

NEUROSCIENCES AND NEUROANAESTHESIA

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 stroke¹¹ or myocardial ischaemia¹² 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 work¹⁶). 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 described²³ (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).

Finally we defined as 'absolute peak' the greatest value observed at any time of the study. This value should reflect the greatest brain injury occurring to the patient. We hypothesized that this value was associated with the long-term outcome.

Statistical analysis

CSF H-FABP and τ values did not follow a normal distribution ($P < 0.05$, Shapiro–Wilks W test) therefore the Mann–Whitney test was used to assess the relationship between H-FABP and τ values with WFNS, ECI, and outcome at 6 months. Data are expressed as median and range. Wilcoxon signed-rank test was used to evaluate temporal changes between H-FABP and τ values and the development of vasospasm. A P -value of < 0.05 was considered significant. Statistical analysis was performed using the Prism 4 software, version 4.01 (Graph-Pad Software, San Diego, CA, USA).

Results

Patients

Thirty-eight patients were included in this study, 31 women and 7 men, with a mean age of 54 (2) years (range 30–78 years). Twenty-four patients (63%), were in poor clinical conditions on admission (WFNS 4–5). Twenty-five patients had an anterior circulation aneurysm, 11 had a posterior circulation aneurysm while two died before ancillary investigation could be performed. Aneurysms were coiled in 28 patients, and clipped in six patients within 24 h of admission. In four patients with GCS of three and absent upper brainstem reflexes after hydrocephalus drainage, aneurysm closure was not performed. All but one patient had extensive subarachnoid blood (Fisher grades 3 or 4, for detailed information see Table 1). Twenty-three patients (60.5%) had ECI related to intracranial haemorrhage (ICH or rebleeding, $n=17$), treatment complications (vascular occlusion during surgery or coiling, $n=6$) or both. During the sampling 11 patients (29%) developed clinical vasospasm and six were eligible for angioplasty. In 10 patients of this group a DCI was detected at the follow-up CT scan.

Pneumonia or urinary infections were diagnosed in 19 and 3 patients, respectively; none of these developed central nervous system infection or severe systemic complication (such as acute respiratory distress syndrome, septic shock, or myocardial infarction).

CSF H-FABP and τ increase in patients after SAH

Median H-FABP levels in controls [357 (range 150–1064) pg ml^{-1}] were significantly lower compared with those observed in SAH patients on Day 1–2 [3682 (212–100 000) pg ml^{-1} ; $P < 0.0001$]. Similarly, median τ levels in controls [152 (60–401) pg ml^{-1}] were significantly lower than those in SAH patients on Day 1–2 [2731 (84–17 222) pg ml^{-1} ; $P < 0.0001$].

CSF H-FABP and τ are associated with acute clinical status and the presence of ECI

Patients with sSAH had significantly higher H-FABP and τ levels on Day 1–2 (acute peak) than those with mSAH (Fig. 1) indicating that the CSF levels of these proteins are related to the initial clinical status. Furthermore, to investigate the relationship between the degree of initial tissue damage and H-FABP and τ we divided patients based on ECI occurrence. ECI was associated with significantly higher acute levels of both proteins (Fig. 1). No difference was observed between patients treated with endovascular coiling and those with surgical clipping (Supplementary Fig. 1).

Cut-off, sensitivity and specificity for SAH severity and ECI are shown in supplementary materials (Supplementary Table 1 and Fig. 2).

CSF H-FABP and τ increase in patients with clinical vasospasm

In the 11 patients (29%) with clinical vasospasm, H-FABP and τ showed a delayed and significant increase with the occurrence of this complication (Fig. 2). This delayed peak corresponded to the highest H-FABP and τ value measured in 10 and 8 of the 11 patients, respectively. Importantly, we noticed that a rise of H-FABP, defined as an increase of the protein level above the acute value, preceded the recognition of clinical vasospasm in 7 of 11 patients (Fig. 3) and was detected simultaneously in 3 patients. In contrast, an increase of τ before vasospasm recognition was observed in 5 of 11 patients (Fig. 3). The time lag between protein increase and vasospasm for both proteins is shown in Fig. 3A. In the group without clinical vasospasm no delayed increases of H-FABP and τ was observed (Fig. 2). Cut-off, sensitivity and specificity for clinical vasospasm are shown in supplementary materials (Supplementary Table 1 and Fig. 2).

