Seizures, CSF neurofilament light and tau in patients with subarachnoid haemorrhage.

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Abstract

Objectives: Patients with severe subarachnoid haemorrhage (SAH) often suffer from complications with delayed cerebral ischemia (DCI) due to vasospasm that is difficult to identify by clinical examination. The purpose of this study was to monitor seizures and to measure cerebrospinal fluid (CSF) concentrations of neurofilament light (NFL) and tau, and to see if they could be used for predicting preclinical DCI.

Methods: We prospectively studied 19 patients with aneurysmal SAH who underwent treatment with endovascular coiling. The patients were monitored with continuous EEG (cEEG) and received external ventricular drainage (EVD). CSF samples of Neurofilament Light (NLF) and total tau (T-tau) protein were collected at day 4 and day 10. Cox regression analysis was applied to evaluate if seizures and protein biomarkers were associated with DCI and poor outcome.

Results: Seven patients developed DCI (37%) and 4 patients (21%) died within the first two months. Six patients (32%) had clinical seizures, and electrographic seizures were noted in one additional patient (4.5%). Increased tau ratio (proportion tau10/tau4) was significantly associated with DCI; Hazard ratio [HR=1.33, 95% confidence interval (CI) 1.055-1.680. p=0.016].

Conclusion Acute symptomatic seizures are common in SAH, but their presence is not predictive of DCI. High values of the tau ratio in the CSF may be associated with development of DCI.

Key words: Continuous EEG monitoring, subarachnoid haemorrhage, acute symptomatic seizures, non-convulsive seizures, cerebrospinal biomarkers, NFL and tau.

Introduction

Subarachnoid haemorrhage (SAH) is a severe condition with high morbidity and mortality with an incidence of 9 per 100 000[1]. The most devastating complication of SAH is delayed cerebral ischemia (DCI). DCI is defined as new focal or global neurological deficits and new cerebral infarction on computed tomography (CT) that is not caused by rebleeding or acute hydrocephalus[2]. Signs of DCI occur in about 30% of patients, typically appear between days 4 and 10 following SAH and are the major causes of morbidity and death[3, 4]. The pathophysiology of DCI is not fully understood, but intracranial arterial vasospasm probably plays a role. A major challenge in the management of SAH is early identification of DCI, thereby enabling therapeutic intervention.

Acute symptomatic seizures (ASS) have been reported to occur in 6-24% of SAH cases [5, 6]. Continuous EEG (cEEG) allows monitoring of patients with acute aneurysmal subarachnoid haemorrhage for detection of non-convulsive seizures and non-convulsive status epilepticus. Both clinical and electrographic seizures (ES) are believed to have a negative impact on the outcome [7]. Continuous EEG has also been used for surveillance and early recognition of DCI[8].

Significant efforts have been made to study different biomarkers as potential tools for predicting vasospasm and outcome after SAH [9, 10]. Cerebrospinal fluid (CSF) biomarkers that reflect neuronal damage have recently been studied. Neurofilament light chain protein is a cytoskeletal constituent of intermediate filaments, and is thought to reflect neuronal and axonal injury when appearing in the cerebrospinal fluid (CSF). High concentrations of NFL and tau have been reported in the first days after the primary injury and extend into the period of DCI to correlate with poor long–term outcome [11, 12]. NFL is a marker that reflects subcortical damage, while tau is more related to cortical brain injury [13].
In this study, we wished to determine whether the occurrence of ASS is a clinical marker of impeding DCI and to study the association between CSF concentrations of NFL and tau and DCI in patients with acute SAH.

Materials and methods

Study population

The study was a prospective observational study. Patients with SAH admitted to the Neuro Intensive Care Unit (NICU) at Uppsala University Hospital between July 2015 and July 2016 were consecutively included.

Inclusion criteria were spontaneous SAH with an aneurysm treated with endovascular coiling. Exclusion criteria were surgical treatment of the aneurysm, a previous history of neurological disease including epilepsy, and severe traumatic brain injury.

