Author’s response to reviews

Title: THE CYTOKINE PROFILE OF WOMEN WITH SEVERE ANXIETY AND DEPRESSION DURING PREGNANCY

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REVIEWER RESPONSES

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CYTOKINE PROFILE IN WOMEN WITH MAJOR DEPRESSION AND SEVERE ANXIETY DURING PREGNANCY
Dear editors

BMC Psychiatry

Reviewer reports:

Michele Okun (Reviewer 1): This is a data rich study intending to examine the association between cytokines and depression scores in pregnant women. I believe that with substantial editing and revising with assistance from a native English speaker, this would be an excellent paper. However, the way it is written makes it very difficult to interpret and follow. I really hope the authors do revisions because it is useful information.

We edited the manuscript and reviewed the English redaction used in the text.

Although the introduction is succinct, it does not really tell a story. It needs more focus and linearity. This applies to the Methods, results and discussion.

Both introduction, methods, results and discussion has been modified in order to adequately match the objective of the study.

The results were very hard to follow. Plus there were a lot of them. Is there a way to portray them more clearly?

Same for Discussion

The results have been amended for a better understanding of the text. Both figures and tables have been reduced, so that the text may be clearer and more specific.
Regarding the discussion, this section has been amended as well, described more clearly.

Linnea Karlsson (Reviewer 2): This study aims at investigating the associations between circulating cytokines and major depression (MDD) and/or anxiety among pregnant women. Overall, the topic is relevant, reports from pregnant populations are scarce, the sample size at larger end of the range of the studies in the field, and the manuscript is coherently written by using acceptable English language. The methods for assessing depressive and anxiety symptoms as well as cytokines are standardized and valid, although only a single blood sample (women at different phases of gestation) was used for the cytokine assessments.

I have several major concerns concerning several aspects of the paper, the most crucial ones being related to the group constructions and the availability of confounders (#1-3):

1. One of major issues is the selection on the phenotype/study groups, if I understood the text correctly. It is very difficult to believe that women without depression would all present with so low levels of each of the selected cytokine that this would be the reason for excluding this comparison group (page 5, lines 33-36). The inclusion on non-depressed, non-anxious comparison group would have been especially important in this group of pregnant women where pregnancy and its phase themselves produce variation in the circulating cytokine concentrations. This statement in page 5 also questions the validity of the cytokine assessment method as it really is hard to figure out how it could be the way it is currently written.

Thank you for the observation, we decided to add the control data for clearer results. The phenotypes are better described using: Severe anxiety and severe depression women, severe anxiety women and healthy controls.

A paragraph was added in the discussion which includes the healthy control group, corresponding to pregnant women (n=40) exhibiting no depression and anxiety, as described in the text, and designated as the control group (CTRL).

Previous paragraph that mentioned the exclusion of healthy pregnant women, as quotes: “Furthermore, the study excluded healthy pregnant women (exhibiting no affective disorders) since they showed blood levels of serum cytokines below the LD” has been omitted in the
amended version of the manuscript, showing the cytokine serum levels of this group in figures 1-3.

2. Related to the above: please, explain, why no group with SD only was included? And what problems does this limitation import to the interpretation of the results? And what is the rationale for the selection of the phenotypes of SA and SA + SD? Why compare these two groups? The rationale is not clear after reading the introduction, which emphasizes depression (which is not independently studied here)?

The phenotypes included were selected due to our consideration in regards that anxiety may be the factor that modifies the circulating levels of cytokines. For that reason, we compared an individual anxiety group and another depression/anxiety group. It has been described the wide interaction between depression and inflammation, nonetheless it is not known it’s interaction when anxiety and depression coexists as comorbidity. Healthy control data has been added.

3. The other major issue is the lack of controlling for the use of psychotropic medication, cigarette smoking, and BMI in these groups of patients. For example, SSRI medication, which might easily be more prevalent in the SD + SA group than SA only, is reportedly related to cytokine concentrations (Latendresse et al, 2013). Further, one would expect that if the SA+ SD is more impaired and more ill than SA only, the prevalence of cigarette smoking would also be higher in that group? What was the proportion of overweight women in each group (and again, here a group free from other SA + SD would have been important).

