. 1). All extracted information was divided according to fibrosis severity, NAFLD *vs.* healthy control, NAFL *vs.* healthy control, NASH *vs.* NAFL and NASH *vs.* healthy control. Only the miRNAs of expression profile studies from the systematic review that had at least one validation study were included in the meta-analysis. The vote-counting strategy, a method of ranking potential molecular biomarkers developed by Griffith *et al.*[28] and Chan *et al.,*[29] was used in our meta-analysis. According to this vote-counting strategy,[28,29] miRNAs were ranked as potential biomarkers based on the following criteria, in order of importance: i) the number of studies reporting an miRNA as differentially expressed in the same direction; ii) total sample size for comparison in agreement; and iii) the mean fold-change from all the studies reporting on differential expression that were in agreement and reported the fold-change data. The total sample size was considered more important than the average fold-change because many studies do not report fold-change. Moreover, a three-step mean fold-change estimation and calculation were performed to increase the reliability and make comparisons between steps (Fig. 1). If the results were very different between the second and third steps, the second step, in which the mean fold-changes were calculated with the histograms, was considered more reliable than the third step, in which the mean fold-changes were estimated and calculated with the box plots and scatter plots.

The diagnostic accuracy meta-analysis included studies only if the miRNA was included in three or more published

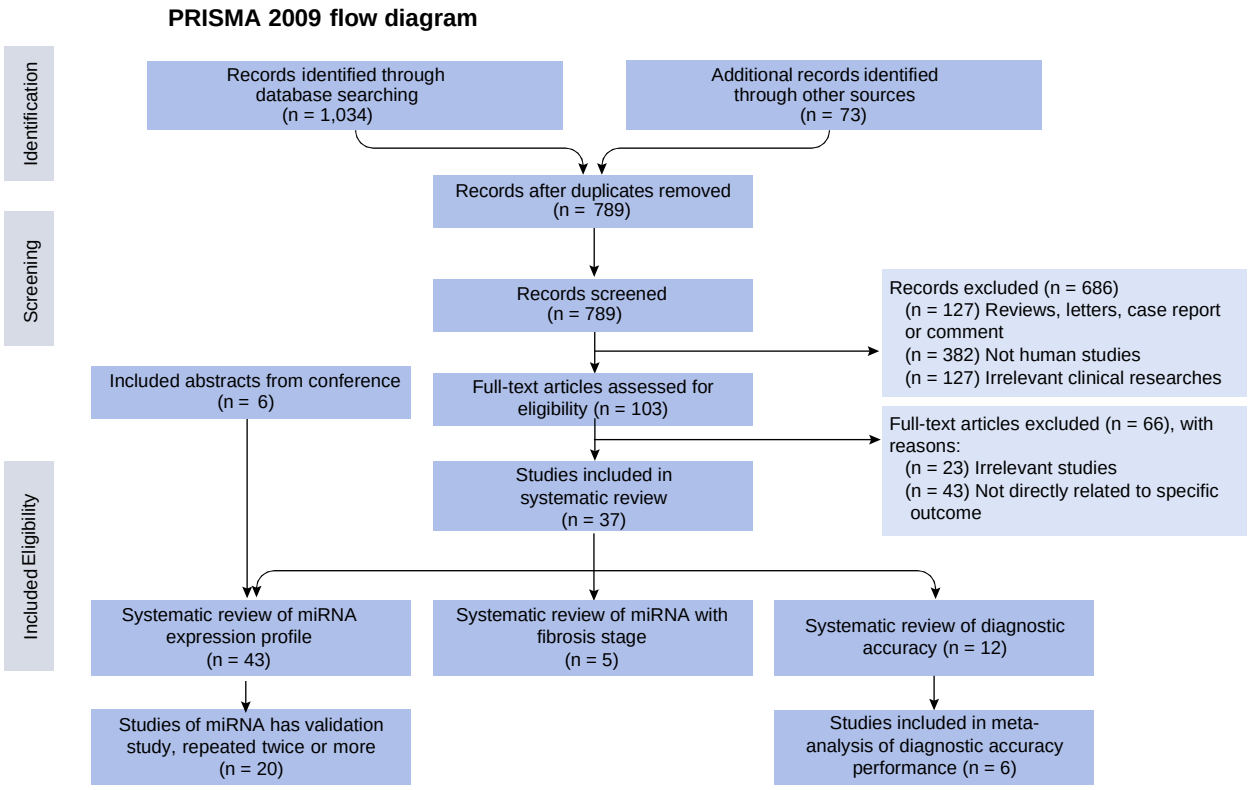

Fig. 2. Flow chart summarizing the selection of eligible studies. NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

Table 1. Database search for studies that reporting miRNA expression profiles in patients with NAFLD.

Studies reporting miRNA expression profiles (part A)					
miRNA	Number and reference	miRNA	Number and reference	miRNA	Number and reference
miRNA-9	1[82]	miRNA-30c	2[43,83]	miRNA-122-5p	2[42,58]
miRNA-10b	1[44]	miRNA-30b-5p	1[58]	miRNA-125	2[22,41]
miRNA-15b	1[84]	miRNA-33a	3[55,56,85]	miRNA-126	1[83]
miRNA-16	2[40,41]	miRNA-33b*	1[56]	miRNA-127	1[86]
miRNA-21	8[40,41,45,49,51,52,54,55]	miRNA-34a	10[33,39–41,48,50,53,54,57,83]	miRNA-130a-3p	1[87]
miRNA-22-3p	1[88]	miRNA-34a-5p	2[38,47]	miRNA-132	1[89]
miRNA-22-5p	1[88]	miRNA-99a	1[44]	miRNA-136	1[86]
miRNA-24-2-5p	1[88]	miRNA-99a-5p	1[42]	miRNA-139-5p	2[58,83]
miRNA-27b	1[41]	miRNA-99b-5p	1[38]	miRNA-141	1[90]
miRNA-27b-3p	1[42]	miRNA-101	1[36]	miRNA-144	1[85]
miRNA-29a	1[44]	miRNA-103	1[91]	miRNA-145	1[54]
miRNA-29a-3p	1[88]	miRNA-122	15[22,33,37,39–41,43,44,51,53–56,58,83]	miRNA-146b	5[33,38,41,44,83]
miRNA-30b	1[83]	miRNA-122b	1[41]	miRNA-146-5p	1[58]
Studies reporting miRNA expression profiles (continue, part B)					
miRNA	Number and reference	miRNA	Number and reference	miRNA	Number and reference
miRNA-148a-3p	1[42]	miRNA-223	2[51,83]	miRNA-423-5p	1[92]
miRNA-150	2[46,83]	miRNA-224	2[34,55]	miRNA-451	2[33,54]
miRNA-155	1[93]	miRNA-224-5p	1[38]	miRNA-495	1[86]
miRNA-181b	1[94]	miRNA-296-5p	1[95]	miRNA-572	1[43]
miRNA-181d	1[44]	miRNA-301a-3p	1[47]	miRNA-574	1[33]
miRNA-192	3[22,41,51]	miRNA-331-3p	1[83]	miRNA-575	1[43]
miRNA-192-5p	1[42]	miRNA-339-3p	1[35]	miRNA-638	1[43]
miRNA-197	1[44]	miRNA-375	2[22,47]	miRNA-642	1[83]
miRNA-199a-5p	1[96]	miRNA-376c	1[86]	miRNA-744	1[43]
miRNA-200a	1[97]	miRNA-379	1[86]	miRNA-1290	1[42]
miRNA-200b	2[34,97]	miRNA-409-3p	1[86]		
miRNA-200c	1[90,97]	miRNA-411	1[86]		
miRNA-221	1[55]	miRNA-422a	1[58]		

NAFLD, non-alcoholic fatty liver disease.

studies. The heterogeneity among these studies was measured by the Q test and Higgins's inconsistency index (I^2). A p value of more than 0.05 and I^2 value more than 50% indicated significant heterogeneity.[30] A subgroup analysis was performed to explore the heterogeneity. A bivariate regression approach was used to estimate the overall sensitivity and specificitywith 95% CIs and a summary receiver operating characteristic (sROC) curve approach by using Meta-DiSc v1.4 (Clinical Biostatistics Unit, Ramón y Cajal Hospital, Madrid, Spain). We also calculated the positive and negative likelihood ratios and the diagnostic odds ratio (DOR).[31] We assessed the poten- tial publication bias by using Deeks' funnel plot asymmetry test, in which p <0.10 indicated statistical significance.[32] We used STATA version 14 (Stata Corp; College Station, TX) with the program ''Midas" for Deeks' funnel plot. All statistical tests were two-sided, with p values ≤0.05 denoting statistical significance.

For further details regarding the methods used, please refer to the CTAT table and supplementary information.