Among cases with clinical vasospasm, DCI was detected at the follow-up CT scan in all but one patient. This patient had an abrupt decrease in the mGCS score from 6 to 1, underwent immediate angiogram which demonstrated vasospasm, and was successfully treated with angioplasty. The treatment was followed by recovery of mGCS to 6 in 2 days. In this patient, H-FABP increased during vasospasm and quickly decreased after angioplasty. On the contrary, τ levels remained below the values of the acute peak. Considering patients with DCI ($n=10$), the protein increases from acute to delayed peak remained significant.

CSF H-FABP and τ are associated with outcome at 6 months

One patient was lost to the follow-up. The outcome was favourable in 11 patients and unfavourable in 26 patients. The absolute peaks of H-FABP and τ were significantly higher in the unfavourable compared with the favourable outcome group (Fig. 4A and B). The H-FABP assay predicts unfavourable outcome at cut-off levels of 4336 pg ml^{-1} with 85% sensitivity and 82% specificity (Fig. 4c). The τ assay

Table 1 Patient clinical characteristics and CSF H-FABP and τ levels. WFNS, World Federation of Neurological Surgeons grade; Clinic. vsp., clinical vasospasm; IPH, intraparenchymal haemorrhage; ECI, early cerebral ischaemia; DCI, delayed cerebral ischaemia; GOS 6 m, Glasgow outcome scale at 6 months; N.A., not available; N.S., not sampled; NT, no treatment

Patient	Age (yr)	Sex	WFNS	Fisher grade	Treatment	Clinic. vsp.	IPH	ECI	DCI	GOS 6 m	H-FABP		τ	
											Acute peak (pg ml ⁻¹)	Delayed peak (pg ml ⁻¹)	Acute peak (pg ml ⁻¹)	Delayed peak (pg ml ⁻¹)
1	56	F	5	3	Coil	No	Yes	Yes	No	3	14 388	12 456	5359	5172
2	51	F	5	3	Coil	No	No	Yes	No	1	74 076		17 222	
3	48	F	5	3	NT	No	No	Yes	No	1	100 000		15 461	
4	65	M	5	3	NT	No	No	Yes	No	1	38 618		13 196	
5	39	F	5	3	Coil	No	No	Yes	No	1	8449		4322	
6	68	F	5	3	NT	No	No	Yes	No	1	23 729		7532	
7	73	F	4	3	Coil	Yes	No	No	Yes	1	3008	28 793	916	11 901
8	68	F	4	4	Coil	Yes	Yes	No	Yes	3	4555	8297	5903	7194
9	58	F	4	4	Clip	No	Yes	Yes	No	3	945	2397	943	1584
10	78	F	4	3	Clip	Yes	Yes	Yes	Yes	1	4326	10 590	2247	5328
11	38	F	1	3	Coil	N.S.	No	No	No	5	3585		270	
12	56	F	5	3	Coil	No	No	No	No	5	476	1334	1121	987
13	47	F	1	4	Coil	N.S.	Yes	No	Yes	1	1800		719	
14	54	F	4	3	Coil	No	Yes	Yes	No	3	2427	425	4795	198
15	62	F	5	4	Clip	No	Yes	Yes	No	3	24 142	28 364	12 296	10 655
16	73	F	1	4	Coil	Yes	No	No	Yes	1	1834	8598	125	3165
17	37	F	1	3	Coil	No	Yes	Yes	No	5	8873	4170	5627	2219
18	63	F	2	4	Coil	No	No	No	No	5	2374	4191	1101	1101
19	63	F	1	3	Coil	No	No	No	No	5	832	2023	125	150
20	30	F	2	3	Coil	N.S.	No	Yes	No	4	1965		2043	1727
21	41	M	2	4	Coil	No	No	Yes	No	5	1334	3658	1842	2086
22	67	F	4	4	Coil	Yes	No	No	Yes	4	1002	6332	2434	676
23	45	F	4	3	Coil	Yes	No	No	Yes	5	1937	4149	710	2448
24	68	F	1	4	Coil	No	Yes	No	No	N.A.	6607	1868	4116	820
25	63	M	5	3	Clip	Yes	Yes	Yes	Yes	1	24 031	77 833	6758	9066
26	49	F	3	4	Coil	No	Yes	Yes	No	3	2447	2457	2855	1159
27	42	M	5	3	Coil	No	No	Yes	No	3	647	37 127	433	8784
28	67	F	5	3	Coil	Yes	No	No	Yes	3	10 226	15 771	3168	5087
29	37	F	1	2	Coil	No	No	No	No	5	212		84	
30	52	F	5	3	Coil	No	Yes	Yes	No	1	44 588		7761	

