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Reviewer Comments

Reviewer A

Comment 1: T The analysis is well structured, but probably there is an error in the calculation of "titles retrieved for full-text screening" in line 117, page 5 (Results Section), in line 407, page 14 (Abstract) and also in figure 1. In fact, the authors talk about "n = 44 Full-text articles assessed for eligibility", but 503-460 = 43.
Please check the calculations.

Reply 1: We thank the reviewer for making this pertinent remark. In fact, 44 full-text articles were assessed for eligibility, but 459 (not 460) were removed. This was corrected in Figure 1, but the description in the results section and abstract remained correct.

Reviewer B

Comment 1: The potential role of disease activity of RA patients. It is well established that CLP levels can fluctuate depending on DA in patients with autoimmune diseases. This aspect should be analyzed and discussed in more detail.

Reply 1: We agree with the reviewer that it has been well established that cCLP levels correlate with disease activity in autoimmune diseases, e.g. RA. As this aspect has been thoroughly analyzed by previous meta-analyses (e.g. Zeng et al., reference 18), we did not re-evaluate or re-described this aspect as we aimed for an alternative and additional evaluation, i.e. the importance of pre-analytical confounders in cCLP analysis. Nevertheless, this aspect has been described in the introduction and discussion section of our paper. (line 88-90)

Comment 2: Why might middle-aged adults with mild to moderate hyperferritinemia be referred to hematologists in the absence of infectious or inflammatory diseases?

Reply 2: The sentence was unclear. Revision has been made as; It is not infrequent that middle-aged adult patients with mild to moderately elevated serum ferritin (500ng/mL~3,000ng/mL) are referred to hematologists as unknown hyperferritinemia. Physicians need to be aware of metabolic hyperferritinemia with or without iron overload.

Comment 3: The analyses are restricted to the difference between RA and HC which does not allow for the assessment if there is heterogeneity in RA patients or in HC.

Reply 3: We agree with the reviewer that the comparisons and heterogeneity analysis is solely focusing on the difference between RA and HC. Importantly to stress is that we focused, in line with other meta-analyses on cCLP in RA, on diagnostic RA samples and healthy controls (HC), excluding resp. RA patients in follow-up and rheumatological diseased patients, thereby minimizing the heterogeneity in RA and HC cohorts. We realize this is global evaluation, which is a first step in objectifying the

possible advantage of the use of cCLP as a biomarker in RA. However, as we fundamentally stress in our paper, the comparison of studies on cCLP in RA study is hampered by pre-analytical factors. So, in future studies, colleagues should first implement strict adherence to pre-analytical requirements, allowing study comparability and more detailed and thorough data analysis.

Reviewer C

Comment 1: The authors indicate that the systematic literature review and meta-analysis were conducted following 2020 PRISMA guidelines.

Reply 1: To convey consistency to the 2020 PRISMA guidelines, we've added the checklist in supplementary materials as described in line 127-128

Comment 2: PRISMA recommends registering the review to allow the assessment of any deviation that may have introduced bias. Was the review and the protocol registered? If so, please include where it can be accessed and provide registration information including register name and registration number.

Reply 2: No, we didn't register the review and the protocol separately, because of the equivalence of the search results to other groups as Zeng et al. (reference 18) and Andalucia C. (Presentation no. POS 1400, EULAR 2022; ICA 2022). As requested by the PRISMA checklist we therefore mentioned that the review was not registered in the Materials and Methods section, line 112-113

Comment 3: he research question is clear and concise.

In the method section, as part of the search strategy, specify all databases used, the date of the last search, the filter applied, and the keywords used. However, PRISMA also recommends providing the full line-by-line search strategy as run in each database or the sequence of terms that were used. I would suggest including them as a supplementary file.

Reply 3: As requested by the reviewer and the PRISMA checklist (item 7), in the Material and Method section, the search strategy, including the terms used, were provided (line 110-113):

"Leukocyte L1 antigen complex", "rheumatoid arthritis", "rheumatic disease", "circulating calprotectin", "blood calprotectin", "calgranulin A/B", "MRP8/14", "S100A8/A9". A time filter was applied, including articles published from the year 2000 onwards.'

Due to the equivalence of the search results with other recent meta-analyses, search strategies revealed to be straightforward and reproducible, not necessitating additional detailed information.

Comment 4: In the method section, the inclusion/exclusion criteria and selection process are clear.

In the method section, as part of the data extraction is recommended to report the number of reviewers that collected data from each report, whether they worked

independently or not, and how disagreements were solved. I would suggest authors to include it.

