Author's response to reviews

Title: Assessment of cerebral circulation in a porcine model of intravenously given E. coli induced fulminant sepsis

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Author’s response to reviews:

Simon T Schaefer
Editor
BMC Anesthesiology

Dear Dr Schaefer,

Thank you very much for allowing us to revise our manuscript entitled „BANE-D-16-00306

Assessment of cerebral circulation in a porcine model of intravenously given E. coli induced fulminant sepsis” (Levente Molnár; Norbert Németh; Mariann Berhés; Endre Hajdú; Lóránd Papp; Ábel Molnár; Judit Szabó; Ádám Deák; Béla Fülesdi). In the following we respond point-by-point to the reviewer’s comments. Responses are marked with bold+italic in the response and are marked with red within the manuscript text.

Reviewer #1:
• How exactly augment the results of the presented study the already existing knowledge?
Please discuss your findings critically on the background of the recent literature.

We added to the introduction section: „The majority of the previous observations were performed in humans or in animals with hyperdynamic sepsis and no information could be found on the cerebral blood flow and cerebral vasoreactivity in hypodynamic sepsis models.” Additionally we added to the discussion section: “Similar to our observations, Pranskunas and co-workers reported on a 2-fold increase of the systemic vascular resistance 5 hours after injection of E.coli, accompanied by a gradual decrease of the cardiac index (20). In a recent review models using continuous infusion of living bacteria are described as hypodynamic sepsis models (Kingsley SMK and Bhat BV: Differential paradigms in animal models of sepsis. Curr Infect Dis rep 2016; 18:26). cerebral blood flow and cerebral autoregulation was so far not tested in hypodynamic sepsis.”

• The authors measured blood flow velocities, which in fact do not necessarily represent CBF. The latter depends on variables not recorded during this study, such as intracranial pressure. This limitation must be critically discussed. It has been demonstrated that increasing TCD PI may be positively or also negatively correlated with CVR, depending on surrounding conditions (see de Riva et al., Neurocrit Care. 2012 Aug;17(1):58-66). However, the authors of the herein presented study assume a strict correlation of increasing PI with increasing CVR. Please revise your discussion accordingly, implementing a variable association between PI and CVR. Additionally, the observed decrease in pH, likewise influencing CVR, should also be considered in the discussion section.

Thank you for this comment. To the discussion section we added: „It has to be noted that although PI index is widely accepted as a descriptor of cerebrovascular resistance (Fülesdi B, Siró P, Molnár C: Neuromonitoring using transcranial Doppler under critical care conditions. In: Csiba-Baracchnini (Eds): Manual of Neurosonology Cambridge University Press 2016) a recent study indicated that in certain conditions –especially in gradually raised intracranial pressure with an exhausted autoregulatory reserve- TCD PI may be positively or negatively correlated with CVR. Therefore changes of the pulsatility index should be interpreted with caution (De Riva N, Boduhoiski KP, Smielewski P et al.: Transcranial Doppler pulsatility index: what it is and what it isn’t. Neurocrit Care 2012;17:58-66). In fact, intracranial pressure measurements were not performed in the present study, but in the animals we could not observe any signs of raised intracranial pressure (elevation of blood pressure, bradycardia) and thus the influencing effect of ICP was considered minimal. Another factor that theoretically could influence vasoreactivity during course of our measurements was the slight, but statistically significant decrease of pH. However, acidosis is a vasodilatory stimulus at the level of the cerebral arterioles that would rather lead to decrease in PI. (Fülesdi B, Siró P, Molnár C: Neuromonitoring using transcranial Doppler under critical care conditions. In: Csiba-Baracchnini (Eds): Manual of Neurosonology Cambridge University Press 2016). Finally, it is known that anesthetic agents may also influence cerebral blood flow and cerebral metabolic rate for oxygen. We used ketamine and xylazine in combination in our animals. It is known that ketamine increases global and regional CBF through a calcium-dependent vasodilation and xylazine does not have any vascular effects in clinical doses (Zeiler FA, Sader N, Gillman LM, Teitelbaum J, West M, Kazina CJ: The cerebrovascular response to ketamine: A systematic review of the animal and
human literature. J Neurosurg Anesthesiol 2016;28:123-140). They were used both in the control and the septic group, therefore their effect on the hemodynamic changes observed in septic animals could be excluded.”

- Please discuss why induction of sepsis led to increasing SVRI, as others have reported contrary results (e.g., see Ridings et al., J Invest Surg. 1995 Mar-Apr;8(2):115-22).

We added to the discussion section: “These observations are in accordance with the results of previous porcine experiments using E. coli (19, 20, 21, 22). Similar to our observations, Pranskunas and co-workers reported on a 2-fold increase of the systemic vascular resistance 5 hours after injection of E.coli, accompanied by a gradual decrease of the cardiac index (20). In a recent review models using continuous infusion of living bacteria are described as hypodynamic sepsis models (Kingsley SMK and Bhat BV: Differential paradigms in animal models of sepsis. Curr Infect Dis rep 2016; 18:26).”

- Is there a reason for including 9 individuals into the control and 10 into the sepsis group? Were there animals excluded from the analysis retrospectively?

There was a technical reason for this. One animal from the control group was exluded before starting the experiment (injured during transport)

- p7, 3rd paragraph: "… at a minimum of xy °C." Please revise.

Corrected, thank you.

