S100B and NSE in cluster headache - evidence for glial cell activation?

Agneta H. Snoer¹, MD, PhD; Anne Luise H. Vollesen¹*, MD; Rasmus Paulin Beske¹, MD; Song Guo¹, MD, PhD; Jan Hoffmann², MD, DMSc; Niklas R. Jørgensen³⁴, Prof, MD, DMSc; Torben Martinussen⁵, prof.; Messoud Ashina¹, Prof, MD, DMSc; Rigmor H. Jensen¹, Prof, MD, DMSc.

¹ Danish Headache Center and Department of Neurology, Rigshospitalet-Glostrup, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark
² Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom
³ Department of Clinical Biochemistry, Rigshospitalet-Glostrup, Denmark
⁴ OPEN, Odense Patient Data Explorative Network, Odense University Hospital/Institute of Clinical Research, University of Southern Denmark, Odense, Denmark
⁵ Section of Biostatistics, University of Copenhagen, Denmark

* The first two authors contributed equally to the study

Word count for main body: 3727
Word count for abstract: 350
Number of tables: 2
Number of figures: 4
Key words: Cluster headache, neuronal specific enolase, NSE, Protein S100B, headache

Corresponding author:
Agneta Snoer, MD, PhD
Danish Headache Center, Department of Neurology, Rigshospitalet Glostrup
Faculty of Health and Medical Sciences, University of Copenhagen
Valdemar Hansens Vej 5, DK-2600 Glostrup, Denmark
Tel: +45 38 63 33 85
E-mail: agneta.henriette.snoer.02@regionh.dk

Financial Disclosure Statement
The authors declare following potential conflicts of interests with respect to research, authorship and/or publication of this article:

Agneta H. Snoer has received honoraria from AstraZeneca for lecturing and is a sub-investigator on clinical trials sponsored by Eli Lilly.

Anne Luise H. Vollesen has received personal fee from Teva Pharmaceutical Industries.

Rasmus Beske reports no disclosures.

Song Guo reports no disclosures.

Jan Hoffmann has consulted for and/or serves on advisory boards for Allergan, Autonomic Technologies Inc (ATI), Chordate Medical AB, Eli Lilly, Hormosan Pharma, Novartis and Teva Pharmaceutical Industries and received honoraria for speaking from Allergan, Autonomic Technologies Inc. (ATI), Chordate Medical AB, Novartis and Teva Pharmaceutical Industries.

Niklas R. Jørgensen reports no disclosures.

Torben Martinussen reports no disclosures.
Rigmor H. Jensen has given lectures for Pfizer, Berlin-Chemie, Norspan, Merck, Autonomic Technologies (ATI), Teva and Novartis; and a principal investigator on clinical trials sponsored by Eli Lilly, Teva and ATI.

Messoud Ashina is a consultant, speaker, or scientific advisor for Allergan, Amgen Inc, Alder BioPharmaceuticals, ATI Technologies, Eli Lilly and Company, Novartis, and Teva Pharmaceutical Industries and is primary investigator for Amgen 20120178 (phase 2), 20120295 (phase 2), 20130255 (OLE), 20120297 (phase 3), Alder ALD403-CLIN-001 (phase 3), Amgen PAC1 20150308 (phase 2a), and GM-11 gamma-Core-R trials.

Funding

The work has been supported by grants from The Lundbeck Foundation (grant R155-2014-171) Novo Nordisk Foundation (grant NNF11OC101433), Tryg Foundation and Research Foundation of Rigshospitalet.

Acknowledgements

The authors would like to thank all participating patients in the study. Without their willingness to subject themselves to a potential cluster headache attack this study would not be possible. The authors would furthermore like to thank study nurses Mette Frank Fisker and Mette Bisgaard for helping with recruitment of patients and handling of blood samples on study days.

Abbreviations

NSE    - Neuronal specific enolase
S100B  - Protein S100 beta
CNS    - Central nervous system
BBB    - Blood brain barrier
CH     - Cluster headache
eCHa   - Episodic cluster headache in bout
Abstract

**Objective:** Neuronal specific enolase (NSE) and protein S100B have gained considerable interest as markers of CNS injury, glial cell activation and/or blood brain barrier (BBB) disruption. No studies have investigated NSE and S100B in cluster headache (CH), but these biomarkers could contribute to understanding of CH.

