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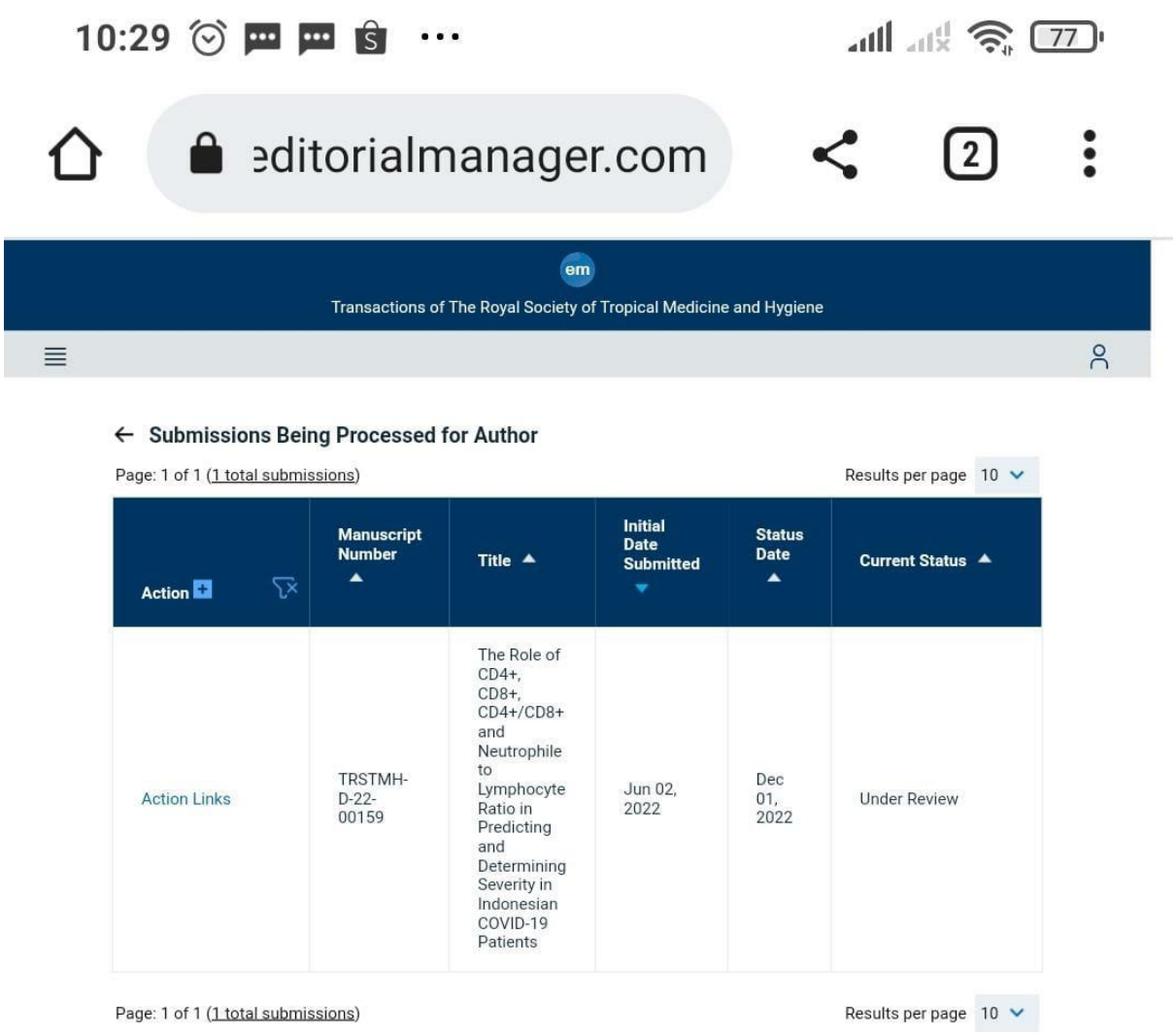

