Author's response to reviews

Title: The correlation between the presence of viremia and clinical severity in patients with enterovirus 71 infection: a multi-center cohort study

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Version: 4 Date: 2 July 2014

Author's response to reviews:

MS: 1513704232118274
Title: The correlation between the presence of viremia and clinical severity in patients with enterovirus 71 infection: a multi-center cohort study

Dear Editor:

Thank you very much for the Editor's and the reviewer's comments, which help us improve the quality of our manuscript.

We have responded to each of the reviewers' comments in a point-by-point fashion in the following section and have revised the manuscript accordingly. We also underline what we have changed and now resubmit the second revised manuscript. If you have further questions or concerns, please feel free to contact us at your convenience.

Sincerely Yours,
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Editor's comment:
- No. 5 even though the results were not significant p=0.41 when comparing mild
grade 1,2, to severe grad 3-4, this should be stated in the results.

Reply:
We added it in the revised manuscript (Lines 203-205, Page 10).

- Manuscript page 4 line 70, the "professionals" should be specified- was this a task force or ministry of health decision.

Reply:
We added it in the revised manuscript (Lines 69-70, Page 4).

Some grammar mistakes:
- Line 73- impact with no s
- Line 76- researched have not identified the mechanism causing ....
- Line 78. This problem impedes the progress...
- Line 145 logistic regression analysis
- Line 151 median age was 2.71 ( cancel of)
- Line 154 mostly mild CNS ( cancel had)
- Line 158 There were only three patients who had ( cancel having) ... and two deaths ( cancel cases with mortality)
- Line 162 collected during the acute stage ( cancel at)
- Line 167 Two -thirds of viremic cases ( cancel total)
- Line 176 Viremia did not have a significant
- Line 181 The risk factor for ( cancel of)
- Line 183 cancel neither
- Line 184 median age 2.60 vs 3.08 (as complicated patients were younger)
- Line 211 one-fourth
- Line 215 in animal models, the viral kinetics
- Line 216 also revealed a similar pattern compared to our
- Line 24 On the other hand
- Line 246 a previous pathogenesis study performed in rhesus monkeys
- Line 247 viral loads in the CNS were
- Line 248 parallel to the viremia. AS a ..... may increase the viral load in the CNS
- Line 268 of one year and viremia persisted beyond 4 days after
- Line 270 but further studies targeted at the interaction between the
- Line 272 will be required

Reply:
All the revision were done and underlined in the manuscript according to the above suggestions.
Title: The correlation between the presence of viremia and clinical severity in patients with enterovirus 71 infection: a multi-center cohort study
Version:3 Date:11 June 2014
Version:3 Date:11 June 2014
Reviewer: H Rogier van Doorn
Reviewer’s report:

The authors have made some effort to address the reviewer's comments, but I'm afraid that this is not sufficient. The new figure 1 is very informative, and taken together if more analyses are done and results and discussion are rewritten accordingly, these data are new and interesting. However, many questions are still unanswered and several issues raised by the reviewers persist.

Specifically (all essential revisions, only referring to my own initial comments)

- day of illness on sampling was not taken into account in the multivariate analysis, while this was my main concern. The authors suggest that patients who have viremia after day four are more likely to have severe disease, but they fail to mention whether patients who present at day four in general are more likely to have severe disease. For a reader to make sense of their data, it is crucial for the authors to present data on severity of illness per day of illness at sampling time. Without this we cannot give any value to potential associations the authors describe.

Reply:

We added the day of illness on sampling in the multivariate analysis and the result showed whether the blood was sampled earlier or later did not have influence on the clinical severity (Lines 190-196, Page 10, New Table 5).

- Similar for children under one having a higher risk of viremia... did they present to hospital earlier and were they thus sampled earlier?

Reply:

To answer this question, we did a multivariate analysis and found that age under one was a significant risk factor of viremia after adjusting the other factors including the day of illness on sampling (Lines 185-186, Page 10, new Table 3).

- There are still a lot of language and grammatical errors and the manuscript may benefit from additional review for this particular purpose.

- The authors should also carefully review their wording: the authors still discuss the viremia as if they have kinetic data (multiple timepoints per patients), this should be avoided. E.g. they should not that viremia rapidly decrease after day four, but that viremia was lower in patients sampled after day four etc. The authors have also not addressed my comments re the phrasing of their results, e.g. in the abstract section the sentence "Two-thirds 48 (68%) of viremia occurred within the first three days of infection" is still there. This sentence should
read: e.g. xx% of patients samples before day 3 of illness, vs yy% of patients sampled after day 3.