**Methods:** Patients with episodic CH in bout (eCHa), in remission (eCHr) and chronic CH (cCH) were included in this randomized, double-blind, placebo-controlled, two-way cross-over provocation study carried out at the Danish Headache Center. The primary endpoints included 1) Differences of NSE and S100B in between groups (eCHa, eCHr and cCH) at baseline; 2) Differences over time in plasma concentrations of NSE and S100B between patient developing an attack and those who did not; 3) Differences in plasma concentrations over time of NSE and S100B between active day and placebo day. Baseline findings were compared to historical data on migraine patients and healthy controls and presented with means ± SD.
Results: Nine eCHa, 9 eCHR and 13 eCH patients completed the study and blood samples from 11 CGRP-induced CH attacks were obtained. There were no differences in NSE levels between CH groups at baseline, but CH patients in active disease phase had higher levels compared with 32 migraine patients (9.1± 2.2 µg/l vs 6.0± 2.2 µg/l, p<0.0001) and 6 healthy controls (9.1± 2.2 µg/l vs. 7.3± 2.0 µg/l, p=0.007). CGRP-infusion caused no NSE changes and, but a slight, non-significant, increase in NSE was seen in patients who reported a CGRP-induced CH attack (2.39 µg/l, 95% CI [-0.26, 3.85], p=0.061). At baseline S100B levels in eCHa patients were higher compared to cCH patients (0.06± 0.02 µg/l vs. 0.04± 0.02 µg/l, p=0.018). Infusion of CGRP and CGRP induced attacks did not change S100B levels. Apart from induced CH-attacks no other adverse events were noted.

Conclusions:

At baseline eCHa patients had higher S100B plasma levels than cCH patients and there was a slight, however not significant, NSE increase in response to CGRP induced CH attack. Our findings suggest a possible role of an ictal activation of glial cells in CH pathophysiology, but further studies are warranted.

Introduction

The pathophysiological mechanisms of CH are not fully elucidated, but the distinct clinical features of attacks implicate the trigemino-vascular system and the hypothalamus. Structural imaging studies using voxel-based morphometry and diffusion tensor imaging have identified changes in several brain areas that seem dynamically influenced by disease state (in bout, remission or chronic) (1). As there are no pathology studies describing the histological correlates to these findings, it is unknown whether these represent morphological changes in neurons or glial cells or merely intra- or extracellular fluid shifts (2). Neuronal specific enolase (NSE) is a cytoplasmic enzyme present in all types of neurons and neuroendocrine cells in the central and peripheral nervous system (3). Within nervous tissues NSE exist in two forms: The \( \gamma \gamma \) isozyme, which is predominantly located in neurons and the \( \alpha \gamma \) isozyme, which is predominantly located in glial cells (4). In contrast, the protein S100 beta (S100B), although not brain specific, is primarily located in the glial cells and most abundantly in astrocytes (5). S100B contributes, among other functions, to neuronal development and brain repair (5). Both NSE and protein S100B have gained considerable attention as markers of CNS
injury, but these markers are also of interest in relation to CH because they may reflect glial cell activation and/or disruption of the blood-brain barrier (BBB) (4). As these aspects have not been studied in CH before, this was the aim of our study. Investigating patients during spontaneous CH-attacks in a hospital setting is extremely difficult given the unpredictable nature of CH. However, CH attacks can be induced in experimental models by nitroglycerin (6–8) and calcitonin gene-related peptide (CGRP) (9), but only in patients in an active disease phase and not in patients in remission.

In the present study we hypothesized that baseline levels of NSE and S100B would be elevated in patients in an active disease state (ie. Episodic patients in bout or chronic patients), compared to patients in remission, and that CGRP-induced CH attacks would cause an increase in NSE and S100B. We conducted a randomized, double-blind, placebo controlled, two-way cross-over study during which we measured NSE and S100B at baseline and during CGRP induced CH attacks (9). In addition, we compared baseline findings with historical data on NSE and S100B collected from migraine patients in an interictal state and healthy controls.