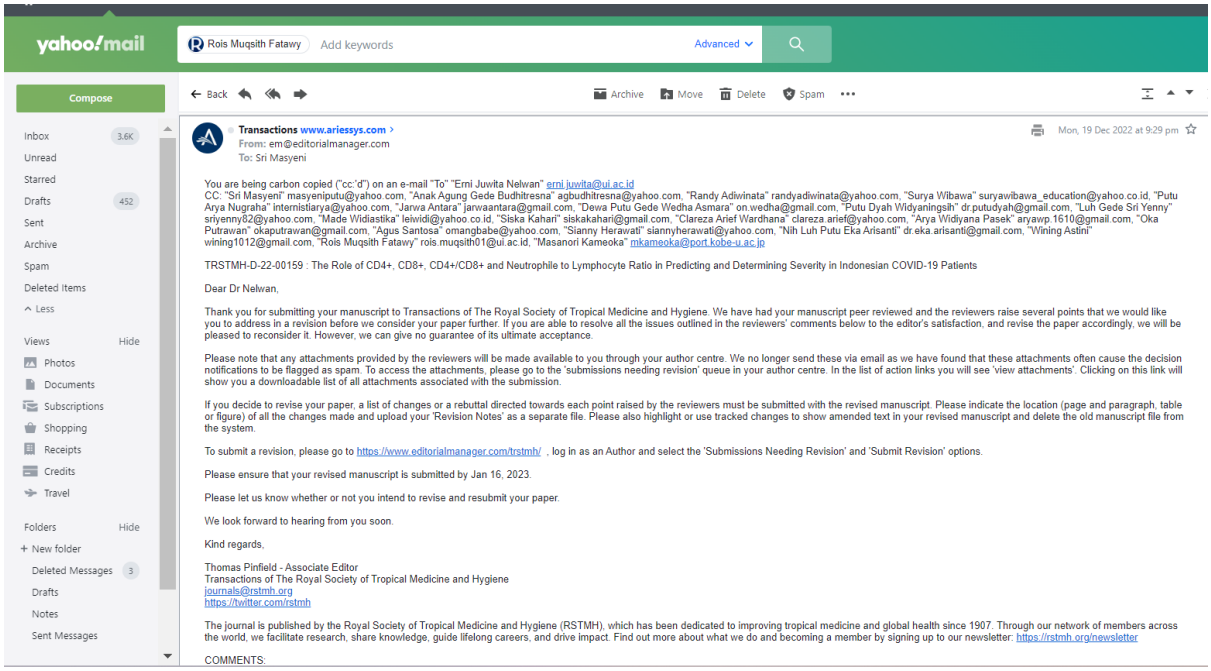

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TRSTMH-D-22-00159 : The Role of CD4+, CD8+, CD4+/CD8+ and Neutrophile to Lymphocyte Ratio in Predicting and Determining Severity in Indonesian COVID-19 Patients

Dear Dr Nelwan,

Thank you for submitting your manuscript to Transactions of The Royal Society of Tropical Medicine and Hygiene. We have had your manuscript peer reviewed and the reviewers raise several points that we would like you to address in a revision before we consider your paper further. If you are able to resolve all the issues outlined in the reviewers' comments below to the editor's satisfaction, and revise the paper accordingly, we will be pleased to reconsider it. However, we can give no guarantee of its ultimate acceptance.

Please note that any attachments provided by the reviewers will be made available to you through your author centre. We no longer send these via email as we have found that these attachments often cause the decision notifications to be flagged as spam. To access the attachments, please go to the 'submissions needing revision' queue in your author centre. In the list of action links you will see 'view attachments'. Clicking on this link will show you a downloadable list of all attachments associated with the submission.

If you decide to revise your paper, a list of changes or a rebuttal directed towards each point raised by the reviewers must be submitted with the revised manuscript. Please indicate the location (page and paragraph, table or figure) of all the changes made and upload your 'Revision Notes' as a separate file. Please also highlight or use tracked changes to show amended text in your revised manuscript and delete the old manuscript file from the system.

To submit a revision, please go to https://www.editorialmanager.com/trstmh/ , log in as an Author and select the 'Submissions Needing Revision' and 'Submit Revision' options.

Please ensure that your revised manuscript is submitted by Jan 16, 2023.

Please let us know whether or not you intend to revise and resubmit your paper.

We look forward to hearing from you soon.

