Author's response to reviews

Title: Cross-sectional study of the relationship of peripheral blood cell profiles with severity of infection by adenovirus type 55

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Author's response to reviews: see over
Dr. Thorsten Wolff  
Editor-in-Chief, *BMC Infect Dis*  
**Manuscript Title:** “Cross-sectional study of the relationship of peripheral blood cell profiles with severity of infection by adenovirus type 55” (formerly “Immunopathological changes associated with adenovirus type 55 infection severity”)  
**MS ID:** 1582984011029585  

Dear Dr. Wolff,  

We thank you and the two reviewers for the many helpful comments on our manuscript (MS ID: 1582984011029585, New title: “Cross-sectional study of the relationship of peripheral blood cell profiles with severity of infection by adenovirus type 55”).  

The manuscript was revised in accordance with the reviewers’ comments and with the assistance of a professional medical editor. All changes were marked by the “Track Changes” feature of MS Word.  

We hope that you find our revised manuscript acceptable for publication in *BMC Infectious Diseases* and we look forward to hearing from you.  

Sincerely,  

Min Zhao, MD
Referee 1:

1. Is the question posed by the authors well defined?

The objectives are defined but no question is posed; this is a descriptive study of some victims of an adenovirus outbreak among military recruits. If there is a hypothesis, it is that there may be differences peripheral blood laboratory markers among adenovirus infection victims with disease of varying severity and between adenovirus victims and uninfected controls. Should differences be found, then these could be used to generate more specific hypotheses and to help design more specific studies.

**Author response:** As requested, we have modified the text by more clearly stating the study hypothesis in the Background (“The objective of the present study was to examine Chinese military trainees from one of the aforementioned HAdV outbreaks and to identify clinical and laboratory markers that have diagnostic or prognostic value.”).

2. Are the methods appropriate and well described?

The hematology, flow cytometry, and cytokine assays are standard and well described. The serologic studies are less well standardized but appear to distinguish between victims of these outbreaks and healthy controls from bases that did not have outbreak. The method for detecting RNA from throat swabs is not well defined nor is the rationale for this test; furthermore, the validity for this test being useful for detecting infection, acute infection, or severe infection is not demonstrated. —discretionary revision

**Author response:** “Detecting RNA” was a typo. In fact, adenovirus is a DNA virus. Viral DNA was detected by PCR in this study.

3. Are the data sound?

The data from the standardized, commercial tests are likely sound. The data from the tests developed internally is harder to evaluate. For example, figure 1 shows the levels of IF intensity for three different groups; they differ but not in a biologically plausible way (silent infection and severe infection are similar but minor infection is far different)

**Author response:** This is a unique and interesting observation, so we have expanded our description of these results. We added the following text to the Discussion: “Furthermore, analysis of the HAdV55-specific IgM IF scores indicated that patients with severe and silent HAdV-55 infections had a significantly higher IgM IF scores than those with minor infections. This may seem paradoxical, although we can propose a simple explanation for this observation. It is possible that patients with silent infections had strong and effective immune responses, leading to high IF scores and prevention of clinical symptoms; patients with minor infections had weak immune responses, leading to low IF scores and some clinical symptoms; and patients with severe infections had strong immune
responses, but maybe a higher viral load that made them develop more severe disease. This explanation is consistent with our observation that more patients with severe disease had evidence of HAdV-55 DNA (Table 1) and became more immunocompromised (Table S2) than the other groups. Further studies will be needed to confirm this explanation.”

4. Are the discussion and conclusions well balanced and adequately supported by the data?
I think the discussion is more extensive than justified by the simple descriptive nature of the study. I suggest it be shortened and focus on generation of hypotheses that could be tested in future studies.-discretionary revision

Author response: As requested, we have reduced the length of the Discussion by ~20%.

5. Are limitations of the work clearly stated?
I disagree with their limitation/conclusion ‘Nevertheless, the characterization of PBL parameters provided a considerable direction for the prediction of disease progression.’ I do not think this advances the prediction of disease progression since they did not provide information about whether the PBL parameters were obtained before, during, or after severe manifestations of disease had become clinically apparent.-compulsory revision

Author response: As requested, we have modified the penultimate paragraph of the Discussion on limitations of this study. In particular, we added the following sentence: “Furthermore, it is necessary to provide more detailed information about whether the PBL parameters changed before, during, or after severe manifestations of disease if alterations in PBLs are to be used to predict disease progression.”

