To the Editor,

**Re:** MS: 2812462873128525

“Clinical and Laboratory Findings Associated with Severe Scrub Typhus”

Dear Editor

Thank you for your review and helpful comments regarding our paper.

*Dear reviewers, on the behalf of all coauthors, I would like to give our sincere thanks to you for your promising comments. We are highly impressed by your valuable comments and suggestions that inspired us to make our manuscript more clear and interesting for the readers of the BMC inf dis. Please see all our responds, and we would be happy to respond to more comments if needed.*

Following is our response to your questions and comments.

**Responses to reviewer #1’s comments**

This is a study attempting to identify the predictors for clinical severe scrub typhus. However, several important minor essential revisions that the authors need to correct and clarify as listed below.

Minor Essential Revisions:
A. Abstract:
1. Page 2, line 12. “absence or presence of eschar” should be “absence of eschar”.

**Responds:** As the reviewer’s comment, we have changed it

2. There are no denotations for abbreviations such as WBC, CRP, OR, and CI.

**Responds:** As the reviewer’s comment, we have spelled out the abbreviations; white blood cell (WBC), C-reactive protein (CRP), confidence interval (CI), odd ratio (OI), active partial thromboplastin time (aPTT)

3. OR>1 indicate the results favor to the variable and OR<1 indicate the reverse results. The reversed OR and CI should be used for “absence of eschar”, and
Responds: We are highly impressed by your valuable comments. We have changed it as your recommendation just like this:

(1) age $\geq$ 60 years (OR=3.13, $P=0.002$, CI=1.53-6.41), (2) the absence of eschar (OR=6.62, $P=0.03$, CI=1.22-35.8, (3) WBC counts $> 10,000/\text{mm}^3$ (OR=3.6, $P=0.001$, CI=1.65-7.89), and (4) albumin $\leq$ 3.0 g/dL (OR=5.01, $P=0.004$, CI=1.69-14.86).

B. Methods:
1. Page 4, line 7. A reference should be cited for the Cockcroft-Gault formula used for definition of renal failure. 

   Responds: As your recommendation, we added the cited reference:
   

2. Definition for “shock” is needed.

   Responds: As your recommendation, we add the definition for shock. We used shock is defined by a systolic blood pressure less than 90 mmHg (or a fall in systolic blood pressure of $> 40$ mmHg).

3. Page 4, line 12. The “effective antirickettsial antibiotic therapy” is not clear. Tetracyclines, macrolides, fluoroquinolones? Did all patients not receive antibiotics effective for scrub typhus before assessment? Because this is a study about clinical characteristics and diseases severity, it is important to make sure that patients did not receive therapy before they were enrolled, or those who had received effective antibiotic treatment should be excluded. Why the author used “5 days” as therapeutic delay, was there any reference? or please explain briefly in one sentence here. For more objective comparison, I would suggest the author provide the data of “duration from disease onset to effective antirickettsial antibiotic therapy (days)” rather than “therapeutic delay” only in table 1.

   Responds: We agree with your comment that therapeutic delay is rather obscure. As your recommendation, we changed that to duration from disease onset to effective antibiotics. Effective antibiotic therapy for scrub typhus was defined as the scrub typhus patients received tetracyclines, macrolides, and rifamycins. All patients who received the antibiotics effective for scrub typhus before enrollment were excluded and assessed for “Mean duration from disease onset to effective antibiotic therapy”, which is presented in Table 2.

4. Page 4, line 17. This is a prospective study and many blood tests are
performed. Were signed informed consents obtained before the patients included in study? Please declare about this.

**Responds:** To clarify that point, we added the comment associated with informed consent. “Signed informed consent was obtained for each patient before the patients were included in the study. This study was approved by the Institutional Review Board of our hospital.”

C. Result and Discussion:

1. Page 5, 1st line of this section. Please provide the number of possible cases and excluded cases according to the exclusive criteria.

   **Responds:**
   To clarify that point, we added following statement in the discussion section.

   A total of 333 patients fulfilled the criteria for possible scrub typhus infection during the study period. Of those, 87 patients did not meet the inclusion criteria (44 patients refused to enroll in our study, 40 patients were confirmed as disease other than scrub typhus, and 3 patients had concurrent infection with hemorrhagic fever with renal syndrome). We could not confirm as scrub typhus in 38 patients due to failure to demonstrate a fourfold or greater rise. Hence, a total of 208 patients were finally confirmed to have scrub typhus by serology.

2. 5th line of this section. Why rhabdomyolysis is presented here? It is not the severe form defined by the author.

   **Responds:** We agree with the reviewer’s comment.
   The scrub typhus patient with rhabdomyolysis had pneuemonitis.
   We deleted it in the result sections.

