Author's response to reviews

Title: Cytokine responses and correlations thereof with clinical profiles in children with enterovirus 71 infections

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Author's response to reviews: see over
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Dear Editor,

Thank you for your letter concerning our manuscript "Cytokine responses and correlations thereof with clinical profiles in children with enterovirus 71 infections" in consideration for publication in your journal. We have addressed the comments raised by the reviewer, and the amendments are highlighted in red in the revised manuscript. Our point-to-point response to the reviewers’ comments is attached below.

I wish that the revised manuscript is acceptable for publication and look forward to hearing from you soon.

I would like to express my sincere thanks to you and the reviewers for the constructive and positive comments.

With best wishes,

Yours sincerely,

Zhao-jun Duan, PhD.,

Professor and Director, Department of Viral Diarrhea
REVIEWER 1:

1. Although it seems to be an interesting report involving multiple parameters, the work has many missing links. Rationale for undertaking the work is not clear and thus need to be strengthened in introduction. For example, why cytokines including “IL-1#, IL-6, TNF-# et al.” were chosen for this study.

Thanks for the reviewer. This study aims to explore the pathogenesis of EV71 infection in the severe cases by comparing the levels of cytokines and chemokines in the plasma and cerebrospinal fluid (CSF) specimens of the EV71-infected individuals in the acute and/or recovery phases with normal controls. This clarification was strengthened in the background section in the revised manuscript (P.3 line.57-61).

2. The English language should be improved.

The revised manuscript has been linguistically polished by the native English speaker.

3. Selection of patients is one of the essential components for any clinical study. Explain the inclusion and exclusion criteria clearly in the methodology section. How many cases were confirmed by detection of EV71 nucleic acid and/or EV71-specific IgM-positivity? What’s the indication of IVIG and/or glucocorticoid administration? Were all the severe or critical cases in present study given both IVIG and glucocorticoid? How many severe and critical cases provided both plasma and CSF specimens? Please described in detail.
In fact, all these data have been included in the methods section of the manuscript. And we have highlighted these in red in our revised manuscript (P.5 line.83-85, line.99-101; P.6 line. 110-112).

4. Fever is, of course, closely tied to cytokines. Why did the authors not include fever as a criterion by which to group the HFMD patients (e.g. no, low, high fever), to determine if the immune response pattern was related to the presence (or to the degree) of fever?

Thank the reviewer and we partially agree with the suggestion. In the present study EV71-infected patients were divided into a mildly ill group, a severely ill group, and a critically ill group. However, there was no significant difference in fever between severe and critical patients in our and other studies. And then it is not necessary to group the HFMD patients by the fever.

5. Authors should compare results obtained against data in published literature and discuss further in details. In Discussion, second paragraph, author stated that “proinflammatory cytokines and chemokines may play both beneficial and harmful roles…” Please elucidate in which status it may be beneficial and in which status it is harmful based on published literature and this study. What’s the possible mechanism of IVIG and glucocorticoid in reducing EV71-induced inflammation?

The different roles of cytokines in circumstances have been discussed in our original manuscript (P.12 line.243-248).

In fact the previous studies have suggested that there is a complex mechanism about IVIG and glucocorticoid in reducing inflammation, which has been addressed in this
manuscript. The present study suggests that they may suppress inflammation by reducing the expression levels of proinflammatory cytokines (P.11 line.225-235).

6. Between- and among-group comparisons should be performed by Man-Whitney rank-sum test and Kruskal-Wallis test.

We agree with the reviewer and we have clarified it in the methods section in our revised manuscript (P.7 line.132).

Minor Essential Revisions:

1. Due to the limited experimental data, the author may add the “(data not shown)” to the “supplemental data”. It would help to provide an unbiased interpretation and better acceptability of the presented data.

All needed data have been displayed in the manuscript. If necessary, we will add more to the supplemental data.

2. The exact p values should be shown in Results, first paragraph, instead of fuzzy expression in Table 1 (e.g. p<0.05).

The exact p values such as cytokines levels and immunological cell counts have been shown in FIG2, 3. We are afraid it may cause confusion if the exact P values (n=20) are shown in table 1.

3. Since the limited number of cases (16 generally, 25 severely and 12 critically ill cases), the conclusion “systemic inflammation, indicated by elevated plasma IL-6 and IL-8 levels and higher WBC counts, may by a good predictor of critical illness” (Conclusion, first paragraph) is overstated.
Based on the result of this study, we indicated that systemic inflammation with elevated plasma IL-6 and IL-8 levels and higher WBC counts may be a predictor of critical illness. We have deleted the “good” of this sentence in the revised manuscript (P.13 line.256).

4. How about the prognosis of EV71-infected patients enrolled in present study? Any sequelae? (Discussion, last paragraph, last sentence).

Except for 3 died patients, the other EV71-infected patients all recovered in the present study and leaved the hospital. We did not carry out follow-up visit.

REVIEWER 2:

Line 31-32: “the acute phase in severe and critical cases treated with intravenous immunoglobulin (IVIG) and glucocorticoids” means that all these patients had been given a combinatory treatment of IVIG and glucocorticoids? If yes, it should be described well in method section.