BMI data has been added. Limitations regarding the absence of smoking evaluation in the study has been added. Exclusion criteria now properly include the presence of psychopharmaceuticals since the study did not include women taking them. The patients were evaluated at hospitalization, for that reason in that moment patients were not receiving any pharmacological treatment the moment they were included in the study.

4. What was the reason for running separate (correlation) analyses in the two groups instead of using a single model with the cytokine concentration as the independent variable and the grouping, Hamilton scores and covariates as the dependent variables? It should be noted that also in the SA group most significant correlations come from the HDRS, not from HARS. Potentially
interactions, between the grouping and the Hamilton scores, adjusted by gestational age (at least) would reveal whether it is actually the depression score that drives these effects regardless of the phenotype grouping (which is not on a very strong ground, re: earlier comments).

Thanks for this observation. A new analysis was performed using a single model with cytokine concentrations as the independent variables and gestation weeks used as the dependent variables in the studied groups, as shown in table 3. Moreover, we showed that different cytokines interact with Hamilton scores mainly in the SA+SD group, as compared to the SA group, after all interacting variables were adjusted by gestation weeks.

5. I may have missed something, but could not find the results were the analyses on cytokine concentrations and psychiatric symptoms were adjusted for the gestational week? It was stated that gwk affected the cytokines (as it should) but no numbers of the adjusted analyses were presented? Or were the parameters of the ANOVA analyses (page 8) those figures - then it should be more clearly stated how the adjustment affected the initial correlation between the cytokines and SA/SA+ SD? This would also limit the number of statistical analyses, albeit multiple comparison was corrected here for.

As stated before, Hamilton scores and cytokine concentrations were adjusted by gestational weeks, as depicted in table 3. However, the table 2, depicts only the bivariate correlations performed between cytokine concentrations and parameters described in the manuscript.

6. I miss having clear hypotheses at the end of the introduction. Why these cytokines were selected? Why these comparison groups? What was expected?

The hypothesis has been included in the introduction. Regarding cytokines, we used a specific kit containing a wide spectrum of cytokines, comprising both Th1, Th2 and Th17-related cytokines as described in the text, so as to detect which sort of cytokines might preferentially interact with Hamilton scores and parameters described herein.

The groups described in the manuscript, comprise the expected populations that we were interested to study during 3rd trimester of pregnancy, in order to find which group displayed
higher interactions between cytokine concentrations, Hamilton scores, and gestation weeks, including anthropometric measures, as described herein.

We expected that the anxiety/depression group would exhibit the higher interaction between cytokines and Hamilton scores, versus the SA or the control group, described herein.

7. The latter half of the conclusions extends too much beyond the findings of this study. No relations between the cytokine profile and pregnancy outcomes are assessed here, so we really do not know, what the clinical implications, especially regarding screening and treatment are as there are no references for an optimal cytokine profile in pregnancy. Please revise this and retain to the results of your study. This also applies to the conclusions in the abstract.

The amended version of the manuscript describes the relationships between the cytokine profile and pregnancy, describing the importance of our findings regarding the interaction between the increase concentrations of Th1/Th17-related cytokines and pregnancy outcomes. We have inserted references describing treatment and cytokine profile in the discussion.

8. The abstract would improve from adding the rationale for the study and clarifying the setting (the comparison groups) in the methods.

The abstract has been amended, adding the rationale of the study, as suggested. Setting of groups have been described in the methods, as well.

9. There are also issues in the discussion, but as the points related to the methods and presentation of the results are likely to lead to subsequent changes in the discussion, it is difficult to comment the current version in greater detail.

As mentioned before, we have refurbished the complete text of the manuscript, which includes the introduction, methods (grouping), results, discussion, conclusions and limitations of the study, respectively.