Author Response 1

Reviewer #1:

Comment 1:

“It’s a little strange that it’s a systematic review of 3000+ papers but then they only include 5. It seems like they could just cut out all or most of the systematic review folderol, and just get to the 5 papers they decided to use. The English is maybe average, but it could use some editing since the meaning is frequently unclear.”

Answer: Thanks for your good suggestions. This is a systematic review and meta-analysis to explore the possibility of miRNAs as diagnostic biomarkers for asthma. We conducted all procedures based on the PRISMA criteria (Moher D. et al., 2009). With the keywords, we retrieved 3091 records from databases. Then, according to our listed inclusion and exclusion criteria by the principle of PICOS, we included 72 articles for qualitative analysis and 5 for quantitative analysis. The detail for the screening process was presented in Figure 1 and the characteristics for all included publications were presented in Table 1. Besides, we have the English been edited by a native speaker, which we do wish meets your approval.

Comment 2:

“Is that this is a prediction paper wherein a predictive model is built and tested, and there’s no information whatsoever about how this is done. There’s also no information about how the predictive models were built in each of the 5 source papers. This is a major weakness, since it’s likely there was no separate training populations and the models were probably learned on the same data they were tested upon. It’s also possible that the models changed through each of the five datasets.” “What kind of predictive models were used and how they were created must be explained. Were the models trained on the entire dataset then tested on the entire dataset? How many datasets does that encompass? Were model parameters retained from the sources? Were model parameters retrained on each separate data source?”

Answer: Thanks for your comment. The objective of this study is to figure out the diagnostic value of microRNAs in asthma through the method of systematic review and meta-analysis. Instead of building or testing a new predictive model, we conducted the analysis based on the bivariate model (Reitsma Johannes B. et al., 2005). The bivariate model is widely used in diagnostic meta-analysis (Reiman MP. et al., 2015) (Fuccio L. et al., 2018) (Xiao G. et al., 2017), which could not only retain the original data but also obtain the negative correlation between sensitivity and specificity. Besides, it could avoid the complexity of parameterization and unstable results in traditional meta-analysis method (Macaskill P, 2004).

Comment 3:

“There’s also a lot of ambiguity of the originally reported effects of the five miRs in each of the five papers. It seems like the authors assume that 1 miR comes from one publication, but the other miRs were available in those datasets.”

Answer: Thanks for your comment. In order to figure out the issue of ignoring other available miRNAs data you pointed out, we have rechecked and extracted all available diagnostic data from

the 5 papers for quantitative analysis (attached Table A). Meanwhile, as shown in the table, we found that there were overlapped participants' data for the same miRNA from the same paper which was conducted in two data sets. For such overlapping cases, we only included and analyzed the largest sample size's cohort data. Based on these, we have revised the detail of included miRNAs for quantitative analysis for Table 2 in the main text. Besides, all diagnostic evaluation related analysis was revised as well.

Comment 4:

"The second major concern is sort of philosophical, but there should be some deeper motivation for a biomarker test of asthma. It'd be a powerful test if you could predict which children will develop asthma before they develop asthma; ie, before they show symptoms, but that's not what's going on here. In the Introduction the authors state: "The FEV1 increased by at least 12% in children with asthma [6], whereas, there is a natural decline for FEV1 in adults [8]." This doesn't make sense. One likely interpretation is that it might be saying that changes in FEV1 throughout the life course invalidate it as a diagnostic measure for asthma, which is false. This issue comes up again later when they say "These results indicate that the combination of these miRNA could greatly increase the diagnosis rates of asthma." This statement must be evaluated against the current standard method of diagnosis for asthma. Since asthma is defined by reversible airflow restriction, diagnosing asthma by spirometry tests - by definition - can't be more accurate."

Answer: Thanks for your good suggestion. We have revised and rewritten the manuscript which should have largely addressed similar concerns. For example, we have rephrased the conclusion as "These results indicate that the combination of these miRNAs could increase the diagnosis rates of asthma and imply the potential application value of these miRNAs in the future."

