Author’s response to reviews

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

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Author’s response to reviews:

Reviewer #1: Michael Hawkes
I thank the authors for their revised manuscript and responses. Most of my comments have been satisfactorily addressed.
I do remain unsatisfied with the authors' responses to the following, but leave these as discretionary revisions, which the authors may choose to ignore:

6. Exclusion of patients with high CRP
Authors' Reply: "The accuracy of an arbitrary value outside the upper limit of detection of the assay, we decided, was unacceptable."
Equally or more unacceptable is disregarding these patients entirely from the analysis!
"The numbers were 19 and therefore would not affect the overall results."
Please provide a sensitivity analysis, including and excluding these patients to show that the overall results are not affected. Can use arbitrary level above cutoff or multiple imputation when including the patients with CRP above upper limit of detection.

A. We have performed a sensitivity analysis of 9 values that were extrapolated –the remainder (10 cases) were missing samples so no result was available. This has shown similar results to the main analysis in both the association of CRP levels and dengue diagnosis and the association of CRP levels and clinical outcomes among dengue patients. All these results have been added in the results section (pages 10-11) and the additional file 9. We have updated the total number tested in the text.

8. Table 3, and indeed entire non-linear analysis of CRP as prognostic marker, not easy to understand
The authors vigorously defend their intricate statistical approach, and oppose a ROC analysis and dichotomization of the CRP.
Accepting their statistically grounded arguments, I maintain that this comes at the expense of clarity and simplicity. Clinicians will have difficulty applying the results to their clinical practice.
I continue to urge the authors to report, in addition to their complex analysis, a single OR for CRP above a certain cutoff as a dichotomous predictor of intermediate/severe dengue.

A. We have added the following cut-offs for CRP and have reported this in the results section on page 10: When categorizing CRP levels into 3 groups (<15; 15-30; and ≥30 mg/L), the 2 latter groups had significantly higher risk of severe or intermediate dengue compared to the lowest CRP level group (ORs [95% CIs] were 1.66 [1.07-2.56] and 1.59 [1.10-2.32] respectively), after correcting for age, DOI at enrolment, plasma viremia, and immune status.

Reviewer #2: Junxiong Pang

I appreciate and applaud the effort of the authors in addressing my comments critically.

I am fine with the comments and revisions. However, i would like to suggest that the authors at least highlight the following pointers in the limitation section of the study for more accurate interpretation of the findings for potential implementation or trials in near future.

1. "A more common intervention is fluid transfusion (other than blood products) but we did not consider it as confounder because there is no apparent evidence that fluid transfusion could affect the CRP level."

2. "As severe/intermediate group may have different demographical characteristics (especially age) to uncomplicated dengue, it is expected that tested and un-tested patients would be different with respect to those features."
3. "Other factors, such as bacteria coinfection, antibiotic usage and comorbidity, had unclear potential relationship with either CRP level and clinical outcome and therefore, they were not considered for the adjustment."

4. "As the sample size was unbalanced between day of illness and DOI 1 was unlikely to have a clinical event, we decided to adjust for DOI rather than to conduct a stratification analysis on this factor."

Thank you.

A. Thank you – we have added points 1,3 and 4 into the statistical analysis section on p7 and added point 3 into the limitations section on P14.

Reviewer #3: Shyam Dumre
Authors have addressed and/or clarified the my concerns.

Reviewer #4: Carol Blair
My comments were all considered and adequately addressed by the authors. Since I am not a clinician or a statistician, but am a scientist who has tried to identify serum biomarkers in dengue patients, my perceptions of the study described were quite different from those of the other reviewers. However, I believe the additional information, particularly in Additional files 1 (and the relevant reference) and 2, was useful and contributed to better understanding and appreciation of the study.

I have only a few minor suggestions for the revised manuscript:

p. 5, last line: change "degrees" to 'C'
This has been changed as suggested.

p. 6, line 3: Change "manufacturers specification's" to manufacturer's specifications'
This has been changed as suggested

p. 12, line 15: Change "multiple sources of possible confounding" to 'multiple possible confounding sources'
This has been changed