Studies of biochemical brain damage markers in patients at a neurointensive care unit

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Physical examination is the basic and most important tool in medical practice. However, at a neurointensive care unit, neurological status can sometimes be difficult to evaluate due to sedation or impaired consciousness. Repeated radiology may not always be feasible. The aims of the present study were to investigate whether Glial Fibrillary Acidic Protein (GFAP) measured in serum could be used as a biochemical brain damage marker. To investigate whether concentrations of CSF-NFL were associated with brain injury severity and long-term outcome after aneurysmal subarachnoid haemorrhage (aSAH). Finally, to compare the two dimers S100A1B and S100BB with S100B, when it came to outcome after severe traumatic brain injury (TBI).

Serum samples were obtained on a regular basis from 116 patients with aSAH and 59 patients with TBI during a two-week period. LP was performed in a subgroup of patients (n=48) with aSAH. The concentrations of the markers were analysed using ELISA methods. Clinical and radiological findings were estimated in the acute phase and outcome was assessed after one year using the Glasgow Outcome Scale.

After aSAH maximum s-GFAP was increased in 81 of the patients and was normal in 35. A normal concentration predicted a favourable outcome. Increased s-GFAP levels were related to focal brain lesions, neurological complications and poor outcome. The concentrations of CSF-NFL were related to focal brain injury and outcome after aSAH.

After severe TBI maximum s-GFAP was increased in all but one of the patients. The five patients with the most pronounced increase died. The serum concentrations of GFAP, S100B, S100A1B and S100BB were all related to outcome.

We conclude that s-GFAP can be used as a biochemical brain damage marker after aSAH and severe TBI. The high npv (32/35) after aSAH is the main finding, which may provide information to complement clinical data. CSF-NFL is a sensitive brain damage marker after aSAH, but for better utility, an analysis method for serum is needed. In the clinical setting in this study the investigated serum markers (GFAP, S100B, S100A1B, S100BB) appeared to predict outcome after severe TBI equally well.

LIST OF ORIGINAL PAPERS

This thesis is based on the following articles, referred to in the text by their Roman numerals.

I   Nylén K, Csajbok LZ, Öst M, Rashid A, Blennow K, Nellgård B, Rosengren L. Serum GFAP is related to focal brain injury and outcome after aneurysmal subarachnoid haemorrhage. In press in *Stroke.* Accepted for publication 070102.

II   Nylén K, Csajbok LZ, Öst M, Rashid A, Karlsson J-E, Blennow K, Nellgård B, Rosengren L. CSF-Neurofilament correlates with outcome after aneurysmal subarachnoid haemorrhage. *Neurosci Lett.* 2006; 404:132-136.

III Nylén K, Öst M, Csajbok LZ, Nilsson I, Blennow K, Nellgård B, Rosengren L. Increased serum-GFAP in patients with severe traumatic brain injury is related to outcome. *J Neurolog Sci.* 2006; 240: 85-91.

IV Nylén K, Öst M, Csajbok LZ, Nilsson I, Hall C, Blennow K, Nellgård B, Rosengren L. Serum levels of S100A1B and S100BB are related to outcome after severe traumatic brain injury. *Submitted for publication.*

CONTENTS

ABBREVIATIONS

aSAH	Aneurysmal subarachnoid haemorrhage
ADL	Activity of daily living
BBB	Blood brain barrier
CNS	Central nervous system
CSF	Cerebrospinal fluid
CPP	Cerebral perfusion pressure
CT	Computed tomography
ELISA	Enzyme linked immunosorbent assay
EVD	External ventricular drainage
GCS	Glasgow coma scale
GFAP	Glial fibrillary acidic protein
GOS	Glasgow outcome scale
GOSE	Glasgow outcome scale-extended
ICP	Intracranial pressure
ISAT	International subarachnoid aneurysm trial
LP	Lumbar puncture
MMSE	Mini-mental state examination
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NICU	Neuro intensive care unit
NIHSS	National institute of health stroke scale
NFH	Neurofilament heavy protein
NFL	Neurofilament light protein
NSE	Neuron specific enolase
RLS 85	Reaction level scale 85
ROC	Receiver operating characteristics
SAH	Subarachnoid haemorrhage
SD	Standard deviation
TBI	Traumatic brain injury
TCD	Transcranial Doppler sonography
TCDB	Traumatic coma data bank
WFNS	World federation of neurological surgeons scale