Comment 5:

"Methods: This section is written a little less like a paper and more like a fill-in-the-blanks worksheet".

Answer: Thanks for your good suggestion. To make it more readable, we have rewritten and rephrased the sentences and languages for the Methods part.

Comment 6:

"Abbreviations should be spelled out the first time they're used."

Answer: Thanks for your good suggestion. We have rechecked the whole text and added an explanation for all abbreviations when they're presented for the first time.

Comment 7:

"Table 2: did each study identify only a single differentially expressed miRNA?"

Answer: Thanks for your comment. There were more than one differentially expressed miRNAs from the 5 quantitative analysis papers. As we stated in the reply to comment 3, we have reorganized

Table 2 in the main text and updated related data included in the evaluation of the diagnostic value of miRNAs for asthma.

Comment 8

“Figure 2 pie chart doesn’t match the text. Blood samples (49%) take up more area than the alternative.”

Answer: Sorry for making your confused. We have revised this information in Figure 2.

Comment 9:

“Figure 2 caption doesn’t match the image. “Forest plots for the diagnostic value of the combined of 5 miRNAs in asthma. Left is the pooled sensitivity analysis. Right is the pooled specificity analysis. And the combined miRNAs contain miR-185-5p, miR-155, Let-7a, miR-21, and miR-1165-3p.”

Answer: Thanks for your comment. We have removed the redundant annotations and revised the caption for Figure 2 as follows: “Profile of miRNAs in asthma. (a). Differentially expressed miRNAs between asthma patients and controls reported in more than two studies. The orange and blue individually represent upregulated and downregulated miRNA in asthma patients compared with controls. (b). Distribution of the specimen for all differently expressed miRNA between asthma patients and controls, and each color standards for a specific sample source. All these data were summarized from 72 included publications according to our listed criteria.”

Comment 10:

“Need to define I-squared.”

Answer: Thanks for your good suggestion. We have described the I-squared according to the research of Higgins JP et al. in 2003 (Higgins JP et al. 2003) in detail and revised as follows “I2 is applied to measure the heterogeneity, which describes the percentage of variation between analyzed studies. If I2 =25%, it means that slight heterogeneity existed; I2 =50% meant moderate heterogeneity; high heterogeneity was present when I2 =75%.” in the Materials and Methods, Section 2.6.

Comment 11:

“Figure 6: need to define ESS. It seems like drawing a regression line through six points is pretty arbitrary, and likely influenced by outliers.”

Answer: Thanks for your comment. ESS refers to the Effective Sample Size, which we used in Deek’s analysis for evaluating publication bias previously. It has the advantages of intuitive and quantitative representation but is seriously limited by the number of researches. According to the review’s comment, we have applied Funnel plot to test publication bias, which has the advantage of not being limited by the number of included studies (Supplemental Figure 4). Besides, we could directly determine whether the publication bias existence or not from the condition of plot, which was described in the Materials and Methods, Section 2.6.

Comment 12:

“Discussion: Some rationale should be provided for which of the many predictive accuracy metrics should be most relevant; why we should consider the other metrics, and under what circumstances the others should take precedence.”

Answer: Thanks for your good suggestion. We have added a paragraph to introduce and evaluate the diagnostic metrics we got in the discussion part as the follows: “According to our results, the combined miRNAs (miR-185-5p, miR-155, Let-7a, miR-21, miR-320a, miR-1246, miR-144-5p, and miR-1165-3p) could be a potential biomarker for the diagnosis of asthma based on a relatively high Sen of 0.87, Spe of 0.84, and high AUC of 0.93. Sensitivity is the ability to correctly find an individual with a specific disease, while specificity is to correctly classify the person as disease-free. When faced with inconsistent trends of Sen and Spe, we need to consider the diseases itself in clinical. For instance, for diseases with high mortality like cancers, biomarkers with high sensitivity are essential for early screening. Meanwhile, in the course of disease diagnosis and treatment, especially for those with obvious side effects, a tool with high specificity is required. Besides, the value of AUC can directly reflect the diagnostic effect. AUC between 0.5-0.7 indicates a low diagnostic value, a value between 0.7-0.9 means good, and above 0.9 is considered very good.”.

