Reviewer's report

Title: Serum IL-10 as a marker of severe dengue infection

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Reviewer: Yadunanda Kumar

Reviewer's report:

Summary:
This manuscript describes a cytokine analysis of sera obtained from a semi longitudinal cohort of dengue patients comprising of severe and non-severe forms of dengue. The authors focus on IL-10, a cytokine cited frequently in literature to be a significant cytokine in distinguishing severe from milder forms of dengue. In addition to IL-10 the authors measured IFN-y, IFN-a and MIF, cytokines previously reported to as potential biomarkers of severe dengue, in a select subset of patients along with laboratory parameters commonly evaluated in the clinic. The authors find that although consistent with literature IL-10 levels were higher in their severe patient subset, ROC analysis indicated poor performance in discriminating severe patients. The authors conclude that IL-10 is unsuitable for use as a predictive biomarker for severe dengue.

General review:
The authors have chosen what appears to be an impressively large clinical cohort of dengue patients to address the question- are cytokines IL-10, IFN-g and MIF, useful biomarkers for prediction of severe dengue? This is an important research focus and in view of previous references to the potential value of these cytokines in literature, a follow up study in a large cohort is an extremely valuable. While the information presented in the manuscript is definitely of significant interest to the dengue research community, the manuscript needs substantial revision in the text and in data presentation for it be of interest and value to the reader. The study appears overall disorganized with different sets of measurements performed on different number of patient samples making it difficult to systematically compare data. The manuscript can be substantially improved via better organization and presentation of the data. More specific comments are provided below.

Major Compulsory Revisions
1. Abstract: The authors need to be cautious in the use of terms ‘severe dengue’ and ‘acute dengue’ and clarify what they mean by acute here. In general I suggest using the terms defined in the WHO 2009 guidelines (used by the authors) namely- DF and DHF or DSS
2. Abstract: Providing p values in the results section does not have any benefit and only serves to clutter the paragraph. I recommend removing this and sticking to a summary description of results.
3. Methods: Paragraph-1: The authors should clarify how they have calculating day of infection (e.g. ay-4 day-5 etc). Is this days since onset of fever (fever days) or is it days since admittance to hospital. This is a very important distinction. Also the authors should provide a clearer definition of severe dengue- The 2009 guidelines call for labeling dengue as DF and DHF or DSS. Why are the authors using the label ‘severe dengue’ which is very confusing to the reader. Also the criteria for classifying their patients as severe dengue (DHF I am guessing) are inadequately described. Overall, a separate table detailing the features of the cohort, exact numbers, age group, primary vs secondary; severe vs non severe will provide clarity.

4. Methods: Paragraph-3: What precise method was used to measure cytokine levels. Is this an ELISA kit? if so what was the serum amounts used and the dilution for the ELISA.

5. Methods: There is no description of how transaminase levels/activity was measured.

6. Results: Para-1: A lot of information here belongs to the figures and tables (and appears to be repeated here). The results indicate platelet counts and white cell counts etc. but in the actual table (table-1) the phrase ‘lowest platelet count’ etc is seen. What is this measurement? (lowest compared to what?). I would be more useful to present this data in the two different time points as potentially bar charts.

7. Results: Para-2: Overall measurements in both febrile and critical phase samples were only done in 65 patients. Why was this and how were these patients chosen? Only 7 of them had severe dengue. Why not chose more from the 40 severe patients to have a balanced set for their analysis. As it is it is impossible to conclude with any degree of confidence that these differences are statistically meaningful as far as severe and non-severe are concerned.

8. Results: Para2: The authors state “Serum IL-10 levels rose in 17/65 of these patients, while serum IL-10 levels fell in the other 8 patients (Fig 1B)”. Do they mean other ‘48’ patients?

9. Results: Para-3: The authors mention they measured interferon levels in only 78 patients. How many of these were severe dengue and why were these subset of patients chosen? It is unclear why the authors chose to examine interferons and why this is not a part of their overall conclusions. Did the levels of interferons differ between severe and non severe dengue patients? Did MIF

10. Results Para4: Although MIF was measured in 65 paired samples it is not mentioned whether the levels reported are from the febrile or critical samples.

11. Results Para5: The authors have mentioned that 50 patients in their cohort had primary infections. In reporting differences in cytokine production between primary and secondary did the authors combine all primary in one grp and secondary in another irrespective if disease severity? Did any of the primary infections result in severe disease?

12. Results Para6: Have the authors tried to combine multiple cytokines and test if this can improve classification of severe patients? A multiple regression
approach can provide greater insight into this.

13. Discussion: Para 3: In the paired samples, number of severe patients is too small to make a significant conclusion.

14. Discussion Para-6: The authors make an interesting suggestion that IL-10 levels maybe a better indicator of liver inflammation. However the correlation coefficients appear to be too low to inspire this confidence. The authors should be careful how they interpret the correlation values.

- Minor Essential Revisions

1. The authors should ensure their figures and figure legends are compatible. As it is the numbering of the legends and figures are do not coincide.

2. Figure legends should provide a concise but complete description of the figures and not just provide a title.

3. There is too much data provided within the text in the form of cytokine levels, p values, etc. The results section should provide descriptions and use figures to display all data. In the current format the reader will find it impossible to follow the authors’ rationale.

4. Result sections does not refer to figures adequately in the text. There is no mention of figures-3-7 in the text at all.

5. Figures or legends should indicate ‘n’ (number of samples evaluated) for each plot, correlation.

- Discretionary Revisions

None

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

YES: I have filed a provisional patent for biomarkers for prediction of DHF which include at least one of the molecules described in this manuscript. No financial gains have resulted from it.

No to all other questions