Kind regards,

Thomas Pinfield - Associate Editor
Transactions of The Royal Society of Tropical Medicine and Hygiene
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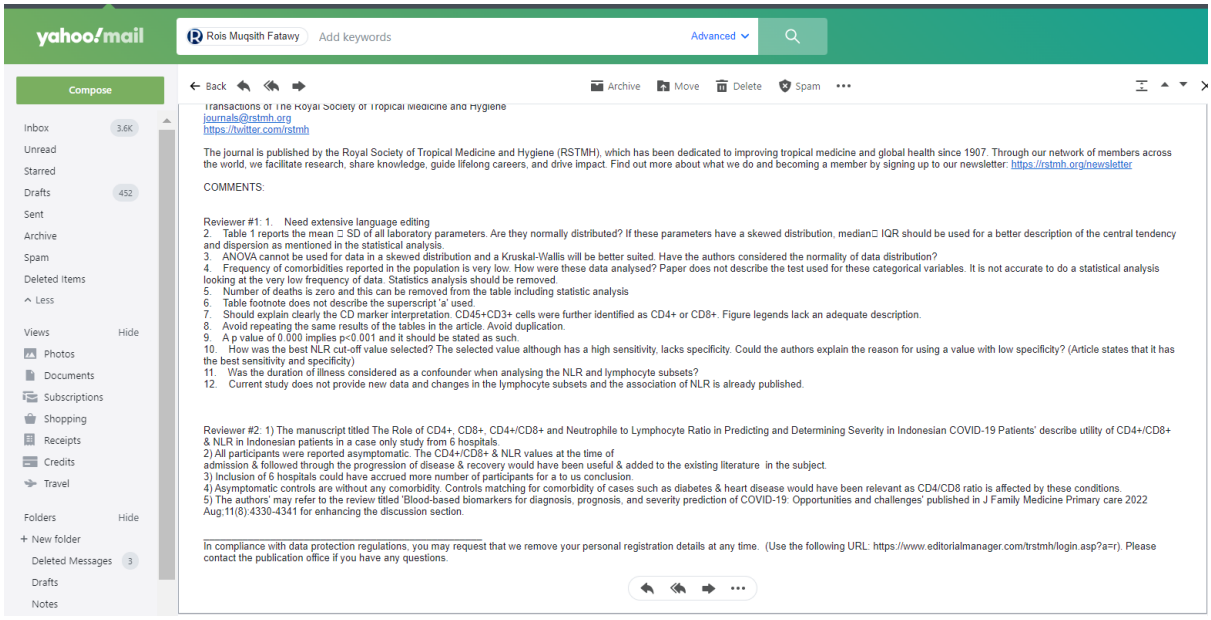
COMMENTS:



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COMMENTS:

Reviewer #1: 1. Need extensive language editing
2. Table 1 reports the mean □ SD of all laboratory parameters. Are they normally distributed? If these parameters have a skewed distribution, median□ IQR should be used for a better description of the central tendency and dispersion as mentioned in the statistical analysis.
3. ANOVA cannot be used for data in a skewed distribution and a Kruskal-Wallis will be better suited. Have the authors considered the normality of data distribution?
4. Frequency of comorbidities reported in the population is very low. How were these data analysed? Paper does not describe the test used for these categorical variables. It is not accurate to do a statistical analysis looking at the very low frequency of data. Statistics analysis should be removed.
5. Number of deaths is zero and this can be removed from the table including statistic analysis
6. Table footnote does not describe the superscript 'a' used.
7. Should explain clearly the CD marker interpretation. CD45+CD3+ cells were further identified as CD4+ or CD8+. Figure legends lack an adequate description.
8. Avoid repeating the same results of the tables in the article. Avoid duplication.
9. A p value of 0.000 implies p<0.001 and it should be stated as such.
10. How was the best NLR cut-off value selected? The selected value although has a high sensitivity, lacks specificity. Could the authors explain the reason for using a value with low specificity? (Article states that it has the best sensitivity and specificity)
11. Was the duration of illness considered as a confounder when analysing the NLR and lymphocyte subsets?
12. Current study does not provide new data and changes in the lymphocyte subsets and the association of NLR is already published.

Reviewer #2: 1) The manuscript titled The Role of CD4+, CD8+, CD4+/CD8+ and Neutrophile to Lymphocyte Ratio in Predicting and Determining Severity in Indonesian COVID-19 Patients' describe utility of CD4+/CD8+ & NLR in Indonesian patients in a case only study from 6 hospitals.
2) All participants were reported asymptomatic. The CD4+/CD8+ & NLR values at the time of admission & followed through the progression of disease & recovery would have been useful & added to the existing literature in the subject.
3) Inclusion of 6 hospitals could have accrued more number of participants for a to us conclusion.
4) Asymptomatic controls are without any comorbidity. Controls matching for comorbidity of cases such as diabetes & heart disease would have been relevant as CD4/CD8 ratio is affected by these conditions.
5) The authors' may refer to the review titled 'Blood-based biomarkers for diagnosis, prognosis, and severity prediction of COVID-19: Opportunities and challenges' published in J Family Medicine Primary care 2022 Aug;11(8):4330-4341 for enhancing the discussion section.

Dear Editor,

Please find our response to the reviewers' comments below.
Thank you so much.

Best Regards,
Authors

Reviewer #1:
1. Need extensive language editing
Our Response: We proofread our manuscript again.

2. Table 1 reports the mean SD of all laboratory parameters. Are they normally distributed? If these parameters have a skewed distribution, median IQR should be used for a better description of the central tendency and dispersion as mentioned in the statistical analysis.
Our Response: Thank you for raising this issue. The data is not normally distributed, we had already used non-parametric analysis, including Kruskal Wallis, however, we have made a mistake at writing it on the method section(data analysis) and presenting it on the table.