Continued

Table 1 Continued

Patient	Age (yr)	Sex	WFNS	Fisher grade	Treatment	Clinic. vsp.	IPH	ECI	DCI	GOS 6 m	H-FBP		τ	
											Acute peak (pg ml ⁻¹)	Delayed peak (pg ml ⁻¹)	Acute peak (pg ml ⁻¹)	Delayed peak (pg ml ⁻¹)
31	45	F	1	3	Coil	No	No	No	No	5	796	135	135	
32	49	F	5	4	Coil	No	Yes	Yes	No	3	2824	2885	3515	
33	42	F	4	3	Coil	Yes	Yes	Yes	Yes	1	4480	2607	1072	
34	55	M	5	3	NT	No	No	Yes	No	1	15 507	4592	504	
35	39	M	2	4	Clip	Yes	No	Yes	No	1	3778	657	504	
36	56	M	5	3	Clip	No	Yes	Yes	No	1	24 952	7346	2015	
37	51	F	1	3	Coil	Yes	No	No	Yes	1	584	376	2015	
38	57	F	5	3	Coil	No	Yes	Yes	No	1	19 970	9309	11 309	

predicts unfavourable outcome at cut-off levels of 2527 pg ml⁻¹ with 85% sensitivity and 90% specificity (Fig. 4D).

Discussion

In this study we observed that SAH is associated with a significant increase of CSF H-FABP and τ concentrations, and is correlated with injury severity. We also observed that the occurrence of clinical vasospasm is associated with a further increase in both proteins and that patients with an unfavourable neurological outcome have higher H-FABP and τ levels.

The rationale behind the research on biomarkers is that they could add information on the mediators of the cellular, biochemical, or molecular cascades involved in mechanisms of injury and repair after cerebrovascular disease, and add diagnostic and prognostic information complementary to those already available from clinical, radiological and physiological monitoring. Previous studies have reported that alterations in H-FABP^{9 11 24 25} and τ ^{13 14 15 26 27} levels are associated with cellular brain damage in patients with acute neurological disorders and neurodegenerative diseases. Similarly, the majority of patients in our study showed increased levels of H-FABP and τ compared with normal values confirming that SAH is a clinical condition characterized by a loss of structural integrity of neuronal cell body and axons and release of cell-specific proteins into the CSF. It should be mentioned that while τ belongs to the group of brain-derived proteins and it is highly expressed only in axons and glial cells,²⁸ H-FABP is expressed not only in the brain but also in myocardial and skeletal muscle, liver, and kidney and has been considered as a biomarker in different conditions such as cardiac, liver, and kidney injury.¹⁰ Although CSF levels of H-FABP could be affected by its systemic release, none of our patients developed severe injury or failure of these organs thus making this explanation less likely.

