Author's response to reviews

Title: Divergent cerebrospinal fluid cytokine network induced by non-viral and different viral infections on the central nervous system.

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Author's response to reviews: see over
To:
Dr Seweryn Bialasiewicz
BMC Infectious Diseases

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Belo Horizonte, June 22\textsuperscript{nd}, 2015

Dear Editor,

We have received your e-mail with the Reviewers’ comments regarding our manuscript, (REF.: MS: 9901075531614645), entitled "\textit{Divergent cerebrospinal fluid cytokine network induced by non-viral and different viral infections on the central nervous system.}" by Bastos and colleagues submitted to the section of BMC Infectious Diseases.

We are presenting below all the queries made by the reviewers as well as the way we have worked to perform all requested changes. The changes requested were clearly outlined in the revised manuscript and marked in yellow. We have prepared a list of answers to the Reviewers’s comments, which are highlighted in “\textbf{bold italic}”. In the response to each query, we are also including the modified part, as it is in the revised manuscript.

\textit{Editor's Comment:}

\textit{The reviewers identified several key areas, which need to be addressed by the authors: The clarity of the writing and spelling/grammar, the inclusion of additional data in the results, and the implications of undiagnosed viral infections on the categorization and analyses of the data.}

We acknowledge the editor’s comment. We have worked on the revised manuscript thoroughly in order to address each and every issue highlighted by the Editor and reviewers throughout the manuscript.

\textit{The CSF samples were screened for a substantial, but not exhaustive, number of viral agents. For example, Polyomaviruses JC and BK and parechovirus have been occasionally shown to be associated with meningitis or other atypical CNS infections. If possible, these agents should be tested for and included in the analyses.}
We acknowledge the editor’s comment. Unfortunately, we are unable to test the patients again because our Ethics committee did not approve a second CSF collection.

Reviewer #2’s main concern is the possibility of the presence of viral agents which have not been tested for in the "virus negative" category confounding the analyses (eg: results where no associations between sample categories are found). This is a valid concern, however it is also appreciated that it would be very difficult to conclusively prove that a sample is free from any etiological viral agent. Therefore, a sufficient compromise would be to change the category definitions from "non-viral" to "virus not detected". So for example, the subtitle on lines 251-252 could read: "Meningoencephalitis evolving with low cellularity in samples where no virus was detected shows an imbricate cytokine network with a strong TNF/IL-17 axis." The change should also be reflected throughout the body of the text.

We acknowledge the editor’s comment and the Reviewer #2’s concern. To solve this query, we have changed the category definition from "non-viral" to "virus not detected" throughout the body of the text as advised by the Editor. In addition, the sentence “presence of virus” was replaced by “detection of virus” throughout the text.

Minor point:
"EV genus" in the results section should be spelled out the first time in-text

We apologize for this error. It is corrected in the Methods section of the revised manuscript as follows:

“The detection of the Enterovirus genus (EV) was performed as described elsewhere [19].”

Reviewers' comments:

Reviewer #1: Major revisions:
1) The abstract needs to be revised substantially. The statement that virus-associated and non-infectious meningoencephalitis are not treatable is not correct. There is treatment for Herpes simplex, Varicella zoster, CMV and for autoimmune encephalitis (NMDA receptor, ADEM, etc). I would clarify. The results section do not state how many patients were studied, how many had the different viruses. The results in the abstracts are not followed by P values.

We acknowledge the Reviewer #1 for the suggestion. We apologize for this error. What we really meant is that in the clinical routine for management of patients with meningoencephalitis
in developing countries such as Brazil, virus-positive meningoencephalitis, as opposed to bacterial meningitis, are usually not well diagnosed due to the cost of tests, and therefore only properly treated in cases in which immunodeficiency is associated. This misdiagnoses may lead to death in a few cases. We corrected the abstract as advised by Reviewer #1 as follows:

“Viral meningoencephalitis differs from bacterial meningitis in several aspects. In some developing countries, bacterial meningitis has appropriate clinical management and chemotherapy is available. Virus-associated and virus not detected meningoencephalitis are treatable, however, they may cause death in a few cases. The knowledge of how mediators of inflammation can induce disease would contribute for the design of affordable therapeutic strategies, as well as to the diagnosis of virus not detected and viral meningoencephalitis.”