INTRODUCTORY REMARKS

Subarachnoid haemorrhage and severe traumatic brain injury are common causes of sudden death and neurological disability. After a subarachnoid bleed the primary brain injury ranges on a continuum from almost negligible to extensive and life threatening. The risk of neurological complications justifies neurointensive care even after treatment of the aneurysm. Following a severe traumatic brain injury the patients are unconscious, often further anaesthetised and treated in a ventilator. Consequently neurological examination may be restricted and repeated radiology not always easy to perform in these critically ill patients. Monitoring the course as well as estimating the scale of the ongoing brain damage and its long-term consequences, can therefore be difficult. No laboratory test will ever replace clinical or radiological assessments, but additional information or alternative ways of obtaining information are required. A study of biochemical brain damage markers in relation to the brain injury severity and long-term outcome may elucidate the future potential for using this adjunct in the clinical setting.

BACKGROUND

A. Subarachnoid haemorrhage

Subarachnoid haemorrhage (SAH) is fatal in up to 50% of patients and causes permanent neurological disability in one third of survivors (van Gijn and Rinkel, 2001). Although subarachnoid haemorrhage comprises only one to seven percent of all strokes, the loss of productive life years is comparable to that caused by cerebral infarctions, because of the relatively young age at onset and poor outcome (Feigin et al., 2005). The haemorrhage is caused by a ruptured aneurysm in 85% of cases. Ten percent occur in patients with non-aneurysmal perimesencephalic haemorrhage. This is an entirely benign, yet somewhat mysterious condition. The angiogram is normal and the patients recover. On rare occasions other vascular pathologies such as arterial dissection are identified (van Gijn and Rinkel, 2001). Women are more frequently affected than men (1.6:1) and age is typically over 40 years. In Finland and Japan the incidence rates are much higher than in other parts of the world. Smoking, hypertension and excessive alcohol consumption are the most important risk factors (Feigin et al., 2005). The clinical hallmark of SAH is a history of explosive headache. A period of unresponsiveness is not uncommon and focal signs may develop at the same time as the headache or soon afterwards. Intraparenchymal haematomas occur in up to 30% of patients with a ruptured aneurysm and not surprisingly their average outcome is poorer than that of patients with purely subarachnoid blood (van Gijn and Rinkel, 2001). Neck stiffness is a well-recognised sign, but it may take many hours to develop and in some cases it never appears (Vermeulen, 1996). Some patients receive attention due to epileptic seizures or confusion. All patients with suspected SAH should have an emergency computed tomography (CT). If the CT is considered normal (uncommon in the first 24 hours after aneurysm rupture), lumbar puncture (LP) for spectrophotometry of the cerebrospinal fluid (CSF) is the next step (Vermeulen, 1996). When a diagnosis is made, the gold standard for detecting aneurysm is conventional angiography, but it is gradually being replaced by the improving CT- and MR-angiography techniques.

Neurological deterioration after the initial bleed is not uncommon. Frequent complications are re-bleeds, hydrocephalus and delayed ischemic events. Re-bleeding occurs in approximately 20% within two days of the initial haemorrhage and is associated with high mortality. Treatment with antifibrinolytic agents reduces the re-bleeding rate (Roos et al.,

2003) and the number of potentially saved lives exceeds those lost to ischemic events (Hillman et al., 2002). To prevent re-bleeding, early treatment by surgical clipping or endovascular coiling is required. Surgical obliteration of the aneurysm has been the mainstay of treatment for decades. Until the 1980s, surgery was deferred until day 10-12 because of the many complications associated with earlier operations. Since then, many neurosurgeons have adopted a policy of early clipping of the aneurysm, i.e. within three days of the initial bleed (van Gijn and Rinkel, 2001). After the introduction of detachable coils for the endovascular packing of aneurysms, this technique has been increasingly used (Guglielmi et al., 1992). The most frequent complications are procedure-related ischemia and aneurysm perforation. In the International Subarachnoid Aneurysm Trial (ISAT), endovascular treatment was compared with neurosurgical clipping in 2,143 patients who were suitable for either treatment. At one year, 23.7% of patients allocated to endovascular treatment were dead or dependent compared with 30.6% allocated to neurosurgical treatment. The risk of re-bleeding from the ruptured aneurysm after one year was 2 per 1,276 patient-years and 0 per 1,081 patient-years for those allocated to endovascular and neurosurgical treatment respectively (Molyneux et al., 2002).