At admission, patients were evaluated clinically and investigated with CT including CT angiography. The severity of the clinical condition at admission was graded according to Hunt and Hess [14]. The amount of bleeding in the first CT was graded according to the Fisher scale[15]. Clinical seizures were systematically recorded and considered acute symptomatic if they appeared during the acute phase of the brain insult [16].

The patients were treated with neurointervention within the first 48 hours in order to eliminate the source of bleeding. The rest of the treatment was performed according to our NICU protocols for SAH [17]. In summary, unconscious patients were given artificial ventilation and external ventricular drainage (EVD) in order to monitor and treat the intracranial pressure (ICP). Sedation was carried out with propofol and opioids. Nimodipine was administered intravenously or in the nasogastric tube.

Delayed cerebral ischemia was clinically suspected if there was an onset of new focal (i.e. hemiparesis, aphasia) or global (decrease in the level of consciousness) neurologic deficits that persisted for at least one hour and were not attributable to other causes such as rebleeding or acute hydrocephalus. CT or MRI were used to identify the ischemic lesions. Other examinations such as transcranial Doppler(TCD) or xenon-CT scans were used to detect vasospasm or hypoperfusion that could later lead to DCI. Infectious parameters were closely monitored in patients with suspected sepsis or meningitis.

Continuous EEG (cEEG) monitoring was started as soon as possible after admission to the NICU, usually at the insertion of the EVD. Nine scalp disc electrodes (ASYS Healthcare/Neuroline EEG) F3, T3, P3, O1, F4, T4, P4, O2 and Cz were used. The first 72 hours recorded were analysed. Amplitude-integrated EEG (aEEG) trend (based on F3, F4, P3 and P4) was used for seizure detection and all suspected seizures were verified or falsified using the raw EEG. Rhythmic or epileptiform activity with spatiotemporal evolution lasting for at least 10 seconds was regarded as electrographic seizure activity[18]. One neurologist made a primary interpretation of the recordings and one of two neurophysiologists subsequently made a second interpretation of the suspected seizures.

Samples of 5 ml CSF were drawn from the EVD at days 4 and 10 after SAH. The samples were mixed, centrifuged and immediately frozen to -80^{0}C. NFL and tau protein concentrations were measured using commercial enzyme-linked immunosorbent assays (NF-Light, UmanDiagnostics, Umeå, Sweden and INNOTEST hTAU, Fujurebio, Ghent Belgium) according to protocols accredited by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC). The measurements were performed in one round of experiments using one batch of reagents by board-certified laboratory technicians who were blinded to clinical data. Intra-assay coefficients of variation were below 10%.

Analysis and statistics

Variables analysed for the study included age, gender, disease severity by Hunt&Hess (H&H) and Fisher scales. Furthermore, tau at days 4 and 10 (tau4, tau10), tau ratio (tau10/tau4), NFL at days 4 and 10 (NFL4, NFL10), electrographic seizure (ES), clinical seizure at onset, clinical seizure during NICU stay, mortality during first month and DCI were examined.

Data was analysed by SAS 9.4 software. Values of mean, median and standard deviation were calculated. Tau ratio was calculated as the ratio tau 10/tau 4. Cox regression analysis was performed using the predictor-biomarkers (those included were H&H, ASS, ES, NFL4, NFL10, tau4, tau10 and tau ratio). DCI was analysed as a dependent value.

Ethical approval

The study was approved by the Uppsala University Ethical Review Board for Human Research. Informed consent was given by the patients or their relatives.

Results

In total 19 patients (14 women) were included. The mean age was 61 years (range 43-79). Clinical seizures were observed in 6 patients (32%). Electrographic seizures were seen in one additional patient (4.5%). One patient did not have cEEG because of technical problems and was therefore excluded from the statistical analysis.
The mean CSF concentrations (±SD) of tau4 were 1672±2687 ng/L (range 75-10000 ng/L) and tau10 2950± 3540 ng/L (range 294-12300 ng/L). The mean value for tau ratio was 3.5±2.4, (range 0.34-10.8). The mean CSF concentrations of NFL 4 were 5612±5366 ng/L (range 340-19100 ng/L) and NFL10 18014±17321 ng/L (range 1850-73100). For details concerning each patient, see Table 1.