8. Do the title and abstract accurately convey what has been found?
No, the title should reflect that this is a descriptive study of peripheral blood among adenovirus 55 infection victims; no immunopathology was demonstrated.-compulsory revision

Author response: As requested, we have changed the title to “Cross-sectional study of the relationship of peripheral blood cell profiles with severity of infection by adenovirus type 55”.

Referee 2:
Major Compulsory Revisions: My major concern is how the etiological diagnosis of the three groups were made.
1. Material and methods-diagnostic criteria: The clinical diagnosis criteria is clearly described, but the etiological diagnosis was not clear for three groups, including silent infections, mild infections and severe infections. In Table 1, only one out of 30 silent patients, and 9 out of 30 in mild cases were tested positive by real-time PCR, how did they diagnosed as infections caused by adenovirus?

**Author response:** The PCR results were not used to prove infection. Throat swabs for viral DNA are not 100% positive, and may be negative even in patients with confirmed HAdV55 infection. The assay with HAdV-55-specific IgM (HAdV55-IgM) with immunofluorescence (IF) was used to confirm virus infection (17). Of course, not all patients with silent or mild infections were identified by the IF assay, so the IF assay was used to confirm the clinical diagnosis.

We clarified this by revisions in the **Methods** ("Silent infection was defined as the presence of no clinical symptoms, but positive results for AdV-specific IgM, as described below.") and **Discussion** ("In the present study, we only observed HAdV-55 DNA in 41% of patients with severe disease and in 30% of patients with minor infections. Therefore, this factor cannot be used as an indicator of infection or disease progression. Analysis of IgG and IgM-specific AdV antibodies was more reliable in identification of patients with AdV infections.").

2. The development of HadV-55 IgM assay is interesting. But did they authors test the sensitivity and specificity of HAdV-55 IgM tests by "gold standard"?

**Author response:** HAdV-55 is a new strain of recombinant virus and there are no available commercial diagnostic kits for anti-HAdV-55 IgM, so there is no “gold standard” assay. Thus, we used the antigen isolated from HAdV-55 to generate our own HadV-55 IgM immunofluorescence (IF) assay.

Without prior information, HadV-55 IgM ELISA, provided by another institution (Academy of Military Sciences), was used as a parallel diagnostic test and the sensitivity and specificity (evaluated by Bayes estimation) were estimated as 0.957 and 0.918, respectively.

**Minor Essential Revisions**

1. Description in "pathogen and serotype identification" can be shortened.

**Author response:** As requested, we shortened this section of the **Methods**.

2. Another limitation is that the authors did not compare the difference between HAdV-55 and other serotypes.

**Author response:** As requested, we have modified the penultimate paragraph of the **Discussion** on limitations of this study. In particular, we added the following sentence:
“Finally, our study was limited to infection by one serotype, so the results should not be generalized to other serotypes.”

3. Discussion also should be shortened to focus on the main findings of the paper.

**Author response:** As requested, we have shortened the Discussion by ~20%.

Editor’s comment.

1. We advise you to seek the assistance of a fluent English speaking colleague, or to have a professional editing service correct your language. Please ensure that particular attention is paid to the abstract.

**Author response:** As requested, we revised the entire manuscript with the help of a professional medical editor.

2. In order to give appropriate credit to each author of a paper, the individual contributions of authors to the manuscript should be specified in this section. An ‘author’ is generally considered to be someone who has made substantive intellectual contributions to a published study. To qualify as an author one should 1) have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) have been involved in drafting the manuscript or revising it critically for important intellectual content; and 3) have given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.

We suggest the following kind of format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

**Author response:** As requested, we have added this information at the end of the manuscript: “WC, JL and MZ conceived the study, participated in its design and coordination, and helped to draft the manuscript. WC carried out the immunoassays and participated the statistical analysis. WN and WX carried out the molecular genetic studies and immunoassays, participated the design of the study, drafted the manuscript, and participated in the sequence alignment. YX, BT, and PZ participated in the clinical data acquisition. EQ and YZ participated in the data analysis XZ, WL and ZZ performed the
statistical analysis. All authors read and approved the final manuscript.”