Hydrocephalus should be considered if the level of consciousness gradually declines, particularly on the first day (Vermeulen, 1996). External drainage of the ventricles is effective but carries a risk of infection. An increased risk of re-bleeding during external ventricular drainage (EVD) or LP has also been suggested, but this was not confirmed in one recent study (Hellingman et al., 2007).

Cerebral vasospasm is a feared complication, with death and disability as a direct result of the ischemia. There is angiographic evidence of vasospasm in two-thirds of cases of SAH; half of these become symptomatic, typically four to 14 days after the initial bleed (Rabinstein, 2006). Monitoring and preventing vasospasm is an important task at the Neuro Intensive Care Unit (NICU). However, research has been hampered by the lack of uniform definition. Clinically symptomatic vasospasm is defined as an acute neurological deterioration in the absence of other causes. Angiographically, vasospasm is defined as vessel narrowing. Many studies have relied on transcranial Doppler sonography (TCD), suggesting increased blood flow velocity as a result of arterial narrowing. The amount of blood in the subarachnoid space on the initial CT, young age and a history of smoking are risk factors for vasospasm, but their predictive value is limited (Rabinstein, 2006). The pathogenesis of secondary cerebral ischemia following SAH has not been completely elucidated. It is believed that an as yet unidentified factor is released into the subarachnoid space after the haemorrhage, which induces vasoconstriction and thereby secondary ischemia. The presence of subarachnoid

blood in itself is not a sufficient factor. Despite this lack of pathophysiological insight, some progress has been made in the prevention of secondary ischemia by increased fluid intake, the avoidance of antihypertensive drugs and the administration of nimodipine. The treatment of delayed cerebral ischemia with hypervolemia, hemodilution and induced hypertension, the so-called triple H-therapy, has become widely used (van Gijn and Rinkel, 2001).

Variables related to outcome

The baseline variables most closely related to poor outcome after aneurysmal SAH (aSAH) are the neurological condition of the patient on admission, age and the amount of subarachnoid blood on the CT. Of these three prognosticators, the neurological condition of the patient, particularly the level of consciousness, is the most important determinant (van Gijn and Rinkel, 2001). Other factors, such as aneurysm size and location, history of hypertension and angiographically demonstrable vasospasm, have been implicated (Rosen and Macdonald, 2004).

Several scoring systems based on the patients' clinical condition have been proposed. Currently, the most frequently used are the Hunt and Hess grading system (Hunt and Hess, 1968) and the World Federation of Neurological Surgeons scale (WFNS; Drake et al., 1988). Grading scales including not only clinical condition but also additional co-morbid factors show higher prognostic efficacy, but they are more complex to use and are accordingly less useful in the day-to-day management of patients (Rosen and Macdonald, 2004). As a result, factors known to correlate with outcome are often reported separately. In ISAT, an algorithm based on age, gender, WFNS grade, Fisher scale (amount of blood on CT), aneurysm size and location was used to ensure balance between the groups (Molyneux et al., 2002). We also used these parameters to describe our study population.

World Federation of Neurological Surgeons scale (WFNS)
The executive committee of the World Federation of Neurological Surgeons worked for six years to devise a simple, reliable, clinically valid scale for grading patients with subarachnoid haemorrhage. The committee took account of the results of the International Cooperative Aneurysm Study (ICAS; Kassell et al., 1990), which showed that the two most important prognostic factors were the level of consciousness (important for prediction of both dead and disability) and the presence or absence of hemiparesis and/or aphasia (important only for

disability in survivors). Because of its worldwide acceptance, the Glasgow Coma Scale (GCS) was used to assess the level of consciousness (Teasdale and Jennett, 1974). The presence or absence of major focal deficit was used to differentiate between grades two and three (Drake et al., 1988; Table 1). When patients present at different points on the axis, clinicians are forced to use their judgement to determine which axis is most important (Rosen and Macdonald, 2005). A patient might present with intact level of consciousness and hemiparesis. We used major focal deficit as the most important axis for conscious patients.