Reply 4: In line with the recommendation of the reviewer, we've described the review more in detail in line 124-126:

'Full-text articles were further assessed for eligibility by two independent reviewers using the National Institute of Health (NIH) quality assessment tool for observational cohort and cross-sectional studies to reduce the risk of bias. Discrepancies were resolved by consensus.'

Comment 5: In the method section, as part of the data items, to avoid duplicating SMD of the same pair RA -HC multiple times (for instance in case of Van Hoovels, 2019), it should be specified when results from multiple commercial tests were presented for the same cohort, which commercial tests would be prioritized and why. I would suggest authors to include it.

Reply 5: Following up the suggestion posed by the reviewer we've explicitly stated in the Materials and Methods section how results from multiple commercial tests were presented for the same cohort (line 136-138):

'For studies evaluating multiple cCLP assays on the same sample cohorts, the specific HC and RA results related to every assay were considered as an unique data set, resulting in an assay- specific SMD value.'

In addition, we also performed the same analyses, only using the Bühlmann cCLP assay, similar comparable results were obtained (Cochran's Q=168.75; I^2 statistic=89.9%; 95%CI= 85.6-92.9%).

Supplemental data Table 2 provides an overview of the study characteristics of the studies included in the meta-analysis, including the assays used.

Comment 6: PRISMA recommends specifying the methods used to assess the risk of bias in the included studies, including how many reviewers did the assessment and whether they worked independently and how discrepancies were solved. I would suggest authors to include it.

Reply 6: We agree with the reviewer and added, in line with PRISMA recommendations (checklist supplemental data 1) both in the abstract (line 46) as in the Materials and Methods section (line 152-154)

Comment 7: Statistical analysis is described appropriately.

In the results section, I would recommend:

- To review numbers of studies included in each box.
- To include main reasons for full-text exclusion
- Number of studies and references comparing RA and HC
- Number of studies and references evaluating pre-analytical conditions.
- Risk of bias results

Reply 7: We've performed the review as suggested by the reviewer and amended Figure 1 accordingly.

Furthermore, we've described the main reasons for study exclusion in the Results section line 159-161:
'Articles including incomplete data, duplicate data, non-diagnostic RA patients or non-healthy control cohorts were excluded, together with meta-analysis, systematic reviews and letters to the editor'.

Comment 8: Which datasets were included to calculate the SMD estimate for RA vs HC? Did you include 4 times the SMD for the same cohort of RA vs the same cohort of HC from van Hoovels, 2019 study? If so, you are counting the same comparison 4 times.
Reply 8: T We refer to our response to C.5 and the overview provided in Supplemental Data1.

Comment 9: In the figure 3a, I would suggest providing information on pre-analytical conditions per study so readers can have an idea of how different conditions could influence results.
Reply 9: An overview of the pre-analytical conditions encountered in the included studies is provided in Supplemental data 1.

Comment 10: Would it be feasible to calculate a SMD estimate for different pre-analytical conditions?
Reply 10: As suggested by the reviewer, we've calculated SMD estimates for the different pre-analytical conditions (line 196-202):
'In 9 out of 21 included studies, time to centrifugation was not specified, neither storage temperature in 6 out of these 9 studies. The total SMD for samples with adherence to centrifugation time (n=12) is 0.750 and 1.084 for samples without any centrifugation time mentioned. When excluding results obtained with Bühlmann assay, the values for SMD declined to 0.648 and 0.892, respectively. For samples for which storage temperature was mentioned (n=15), the total SMD was 0.846 compared to a total SMD of 0.892 for samples without any storage temperature information.'

Comment 11: In the introduction was mentioned that sample type influences results. Where are the number of studies including different matrices and data analysis included in the results section?
Reply 11: As mentioned in the discussion section (line 295-296),
'The matrix effect on cCLP was not evaluated as only a minority of studies were performed on plasma, which may cause bias in subgroup analysis.'

Comment 12: I would kindly ask you to check the format of the text. I have observed some extra space. If cCLP is the acronym for circulating calprotectin, why do you use CLP sometimes?
Reply 12: We apologize for the formatting errors. We've reviewed the text and removed several extra spaces.

We use CLP to discriminate between the ‘biomarker’ calprotectin and the specific systemic form ‘circulating calprotectin’, but we’ve replaced CLP as much as possible by cCLP.

Comment 13: I would recommend including how findings from this meta-analysis would impact the clinical practice and what recommendations we can get from it.
Reply 13: We’ve included the main goal of our meta-analysis in the highlight box: ‘Adherence to pre-analytical recommendations is primordial in studies on cCLP in RA and significantly reduces inter-study heterogeneity.’