Reviewer’s report

Title: Selected serum cytokines and nitric oxide as potential multi-marker biosignature panels for Parkinson disease of varying durations: A case-control study

Version: 0 Date: 13 Sep 2018

Reviewer: Johannes Schlachetzki

Reviewer’s report:

In the present manuscript, Rathnayake et al. determine serum levels of four immune mediators (IFNγ, IL-10, NOx, and TNFa) with the question, whether these markers alone or in combination change according to disease duration. A multicenter, cross-sectional study design was chosen. Altogether, 72 patients with PD were enrolled and placed into three categories according to disease duration (<1 year, 1-3 years, and > 3 years). 26 age-and gender healthy matched controls, and 30 with other neurological diseases were recruited.

There is an extensive effort to identify serum biomarkers in PD. Thus, the study is of interest, however, the data as presented here are inconclusive. Furthermore, statistical analysis is insufficiently described. There are major points the authors need to address.

Major points:

* The first clinical appearance of motor symptoms can be challenging to determine and relies on the patient's or relative's recollection. Was the disease duration based on the clinical history or when the diagnosis was made? Furthermore, what was the rational to divide the PD patients into these three categories? A more objective variable would be Hoehn and Yahr staging. The authors need to additionally use H&Y as categories.

* Figure 1 is difficult to interpret. Instead of a line plot with error bars, the authors need to show their data with a categorical scatter plot with mean segments or box plot. This enables the reader to better interpret the data and demonstrates more clearly the spread of the data. Since this is a cross-sectional and not a longitudinal study, a line plot is misleading.

* It is unclear, how these biomarkers can be of prognostic value. To address this question, a longitudinal study would be needed. Thus, the statement that these immune mediators may be used as prognostic biomarkers can be discussed, but should be removed from the abstract.

* According to Table 1, only eleven patients were included with a disease duration less than one year, resulting in a total of 71 PD patients enrolled. However, 72 PD are reported throughout the manuscript.
Medication may influence serum levels, did the authors take this into account in their analysis?

It is crucial to report the statistical test used to determine significance. This is missing throughout the manuscript. In addition, within the text, the authors need to report not only significance values, but also mean, standard deviation, F-ratio, etc.

Figure 2 shows a bar graph displaying a IFNγ:IL-10 ratio. The ratio for healthy controls is given as less than 0.4. According to figure 1, IFNγ is about 20pg/ml, and IL-10 about 5pg/ml resulting in a ratio of about 4 and not 0.5. What was the statistical test used to determine significance?

The legends need to be extended.

Figure 3 and 4 are difficult to understand and are insufficiently explained in the manuscript.

Minor points:

The authors need to explain what SE/SP is.

Did the authors observe any sex differences with regards to the four measured proteins?

Did the authors perform another blood draw from the same patients to evaluate how consistent the serum concentrations are?

Levels of these serum proteins may change depending on whether the patients were eating or the time of day blood was drawn or food. Did the authors take this into account?

The authors need to discuss more recent studies aiming to identify serum based biomarkers.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.
No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

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Please indicate the quality of language in the manuscript:

Acceptable

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