Recently the term early brain injury⁴ has been introduced in SAH literature, referring to brain damage that occurs within the first 72 h (preceding the occurrence of vasospasm) and that is considered the primary cause of mortality in these patients. Mechanisms of early brain injury include global cerebral ischaemia because of the sudden intracranial hypertension at the time of bleeding and regional ischaemia associated with intraparenchymal haematoma or with complications of the aneurysm treatment. We have observed a relationship between early brain injury, measured by WFNS and by the occurrence of ECI, and acute levels of H-FABP and τ . Thus, despite the relatively small sample size, our data suggest that these two proteins may reflect initial injury severity.

Sixty-three per cent of our patients were in poor clinical condition on admission and were difficult to follow clinically because they were comatose, required sedation for intracranial pressure control, or both. In these patients, it is difficult to quickly identify subtle signs of neuro-worsening associated with vasospasm and the tools available in ICU (i.e. transcranial doppler, EEG, regional monitoring of cerebral

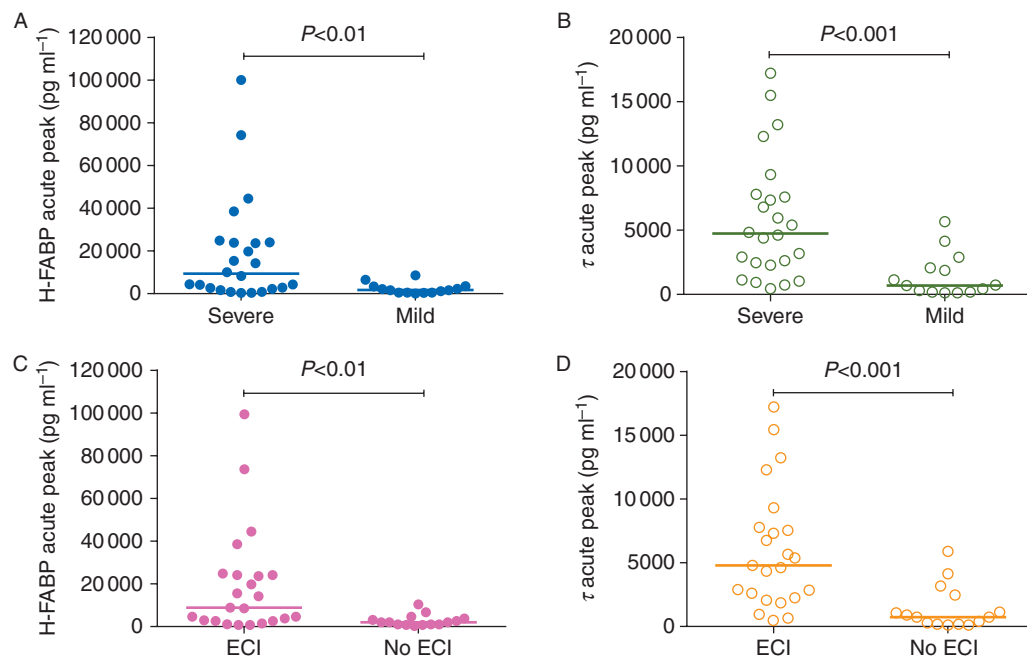


Fig 1 The relationship of CSF H-FABP and τ levels to acute clinical status and ECI. H-FABP (A) and τ (B) acute peak in patients with mild SAH were significantly lower than those in patients with severe SAH. H-FABP (C) and τ (D) concentrations were significantly higher in patients with ECI compared with those without ECI. Data are presented as median and range and statistically analysed by Mann–Whitney U -tests.

