Reviewer’s report

Title: Interleukin-6 is Associated with Acute Concussion in Military Combat Personnel

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Reviewer: Eric P. Thelin

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Title: Interleukin-6 is Associated with Acute Concussion in Military Combat Personnel

Main message: The authors measured IL-6, IL-10 and TNFa in military personnel. In n=94 participants, n=45 sustained a concussion while n=49 did not. Blood was drawn at <8h after injury and at 24 hours after injury. The authors found that IL-6 was elevated at the first time point as compared to the later time point.

English language: Adequate throughout.

Statistical approach: Please see comments below, presumably adequate methods have been made. However, the authors need to better highlight the non-parametric distribution of the data.

Relevance: There is a need for better blood biomarkers of mild brain injury early after trauma as well as a better understanding of the systemic inflammatory response following injury.

Main issues:

1. While the levels of IL-6 are slightly elevated during the first time point, it is difficult to draw any clinical relevance to these levels. You need to be humbler with your interpretations of the IL-6 results as the risk of a "false positive" is fairly high and while there might be a significant p-value, it is very questionable if it is clinically relevant. The narrative also seems to be that IL-6 elevation is some sort of "immunological dysregulation". Several of these cytokines act as acute phase reactants to distress and if you are exposed to injury, so I don't think at all that an elevated IL-6 is abnormal in any way, neither is it impossible to say from your current study if it is detrimental (it is perhaps a helpful step for the body to cope with the blast?). Please elaborate further and tone down the IL-6 significance in the Discussion.

2. The authors have three outcome metrics, levels at time point 1, levels at time point 2 and the delta from time point 1 and 2. Considering the results form IL-6 at time point 1, I'd say that it is obvious that there will be a delta difference. I wouldn't consider this a major finding, but group it together as a general increase in IL-6 as a main finding and thus be humbler with your result interpretation.

3. The effective half-life of cytokines in serum is very short, thus if the authors believe that IL-6 is released at the same time as the impact causing the concussion, it is presumably a
huge difference between levels acquired at 1 hour and 7 hours after injury. Did you conduct any post-hoc analysis to see if the very high levels of some cytokines seen in about 10 patients were due to very early sampling? Or did they have other injuries (see additional comment)?

4. Why would you want a very unspecific, non-brain enriched cytokine (short half-life, elevated if the patient has an infection etc.) as a marker for brain injury as compared to brain-enriched protein such as glial fibrillary acidic protein (GFAP), S100B or neurofilament-light (NF-L)? Why IL-6 should be a preferable biomarker of concussion is not adequately described in the manuscript.

5. While you highlight this in the "limitations" it should be better elaborated that the lack of CT scans could indicate that some patients actually had CT verifiable traumatic lesions (which in theory could explain some of the very high samples?). I assume that other traumatic radiology scans were not performed? For blast injuries, more sensitive radiology such as magnetic resonance imaging would probably be required. Please elaborate more extensively on this limitation.

6. IL-6 is very unspecific to brain injury, could there have been other extracranial injuries as well? How many of your soldier also suffered other injuries? Please provide this data as well. While there might not be enough power/low sample size to conduct any analyses/adjust for this, it would be good to get an understanding of your cohort.

7. Another limitation that should be acknowledged is the lack of baseline cytokine levels. Further research should measure these levels prior to exposure as to certify that it was the injury exposure itself that caused the elevations.

Specific issues:

8. You should treat all parameters throughout the manuscript as non-parametric as they are clearly non-normally distributed. Thus, use median and interquartile ranges instead of mean and SD throughout the manuscript, including the abstract.

9. How did you define "concussion"? This is not at all described in the Material and Methods section.

10. In Fig 1 and 2, it is not obvious what the box plot indicates, but I assume that it is median and interquartile range? This should be highlighted.

11. In Figure 2, (as per my previous comment), "mean change" is probably wrong to say as the data is not normally distributed and driven heavily by a few outliers. Please change to median change.

12. Page 12: "Both pro- and anti-inflammatory cytokines are produced by microglia following insult to the brain…” - I’d be very careful writing this as astrocytes and other cells of
the CNS have been shown to be immunologically active, as well as migrating cells from the systemic circulation.

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

Unable to assess

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 am able to assess the statistics

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