Author's response to reviews

Title: IgG-index predicts neurological morbidity in patients with infectious central nervous system diseases

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Author's response to reviews: see over
To the Editor in Chief of BMC Infectious Diseases

Revision of the manuscript: “IgG-index predicts neurological morbidity in patients with infectious central nervous system diseases” by Lackner P. et al.

Dear Professor Norton,

thank you very much for giving us the opportunity to improve our manuscript. We have responded in detail to the very helpful comments of the reviewers. We feel that the manuscript is now substantially improved and ready for a re-evaluation. We would like to thank you very much for reading and judging our manuscript. Please do not hesitate to contact us if we can be of any further help regarding the review process.

Yours sincerely,

Peter Lackner, MD
Bettina Pfausler, MD
Reviewer 1

Major Compulsory Revisions

1. Would this be allowed by an ethics panel in other EU countries? This is not presented as an audit, therefore as research it ought to have ethical scrutiny. Perhaps mentioning data was anonymised etc. might clarify this.

The original version of our manuscript might have been ambiguous on this important issue. We have now clearly pointed out that all data analyses was performed in a retrospective nature on anonymized patient data (page 5, lines 3-4). Therefore IRB approval is waived due to Austrian regulations.

2. In LR were data log transformed to ensure normality before entering in model? How did they decide which variables were entered initially, did they use non-parametric test results to decide what to enter or was all entered initially? Did the use forward or backward methodology?

Raw data of all parameters were entered into the model on a non selective basis. A forward conditional stepwise logistic regression model was applied. Good model fitting was achieved by this procedure. This is now mentioned in the revised version of our manuscript (page 5, lines 10-14).

3. Diagnosis – LM was by cytology only – any immunohistochemistry? Why such a low yield in diagnosis for VM, e.g. tests not done?

Confirmed etiology required definite diagnosis of LM by cytology and/or in some patients immunohistochemical staining. In patients with viral meningitis as shown in table 2 the most frequent etiology was TBEV diagnosed by serology. We agree with the reviewer that the percentage of patients with confirmed etiology is low compared to reports by others (e.g. Kupila L – 2006 – Neurology). However all patients under the suspicion of VM received serological and PCR workup for common viruses. Due to lack of therapeutic consequence patients who are quickly recovering are not routinely followed-up serologically due to economic reasons. This policy might explain the discrepancy and is now discussed in the revised version of our manuscript (page 11, lines 16-20).
4. Heavy bias with poor outcome by LM – what diagnosis did these patients have? e.g. B-cell lymphoma might influence IgG index or T-cell lymphoma would not produce increase in IgG index. Table 2 could include this data.

We fully agree with the reviewer that the primary tumor of patients with LM is of interest. In the revised version of our manuscript this information has been included in table 2. Of course outcome is different in the three patient groups. However primary diagnosis is frequently unclear in the initial clinical workup. Therefore all patient groups were analyzed together in the stepwise LR model. Subsequently, the selected parameters were analyzed in each patient group separately. Hence we are convinced that this bias does not adversely affect the interpretation of our data.

5. Are all CSFs lumped together for analysis? Day 0 CSF might be better predictor or change between paired samples. It is not clear to me that this was done. For outcome prediction only day 0 CSF samples entered the regression model as suspected by the reviewer. This is now clarified in the revised version of our manuscript (page 5, line 12).

6. Figure 1 A-G has no clear statistical test results on graphs, with only a comment in legend. The authors note that the K-W test gives a statistical result, but it cannot differentiate between the groups. In addition when data are log transformed the appropriate test to use is an ANOVA with a post test correction (e.g. Bonferroni) assuming the log transformation normalises the data. In addition they ought to give y-axis a log scale. Otherwise it might be better to present data non-log transformed and do simple MWU tests between groups. I suspect between many of the groups results are not statistically different.

On request of reviewer 2 and for clarity reasons, figure 1 is omitted in the revised version of our manuscript, since the discriminatory potential of CSF analyses was not the primary focus of the current manuscript and has been studied extensively elsewhere.