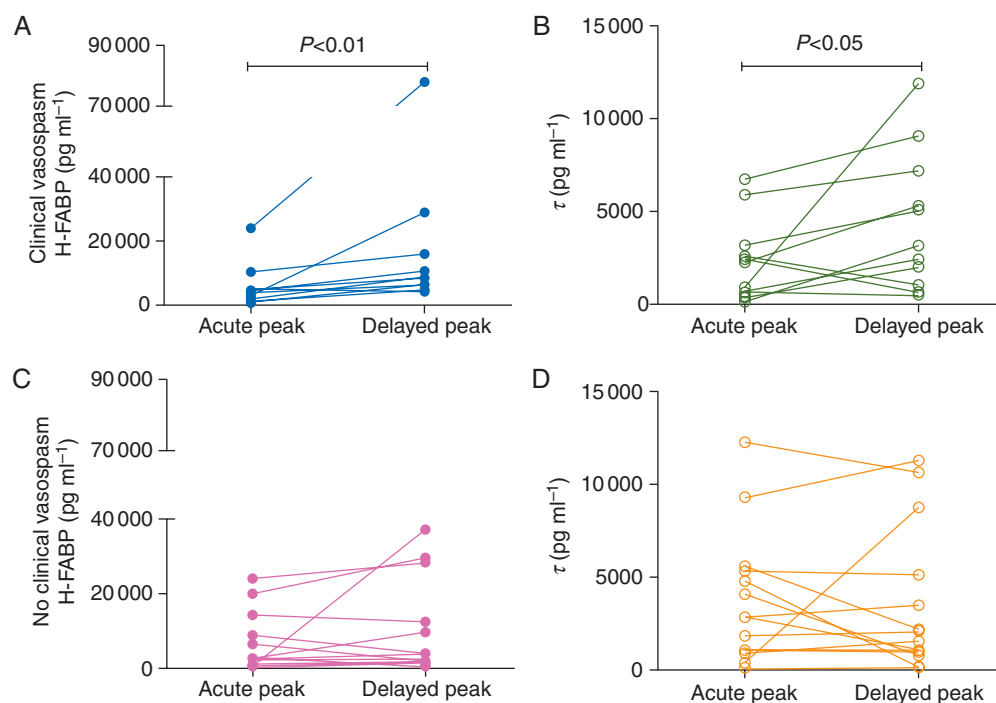
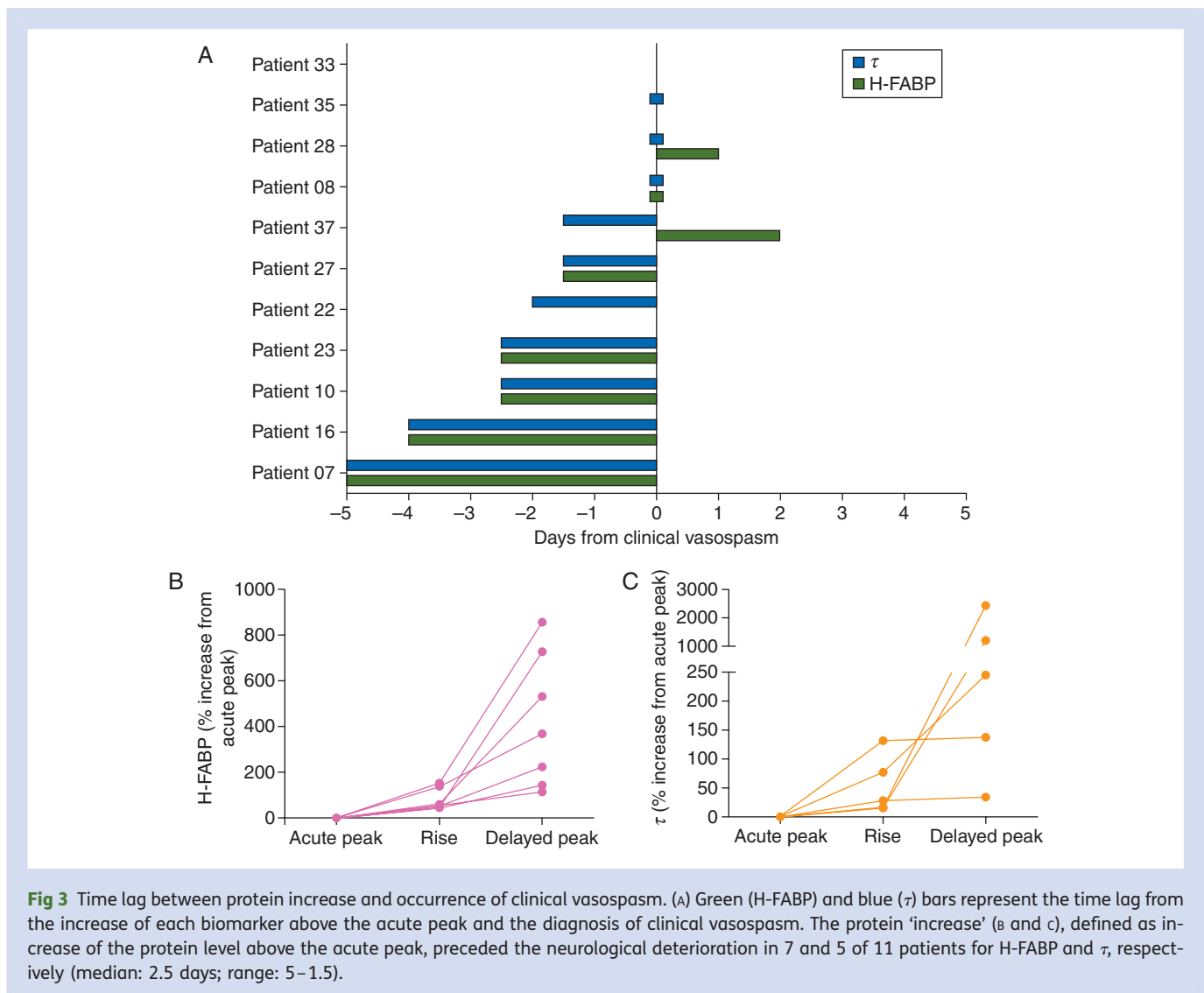


