Author’s response to reviews

Title: Clinical characteristics and managements of severe hand, foot and mouth disease caused by Enterovirus 71 and Coxsackievirus A16 in Shanghai, China

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Author’s response to reviews:

Dear Prof. Yoke-Fun Chan,

Many thanks for the letter dated November 21, 2018, regarding our manuscript entitled “Clinical characteristics and managements of severe hand, foot and mouth disease caused by Enterovirus 71 and Coxsackievirus A16 in Shanghai, China” (Manuscript ID INFD-D-18-01940). We appreciate the valuable comments of the editor and reviewers. Based on the editor and reviewers’ comments and suggestions, we have revised the manuscript. Our specific point-by-point responses to the editor and reviewers’ comments and suggestions are as follows:

Editor Comments:

I have read the paper. The study is interesting but suffers from some major flaws which must be addressed. Here are my major comments:
1. Table 2, 3 and 4. Without non-severe HFMD as control, it is difficult to conclude that the cytokines are elevated because of severe HFMD or mild HFMD. Please include cytokine profiles of non-severe HFMD.
Response: We thank for the valuable comments from the editor, and we have included cytokine profiles of non-severe HFMD cases in the revised manuscript (see page 5, line 14-16; Table 2, 3 and 4).

2. Can authors also describe the selection process of antiviral treatment for HFMD patients, who will be treated with ribavirin or IVIG? Is this double-blind, random, placebo-controlled etc? Is this a trial, and has registration? Also should include STROBE statements.
Response: Our current study was not a double-blind, random, placebo-controlled study, the selection process of antiviral treatment for HFMD patients was according to the guidelines for diagnosis and treatment of HFMD in China (World J Pediatr. 2018 Oct;14(5):437-447). The hospitalized HFMD who treated with ribavirin or IVIG were depended on the clinical symptoms: HFMD children with fever, rashes on hands, feet, mouth and buttocks (macular papules, papules, small herpes), and accompanied with cough, runny nose, loss of appetite were treated with ribavirin; children with prolonged hyperthermia (>39°C), obvious neurologic symptoms, such as irritability, drowsiness, startle reaction, or myoclonic twitching were treated with IVIG (see page 5, line 20-23; page 6, line 1-5).

3. The Ribavirin aerosol treatment was also poorly discussed in the paper. Pls give appropriate citations and discussions. The authors have done some studies?
Response: We have added information of ribavirin treatment of HFMD in the discussion part, and cited several appropriate references (see page 11, line 10-16). We have not done study focusing on the ribavirin treatment of HFMD.

4. Table 5. Only small differences were observed. Really significant?
Response: Yes, it is significant.

5. Pg, line 47. The only inclusion criteria for severe HFMD is aseptic meningitis or admission to hospital? Pls outline the criteria clearly.
Response: The inclusion criteria for severe HFMD is admission to the hospital, including HFMD with aseptic meningitis (see page 4, line 20-23).

Minor comments:
Pg3, line 53. EV-G18 is only in pigs (http://www.picornaviridae.com/enterovirus/ev-g/ev-g.htm). It really caused HFMD?
Response: We appreciated for this important concern, and we have deleted EV-G18 in the revised manuscript (see page 4, line 15-18).

Koh Mia Tuang (Reviewer 1):

1. Overall this study is of major interest and clinical importance and merits to be published.
2. However, there are far too much corrections needed from the point of the English grammar and phrasing of the sentences to reflect their true meaning. I have attempted to make suggestions for this but there were others which need fairly extensive correction.
Response: We thank for the valuable comments of reviewer 1, and have corrected the grammar mistakes accordingly. Furthermore, the writing of revised manuscript has been edited by Prof. Wenzhe Ho (Department of Pathology and Laboratory Medicine, Temple University Lewis Katz School of Medicine).
However, neurologic and systemic complications, such as encephalomyelitis, aseptic meningitis, acute flaccid paralysis, and even brainstem encephalitis, can develop rapidly in a small minority of cases. Response: We have re-phrased the sentence as suggested (see page 3, line 10-12).

