

# Neurofilament light chain levels in ventricular cerebrospinal fluid after acute aneurysmal subarachnoid haemorrhage

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## ABSTRACT

**Purpose** The contribution of axonal injury to brain damage after aneurysmal subarachnoid haemorrhage (aSAH) is unknown. Neurofilament light chain (NF-L), a component of the axonal cytoskeleton, has been shown to be elevated in the cerebrospinal fluid of patients with many types of axonal injury. We hypothesised that patients with aSAH would have elevated cerebrospinal fluid (CSF) NF-L levels and sought to explore the clinical correlates of CSF NF-L dynamics.

**Methods** Serial ventricular CSF (vCSF) samples were collected from 35 patients with aSAH for up to 15 days. vCSF NF-L measurements were determined by enzyme-linked immunosorbent assay. NF-L levels were analysed in relation to acute clinical status, radiological findings and 6-month outcomes.

**Results** vCSF NF-L concentrations were elevated in all patients with aSAH. Patients with early cerebral ischaemia (ECI), defined as a CT hypodense lesion visible within the first 3 days, had higher acute vCSF NF-L levels than patients without ECI. These elevated NF-L levels were similar in patients with ECI associated with intracranial haemorrhage and ECI associated with surgical/endovascular complications. vCSF NF-L levels did not differ as a function of acute clinical status, clinical vasospasm, delayed cerebral ischaemia or 6-month Glasgow Outcome Scale.

**Conclusions** Elevated vCSF NF-L levels may in part reflect increased injury to axons associated with ECI. However, our results suggest that axonal injury after aSAH as reflected by release of NF-L into the CSF may not play a major role in either secondary adverse events or long-term clinical outcomes.

Aneurysmal subarachnoid haemorrhage (aSAH) is a devastating disease associated with long-term cognitive impairment in nearly 50% of survivors.<sup>1</sup> Key factors associated with unfavourable outcome include older age, poor presenting neurological condition, extent of aSAH on head CT, intraparenchymal haemorrhage, vasospasm and cerebral infarction.<sup>2</sup> Earlier and more refined methods for recognition of patients at high risk of the secondary sequelae would permit early and more aggressive initiation of appropriate therapy, whereas identification of patients likely to suffer poor outcomes would allow for more informed discussions with family regarding patient prognosis. Aside from clinical examination and radiographic imaging, no diagnostic study is currently used for the early recognition of patients susceptible to ischaemic secondary events.

In recent years there has been considerable effort towards identifying biomarkers (ie, cardiac troponin, S100-B,  $\alpha$ -II spectrin) that might prove useful in assessing brain injury after aSAH<sup>3–5</sup>; however, these biomarkers have not been widely used in clinical practice to date.

Neurofilaments constitute a major component of the axonal cytoskeleton and are composed of neurofilament heavy chain (NF-H 190–210 kDa), neurofilament medium chain (160 kDa), neurofilament light chain (NF-L 68 kDa) and  $\alpha$ -internexin (66 kDa).<sup>6–7</sup> Their function is to maintain axonal structural integrity.<sup>8</sup>

Neurofilaments are normally restricted to intracellular compartments. Disruption of axonal membrane integrity could result in neurofilament proteins being released into the extracellular space, from which they may diffuse into the cerebrospinal fluid (CSF). As such, neurofilament subunits are candidate CSF biomarkers of axonal injury.

Petzold *et al*<sup>9</sup> reported a correlation between CSF levels of NF-H and outcome in 17 patients with aSAH. However, they did not specify whether patients developed secondary insults. More recently, Lewis *et al*<sup>10</sup> demonstrated that elevated CSF and blood NF-H levels in patients with aSAH were associated with poor outcome and that patients with vasospasm had higher CSF NF-H levels compared to those without vasospasm, suggesting that NF-H may be a useful marker of axonal injury in aSAH.<sup>9–11</sup>

NF-L may be a complementary marker of axonal injury because it has been detected in the CSF of patients with traumatic brain injury, HIV infection and neurodegenerative disease. It has also been detected in the lumbar CSF of patients with aSAH.<sup>6–8–12–13</sup> However, no study has investigated vCSF levels of NF-L after aSAH. The aims of this study were to examine the relationship between vCSF NF-L dynamics and relevant clinical outcomes.

