Reviewer’s report

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

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Reviewer: Ondrej Dolezal

Reviewer's report:

I reviewed article 'Peripheral blood lymphocytes immunophenotyping predicts disease activity in clinically isolated syndrome patients' by Posová Helena, Horáková Dana, Čapek Václav, Uher Tomáš, Hrušková Zdenka, Havrdová Eva.

I must admit that authors provided thorough longitudinal data, when they followed-up 181 patients with CIS (151 for four years). This longitudinal long term approach is from my point of view the most useful way how to reach convincing conclusion in MS. Authors try to identify blood marker of neurological progression in MS patients. Statistics level seems sufficient according to patients' population needs. Language quality is suitable for publication.

Authors formulated some hypotheses and their data in many ways supported already published studies. I believe that paper needs some changes to be more understandable and less contradictory.

1. Patients were very early in disease course started on DMT (INF Beat 1a), which is admirable but from the context of table annotations ('The results at 48 months of follow-up should be influenced by treatment (natalizumab, fingolimod, copaxone)' I understood that variable switches between treatments were made over the period of 4 years. This seems to be major downfall. All treatment mentioned above would inevitably influence levels of lymphocyte subpopulations. I believe that authors should be paying attention mostly to first year or two as I believe that population of CIS patient was at that point the most homogeneous from treatment point of view. This subgroup would be the best to study differences between future "MS converters" or "CIS remainers". Authors also admit that steroid treatment before baseline (which I believe was used in all of patients to treat initial CIS?) skewed FBC (full blood count results) and subpopulation results.

2. Authors are mentioning role of NK cells which were assumed to play rather positive role in MS parthenogenesis (authors mentioned that 'MS relapses and new brain lesions detected by magnetic resonance imaging are often preceded by a reduction in NK cell functional activity') however authors mention in Results section that 'The decrease of NK cells below 10.8 % increased the Clinically defined progression (CDP)…..but increase (of more than
28.7%) led to EDSS worsening. These statements are contradictory or authors did not explain their conclusions properly.

3. Authors should put accent on naive cells (including helpers (CD45RA+ in CD4)) as those seem to be more stable and statistically related to clinical course (CDP)

Other contradiction was identified in Discussion section as authors mentioned 'increase of CD5+ memory B cells in remitting stage of the disease....other authors concluded that increased percentages of blood CD5+ B cells were associated with further elevated risk of conversion to MS and increase in relapse'.

These contradictions are originating from author's effort to cover every angle however they are doing their work within 'quick sand of MS blood biomarker's field' and you can not address all contradictions/theories as a part of original study.

I would recommend to change even paper's name to e.g. 'Possible role of naïve cells/lymphocytes and NK cells in CIS/MS conversion' and I would try to eliminate medication/DMT bias from the study (paying more attention to lymphocyte levels during initial year 1 or 2 and its influence on further clinical outcome (year 3 and 4). Authors also should pay special attention to group of 37 pts who remained CIS (special section about this group).

**Are the methods appropriate and well described?**

If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**

If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**

If not, please explain in your comments to the authors.

No

**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.

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I acted as a member of prof. Havrdova's team between 2002-2011 however I was not involved in project discussed in the paper and I do not know principal author, otherwise I declare that I have No other competing interests.

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