PEER REVIEW HISTORY

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ARTICLE DETAILS

| TITLE (PROVISIONAL) | Aluminium adjuvants versus placebo or no intervention in vaccine randomised clinical trials. A systematic review with meta-analysis and Trial Sequential Analysis |
|---------------------|----------------------------------------------------------------------------------------------------------------------------------|
| AUTHORS             | Krauss, Sara; Barmateskovic, Marija; Klingenberg, Sara; Djurisic, Snezana; Petersen, Sesilje; Kenfelt, Mette; Kong, De Zhao; Jakobsen, Janus; Gluud, Christian |

VERSION 1 – REVIEW

| REVIEWER            | Fang, Chi-Tai |
|                     | National Taiwan University |
| REVIEW RETURNED     | 19-Nov-2021 |

GENERAL COMMENTS

This is a timely important review on aluminum as adjuvant, particularly in the current time of COVID-19 pandemic. However, some aspects of this work need to be substantially improved before acceptable for publication:

1. The type of pathogens targeted by the vaccines is a very important variable in such systematic review and meta-analysis. Specifically, aluminum adjuvant elicits a T helper cell response of type 2 (against extracellular bacterial pathogens), rather than the type 1 response critical for controlling intracellular viral pathogens. Therefore, authors need to describe the targeted pathogens for vaccine trials included in their review. Furthermore, subgroup analyses (extracellular bacterial pathogens vs intracellular viral pathogens) need to be performed to look for any difference between these subgroups.

2. Please delete the unjustified claim in page 16, ll. 10-13: "We seem to be the first to conduct a systematic review comparing aluminum adjuvants versus placebo or no intervention in combination with vaccines." At least three teams had explored this topic, although previous works had been focused on specific types of vaccines.

| REVIEWER            | Hejazi, Nima |
|                     | Weill Cornell Medical College, Population Health Sciences |
| REVIEW RETURNED     | 24-Jan-2022 |

GENERAL COMMENTS

Statistical review. This appears to be a thorough and ambitious meta-analysis of a large number of trials and on a topic of present importance. While I am unable speak to the scientific claims made, the statistical techniques used (e.g., random/mixed effects models) appear appropriate for the design of this study. A wealth of information is given in the Supplementary Materials, but the
exposition on methodology could be clarified by presenting exactly the forms of the models used (e.g., did these correct for the number of individuals in each study? How was the recency of different trials accounted for?). In general, descriptive statistical approaches --- such as concise visualizations of how adverse event rates and the like differed between the adjuvant-inclusive and adjuvant-free conditions across trials --- would allow the reader to more easily come to the same conclusions as the authors than the relatively heavy reliance of hypothesis testing procedures with differing sets of assumptions.

**VERSION 1 – AUTHOR RESPONSE**

1. Please revise the formatting of your abstract so that it includes the following sections: Objectives >> Design >> Data Sources >> Eligibility Criteria >> Data extraction and synthesis >> Results >> Conclusions.

Response: thank you for pointing out this shortcoming. In our primary submission we wrote “Objectives >> Data sources >> Main outcome measures >> Results >> Conclusions. We have amended this text in this second submission into Objectives >> Design >> Data Sources >> Eligibility Criteria >> Data extraction and synthesis >> Results >> Conclusions.

1. Please go through the PRISMA extension for abstracts (http://www.prisma-statement.org/Extensions/Abstracts.aspx) and check that items 1-10 are reported in your abstract.

Response: we now made sure that items 1-10 are reported in our abstract.

1. The reporting of the methods could be improved in this section e.g. how was risk of bias assessed? Also make it clear you used GRADE.

Response: we now provide more explanation on how the risk of bias was assessed and about the GRADE assessment (page 8 lines 13 to 22 and page 9 lines 1 to 2). In our primary submission we wrote “The review author pair (SRK, SLK and MB) independently assessed the risk of bias of each included trial according to the recommendations in the Cochrane Handbook for Systematic Reviews of Interventions”; and “We used the GRADE system to assess the certainty of the body of evidence associated with each of the outcomes”. We have amended this text in this second submission into “The review author pair (SRK, SLK and MB) independently assessed the risk of bias (RoB 1) of each included trial according to the recommendations in the Cochrane Handbook for Systematic Reviews of Interventions. We used the following bias risk domains: ‘allocation sequence generation’; ‘allocation concealment’; ‘blinding of participants and treatment providers’; ‘blinding of outcome assessment’; ‘incomplete outcome data’; ‘selective outcome reporting’; and ‘other bias’. We assessed the domains ‘blinding of outcome assessment’, ‘incomplete outcome data’ and ‘selective outcome reporting’ for each outcome. The trial was classified at overall ‘low risk of bias’ only if all the bias domains described in the above paragraphs were classified at low risk of bias, or at ‘high risk of bias’ if any of the bias risk domains described above were classified at ‘unclear’ or ‘high risk of bias’. We used the GRADE system to assess the certainty of the body of evidence associated with each of the outcomes. We constructed the ‘Summary of findings’ tables using the GRADEpro software. The GRADE system appraises the
certainty of evidence based on the extent to which one can be confident that an estimate of effect or association reflects the item being assessed.

