Author's response to reviews

Title: A putative role for homocysteine in the pathophysiology of acute bacterial meningitis in children

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Author's response to reviews: see over
To Ms Ma. Celine Zapanta
on behalf of Dr Magdalena Morawska
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A putative role for homocysteine in the pathophysiology of acute bacterial meningitis in children
Roney S. Coimbra Bruno, F. A. Calegare, Talitha M. S. Candiani, and Vânia D'Almeida

Dear Ms. Zapanta,

Thank you for the opportunity to improve our manuscript with the suggestions of Dr. Ken Sakushima and Dr. Thomas Bleck. Below in this letter we answered each question raised by the two referees and presented the actions taken. We hope you will consider this revised version of our manuscript publishable by BMC Clinical Pathology.

Referee 1 – Dr. Ken Sakushima

- To support their suggestion of a role of homocysteine in bacterial meningitis, the authors should include any additional cerebrospinal fluid examination information or sufficient background information of subjects
The cytochemical parameters routinely used for the diagnosis of meningitis were quantified in the cerebrospinal fluid samples and these results are presented in table 1. There was no correlation between the cerebrospinal fluid levels of these canonical cytochemical parameters and the cerebrospinal fluid concentrations of homocysteine, suggesting that this sulphur amino acid is produced intrathecally in acute bacterial meningitis, but not in viral meningitis, as stated and discussed in page 7, lines 155 – 165:

“These results indicate that the HCY and CYS levels were increased during acute BM, dropping to normal levels after cure. No correlations were found between HCY or CYS levels and the standard CSF cytochemical parameters.

This is the first work reporting the association between increased HCY levels within the CNS and the pathophysiology of acute BM. The specific increase in HCY concentrations in the CSF of children with acute BM, and the absence of correlation between HCY and CYS or the standard CSF cytochemical parameters indicate that the local production of HCY in the intrathecal space is part of the host response to the pneumococci or meningococci invasion of the CNS.”

At the time the patients were recruited to this study, CSF lactate testing was not included in the routine of the Children’s Hospital João Paulo II - FHEMIG.

*Action taken:*

No action required.

1. Pivotal role in the pathophysiology of acute bacterial meningitis

In this study, we analyzed cerebrospinal fluid samples collected from children attending the hospital with symptoms and signs suggestive of meningitis. As stated in
page 6, lines 123 to 125, all children survived and only one had permanent hearing loss. There was no correlation between the CSF levels of the canonical cytochemical parameters, including white blood cell count, and the CSF concentrations of homocysteine. We agree with the referee that additional studies are necessary to explain the suggested pivotal role of homocysteine in brain damage caused by acute bacterial meningitis. However, in order to assess the possible association between elevated HCY concentrations and microglia activation and the modulation of other critical pathways in the brain parenchyma, studies using animal models would be required, and this is beyond the scope of this work.

Action taken:

We changed the following sentence of the Conclusions:

Lines 184 – 187: “Further investigations with larger cohorts and additional studies using animal models, are still needed in order to assess the association between increased HCY levels within the CNS and the development of brain damage and permanent neurological sequelae after acute BM.”

2. Concentration dependency of homocysteine

As explained above, in order to assess the association between the elevated concentrations of HCY and neuronal apoptosis and brain damage in acute bacterial meningitis, additional studies using animal models would be required, which is beyond the scope of this work. However, we found that the median concentration of HCY in the CSF of patients with acute bacterial meningitis was higher than the lowest concentration previously reported to induce apoptosis in cultured hippocampal neurons, as we stated and discussed in pages 7 and 8:
Lines 150 - 152: “The median concentration of HCY in the CSF of patients with BM (0.69 µM) was higher than the lowest concentration reported to induce apoptosis in cultured hippocampal neurons (0.5 µM) [16].”

Lines 165 – 168: “The intrathecal accumulation of HCY at potentially neurotoxic levels [16] strongly support the hypothesis that this sulfur amino acid may play a pivotal role in the pathophysiological processes that lead to neuron death and brain damage associated with acute BM.”

Action taken:

No action required

3. Concentration of homocysteine in viral meningitis and controls

This is a very interesting question and we thank referee 1 for raising this point. The differences between the HCY concentrations in patients with aseptic meningitis and in control subjects reported by Qureshi et al. and those that we reported herein may be explained by the fact that Qureshi et al. studied adults while we studied children. Indeed, previously reported reference values for HCY in the CSF from healthy adults ranged from 1.28 to 0.66 µM, while for healthy children these reference values were lower than 0,10 µM.

Action taken:

We added the following sentence to the Discussion, on page 8, lines 172 – 178.

“The HCY concentrations in adult patients with aseptic meningitis and control adults reported by Qureshi et al were higher than those reported herein for children. This may be explained by the differences in the average age of the patients enrolled in these two
studies. Indeed, previously reported reference values for HCY in the CSF from healthy adults ranged from 1.28 to 0.66 µM [20], while for healthy children these reference values were lower than 0,10 µM [21]."

We also added references 20 and 21:


Referee 2 – Dr. Thomas Bleck

1. Line 35: "The excess of homocysteine also induces this cascade of events in hippocampal neurons." I believe that the authors meant to state that "an excess of homocysteine can also induce this cascade...." As stated, it implies that an excess of homocysteine is already known to be present in bacterial meningitis.

   Indeed, we meant that "an excess of homocysteine can also induce this cascade...." and this is the first work to report an excess of homocysteine in the central nervous system during acute bacterial meningitis. We thank the referee for this kind suggestion.

   Action taken:
We have changed the sentence at lines 34 and 35 following the referee’s suggestion.

2. Line 131: What is the lower limit of detection of the assay?

Calibration curves were linear up to 200 µM and 800 µM for homocysteine and cysteine, respectively. The limit of detection for homocysteine was 0.16 µM.

Action taken:

We added the following sentence to the Methods section, page 5, Line 131 and 132:

“Calibration curves were linear up to 200 µM and 800 µM for homocysteine and cysteine, respectively. The limit of detection for homocysteine was 0.16 µM.”

Sincerely yours,

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