7. IgG index will not help clinicians determine who has viral and who has bacterial disease. In those patients with LM they will be older and possibly have disease elsewhere (we are not told this) so I cannot see this being as useful a laboratory test as the authors conclude.
We fully agree with the reviewer that the IgG-index does not differentiate between the different patients groups. Differential diagnosis of inflammatory CNS diseases was not the primary focus of this paper. As suggested by reviewer 2 figure 1 has been omitted for reasons of clarity. We are convinced that the main result of this paper — the prognostic value of the IgG-index — is now strengthened.

8. The authors conclude that there is a wide consensus that steroids should be used as an adjunct for treatment, but a recent Lancet Neurology meta-analysis has challenged this. In addition they do not state how many of their patients have been treated with steroids, which may (or may not) influence CSF parameters and so ought to be a co-variate in the analysis.

The mentioned paper was not available prior to submission of the original version of this manuscript. This important meta-analysis is now discussed in the revised version of our manuscript (page 9, lines 23-26; page 10, lines 1-2). In the light of this new data status of treatment with steroids should not influence outcome.

Minor Essential Revisions

1. Retrospective data - 592/835 patients excluded due to insufficient data/inconclusive diagnosis – does this skew their results? When was clinical outcome measured (e.g. death at d28?).

Clinical outcome was measured at first discharge from hospital (page 4, lines 20-21). Length of stay is given in table 1.

2. Why have the authors included TBM and Lyme disease as “bacterial meningitis” when the pathogenesis of these conditions is very different to pyogenic organisms. The primary intent of this paper was to analyse the predictive value of basic CSF parameters in patients with inflammatory CNS disease. The diagnostic time window is variable in some conditions. This is especially true for patients with TB meningitis. However early risk assessment is a critical issue for all patients. Therefore we did not exclude patients with TB or Lyme disease from the analysis, albeit we agree with the reviewer that these disease entities differ from acute pyogenic meningitis.

3. Only 243/1675 analysed (14.5%).
We agree with the reviewer that this percentage seems low. However it has to be kept in mind that this number comprises all patients requiring lumbar puncture for differential diagnosis of neurological diseases (e.g., subarachnoid hemorrhage, normal pressure hydrocephalus).

4. Glasgow Outcome score reference. Maximum/minimum and how calculated could be a little better explained in the text (it is done well in one figure). GOS is now explained in the methods section (page 4, lines 21-26).

5. Why were age and sex included as mandatory in LR – is gender associated with outcome? If so then needs a ref. We agree with the reviewer that to our knowledge sex has no reported association with outcome and was therefore omitted from the models in the revised version of our manuscript.

6. Did they perform LR with just death as an outcome rather than GOS 5/6? If so this is not clear from the text. For the LR models outcome was dichotomized with death (GOS=1) and any significant neurological deficit (vegetative state GOS=2, severe disability GOS=3, moderate disability but unable to return to work GOS=4) as unfavorable outcome and good recovery (only slight or no neurological deficits, able to return to work GOS=5) as favorable outcome. This categorization was chosen in accordance with previous studies in bacterial meningitis (de Gans J – 2002 – N Engl J Med). This is now clarified in the revised version of our manuscript (page 4, lines 21-26).

Discretionary Revisions
1. Figure 2 might be better presented as a table with univariate and then multivariate OR (95% CI) and P-value. In the revised version of our manuscript table 3 has been extended with the data of univariate models as suggested by the reviewer.
Reviewer 2

Although this research question is well defined, it however warrants some additional justification.

1. It seems that the 3 disease groups were pooled in order to evaluate the differential diagnostic rather than the prognostic value of CSF parameters. From a clinical standpoint, it is questionable whether pooling the groups is useful in the evaluation of prognostic markers only.

The 3 disease groups were pooled for the evaluation of the prognostic value of the respective CSF parameters. Primary diagnoses were entered in the model to control for outcome differences in the patient groups. In the revised version of our manuscript an additional statistical approach is shown using univariate followed by multivariate logistic regression models for each patient group separately. The IgG-index remained the only significant predictor of outcome in this approach.