Regarding the number of patients in each group and P values, these information are now included in the abstract.

2) Sentence in lines 73-75 is awkward and needs to be rewritten and clarified.
We thank the Reviewer #1 for the suggestion. The sentence was rewritten as follows:

“Howeber, some meningoencephalitis patients may test negative for any virus known and are, therefore, considered as virus not detected meningoencephalitis.”

3) Sentences 98-102 regarding the use of biomarkers is unclear and not referenced.
We thank the Reviewer #1 for the correction. We apologize for this error. The reference to this sentence corresponds to references #8 and #9. The sentence was referenced as follows:

“Using these molecules as biomarkers of disease morbidity could improve the prognosis of patients with meningoencephalitis, allowing for a more rapid and accurate follow-up of these patients [8,9].”

4) Line 135, what is ROCV
Rocio virus (ROCV) is an encephalitic flavivirus endemic to Brazil. We have included the full name of the virus in the corrected manuscript as follows:

“For detection of flaviviruses (DENV-1, DENV-2, DENV-3, DENV-4, yellow fever virus (YFV), Rocio virus (ROCV), Ilheus virus (ILHV) and SLEV and alphaviruses (Western equine encephalitis virus (WEEV), Eastern equine encephalitis (EEEV), Venezuelan equine encephalitis virus (VEEV) and Mayaro virus (MAYV)), the methods described by Bronzoni et al. were used [16,17].”

5) **The results do not mention how many patients were diagnosed with the different viral infections**

We appreciate the Reviewer #1’s comment. We have inserted the number of patients who were diagnosed as virus-positive or virus not detected. We have also inserted the number of patients with each of the different viral infections in the results section as follows:

Line 176-177 - “In order to understand the biochemical profile of patients with meningoencephalitis with virus-positive (n=43) or virus not detected (n=80) patients, the levels of protein, glucose and lactate along with cellularity were tested in the cerebrospinal fluid samples of those patients and healthy controls.”

Line 188-190 – “Meningoencephalitis patients with viral infections were subdivided by the viral family - Herpesvirus (Herpes; n=15), Enterovirus (Entero; n=13), Arbovirus (Arbo; n=5) and Lentivirus (Lenti; n=10)”

6) **There are several typos throughout the manuscripts and mistakes (e.g. line 161, meningitis is not capitalized, line 217 "bellow" should be below, change "segregate" to differentiate in line 219, add "and" between mumps and echovirus in line 298-299, line 531 "virus negative" should be "virus positive"**

We thank the Reviewer #1 for the corrections. We apologize for these errors. We corrected all the typos in the revised version of the manuscript.

7) **The manuscript and figures should be revised for clarity.**
We acknowledge the Reviewer #1 for the suggestion. We revised the manuscript thoroughly and we corrected several sentences in order to clarify the text, which are highlighted in yellow in the revised version of the manuscript.

Reviewer #2: Reviewer's report:
The authors described cytokine profiles in CSF from patients with viral vs non-viral meningoencephalitis. Non-infectious etiology was defined as negative CSF PCR for selective viruses (in addition to negative CSF bacterial cultures), but validity of this definition remains unclear. Many patients with presumed viral meningoencephalitis have negative CSF PCR for selective viruses and investigation of infectious etiology for those patients remains a challenge. It is, therefore, not surprising to see overlaps in CSF chemistry, cell counts and cytokines between their virus positive and negative groups.

We appreciate the Reviewer #2’s comment. As highlighted by the Editor, this is a valid concern. In order to solve this query, we have changed the category definition from "non-viral" or “non-infectious” to "virus not detected" meningoencephalitis as suggested.

At last, we believed that the new changes have improved significantly the quality of our manuscript. We would like to thank BMC Infectious Diseases members and reviewers for your attention in revising this article. We sincerely hope that the revised version of our manuscript is now compatible with the high quality of the BMC Infectious Diseases publications and is, therefore, acceptable for publication in this journal.

Kindest regards,

[Signature]

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