Author's response to reviews

Title: Transcranial Doppler to Assess Sepsis Associated Encephalopathy in Critically Ill Patients.

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Author's response to reviews: see over
Answers to reviewers

Reviewer 1

- The authors need to position their contribution more appropriately. There are recent contributions (e.g., http://www.biomedcentral.com/content/pdf/2110-5820-3-28.pdf or http://www.biomedcentral.com/content/pdf/cc8856.pdf) describing very similar ideas. There are other similar contributions. How does this contribution differ from those?

**Answer:** Previous experimental and clinical studies have shown that sepsis is associated with disturbances of cerebral microcirculation. However, the effects of cerebral microcirculation alterations on cerebral function are not clear. The results of our study, as a result of confirming cerebral microcirculation disturbances in septic patients, suggest their correlation with clinical signs of SAE. Importantly, only changes that appeared early were related to clinical signs of SAE. (We added this fact in the discussion section: page 9, last paragraph). Additionally, based on the results of our study, we propose a cut-off PI value that can be used in future studies or even in clinical practice. (We added this fact to the conclusions section: page 12).

- The second major issue is that your two groups have different age distributions. Age is known to affect cerebral blood flow velocities measured by TCD. How do you know that the obtained results are not the side effects of age differences?

**Answer:** The aim of this study was to correlate PI value with the presence of clinical signs of SAE. Even though it was suggested that PI was higher in septic patients compared with non-septic patients, no cut-off value was proposed. Consequently, when we designed the study, it was not possible to form equivalent groups. The analysis of the characteristics of the patients was made based on the results of the ROC curve analysis, where we found that PI>1.3 can predict a positive CAM-ICU test in a non-selected, non-homogeneous population. We agree with the reviewer that differences in age between the two groups of patients is a limitation of our study, since age can affect cerebral blood flow velocities and PI. However, we believe that this factor cannot explain our results since we did not find any correlation between PI and age on the first and third days ($r^2=0.09$, $p=0.06$; $r^2=0.05$, $p=0.16$, respectively). (We added this fact to the results: page 7, first paragraph). Additionally, multivariable analysis using CAM-ICU test as an independent factor revealed that PI on the first day was related to a positive CAM-ICU test, independent of age and APACHE II score. (We added this comment to the discussion of the limitations of the study: page 11, last paragraph).

- The authors should check the appearance of their manuscript before they submit it. Random spacing in sentences and words made very difficult to read.
Reviewer 2

Using transcranial Doppler (TCD) the authors investigated 40 patients with sepsis and found that using a pulsatility index of 1.3 or higher on day one had a sensitivity of 95% and specificity of 88% to predict sepsis associated delirium (SAD). I have several major reservations regarding this manuscript:

• I do not quite understand the authors’ hypothesis. The link between cerebral perfusion and SAD is unclear. Please explain why a higher value of PI on day 1 or 3 should predict SAD?

Answer: We explained our hypothesis better in the introduction section of the paper. Cerebral microcirculation alteration in sepsis is characterized by a decrease in the density of microvessels. Possible consequences of this decrease include neuron dysfunction because of the lack of an adequate oxygen supply and an increase in cerebral resistance. Therefore, cerebral PI is possibly an indicator of cerebrovascular resistances related to clinical signs of SAD.

What is the reasoning for the temporal relationship that you report? Why should a PI on day 1 (or 3) be related to the development of SAD during the first 10 days of sepsis? (p.5, 1st paragraph)

Answer: We evaluated static PI, when the patient was considered to be stable. Considering that PI is related to microcirculation disturbances, an increase in static PI is expected to be related to prolonged microcirculation disturbances and consequently prolonged cerebral hypoxia. Therefore, we expected that clinical effects of this hypoxia would be persistent or even presented later than the time of examination. (We added this fact to the methods section of the paper: page 5, first paragraph).

I would not be able to replicate your experiments. The methods are insufficiently described:

For how many minutes was TCD performed in each patient?

Answer: We performed the TCD evaluation for 10 seconds. (We added this information to the methods section of the paper: page 4, third paragraph).

What were the PaCO2 values during the TCD measurements (when were the values measured shown in table 1?)

Answer: The values shown in Table 1 are the pCO2 values on the first day at the time of examination with TCD. In order to avoid this confusion, we eliminated the values of pCO2 from Table 1 and we added the values of pCO2 on the first and third days to Tables 2 and 3, respectively. We did the same procedure with the MAP values.
How did you perform the multivariate analysis assessing the effects of age and APACHE II. How did you select the variables that you included in your logistic regression? Please show the results of this analysis.

**Answer:** We performed the logistical regression analysis using delirium as a dependent factor (the results of the CAM-ICU test). PI, age, and APACHE II score were evaluated as risk factors. Age and severity of the disease of patients are two factors that could affect cerebral microcirculation but may also be related to SAD. (We added this explanation to the methods section of the paper: page 6, first paragraph). The results of our study showed that PI is a risk factor for a positive CAM-ICU test independent of severity of the disease and age of the patient. We added a table (Table 4) with the results of our analysis.

In how many patients was SAD present at the time of the first determination of PI?