Delayed cerebral ischemia was observed in 7 patients (37%). Four patients (21%) died during the first two months after development of DCI. Sepsis and bacterial meningitis were the most frequent observed complications. Complete clinical data are shown in Table 1.

Cox regression analysis between tau ratio and DCI showed a Hazard ratio [HR]=1.33 per point increase in tau ratio, [(CI) 1.055-1.680. p=0.016].
There was no statistically significant association between DCI and any of the other variables (H&H, ASS, ES, NFL4, NFL10, tau4 and tau10), see Table 2.

Discussion

Many attempts to predict DCI depending on a single factor have failed; therefore, we carried out a prospective study to examine the use of cEEG and CSF concentrations of NFL and T-tau.
Convulsive and non-convulsive seizures are common complications of SAH. The true frequency of NCS is unknown but has been described in 8% to 30% [19, 20]of comatose patients. It has been shown that seizures (clinical and electrographic) independently predict poor outcome after SAH but a significant relation to vasospasm has never been established. In the present study we used continuous EEG monitoring for 72 hours early in the disease course and only found one patient with electrographic seizures. Altogether, seizures were noted in 36% of the patients but their presence could not predict impending DCI.

Reports of NFL in relation to SAH and DCI are very scarce in the literature [9] .One study[11] reported a significant increase in CSF-NFL in SAH patients with secondary events. In our study, NFL showed an increase in all patients, but we were not able to detect any association between levels of concentrations of NFL and poor outcome with DCI.

In previous studies[21, 22] it has been shown that SAH was associated with a significant increase of CSF tau measured at days 1 and 2 and that the increase correlated to extent of brain injury and poor clinical outcome. A delayed peak, however, preceding the occurrence of clinical vasospasm, did not follow this acute peak. We could not confirm these findings. On the contrary, there was no correlation between tau at day 4 and severity of SAH. This discrepancy could be attributed to the rather small numbers of patients in both studies and measurements at different time points. Using tau ratio, a factor that may reflect ongoing brain damage and risk of DCI, an increase was noted in a majority of the patients who developed DCI (5 out of 7) during the first two weeks. We believe that changes in tau levels between days 4 and 10 could thus be a sign of excessive cortical damage related to development of DCI. The exact relationship between tau levels and brain damage is not possible to distinguish with our data and several explanations are possible. The primary brain damage may cause both increase in tau and provoke DCI. Furthermore, DCI alone may cause an extra increase in tau and finally DCI may cause secondary brain damage resulting in tau elevation.
We could not detect an increase in tau ratio in the patients that developed DCI later than day 12, most probably due to the limitation of sampling time.

The pathophysiologic mechanism underlying DCI is probably multifactorial, and is not fully understood. It has long been argued that cerebral vasoconstriction is the major contributing factor. Other additional mechanisms include spreading depression and “early brain damage”, referring to the damage that occurs to the brain in the first 72 hours after the initial bleeding[23]. While this takes place before the onset of DCI, it seems probable that the physiological changes increase the energy demand and thereby influence the likelihood of secondary complications such as DCI. With this view, a sensitive CSF biomarker for cortical or neuronal damage would be a valuable tool in treatment aimed at avoiding DCI. There were 3 cases with bacterial meningitis and 2 with sepsis. Bacterial meningitis may increase NFL concentration in CSF but CSF T-tau is unaffected which corroborates with our data [24, 25] . The increase in CSF NFL in meningitis has been interpreted as a sign of neuronal injury regardless of the presence or absence of neurological sequelae. There are to our knowledge no studies on CSF NFL or tau in sepsis without meningitis.