Reviewer #2:

Comment 1:

“The manuscript requires proofreading and minor sentence revisions to improve the quality of English. For example, sentences in the Results section such as “We found that there were four kinds of miRNAs” and ”greatly supported they may be good diagnosis biomarker candidates” need to be restructured. Additionally, the mixed use of “FEV1” and “FEV1” in the introduction, and typos such as “quantiative” on Figure 1, and “hot differently” in the Results, should be corrected. The usage of the word “like” should also be greatly reduced. These types of errors occur throughout the manuscript”.

Answer: Thanks for your good suggestions. We have revised the sentences and rewritten the manuscript according to your comment. Besides, we also have the English been edited by a native speaker, which we do wish meets your approval.

Comment 2:

“Material and Methods, Section 2.2: please fully describe which “other accepted criteria” (line 48), “other acceptable methods” (line 49), and “other cohorts design” (line 56) were used”.

Answer: Thanks for your comment. In order to make it more clear, we have added and listed more details of diagnostic criteria, detection methods, and cohort designs instead of “other accepted criteria”, “other acceptable methods” and “other cohorts design” in the Materials and Methods, Section 2.2.

Comment 3:

“Figure 1: Judging by the position of the penultimate box in the figure, it is not clear if the studies used for qualitative are part of the “Eligibility” or “Included” steps.”

Answer: Sorry for making you confused. According to our research goal, we included both qualitative studies and quantitative researches. We have revised the flowchart to make it more clear for the screening process which was conducted according to PRISMA (Figure 1).

Comment 4:

“Table 1: Please explain why studies that display a diagnostic criterion of “NA” were included in the qualitative analysis. Additionally, there’s a hidden value after miR-155 in the “Differentially expressed miRNAs’ column on the second page of Table 1 (line 19, Tang X. 2018), this is easily corrected by increasing the row height.”

Answer: Thanks for your comment. The reason why we stated as “NA” previously was because we could not directly extract the diagnostic criteria from the paper. When we rechecked these papers, we found the diagnostic criteria for the so-called “NA” were still available though not directly described. For example, the diagnostic criteria of patients from the paper of Tsai, M. Ju et al. in 2019 (Tsai, M. Ju. et al. 2019) has been stated in previous studies (Tsai, M.J. et al. 2018, Sheu, C.C. et al. 2017 and Chang, W.A et al. 2018). Thus, we have revised responsive diagnostic criteria in Table 1. Besides, we also have revised the format of Table 1 according to your suggestions.

Comment 5:

“Table 2: To make it clearer, I would suggest replacing “ meta-analysis” with “quantitative analysis”.

Answer: Thanks for your good suggestions. We have replaced “meta-analysis” with “quantitative analysis” in Table 2.

Comment 6:

“Results, Section 3.2:

- Line 36: Missing reference at “and et al.”.

- Lines 37 and 38: please explain the intended meaning of the sentence “any change from the above samples could be explained by the disease itself other than statistical difference”.

- Line 8, Page 9: miR-21 is listed as being present in Figure 2a, however, this figure shows the miR-21 family. The text should be corrected to reflect it refers to the miR-21 family.

- Line 11, Page 9: maybe a typo on “PBMCs than controls’”, since the comparison to controls doesn’t fit in the list”.

