Author’s response to reviews

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

Authors:
Helena Posova (hmare@lf1.cuni.cz)
Dana Horakova (dana.horakova@lf1.cuni.cz)
Vaclav Capek (venca@cicconia.cz)
Tomas Uher (tomas.uher@vfn.cz)
Zdenka Hruskova (zdenka.hruskova@vfn.cz)
Eva Havrdova (ehavr@lf1.cuni.cz)

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Author’s response to reviews:

Reviewer reports:

Reviewer #2: 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 worth 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.

The threshold was the same for all the curves, this piece of the information has been added in the Manuscript:
The same threshold was applied to measurements before baseline, at baseline, at 6M, etc.

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.

Thank you for the comment, we have transferred this part to the Methods section.

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?).

The threshold was 9.5% of total lymphocytes – we have amended that in the manuscript.

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 were all patients be followed up? Please clarify the study nature (retrospective or prospective?)

This was a prospective study. The follow-up lasted for 48 months, but only data until a treatment change (relapse) were included in the statistical analysis.

We have transferred the explanation of the statistics from the Discussion to the Methods section as suggested. Hopefully, this will make it easier for readers to understand the statistical approach used.

Thanks for author's explanations.
Reviewer #3: Authors addressed majority of my propositions and answered my questions. I am satisfied with their explanation of NK cells/ disease progression mismatch. Their study (despite being negative) provides further, potentially important, data in MS immunology field. Minor language corrections (MS Word revision) needed. Graphs are probably too busy and will be difficult to interpret for medical professionals but otherwise text is understandable.

Comments from editor:

Proofreading by native English speaker should be considered before being published.

We tried to make also linguistic corrections.