In past decades HFMD outbreaks worldwide causing epidemics were reportedly due to EV71 and CV-A16. Response: We have re-phrased the sentence as suggested (see page 3, line 19-20).

Severe and fatal cases of HFMD caused by CV-A16 were also reported [18]. Response: We have re-phrased the sentence as suggested (see page 4, line 7-8).

Inclusion criteria were children aged 1 month to 14 years, with severe EV71 or CV-A16 HFMD which required hospital admission under the Pediatric Department of Infectious Diseases at Xinhua Hospital. Response: We have re-phrased the sentence as suggested (see page 4, line 15-18).

Children with significant underlying disease, and children with mild EV71 or CVA16, HFMD who do not require admission were excluded from the study. Response: We have re-phrased the sentence as suggested (see page 5, line 2-5).

Possible HFMD and/or herpangina and/or aseptic meningitis would be better to replace "and/or aseptic meningitis" with "with or without aseptic meningitis" Response: We have re-phrased the sentence as suggested (see page 7, line 5-6).

For definition of aseptic meningitis these two lines could be merged and better defined in terms of both clinical and CSF laboratory criteria instead of separating them into different lines for easier reading. Response: We have re-phrased the sentence as suggested (see page 5, line 5-6).

It should be "Indications for antiviral treatment" 0.5 mg/time should be 0.5 mg per dose; IVIG at 1 g/kg 2 times per day should be IVIG 1 g/kg given twice daily. Response: We have re-phrased the sentence as suggested (see page 5, line 21-23; page 6, line 1-3).

It is not clear in the section on "Antiviral treatments" whether all patients were given ribavirin and those with severe disease were given IVIG in addition to the ribavirin. Response: All patients were given ribavirin. However, only partial HFMD children with neurologic complications were given IVIG in addition to the ribavirin because of the high price and safety concern of IVIG (see page 6, line 3-5).

Serum cytokine levels were measured using IMMULITE-1000, an automated immunoassay analyzer. Reference was cited in the revised manuscript (see page 6, line 7-11).

"length of stay" instead of "hospitalization duration" Response: We have re-phrased the sentence as suggested (see page 6, line 20).

Do the authors mean "treatment outcome" when they refer to "outcomes to
identify"?  
Response: We have re-phrased the sentence as suggested (see page 6, line 21).

12) Pg 6 Line 36: ..... and H test was used to compare the difference between multiple groups  
Response: We have re-phrased the sentence as suggested (see page 6, line 22-23).

13) Pg 7 Line 7: None of the children required pediatric intensive care unit (PICU) care  
Do the authors have a different criteria for severe HFMD cases and those who required intensive care?  
If so, what are the indications for intensive care?  

14) Pg 4 Lines 47 and 52: Can authors please clarify what constitutes severe and mild HFMD; i.e their definitions.  
What constitutes "significant underlying disease"?  
Response: Mild HFMD was defined as oral ulcers, maculopapular or vesicular rash on the hands, feet and buttock, accompanied with or without fever. Patients were classified as severe if they had any neurological complications (aseptic meningitis, encephalitis, encephalomyelitis, acute flaccid paralysis, or autonomic nervous system dysregulation), or cardiopulmonary complications (pulmonary edema, pulmonary hemorrhage, or cardiorespiratory failure), or both. In this study, significant underlying disease included 5 children with congenital heart disease, 20 with iron deficiency anemia, and 2 with cerebral palsy (see page 5, line 2-5).

15) Pg 4 Line 52: Please clarify if subjects were enrolled into study only if, in the opinion of the investigators, admissions were based on "severe HFMD" due solely to EV71 and CA-16 (subsequently confirmed by RT-PCR). Also please confirmed that if in the opinion of the investigators, if a subject has severe clinical disease but has a "significant underlying disease" deemed to contribute to the severity of disease, the subject is excluded from study.  
Response: In the current study, we only included admitted HFMD cases due to EV71 and CA-16, and subjects with underlying disease were excluded from the study (see page 4, line 15-23; page 5, line 1-5).

