Author's response to reviews

Title: Hypogelsolinemia, a disorder of the extracellular actin scavenger system, in patients with multiple sclerosis

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Author's response to reviews:

Editor, BMC Neurology

Dear Professor Drulovic,

Thank you for your e-mail regarding our manuscript: Hypogelsolinemia, a disorder of the extracellular actin scavenger system, in patients with multiple sclerosis” MS:1718012066383170. We are pleased to respond to the suggestions of the reviewers. To address the reviewers' concerns we have included a more detailed description of the MS patients and the control group. Additionally, to meet the request of Reviewer 2, in table 1 we have included the albumin CSF/serum quotient (QAlb). We would like to thank the reviewers for their help and hope that the included new data and modifications adequately address all concerns.

Thank you,

Robert Bucki

Response to Reviewer 1

Because, GSN and DPB concentrations in CSF did not significantly differ between those two groups, the sentence “average CSF gelsolin concentrations in MS were lower (2.9±1.8 versus 4.0±1.7 µg/ml) and DPB levels were higher (1.6±0.9 versus 1.4±0.8 µg/ml) compared to the control group” must be remove from the abstract.

As requested, the sentence “average CSF gelsolin concentrations in MS were
lower (2.9±1.8 versus 4.0±1.7 µg/ml) and DBP levels were higher (1.6±0.9 versus 1.4±0.8 µg/ml)” was removed from the abstract

24 CSF samples from MS disorder? And how many controls? Age, sex of patients? Are both groups matched on age and sex?

Information regarding the numbers of patients included in this study, their age and sex is included in Table 1. We have collected CSF and blood samples from 56 subjects suffering from MS. However due to volume limitation (as we mention on page 6 of the manuscript) we were able to perform simultaneous analysis of gelsolin and DBP concentration in 24 samples only, which explains the lower number of MS subjects used for correlation analysis (Figure 3). According to previous studies, aside from sex hormone concentration, the sex of the subject is not an important factor accounting for the CSF composition. (Reiber H. 1998 Cerebrospinal fluid-physiology, analysis and interpretation of protein patterns for diagnosis of neurological diseases. Mult Scler 4, 99-107). On the other hand, the blood-brain barrier permeability increases during adolescence, but there were not significant differences between average age of MS and control group subjects included in our study and the QALB values do not indicate any dysfunction of the blood/CSF barrier in any group of the included subjects.

24 CSF samples and how many blood samples, 56 samples from MS? And how many controls?

From each person included in the study we collected CSF and blood samples on the same day. This information is provided in Table 1.

The author may include recent data about depletion on geloslin levels in CSF from Alzheimer’s disease patients (Antequera et al, Neurobiol Dis. 2009 Oct;36(1):42-50.)

The data from the recent study by Antequera et al. (Neurobiol Dis. 2009 Oct; 36(1): 42-50.) are discussed in the new version of the manuscript (page 11).

Response to Reviewer 2

In this manuscript, Kulakowska et al. measured gelsolin (GSN) and Vitamin D binding protein (DBP) as indicators of the actin scavenger system in blood and cerebrospinal fluid (CSF) of patients with multiple sclerosis (MS).

The authors used Western blot for analysis of GSN and ELISA for DBP.

The authors found that decreased serum levels of GSN are associated MS, and conclude that this protein may be involved in chronic inflammation associated with neurodegeneration.

Although the preliminary character of the study does not allow drawing a meaningful conclusion yet, the findings of this study warrant systematic
longitudinal and prospective studies to further understand the role of GSN in the pathogenesis of MS.

Major concerns:

Main methodological limitations of this study are

1. cross sectional nature (longitudinal changes can not be excluded),

We agree that over the course of MS progression the concentration of plasma gelsolin in blood and CSF may differ. Indeed, up-regulation of gelsolin was reported for a MS pediatric group (ref 33 in current version of manuscript) suggesting that during MS development, this protein may increase at some stage of the disease. However, in the context of our study, evaluation of blood and CSF gelsolin concentration to assess longitudinal changes will be very challenging as lumbar puncture is performed usually only in the “first diagnosed” patient group. Therefore the study with repeated lumbar puncture is very unlikely to be approved.

2. small cohort of patients (MS is a heterogenic disease),

We agree that MS is a heterogenic disease, however our study included a defined population of patients at an early stage with the same clinical type of MS. There is not a clear definition about what group of patients can be considered small in a MS study. The group of 56 subjects is sufficient for the conclusions of our study, which should be considered as a preliminary report.

