Reviewer’s report

Title: Peripheral blood lymphocytes immunophenotyping predicts disease activity in clinically isolated syndrome patients

Version: 2 Date: 11 Nov 2016

Reviewer: Ming-Feng Liao

Reviewer's report:

I think the statistical analysis methods were better understood after the author's explanations. The author gave us a new idea that we can try monitor the disease activity of MS by compare the individual difference of subgroups of lymphocyte, and this idea is wroth to be published. However, In my opinion, some methodology remain need to be clarify before publish. Moreover, I remain have some suggestions and questions.

1. The author made different survival curve at different time point, and use different indicators. From author's answers, I understood the thresholds of different survival curve at different time point are chosen form the ROC curve at that time. So the different survival curve at different time point should have different thresholds. For example, in Figure 1, threshold at Ratio vs. LY in 12M = 0.808, threshold at Ratio vs. LY in 24M should be another value. I suggested authors can add the threshold values at different time points in the figures, or the readers may have the misunderstanding like me that the author use the same threshold in the different time point. Or the author may change the layout, and try show all ROC curve in different time points in one figure.

2. I suggested author can add those details in the statistical analysis methods, rather than just write "An ROC curve to predict the clinical outcome at one year was constructed and an optimal threshold was chosen." The author wrote some detailed methods in the discussions. I think the author can move those sections to statistical analysis methods.

3. I suggested the author can write down and specify the unit of threshold in the figure (should be %, for example, in Figure 1, B lymphocyte > 9.5% of total leukocyte?).

4. I remain not understand the meaning of the survival curve at 48 months. In my original understanding from the manuscript: the author checked blood tests of patients at different time point (12M, 24M, 36M, 48M, et al.), and all patients received follow up at least 48 months. Then the author draws the survival curve at different time point (based on the different lab data and threshold from ROC curve at that time) to predict the disease condition at final (at least 48 months). For example, the follow up duration of the survival curve at 12M should be at least 36 M, and survival curve at 24M should be at least 24M, et al. However, in the replay latter, the author said "survival curve is drawn from the baseline
(not at 12M, 24M, 36M, 48M)”? and the X axis of every figures (at 12M, 24M, 36M, 48M) is the same (all 2500 days, around 80 months, over 48 months)? How long did all patients follow up? the author may summary in the results. This is a retrospective or prospective study?

5. I also suggest the author may consider native English proofreading before publish.

Thanks for author's explanations.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Unable to assess

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

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