Results

Research results and data extraction

The manual search of conference abstracts was from the year 2008 to 2018 because the first original article[33] on the current topic was published earlier in 2008. As seen in the flow diagram of article selection, a total of 702 studies were excluded. Thirty-seven original articles and six conference abstracts containing expression profile studies were included in the systematicreview, and 20 studies were ultimately included in the meta-analysis. Five studies that utilised miRNA expression profiles to distinguish fibrosis stage and 12 studies that performed diagnostic accuracy experiments were included in the systematic review, with six studies ultimately included in the meta-analysis. (Fig. 2) Four clinical setting scenarios were used, and the patients were divided as follows: NAFLD *vs.* healthy controls (833 total patients), NAFL *vs.* healthy controls (358 total patients), NASH *vs.* NAFL (406 total patients) and NASH *vs.* healthy controls (462 total patients). Studies that did not report the specific experimental design were not included in this meta-analysis.[34–39] In the diagnostic accuracy meta-analysis, three studies reported miRNA-122, and three studies addressed miRNA-34a. With regard to data extraction, six studies[22,40–44] directly reported the fold-changes in the original papers, we extracted the fold-changes from the histograms of eight studies,[41,45–50] the fold-changes from the box plots of two studies,[51,52] and the fold-changes from the scatter plot of one study.[53]

Data analyses: meta-analysis of miRNA expression profiles

Thirty-seven studies reporting miRNA expression profiles were analysed (Table 1). Twenty studies with miRNAs that had been included in validation studies in each subcategory of the different clinical scenarios were included in the meta-analysis (Table 2). The correct method of extracting data from the graphical plots was confirmed by our results, because the mean fold-changes in the third step were very similar to those in the second step. Therefore, all the fold-changes were interpreted along with the mean fold-changes determined in the third step (if available) to combine the greatest number of published

Table 2. Meta-analysis of expression profile studies that had at least one validation study.

Meta-analysis of expression profiles in NAFLD and NASH patients																			
NAFLD patients *vs.* Healthy controls																			
miRNA	Tissue type	Studies and years	Expression direction	Fold-change	Sample size (Healthy control/NAFLD/NAFL/NASH)	Total sample size	First step				Second step				Third step			
							No. of studies	Sample size	Mean fold-change	Range	No. of studies	Sample size	Mean fold-change	Range	No. of studies	Sample size	Mean fold-change	Range
miR-16	Serum	Cermelli, 2011	↗	5.50	19/34/18/16	138	1	53	5.50	–	2	138	3.88	2.25–5.50	–	–	–	–
		Liu, 2016	↗	2.25	37/48/17/31													
miRNA-21	Serum	Liu, 2016	↗	2.04	37/48/17/31	85	–	–	–	–	1	85	2.04	–	–	–	–	–
		Cermelli, 2011	⟷		19/34/18/16	53	–	–	–	–	–	–	–	–	–	–	–	–
		Sun, 2015	↙	0.63	12/25/–/–	37	–	–	–	–	1	37	0.63	–	–	–	–	–
	Liver	Lendvai, 2014	⟷		12/18/–/–	30	–	–	–	–	–	–	–	–	–	–	–	–
miRNA-34a	Serum	Yamada, 2013	↗		311/92/–/–	605	1	85	2.80	–	1	85	2.80	–	2	149	4.42	2.80–6.04
		Liu, 2016	↗	2.80	37/48/17/31													
		Salvoza, 2016	↗	6.04	36/28/–/–													
		Cermelli, 2011	↗		19/34/18/16													
miRNA-122	Serum	Salvoza, 2016	↗	5.10	36/28/–/–	605	2	138	7.55	7.20–7.90	2	138	7.55	7.20–7.90	3	202	6.73	5.10–7.90
		Cermelli, 2011	↗	7.20	19/34/18/16													
		Liu, 2016	↗	7.90	37/48/17/31													
		Yamada, 2013	↗		311/92/–/–													
		Zarrinpar, 2016	↗		62/18/–/–	80	–	–	–	–	–	–	–	–	–	–	–	–
	Liver	Lendvai, 2014	⟷		12/18/–/–	30	–	–	–	–	–	–	–	–	–	–	–	–
miRNA-122-5p	Serum	Tan, 2014	↗	9.27	20/20/–/–	40	1	40	9.27	–	–	–	–	–	–	–	–	–
	Liver	Latorre, 2017	↗		19/17/–/–	36	–	–	–	–	–	–	–	–	–	–	–	–
miRNA-139-5p	Serum	Zarrinpar, 2016	↗		62/18/–/–	80	–	–	–	–	–	–	–	–	–	–	–	–
	Liver	Latorre, 2017	↗		19/17/–/–	36	–	–	–	–	–	–	–	–	–	–	–	–
miRNA-146b	Serum	Zarrinpar, 2016	↗		62/18/–/–	165	–	–	–	–	1	79	1.96	–	–	–	–	–
		Liu, 2016	↗	1.96	37/48/17/31													
miRNA-150	Serum	Zarrinpar, 2016	↗		62/18/–/–	135	–	–	–	–	1	55	3.54	–	–	–	–	–
		Zhuge, 2017	↗	3.54	22/33/–/–													
NAFL patients *vs.* Healthy controls																			
miRNA	Tissue type	Studies and years	Expression direction	Fold-change	Sample size (Healthy control/NAFLD/NAFL/NASH)	Total sample size	First step				Second step				Third step			
							No. of studies	Sample size	Mean fold-change	Range	No. of studies	Sample size	Mean fold-change	Range	No. of studies	Sample size	Mean fold-change	Range
miRNA-16	Serum	Cermelli, 2011	↗	5.30	19/34/18/16	37	1	37	5.30	–	1	37	5.30	–	–	–	–	–
		Liu, 2016	⟷	1.88	37/48/17/31	54	–	–	–	–	1	54	1.88	–	–	–	–	–
miRNA-21	Serum	Cermelli, 2011	⟷		19/34/18/16	202	–	–	–	–	1	54	1.72	–	2	165	1.33	0.94–1.72
		Becker, 2015	⟷	0.94	61/137/50/87													
		Liu, 2016	⟷	1.72	37/48/17/31													
	Liver	Loyer, 2016	⟷	1.31	6/19/8/11	14	–	–	–	–	–	–	–	–	1	14	1.31	–
miRNA-34a	Serum	Cermelli, 2011	↗		19/34/18/16	37	–	–	–	–	–	–	–	–	–	–	–	–
		Liu, 2016	⟷	1.39	37/48/17/31	54	–	–	–	–	1	54	1.39	–	1	54	1.39	–
		Muangpaisarn, 2017	⟷		23/50 /17/33	40	–	–	–	–	–	–	–	–	–	–	–	–

(*continued on next page*)

Table 2 (*continued*)