Reply:
We rephrased the wording use in the abstract (Lines 48, 51-52 Page 3) and result section (Line 173-174, Page 9). We also tried our best to reduce language and grammatical errors.

- The revision of the background section was minimal. As said, the background section should be to the point and be less vague, describe well what we know, and what assessment of viremia may add.

Reply:
We revised the section (Lines 79-87, Pages 4-5) and tried to be more precise.

- In the results section, classify patients by grade first and then discuss their symptoms. x patients were grade 1, y patients were grade 2 etc...

Reply:
We revised the text in the manuscript (Lines 159-166, Page9). Also Table 1 shows the detailed number and percentage of different grades.

Title: The correlation between the presence of viremia and clinical severity in patients with enterovirus 71 infection: a multi-center cohort study

Reviewer: Kulkanya Chokephaibulkit

Reviewer's report:

Major compulsory revision:
1. The authors has made to responses to the comments well. However, the response to the critical comments on the conclusion that prolonged viremia was associated with severe complications is not satisfied. The day of the blood drawn depended on the day that the patients hospitalized. It could be that all the milder case were admitted earlier by any reasons, then got the blood drawn earlier, while the sicker patients did not recovered by day 3 of illness showed up and hospitalized later, and therefore got the blood drawn later. But because the second blood drawn was not performed therefore the milder cases who may also have prolonged viremia will never be discovered. Although it is suggestive from Fig 1 that the chance of detecting the virus was less in later days, one may assume the milder case will not have a viremia in the subsequent blood drawn if it was done. But this has to be proven. It is likely because the sicker patients tended to be hospitalized after day 3 that led to the higher proportion of more severe cases in viremic population after day 3. The milder cases probably already recovered by day 3 while the grade 3-4 CNS symptoms usually presented after day 3. Therefore what found in Fig 3 may be just reflecting the proportion of sicker patients hospitalized before or after day 3. This figure may be
misleading from selection bias (sicker hospitalized later), not the that the sicker had higher chance of viremia in the later days, and should not include in the manuscript. If Fig 3 is to be presented, it should present the proportion of viremia in patient with grade 1-2 vs grade 3-4 on before and after day 3 of illness.

Reply:
We replaced the figure with new Figure 3 according to the suggestion and revised the result and discussion section according to it. The New Figure 3 shows that the percentage of viremic cases did not decrease beyond 3 days of illness compared to those who had milder EV71 diseases (grade 1 to 2) (Lines 197-199, Page 10).

- In order to clarify this, I suggest that the proportion and number of patients with severe complications (grade 3-4) should be added at the bottom of Fig 1. If the proportion of viremia in patients with grade 3-4 when they get blood drawn after day 3 of illness was higher than the proportion of viremia in the patients with grade 1-2 on the similar days, then prolonged virmemia in grade 3-4 can be concluded.

Reply:
This is really a very helpful suggestion. However, the cases of severe complication was too few to make the analysis as we showed below. As a result, we did not put it into the manuscript in case of distraction.

Day 1 2 3 4 5 6 7
Viremic cases with severe complications (grade 3-4) 0 2 0 3 2 0 0
Total grade 3-4 cases 0 2 5 11 8 6 3
Percentage of viremia among grade 3-4 cases 0 100% 0.00 27% 25% 0.00 0.00

- The conclusion at this point can only say that the proportion of grade 3-4 patients was higher in viremic patients who presented after day 3. It cannot generalize that the grade 3-4 patients had a longer period of viremia.

Reply:
We revised the abstract (Lines 51-52, 54-55, Page 3), result (Line 197-199, Page 10) and discussion section according to the suggestion.

- 2. The Method on page 6, as well as in Abstract are not quite similar. Please clarify this that the blood drawn for virologic test was performed on the first day of admission. If not, how delayed it is from the point of admission.

Reply:
We revised it in the manuscript (Line 42, Page 3, Line 177, Page6).

- 3. In Table 3 and 4, the authors added Fever in the variables. Please clarify the fever, eg. the peak temperature after hospitalization, or any fever T.37.8 degree celcius?
Reply:
We added the description in the tables.

- 4. In the Results in the Abstract stated "2/3 of viremia occurred within the first 3 days of infection." This should be revised to "2/3 of viremic cases were detected in the first 3 days of illness.

Reply:
We've revised it in the manuscript (Line 48, Page 3).