Author's response to reviews

Title: Dengue NS1 antigen as a marker of severe clinical disease

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Author's response to reviews: see over
Prof. Philippa Harris,
Executive Editor,
BMC Infectious Diseases.

Dear Prof. Harris,

We wish to thank you again and the reviewers for the positive comments and for the careful review of our manuscript. We have highlighted the changes in yellow in the re-revised manuscript. We have addressed each of the points below:

1. **Reviewer 1**
   **Major comments:**

   1. The figure 1B was not included in the revised manuscript. Also, the correlative figure legend should be reconsider, how can we predict “shock” when there is no concrete evidence about the time that “shock” happened (line 504, page 21). (line 504, page 21).
   
   **Answer:** We are sorry if Fig 1B is not seen in the revised manuscript, but it appears in the version we submitted. We have used to-NS1 antigen level on admission as a predictor of shock. None of the patients had shock before admission, and all patients who developed shock did so in hospital.

   2. **Figure 2 needs correction with a line demonstrating the median value.**
   **Answer:** We thank the reviewer for this comment. We have changed the figure accordingly.

   3. **A multivariable analysis should be performed to control confounder, if any.**
   **Answer:** We have done this to determine any confounders as requested.
4. There were only 25 cases in total which had Dengue Shock Syndrome (DSS). I wonder how many cases had DSS before being hospitalized and how many cases actually developed DSS later. Then the fact is the magnitude is possibly small, we have to avoid “over-interpret statistical significance”. Therefore, it would be better to put some limitations in the discussion sentence at line 328 to 330, page 14.

Answer: We are sorry for not being clear. None of the patients had shock before admission or during admission. Our purpose was to determine if the NS1 antigen levels on admission was a predictor of the development of shock later in the illness. All patients developed shock while in hospital.

5. The discussion section: (1) Please add some more sentences discussing the results of NS1 from previous studies if any, and make a comparison (2) A limitation paragraph should be included. E.g.: Study design (no control group, not-blinded study, and different criteria for severe and non-severe dengue...).

Answer: Thank you for this comment. We have re-revised this manuscript as suggested by the reviewer.

Minor comment:

We have addressed these minor comments in the manuscript.

Reviewer 2:

1. The overall proportions of NS1 antigen positivity in severe dengue and non severe dengue groups were not much different (55.3% vs 50%). I’m still not clear why the authors didn’t include day 3 and 4 of illness for the analysis of dengue prediction (there was quite many patients in day 4 of illness - 43 patients). I think if we do this, the association between NS1 antigen positivity and dengue severity will be lower (the proportions of NS1 antigen positivity in severe dengue and non severe dengue groups in day 4 were quite similar, Figure 1).

Answer: We wish to thank the reviewer for raising this very important point. On admission the NS1 positivity was not significantly different between those with severe dengue and non severe dengue. We have highlighted this in the re-revised version. What we wanted to point out is that,
NS1 positivity at a later stage of illness, i.e. after day 5, was associated with a higher risk of severe dengue. For instance on day 1, approximately >90% of patients would be NS1 antigen positive and chance of positivity falls drastically from day 1 to 4 due to the decline in viraemia. Only those who have prolonged viraemia would have prolonged NS1 antigen positivity. Therefore, this was the basis of choosing day 5-6 for using NS1 positivity as a predictor of severe disease. We have highlighted in our manuscript that NS1 positivity is only a risk factor if it is still positive after day 5. For instance between day 5-6, those who developed severe dengue were twice as more likely to be NS1 antigen positive than those with non severe dengue.

2. The authors compared the sensitivity and specificity of NS1 ELISA and NS1 rapid tests in terms of dengue diagnosis not in terms of prediction of severe dengue. I think it is careful when we conclude that NS1 rapid test can be used to predict severe dengue (The conclusion of the abstract and the last sentence of the manuscript).

Answer: Thank you for this comment. We found that the NS1 rapid antigen test was of comparable sensitivity and specificity as the NS1 ELISA. Since the rapid test is of comparable sensitivity and specificity we have mentioned that this can be used as a warning sign of severe dengue. However, as NS1 positivity only appears to be a warning sign beyond day 5 of illness, we have highlighted this in the revised manuscript.

Minor comment:
We have addressed these minor comments in the manuscript.

Yours sincerely

Dr. Neelika Malavige