Materials and methods

Patients were eligible for inclusion if they were between 18 – 65 years and had a verified diagnosis of either episodic or chronic CH according to the ICHD-3 (beta) criteria (10). All patients were recruited from the outpatient clinic at the Danish Headache Center (Rigshospitalet-Glostrup) in the period from December 2015 to April 2017. At inclusion episodic CH had to be either in: Current bout (eCHA) defined as occurrence of typical CH attacks within the last 30 days; or in remission (eCHr), defined as attack free for at least 30 days. Chronic patients (cCH) were included if they did not have attack free periods exceeding 30 days in the last 12 months according to ICHD-3 (beta) criteria (10). Additional inclusion criteria were: Weight 50 – 100 kg and use of safe contraception if woman of child-bearing potential. Patients were excluded if they suffered from: Other primary or secondary headache disorders except from episodic tension-type headache ≤ 5 days per month; serious somatic or psychiatric disease; substance abuse; or if they were pregnant or lactating. Episodic patients could participate twice, in remission and in active disease, if they so wished.

The current study was a predefined part of a larger parent protocol, investigating the ability of CGRP to induce CH attacks (11,12). The study was approved by the Regional Committee on Health Research Ethics of the Capital Region (H-15006836) and was registered at clinicaltrials.gov (identifier NCT02466334) and approved by the Danish Data Protection Agency. All participants received oral and written information about the study and were given reasonable time for...
consideration prior to giving the written informed consent for participation. Data for post hoc analyses comparing current findings with migraine patients and healthy controls derived from a study previously conducted at the Danish Headache Center in 2013 (ClinicalTrials.gov identifier NCT01841827). Blood samples from the historical data had been analyzed in the same laboratory facilities using the same assays (13).

Design and experimental protocol

The study was conducted as a randomized, double-blind, placebo controlled, two-way cross-over study. Patients were randomized to receive a 20-minute continuous infusion with either 30 µg CGRP (1.5µg/min) (Calbiochem® and PolyPeptide group) or placebo (isotonic saline) on two separate study days scheduled at least 7 days apart. CGRP and placebo were prepared in identical vials and randomized without restriction by the regional central pharmacy prior to initiation of the study. Allocation was balanced to ensure approximately even numbers of participants receiving CGRP first and placebo last or vice versa. Both patients and investigators were blinded with regards to which type of infusion (CGRP or placebo) a patient would receive on a given study day. At the inclusion visit all patients underwent a full medical examination and medical interview to confirm diagnosis and secure relevant demographical data. On both study days, eCHa and cCH patients had to arrive headache/attack free for 3 and 8 hours respectively and female participants underwent pregnancy testing. An 8-hour headache-free-interval prior to provocation was initially set for both eCHa and cCH patients, but due to feasibility concerns, a revised 3-hour headache-free-interval was set in order to include episodic patients with a high mid-cluster attack burden. The timing of the participants last attack was documented on both study days.

Patients where then placed in a supine position and connected to a cardiac monitor. A venous catheter (Venflon®) was placed in the right or left cubital vein for drawing of blood samples and CGRP infusion. Baseline status (T-10 and T0) was obtained after 15 minutes of rest. At baseline and every 10 minutes throughout the experiment the following variables were collected: pulse; blood pressure; headache intensity on a verbal rating scale (VRS) from 0 to 10 (0; no headache, 1; very mild headache, 10; worst imaginable headache); quality of pain (stabbing, throbbing, pulsating or resembling usual CH attack); headache localization. Symptoms outside of these intervals these were recorded separately. Attacks were aborted using the patients preferred treatment, when the pain was considered severe enough to require treatment. Overview of experimental protocol is
depicted in figure 1 and described in our previous article on effect of CGRP-infusion in CH patients (9).

**Blood collection and processing**

Blood samples for analyses of NSE and S100B were drawn at fixed time points: At baseline (T0), after infusion (T20), 10 minutes (T30) and 70 min after infusion (T90). If the patient in the observation period developed a CH attack, blood was drawn at the beginning of the attack (Ta0), after 15 min (Ta15) and at 30 min after start attack (Ta30). All Ta0 samples were drawn prior to eventual abortive treatment.

Samples were drawn through the venous catheter and connector using 20 ml syringes, after discarding the first 5 ml of blood, and transferred to a 9 ml serum tube (BD Vacutainer®). The catheter was flushed with isotonic saline after each sampling. Tubes were inverted several times and stored at room temperature 20 minutes before being centrifuged at 1851g at 4 °C for 10 minutes. Serum was thereafter transferred to a polypropylene tube (Greiner Cryo.s™) and stored at -25°C until later analysis.

Plasma concentration of NSE was determined using the NSE Elecsys assay (Roche Diagnostics GmbH, Mannheim, Germany). The antibody used in the assay is directed towards the γ subunit of enolase. Plasma concentration of S100B was determined using the S100 Elecsys assay (Roche). Both assays are electrochemiluminescence immunoassays (ECLIA) and were both measured on the automated Cobas e411 analyzer (Roche). The intermediary precisions (CV%) for NSE and S100B were 5% and 6%, respectively.

