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Cytokine CXCL2 concentration in the cerebrospinal fluid of patients with aneurysmal subarachnoid hemorrhage is associated with patient-reported headache pain

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Introduction

Subarachnoid hemorrhage (SAH) is a severe type of stroke categorized by a sharp, sudden, and persistent headache.

The mechanism of headache after SAH is currently poorly understood but the neuro-inflammatory response has been identified as a target in understanding the causes of headache after SAH.

A better understanding of this mechanism may lead to identification of molecular targets for therapeutic reduction of headache pain and improving outcomes after SAH.

In this study, we collected cerebrospinal fluid (CSF) from patients hospitalized with SAH and conducted a cytokine array to screen for soluble factors involved in SAH-associated headache

Hypothesis

We hypothesized that analysis of associations between headache pain after SAH and soluble inflammatory factors in CSF samples will lead to novel targets to explore for future treatment of headache after SAH

Methods

- Clinical collaborators drained waste CSF from an external ventricular drain (EVD) from n=12 patients hospitalized with SAH on post-bleed days (PBD) 1, 3, 5, 7, and 10
- The sample population was an average age of 63 (52-69) years and 75% female.
- Time between collection and processing of samples was minimalized to reduce sample degradation. Processed CSF was stored at -70°C for further testing
- Headache severity was calculated using the Numerical Rating Scale scores (ranges 1-10 with 10 being the most severe), scores were provided by MMC clinical collaborators.
- Frozen PBD5 CSF samples were shipped to an outside laboratory for quantitative proteomics analysis to determine the concentration of 640 human cytokines
- CSF cytokine targets identified by screen were analyzed by Enzyme-linked immunoassay (ELISA) to validate correlation results
- Spearman correlation was determined using median NRS score on PBD 5. Data analysis was performed using GraphPad Prism v7 and a p-value <0.5 was significant.

Results

- High-throughput quantitative analysis of CSF samples of patients with SAH suggest that growth-regulated oncogene (GRO) cytokines including CXCL1, 2, and 3 may be a factor in headache pain (Fig. 1).

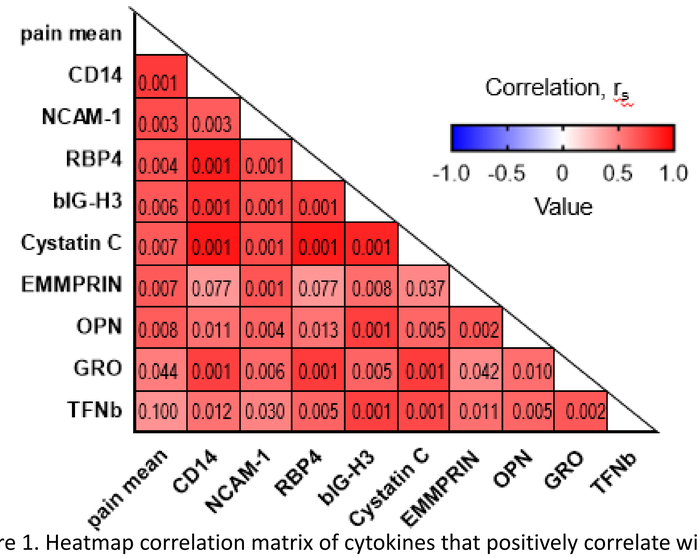

Figure 1. Heatmap correlation matrix of cytokines that positively correlate with pain scores. Data was collected from high-throughput quantitative analysis for samples collected on PBD5. P-value shown in each box n=20

- We found a positive correlation between CXCL2 levels in CSF and NRS pain scores on PBD5 (r_s=0.622; p=0.031) (Fig. 2) upon ELISA verification

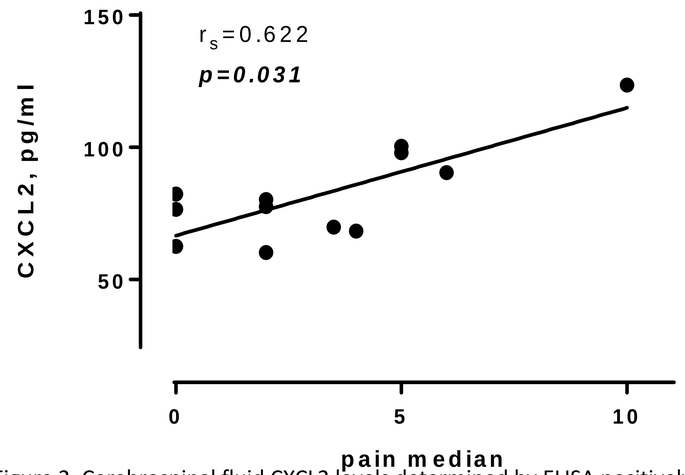

Figure 2. Cerebrospinal fluid CXCL2 levels determined by ELISA positively correlate with NRS pain score median at PBD5. Spearman correlation coefficient and P-value, n=12

- ELISA analysis indicated that CSF CXCL2 concentrations were dynamic throughout the 10 day period and peaked on PBD5 compared to PBD 1 (p=0.015), PBD 3 (p<0.001), and PBD 7 (p<0.001) (Fig. 3).

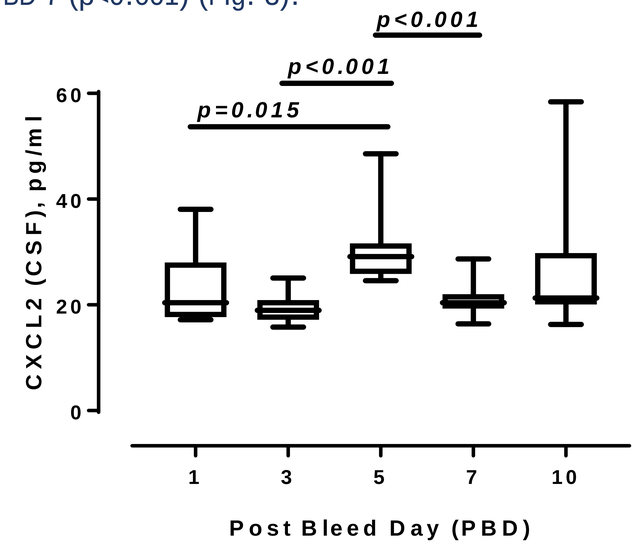

Figure 3. Cerebrospinal fluid CXCL2 levels determined by ELISA increase significantly on day 5, compared to days 1, 3, and 7 . n=20

Conclusions and Discussion

- This study suggests CXCL2 concentration may be associated with headache severity after SAH in patients treated with an EVD. It also supports the hypothesis that headache may be caused by neutrophil-mediated neuro-inflammation.
- CXCL2, also known as GRO beta, is a chemokine primarily produced by neutrophils that induces neutrophils into inflamed tissue.
- Previous analysis of CSF samples from PBD5 showed elevated protein levels, compared to PBD 1,3,7, and 10. Further analysis of white blood cell subpopulations to evaluate the associations between neutrophil populations and CSF CXCL2 is needed
- Medications that suppress GRO CXCL mediated neutrophil chemotaxis, including colchicine, represent a promising way to target the underlying mechanism of SAH headache.

Acknowledgements

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