## MATERIALS AND METHODS

### Patients

The study was approved by the research ethics committees of the Ospedale Maggiore Policlinico, Milano and Washington University, Saint Louis. Thirty-five patients with aSAH were enrolled (Supplementary table 1). Written informed consent was obtained from patients or, in the comatose patients, from the next of kin. Clinical management was performed as previously described<sup>14</sup>;

## Short report

clinical outcome was assessed at 6 months post-injury using Glasgow outcome scoring system.<sup>15</sup>

## Neuroradiologic monitoring

All patients received a first head CT on admission. A second CT was performed within 48 h after aneurysm treatment to identify procedural complications, and a later CT was performed between days 21 and 28 after aSAH for follow-up. Additional scans were performed in cases of neurological deterioration such as the loss of one point of the Glasgow Coma Scale motor component and/or the presence of new focal deficits.

All CTs were reviewed by two investigators blinded to the clinical history who independently assessed the occurrence of ischaemic events. Early cerebral ischaemia (ECI) was defined as a hypodense lesion that was visible on the CT performed within the first 48 h after aneurysm treatment. Delayed cerebral ischaemia was defined as the appearance of a new hypodense lesion detectable on the 21- to 28-day follow-up CT that was not present on the CT performed within 48 h after aneurysm treatment.

## Criteria for evidence of vasospasm

Clinical vasospasm was defined as neurological deterioration associated with angiographic confirmation of vasospasm, defined as an arterial diameter narrowing >20% from baseline, by a neuroradiologist blinded to the clinical history.

## CSF sampling

CSF was collected twice daily from an external ventricular drain in all patients. The median number of samples per patient was 9 (range 1–21). CSF samples were immediately treated with 10 mM ethylenediaminetetraacetic acid and 0.125% polybrene (Sigma) to prevent admixed blood from clotting. Supernatant was separated by centrifugation (10 min at 2500×g at 21°C) and stored at –80°C. Additionally, CSF samples of 13 patients free of neurological diseases who underwent lumbar puncture as part of a study protocol at Washington University, Saint Louis, and Ospedale Maggiore Policlinico, Milano, served as negative controls. NF-L levels were analysed by enzyme-linked immunosorbent assay. The protocol was adapted from Van Geel *et al*<sup>6</sup>; for details on analysis and assay procedure, please refer to supplementary materials.

## Statistics

NF-L values did not follow normal distributions. Therefore, Mann–Whitney U tests were used to assess the relationship between NF-L values and clinical outcomes. Kruskal–Wallis test followed by Dunn’s multiple comparison test was used to assess the relationship between NF-L and ECI. The Wilcoxon signed rank test was used to investigate the relationship between changes in NF-L values over time and the occurrence of vasospasm.

## RESULTS

All 35 patients with Fisher grade 3–4 aSAH had elevated acute vCSF NF-L levels (median 643 pg/ml, range 60–2688 pg/ml). In two of these patients with aSAH, lumbar CSF was additionally obtained and also found to have elevated NF-L levels. In contrast, 0 of 13 non-neurological patients (age range 36–72 years) had detectable lumbar CSF NF-L levels (<12 pg/ml).

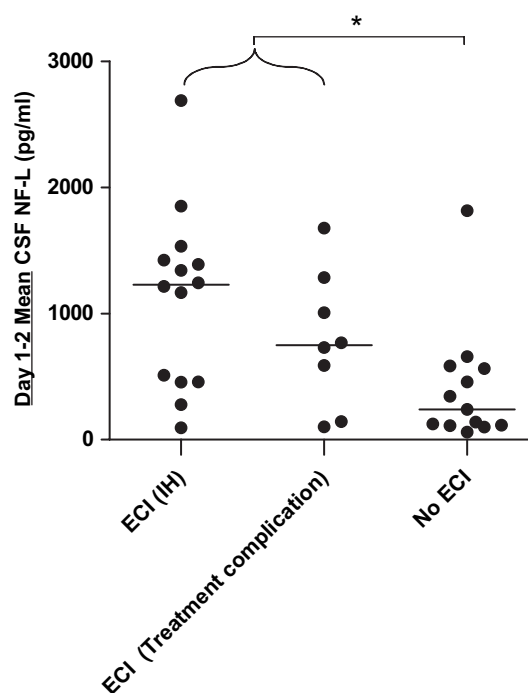
To explore the relationship between vCSF NF-L levels and initial clinical status after aSAH, we divided the patients into good (1–3) versus poor (4–5) World Federation of Neurosurgical