**Statistical analysis**

We had defined the following primary endpoints: 1) Differences of biochemical variables (NSE and S100B) in between groups (eCHa, eCHr and cCH) at baseline; 2) Differences over time in plasma concentrations of biochemical variables between patient developing an attack and those who did not; 3) Differences in plasma concentrations over time of biochemical variables between active day and placebo day.

As this study was part of a larger protocol investigating the CH inducing capabilities of CGRP, sample size was calculated to specifically for this purpose. Based on previous similar studies in migraine we assumed a 60-70% induction rate to CGRP and 20% on placebo and furthermore set a
with 80% power. With these assumptions we calculated that 15 patients in each group (eCHa, eCHR and cCH), would be sufficient to show a difference between the two study days.

In exploratory post hoc analyses we compared baseline samples from CH patients in an active phase (eCHa and cCH) with historical data from migraine without aura (MO) (n=32) and healthy controls (HC) (n=6). Asides from different male:female ratios, CH patients were comparable to MO and healthy controls with regards to demographical data (13). We furthermore compared baseline values of NSE and S100B on active day between patients who developed a CGRP induced attack, to those who did not. All samples from the historical data had been analyzed in the same laboratory facilities using the same assays.

Group comparisons of demographs data were analyzed using one-way ANOVA or Kruskal-Wallis test depending on distribution of data determined by D’Agostino and Pearson normality test. Group comparisons of baseline data from both study days was calculated using a generalized linear model with repeated measurements. All absolute values are presented as mean ± standard deviation or median and interquartile ranges depending on the distribution of data.

For effect of CGRP infusion on biochemical variables we used repeated measurements analysis with random effect of subjects and further of subject times day. By this we allow for correlation between measurements on the same individual, and additional correlation between measurements on same individual on the same day. Main effects investigated were baseline measurement, time, diagnose, with latter being categorical with 3 levels (no CGRP, CGRP + no attack, CGRP + attack). The measurement taken at time zero was used as baseline variable in the repeated measurements model.

We used GraphPad Prism 7.02, SAS Enterprise and R 3.4.3 for statistical analyses. All p-values were two-sided and considered significant if <0.05.

**Results**

In total 37 patients completed study days 1 and 2, but only 31 were included in the final analysis of biochemical data. Two patients were excluded as they had mild pain in their usual attack area prior to infusion; one patient went in bout between study days 1 and 2; two patients reported comorbid migraine diagnosis after their primary interview; in one patient blood samples were not drawn due
to technical issue (Fig. 2). The remaining included patients (9 eCHa, 9 eCHr and 13 cCH) were on average 37.0 years (range 19 – 59), with a 4:1 male to female ratio and mean BMI 24.9 ± 3.9 kg/m². Apart from chronic patients being older than episodic patients (p=0.029), there were no difference in baseline characteristics (Table 1). Median latency, in hours, from last attack was higher for cCH patients (48.0 (14.5 – 150.0)) compared to eCHa patients (9.5 (4.75 – 66.0, p=0.004). ECHr patients had been outside bout on average 6.6 (range 1.3 – 18.0) months prior to participation in the study. We provoked 16 CH attacks, but only obtained blood samples in 11 of these attacks, because symptoms in the remaining 5 were mild and subsided before we had the chance to engage attack sampling protocol. Of patients in an active disease phase (eCHa and cCH), there was no difference in the proportion of patient using prophylactic treatment (p=0.666). Likewise, we found no difference in proportion of patients using prophylactic treatment in patients who developed a CGRP induced attack vs. those who did not (p>0.999). All attacks observed were on CGRP day, no attacks occurred on placebo day. The clinical characteristics of the 11 attacks are listed in table 2. Apart from expected discomforts related to study procedures (insertion of venous catheter and induced CH attacks), no adverse events took place during the study.

**NSE**

At baseline we found no differences in NSE levels between CH groups (Fig.3). Of main effects investigated in the repeated measurements analysis (baseline measurement, time and diagnose) we found a slight, however not significant, increase in NSE in patients who reported a CH attack after provocation with CGRP (2.39 µg/l, 95% CI [-0.26, 3.85], p=0.061) (Fig. 4). Other main effects investigated were not significant. Analysis of all 36 patients with available biochemical data (including two additional CGRP-induced plus one spontaneous attack) a significant increase in NSE as response to attack was noted (2.37 µg/l, 95% CI [0.14, 3.75], p=0.049). Infusion of CGRP did not cause changes in NSE levels compared to placebo (p=0.266) (Fig. 4).