**Answer:** The majority of the patients (16 out of 21) had a positive CAM-ICU test on the day of the first examination. Three patients could not be evaluated on the first day because of sedation. Two of these patients was first evaluated three days later for the first time and one patient was evaluated five days later, where a positive CAM-ICU test was found. Only two patients showed a positive CAM-ICU test on the second day after the first evaluation. (We added this finding to the results section: page 6, third paragraph).

The GCS was significantly lower in the group with a PI > 1.3. Please explain.

**Answer:** GCS, which is presented in Table 1, refers to the first neurological evaluation at the time of the diagnosis of sepsis. GCS values were recorded before intubation in cases where patients needed support from mechanical ventilation. (We added this explanation to the legend of Table 1.) Independent of the reason (hypotension, inflammation, etc.), a lower GCS in patients with high PI compared to other patients may imply a more severe cerebral affection by sepsis. It is possible that these patients showed more severe microcirculatory disturbances earlier, which contributed to persistent cerebral tissue hypoperfusion. (We added this comment to the discussion section of the text: page 10, first paragraph).

Did SAD develop after TCD determination or was it already present at the time of TCD on day 1?

**Answer:** As we discussed above, SAD was already present at the time of TCD in the majority of the patients.

The discussion is superficial: What effects would you expect form mechanical ventilation (control of PaCO2!) and sedation or vasoactive drugs and blood pressure on PI?
**Answer:** The aim of this study was to correlate static values of PI with clinical signs of SAE in critically ill septic patients. We did not aim to investigate the factors that may be involved in the pathophysiology of cerebral microcirculation disturbances. We believe that a differently designed study is needed to evaluate all of these factors related to PI and cerebrovascular microcirculation.

You investigated a heterogeneous group of patients as far the severity of sepsis is concerned. How do you think this influenced your results?

**Answer:** We explored the efficiency of PI at predicting delirium in septic patients. For this reason, we evaluated consecutive patients. Obviously, as a result of our methodology, the group of patients is heterogeneous. We believe that this heterogeneity is the power of our study as PI was found to be efficient at predicting delirium in a non-selected, heterogeneous group of septic patients.

Patients with a PI > 1.3 (Table 1, on which day?) had septic shock more often. How can you be sure, that SAD is not a reflection of more severe disease and the higher PI an epiphenomenon or even unrelated to SAD? Please discuss.

**Answer:** Similar to the case of age, the results of this study cannot exclude the possibility that an increase in PI is a co-founder. However, multivariable analysis revealed that PI is a risk factor for delirium, independent of age and APACHE II score. (We added this to the limitation of the study: page 11, last paragraph).

Your statement that increased PI is indicative of increased cerebrovascular resistance has been challenged repeatedly (e.g. de Riva N, et al. Transcranial Doppler pulsatility index: what it is and what it isn’t. Neurocrit Care. 2012 Aug;17(1):58-66).

**Answer:** We thank the reviewer for making this very important point. Even though PI depends on peripheral resistances, possibly it depends also on the mechanoeelastic properties of the vascular bed (compliance of large cerebral arteries). It may be for this reason that, in cases of increase intracranial pressure, when there is a decrease of cerebral perfusion pressure (CPP), even though we expect a decrease in cerebral resistances, we usually observe an increase in PI. However, when there are no significant decreases in CPP, changes of PI reflect changes in cerebrovascular resistances (Czosnyka M. et al JN 1996, Riva N et al NC 2012). In our study, we evaluated static PI in hemodynamically stable patients; we expected a CPP much higher than the lower autoregulation limit. Consequently, we believe that in our study high PI is an indicator of increased peripheral resistances, possibly because of microcirculation disturbances. (We added this comment to the discussion section of the paper: page 8, second paragraph).

**Reviewer: 3**

This is a study using TCD to exam how cerebral perfusion is altered in sepsis and septic delirium and how the properties of TCD such as CBFl and PI are
altered by the clinical entity. The authors are to be congratulated for their efforts

Writing: the grammar and spelling are appropriate and within norms. The tables and figures are appropriate.

Methods: The sample size is too small. There are no age matched controls.

**Answer:** We agree with the reviewer that the sample size is small. However, the primary goal of the study was to assess the efficiency of PI to predict delirium in septic patients. Therefore, the character of our study is exploratory. We believe that the sample of the study was suitable for the aim of the study, as the prevalence of delirium was not unacceptably high (55%), similar to what has been observed in other studies (Ebersoldt M. ICM 2007).

The study is limited to an observational study only.

**Answer:** We agree with the reviewer that our study is only observational. Nevertheless, we believe that the results of our study are still important as they can contribute to better use of TCD in evaluating septic patients. Additionally, the results highlight the importance of the timing of cerebral microcirculation changes; possibly only early microcirculation disturbances have deleterious effects on cerebral function.

The statistical analysis are appropriate and well executed.

Results: The results are well portrayed and very relevant.

Discussion:
The discussion is overreaching. There are no data to support the conclusion of altered nitric oxide in these patients nor mitochondrial involvement.

**Answer:** We agree with the reviewer. We have eliminated these comments.

The authors should focus on building a case for more study of relevant issues such as correlating findings to cognitive impairment. Future measurements after recovery. The authors should put forward a plan to validate these measures in a future study with an adequate control population.

**Answer:** We totally agree with the reviewer. We think that the results of our study will help in the design of future studies that will correlate cerebral perfusion alterations with long term cognitive impairment.