16) Pg 7 Line 36: "Laboratory tests showed that white blood cell (WBC), C reactive protein (CRP), creatinekinase MB (CKMB) were evaluated in most of those hospitalized subjects. Blood sugar (BS) and alanine aminotransferase (ALT) were evaluated in some of subjects"  

17) Please clarify if the authors mean "elevated" rather than "evaluated"  
Response: We mean "elevated" rather than "evaluated" (see page 7, line 23; page 6, line 1).

18) Pg 8 Line 24: Please explain what "critical illness" the investigators were trying to prevent with IVIG and RBV  
Response: We try to prevent serious neurological and cardiopulmonary complications, including aseptic meningitis, encephalitis, encephalomyelitis, acute flaccid paralysis, or autonomic nervous system dysregulation, pulmonary edema, pulmonary hemorrhage, and cardiorespiratory failure (see page 8, line 17-19).
19) Table 5: The authors are comparing the "clinical and laboratory outcomes" among subjects with severe HFMD with and without treatment with IVIG. The original title of Table 5 does not reflect this objective. 
Response: We have changed the title of Table 5 (see Table 5).

20) Pg 10 Line 41: In addition, the subjects with more severe symptoms which were treated with IVIG showed no statistical differences of cytokine levels with subjects without IVIG ……
Re-phrase suggested: Among subjects with severe HFMD there was no statistical differences in their cytokine levels irrespective of whether they were given IVIG, suggesting that cytokine levels may not be an indicator for severe HFMD due to EV71 and CA-A16.
Response: We have re-phrased the sentence as suggested (see page 11, line 4-6).

21) Pg 11 Line 58: representational with respect to severity factors. 
Do authors mean "several" instead of "severity" and "representative" instead of "representational"? 
Response: We have re-phrased the sentence as suggested (see page 12, line 15-16).

22) Pg 12 Line 4: Authors should admit there is selection bias of cases confirmed to be caused by EV71 and CA-A16 only.
Response: We have added the information in the revised manuscript (see page 12, line 16-17).

Veasna DUONG, PhD (Reviewer 2):

Major comments
1. How healthy children were enrolled? According to page 5, the healthy children were enrolled from children who came to Xinhua Hospital for health checkup. Is this a regular normal health checkup?
Response: We thank for the valuable comments of reviewer 2. Healthy children were enrolled from children who came to Department of Children Healthcare of Xinhua Hospital for health checkup. Normally, blood samples of healthy children were collected for blood routine examination. Written informed consent was obtained from parents or legal guardians of healthy children eligible for the study.

2. The control group was not well described although it was stated that it was matched by gender and age but there was not data on the control group.
Response: Based on the gender ratio (male: 134/234, 58%) HFMD cases, 55 (55%) healthy boys and 45 (45%) healthy girls were selected as control group. In order to match the age, 10 (10%) healthy children aged less than 1 year, 50 (50%) healthy children aged from 1-3 years, 30 (30%) healthy children aged from 3-5 years, and 10 (10%) healthy children aged from 5-14 years were enrolled.

3. The healthy control group might not be appropriate for comparison with severe HFMD cases. The author should compare the severe vs. non severe HFMD cases. Hence, we can't draw any useful conclusion from the result particularly the comparison of cytokines levels among the 3 groups.
Response: We compared the cytokine levels of severe vs. non severe HFMD cases in the revised manuscript (see page 5, line 14-16; Table 2, 3 and 4).

4. Although the author stated in the limitation of the study, the randomization of the treatment is crucial to draw any substantial conclusion of the efficacy of the treatment by IVIG. Additionally, the efficacy of Ribavirin was not presented.
Response: We fully agree this important concern. The study design of our study limited the result of the efficacy of IVIG for severe HFMD. It is very important design a prospective, multicenter, randomized,
double-blind and placebo-controlled trial to investigate the efficacy, safety of IVIG for severe HFMD treatment. Several previous studies indicated that ribavirin was effective for HFMD (see page 11, line 10-16). In this study, all the HFMD cases were treated with ribavirin, partial HFMD children with neurologic complications were given IVIG in addition to the ribavirin. We only compared the efficacy of ribavirin with or without IVIG in HFMD children with prolonged hyperthermia and/or neurologic complications.

We hope that our revised manuscript is now suitable for publication in BMC Infectious Diseases.

Sincerely Yours,

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