**S100B**

At baseline we found statistically significant higher S100B levels in eCHa patients (0.06 ± 0.02 µg/l) compared to cCH patients (0.04 ± 0.02 µg/l, p=0.017) (Fig.3). We found no increase of plasma S100B in patients who reported a CH attacks after provocation with CGRP (p=0.102) (Fig.
4). Infusion of CGRP did not cause changes in S100B levels compared to placebo (p=0.563) (Fig. 4). Other investigated main effects were similarly not significant.

**Post hoc analyses**

At baseline CH patients in an active disease phase (eCHa and cCH) had statistically significant higher NSE levels compared to migraine patients (9.1± 2.2 µg/l vs. 6.0 ± 2.2 µg/l, p<0.0001) and healthy controls (9.1± 2.2 µg/l vs. 7.3 ± 2.0 µg/l, p=0.007) (Fig.3). There was no difference in baseline levels of NSE on active day between patients (eCHa and cCH) who later developed an attack compared to those who did not (9.9 ± 2.1 µg/l vs. 9.1 ± 2.0 µg/l, p=0.356).

At baseline there was no difference in S100B levels between CH patients in an active disease phase (eCHa and cCH) compared to migraine patients (0.04 ± 0.02 µg/l vs. 0.04± 0.02 µg/l, p=0.412) or healthy controls (0.04 ± 0.02 µg/l vs. 0.04± 0.02 µg/l, p=0.642) (Fig.3). Baseline levels of S100B were statistically significant higher on active day in patients (eCHa and cCH) who later developed an attack compared to those who did not (0.05 ± 0.03 µg/l vs. 0.04 ± 0.02 µg/l, p=0.046).

**Discussion**

The main findings of our study were that baseline levels of S100B were higher in eCHa patients compared to cCH patients and that there was a slight, however not significant, increase in NSE in response to CGRP-induced CH attacks. In addition, baseline levels of S100B were statistically significant higher in patients who developed an attack in response to CGRP infusion compared to patients who did not. Furthermore we found that NSE, but not S100B, was higher in CH than in migraine patients and in healthy controls.

With multiple cellular functions of both S100B and NSE, these findings raise the important question: What does altered expression in these peptides indicate in CH and which structures represent a potential source? NSE is expressed in small amounts in platelets, but levels are negligible in most other healthy non-nervous tissues (3). As NSE concentrations are high in cytoplasm of neurons, elevated levels could theoretically indicate neuronal damage, which is consistently reported after ischemic stroke and traumatic brain injury as expected. Regardless of the severity of a CH attack, there are, however, no clinical indications of neuronal damage in CH explaining a potential attack-related increase. This renders the possibility that other NSE-related mechanisms are implicated in CH pathophysiology. Bearing in mind, that the assay used in this
study is aimed at the γ subunit of enolase, the hybrid αγ isozyme specific for glial cells might contribute to observed baseline and CGRP induced findings (14). NSE is found in several types of glial cells, but expression of NSE is dependent of type of glial cell and inducing stimulus (4). Cell surface expression of enolase has been detected on activated microglia and astrocytes resulting in, among other functions, production of pro-inflammatory cytokines and chemokines in sites of injury (4). The release of cytokines and chemokines represent a central element in induction and maintenance of neuropathic pain, but also contributes to processes leading to long-term changes in excitability of sensory neurons and ultimately in development of peripheral and central sensitization (15). Inhibition of enolase/NSE in animal models of spinal cord injury has, interestingly, resulted in decreased levels of these pro-inflammatory mediators (16).

Considering S100B is a marker of glial cell activation we found higher S100B levels in eCHa patients compared with cCH patients. As they both represent patients in an active disease phase this is of course thought provoking. However, this might be attributed to a difference in attack burden leading up to provocation. The latency from last attack was statistically significant higher in cCH patients, indicating that attack burden measured in attack frequency in the eCHa patients were higher than in cCH patients. This might explain why we only saw elevated levels of S100B in eCHa patients and not in cCH patients. It might be speculated, that use of preventive treatment influenced baseline data, but we did not see any difference in the proportion of patients using preventives between eCHa and cCH patients, nor between patients in active disease phases (eCHa and cCH) developing a CGRP induced attack vs. those that did not.