NAFL patients *vs.* Healthy controls																			
miRNA	Tissue type	Studies and years	Expression direction	Fold-change	Sample size (Healthy control/NAFLD/ NAFL/NASH)	Total sample size	First step				Second step				Third step			
							No. of studies	Sample size	Mean fold-change	Range	No. of studies	Sample size	Mean fold-change	Range	No. of studies	Sample size	Mean fold-change	Range
miRNA-122	Serum	Becker, 2015	↗	2.72	61/137/50/87	202	1	37	5.70	–	2	91	5.10	4.50–5.70	3	202	4.31	2.72–5.70
		Liu, 2016	↗	4.50	37/48/17/31													
		Cermelli, 2011	↗	5.70	19/34/18/16													
		Auguet, 2016	⟷		22/40/18/22	89	–	–	–	–	–	–	–	–	–	–	–	–
		Pirola, 2015	⟷		19/77/30/47													
	Liver	Pirola, 2015	↗		19/77/30/47	49	–	–	–	–	–	–	–	–	–	–	–	–
miRNA-192	Serum	Liu, 2016	↗	2.39	37/48/17/31	54	–	–	–	–	1	54	2.39	–	–	–	–	–
		Becker, 2015	⟷	1.26	61/137/50/87	160	–	–	–	–	–	–	–	–	1	111	1.26	–
		Pirola, 2015	⟷		19/77/30/47													
	Liver	Pirola, 2015	↗		19/77/30/47	–	–	–	–	–	–	–	–	–	–	–	–	
miRNA-375	Serum	Pirola, 2015	⟷		19/77/30/47	49	–	–	–	–	–	–	–	–	–	–	–	–
	Liver	Guo, 2016	↙	0.54	10/23/12/11	22	–	–	–	–	1	22	0.54	–	1	22	0.54	–
NASH *vs.* NAFL																			
miRNA	Tissue type	Studies and years	Expression direction	Fold-change	Sample size (Healthy control/NAFLD/ NAFL/NASH)	Total sample size	First step				Second step				Third step			
							No. of studies	Sample size	Mean fold-change	Range	No. of studies	Sample size	Mean fold-change	Range	No. of studies	Sample size	Mean fold-change	Range
miRNA-16	Serum	Liu, 2016	⟷	1.34	37/48/17/31	82	–	–	–	–	1	48	1.34	–	1	48	1.34	–
		Cermelli, 2011	⟷		19/34/18/16													
miRNA-21	Serum	Becker, 2015	↗	1.55	61/137/50/87	137	–	–	–	–	–	–	–	–	1	137	1.55	–
		Liu, 2016	⟷	1.30	37/48/17/31	82	–	–	–	–	1	48	1.30	–	1	48	1.30	–
		Cermelli, 2011	⟷		19/34/18/16													
	Liver	Loyer, 2016	↗	3.44	6/19/8/11	19	–	–	–	–	–	–	–	–	1	19	3.44	–
miRNA-34a	Serum	Cermelli, 2011	↗	2.80	19/34/18/16	82	1	34	2.80	–	2	82	2.90	2.80–2.99	–	–	–	–
		Liu, 2016	↗	2.99	37/48/17/31													
		Muangpaisarn, 2017	⟷		23/50 /17/33	50	–	–	–	–	–	–	–	–	–	–	–	–
	Liver	Castro, 2013	↗	2.66	0/28/15/13	28	–	–	–	–	1	28	2.66	–	–	–	–	–
miRNA-122	Serum	Pirola, 2015	↗	3.10	19/77/30/47	336	2	111	2.55	2.00–3.10	3	159	2.51	2.00–3.10		296	2.61	2.00–3.10
		Becker, 2015	↗	2.94	61/137/50/87													
		Liu, 2016	↗	2.42	37/48/17/31													
		Auguet, 2016	↗		22/40/18/22													
		Cermelli, 2011	↗	2.00	19/34/18/16													
	Liver	Pirola, 2015	↙	10.00	19/77/30/47	77	1	77	10.00	–	–	–	–	–	–	–	–	–
miRNA-192	Serum	Becker, 2015	↗	1.93	61/137/50/87	262	–	–	–	–	1	48	2.28	–	2	185	2.10	1.93–2.27
		Liu, 2016	↗	2.28	37/48/17/31													
		Pirola, 2015	↗		19/77/30/47													
	Liver	Pirola, 2015	↙		19/77/30/47	77	1	77	2.00	–	–	–	–	–	–	–	–	–
miRNA-375	Serum	Pirola, 2015	↗		19/77/30/47	77	–	–	–	–	–	–	–	–	–	–	–	–
	Liver	Guo, 2016	⟷	1.15	10/23/12/11	23	–	–	–	–	1	23	1.15	–	1	23	1.15	–

NASH patients *vs.* Healthy controls																			
miRNA	Tissue type	Studies and years	Expression direction	Fold-change	Sample size (Healthy control/NAFLD/ NAFL/NASH)	Total sample size	First step				Second step				Third step			
							No. of studies	Sample size	Mean fold-change	Range	No. of studies	Sample size	Mean fold-change	Range	No. of studies	Sample size	Mean fold-change	Range
miRNA-21	Serum	Becker, 2015	↗	1.46	61/137/50/87	216	–	–	–	–	1	68	2.23	–	2	216	1.85	1.46–2.23
		Liu, 2016	↗	2.23	37/48/17/31													
	Liver	Loyer, 2016	↗	4.51	6/19/8/11	23	–	–	–	–	1	6	4.34	–	2	23	4.43	4.31–4.51
		Dattaroy, 2015	↗	4.34	3/3/0/3													
miRNA-34a	Serum	Liu, 2016	↗	4.16	37/48/17/31	68	–	–	–	–	1	68	4.16	–	1	68	4.16	–
		Muangpaisarn, 2017	↗		23/50 /17/33	56	–	–	–	–								
		Celikbilek, 2014	⟷		20/20/0 /20	40	–	–	–	–	–	–	–	–	–	–	–	–
	Liver	Xu, 2015	↗	2.30	–/8/0/8	–	–	–	–	–	1	–	2.30	–	–	–	–	–
miRNA-122	Serum	Pirola, 2015	↗	7.20	19/77/30/47	410	2	110	5.12	3.04–7.20	3	178	7.04	3.04–10.87	4	326	7.28	3.04–10.87
		Becker, 2015	↗	8.00	61/137/50/87													
		Liu, 2016	↗	10.87	37/48/17/31													
		Zhang, 2012	↗	3.04	24/20/0/20													
		Auguet, 2016	↗		22/40/18/22													
		Celikbilek, 2014	⟷		20/20/0 /20	40	–	–	–	–	–	–	–	–	–	–	–	–
miRNA-146b	Serum	Liu, 2016	↗	2.23	37/48/17/31	68	–	–	–	–	1	68	2.23	–	–	–	–	–
		Celikbilek, 2014	↙	1.52	20/20/0 /20	40	1	40	1.52	–	–	–	–	–	–	–	–	–
miRNA-192	Serum	Becker, 2015	↗	2.43	61/137/50/87	282	1	66	4.40	–	2	134	4.92	4.40–5.44	3	282	4.09	2.43–5.44
		Liu, 2016	↗	5.44	37/48/17/31													
		Pirola, 2015	↗	4.40	19/77/30/47													
miRNA-375	Serum	Pirola, 2015	↗		19/77/30/47	66	–	–	–	–	–	–	–	–	–	–	–	–
		Guo, 2016	↙	0.62	10/23/12/11	21	–	–	–	–	1	21	0.62	–	1	21	0.62	–

Orange, fold-change already reported in original papers. Green, fold-change directly extracted and calculated with reported box plot (mean ± standard deviation). Yellow, fold-change extracted, estimated, calculated with reported histogram (median ± interquartile). Red, fold-change extracted, estimated and calculated with reported scatter plot. By using the vote-counting strategy, the total sample size was considered more important than the average fold- change. Three steps of combining mean fold-change were used. First step: studies reported fold-change directly; second step: studies together with fold-change extracted with box plot; third step: studies together with fold-change extracted with histogram and scatter plot.

NAFL, non-alcoholic fatty liver; NAFLD, NAFL disease; NASH, non-alcoholic steatohepatitis.

studies, which included eight studies on miRNA-34a and 11 studies on miRNA-122. Thus, the results on those two miRNAs were considered more reliable because they were included inthe greatest numbers of reported studies. miRNA-122 had the most consistent upregulation in every scenario used to distin- guish different clinical settings, such as NAFLD *vs.* healthy con- trols with a total sample size of 605 and a mean fold-change of 6.73 (range 5.10–7.90), NAFL *vs.* healthy controls with a total sample size of 202 and a mean fold-change of 4.31 (range 2.72–5.70), NASH *vs.* NAFL with a total sample size of 336 anda mean fold-change of 2.61 (range 2.00–3.10) and NASH *vs.* healthy controls with a total sample size of 410 and a mean fold-change of 7.28 (range 3.04–10.87). miRNA-34a demon- strated a consistent upregulation in scenarios of NAFLD *vs.* healthy controls, with a total sample size of 605 and a meanfold-change of 4.42 (range 2.80–6.04). The upregulation of miRNA-192 showed consistency and usefulness in distinguish- ing NASH from NAFL, with a total sample size of 262 and a mean fold-change of 2.10 (range: 1.93–2.27). The upregulated miRNA- 21 could be used to distinguish NASH from healthy controls with both serum samples (a total sample size of 216 and a mean fold-change of 1.85 [range 1.46–2.23]) and liver samples (a total sample size of 23 and a mean fold-change of 4.43 [range 4.31– 4.51]). Notably, there was almost always an inconsistent or even inverse correlation between the direction of miRNA expressionin the serum and in the liver. For example, serum miRNA-122was always upregulated in NAFLD *vs.* healthy controls[40,41,53,54]but was unchanged in liver tissue,[55] and miRNA-122 was upreg- ulated in NASH *vs.* NAFL in serum[22,40,41,51,56] and downregu- lated in liver samples.[22]

Systematic review of miRNAs used to distinguish fibrosis stage, diagnostic accuracy of studies and quality assessment of meta-analysis

Five studies that reported miRNAs to distinguish fibrosis stage (Table 3) and 12 studies that reported diagnostic accuracy (Table 4) were included in the systematic review. Quality assessment was performed in accordance with the modified QUADAS-2 criteria (Table 5). Details of the quality assessment are also reported (supplementary information). The major biases identified in the studies were in ''patient selec- tion''[40,53,56,57] and ''flow and timing''.[40,41,56] None of the studies reported the interval between the index text and reference stan- dard because diagnostic performance tests were not the main objective of those studies.[40,41,53,56–58]

Data analyses: meta-analysis of diagnostic accuracy

miRNA-122 showed an AUC of 0.82 (95% CI 0.75–0.89) and DOR of 9.1 (95% CI 4.63–17.96) in the diagnosis of NAFLD *vs.* healthy controls. (Fig. 3A) miRNA-34a showed an AUC of 0.78 (95% CI 0.67–0.88) and a DOR of 6.248 (95% CI 2.69–15.34) for the diagnosis of NASH *vs.* NAFL. (Fig. 3B). There was no heterogeneity.