3. 8th line of this section. Important underlying disease should be included such as DM, liver cirrhosis, COPD, ------ etc, especially older patients were included in this study. Liver cirrhosis had been identified to be associated with mortality in recent publication (Kim et al. Scrub typhus in patients with liver cirrhosis: a preliminary study. Clin Microbiol Infect. 2009 Jul 14. [Epub ahead of print]).

   **Responds:**
   As your recommendation, we assessed the underlying diseases such as diabetes, liver cirrhosis, COPD etc. except hypertension.
   But in our study there was no statistic significance.
   We added that result in our tables.

4. Please denote the abbreviations in texts.

   **Responds:** As the reviewer’s comment, we have spelled out the abbreviations; aspartate aminotransferase(AST), alanine aminotransferase(ALT), lactate dehydrogenase(LDH), creatine phophokinase(CPK), adenosine deaminase(ADA), erythrocyte sedimentation rate(ESR), prothrombin time(PT), C-reactive protein (CRP), and active partial thromboplastin time (aPTT)

5. Where are the table 2 and 3 cited in text?

   **Responds:** We apologize for our carelessness. It has been cited properly as your recommendation.
6. Page 6, line 5. The “absence or presence of eschar” should be “absence of eschar”.

Response: As the reviewer’s comment, we have changed it

7. Page 6, line 15. The sentence “Scrub typhus…its incidence being highest in Korea.” needs citation of reference.

Response: As the reviewer’s comment, we have added it


8. Page 6, last line to page 7, line 5. I could not understand the association between presence of eschar and the length of time from onset of symptoms to hospital visit that the author discussed. Patients visit hospital mainly due to fever or other discomforts rather than eschar found by themselves. According to the result in this study, therapeutic delay is not different between the two groups (severe and non-severe cases). However, the duration (days) from disease onset to effective antirickettsial antibiotic therapy or therapeutic delay might be different between patients with and without eschar. That indicates the time of effective treatment rather than presence or absence of eschar is associated with severe scrub typhus. The author must clarify this. A recent publication comes from Korea also mentioned about absence of eschar is associated with mortality (Lee CS et al. Risk factors leading to fatal outcome in scrub typhus patients. Am J Trop Med Hyg. 2009 Sep;81:484-8.).

Response: We agree with the reviewer’s comment. In our study, therapeutic delay is not different between the scrub typhus patients with and without severe severity. However, the days from disease onset to the hospital visit was different between scrub typhus patients with or without eschar. Most scrub typhus patient with eschar are presented with rash. Patients with rash or eschar tend to go see a doctor earlier in the disease course.

In a Mediterranean spotted fever report (JID 2008;198:576-85), a fatal outcome was significantly more likely for patients infected with the ISF strain than Malish strain. Eschar was observed in a significantly higher percentage of patients infected with the Malish strain(60%), compared with patients infected with the ISF strain (38%). In that study, even though ISF strain infected patients received effective antirickettsial treatment earlier in the course of the disease, fatality was much higher. In scrub typhus patients, there is a possibility of the differences in severity or clinical features like rash or eschar according to the serotypes or genotypes of *O. tsutsugamushi*. It is possible that scrub typhus patients contracted with more virulent strain have lower incidence of rash or eschar. Large scale studies are needed to confirm the difference in virulence according to their genotypes or serotypes.

The following study is now in progress to clarify that (Study about Genotype and DNA copy count using real time PCR): In our hospital, there were 3 mortality cases among the 49 scrub
typhus patients in 2007. The intervals between onset of clinical symptoms and hospital admission were 2 to 5 days in the patients who died, whereas the average time among the survivors was 7.18±4.32 days, which suggests that there may be no significant treatment delay between the onset of clinical symptoms and hospital admission in fatalities.

To clarify that point, we revised our manuscript in the discussion section


Another explanation is that scrub typhus patients enrolled in our study were infected with O. tsutsugamushi of relatively lower virulence.

9. Page 7, line 7. The references cited by the author for the statement about frequency of presence of eschar “ but rare in Thailand and Taiwan [8,12]” are not appropriate. Ref. 8 is about 50 patients in south India and ref. 12 is about “pediatric” patients in Thailand. None of the references represent the adult scrub typhus in Thailand or Taiwan. In the study of Lai et al.(Int J Infect Dis. 2009 May;13:387-93), they reviewed literature about scrub typhus in Taiwan and stated that eschar could be found in 23-67% of patients with scrub typhus. The author should re-cite relevant references for this statement.

Responds: We apologize for our carelessness. It has been cited properly as your recommendation.


