Reviewer’s report

Title: Posttraumatic stress disorder is associated with an enhanced production of pro-inflammatory cytokines by peripheral blood mononuclear cells

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Reviewer: Andelko Vidovic

Reviewer’s report:

The present paper addresses a very important question of possible chronic low-grade inflammation in PTSD. To address this question, authors did not only compare basal plasma cytokine levels between PTSD patients and healthy controls, but also production of cytokines by isolated peripheral blood mononuclear cells. Measurements of basal cytokine levels were even repeated after a one-week interval to test the stability of the measures which is, in my opinion, very interesting approach (because of the high variability of cytokine levels). However there are some major methodological issues that must be addressed.

Major Compulsory Revisions

1. As presented in Participants section (page 5), considerable differences exist between groups in gender, age, smoking and drug intake. Authors should put participants’ characteristics in table and compare them between groups with appropriate statistical methods. Additional potential modifiers (confounders) such as alcohol use and physical activity should have been assessed. If not, authors should point it out while discussing limitations of the study.

2. In Participants section (page 6, first paragraph) authors list exclusion criteria suggesting that they enrolled more participants than reported for the purpose of this study. They even mention 6 participants that were excluded due to the hepatitis. Authors should explicitly state how many subjects were enrolled and how many were excluded due to the exclusion criteria.

3. While listing exclusion criteria authors mentioned “stress-related affective or anxiety disorders”. Neither DSM-IV (which was btw. used for diagnosis), nor ICD-10 clearly defines such disorders (disorders related to trauma/environmental stress are scattered through multiple diagnostic groups). Authors should explicitly list mental disorders that were considered exclusion criteria.

4. Main findings of the study (differences between groups in spontaneous and LPS-induced cytokine production by cultured PBMCs) are based on analyses of a subsample of 16 PTSD patients and 18 control subjects. Authors don’t provide any information regarding demographic and/or clinical characteristics of these specific subsamples and they didn't control for possible influence of these variables in their analyses.

5. Statistical analyses section (page 8) should be more elaborate. I am not sure which methods authors actually used: parametric or nonparametric. Most of the
results that are reported have emphasized F-values (where appropriate) or even t-values (Table 1, supplement 1). This suggests that authors used parametric tests (ANOVA, student t-test). If so, authors should state how they tested for assumptions that should had been met. They should also address the problem of multiple comparisons. On the other hand, authors state that “statistical significance for the immune measures was assessed by nonparametric permutation tests” (page 8). In my opinion, this indeed would be appropriate method for the purposes of this study. However, no p-values derived from permutation tests are emphasized throughout the manuscript.

6. I don’t think that Figure 1 is appropriate way to present cytokine data. Authors don’t provide any information regarding distribution of their data. Even if we assume that depicted variables are normally distributed, standard errors (whiskers) underestimate dispersion of the data (standard deviation should be used). On the other hand, if nonparametric tests were used (which is not clear), this would imply that data was not normally distributed and in that case other type of diagram should be used (i.e. box and whiskers).

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.