Answer: Thanks for your comment. Firstly, we have revised the typo of “and et al.”. Secondly, following the description of sample sources from the upper or lower airway, the meaning of the sentence you cited is to clarify the advantages of research results from the respiratory system derived specimens compared with other sample sources. To make it more clear, we have rephrased

this part as follows: “Since asthma is a kind of respiratory system disease, upper or lower airway derived sample sources containing bronchial epithelial cells (BECs) (13%), airway biopsies (7%), nasal mucosa (6%), sputum (5%), airway smooth muscle cells (ASMCs) (4%), bronchoalveolar lavage fluid (BALF) (13%), bronchial biopsy (2%) and lung tissue (1%), could directly reflect the pathologic change of the disease and have a good representative role (Figure 2b).” in Results, Section 3.2. Thirdly, we have revised the expression as miR-21 family in Figure 2a to keep it consistent with the main text. Fourthly, we also have revised the typo on “PBMCs than controls”.

Comment 7:

“- Make it clearer that Figure 2a displays both miRNA families and individual miRNAs. The current text omits the fact that some of the bars are miRNA families.

- Line 60: please remove what seems to be the title for Figure 3 at the end of the title for Figure 2.”.

Answer: Thanks for your good suggestions. Firstly, we have rechecked and modified the expression of individual miRNAs and miRNA families through the full text to make sure which were consistent with what displayed in Figure2a. Besides, we have also revised the legend of Figure 2.

Comment 8:

“Figure 3: I would recommend adding the miRNA names to their respective publications in Figure 3.”

Answer: Thanks for your good suggestions. We have added the names of researched miRNAs followed the study-ID of each article in Figure 3.

Comment 9:

“Figure 4:

- Please include in the figure key what each number in the “observed data” circles represent.

- The sentence “The red dot standards” could be replaced with “The red diamond represents”. Also consider rephrasing the remaining of this sentence on Line 26.”

Answer: Thanks for your comment. We have rephrased the legend of Figure 4 according to your suggestion. Firstly, we have illustrated the meaning of each numbered black circle. Secondly, we have rephrased the remaining sentences.

Comment 10:

“Results, Section 3.3, Line 30: please explain the usage of pre-test probabilities in this context.”

Answer: Thanks for your comment. To show the clinical utility of the analyzed miRNAs for asthma, Fagan analysis was used to prove the relationship among prior probability, likelihood ratio, and posterior probability under the pre-test probabilities of 25%, 50%, and 75% which presented for clinical suspicion of asthma at 25%, 50%, and 75% respectively. According to our results, we found that the combination of these miRNAs (miR-185-5p, miR-155, Let-7a, miR-21, miR-320a, miR-1246,

miR-144-5p, and miR-1165-3p) could increase the diagnosis of asthma to 65%, 85% and 94% separately (Figure 5a-c) when setting the Pre-test Prob at 25% (low clinical suspicion of asthma: 25%), 50% (clinical suspicion of asthma: 50%) or 75% (high clinical suspicion of asthma: 75%) individually. These results indicated that the combination of these miRNAs could increase the diagnosis rates of asthma and imply the potential application value of these miRNAs in the future. We have added more detail in the Results, Section 3.3.

Comment 11:

"Results, Section 3.4:

- Lines 11 and 12: This sentence about heterogeneity is a little confusing and needs to be rewritten to improve clarity.

- Lines 17 and 18: This sentence about heterogeneity is a little confusing and needs to be rewritten to improve clarity.

- Line 21: Here it is mentioned that regression analysis was applied. Please include details of the model and statistical analysis in the methods section. Additionally, it is not clear what the authors mean by "age and sample on heterogeneity respectively", please clarify."

