Reviewer’s report

Title: The effect of acute stress on salivary markers of inflammation: A systematic review protocol

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Reviewer: Emma Soneson

Reviewer’s report:

This systematic review protocol concerns the effects of acute stress on eleven different biomarkers (TNF-α, IL-1β, interleukin receptor 1 antagonist, interleukin-2, interleukin-4, IL-6, interleukin-8, IL-10, CRP, immunoglobulin-A, and fibrinogen). The manuscript is well written and the topic is important. Slavish and Szabo have given careful consideration to how they will conduct the review and are generally very thorough in presenting their methodology. Pending additional detail for inclusion/exclusion criteria, quality assessment, and meta-analysis methodology, I would be glad to see this protocol published in Systematic Reviews.

1. The authors should indicate in the Methods section of the abstract how they will assess moderators (i.e. through meta-regression). (Essential)

2. The authors present four aims of the study. It would be very helpful for readers if they could explicitly refer to these aims throughout the protocol (e.g. random effects meta-analysis of effect sizes will address Aim X, meta-regression will address Aim Y). (Discretionary)

3. The authors should elaborate on the inclusion/exclusion criteria, and should consider using the PICOS approach as guidance. Population: authors should address what they will do if studies include both adults and children/adolescents, and whether there is any other restriction on population other than age (e.g. clinical vs. non-clinical populations; adults with certain medical conditions). Intervention: greater justification is needed for why longitudinal studies of post-stressor inflammation will not be included, and authors should provide a maximum time-point for response measurement for inclusion in the review. Comparator: authors should specify whether a comparator group is needed for inclusion in the review. Outcomes: authors should discuss any criteria regarding how outcomes are measured (or state that there are none). Setting: authors should indicate if there is any restriction on study setting. Study design: authors should specify which study designs will be included in the review (e.g. only controlled trials? All study designs including pre-post measurements?) (Essential)

4. The authors state that in the first author's past review, they 'found more of their articles through reference treeing than original searches.' While their plan to use reference treeing is thorough, I worry that the relative simplicity of the search strategy may be limiting their electronic database results. First, the authors include only two databases - searching additional databases (e.g. MEDLINE, Embase) would greatly strengthen the search. In addition, the search itself could be improved (e.g. through addition of MeSH terms). Furthermore, authors should consider including foreign language publications for which there exists an English abstract, both
to increase number of included studies and avoid bias (see Cochrane handbook section 10.2.2.4 Language bias). (Discretionary)

5. The authors should elaborate on which 'study protocol details' they will extract from the included studies. They should further ensure that they have included all information of interest when describing extraction (e.g. on p. 10, line 59, authors say they will report on baseline levels of biomarkers, but have not indicated in the data extraction section that they will extract this information). Clearly presenting all extraction variables in the protocol will help ensure against appearing as if some extracted variables have been chosen post hoc. (Essential)

6. Throughout the document, the authors should specify how they will resolve disagreements not resolved by discussion (e.g. refer to a third reviewer). (Essential)

7. Further justification is needed for why the authors plan to use a bespoke quality appraisal tool, as well as greater detail on tool development (the references cited are not for standardised tools, e.g. the Cochrane Risk of Bias Tool, but rather for bespoke tools from other systematic reviews - this is not very convincing). There are some key factors that are missing that are found in the more commonly-used tools, e.g. randomisation procedures, blinding, attrition, appropriateness of analyses, etc. I am also not convinced about some of the numerical ratings. For example, higher scores seem to be beneficial and will be used in moderator analyses, yet a score of '1' in category 6 ('Quality of sample handling/processing') only means that procedures were documented, not that they were appropriate. Similarly, a score of '1' in category 9 ('Rates of missing data') refers only to whether missing data is reported, rather than to the quantity of missing data. In sum, I am not convinced about the utilisation of this bespoke tool, and highly recommend either using a standardised tool instead of or in addition to this tool. (Essential)

8. The authors indicate that they will use narrative review to present results of studies not eligible for inclusion in meta-analysis. An additional sentence explaining what results will be presented would be helpful. It is furthermore unclear what is meant by 'For any parameter that is coded, but insufficiently reported across studies, we also will provide a narrative review' (p. 11, lines 12-17). Authors should clarify what they mean by insufficient reporting. If this indicates that it is possible to have 2 or more studies that measure the same outcome but not analyse them through meta-analysis, then additional information is needed on how authors will narratively synthesise results. (Essential)

9. From the proposed aims, it is not currently clear to me what the meta-analysis part of the review aims to do. The authors should be clearer in this regard; for example, are they looking to see which biomarkers have significant (non-zero) pre-post changes? Or are they trying to quantitatively synthesise the effects of acute stress on each of these, e.g. to see which have larger changes (see p. 11, lines 22-27)? I believe that the meta-analysis aims to address Aim #1 in the Introduction, but the wording ('What changes...') does not make this very clear. (Essential)

10. Currently, it is not evident which study designs will be included in the meta-analysis (see also the comment about inclusion/exclusion criteria). Authors should state this explicitly. (Essential)
11. It would be helpful if authors indicated the maximum number of analyses that may possibly be performed to ensure transparency. Currently, it appears that they will do 11 meta-analyses (one per biomarker), and that the number may be increased by studies measuring response at different time points (though the number of different time categories is not specified—see next comment). The increased number of meta-analyses generated by sub-dividing studies will limit the power of the analyses, so authors might seek to address this in the protocol (e.g. by using sub-group analyses instead of entirely separate meta-analyses). (Discretionary)

12. The section on how timing will be addressed in the meta-analyses (p. 11, lines 27-49) is, in my opinion, the section that needs greatest clarification. First, the authors should be more explicit in linking to Aim #2 from the Introduction. Second, the point about creating a new categorical variable is not clear. Is this saying that, in order to perform a meta-analysis, two studies must measure outcomes within the same time category? If this is the case, it seems that the authors could miss out on important information, and that they should seek to meta-analyse all of the studies that measure the same outcome, and account separately for timing (however, I am not an expert on this topic, so I may be mistaken in this). Either way, authors need to define the time categories up front to improve transparency (currently, authors only mention groups 20-40 minutes and 120-180 minutes; why these categorisations? What about other timings?). Third, for the meta-regression, it is currently unclear which extracted outcomes will be used as the meta-analysis outcomes (i.e. at which time points) and how sample timing will provide useful information as a moderator in these analyses. (Essential)

13. The authors mention using random-effects meta-analysis for subgroup analyses (p. 9, line 39), but do not anywhere describe which subgroup analyses they plan to conduct. All planned sub-group analyses should be explicitly described. (Essential)

14. The section concerning confidence in cumulative evidence needs further elaboration and clarification. The authors clearly explain how they will assess the quality of individual studies, but do not discuss how they will rate the strength of evidence as a whole. Authors should consider using the GRADE or other established and validated approach, or else justifying why they will not do so (see PRISMA-P Elaboration & Explanation). (Essential)

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