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
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Prof. Philippa Harris,
Executive Editor,
BMC Infectious Diseases.

Dear Prof. Harris,

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

- **Reviewer 1**
  
  **Major comments**
  
  **Question 1. There are some limit of the study design:**
  
  **a. Was it a case control study?**
  
  Answer: No
  
  **b. Year of sampling?**
  
  Answer: 2013. We have made these amendments in the revised manuscript.
  
  **c. The name of the hospital, City and the number of beds?**
  
  Answer: Colombo South Teaching Hospital with bed strength of over 1000 beds. Added to manuscript.
  
  **d. Details of inclusion and exclusion criteria?**
  
  Answer: We have included this in the revised manuscript
  
  **e. Descriptive statistics: Characteristic of patients?**
  
  Answer: We have added more descriptive details as requested.
  
  **f. What is “bleeding manifestations”?**
  
  Answer: we apologies for not explaining this. We have done so in the revised manuscript.
Question 2. In the method section, why did the authors choose those parameters as criteria for severity? According to WHO criteria or what else?

Answer: We wish to thank the reviewer for this very important question. Based on the 2011 WHO criteria, and previous criteria there should be evidence of fluid leakage such as the presence of pleural effusions or ascites or a rise in the haematocrit of 20% from baseline. However, we felt that if a patient had a platelet count of <25,000/mm3 or if their liver enzymes are >500 IU (>12 times the upper limit of normal) they do need extensive monitoring as there is a very high probability that they will develop shock or severe organ impairment. However, the reduction of platelet counts to <25,000/mm3 has not been listed as a feature of severe disease. In the WHO 2009 guidelines severe organ impairment is defined as liver transaminases rising above 1000 IU. However, we considered that liver transaminases above 500 IU could be an indicator of severe liver involvement although it would not indicate severe liver impairment.

3. The manuscript did not mention the blindness of the method. Who did analyze the NS1 ELISA and measured bedside test? The outcome might be affected because doctors/researchers might be aware of NS1 results. So it is important to make it clear and reconsider one more limitation if the study design was not blind.

Answer: Thank you again for this very valid comment. The NS1 rapid antigen test was not done at bed side but on the blood samples that were brought to the Centre for Dengue Research. However, the results of the NS1 antigen (rapid) tests were made available to the hospital as it was required for patient management. i.e. the medical staff had some difficulty to differentiate leptospirosis and dengue in some patients. Therefore, it was not ethical to blind the ward staff of the results of the NS1 antigen tests as there was a co-existing leptospirosis outbreak at the same time and the NS1 results were required in the management. At the time the NS1 results were made available to the ward staff, none of the patients had developed shock. The NS1 ELISA was only carried out at a later point. We have made amendments to the manuscript.

4. The statistical usage of results, it is not a normal distribution for all data (as mentioned in line 180-181, page 8), then non-parameter method should be used, not “difference in means”. Therefore, it is better to use median (and range or IQR) instead of mean (and SD).

Answer: We totally agree with the reviewer and we have used median and ranges instead.
5. The authors should consider revising the table 1, because:
   a. It is better to use footnotes to explain statistically significant differences and statistical test.
   b. It is better to use the unique decimal number in the result columns (Eg. 170.4 ± 196.9 or 170 ± 197).
   c. Word should not be exist in the result columns but in the headings (eg. Mean ± SD).
   d. Publishers prefer to use three horizontal lines: One above the column headings, one below the column headings, and one below the data. The grid line between columns should not be included.
   e. It is better to clarify the experimental details or abbreviations at footnote.
   f. In case of the Non-normal distributed data, it is better to use Median ± range

Answer: We wish to thank the reviewer for the above comments and we have made amendments of the revised manuscript as suggested by the reviewer.

6. Figure 1: N total = ? for each day? It is better to use “*” for significant difference and the day nomenclature should be “Day -3, -2, -1, 0 (defervescence day), +1, +2 (for prediction).

Answer: Thank you for this comment. We apologize for not mentioning the total for each day which we have done in the results section (line 231). It would not be practical to include nomenclature as day -3, -2, -1 and 0, +1 etc… because some of the patients were only hospitalized for 2 days and the majority presented to hospital on day 4 or 5 of illness. For instance 94/186 presented to hospital on day 5 of illness.

7. There was no figure for ROC curves.

Answer: We have included this in the revised manuscript.

8. When did the researcher perform platelet count? Why did the author use the lowest lymphocyte count instead of the value at admission day (line 253, page 11)? The lowest lymphocyte count would be useless for prediction.