3. ANOVA cannot be used for data in a skewed distribution and a Kruskal-Wallis will be better suited. Have the authors considered the normality of data distribution?
Our Response: Thank you for mentioning this. Again, we had tested the normality distribution and the data was not normally distributed. We had already used non-parametric analysis including Kruskall Wallis, however, we need to correct a mistake at writing it on the method section(data analysis) and presenting it on the table.

4. Frequency of comorbidities reported in the population is very low. How were these data analysed? Paper does not describe the test used for these categorical variables. It is not accurate to do a statistical analysis looking at the very low frequency of data. Statistics analysis should be removed.
Our Response: Thank you so much. We have removed the statistical analysis of comorbidities.

5. Number of deaths is zero and this can be removed from the table including statistic analysis
Our Response: We have already removed the number of death from the table

6. Table footnote does not describe the superscript 'a' used.
Our Response: We have already made the new footnote description of the first table.

7. Should explain clearly the CD marker interpretation. CD45+CD3+ cells were further identified as CD4+ or CD8+. Figure legends lack an adequate description.
Our Response: We have already improved the description of Figure 1.

8. Avoid repeating the same results of the tables in the article. Avoid duplication.
Our Response: Our manuscript overall percentage of variables such as comorbidities and lab examination were mentioned the detailed information among each group in the presented table.

9. A p value of 0.000 implies $p<0.001$ and it should be stated as such.
Our Response: Thank you. We have already revised as suggested.

10. How was the best NLR cut-off value selected? The selected value although has a high sensitivity, lacks specificity. Could the authors explain the reason for using a value with low specificity? (Article states that it has the best sensitivity and specificity)
Our Response: Thank you for highlighting this issue. We agree that we choose the optimal cut-off with the highest sensitivity with the intention that we want to classify patients with the worse

prognostic outcome. In addition to that, we have explained the fifth paragraph that the NLR cut off is for prognostic marker instead of diagnostic tool.

11. Was the duration of illness considered as a confounder when analysing the NLR and lymphocyte subsets?
Our Response: We understand the reviewer's concern about the duration of the illness. However, among the covid-19 patients seems more reliable for the progression of illness rather than duration. Duration of COVID-19 illness cannot be considered as a confounder when analyzing the NLR (neutrophil-lymphocyte ratio) and lymphocyte subsets because it is a variable that is directly related to the outcome of interest, rather than a variable that is associated with both the exposure and outcome. The duration of illness for COVID-19 patients is often not well defined. COVID-19 patients may have a wide range of symptoms and disease progression, and the duration of illness can vary greatly between patients.

12. Current study does not provide new data and changes in the lymphocyte subsets and the association of NLR is already published.
Our Response: We believe that the report comparing the disease classification, including asymptomatic, mild, moderate, severe and critical, the published data on lymphocyte subsets and NLR ratio only mentioned the critical and severe group.

Reviewer #2:
1) The manuscript titled The Role of CD4+, CD8+, CD4+/CD8+ and Neutrophile to Lymphocyte Ratio in Predicting and Determining Severity in Indonesian COVID-19 Patients' describe utility of CD4+/CD8+ & NLR in Indonesian patients in a case only study from 6 hospitals.
Our Response: This study's severe, mild-moderate characteristics are similar to the national standard. Therefore, we can generalize the results of our study as a whole.

2) All participants were reported asymptomatic. The CD4+/CD8+ & NLR values at the time of admission & followed through the progression of disease & recovery would have been useful & added to the existing literature in the subject.
Our Response: We have total 95 patients, and 35.8% is asymptomatic. The patients were classified on the recruitment day as presented in the method. This is a cross-sectional study. Therefore, we did not follow up with the patients.

3) Inclusion of 6 hospitals could have accrued more number of participants for a to us conclusion.
Our Response: This study was conducted in the first wave of the COVID-19 pandemic. The confirming test/PCR for COVID-19 was still limited in Indonesia. And we only analyzed the confirmed patients.

4) Asymptomatic controls are without any comorbidity. Controls matching for comorbidity of cases such as diabetes & heart disease would have been relevant as CD4/CD8 ratio is affected by these conditions.
Our Response: We agree that the asymptomatic group has no comorbidities. We take this as an advantage because the number of comorbidities among the critical or severe group is also very small; therefore, this group is comparable.

5) The authors' may refer to the review titled 'Blood-based biomarkers for diagnosis, prognosis, and severity prediction of COVID-19: Opportunities and challenges' published in J Family Medicine Primary care 2022 Aug;11(8):4330-4341 for enhancing the discussion section.
Our Response: We have already added it to the discussion section.