Publication bias

The Deeks' funnel plots[32] failed to reveal any publication bias for miRNA-122 (p = 0.53) or miRNA-34a (p = 0.95) (Fig. 3C and 3D).

Discussion

In the present study, the potential biomarkers miRNA-122 and miRNA-192 were consistently upregulated in NASH *vs.* NAFL, fulfilling a principle requirement for use as biomarkers in clini- cal practice. miRNA-122 showed a consistent upregulation in all stages of disease severity, indicating that this miRNA should be further researched. An inconsistent or inverse correlation was found between circulating and liver-expressed miRNAs. Although a limited number of studies were included, the sROC curves showed a moderate diagnostic accuracy (0.7–0.9) of miRNA-122 in distinguishing NAFLD *vs.* healthy control patients and of miRNA-34a in distinguishing NASH *vs.* NAFL patients. To our knowledge, this is the first meta-analysis to report either

Table 3. Systematic review of miRNA expression profiling in patients with NAFLD with different fibrosis stages.

miRNA	Tissue type	Patient	Study and year	Expression direction	Fibrosis	p values
miRNA-34a	Serum	36 Healthy controls/28 NAFLD	Salvoza, 2016	G	Healthy controls *vs.* F0	p = 0.01–0.05
				G	Healthy controls *vs.* F1	p = 0 0.01–0.05
				G	Healthy controls *vs.* F2	p = 0.01–0.05
				↔	Healthy controls *vs.* F3	p = n.s.
				↔	Healthy controls *vs.* F4	p = n.s.
				G	Healthy controls *vs.* F0-F2	p = 0.001–0.01
				G	Healthy controls *vs.* F3-F4	p <0.001
		67 NAFLD	Miyaaki, 2014	D	F0-1 *vs.* F2-4	p = 0.0191
		23 Healthy controls/50 NAFLD	Muangpaisarn, 2017	↔	Healthy controls *vs.* F0-1	p = 0.07
				G	F0-1 *vs.* F2-4	p = 0.022
miRNA-122	Serum	36 Healthy controls/28 NAFLD	Salvoza, 2016	G	Healthy controls *vs.* F0	p <0.001
				G	Healthy controls *vs.* F1	p <0.001
				G	Healthy controls *vs.* F2	p = 0.001–0.01
				G	Healthy controls *vs.* F3	p = 0.01–0.05
				↔	Healthy controls *vs.* F4	p = n.s.
				G	Healthy controls *vs.* F0-F2	p = 0.001–0.01
				G	Healthy controls *vs.* F3-F4	p <0.001
		19 Healthy controls/77 NAFLD	Pirola, 2015	G	F0-1 *vs.* F2-3	p <0.014
miRNA-192	Serum	19 Healthy controls/77 NAFLD	Pirola, 2015	↔	F0-1 *vs.* F2-3	p = n.s.
miRNA-221	Liver	26 NASH	Ogawa, 2012	G	F1 *vs.* F4	p <0.01
miRNA-222	Liver	26 NASH	Ogawa, 2012	G	F1 *vs.* F4	p <0.01
miRNA 375	Serum	19 Healthy controls/77 NAFLD	Pirola, 2015	↔	F0-1 *vs.* F2-3	p = n.s.

NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; n.s., not significant.

Table 4. Systematic review of the diagnostic performance of miRNAs in patients with NAFLD or NASH.

Systematic review of the diagnostic performance of miRNA in patients with fibrosis, NAFLD and NASH (part A)

Significant fibrosis (F ≥2)				NAFLD vs. Healthy controls				NAFL vs. Healthy controls			
miRNA	Study	Sample size (HC/P)	AUC	miRNA	Study	Sample size (NAFLD/HC)	AUC	miRNA	Study	Sample size (NAFL/HC)	AUC
miRNA-34a	Liu, 2016	48/37	0.716	miRNA-21	Salvoza, 2016	28/36	0.697	miRNA-16	Cermelli, 2011	18/19	0.927
miRNA-122	Pirola, 2015	77/19	0.666	miRNA-27b-3p	Tan, 2014	152/90	0.693	miRNA-122	Cermelli, 2011	18/19	0.926
	Miyaaki, 2014	67 total	0.82	miRNA-30b	Latorre, 2017	41/19	0.71				
				miRNA-34a	Salvoza, 2016	28/36	0.781				
				miRNA-99a-5p	Tan, 2014	152/90	0.559				
				miRNA-122	Salvoza, 2016	28/36	0.858				
					Auguet, 2016	61/61	0.82				
					Latorre, 2017	41/19	0.68				
				miRNA-122-5p	Tan, 2014	152/90	0.729				
				miRNA-125b	Salvoza, 2016	28/36	0.661				
				miRNA-139-5p	Latorre, 2017	41/19	0.74				
				miRNA-146b	Latorre, 2017	41/19	0.67				
				miRNA-148a-3p	Tan, 2014	152/90	0.54				
				miRNA-192-5p	Tan, 2014	152/90	0.652				
				miRNA-442a	Latorre, 2017	41/19	0.7				
				miRNA-1290	Tan, 2014	152/90	0.629				

Systematic review of the diagnostic performance of miRNA in patients with fibrosis, NAFLD and NASH (continue, part B)

NASH vs. NAFL				NASH vs. Healthy controls			
miRNA	Study	Sample size (NASH/NAFL)	AUC	miRNA	Study	Sample size (NASH/HC)	AUC
miRNA-34a	Liu, 2016	31/13	0.811	miR-99a	Celikbilek, 2014	20/20	0.76
	Cermelli, 2011	16/18	0.764	miR-122	Zhang, 2012	20/34	0.8
	Muangpaisarn, 2017	33/17	0.67	miR 146b	Celikbilek, 2014	20/20	0.75
miRNA-122	Pirola, 2015	47/30	0.635	miR-181d	Celikbilek, 2014	20/20	0.86
	Cermelli, 2011	16/18	0.698	miR-197	Celikbilek, 2014	20/20	0.77
miRNA-192	Pirola, 2015	47/30	0.676	miR-572	Zhang, 2012	20/34	0.85
miRNA-375	Pirola, 2015	47/30	0.72	miR-575	Zhang, 2012	20/34	0.9
Combination	Becker, 2015	87/50	0.81	miR-638	Zhang, 2012	20/34	0.87
				miR-744	Zhang, 2012	20/34	0.96

AUC, area under the curve; SF, significant fibrosis; HC, healthy controls; NAFL, non-alcoholic fatty liver; NAFLD, NAFL disease; NASH, non-alcoholic steatohepatitis; Combination: combination of miRNA-21, miRNA-122 and miRNA-192.

Table 5. Quality assessment with QUADAS-2 criteria.

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
miRNA-122: NAFLD *vs.* Healthy controls							
Salvoza, 2016	unclear	low risk	low risk	low risk	low risk	low risk	low risk
Auguet, 2016	high risk	unclear	low risk	unclear	high risk	low risk	low risk
Latorre, 2017	low risk	low risk	low risk	high risk	low risk	low risk	low risk
miRNA-34a: NASH *v*:	NAFL						
Cermelli, 2011	high risk	high risk	low risk	unclear	low risk	high risk	low risk
Liu, 2016	low risk	low risk	low risk	unclear	low risk	low risk	low risk
Muangpaisrn, 2014	high risk	low risk	high risk	low risk	high risk	low risk	high risk

NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

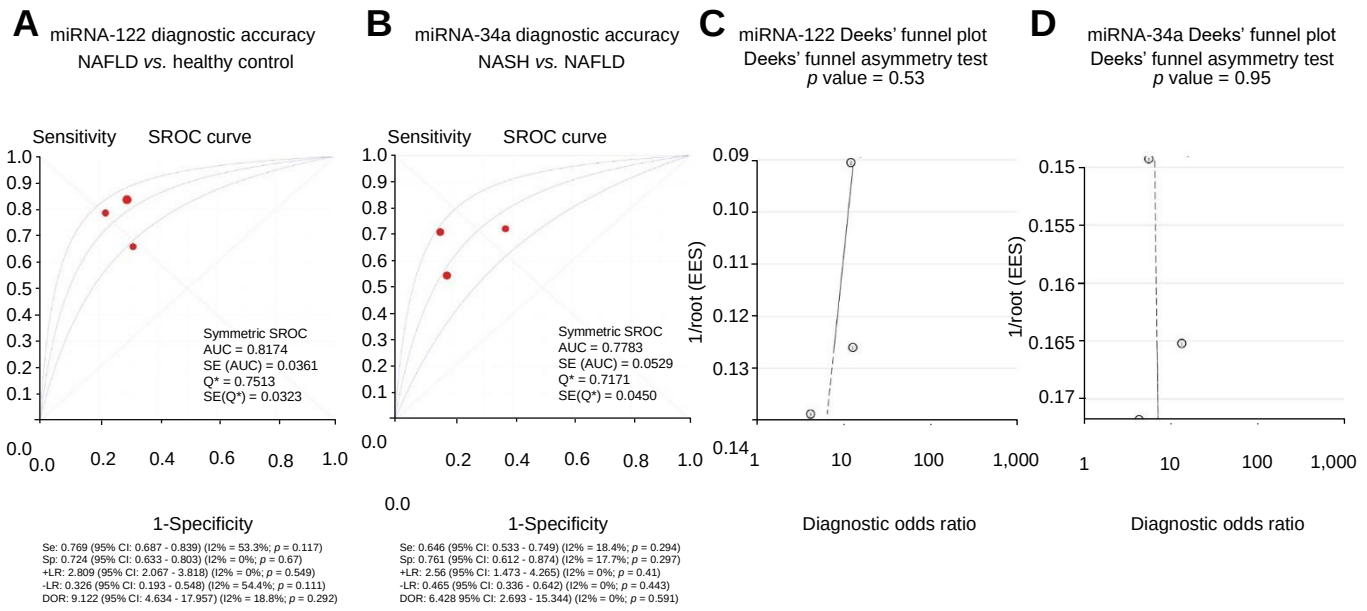

Fig. 3. Diagnostic accuracy of miRNAs. (A) miRNA-122 for NAFLD *vs.* healthy controls and (B) miRNA-34a for NASH *vs.* NAFL. Publication bias assessment by Deeks' funnel plot. (C) miRNA-122 and (D) miRNA-34a. NAFL, non-alcoholic fatty liver; NAFLD, NAFL disease; NASH, non-alcoholic steatohepatitis. The heterogeneity among these studies was measured by Q test and Higgins's inconsistency index (I^2). A bivariate regression approach was used to estimate the sensitivtiy and specificity with 95% confidence intervals (CIs) and a summary receiver operating characteristic (sROC) curve. The publication bias were assessed by using Deeks' funnel plot asymmetry test, and A p value of 0.10 indicated statistical significance. (This figure appears in colour on the web.)

miRNA expression profiles or diagnostic accuracy in patients with NAFLD. We found consistency in each miRNA expression direction and demonstrated combined mean fold-changes according to disease severity. We also illustrated the diagnostic performance by using sROC curves. To avoid publication bias, we also included conference abstracts and posters because negative results are not usually published in international jour- nals as original articles. The vote-counting strategy we used helps to avoid publication bias by including studies with both negative and positive results. Compared with traditional vote- counting strategy studies, we further used reliable graphical data extraction software to reverse-engineer raw numerical data from images of data visualizations. The three-step compar- ison of the mean fold-changes was used to maximise the sample size and further strengthened the power of the reliability.

Most of the differences found in the miRNAs assessed in this study show biological plausibility according to the main pathogenic mechanisms for NAFLD. miRNAs are involved in the regulation of lipid metabolism, generation of reactive oxygen species, cell differentiation and also in controlling cell inflam- mation, proliferation, apoptosis and fibrosis, which were all key events in the pathogenesis of NASH.[23,33,59] To date, miRNA-34a and miRNA-122 have been the most investigated miRNAs in patients with NAFLD. miRNA-34a expression showed an association with susceptibility to NAFLD in mice fed a high-fat diet.[60] Anti-sense inhibition of miRNA-34a in obese mice improved metabolic gene expression and metabolic outcome.[61] miRNA-34a were upregulated in serum samples of patients with NAFLD,[40,41,53,54] and its levels of expression were associated with disease severity.[48] miRNA-122 may play an essential role in the regulation of hepatocyte differentiation and liver development.[62] Serum miRNA-122 was upregulated in patients with NAFLD.[40,41,53] Its concentration was correlated with liver histological stage, severity of liver steatosis,[54,63] inflammation grade, and ALT activity.[64,65] miRNA-122 was also associated with cholesterol- and lipid-metabolising enzymes,[64] and it regulated metabolic pathways (including cholesterol biosynthesis).[65,66] Furthermore, serum-obtained miRNAs are more tissue-specific than tumour-specific,[67] supporting our principle objective of NASH and fibrosis screening by using circulating miRNAs. However, downregulation of miRNA-122 has been observed in HCC, often in advanced tumours associated with a poor prognosis.[68] Boeri *et al.* found that a partially different set of miRNAs were dysregulated in plasma before and during the disease course, suggesting that genes and pathways necessary in the earlier phases of disease development are different from those required for tumour maintenance and progression.[69] Interestingly, we found that several miRNAs had an inconsistent or inverse correlation between circulating and liver tissue expression. Pirola *et al.* also described the same phenomenon with

1344

miRNA-122 in patients with NAFLD, suggesting that the lower expression of miRNA-122 in the liver is a consequence of the dynamic regulation of the biological process that produces a high rate of miRNA-122 release into the circulation, supporting our results.[22] Furthermore, the same inverse correlation of EVs and liver sample were found by Povero *et al.*,[70] suggesting thatthe miRNAs might have completely different functions and roles according to the compartment they are transported in. This new insight may enable the future refinement of miRNA in biomarkers, where the miRNA in the different compartments, such asEVs, are better than whole blood miRNA measurements. The same inverse correlation was also found for miRNA-101 expres- sion in patients with HCC[71] and for miRNA-139-5p in patients with primary biliary cirrhosis.[72] Moreover, Boeri *et al.* found that miRNAs that were dysregulated in liver tissue specimens were rarely detected in plasma samples in patients with lungcancer, further strengthening the high tissue-specificity of miRNAs and suggesting a predictive role of plasma miRNAs independent of tissue specimens.[69] Future studies need to investigate why this occurs and what mechanism is involved. However, a single miRNA can have many targets, potentially providing simultaneous regulation of many genes; furthermore, many different miRNAs can take action synergistically at multi- ple target sites of a single miRNA.[73] Thus, the approach used in the current study cannot overcome this complication. Another investigatory method, such as the integrated analysis used byZhu *et al.*,[74] may yield a better understanding of gene regulation and improve systems-level modelling. Moreover, if a single bio- marker is not sufficient to precisely distinguish NASH from NAFL, those miRNAs combined with clinical and laboratory composite scores and metabolomics could further improve the diagnostic and prognostic value.

A problem associated with miRNA expression profiling studies is that they lack agreement. The present study helped us to address and select potential biomarkers. However, the following concerns emerged. First, data normalization used to remove variation across samples can minimise systematic technical or experimental variation and thus inappropriate normalization of the data can lead to incorrect conclusions. Several normaliza- tion methods have been proposed for miRNA expression profiling analyses including the following: a) one or a few endogenous control miRNAs largely invariant in a given sample set (the most commonly used); b) global measure of the miRNA expression data as the normaliser for large-scale miRNA- profiling data sets; and c) spiked-in synthetic control miRNAsthat are introduced into the RNA sample at a range of known input amounts (more advanced and rigorous quality control). To date, there is no consensus regarding the use of control microRNAs for either RT-qPCR, microarray, or RNA-seq; the studies that were included in the present meta-analysis used different housekeeping genes for normalization. Standard nor- malization methods for miRNA need to be established carefully and to be scrutinised in specific biological contexts. Second, the results of a single study may be influenced by variability in sample collection and processing, RNA isolation, the expression profiling platform (the inter-platform reproducibility is rela- tively low between different platforms)[75] and the method of detection.[76] For example, Pirola *et al.*[22] used the MIHS 106Z PCR array, which interrogates 84 miRNAs; however, of the twelve validated miRNAs in the previous study, only four (miR-17, miR-150, miR-200a and miR-224) were reproduced in the study performed by Leti *et al.*[76] In terms of choosing the platforms, miRNA microarrays are less expensive but tend to have a lower sensitivity and dynamic range and are therefore best used as discovery tools; however, RT-qPCR generally has the widest dynamic range, highest accuracy and is the only method that can easily provide absolute miRNA quantification. Third, depending on the goals of miRNA-profiling experiments, measurement of different specimens (liver tissue, serum, plasma, blood, microvesicle, apoptotic body and exosome, *etc.*) may also contribute to poor reproducibility due to completely different functions and roles according to the compartment the miRNA is transported in, which must be considered. For example, human blood plasma is a challenging specimen type because miRNA measurements were impacted by high levels of endogenous RNase activity, centrifugation conditions, white blood cell counts and red blood cell hemolysis.[77] Fourth, although body mass index, age and diabetes are known to affect miRNA expression, none of the included studies used multivari- ate Cox logistic regression to adjust for potential confounders. Fifth, genetic, environmental and clinicopathological differences in donor samples were not considered. Sixth, most of the included studies did not use or report *a priori* significant thresh- olds (*e.g.*, cut-off ≥2 or cut-off ≥2.5). Finally, although RT-qPCR is the gold standard of gene expression measurement, the pres- ence of inhibitors in serum samples may limit the ability to extract RNA or the ability to accurately measure serum miRNAs by RT-qPCR.[78] Thus, future miRNA expression profiling experi- ments need to address those considerations and avoid those limitations. For a deeper discussion of miRNA characteristics, sample considerations, miRNA-profiling methods and miRNA data analysis and interpretation, we point readers to a recent review[77] by Pritchard *et al.*