Supposing that glial cells are activated in active phases of CH prompts the question which structures may represent a potential source. As neither S100B nor NSE cross the BBB under normal circumstances and as we did not see an attack-related increase of S100B, which has been proposed a marker of early BBB disruption, we may assume that BBB remains intact in CGRP-induced CH attacks and that the potential source is likely to be located outside the BBB (17). Possible peripheral structures that contain glial cells and are known to be involved in CH pathophysiology include the trigeminal (TG), otic and sphenopalatine ganglia (18). As has been demonstrated in other nervous tissues, the application of noxious stimuli to the TG with capsaicin in experimental animal models has been shown to induce the release of CGRP and S100B (19). Thalakoti et al. also found that CGRP causes release of the inflammatory cytokines IL-1β, IL-6, IL-10, cin-3, CNT-F and fractalkine in TG cell cultures (19). Furthermore in another study S100B has also been shown to
induce expression and secretion of IL-6 in neurons (20). Interleukin 1β has been demonstrated to induce CGRP release in TG cell cultures, an effect blocked by administration of methylprednisolone (21). Thus, CGRP can induce release of pro-inflammatory cytokines and these can in turn induce the release of CGRP implying that paracrine signaling between glial cells and neurons in the TG are subjected to a positive feedback loop. Supporting the role of inflammatory mechanisms in CH pathophysiology are reports of elevated IL-1β and IL-2 in CH patients during attacks and interictally by Marteletti et al. and Steinberg et al. (22,23).

In the main study evaluating the CH inducing capabilities of CGRP, we found that only 7/14 chronic patients developed an attack (9). The interesting difference here was, that with regards to attack burden in the month prior to provocation, those who developed an attack had a median of 33 attacks, whereas those who did not only had a median of 7.5 attacks (9). In a post-hoc analysis we found that baseline level of S100B was significantly higher in patients who developed an attack in response to CGRP infusion compared to patients who did not. These observations suggest that provocability may be dependent on the degree of activation of TG cells. In future studies it might be relevant to investigate S100B as a marker for disease activity of CH or provocability of attacks.

In exploratory post-hoc analyses we compared baseline levels of NSE and S100B in CH patients with that of migraine patients and healthy controls. We found that CH patients in an active disease phase had higher levels of NSE, but not S100B, compared with migraine patients and healthy controls. NSE and S100B have been investigated in relation to migraine previously, but findings have been inconsistent and NSE and S100B mostly been interpreted as markers of neuronal damage or BBB disruption (24–29).

Although the current results should be interpreted with caution, as appropriate with historical data, the observed differences between CH, migraine and healthy controls might be relevant.

**Strengths and limitations**

There are some limitations that must be taken into consideration. Both NSE and S100B serum levels might be influenced by age, sex and BMI, although studies on this topic have been inconsistent (30–33). However, apart from cCH patients being older than episodic patients, we did not find any differences in the clinical characteristics of patients, limiting the effect of this bias in our results. Another limitation is our sample size, which was relatively small and calculated primarily to investigate the CH inducing capabilities of CGRP. We had to exclude patients in the final analysis, which rendered the finding of attack related NSE increase insignificant. The findings
from our study should therefore be reproduced in a larger cohort of CH patients as well as during spontaneous or experimentally induced attacks, if possible. The design of the study and application of a provocation model is, though a considerable strength, as it allows for investigation of these biomarkers in a controlled setting. Limiting the value of the historical data is the fact that although collected in a standardized protocol, samples were analyzed in different batches and collected by different investigators.

**Conclusion**

In conclusion we found that at baseline S100B levels are higher in eCHa patients compared with cCH patients and furthermore that there was a small, however not significant, NSE increase in CGRP-induced CH attacks. As S100B was not associated with an attack-related increase we can hypothesize that: The integrity of the BBB is not disrupted by CH attacks and that a potential source of NSE and S100B is structures outside the BBB. Our findings suggest that glial cells, possibly in the TG, are activated in CH and that the resulting inflammatory response may pose a role in CH pathophysiology.

**References**


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Tables and figures:

Figure 1. Overview of experimental protocol on study days.