We agree with the reviewer and we have clarified it in the methods section in the revised manuscript (P.5 line.99-101).

Line 36-38: the sentence is too long and confusion, better to rephrased it to two sentence.

Thanks and we have rephrased it in the revised manuscript (P.2 line.34-37).

Line 73-75: it is confusing too. “on the other”

Thanks and we have corrected it in the revised manuscript.

Line 104-105: “Nine acute- and five recovery-phase CSF samples were obtained from
two patients who died, and all were negative upon bacterial culture.” How could they can get the recovery-phase CSF samples from died patients?

We have clarified it in the methods section in the revised manuscript (P.6 line.107-108).

Line 108: the author stated “not all such specimens came from the same patients because of difficulties associated with specimen collection”. The statement should be more precise, e.g. 10 paired-samples from the same patients.

We agree with the reviewer and we have clarified it in the methods section in the revised manuscript (P.6 line.110-112).

Line 169: “sought correlations between” should be “sought to analyze the correlation between”

Thanks and we have corrected it in the revised manuscript.

line 180: “any other inflammatory marker” should be “any other inflammatory markers”

Thanks and we have corrected it in the revised manuscript.

REVIEWER 3:

P2 Line21: Severe complications are NOT commonly associated with EV71 infections. Many patients are infected with no or mild symptoms. Pathogenesis of EV71 infection in the severe cases remained poorly understood.

We agree with the reviewer and we have revised this in the revised manuscript (P.2 line.20-21).
P2 Lines 36-38: The second half of the conclusion may not be correct. Even though high cytokine levels in CSF correlate with severe cases, but not with critical cases, it doesn't mean the cytokines in CSF play a less role in critical illness. High cytokine levels in CSF may be transient, and their levels become lowered when the patient progresses to the critical illness stage. It may have nothing to do with “changing battlefield” from CSF to plasma with disease progression. On the other hand, neither CSF nor plasma may be the original site of induced inflammation. Cytokines/chemokines could be induced in some tissues/organs that are secreted into CSF and plasms. Organs or tissues, in periphery or CNS, may be the true “battlefield”.

Remove or replace the word “battlefield”: it is too dramatic.

We agree with the reviewer and we have revised this in the revised manuscript (P2 line.34-37).

P3 Line 60: A vaccine has been in the market in China since 2014. Virus vaccine has successfully developed in 2014, but so far has not yet entered the market.

P4 Line 74-75: The sentence needs to be rephrased. Semicolons should be removed; neutrophils, lymphocytes, and monocytes are part of WBC. Suggestion: Moreover, we also determined the correlations between cytokine levels and markers of inflammation including temperature, white blood cell (WBC) counts or individual counts of neutrophils, lymphocytes, or monocytes.
Thanks and we have rephrased this in the revised manuscript (P.4 line.74-76).

P4 Line 78: Spelling: Enrollment

Thanks and we have corrected it in the revised manuscript.

P5 Line 92, 93 & P7 Line 142 & thereafter: “generally” and “general” could be replaced by “mildly” and “mild”.

Thanks and we have corrected it in the revised manuscript.

P5 Line 98: “examination” – wrong spelling?

Thanks and we have corrected it in the revised manuscript.

P8 Line 153: “subjected to repeat lumbar puncture” > “subjected to repeated lumbar puncturing”

Thanks and we have corrected it in the revised manuscript.

P9 Line 187: Remove the word “rather”.

Thanks and we have corrected it in the revised manuscript.

P10 Lines 193-194: Comment: It’s unlikely that peripheral blood is a major site of inflammation. More likely, cytokines are produced in organs, which are secreted in the blood. And the organ(s) or tissue(s) is the site of inflammation. In another word, inflammatory cytokines/chemokines may not be produced much in both severe and mild patients, resulting in less or little being secreted into blood.

By the same token, higher levels of cytokines in plasma may not be the cause of the critically illness. They could be the outcome of it.

We agree with the reviewer and we have revised this in the revised manuscript (P.10 line. 195-197).
P10 Line 197: “tested” should be replaced by “detected”.

Thanks and we have corrected it in the revised manuscript.

P11 Line 210: “high quality” should be replaced by “solid”.

Thanks and we have corrected it in the revised manuscript.

P11 Line 216-7: “development of a CNS inflammatory response was important in terms of induction of CNS disease in EV71-infected patients”.

Suggested change: “development of a CNS inflammatory response may be essential in induction of CNS complications in EV71-infected patients”.

Thanks and we have changed it in the revised manuscript (P.11 line. 218-219).

P11 Line 218: Remove “However”

Thanks and we have corrected it in the revised manuscript.

P11 Line 223: EV71 infection may be “prevented” only by vaccines, not by anti-inflammation therapy. Manage clinical cases? Yes.

Thanks and we have corrected it in the revised manuscript.

P12 Line 234# “Further work is needed and optimal therapeutic doses and timing of intervention require attention”

Suggested change: “Further study is needed to determine optimal therapeutic doses and timing of intervention clinically”.

Thanks and we have changed it in the revised manuscript.

P12 Line 243: Replace “agents” by “molecules”

Thanks and we have corrected it in the revised manuscript.