Fibrosis is a major risk factor for the development of HCC; however, we could not draw conclusions on whether miRNAs are useful diagnostic tools with which to distinguish fibrosis stages because of the limited number of published studies. Nevertheless, an accurate diagnostic tool based on miRNAs will not only aid in NASH diagnosis but will also help mitigate the healthcare burden and improve cost-effectiveness, as medical resources will be diverted toward 'high-risk' populations instead of whole populations to perform further investigations. More importantly, the screening of high-risk populations will ensure a high pre-test probability of identifying clinically signif- icant liver disease because a lower prevalence of the disease decreases the accuracy of the diagnostic methods.[79] Moreover, experimental populations should be extended from tertiary referral centres to populations that attend primary care (better reflecting the populations at risk who need to be screened) to avoid selection bias.[80] Finally, determination of potential miR- NAs to be used as biomarkers could facilitate the development of miRNA therapeutic target drugs.

There were several limitations of the present study. First, miRNA expression profiling investigations involve several heterogeneities due to their use of different profiling platforms, qPCR normalization controls and various statistics and bio-computational analysis methods. To resolve this problem, our use of the vote-counting strategy, a ranking system to explore candidate molecular biomarkers, and the inclusion of more than just statistical significance calculations helped us identify the consistencies in miRNA expression from all the findings and avoid bias. Second, a disadvantage of the vote- counting strategy is that weights of the studies' contributions were ignored, as there were primary studies that did not report

non-significant results. Even though the use of aggregated raw profiling datasets is considered the ideal method of analysing miRNA, it is usually unrealistic to accomplish due to the unavailability of raw datasets, the low concordance of inter-platform results and the large heterogeneity. Therefore, we still believe the vote-counting strategy is the best option for the meta-analysis of miRNA expression profiling studies. Third,the different diagnostic criteria were used to diagnostic NASH among the included studies and previous study[81] clearly indicated the importance of reference standards, study designs and cut-off values on the results of diagnostic accuracy experiments. Finally, a relatively small number of patients were included in the individual studies, limiting the strength of the conclusions of our meta-analysis, but we hope our meta-analysis may have provided a basis for future studies.

In conclusion, miRNA-34a, miRNA-122 and miRNA-192 were identified as potential biomarkers with which to distin- guish NASH from NAFL. miRNA-122 demonstrated its useful- ness in every scenario in distinguishing disease severity. Both miRNA-122, in distinguishing patients with NAFLD fromhealthy controls, and miRNA-34a, in distinguishing patients with NASH from those with NAFL, showed moderate diagnos-tic accuracy.

Financial support

The authors received no financial support to produce this manuscript.

Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Authors' contributions

Guarantor of the article: MRG. Study design: MRG. Drafting the manuscript: CHL, MRG. Statistical analyses and interpretation: CHL, MRG. Data acquisition of expression profile part: CHL, AGG, JA. Data acquisition of fibrosis part: CHL, RMV, RMH. Data acquisition of diagnostic accuracy part: CHL, AR, RGD. Critical revision of the manuscript: MRG, JA, AGG.

Acknowledgements

FIS 2016-01842 from ISCIII, MINECO, Spain.

References

Author names in bold designate shared co-first authorship

[1] Konerman MA, Jones JC, Harrison SA. Pharmacotherapy for NASH: current and emerging. J Hepatol 2018;68:362–375.

[2] Cholankeril G, Perumpail RB, Pham EA, Ahmed A, Harrison SA. Nonalcoholic fatty liver disease: epidemiology, natural history, and diagnostic challenges. Hepatology 2016;64:954.

[3] Harrison SA. NASH, from diagnosis to treatment: where do we stand? Hepatology 2015;62:1652–1655.

[4] Spengler EK, Loomba R. Recommendations for diagnosis, referral for liver biopsy, and treatment of nonalcoholic fatty liver disease and nonalco- holic steatohepatitis. Mayo Clin Proc 2015;90:1233–1246.

[5] Blachier M, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. J Hepatol 2013;58:593–608.

[6] Agrawal S, Duseja AK. Non-alcoholic fatty liver disease: east versus west. J Clin Exp Hepatol 2012;2:122–134.

[7] Anstee QM, Day CP. The genetics of NAFLD. Nat Rev Gastroenterol Hepatol 2013;10:645–655.

[8] Romero-Gomez M, Zelber-Sagi S, Trenell M. Treatment of NAFLD with diet, physical activity and exercise. J Hepatol 2017;67:829–846.

[9] Oni ET, Agatston AS, Blaha MJ, Fialkow J, Cury R, Sposito A, et al. A systematic review: burden and severity of subclinical cardiovascular disease among those with nonalcoholic fatty liver; should we care? Atherosclerosis 2013;230:258–267.

[10] De Minicis S, Day C, Svegliati-Baroni G. From NAFLD to NASH and HCC: pathogenetic mechanisms and therapeutic insights. Curr Pharm Des 2013;19:5239–5249.

[11] Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. Hepatology 2010;51:679–689.

[12] Non-alcoholic Fatty Liver Disease Study G, Lonardo A, Bellentani S, Argo CK, Ballestri S, Byrne CD, et al. Epidemiological modifiers of non- alcoholic fatty liver disease: focus on high-risk groups. Dig Liver Dis 2015;47:997–1006.

[13] Wong RJ, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. Gastroenterology 2015;148:547–555.

[14] Rafiq N, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, et al. Long-term follow-up of patients with nonalcoholic fatty liver. Clin Gastroenterol Hepatol 2009;7:234–238.

[15] Sanyal AJ, Brunt EM, Kleiner DE, Kowdley KV, Chalasani N, Lavine JE,et al. Endpoints and clinical trial design for nonalcoholic steatohepatitis. Hepatology 2011;54:344–353.

[16] Sanyal AJ, Friedman SL, McCullough AJ, Dimick-Santos L, American Association for the Study of Liver D, United States F, et al. Challenges and opportunities in drug and biomarker development for nonalcoholic steatohepatitis: findings and recommendations from an American Association for the Study of Liver Diseases-U.S. Food and Drug Administration Joint Workshop. Hepatology 2015;61:1392–1405.

[17] Brunt EM, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA, Network NCR. Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. Hepatology 2011;53:810–820.

[18] Kleiner DE, Makhlouf HR. Histology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in adults and children. Clin Liver Dis 2016;20:293–312.

[19] Arrese M, Eguchi A, Feldstein AE. Circulating microRNAs: emerging biomarkers of liver disease. Semin Liver Dis 2015;35:43–54.

[20] Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A 2008;105:10513–10518.

[21] Weiland M, Gao XH, Zhou L, Mi QS. Small RNAs have a large impact: circulating microRNAs as biomarkers for human diseases. Rna Biol 2012;9:850–859.

[22] Pirola CJ, Fernandez Gianotti T, Castano GO, Mallardi P, San Martino J, Mora Gonzalez Lopez Ledesma M, et al. Circulating microRNA signature in non-alcoholic fatty liver disease: from serum non-coding RNAs to liver histology and disease pathogenesis. Gut 2015;64:800–812.

[23] Povero D, Feldstein AE. Novel molecular mechanisms in the develop- ment of non-alcoholic steatohepatitis. Diabetes Metab J 2016;40:1–11.

[24] Sato K, Meng F, Glaser S, Alpini G. Exosomes in liver pathology. J Hepatol 2016;65:213–221.

[25] Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg 2010;8:336–341.

[26] Higgins JPT, Green S. Cochrane handbook for systematic reviews of interventions. Cochrane Book Series; 2008. p. 1–674.

[27] Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011;155:529–536.

