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Heart-fatty acid-binding and tau proteins relate to brain injury severity and long-term outcome in subarachnoid haemorrhage patients

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Editor's key points

- Biomarkers offer promise as objective and valuable measures of risk status, early indicators of organ dysfunction or both.
- Subarachnoid haemorrhage (SAH) patients are at high risk of delayed neurological injury.
- Heart-fatty acid-binding protein (H-FABP), an intracellular carrier protein, and microtubule-associated tau protein (τ), leak into the cerebrospinal fluid of patients with SAH.
- H-FABP and τ concentrations correlate with the extent of brain injury, secondary neurological deficits, and long-term neurological outcome.

Background. Vasospasm and other secondary neurological insults may follow subarachnoid haemorrhage (SAH). Biomarkers have the potential to stratify patient risk and perhaps serve as an early warning sign of delayed ischaemic injury.

Methods. Serial cerebrospinal fluid (CSF) samples were collected from 38 consecutive patients with aneurysmal SAH admitted to the neurosurgical intensive care unit. We measured heart-fatty acid-binding protein (H-FABP) and tau protein (τ) levels in the CSF to evaluate their association with brain damage, and their potential as predictors of the long-term outcome. H-FABP and τ were analysed in relation to acute clinical status, assessed by the World Federation of Neurological Surgeons (WFNS) scale, radiological findings, clinical vasospasm, and 6-month outcome.

Results. H-FABP and τ increased after SAH. H-FABP and τ were higher in patients in poor clinical status on admission (WFNS 4–5) compared with milder patients (WFNS 1–3). Elevated H-FABP and τ levels were also observed in patients with early cerebral ischaemia, defined as a CT scan hypodense lesion visible within the first 3 days after SAH. After the acute phase, H-FABP, and τ showed a delayed increase with the occurrence of clinical vasospasm. Finally, patients with the unfavourable outcome (death, vegetative state, or severe disability) had higher peak levels of both proteins compared with patients with good recovery or moderate disability.

Conclusions. H-FABP and τ show promise as biomarkers of brain injury after SAH. They may help to identify the occurrence of vasospasm and predict the long-term outcome.

Keywords: brain injury; cerebral vasospasm; H-FABP; subarachnoid haemorrhage; τ protein

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Aneurysmal subarachnoid haemorrhage (SAH) is an important cause of premature death and disability worldwide, affecting ~10 per 100 000 individuals every year. The mortality rate in SAH is ~40% and even with aggressive treatment, good recovery is restored in <30% of patients.[1 2] Brain damage secondary to cerebral ischaemia is a major concern in these patients.[3] It may occur abruptly at early post-haemorrhagic stages, as a consequence of acute intracranial hypertension, focal compression, or as complication of the endovascular, or surgical procedure.[3 4] In addition, in ~25% of these patients a delayed cerebral ischaemia (DCI) is observed from 3 to 14 days after SAH.[5] DCI is thought to represent a consequence of vasospasm, which is the most important potentially treatable cause of mortality and morbidity in these patients.[6]

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The delayed nature of vasospasm provides an opportunity to detect this complication at an early stage. However, the diagnosis of vasospasm-associated cerebral ischaemia can be difficult in comatose and sedated patients in whom accurate clinical evaluation is not feasible.[7] Thus, biomarkers could be an additional useful tool to investigate mechanisms of secondary brain damage and to identify new cerebral complications.[8] Ideal markers in SAH patients should help the clinician to stratify patients' severity, quantify early brain damage, and provide timely information on impending delayed ischaemic damage.

In the present study, we focused on H-FABP and τ hypothesizing that together these two molecules could fulfil the characteristics mentioned above. H-FABP is a low-molecular-weight (MW, 15 kDa) lipid-binding protein, highly expressed in the cytoplasm.[9 10] These two features allow its rapid release into the extracellular space within 3 h after stroke[11] or myocardial ischaemia[12] and suggest its potential as indicator of cellular injury even in the case of a transient ischaemic insult. τ (MW 48–67 kDa) is a microtubule-associated protein highly concentrated in axons, which has been shown to be related to the degree of damage after traumatic brain injury and stroke.[13–15]

Our preliminary results showed an association between the two proteins and SAH mortality at discharge from ICU.[16] The present study was designed to evaluate the relation of H-FABP and τ with early and delayed brain injury after SAH.