Answer: Thanks for your comment. Totally, to explore the sources of heterogeneity (I2=79.3%) for the combined effect size, we performed subgroup analysis and Meta-regression analysis from the aspect of sample sources (serum vs plasma) and ages (adults vs children) respectively. We have rephrased the Results, Section 3.4 of Subgroup analysis and Meta-regression analysis to make sure that all information is clear. Besides, we also have added the details for procedures of Subgroup analysis and Meta-regression analysis in the Materials and Methods, Section 2.6 as follows: " Next, subgroup analysis and Meta-regression analysis were used to find the sources of heterogeneity, which we performed from the aspect of sample sources (serum vs plasma) and ages (adults vs children) respectively. If there was a significant decrease in heterogeneity in either subgroup, it would be considered as the source of heterogeneity. Additionally, Meta-regression analysis was carried out by taking age and sample sources as covariables respectively and using the Restrictive maximum likelihood method (REML) to establish the regression model of effect size to a single covariable. When the tau, which represented the estimate of between-study variance, decreased significantly in a covariable (sample sources or ages), this covariable would be considered as the source of heterogeneity.".

Comment 12:

"Figure 6: I would recommend adding the miRNA names to their respective publications in Figure 6."

Answer: Thanks for your good suggestion. Considering the number of articles included, we used Funnel plot to replace the Deek method to analyze the publication bias in this revised version. At the same time, we have rephrased the legend of the updated Figure 6.

Comment 13:

"Discussion:

- Lines 42 to 45: This paragraph is a little confusing and should be rewritten for clarity.

- Lines 52 to 54: A hypothesis should be contained to a pre-determined set of variables; therefore, it is unclear why this paragraph ends with “and so on”. Please explain the reasoning or amend it.

- Line 55: Consider replacing “meta-analysis” with “quantitative analysis”, in order to make this sentence clearer.”,

- Lines 56 to 59: The sentence “other related miRNAs of good biomarker candidate features” is too vague. What would be considered good biomarker features? Furthermore, consider rewriting this paragraph as it doesn’t clearly explain the limitations in the present study.

- Lines 3 to 5, Page 13: Consider rewriting these sentences as they don’t clearly explain the limitations in the present study.”

Answer: Thanks for your good suggestions. We have amended and rewritten the part of heterogeneity in Discussion as follows: “However, there was some heterogeneity in the pooled quantitative statistics for the combination of above 8 miRNAs. From the results of subgroup analysis and Mete-regression analysis, we found that ages may be one of the main causes of heterogeneity, which suggested that it would be better to perform analysis in different age groups to explore the diagnosis role of miRNAs for asthma in future research. Besides, the qualitative analysis also provided some evidence for understanding the origin of heterogeneity to some extent. For instance, the expression level for miR-155 could go in a different direction when compared with different control groups. Moreover, depending on the cut-off of expression value of miRNAs, the Sen and Spe for miR-155 could be as high as 100% or go down to 80% and 89% respectively. Similar situations of different region derived samples or cut-off also affect the results of miR-21 for asthma. These indicate that further researches could be optimized from the above aspects to increase the homogeneity”. Besides, we have replaced “meta-analysis” with “quantitative analysis” in the appropriate places in the paper.

What’s more, we also rewritten the paragraph of the limitation statement in Discussion part as follows: “There are still some limitations to this analysis. Firstly, there were only 5 studies available for quantitative analysis resulting in a small sample size of patients and controls included in the study, which may weaken the diagnostic value of miRNAs in asthma. Besides, due to the limited amount of available data, we were unable to explore the diagnostic value of individual miRNAs for asthma. Nor can we compare the diagnostic power between a single miRNA and the combination of specific miRNAs in asthma. Additionally, we did not explore the relationship between miRNAs and related clinical phenotypes.”

Comment 14:

“Supplemental Figure 1: In the title, replace “Quality assessment of five quantitative studies” with “Quality assessment of five studies selected for quantitative analysis”

Answer: Thanks for your good suggestion. We have changed the title of supplemental Figure 1 from “Quality assessment of five quantitative studies” to “Quality assessment of five studies selected for quantitative analysis” in the legend.

Comment 15:

“References: Some of the references are missing a title or journal, others are formatted differently from the rest, and two seem to have been joined in the same line. Please review all references and formatting.”

Answer: Thanks for your comment. We have revised the format of all the references to fulfill the requirements of the journal.