3. lack of detailed clinical description of relapse activity,

Clinically, all MS patients included in the study represent category 1 or 2 according to McDonald criteria. All of them have a history of at least two neurological attacks (indicative of dissemination in time). Therefore, the lumbar puncture was performed to receive a definitive diagnosis of MS.

4. lack of data on MRI brain lesions,

Single MRI shows T2-hyperdense lesions in different parts of the brain. In our study we investigate changes in gelsolin concentration in relation to inflammation (indicated by the presence of oligoclonal IgG bands) rather than correlation of gelsolin concentration with MRI images.

5. lack of other MS subtypes representing early (first attack) and advanced disease (secondary progressive MS) stages, and finally

As we stated in response to point 2, our study included patients first being diagnosed with MS. A more detailed description of MS subjects is now included in the revised version of the manuscript (please see page 11). Usually there is no indication to perform the lumbar puncture in subjects with secondary progressive MS.

6. lack of other inflammatory disease controls.
Based on several previous clinical reports showing a decrease of plasma gelsolin in association with the inflammatory response we can assume that such a decrease will occur in other neurological conditions. Indeed in our previous study (Eur J Neurol 2008, 15(6): 584-588) we observed lower levels of plasma gelsolin in patients with lymphocytic meningitis and neuroborreliosis.

Minor concerns:

1. Numbers of samples or patients should be given in the abstract.

As requested we included those numbers in the new version of the abstract

2. To get an impression of the clinical activity presence of relapse or time between sampling and last relapse would be of interest.

All included MS patients were at least 30 days from their last neurological attack.

3. In Table 1, presence or prevalence of oligoclonal IgG bands should be included, since this diagnostic parameter is the only indicator of the inflammatory process found in the CSF of MS patients.

As requested this information is included in table 1

4. If possible data on the CSF/serum ratio of albumin to assess the state of the blood-CSF-barrier function in MS and control patients should be given in Table 1. This would be more appropriate than total protein, especially since mobilization effect of the actin scavenger system across the blood-CSF barrier is discussed as a potential mechanism for the reduced GSN in blood of MS.

Albumin CSF/serum quotient (QAlb) values are included in table 1. Based on QAlb values we conclude that the function of the blood-CSF barrier was not compromised in the MS patients included in the study.

5. Potential causes for the reduced GSN should be discussed more cautiously, since there is no convincing evidence from the data presented or from the literature. In contrast, the authors offer an argument against by “However, accumulation of EASS protein in the CNS would probably result in the presence of significantly higher intrathecal gelsolin and DBP levels, which was not observed."

We agree with the Reviewer’s opinion and in the revised version of the manuscript we have omitted the above sentence.

6. Please discuss the limitations of Western blot assay, as it is more difficult than ELISA to standardize. Furthermore Western blot data have to be regarded
Determination of gelsolin concentration was performed using WB technique according to previously described techniques (Blood 2002, 100(13):4367-4371). There is not a commercially available ELISA kit that can be used for this purpose. For unknown reasons the efforts to develop a gelsolin ELISA undertaken by Critical Biologics Inc. were not successful, likely due to low reactivity of anti-gelsolin antibody after its attachment to the detection plates.

Response to Reviewer 3

The role of actin-related pathology and of the actin-scavenger system is emerging as a new treatment target for neurodegenerative and neuropsychiatric disease. The study by Kulakowska et al. on gelsolin levels in MS patients is therefore a timely and interesting report.

My major concern is that the methods used for measuring F-actin and especially for measuring gelsolin seem rather crude. The authors should comment on why they did not use/establish an ELISA to measure gelsolin in blood and CSF? Could this still be done with the available samples? In the methods, they do not report on essential control experiments ('spiking' of samples; replicate analyses; reference materials etc.).

It would be interesting to test a possible correlation between blood and CSF levels (F-actin, DBP, gelsolin). It would also be interesting to test the correlations between each of these measurements (especially gelsolin with F-actin and gelsolin with DBP in both blood and CSF).

Determination of gelsolin concentration was performed using WB technique. There is not a commercially available ELISA kit that can be used for this purpose. For unknown reasons the efforts to develop a gelsolin ELISA undertaken by Critical Biologic Inc. were not successful, likely due to low reactivity of anti-gelsolin antibody after its attachment to the plates. The presence of F-actin was evaluated qualitatively, based on fluorescent phalloidin signal detected using a microscope in a limited number of CSF samples. As indicated in the methods section this evaluation required at least 250 µl of CSF. For this reason at this point it is not possible to perform a correlation analysis.