Methods

Patients

The study was approved by the Local Research Ethics Committee of the Ospedale Maggiore Policlinico, Milano. Eligible patients had aneurysmal SAH and need of cerebrospinal fluid (CSF) withdrawal within the 48 h from SAH for clinical purposes [i.e. clinical or radiological signs of hydrocephalus or intracranial pressure (ICP) control]. Additional criteria included age >18 years.

Thirty-eight consecutive SAH patients admitted to our Neurosurgical Intensive Care Unit (ICU) were enrolled between 2005 and 2007 (including 27 patients presented in a preliminary work[16]). Written informed consent was obtained from the patient or, in the comatose patients, from the next of kin. CSF samples from 16 patients free of neurological diseases served as negative controls; in these patients lumbar puncture was done during spinal anaesthesia for elective surgery (saphenectomy or transurethral urologic surgery) at Ospedale Maggiore Policlinico, Milan.

Clinical management was performed as previously described[16 17] according to international guidelines.[18] Briefly, management goals included the early clipping/coiling of the aneurysm and evacuation of an intracranial haematoma, where indicated. Symptomatic hydrocephalus was treated by external drainage of the CSF through an intraventricular catheter and ICP was monitored with the goal of maintaining ICP levels <20 mm Hg and cerebral perfusion pressure ~60–70 mm Hg. The severity of SAH was recorded according to the World Federation of Neurological Surgeons (WFNS) grading scale.[19] The initial CT scans were classified using the Fisher scale.[20]

Based on WFNS grade, patients were grouped into the following categories: (i) severe SAH (sSAH), WFNS score 4–5; (ii) mild SAH (mSAH), WFNS 1–3. The clinical outcome was assessed at 6-month post-SAH, using the Glasgow outcome scale (GOS).[21] The outcome was defined as (i) unfavourable (GOS 1–3) or (ii) favourable (GOS 4–5).

Criteria for evidence of clinical vasospasm and of ischaemic lesions

Clinical vasospasm was defined as neuro-deterioration associated with angiographic confirmation of vasospasm (arterial diameter narrowing >20% from baseline). In our patients, neurological status was monitored at least six times a day by the medical staff until discharge from the ICU. Neuro-deterioration was defined as a decrement of one point on the Glasgow coma scale (GCS, evaluating both side of the body for the motor component), the presence of new focal deficits, or both. If neuro-deterioration could not be attributed to any systemic complication, patients underwent a new CT scan or magnetic resonance imaging (MRI) to rule out hydrocephalus/rebleeding, and then angiography.

In order to evaluate all the ischaemic brain lesions, including those unrelated to vasospasm, all patients received an additional CT scan within 72 h post-SAH to identify early complications. We defined 'early cerebral ischaemia' (ECI) as a hypodense lesion that was visible on the CT performed within the first 72 h after SAH. We defined DCI as the appearance of a new hypodense area in one or more arterial-dependent territories detectable on the 21–28 day follow-up CT scan.[22]

All CTs were reviewed by two investigators blinded to the clinical history that independently assessed the occurrence of new hypodense lesions. All clinical and radiological assessments were performed blinded to the biochemical determinations.

CSF sampling and assay

CSF samples were collected beginning on Day 1 and thereafter twice daily up to the removal of the ventricular catheter or discharge from ICU. Blood clotting was prevented by collecting samples in 10 mM ethylenediaminetetraacetic acid and 0.125% polybrene (Sigma-Aldrich, St. Louis, MO). Supernatant was separated by centrifugation (10 min at 2500$\times g$ at 21°C) and stored at −80°C. Samples (n=139) were analysed using specific ELISA kits for H-FABP and τ as described[23] (intra- and inter-assay coefficient of variations: 5%; detection limits were 150 pg ml^{-1} for H-FABP and 60 pg ml^{-1} for τ).

In all patients, we defined as 'acute peak' the highest values of CSF H-FABP and τ obtained during the first 48 h post-SAH. This time-window was chosen to directly reflect H-FABP and τ release because of acute brain injury after SAH.[3] We defined as 'delayed peak' the highest value measured during the remaining period of the study, with the hypothesis that a further increase should reflect a superimposed additional brain damage (i.e. a new ischaemia).