Table 1. The World Federation of Neurological Surgeons subarachnoid haemorrhage scale

WFNS	GCS	Major focal deficit
I	15	Absent
II	14-13	Absent
III	14-13	Present
IV	12-7	Present or absent
V	6-3	Present or absent

Fisher scale

In 1980 Fisher proposed a scale based on the pattern of blood visualised on the initial CT (Fisher et al., 1980; Table 2). Although the scale was originally designed to predict cerebral vasospasm, correlation with outcome has been reported and it is used in comprehensive scales to predict outcome (Ogilvy and Carter, 1998). The scale is still widely used, but, since there have been significant advances in both neurological imaging and patient care, it is suggested that the scale requires revision (Smith et al., 2005). The scale has also been criticised due to the fact that it does not differentiate between intracerebral clots and intraventricular haemorrhage. We used the Fisher scale since it is well known, and allows comparable description of the severity of the initial haemorrhage.

Table 2. The Fisher scale

	Subarachnoid blood
1	None
2	Diffuse only
3	Clot or thick layer ($\geq$ 1mm)
4	Diffuse or none, with cerebral or ventricular blood

B. Severe traumatic brain injury

Severe traumatic brain injuries are the most common cause of morbidity and mortality among children and young adults in the western countries. Men are more frequently affected (4:1) and road traffic accidents are the most common cause. Two important developments in the evolution of head trauma care were the introduction of the CT scan and the Glasgow Coma Scale (GCS; Teasdale and Jennett, 1974) in the 1970s. CT scanning has a major impact on the early care of severely head-injured patients by providing a quick diagnosis and enabling the prompt evacuation of intracranial mass lesions. The severity of the brain injury is graded as mild, moderate or severe on the basis of level of consciousness or GCS after resuscitation. The vast majority of traumatic brain injuries (TBI) are mild (GCS 15-13) and in most cases the patients suffer from a concussion. Patients with moderate injuries (GCS 12-9) are lethargic or stuporous and patients with severe injury (GCS 8-3) are comatose. Sometimes further sub categorisations are made and GCS 3-4 is denoted as a critical injury (Stein and Spettell, 1995). Approximately 10% of all closed head injures are severe (Tagliaferri et al., 2006). The ability rapidly to diagnose and accurately describe injuries has been extremely beneficial to trauma patients. Head injury is a heterogeneous diagnosis, encompassing a wide range of pathologies, including diffuse axonal injury, focal contusions and space-occupying intra- and extradural haematomas. After the primary damage due to the initial impact, secondary brain damage ensues. Most secondary brain injuries are caused by brain swelling, with an increase in intracranial pressure (ICP) and a subsequent decrease in cerebral perfusion pressure (CPP) leading to ischemia. Several pharmacological agents have been investigated in an attempt to prevent the secondary brain injury, but none has proven effective.

The mortality from severe head injuries has been reduced by improved prehospital care, early surgical intervention, improved monitoring and treatment in dedicated neurosurgical intensive care units. Since the actual brain damage that occurs at the time of injury cannot be modified, the maximisation of neurological recovery is dependent upon minimising secondary insults to the brain. Several modern and advanced forms of brain monitoring have been studied and compared with more traditional techniques, but intracranial pressure and mean arterial blood pressure remain important factors for neurocritical care monitoring. High ICP is strongly associated with fatal outcome (Balestreri et al., 2006). The volume targeted "Lund concept" combines a gradual increase in medical treatment with neurosurgery in patients with severe head injuries and raised intracranial pressure. The primary goal is the reduction of the interstitial oedema while simultaneously maintaining adequate blood flow. The main

principles of the therapy are to reduce the hydrostatic capillary pressure, preserve the transcapillary colloid osmotic force and preserve blood flow to regions with compromised circulation suffering from hypoxia. The authors report a gradual decrease in mortality from almost 50% before the start of the therapy to close to zero in patients with the most severe head injuries (Grände et al., 1997).

Variables related to outcome

The early prediction of outcome cannot reliably be made in individual patients with severe head injuries. However, for the group as a whole, consideration of age, initial GCS scores, severity of associated injuries, blood pressure and ventilatory status, predicted outcome for fewer than two-thirds in an early study of 306 patients between 1985-1987 (Vaxman et al., 1991). The strongest indicators at the initial judgment include age, GCS score and pupillary reactivity. After hospitalisation, the results of CT scanning and measurements of ICP provide additional information.

Age

Age is one of the most important predictive factors of outcome for patients with head injuries. In a retrospective study, Mosenthal and co-workers stratified the head injuries by the GCS into mild, moderate and severe. The mortality rate was higher in the elderly (≥65 years) for all levels of injury. Although some of the increased mortality may be explained by complications or the type of head injury, age itself was an independent predictor of mortality. Elderly survivors were also more likely to have a poor functional outcome than young patients (Mosenthal et al., 2002). Older patients were more likely to develop mass lesions and, when they did, the lesions became larger (Vollmer et al., 1991).