[28] Griffith OL, Melck A, Jones SJ, Wiseman SM. Meta-analysis and meta- review of thyroid cancer gene expression profiling studies identifies important diagnostic biomarkers. J Clin Oncol 2006;24:5043–5051.
[29] Chan SK, Griffith OL, Tai IT, Jones SJ. Meta-analysis of colorectal cancer gene expression profiling studies identifies consistently reported candi- date biomarkers. Cancer Epidemiol Biomarkers Prev 2008;17:543–552.
[30] Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557–560.
[31] Reitsma JB, Glas AS, Rutjes AW, Scholten RJ, Bossuyt PM, Zwinderman AH. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. J Clin Epidemiol 2005;58:982–990.
[32] Deeks JJ, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. J Clin Epidemiol 2005;58:882–893.
[33] Cheung O, Puri P, Eicken C, Contos MJ, Mirshahi F, Maher JW, et al. Nonalcoholic steatohepatitis is associated with altered hepatic Micro- RNA expression. Hepatology 2008;48:1810–1820.
[34] Gallego-Durán R, del Campo J, Ampuero J, García-Valdecasas M, Rojas L, Pastor-Ramírez H, et al. P0969: Role of epigenetic factors in non- alcoholic steatohepatitis development. J Hepatol 2015;62:S709.
[35] Simao AL, Afonso MB, Rodrigues PM, Amaral AJ, Gama-Carvalho M, Borralho PM, et al. Mirna-339-3p is increased in human and experi- mental models of Nafld and targeted by Tudca in insulin-resistant muscle cells. Hepatology 2015;62:681A.
[36] Elfimova N, Ommer K, Schlattjan M, Sowa JP, Wedemeyer I, Vantler M, et al. 1255 miR-101 in steatosis and steatohepatitis of non-alcoholic fatty liver disease (NAFLD). J Hepatol 2013;58:S508.
[37] Handa P, Morgan-Stevenson V, Juran BD, Lazaridis K, Kowdley KV. MicroRNA profiling in plasma of patients uncovers unique microRNA signatures associated with liver inflammation, cirrhosis, nonalcoholic steatohepatitis and hepatocellular carcinoma that predict the progres- sion of Primary Sclerosing Cholangitis. Hepatology 2017;66, 164A-164A.
[38] Younossi ZM, Karrar A, Tokarz K, Ulyanov A, Stepanova M, Younoszai Z, et al. Hepatic signature expression of miR-199a-5p, miR-21-3p, miR- 224-5p, and miR-150-5p detected by NextSeq technology are indepen- dently associated with fibrosis in non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD). Hepatology 2017;66:1061A–1062A.
[39] Srivastava R, Wang X, Lin J, Wei R, Chaturvedi P, Chalasani NP, et al. Role of integrated microrna-mRNA network in human hepatic fat accumula- tion and non-alcoholic fatty liver disease. Hepatology 2013;58:525A.
[40] Cermelli S, Ruggieri A, Marrero JA, Ioannou GN, Beretta L. Circulating microRNAs in patients with chronic hepatitis C and non-alcoholic fatty liver disease. PLoS One 2011;6 e23937.
[41] Liu XL, Pan Q, Zhang RN, Shen F, Yan SY, Sun C, et al. Disease-specific miR-34a as diagnostic marker of non-alcoholic steatohepatitis in a Chinese population. World J Gastroenterol 2016;22:9844–9852.
[42] Tan Y, Ge G, Pan T, Wen D, Gan J. A pilot study of serum microRNAs panel as potential biomarkers for diagnosis of nonalcoholic fatty liver disease. PLoS One 2014;9 e105192.
[43] Zhang H, Li QY, Guo ZZ, Guan Y, Du J, Lu YY, et al. Serum levels of microRNAs can specifically predict liver injury of chronic hepatitis B. World J Gastroenterol 2012;18:5188–5196.
[44] Celikbilek M, Baskol M, Taheri S, Deniz K, Dogan S, Zararsiz G, et al. Circulating microRNAs in patients with non-alcoholic fatty liver disease. World J Hepatol 2014;6:613–620.
[45] Sun C, Huang F, Liu X, Xiao X, Yang M, Hu G, et al. MiR-21 regulates triglyceride and cholesterol metabolism in non-alcoholic fatty liver disease by targeting HMGCR. Int J Mol Med 2015;35:847–853.
[46] Zhuge B, Li G. MiR-150 deficiency ameliorated hepatosteatosis and insulin resistance in nonalcoholic fatty liver disease via targeting CASP8 and FADD-like apoptosis regulator. Biochem Biophys Res Commun 2017;494:687–692.
[47] Guo Y, Xiong Y, Sheng Q, Zhao S, Wattacheril J, Flynn CR. A micro-RNA expression signature for human NAFLD progression. J Gastroenterol 2016;51:1022–1030.
[48] Castro RE, Ferreira DM, Afonso MB, Borralho PM, Machado MV, Cortez-Pinto H, et al. MiR-34a/SIRT1/p53 is suppressed by ursodeoxycholic acid in the rat liver and activated by disease severity in human non-alcoholic fatty liver disease. J Hepatol 2013;58:119–125.
[49] Dattaroy D, Pourhoseini S, Das S, Alhasson F, Seth RK, Nagarkatti M, et al. Micro-RNA 21 inhibition of SMAD7 enhances fibrogenesis via leptin-mediated NADPH oxidase in experimental and human nonalcoholic steatohepatitis. Am J Physiol Gastrointest Liver Physiol 2015;308: G298–G312.
[50] Xu Y, Zalzala M, Xu J, Li Y, Yin L, Zhang Y. A metabolic stress-inducible miR-34a-HNF4alpha pathway regulates lipid and lipoprotein metabo- lism. Nat Commun 2015;6:7466.
[51] Becker PP, Rau M, Schmitt J, Malsch C, Hammer C, Bantel H, et al. Performance of Serum microRNAs -122, -192 and -21 as Biomarkers in Patients with Non-Alcoholic Steatohepatitis. PLoS One 2015;10 e0142661.
[52] Loyer X, Paradis V, Henique C, Vion AC, Colnot N, Guerin CL, et al. Liver microRNA-21 is overexpressed in non-alcoholic steatohepatitis and contributes to the disease in experimental models by inhibiting PPARalpha expression. Gut 2016;65:1882–1894.
[53] Salvoza NC, Klinzing DC, Gopez-Cervantes J, Baclig MO. Association of circulating serum miR-34a and miR-122 with dyslipidemia among patients with non-alcoholic fatty liver disease. PLoS One 2016;11 e0153497.
[54] Yamada H, Suzuki K, Ichino N, Ando Y, Sawada A, Osakabe K, et al. Associations between circulating microRNAs (miR-21, miR-34a, miR-122 and miR-451) and non-alcoholic fatty liver. Clin Chim Acta 2013;424:99–103.
[55] Lendvai G, Jarmay K, Karacsony G, Halasz T, Kovalszky I, Baghy K, et al. Elevated miR-33a and miR-224 in steatotic chronic hepatitis C liver biopsies. World J Gastroenterol 2014;20:15343–15350.
[56] Auguet T, Aragones G, Berlanga A, Guiu-Jurado E, Marti A, Martinez S, et al. MiR33a/miR33b́ and miR122 as possible contributors to hepatic lipid metabolism in obese women with nonalcoholic fatty liver disease. Int J Mol Sci 2016;17.
[57] Muangpaisarn P, Jampoka K, Payungporn S, Wisedopas N, Bunchorn-tavakul C, Tangkijvanich P, et al. Serum microRNA-34a is potential biomarker for inflammation in nonalcoholic fatty liver disease. Asian Biomed 2017;10:10.
[58] Latorre J, Moreno-Navarrete JM, Mercader JM, Sabater M, Rovira O, Girones J, et al. Decreased lipid metabolism but increased FA biosyn- thesis are coupled with changes in liver microRNAs in obese subjectswith NAFLD. Int J Obes (Lond) 2017;41:620–630.
[59] Povero D, Panera N, Eguchi A, Johnson CD, Papouchado BG, de Araujo Horcel L, et al. Lipid-induced hepatocyte-derived extracellular vesicles regulate hepatic stellate cell via microRNAs targeting PPAR-gamma. Cell Mol Gastroenterol Hepatol 2015;1 e644.
[60] Pogribny IP, Starlard-Davenport A, Tryndyak VP, Han T, Ross SA, Rusyn I, et al. Difference in expression of hepatic microRNAs miR-29c, miR-34a, miR-155, and miR-200b is associated with strain-specific susceptibility to dietary nonalcoholic steatohepatitis in mice. Lab Invest 2010;90:1437–1446.
[61] Fu T, Choi SE, Kim DH, Seok S, Suino-Powell KM, Xu HE, et al. Aberrantly elevated microRNA-34a in obesity attenuates hepatic responses to FGF19 by targeting a membrane coreceptor beta-Klotho. Proc Natl Acad Sci U S A 2012;109:16137–16142.
[62] Xu H, He JH, Xiao ZD, Zhang QQ, Chen YQ, Zhou H, et al. Liver-enriched transcription factors regulate microRNA-122 that targets CUTL1 during liver development. Hepatology 2010;52:1431–1442.
[63] Miyaaki H, Ichikawa T, Kamo Y, Taura N, Honda T, Shibata H, et al. Significance of serum and hepatic microRNA-122 levels in patients with non-alcoholic fatty liver disease. Liver Int 2014;34:e302–e307.
[64] Iino I, Kikuchi H, Miyazaki S, Hiramatsu Y, Ohta M, Kamiya K, et al. Effect of miR-122 and its target gene cationic amino acid transporter 1 on colorectal liver metastasis. Cancer Sci 2013;104:624–630.
[65] Lewis AP, Jopling CL. Regulation and biological function of the liver- specific miR-122. Biochem Soc Trans 2010;38:1553–1557.
[66] Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, et al. MiR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab 2006;3:87–98.
[67] Babak T, Zhang W, Morris Q, Blencowe BJ, Hughes TR. Probing microRNAs with microarrays: tissue specificity and functional inference. RNA 2004;10:1813–1819.
[68] Filipowicz W, Grosshans H. The liver-specific microRNA miR-122: biology and therapeutic potential. Prog Drug Res 2011;67: 221–238.
[69] Boeri M, Verri C, Conte D, Roz L, Modena P, Facchinetti F, et al. MicroRNA signatures in tissues and plasma predict development and prognosis of computed tomography detected lung cancer. Proc Natl Acad Sci U S A 2011;108:3713–3718.
[70] Povero D, Eguchi A, Li H, Johnson CD, Papouchado BG, Wree A, et al. Circulating extracellular vesicles with specific proteome and liver microRNAs are potential biomarkers for liver injury in experimental fatty liver disease. PLoS ONE 2014;9 e113651.