10. Page 7, line 10. Citation of references and more clear description is needed for the statement of “Despite the fact that this study included older patients than other studies, the mortality rate was lower.”.

Responds: As you know, in the Taiwan study of Lai et al.(Int J Infect Dis. 2009 May;13:387-93) mean age of scrub typhus patients was 42.5. Study from India, median age of scrub typhus patients was 36.5 years (range: 12–75 years) they also report that case fatality rate was 14%. (J Infect. 2006 Jan;52(1):56-60.)

Whereas in Korea the population of the farming villages in rural areas is getting old, the greatest number of scrub typhus patients in Korea was in the age group 50–69 years. (Kweon SS, Choi JS, Lim HS, Kim JR, Kim KY, Ryu SY, Yoo HS, Park O. Rapid increase of scrub typhus, South Korea, 2001-2006. Emerg Infect Dis. 2009 Jul;15(7):1127-9.) Many papers reporting scrub typhus in Korea have presented mortality much less than 10%. “Wie SH, Chang UI, Kim HW, Hur J, Kim SI, Kim YR, Kang MW. Clinical features of 212 cases of scrub typhus in southern region of Gyeonggi-do and the significance of initial simple chest x-ray. Infect Chemother 2008;40:40-5”

To clarify that point, We have changed that part and added the references

11. Page 7, line 18. Because hypoalbuminemia is also a complication of liver cirrhosis and cirrhosis is associated with disease mortality, the author should provide evidence about there is no different frequency of diseases (particularly liver cirrhosis) associated with hypoalbuminemia between the two groups.

Responds: To clarify that point, We have added following sentence.

Because hypoalbuminemia could be a complication of liver cirrhosis, we reassessed the serum albumin level excluding the patients with liver cirrhosis; But serum albumin level was still decreased significantly in the severe scrub typhus group.


Responds: As your recommendation, we changed the cited reference. Thank you so much for your consideration.


D. Table 1. Add variables of important underlying disease (DM, COPD, liver cirrhosis, …etc.) and duration from disease onset to effective antirickettsial antibiotic therapy. Table 3. The title is for severe scrub typhus. So, the values of OR and CI are wrong for albumin#3 g/dl. They should be reverse values

Responds: As your recommendation, we added the the recommended variables, and also changed the OR and CI values for albumine.
Responses to reviewer #2’s comments

Reviewer 2:

Minor essential corrections:
1. Abstract is clear.
   a. Line 3 read as factors associated with severe condition....
2. Background
   a. The study objective is clear and justified
   b. Check spelling in line 3 – interstitial?

Responds:

3. Methods
   a. Study design is appropriate
   b. There is no rationale for Sample size for cases and controls

Responds: In our institution review board, a certain amount of sample size was not required since this study is not a clinical trial. We did not consider the sample size when we designed this study. As the reviewer referred to this matter, evaluation of the sample size for the cases and controls before we started this study would have been better. I am sorry for not describing the rational for the sample size. I am thankful for your good suggestion. We will consider the statistics method before we start to design a new study.

   c. It is not clear whether all the study subjects – cases and controls are lab confirmed cases of scrub typhus in the first paragraph of methods. Definition of severe cases of scrub Typhus was given in Para 2 and laboratory confirmation was stated in the third paragraph. Re-alignment of paragraphs would render better readability.

   Responds: To clarify that point, We have added following sentence

   “A total of 333 patients fulfilled the criteria for possible scrub typhus infection during the study period. Of those, 87 patients did not meet the inclusion criteria (44 patients refused to enroll in our study, 40 patients were confirmed as disease other than scrub typhus, and 3 patients had concurrent infection with hemorrhagic fever with renal syndrome). We could not confirm as scrub typhus in 38 patients due to failure to demonstrate a fourfold or greater rise. Hence, a total of 208 patients were finally confirmed to have scrub typhus by serology.”

4. Results
   a. Distribution severity symptoms of severe scrub typhus cases do not add up to
89 cases. Probably there were cases with multiple symptoms. Instead of writing a paragraph, the authors can present them in a table and mention just the top conditions in the text.

**Responds:**
Actually there were cases with multiple symptoms. We presented it in table 1 as your suggestion.

"Severe scrub typhus such as pneumonia, renal failure, meningoencephalitis, shock, gastrointestinal bleeding, myocarditis, transient cranial nerve palsy and death was observed in 89 of 208 (42.8%) patients with a definitive diagnosis of scrub typhus. This content is summarized in table 1. Patients were divided into two groups: case group with severe scrub typhus (n=89) and control group without severe scrub typhus (n=119)."

b. In multivariate analysis, variables 1, 3 and 4 the factors considered as risk factor is mentioned and the complementary of it could be assumed as a reference class. For variable 2, namely, eschar, both presence and absence are mentioned and it is not clear which is compared with what. It is better to state the reference class of each category.