1. The introduction section is very brief. Can you please expand this section to provide a more thorough overview of the background literature and rationale for carrying out this study?

Response: thank you for your relevant comment on our introduction. It has now been amended as follows:

   a. Why is a systematic review needed on this topic?

   Response: please see page 4 lines 7 to 23, page 6 lines 2 to 8

   What gap in the literature is it addressing?

   Response: please see page 5 lines 19 to 24 and page 6 lines 1 to 7

   a. Are there other systematic reviews published on this topic and if so, how is this review adding to those?

   Response: please see page 5 lines 18 to 24 and page 6 lines 1 to 7

   a. It's not entirely clear what the problem is. No safety concerns were raised in the introduction and there was no mention of any other emerging adjuvants that may be better.

   Response: please see page 4 line 7 to 23, page 5 line 23, 24, page 6 lines 1 to 7

1. Please work on improving the presentation of the methods section. Can you use side headings to guide the reader? (e.g. inclusion criteria, search strategy, data extraction, data synthesis, risk of bias assessment etc.)

Response: thank you for your relevant comment on our methods section. We have now amended the methods section by adding side headings.

1. Please ensure 'Table 1. Summary of findings table' is placed on top of the table.

Response: thank you for this comment. We have now corrected this.

1. Aluminum adjuvant elicits a T helper cell response of type 2 (against extracellular bacterial pathogens), rather than the type 1 response critical for controlling intracellular viral pathogens. Therefore, authors need to describe the targeted pathogens for vaccine trials included in their review. Furthermore, subgroup analyses (extracellular bacterial pathogens vs intracellular viral pathogens) need to be performed to look for any difference between these subgroups.

Response: We are grateful that the peer reviewer pointed out this aspect related to the mechanism of action of the aluminium adjuvants. We have now given a description of the targeted pathogens for vaccine trials included in our review in page 11 lines 19 and 20. We have also performed subgroup analyses (extracellular bacterial pathogens vs intracellular viral pathogens) for all our outcomes (supplementary figures 11, 21, 34, 35) and the test for subgroup differences showed no differences when analyzing serious adverse events, all-cause mortality, non-serious adverse events and serology.
1. Please delete the unjustified claim in page 16, ll. 10-13: “We seem to be the first to conduct a systematic review comparing aluminum adjuvants versus placebo or no intervention in combination with vaccines.” At least three teams had explored this topic, although previous works had been focused on specific types of vaccines.

Response: We have now amended the sentence into ‘We seem to be the first to conduct a systematic review comparing aluminum adjuvants versus placebo or no intervention regardless the type of vaccine’.

1. A wealth of information is given in the Supplementary Materials, but the exposition on methodology could be clarified by presenting exactly the forms of the models used:
   . did these correct for the number of individuals in each study?

Response: Intervention effects were assessed and presented with both random-effects model (DerSimonian 1986) and fixed-effect model meta-analyses (DeMets 1987). The more conservative point estimate of the two, comprised by the estimate closest to zero effect (the analysis with the highest P value), was chosen for assessment of significance (Jakobsen 2014). In the presence of small heterogeneity, the two approaches give similar results Random-effects meta-analysis gives more weight to imprecise (or small) studies compared to a fixed effect meta-analysis. Random-effects meta-analysis gives more conservative results unless there are small study effects. To clarify if the model used in our analysis corrected for the number of individuals in each study, we have now amended our reporting by clearing specifying the model used in each analysis (we added the wording random-effects or fixed-effect under each forest plot figure, in addition to the reporting that was originally present at the top of the figure).

Moreover, we have amended the supplementary material that now more clearly defines the best-worst and worst-best analysis to assess the potential impact of missing data (see pages 17, 18, 29, 30).

a. How was the recency of different trials accounted for?

Response: We performed our analysis using Review Manager 5.4. In this software, we used the inbuilt function of sorting the forest plots entries by year and we visually inspected the forest plots to account for the recency of different trials. Together with the inspection of the I², we did not observe an influence on the analysis stemming from the date of when the studies were performed. This has now been described in supplementary Analysis S4.

a. In general, descriptive statistical approaches --- such as concise visualizations of how adverse event rates and the like differed between the adjuvant-inclusive and adjuvant-free conditions across trials --- would allow the reader to more easily come to the same conclusions as the authors than the relatively heavy reliance of hypothesis testing procedures with differing sets of assumptions.

Response: We thank the reviewer for this relevant suggestion. We decided not to add additional figures to this manuscript.

VERSION 2 – REVIEW

| REVIEWER       | Fang, Chi-Tai             |
|----------------|---------------------------|
| National Taiwan University |

| REVIEW RETURNED | 04-Apr-2022 |
| GENERAL COMMENTS | No further comments. |