Societies (WFNS) grade, as well as good (5–6) versus poor (1–4) Glasgow Coma Scale motor component. Acute NF-L levels did not differ between good versus poor initial clinical status groups (Supplementary fig 1). This indicates that vCSF NF-L levels do not reflect initial injury severity as reflected by clinical status.

ECI may cause injury to axons in both grey and white matter. We found that vCSF NF-L levels were significantly higher in patients with ECI compared to patients without ECI ( $p<0.01$ ), although there was considerable overlap between groups (figure 1 and Supplementary fig 2). vCSF NF-L levels on days 1–2 were elevated most prominently in patients with ECI related to intracranial haemorrhage (median 1229 pg/ml, range 93–2688 pg/ml,  $n=14$  patients,  $p=0.01$  vs no ECI) but also elevated in patients with ECI related to complications of aneurysm treatment (median 749 pg/ml, range 100–1678 pg/ml,  $n=8$  patients;  $p=0.06$  vs no ECI). Thus, higher NF-L levels may reflect increased injury to axons associated with ECI.

We next analysed the vCSF NF-L levels in patients who developed clinically significant vasospasm or delayed cerebral ischaemia. There were no statistically significant differences in vCSF NF-L levels between groups (Supplementary fig 3). Neither were there changes in vCSF NF-L levels over time that reflected the development of clinical vasospasm (Supplementary fig 4).

Finally, we assessed whether acute vCSF NF-L levels would be useful predictors of 6-month clinical outcome. Although there were trends toward worse outcomes in patients who had the highest vCSF NF-L levels, there was considerable overlap between groups and no statistically significant differences were detected (Supplementary fig 5).



**Figure 1** Relationship between vCSF NF-L levels and ECI. The presence of ECI was associated with higher NF-L levels (Kruskal–Wallis test,  $*p=0.02$ ). Patients with ECI related to IH showed higher NF-L levels compared to patients without ECI ( $p=0.01$ , Dunn’s multiple comparison test). A more modest increase was observed in case of ECI related to treatment complications ( $p=0.06$ ). ECI, early cerebral ischaemia; IH, intraparenchymal haemorrhage; NF-L, neurofilament light chain; vCSF, ventricular cerebrospinal fluid.

**DISCUSSION**

Our results demonstrate that NF-L, a major component of the axonal cytoskeleton, can be detected in vCSF as early as 24 h post-aSAH. The levels of NF-L were higher in patients with CT evidence of ECI. This occurred in the context of intracranial haemorrhage or complications of endovascular/surgical aneurysm treatment. However, vCSF NF-L levels were not consistently related to acute clinical status, vasospasm, delayed cerebral ischaemia and 6-month outcomes.

What underlies the release of NF-L into the vCSF even in patients without ECI? This has not been determined definitively, but possibilities include axonal shear injury due to a rapid pressure wave occurring at the time of aneurysm rupture, an acute rise in intracranial pressure causing mechanical deformation of axons, transient global ischaemia or ischaemic white matter injury due to microvascular dysfunction. All of these would be difficult to detect using current clinical methods. It is not clear whether vCSF NF-L elevations indicate specific axonal injury; more general brain injury processes such as ischaemia that affect axons indiscriminately along with other brain tissues appear also to result in NF-L release into the CSF.

An alternative explanation is that the ventricular drain placement itself was responsible for the high levels of vCSF NF-L measured. The drain insertion through the brain parenchyma could release neurofilament from injured axons in the frontal cortex and white matter.

**CONCLUSION**

Ventricular CSF NF-L levels are elevated after aSAH. A relationship between ECI and NF-L levels could be detected suggesting that NF-L levels may reflect increased injury to axons associated with intracranial haemorrhage and/or surgical or endovascular complications.

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**Competing interests** None.

**Ethics approval** This study was conducted with the approval of the University of Milan and Washington University.

**Contributors** ERZ and DR contributed equally.

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