Our study has several limitations. First, the small number of patients restricts the statistical power of the results. Nonetheless there was statistically significant data supporting the hypothesis that increased tau ratio could be a valuable biomarker for DCI. However, verification of the results in a larger cohort is required in order to be able to rely on the clinical utility of this approach. Other constraints include the presence of comorbidities and that the use of sedatives and antiepileptic medication was not controlled. This most certainly influenced the EEG-results and theoretically could have affected levels of tau and NFL. In the literature, however, there are no data on the impact of sedatives and antiepileptic drugs on biomarkers such as NFL and tau. Furthermore, the optimal timing of measuring CSF biomarkers is not fully established. We chose days 4 and 10 since DCI typically appears between 4 and 10 days after SAH but more frequent sampling might have disclosed important changes.

In summary, acute symptomatic seizures are common in SAH, but their presence does not predict DCI. It may be important to monitor continuous measurements of CSF tau concentrations in patients with SAH during intensive care.

Conflict of interest and sources of funding statement

HZ is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg, and has served on the advisory boards of Eli Lilly, Roche Diagnostics and Pharmasum Therapeutics. The other authors have no conflicts of interest to disclose. The authors' institution and the Selander and Thureus Foundation funded the study.

Table 1. Clinical data on patients with SAH

Patient	Age/sex	H&H	Fisher	ASS	ES	Co-morbidity	Day of DCI	Death < 2 month	NFL4 ng/L	NFL10 ng/L	Tau4 ng/L	Tau10 ng/L	Tau ratio
1	55/F	3	2	-	-	meningitis		-	2300	6750	148	312	2,1
2	50/F	3	3	+	-		9	-	340	3760	136	816	6,0
3	67/M	2	3	-	-			-	800	2100	75	294	3,9
4	60/F	2	3	-	+			-	860	8430	222	844	3,8
5	65/F	3	4	-	-		12	-	1220	6030	116	1250	10,8
6	61/F	4	4	-	-			-	3520	24300	1070	3660	3,4
7	56/F	2	4	+	-	meningitis		-	5120	8530	2450	1230	0,5
8	43/F	2	3	-	-		4	-	7280	24300	2290	12300	5,4
9	76/F	4	4	-	-			-	14000	73100	2530	6600	2,6
10	66/F	3	4	+	-			-	390	1850	288	450	1,6
11	50/M	3	2	-	-			-	4820	9150	886	1700	1,9
12	68/M	3	4	-	-	sepsis	2	yes	2320	23800	551	2860	5,2
13	70/F	3	4	+	-			-	4720	12700	771	1820	2,4
14	58/F	3	4	-	-			-	4110	10500	387	1720	4,4
15	44/F	1	3	-	-		4	yes	1920	8070	181	902	5,0
16	70/F	5	4	-	-	meningitis	15	yes	12700	29800	10000	11800	1,2
17	63/F	3	4	+	-			-	10100	40900	439	1510	3,4
18	54/M	4	4	+	-	sepsis	18	yes	11000	30600	1500	3350	2,3
19	79/M	3	4	-				-	19100	17600	7730	2640	0,3

Abbreviations: Acute Symptomatic Seizure (ASS), Delayed Cerebral Ischemia (DCI), Electrographic Seizure (ES), Fisher Grading Scale (Fisher), Hunt and Hess grading scale (H&H).
Reference values for lumbar CSF NFL; 40 – 59 years < 890 ng/L ≥ 60 years <1850 ng/L.3
Reference values for lumbar CSF tau; 18 – 44 years < 300 ng/L > 45 years <400ng/L.

Table 2. Association between clinical parameters, CSF concentrations of biomarkers and DCI.

Variable	No of counts	Hazard Ratio (95% CI)	p-value
ASS	19	1.4	0.72
ES	18	-	-
H&H	19	0.9	0.90
NFL4	19	1.0	0.61
NFL10	19	1.0	0.89
tau4	19	1.0	0.81
tau10	19	1.0	0.10
tau-ratio	19	1.3	0.01*

A Cox regression model was applied to evaluate the significance of acute symptomatic seizures (ASS), electrographic seizures (ES), Hunt and Hess severity scale (H&H), concentrations of neurofilament light (NFL), tau and tau-ratio and development of delayed cerebral ischemia (DCI).
*$p < 0.05$

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