Reviewer’s report

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

Version: 0 Date: 22 Sep 2019

Reviewer: Shyam Dumre

Reviewer's report:

Summary:

In the report the authors have attempted to assess whether CRP at early acute phase can serve as a prognostic marker for dengue severity. They also examined whether it could differentiate dengue and OFI. They employed a nested case-control study using samples collected from Asian and L. American countries. The topic of the study is certainly of great interest since having a reliable prognostic biomarker/predictor of severe dengue could drastically reduce the unwanted hospitalizations based on warning signs, and this would be a game changer in dengue management. Although the studies on biomarkers have been notably increased in recent years, such a large sample size in this study (enrolled) from different countries is quite impressive. Despite this, there are several issues need to be clarified and modified as highlighted below:

Major comments:

1. The authors of this manuscript preferred the clinical definitions - uncomplicated, intermediate and severe dengue; and used the intermediate or severe as primary endpoint. However, it is not very clear whether it is similar to the WHO-2009 classification (dengue without warning signs, with warning signs and severe dengue) specifically the severe dengue group. If it is different, more clarification is required why they did not consider WHO-2009. This is important because, there is huge number of unnecessary hospitalizations based on the warning signs, and the prognostic marker should be able to detect the most severe cases (severe dengue) among the total dengue cases so that the majority of those with mild dengue (with and without warning signs) need not to be hospitalized. Authors even combined the intermediate/ severe dengue as primary endpoint, what was the criteria considered for hospital admission?

2. In the sampling strategy, it is not clear- how many patients were enrolled from each site (country/continent). Also the how many were children and adults from each site and in total? Without this information, it is difficult to follow tables.

3. What was the just criteria to include 400 OFI (from 2540), 1133 (from 2694) for CRP testing? Why the rest were excluded. In the results, authors had subgroup analysis too (Fig-3; bacterial, viral, confirmed dengue), why the patients with other viral infections (flaviviral) were excluded (Fig-1). This would add more value to the analysis. It has to be justified/clarified. Because a major confusion in peripheral health facilities is in differentiating between dengue/ other viral infections (compared to dengue vs bacterial infection).
4. There is no sub-group analysis of Asian and L-American population. It would be interesting to look at it, if there is any difference.

5. In many places, authors expressed the terms like "younger age, highest secondary infections in severe dengue"; "Less OFI hospitalized", "Lower CRP among clinically viral patients" "median CRP higher than in uncomplicated dengue" etc., in results section Paragraphs-2, 3, 4. But, no statistical differences (p-value) is mentioned. It is suggested to apply Mann-Whitney/ Kruskal-Wallis or chi-square tests as appropriate and supply these values.

6. Despite impressive sample size and multi-country design, CRP is actually lower in the "severe dengue", and it may not serve as a good prognostic biomarker for "severe dengue". Differentiating dengue and OFI may not be of immense value, since the POC tests are available for dengue. The authors are required to discuss on its clinical applicability. Since the OR (odds of being severe or being dengue) is relatively small despite statistically significant, this should also be discussed clearly on the clinical significance (real world scenario) while claiming the prognostic ability. Nevertheless, early acute CRP may also aid in the existing WHO-2009 classification, but for that the sensitivity/specificity after inclusion of CRP has to be determined.

Other comments:

Methods:

1. Line 39. Please provide age range, and also consider comment#2 here.

2. Case definitions, please provide Reference for the method to primary/secondary dengue determination.

3. Laboratory evaluation: The follow up samples were from day 10-14 post onset of illness. However in the figure-2 it is mentioned as ≥7. Why not to write as ≥10 or 10-14 day post onset?

4. Statistical analysis: It is not clear which variables were significant in crude OR analysis, and of those which parameters were included in the final model of multi-variate analysis. CRP is increased in many other conditions like cardiovascular conditions, blood pressure, liver failure, lipid levels, weight, hormone replacement, etc. Apart from the age, immune response, DOI, whether any of these variables were considered while adjusted? If not why?

Results:

People with higher CRP are more likely to be hospitalized longer. It seems contradictory in the case of "severe dengue" which has lower CRP in this study. In reality, often the "severe dengue" patients are hospitalized longer. How the authors will clarify it.
Figure-2: In the OFI group, number of Follow up patients seems few, please indicate it in the legend. And also show p-value is there is any statistical significance.

Figure-3: Lower panel. Mention in the legend - statistical test/significance (p-value) between/among subgroups. How and why was the CRP cut off 30 mg/l derived/used? Please, indicate it in methods. Additionally, would it be possible to analyse the sensitivity/specificity of CRP at an cutoff of 30 mg/l in identifying worse dengue outcomes, delayed fever clearance and longer hospital stay?

Figure-4: It is be better to do subgroup analysis for correlation of CRP and WBC/Neutro/Lympho (and other parameters of Appendix-8) in different levels of dengue severity. Another point - in the figure/text, only the "r" is mentioned. These are only moderately or weakly correlated; therefore it is suggested to include the p-value (statistical significance) for each correlation performed by Pearsons method.

Tables 1, 2: Please, indicate the statistically significant difference.

Table-3: Please, mention reference (for multivariable logistic regression) in the footnote/caption. Appendix-4: Please include severe dengue also (as in Table 3)

**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.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

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