Fig 2 CSF H-FABP and τ levels and clinical vasospasm. CSF H-FABP and τ levels showed a significant delayed increase in patients with clinical vasospasm (A and B) (Wilcoxon signed-rank test), but not in those without this complication (C and D).



blood flow, metabolism and oxygenation, and perfusion CT) have low-predictive value and present some limitations.⁵ As biomarkers could potentially be useful to identify impending secondary adverse events, we analysed the relationship of H-FABP and τ with vasospasm.

Although we observed an increase of both proteins with the occurrence of clinical vasospasm, only H-FABP increases preceded the occurrence of clinical vasospasm in the majority of the patients (64%). H-FABP is a key fatty acid carrier protein, with a low-molecular-weight (15 kDa) and is abundant in cytoplasm.⁹ Given the low molecular weight and the cellular localization, it can be hypothesized that even a transient loss of cell membrane integrity may cause H-FABP to leak into the CSF. In contrast, τ is a microtubule-associated protein with a higher molecular weight (48–67 kDa)²⁷ and a more severe ischaemic insult is needed to cause a cellular release of this protein in the extracellular compartment. Notably, a selective secondary increase of H-FABP occurred in the patient in which vasospasm, successfully treated, did not lead to DCI. This secondary release of H-FABP into the

CSF even in the case of a reversible ischaemic insult is a novel finding and could provide additional complementary information to the early diagnosis of vasospasm in the clinical setting. It must be acknowledged that plotting H-FABP and τ values over time did not reveal any relationship with vasospasm. This was not surprising given the high variable time lag between aneurysmal bleeding and occurrence of vasospasm (median: 6.5; range: 2.5–12.5) and the relatively low number of patients. In this condition, we believe that longitudinal analysis of protein values does not help to clarify their behaviour in response to a given complication (data not shown).

The relationship of CSF τ and H-FABP levels with outcome indicates that these two proteins may represent potential predictors of the unfavourable outcome in the clinical setting. Our finding extends the work by Kay and colleagues²⁶ who showed a significant correlation between CSF τ and 3-month outcome after SAH and that by Turck and colleagues²⁴ reporting a significant correlation between blood H-FABP and outcome in a larger cohort of SAH patients.