2. The authors should justify the need for additional markers in the “complex scores” that already exist.

In contrast to clinical presentation and basic CSF parameters (Weisfelt M, 2008, Ann Neurol) immunoglobulin indices to our knowledge were only studied in one small patient group (Giasuddin AS, 1998, Br J Biome Sci). Therefore this study was conducted to evaluate their predictive potential. The role of the IgG-index in future predictive scores remains to be resolved. A statement has been added in the revised version of our manuscript (page 12, lines 4-6).

3. “Patients were stratified into 3 diagnostic groups (bacterial meningitis, viral meningoencephalitis, LM) diagnosed by commonly accepted clinical and / or microbiological and pathological / cytological criteria”. These diagnostic criteria include basic CSF parameters. It is not clear what the sensitivities and specificities are. This is an essential weakness of the study and should be mentioned in the paper.

We fully agree with the reviewer that the span of most CSF parameters in inflammatory CNS diseases are overlapping and no single discriminating parameter could be found yet. In patients without definite etiology clinical presentation, laboratory values and also CSF analyses influenced the diagnosis. To eliminate this source of bias we have calculated logistic regression models including only patients
with microbiologically confirmed etiology (page 7, lines 8-11) which yielded a similar result. In the revised version of our manuscript this important critique is now discussed (page 11, lines 20-22).

4. Was lactate not included in CSF measurements?
Lactate is not routinely measured in our laboratory.

5. Why were the primary tumors for LM not included in table 2?
Figure 2 now includes the primary tumors of patients with LM.

6. CSF parameters for differential diagnosis of the 3 disease entities that the authors include in the study have been well described in the past. In that sense this study is not innovative. It may improve the clarity of the paper if these results are omitted and add a short comment on this part of the study in the discussion.
We fully agree with the reviewer that the role of CSF parameters in the differential diagnosis of inflammatory CNS diseases has been extensively studied. Since this aspect of CSF analyses was not the primary focus of the current manuscript, the data (figure 1, results paragraph 1) is omitted in the revised version of our manuscript. A short comment has been added to the discussion (page 9, line 9).

7. Figure 2 and table 3 could include the other CSF parameters.
As suggested by the reviewer we have included the results of the univariate analyses of the other parameters in table 3 in the revised version of our manuscript.

8. Was the choice of cut-off point of 0.75 for IgG index based on clinical or statistical reasons?
The choice of the cut-off value is based on statistical reasoning. Receiver operating characteristic curves were drawn and the cut-off value was chosen to reach maximum specificity.

9. It does not become clear how the IgG-index develops over the course of disease in the follow-up lumbar punctures.
Due to that valuable comment we have reevaluated the data analyzed the course of the IgG-index in all patient groups. Interestingly, patients with bacterial meningitis
showed gradually decreasing levels of the IgG-index in follow up CSF samples. In contrast patients with viral meningoencephalitis showed an increase in the third CSF sample. No differences were observed in patients with LM. In the revised version of our manuscript a figure has been added showing the course of the IgG-index (figure 2). These findings are also discussed in the revised version of our manuscript (page 10, lines 10-14).

10. It would be interesting to include ideas on how IgG index may be incorporated in existing clinical work-up for bacterial and viral meningitis. This critique has been discussed in the revised version of our manuscript (page 11, lines 9-13).

11. As early detection of markers is necessary for rapid initiation of appropriate treatment, the paper should mention the time to result of the significant CSF parameters. Immunoglobulin indices are now usually done by fully automated analyzers. The average time to result is about 15-20 minutes. Therefore this very rapid test is easily applicable in the emergency setting. We have discussed this important information in the revised version of our manuscript (page 11, lines 9-13).

12. The conclusion may be moderated by adding the need for prospective studies to consolidate the findings in this paper. Added (page 12, lines 4-6).

minor essential revisions
1. In table 2, the causes of bacterial meningitis add up to 100.2% This is due to a rounding error.