

RESPONSE TO REVIEWERS

Manuscript No:

Title: Salivary MicroRNA signature for Diagnosis of endometriosis

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By Sofiane Bendifallah, Stéphane Suisse, Anne Puchar, Léa Delbos, Mathieu Poilblanc, Philippe Descamps, Francois Golfier, Ludmila Jornea, Delphine Bouteiller, Cyril Touboul, Yohann Dabi, Emile Daraï.

First of all, we would like to thank the Editor and all reviewers for all their constructive and relevant comments. All comments were considered carefully. A detailed response has been formalized for each of them in the above document. We hope that these substantial changes, considering their relevance and their clinical impact, will find a favorable issue.

Reviewer 1

This is the first report linking salivary miRNA with endometriosis. If translated, this could be used as a diagnostic tool for endometriosis. This manuscript can be accepted in its current form.

We sincerely thank the reviewer for this very positive comment.

Reviewer 2

In this study the authors investigated the human miRNAome to define a saliva-based diagnostic miRNA signature for endometriosis in 200 women with chronic pelvic pain suggestive of endometriosis between January and June 2021. The aims of this study were the identification of a biomarker based on genome-wide miRNA expression profiling by small RNA sequencing using next generation sequencing (NGS), and the development of a saliva-based miRNA diagnostic signature according to expression and accuracy profiling using a Random Forest algorithm. Here, the first exhaustive sequencing of the human saliva miRNAome in the specific context of endometriosis has been reported resulting in the selection of a set of 109 miRNAs robustly tested.

- **Main points:**

1. The authors may improve the introduction section by adding more previous studies reporting the miRNA previously reported in patients with endometriosis

We have considered the comment. Introduction was modified according and mentioning more specifically studies that addressed this comment.

For endometriosis diagnosis, Maged et al. have shown that serum miR-122 and miR-199a had a sensitivity of 95.6 and 100 % and a specificity of 91.4 and 100%, respectively [30]. Moustafa et al. have reported an up-regulation of miR-125b-5p, miR-150-5p, miR-342-3p, and miR-451a, while a down-regulation of miR-3613-5p and let-7b in serum of patients with endometriosis compared with controls [31]. The area under curve (AUC) values in receiver operating characteristic (ROC) curves ranged from 0.68 to 0.92 for these miRNAs. However, these previous studies did not investigate the entire miRNAome and often had inadequate control groups. In recent systematic reviews, Ghafouri-Fard et al [32] and Agrawal et al [33] highlighted the lack of clearly identified diagnostic marker of endometriosis and found that no single miRNA or any panel composed of few miRNAs can reflect the heterogeneity of endometriosis.

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2. In materials and methods section, and specifically in the paragraph 2.1 (“Ethics Statement”) at line 97, the authors could insert the full form for the abbreviation “ML” and could explain better this algorithm in the appropriate paragraph.

We clarified the abbreviation ML. Machine Learning is basically described in the method section “statistical analysis” with the appropriate references if more precision is needed.

“ and (ii) development of a saliva-based miRNA diagnostic signature according to expression and accuracy profiling using an Machine Learning (ML) algorithm ”

In the statistical section we gave the following detail concerning the Machine Learning (ML) based on Random Forest (RF) algorithm.

“ Random Forest (RF) is considered to design the saliva signature [35–38,55–57]. F1-score, sensitivity, specificity, and the ROC AUC were calculated to assess and compare the diagnostic performance of the diagnostic signature [58,59]. “

3. In the paragraph 2.2 (“Study population”), the authors could better describe the collection of demographic characteristics of the patients included in the cohort and the methods used to measure them. Specifically, the authors could explain the methods used to measure some parameters such as the pains described. The authors could also insert the reference where the parameters used have been previously described

We have considered the comment and agree with it. We agree with this comment. Therefore, we clarify in the paragraph 2.2 (“Study population”), the collection of demographic characteristics of the patients included in the cohort and the methods used to measure them.

“Data have been added in the revised version of the manuscript. “All patients filled an electronic Case Report Form (eCRF) to assess their symptoms and their importance. The importance of each symptom was evaluated using standard Numeric Rating Scale as recommended and validated “

4. **Moreover, in the flow chart of the study are indicated the eligible patients after screening (247). Subsequently, 47 patients have been excluded by the study and the authors could better explain the not meeting inclusion criteria used.**

We have considered the comment and agree with it.

Indeed, a strength of this work lies in the attention paid for inclusion criteria, restricting the cohort to patients with pain suggestive of endometriosis and no comorbidities that could affect miRNAs expression. The full list of inclusion and exclusion criteria can be found on the online registration form ClinicalTrials.gov Identifier: NCT04728152) on the ENDOmiARN trial. Among the 47 patients who do not meet the inclusion criteria, (n=7) declined to participate, 15 due to significant difficulties in reading or writing the French language, 2 has objected to the collection of their data and 23 declare to be unable to comply with study and/or follow-up procedures. These information have been added to the flow chart to improve the clarity of our manuscript.

5. **In the Results section, and specifically in the paragraph 3.1 (“Description of the ENDO-miRNA cohort”), the authors could describe the statistically significant differences in the demographic characteristics between the Control and Endometriosis groups.**

We have considered the comment and agree with it. The following section was added in the results section of the revised version of the manuscript.

“

Patients with endometriosis had significantly more often other symptoms associated with endometriosis. Pain suggesting sciatica, dyspareunia, lower back pain outside menstruation, painful defecation or urinary pain during menstruation were significantly increased in patients with endometriosis.

“

6. In the Table 1, the authors could replace the commas with dots and add the full form for rASRM classification, as they already did for BMI abbreviation.

We have considered the comment and agree with it. The correction was added

7. Moreover, the authors could check the mean and SD values for some characteristics, inserting only the SD values in the brackets; for example, Mean of Dyspareunia ($\pm$ SD).

This was corrected in the revised version.

8. In the paragraph 3.3 (“Feature selection of miRNAs relevant for a diagnosis of endometriosis”) the authors could check the symbols used for values higher or lower than 60% and 80, from 200 to 204 line.

We have considered the comment and checked for these symbols. We added a detail specifying none of the miRNAs of the signature had an AUC > 70%.

9. In the Discussion section, at line 232, the authors could insert the full form for “AI”, since this word appears here for the first time.

We have considered the comment and agree with it. The correction was added

10. Finally, the authors could discuss more about the differences into the characteristics found between the two groups analyzed.

We have considered the comment and agree with it.

We added the following sentence in the limit section

“ Moreover, although all the patients with and without endometriosis had dysmenorrhea, some differences in the percentage with other symptoms suggestive of endometriosis have been observed suggesting that further studies are required to evaluate the value of combined symptoms to suspect endometriosis on questionnaire”

Minor points

1. In the abstract section, the authors could insert the full form for the abbreviation “AUC” and could explain better this parameter and the percentage assigned to the diagnostic miRNA signature.

The abbreviation was clarified. Unfortunately, the limited number authorized in the abstract section does not permit further explanation in this part of the manuscript.

Reviewer 2

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