- Please explain when exactly the second measurement (T2) was performed (in relation to the infusion of bacteria).

For sake of clarity and also based on the suggestion of reviewer 3 time points are now marked with minutes after starting the infusion of E. coli or saline.

- Please indicate if baseline parameters between control and sepsis group differed significantly. There seem to be differences in, e.g., SVRI (4084 vs. 2658) and SVV (15 vs. 10). If so, please explain why. Were control animals possibly volume depleted prior to beginning of the experiment?

Animals were fed and kept under the same circumstances, their selection was based on randomisation on the day of the experiment. We do not have explanation for this. Possibly the individual variation in this small sample size influence these results.

- Table 1 and 2 should be presented in the same way, with pulse rate values at the top. Corrected, thank you.

- p10, 3rd paragraph: according to Table 2, SVV showed no decline but rather an increase during development of septic shock.
Corrected, thank you.

Reviewer #2:

- Please explain why you have used three different statistical tests for continuous variables? why not just using Mann-Whitney Test (also safe when data is not normally distributed)?

Statistical analysis was performed by involving a statistician. According to his suggestion, we performed normality tests first, and t-test was used for normally distributed whereas Mann-Whitney for nonparametric values as was suggested by him. We wanted to ensure the methodological correctness of our analysis.

- Please discuss the possibility that the observed effect on cerebral vasoconstriction of arterioles may be specific to the used anesthetic drugs.

Thank you for this suggestion. To the discussion section we added: „Finally, it is known that anesthetic agents may also influence cerebral blood flow and cerebral metabolic rate for oxygen. We used ketamine and xylazine in combination in our animals. It is known that ketamine increases global and regional CBF through a calcium-dependent vasodilation and xylazine does not have any vascular effects in clinical doses (Zeiler FA, Sader N, Gillman LM, Teitelbaum J, West M, Kazina CJ: The cerebrovascular response to ketamine: A systematic review of the animal and human literature. J Neurosurg Anesthesiol 2016;28:123-140). They were used both in the control and the septic group, therefore their effect on the hemodynamic changes observed in septic animals could be excluded.”

- The author should also state that these results may not be verifiable in humans, since the animals were healthy and young in contrast to patients with sepsis, who represents a heterogeneous cohort of critically ill patients with co-morbidities and concomitant medications.

We added to the discussion as suggested: „The results should be interpreted with caution as results gathered from animal models may not be verifiable in humans, since animals are usually young and healthy in contrast to patients with sepsis who represent heterogeneous cohort of critical illnesses with wide co-morbidities and concommittant medications.”

Reviewer #3:

- The control group should have received culture medium without bacteria, saline is not optimal for such experiment.

For suspension purposes a product from the bioMérieux was used that contains physiological saline infusion (now added to the text in the Methods section). This is the explanation why we decided to use saline in the control group.
Each time before administrating E.coli, blood should have been drawn for analysing few inflammatory markers also. This was the part where author missed opportunity to detect any possible interactions between induced infection and host immune system.

Thank you for this comment. Originally the study was started for assessing the hemodynamic changes (especially cerebral hemodynamics) during experimental sepsis. Additionally we also performed laboratory tests for the assessment of hemorheological factors. However, as they were beyond the main topic of the present study, they were published in a separate manuscript elsewhere (Németh N, Berhé M, Kiss F, Hajdú E, Deák A, Molnár Á, Szabó J, Fülesdi B: Early hemorheological changes in a porcine model of intravenously given E coli induced fulminant sepsis. Clin Hemorheol Microcirc 2015;61:479-496).

Author should have mentioned that if he evaluates any sign for bacterial/medium accumulation which could easily cause death in septic animals.

We did not evaluate bacterial/medium accumulation.

Author reported that out of 10 septic animals, only 7 died, but did not provide possible explanations for the ones which survive the similar treatment. Table 2 provide summary of the whole septic group. In my opinion, the ones which survive septic shocks harbour the great interest and should have been checked at least for Haematological/Liver dysfunction marker.

Thank you for this suggestion. In fact, we missed this opportunity and hemodynamic parameters may not give a good explanation for their survival. Again, the main topic of the present study was the assessment of cerebral and systemic hemodynamic changes during induced sepsis and therefore many important immunological and non immunological laboratory parameters were not tested. We do regret this.

I would suggest writing time points of measurements as T0, T60, T120, T180… instead of T1, T2, T3…otherwise it's misleading as sample numbers.

Corrected, according to your suggestions.

Although not necessary but could attract more interest, if author would check if there was any viable E.Coli at early and late administrating phase.

Thank you for this suggestion: We checked it and added a sentence to the Methods section: „According to our laboratory tests, at 3 hours after suspending the E.coli, the number of the living bacteria remained stable.”

For the reader outside of clinical sciences, normal ranges for the observed parameters should be mentioned in tables.

We are not aware of normal values of these hemodynamic parameters published in pigs. For instance, for the PiCCo measurements a medium-sized catheter was used that is usually suitable for axillary artery measurements in human PiCCo measurements. Thus, we did not provide the
usual human normal values. In a previous study by Pranskunas (ref 20) using PiCCo also used self-control data. We therefore thought that the values gathered from control animals could serve as normal (or reference) values.

Again, thank you very much for the thorough work on our manuscript. We hope that in its present form the manuscript will meet the requirements of the journal.

With compliments

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