Answer: We performed serial platelet counts, haematocrits and white cell counts throughout the course of the illness (line 157). The lymphocyte count and neutrophil that is reported in this study was not used for prediction of severity. We have reported the lowest lymphocyte count and the lowest neutrophil count rather than the counts on admission as we used them as an indicator of severe disease. Our earlier studies using flowcytometry have shown that T cell counts (CD3+ T
cells) were significantly lower in those who develop shock (Malavige et al, PLOS one 2012). Others have also shown that there is massive T cell apoptosis in severe dengue (Mongkolsapaya et al, J Immunology, 2006). In addition, secondary bacterial infections or co-infections are frequently reported in patients with DHF which is thought to be related to the neutropenia. Therefore, as the purpose of this study was not to evaluate the value of lymphocyte counts or neutrophil counts to predict severity we have not included the values on admission.

9. Line 226-230, page 10, the sentence should be reconsider.
Answer: Thank you for this comment. We have rephrased this sentence.

10. The purpose of this study is to prove that whether bedside quick test is reliable and good agreement with standard ELISA test. Therefore, the authors should show the correlation between two methods before stating the role of NS1 in the prediction of severity.
Answer: thank you for this very relevant comment. We have included this in the revised manuscript.

11. Bedside tests are mainly used for early detection, the author did not mention the time at the day NS1 test were performed (day 0).
Answer: We are extremely sorry for this omission. We have included this in the revised manuscript.

Minor comments:
Answer: we wish to thank the reviewer for all these comments and we have made amendments to all his/her suggestions in the revised manuscript.

Reviewer 2
1. NS1 levels were measured on admissions for all patients recruited, corresponding with the days of illness between day 3 and 8. However, the analysis for the association between NS1 levels and disease severity only included patients admitted on day 5 and 6 of illness. I wonder how well the associations were in other days, especially in day 3 to 4 of illness. I think days 5 and 6 are late for predicting disease severity since complications of dengue usually occur on these days. In addition, it is better if authors could give the number of patients in each day of illness in Figure 1.
Answer: We wish to thank the reviewer for this question. As we did this study in adult patients, the majority of adult patients present to hospital in Sri Lanka between day 5-6 of infection. In this study 111/186 (59.7%) of those presented during these days. Therefore, we analysed the data with special relevance to these days. In fact 23/186 (12.4%) of patients presented after day 6 of illness and therefore, if we had included them in the analysis as well, the sensitivity of the NS1 test become less significant. We have added the numbers of patients to the figure legend.

2. Definition for disease severity: authors didn’t use the WHO dengue disease severity classification and defined their own classification. I think the classification is not thorough and clear enough. For example, other organ dysfunction rather than liver dysfunction such as encephalopathy, myocarditis was not included in the severe dengue. Authors should also give more details about what was the baseline of haematocrit for patients. In addition, criteria such as presence of bleeding manifestations or liver enzyme > 500 IU/mL are not really severe and the group “severe dengue group” should be defined as “more severe dengue group”.

Answer: Thank you for highlighting this point. We apologise for lack of clarity. We have made the suggested amendments in the revised version of the manuscript. None of our patients had myocarditis or encephalitis and therefore, we did not list them. But we have done so in the revised manuscript. We agree with the reviewer that the mere presence of liver transaminases >500 IU is not really severe, but we have only included one patient with liver enzymes of 764 (AST) who did not have clinically detectable fluid leakage or a rise of the HCT of >20%. All other patients with liver enzymes between 500-1000 IU also had some other criteria we have defined.

3. At the beginning of the second and fourth paragraphs of the results section, authors wrote “Dengue NS1 and clinical parameters” or “Dengue NS1 positivity and laboratory parameters”. It was not clear whether this is NS1 ELISA or NS rapid tests. About NS1 antigen rapid test, authors didn’t show whether this test was done at the same time (on admission) with NS1 ELISA test in the methods section. In the results section, authors showed “NS1 rapid antigen detection test had a comparable sensitivity and specificity as the Panbio commercial capture NS1 antigen detection ELISA”. It was not shown in details and it seems to me that authors showed comparison of the sensitivity and specificity of these two
tests in terms of dengue diagnosis (not in terms of comparison of predicting severe dengue), but authors concluded that NS1 rapid test could be used to predict more severe disease group at the end of the manuscript.

Answer: we apologize for these omissions. We have included all these suggestions in the revised manuscript.

4. Since data were not normally distributed, they should be presented as median (range) rather than mean and SD.

Answer: we totally agree with the reviewer and have done changes accordingly.

Yours sincerely

Dr. Neelika Malavige