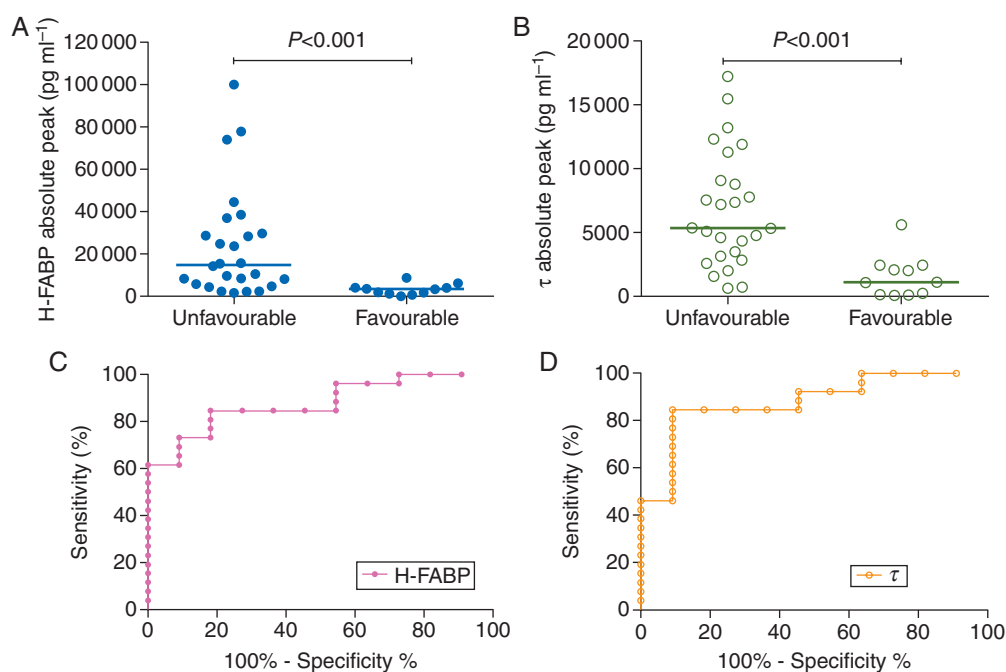


Fig 4 CSF H-FABP and τ peak values and 6-month outcome. 'Absolute peaks' of H-FABP (A) and τ (B) in patients with the unfavourable outcome were significantly higher than those in patients with the favourable outcome. Data are presented as median and range, and statistically analysed by Mann-Whitney *U*-test. ROC curves of CSF 'absolute peak' for H-FABP (C) and τ (D) as discriminator for the unfavourable outcome (6 months GOS 1-2-3).

Our study has some limitations. As DCI aetiology is multifactorial and not completely understood³ we cannot exclude that beyond vasospasm other mechanisms such as cortical spreading depression or microthrombosis are involved in H-FABP and τ modifications.²⁹ Moreover, we based our radiological analysis on CT-imaging for practical reasons. A further study based on MRI-imaging could be helpful to quantify brain damage in greater detail and to analyse the relationship between the two markers and ischaemic insult localization. Finally, CSF is not routinely available after SAH. However, CSF rather than blood protein concentrations more closely reflect brain changes and are less affected by systemic events. The vast majority of SAH patients admitted to ICU needs hydrocephalus drainage, ICP monitoring, or both, making CSF sampling reasonable, and safe. The relevance of these markers in milder patients and in plasma still needs to be assessed.

Conclusion

H-FABP and τ provide information complementary to clinical data for early vasospasm diagnosis and offer a possibility to improve stratification of severity and prediction of functional outcome after SAH.

Authors' contributions

E.R.Z. and T.Z. carried out the design, sample collection, clinical data collection, data analysis and interpretation, and

manuscript writing; L.L. and A.B. carried out design, sample collection, data interpretation and revision of manuscript; N.S. carried out design, data interpretation and final approval of manuscript; V.B. carried out data analysis; S.M., L.C., M.D.B., and M.F. carried out ELISA measurement; S.M. and M.G.D.S. carried out manuscript revision. E.R.Z. and T.Z. contributed equally.

Supplementary material

Supplementary material is available at *British Journal of Anaesthesia* online.

Declaration of interest

None declared.

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