**Reviewer’s report**

**Title:** C-reactive protein as a potential biomarker for disease progression in dengue: A multicountry observational study

**Version:** 0  **Date:** 16 Sep 2019

**Reviewer:** Michael Hawkes

**Reviewer's report:**

The authors are to be congratulated on this interesting analysis of CRP as both a diagnostic and a prognostic marker in a large cohort of patients with dengue-like illness in Asia and Latin America. Below, I suggest a simplified analysis of CRP as a prognostic marker (dichotomize the predictor into "high" and "low" with a single cutoff value) - please give this serious consideration as it would greatly improve the clarity and "readability" of the study. Along with other comments, questions, and criticisms below.

1. Is the dengue serotype available?

2. Error?
   Results: Association of CRP level and clinical outcomes among dengue patients, line 45
   "CRP levels at enrolment in patients with severe or intermediate dengue was higher than in patients with uncomplicated dengue, with median levels (interquartile range) of 28.6 mg/L (10.5-58.9 mg/L) and 34.0 (17.4-71.8 mg/L), respectively"
   Did the authors reverse the order of the levels in this sentence? I.e., 34.0 is higher and may correspond to severe or intermediate dengue and 28.6 may correspond to uncomplicated dengue (Table 2).

3. Typographical error: Appendix 2 : Tonsillitis

4. Matching procedure
   Methods: Study population, lines 9 and 15
   Patients of "similar geographics, demographics and day of illness" were selected from both the uncomplicated dengue group (in a 1:3 ratio). Were 3 controls matched to each case? If so, should the matching be taken into account in the analysis (e.g., conditional logistic regression)?
   Patients of "similar geographics, demographics and day of illness" were selected from OFI group (400 controls). It would appear that this is not selection of controls for each case; not entirely clear how the "matching procedure" was performed.

5. Inclusion and exclusion criteria
   Methods: Study design, line 40
   It appears that patients were eligible for inclusion in the parent IDAMS study if they had a dengue-like illness with "no localizing features suggesting an alternative diagnosis, e.g., pneumonia." However, a number of patients in this report have OFI, suspected bacterial infection, and Appendix 2 lists several
focal bacterial infections (abscess, urinary tract infection, pneumonia, infected wound). Why were these patients included in IDAMS, given the stated inclusion criteria?

6. Exclusion of patients with high CRP

Figure 1 and Results line 21
I don't think it is appropriate, in a study of CRP as a diagnostic and prognostic disease biomarker, to exclude patients with high CRP values (&gt;LOD). I would much more readily accept (arbitrarily) assigning the value of the upper limit of detection of the assay than excluding these patients. Alternatively, a dilution of the patient plasma to bring the sample within the dynamic range of the assay.

7. Insufficient detail on CRP measurement
Methods: Laboratory evaluation line 52
The accuracy of the CRP measurement is critical/central to this study. Much more detail is warranted to explain the measurement of the biomarker. Sample collection in the field, plasma separation and time from collection to centrifugation, storage (temperature -80°C?), transport to analysing lab, temperature control during transport, time from collection to analysis, description of commercial Luminex assay, standard curve, quality control measures used. These details are important to assess the validity of the reported quantitative CRP levels.

8. Table 3, and indeed entire non-linear analysis of CRP as prognostic marker, not easy to understand
I found Table 3 difficult to understand/interpret until I saw the Figures in the Appendix 5, 6 and 7. I much prefer the Figures and would recommend that they go in the main manuscript, in preference to the Table.

However, I would strongly urge the authors to consider a simpler analysis (dichotomize the CRP, for the purpose of the prognostic biomarker). I think this may make their findings much more clinically usable and easier to interpret by the general clinical readership of BMC medicine.

If I have understood correctly, there is a non-linear relationship between CRP and risk of severe/intermediate dengue, time to fever resolution, and risk of hospitalization. This is interesting, and I think fine to report in the Appendix. But, it makes the interpretation of the ORs and HRs very complicated: for every two times increase in CRP below 30, the odds increases/decreases by x, and for every two times increase in CRP above 30, the odds decreases/increases by y.

Can the authors not define a CRP cutoff (e.g., ROC curve analysis) above which the risk of severe/intermediate dengue is higher than uncomplicated dengue (baseline group)? And similarly for time to fever resolution and hospitalization? Perhaps a cutoff of 30 would work parsimoniously for all 3 outcomes, based on inspection of the figures (Appendix 5, 6 and 7). Then report the ORs or HRs as a single value, representing the risk in the "high CRP" group relative to the "low CRP" group. Although the utility of a statistical approach which accounts for non-linear relationship of CRP and risk is clear when CRP is used as a diagnostic marker (intermediate levels around 30 are associated with highest odds of dengue versus other virus or versus bacterial infection), it is not obvious to me that there is any clinical value when assessing CRP as a prognostic marker. I appreciate that statistically, there is significant non-linearity; however, dichotomizing the CRP would simplify the analysis and
interpretation, would be a more standard approach, and would make it clear how the CRP could be used in practice (above cutoff - higher risk of severe dengue).

9. Possible overstatement of clinical utility of CRP

Discussion, Line 16

Although the authors claim that CRP as a prognostic marker could have "significant implications for patient management," the odds ratio(s) are quite modest in magnitude. I'm not convinced that CRP would meaningfully affect management decisions (admit, give IV fluids, etc.) based on the small changes in pre- and post-test probability (likelihood ratios) that would be associated with this test. For the general medical readership of BMC Medicine, I think the authors should be more circumspect about the potential clinical utility, although CRP may be one of a constellation of clinical and laboratory criteria that could improve the accuracy of clinical decision-making.

**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.

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