[71] Fu Y, Wei X, Tang C, Li J, Liu R, Shen A, et al. Circulating microRNA-101 as a potential biomarker for hepatitis B virus-related hepatocellular carcinoma. Oncol Lett 2013;6:1811–1815.
[72] Katsumi T, Ninomiya M, Tomita K, Sato C, Okumoto K, Nishise Y, et al. Discrepant expression of mir-139-5p between serum and liver in patients with primary biliary cirrhosis, and its possible cellular origin. Hepatology 2014;60:362A.
[73] Krek A, Grun D, Poy MN, Wolf R, Rosenberg L, Epstein EJ, et al. Combinatorial microRNA target predictions. Nat Genet 2005;37: 495–500.
[74] Zhu M, Wang Q, Zhou W, Liu T, Yang L, Zheng P, et al. Integrated analysis of hepatic mRNA and miRNA profiles identified molecular networks and potential biomarkers of NAFLD. Sci Rep 2018;8:7628.
[75] Sato F, Tsuchiya S, Terasawa K, Tsujimoto G. Intra-platform repeatability and inter-platform comparability of microRNA microarray technology. PLoS ONE 2009;4 e5540.
[76] Leti F, Malenica I, Doshi M, Courtright A, Van Keuren-Jensen K, Legendre C, et al. High-throughput sequencing reveals altered expression ofhepatic microRNAs in nonalcoholic fatty liver disease-related fibrosis. Transl Res 2015;166:304–314.
[77] Pritchard CC, Cheng HH, Tewari M. MicroRNA profiling: approaches and considerations. Nat Rev Genet 2012;13:358.
[78] Mestdagh P, Van Vlierberghe P, De Weer A, Muth D, Westermann F, Speleman F, et al. A novel and universal method for microRNA RT-qPCR data normalization. Genome Biol 2009;10:R64.
[79] Ampuero J, Romero-Gomez M. Editorial: looking for patients at risk of cirrhosis in the general population-many needles in a haystack. Aliment Pharmacol Ther 2018;47:692–694.
[80] Romero-Gomez M, Cortez-Pinto H. Detecting liver fat from viscoelasticity: how good is CAP in clinical practice? The need for universal cut-offs. J Hepatol 2017;66:886–887.
[81] Chang-Hai L, Antonio G-G, Javier A, Manuel R-G. Diagnostic accuracy of SCCA and SCCA-IgM for hepatocellular carcinoma: A meta-analysis. Liver Int 2018;00:1–12.
[82] Ao R, Wang Y, Tong J, Wang BF. Altered microRNA-9 expression level is directly correlated with pathogenesis of nonalcoholic fatty liver disease by targeting Onecut2 and SIRT1. Med Sci Monit 2016;22: 3804–3819.
[83] Zarrinpar A, Gupta S, Maurya MR, Subramaniam S, Loomba R. Serum microRNAs explain discordance of non-alcoholic fatty liver disease in monozygotic and dizygotic twins: a prospective study. Gut 2016;65:1546–1554.
[84] Zhang Y, Cheng X, Lu Z, Wang J, Chen H, Fan W, et al. Upregulation of miR-15b in NAFLD models and in the serum of patients with fatty liver disease. Diabetes Res Clin Pract 2013;99:327–334.
[85] Vega-Badillo J, Gutierrez-Vidal R, Hernandez-Perez HA, Villamil-Ramirez H, Leon-Mimila P, Sanchez-Munoz F, et al. Hepatic miR-33a/miR-144 and their target gene ABCA1 are associated with steatohepatitis in morbidly obese subjects. Liver Int 2016;36:1383–1391.
[86] Okamoto K, Koda M, Okamoto T, Onoyama T, Miyoshi K, Kishina M, et al. A series of microRNA in the chromosome 14q32.2 maternally imprinted region related to progression of non-alcoholic fatty liver disease in a mouse model. PLoS One 2016;11 e0154676.
[87] Wang Y, Du J, Niu X, Fu N, Wang R, Zhang Y, et al. MiR-130a-3p attenuates activation and induces apoptosis of hepatic stellate cells innonalcoholic fibrosing steatohepatitis by directly targeting TGFBR1 and TGFBR2. Cell Death Dis 2017;8 e2792.
[88] Lopez-Riera M, Conde I, Tolosa L, Zaragoza A, Castell JV, Gomez-Lechon MJ, et al. New microRNA biomarkers for drug-induced steatosis and their potential to predict the contribution of drugs to non-alcoholic fatty liver disease. Front Pharmacol 2017;8:3.
[89] Hanin G, Yayon N, Tzur Y, Haviv R, Bennett ER, Udi S, et al. MiRNA-132 induces hepatic steatosis and hyperlipidaemia by synergistic multitarget suppression. Gut 2017.
[90] Tran M, Lee SM, Shin DJ, Wang L. Loss of miR-141/200c ameliorates hepatic steatosis and inflammation by reprogramming multiple signal- ing pathways in NASH. JCI Insight 2017;2.
[91] Xu Q, Li Y, Shang YF, Wang HL, Yao MX. MiRNA-103: molecular link between insulin resistance and nonalcoholic fatty liver disease. World J Gastroenterol 2015;21:511–516.
[92] Yang W, Wang J, Chen Z, Chen J, Meng Y, Chen L, et al. NFE2 induces miR-423-5p to promote gluconeogenesis and hyperglycemia by repressing the hepatic FAM3A-ATP-Akt pathway. Diabetes 2017;66:1819–1832.
[93] Wang L, Zhang N, Wang Z, Ai DM, Cao ZY, Pan HP. Decreased MiR-155level in the peripheral blood of non-alcoholic fatty liver disease patients may serve as a biomarker and may influence LXR activity. Cell Physiol Biochem 2016;39:2239–2248.
[94] Wang Y, Zhu K, Yu W, Wang H, Liu L, Wu Q, et al. MiR-181b regulates steatosis in nonalcoholic fatty liver disease via targeting SIRT1. Biochem Biophys Res Commun 2017;493:227–232.
[95] Cazanave SC, Mott JL, Elmi NA, Bronk SF, Masuoka HC, Charlton MR, et al. A role for miR-296 in the regulation of lipoapoptosis by targeting PUMA. J Lipid Res 2011;52:1517–1525.
[96] Li B, Zhang Z, Zhang H, Quan K, Lu Y, Cai D, et al. Aberrant miR199a-5p/caveolin1/PPARalpha axis in hepatic steatosis. J Mol Endocrinol 2014;53:393–403.
[97] Guo J, Fang W, Sun L, Lu Y, Dou L, Huang X, et al. Reduced miR-200b and miR-200c expression contributes to abnormal hepatic lipid accumula- tion by stimulating JUN expression and activating the transcription of srebp1. Oncotarget 2016;7:36207–36219.