**Responds:** We are highly impressed by your valuable comments. We have changed it as your recommendation as the following.

(2) the absence of eschar (OR=6.62, \( P=0.03 \), CI=1.22-35.8)

c. Tables 1 and 2 need improvement in the format and title. Many confidence intervals are very wide – Need to discuss.

**Responds:** We added some variables.

Many confidence intervals in variables of Table 1, 2 are very wide
As your recommendation, we added that in the result and discussion section.

We changed the title of Table 1

Demographic, clinical characteristics and laboratory findings of the scrub typhus patients evaluated in terms of severe scrub typhus →

Demographic, clinical characteristics and laboratory findings on admission of the 208 scrub typhus patients

We changed the title of Table 2

Unadjusted relative risk of each selected factor for severe scrub typhus.

→ Unadjusted relative risk for each selected factor on severe scrub typhus.
5. Conclusions
a. ’Patients without eschar’ instead of ‘presented with the absence of eschar’ (line 2).
   
   **Responds:** As the reviewer’s comment, we have changed it

Major compulsory revision

Discussion
a. There is no separate section. There are several repetitions of results. It should be more a discussion on the interpretation of the results. There must be a paragraph on internal and external validity (bias, confounders, how far the results are generalised etc.) and their implications on the study results
b. Limitations of the study

As your recommendation we revised our manuscript.

**Responses to reviewer #3’s comments**

Minor essential revisions.

Some minor corrections to grammar and spelling are suggested:

Background.
Page 3, line 5. Grammar: “…pay our attention…” should read “…pay close attention…” or otherwise delete “our.”
   
   **Responds:** As the reviewer’s comment, we have changed it

Page 3, line 13. Insert “a” to read “…admitted earlier to a hospital…”
   
   **Responds:** As the reviewer’s comment, we have changed it

Methods.

Have you established [in other studies] that such IgM titers rarely occur in your non-patient population, i.e., normal people living in the endemic area? Yours is a hyperendemic region for this disease.


The 2007 Korea Center of Disease control and Prevention(KCDC) guidline for diagnosis of infectious disease specify cutoff values ≥ 1:16 for IgM antibody. And Cutoff value of ≥1:160 for IgM antibody could differentiate between previous and current infection in Korea and Japan. IFA was performed to determine the seroprevalence of scrub typhus in 273 healthy subjects who attended a health screening programm. There are no normal people who show ≥1:160 for IgM antibody.
Page 4, line 4. “increased four-fold or more…” [This is the generally accepted way of stating increases in titer].
   Responds: As the reviewer’s comment, we have changed it

Page 4, line 5. “Severe scrub typhus ‘was’ defined…”
   Responds: As the reviewer’s comment, we have changed it

Page 4, line 14. “At presentation, ‘a’ thorough history ‘was taken’…”
   Responds: As the reviewer’s comment, we have changed it

Results and Discussion.
Page 5, line 9. “Severe scrub typhus ‘was’ observed…”
   Responds: As the reviewer’s comment, we have changed it

How large was your primary cohort, from which you selected the scrub typhus patients for your study, i.e., what percent of presenting patients were diagnosed with scrub typhus during that time period?
   Responds: We have added following sentence in result section.
   “A total of 333 patients fulfilled the criteria for possible scrub typhus infection during the study period. Of those, 87 patients did not meet the inclusion criteria (44 patients refused to enroll in our study, 40 patients were confirmed as disease other than scrub typhus, and 3 patients had concurrent infection with hemorrhagic fever with renal syndrome). We could not confirm as scrub typhus in 38 patients due to failure to demonstrate a fourfold or greater rise. Hence, a total of 208 patients were finally confirmed to have scrub typhus by serology.”

Page 7, line 6. “An eschar could be…frequently 'in' reports…”
   Responds: As the reviewer’s comment, we have changed it

Page 7, line 13. 90% of our patients had ‘an’ eschar…”
   Responds: As the reviewer’s comment, we have changed it

Conclusions.
Page 8, line 9. “…when ‘the’ scrub typhus patient was old age, or presented with…” Delete “were”. The phrase “old age” is relative and imprecise. Suggest you insert “older” patient for “old age” (> or = 60?) (including the abstract) and an age breakpoint.[This reviewer is 62 years old and does not consider himself "old."
   Responds: As the reviewer’s comment, we have changed it
We have revised our paper. Please see attached one main text file. We believe that we have addressed all questions and comments, but we would be happy to provide further information or revision if necessary. Thank you for your consideration and please feel free to contact us if we can help you in any way.

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