**Reviewer’s report**

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

**Version:** 0  **Date:** 27 Dec 2019

**Reviewer:** Breton M Asken

**Reviewer's report:**

This study evaluates a panel of serum-based cytokines at two acute time points after diagnosed concussion. As stated throughout, the project includes a unique and important sample of military personnel. The study is appropriate for the journal and the novelty of this research counterbalances the limitations associated with the small sample and relatively simple analyses. Results are interpreted appropriately in these contexts as well. That being said, the lack of clinical data in the project is a major downside, which leaves me wondering what the implications of these findings really are. Presumably, there are symptoms scales and/or other tests incorporated into the concussion evaluations. If these exist and there is any way to incorporate these into analyses, the study would be strengthened significantly, even if limited to symptom severity or recovery duration.

I have some general and specific comments below for the authors to consider during the revision of the manuscript.

**Introduction**

General: Inflammatory cytokines are increasingly studied across several areas of neurologic research and are relatively novel to concussion research. The introduction might benefit from some elaboration on the important concepts of benefits vs. risks associated with inflammation after brain injury, and the proposed mechanisms for these effects.

P5 L6-7 (…is crucial to identify people who may be at the most risk,…“): At the most risk of what? And can you elaborate on how biomarkers would help identify individuals who "require increased monitoring and preventive interventions"? In general, these statements meant to highlight the importance of biomarkers would benefit from some more specificity.

P6 L1: The basis for stating most studies include moderate to severe patients rather than mTBI patients is unclear - especially over the last decade where the frequency of blood biomarker concussion studies has risen dramatically. The authors ironically also cited more studies when referring to "fewer studies include mild patients" than moderate to severe patients.

P7 L7: Is it necessary to describe the biomarkers as "peripheral blood samples," or is "blood samples" sufficient?
Methods

P7, 2.1 Participants: Readers would benefit from more details about the diagnostic process in this unique environment. Can the authors elaborate on who the providers typically are, how the diagnosis is made, etc.? At a minimum, please provide the definition of concussion used in this sample and the typical tools available for informing the diagnosis.

P8, 2.4 Statistical methods: Is there a reason for not conducting within-subject comparisons to determine statistical significance of biomarker change between T1 and T2? It appears only between-group comparisons were made.

Results

General: While the group-level comparisons are informative, I am particularly interested in learning more about translation to the individual level. Can the authors comment on how many of the concussed participants had similar cytokine concentrations to controls (e.g., arbitrarily, within .5SD of the controls? Or within the IQR)? I think it also would be great to know which concussed participants showed evidence of elevated cytokines within 8hrs, but then did not show the decrease at 24hrs seen at the group level. Presumably, some aspect of the 67% decrease from 8hrs to 24hrs reflects progression to the mean associated with normal variability of repeated sampling in the concussed group, but since the authors correctly highlight the potential importance of chronic inflammation, readers may want to know some details about participants who showed evidence of this. Relatedly, how about those who did not show evidence of elevated cytokines like IL6, which the authors might hypothesize also would predict poor outcome?

P9, 3.1 Demographics: The study employs a unique control group comparison of active duty military personnel. Was there any documentation of whether the control groups were also exposed to blast waves prior to providing blood samples? Given 73% of the concussed sample was exposed to a blast at their time of injury, it seems reasonable to assume controls recruited from the same environment might also have been exposed even if asymptomatic.

P9, 3.2 Inflammatory protein differences following mild concussion: I suspect "mild concussion" will leave a sour taste in some readers' mouths. I recommend simply using "concussion" throughout.

Discussion:

1) The background on pro vs. anti inflammatory markers was especially appreciated, and I believe this will help spark ideas for other researchers. I think the discussion would benefit from some additional commentary on translating these findings to individual participants since there was significant group overlap in the biomarker concentrations.

2) Inflammatory markers appear to have greater utility for modeling the recovery process, predicting outcomes, etc. rather than diagnosis per se, which is the focus of so much other
concussion biomarker work. I consider this a strength of the study and inflammatory biomarker research in general. The discussion would be enhanced by explicitly stating the proposed context of use for inflammatory biomarkers in concussion management, and how this differs from other ongoing concussion biomarker work.

Figures: These figures are very helpful and highlight the significant overlap between healthy and concussed participants. The results also noted how much more variability in IL6 was seen in concussed participants vs. healthy participants. I encourage the authors to consider the following, while acknowledging the sample size is small and precludes advanced analyses:

1) There were some participants who did show high levels of IL10 and TNFα. Did these folks also show highest (or lowest) IL6?

2) Can some exploratory analyses be conducted to see if any factors predicted these "outliers" in the concussed group that seem to drive some of the group level effects?

3) Any attempt to explore these biomarkers as a panel, rather than isolating their individual results, would be greatly appreciated. This is one of the primary benefits of multiplex assays providing multiple biomarker concentrations at once, yet these analyses and results essentially treat each biomarker independently. The authors hint at potential importance of discrepancies between biomarkers in the discussion. This is a fascinating concept and I feel could be explored to some degree, even if just descriptively, in this study.

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

Yes

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

Unable to assess

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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