All patients came in on two separate study days and in a randomized manner received a 20-minute infusion of CGRP one day and placebo (isotonic saline) the other. Blood samples for measurements of NSE and S100B were drawn at fixed time points: At baseline (T0), after infusion (T20), 10 minutes (T30) and 70 min after infusion (T90). If the patient in the observation period developed a CH-like attack, blood was drawn at the beginning of the attack (Ta0), after 15 min (Ta15) and at 30 min after start attack (Ta30). Vital signs and headache symptoms were recorded at 10-minute intervals throughout the experiment and in relation to blood samples if the patients developed a CH-like attack.

Figure 2. Flow chart of recruitment and inclusion of patients
**Figure 3.** Baseline levels of NSE and S100B presented as means and SD.

Dashed lines divide present data from historical data. Significant findings according to primary endpoints are marked. Significant findings in post hoc analyses comparing CH patients to data from historical data: NSE: eCHa+cCHr > MO (p<0.001), eCHa+cCH > HC (p<0.007).

cCH = chronic cluster headache; eCHa = episodic cluster headache in cluster bout; eCHr = episodic cluster headache in remission; MO = Migraine without aura; HC = Healthy controls; NSE = Neuronal Specific Enolase; S100B = Protein S100 B.

**Fig. 4.** Levels of NSE and S100B presented as means and SD.

Effect of infusion is represented as mean NSE/S100B levels at T20 at the end of infusion. Effect of attack is represented as NSE/S100B levels at Ta15 (second attack sample drawn). There are no attack samples on placebo days and no attack samples in remission eCHr patients on CGRP day.

eCHr = episodic cluster headache in remission cCH = chronic cluster headache; eCHa = episodic cluster headache in cluster bout.

**Table 1.** Clinical data on patients with episodic cluster headache in active phase (eCHa), remission (eCHr) and chronic cluster headache (cCH).

M/F = Male/Female, GON blockade= Greater Occipital Nerve blockade, SPG = The Pulsante SPG Microstimulator System
Table 2. Clinical characteristics of the CGRP-induced CH-like attacks in 11 patients.

<table>
<thead>
<tr>
<th>Headache intensity: 0 – 10, where 0 is no pain and 10 is maximum pain on a Verbal response scale.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ta0: Attack onset, prior to acute therapy. Ta15 and Ta30: 15 and 30 minutes after attack onset respectively. Acute therapy: Suma: sumatriptan 6mg sc; Oxy: oxygen 15L/min Optimask; SPG: The Pulsante SPG Microstimulator System; Dic: Diclofenac 25mg sc. Accompanying symptoms: lac: lacrimation; pto: ptosis; mio: miosis; con: nasal congestion; inj: conjunctival injection; swe: forehead and facial sweating; res: restlessness; rhi: rhinorrhea; ede: eyelid edema</td>
</tr>
</tbody>
</table>

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<table>
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<th></th>
<th>Sex</th>
<th>Age (years)</th>
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<tr>
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<td>36</td>
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<td>29.7</td>
<td>11</td>
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<td>Yes</td>
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</table>

Microstimulator System

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<p>| | | | | | |</p>
<table>
<thead>
<tr>
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Table 2. Clinical characteristics of the CGRP-induced CH-like attacks in 11 patients.

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<tr>
<th>ID</th>
<th>Time to onset</th>
<th>Headache intensity Ta0</th>
<th>Peak Headache intensity attack</th>
<th>Acute therapy</th>
<th>Headache intensity Ta15</th>
<th>Headache intensity Ta30</th>
<th>Accompanying symptoms reported</th>
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<td>eCHa01</td>
<td>70</td>
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<td>7</td>
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<td>Suma/oxy</td>
<td>3</td>
<td>0</td>
<td>Con/pto/res</td>
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<td>Lac/pto/res</td>
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<td>10</td>
<td>Oxy/SPG</td>
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<td>2</td>
<td>0</td>
<td>Inj</td>
</tr>
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</table>

Headache intensity: 0 – 10, where 0 is no pain and 10 is maximum pain on a Verbal response scale.
Ta0: Attack onset, prior to acute therapy. Ta15 and Ta30: 15 and 30 minutes after attack onset respectively. Acute therapy: Suma: sumatriptan 6mg sc; Oxy: oxygen 15L/min Optimask; SPG: The Pulsante SPG Microstimulator System; Dic: Diclofenac 25mg sc. Accompanying symptoms: lac: lacrimation; pto: ptosis; mio: miosis; con: nasal congestion; inj: conjunctival injection; swe: forehead and facial sweating; res: restlessness; rhi: rhinorrhea; ede: eyelid edema