Level of consciousness

Teasdale and Jennett introduced the Glasgow Coma Scale in 1974. The scale was evolved to assess the depth and duration of impaired consciousness and coma. Three aspects of behaviour, motor responsiveness, verbal performance and eye opening were independently measured and totalled to provide the GCS (Teasdale and Jennett, 1974), Table 3.

GCS alone can provide a good idea of mortality. In the Traumatic Coma Data Bank (TCDB) 78% of patients with a GCS of 3 died as opposed to only 11% of patients with a GCS

of 8 (Marshall et al., 1991a). On the GCS, motor score has been found to be the most important (Healey et al., 2003). Eye opening and verbal score can be difficult to estimate due to facial injures and early intubation. A full assessment of the GCS was not possible in at least a quarter of patients in the European Brain Injury Consortium survey of head injuries (Murray et al., 1999). In Sweden the Reaction Level Scale (RLS 85; Starmark et al., 1988), which is based on motor response, is often used instead of the GCS in ambulances and at trauma centres, Table 3.

Table 3. The Glasgow Coma Scale and the Reaction Level Scale 85

Glasgow Coma Scale			Reaction Level Scale 85
Best motor response	Best verbal response	Eye opening	1. Alert
Obeys commands (6)	Oriented speech (5)	Spontaneous (4)	2. Drowsy or confused
Localises pain (5)	Confused speech (4)	To command (3)	3. Very drowsy or confused
Flexor withdrawal (4)	Words only (3)	To pain (2)	4. Localises pain
Abnormal flexion (3)	Sounds only (2)	None (1)	5. Withdrawing movements
Extension (2)	None (1)		6. Stereotype flexion movements
None (1)			7. Stereotype extension movements
			8. No response to pain stimulation

Marshall classification

This CT classification (Table 4) was designed on the basis of experiences acquired in the pilot study of the TCDB study. The intention was to enable a classification of severe head injury so that patients at particular risk of deterioration could be identified and to enable early predictive statements regarding the likelihood of a fatal or nonfatal outcome (Marshall et al., 1991b). In the TCDB the risk of dying for patients with abnormal basal cisterns was approximately three times that of patients with normal cisterns with or without a mass lesion. Abnormal cisterns were also associated with an almost threefold risk of increased ICP (Eisenberg et al., 1990). The variability of timing of the CT after injury and the retrospective evaluation of evacuated versus non-evacuated lesions limits the usefulness of the Marshall categorisation in predicting outcome (Wardlaw et al., 2002). Furthermore the policy relating to the evacuation of haematomas may differ between surgeons (grade V or VI).

Table 4. The Marshall classification

Category	Definition
Diffuse injury I	No visible intracranial pathology
Diffuse injury II	Cisterns are present with midline shift of 0-5mm and/or lesion densities present, no high- or mixed- density lesion of > 25 mL
Diffuse injury III	Cisterns compressed or absent, with midline shift of 0-5 mm, no high- or mixed-density lesion of > 25 mL
Diffuse injury IV	Midline shift of > 5mm, no high or mixed density lesion of > 25 mL
Evacuated mass lesion (V)	Any lesion surgically evacuated
Non-evacuated mass lesion (VI)	High or mixed density lesion of > 25 mL, not surgically evacuated.

Pupils

Fixed and unreactive pupils are important predictors of mortality. In the TCDB, seventy-four per cent of patients with bilaterally unresponsive pupils after resuscitation died or were left vegetative (Marshall et al., 1991a). However, these changes do not occur if the injury is not extremely severe and they do not occur in a large percentage of head-injured patients with a GCS of less than 8. So, although absence of pupillary response are predictive of significant mortality, normal reflexes are not necessarily predictive of a good outcome.

Hypotension

Hypotension is one of the predictors that are amenable to early therapeutic modification. In one analysis of patients from the TCDB, early hypotension (systolic blood pressure < 90 mmHg) was associated with a doubling of mortality (Chesnut et al., 1993). The avoidance or minimisation of hypotension during the entire acute and post-injury period has the highest likelihood of improving outcome. In addition to avoiding hypoxia and hypotension one major therapeutic goal is the maintenance of adequate cerebral perfusion pressure (CPP). The two key hemodynamic factors associated with CPP are the ICP and blood pressure (CPP = mean arterial pressure minus ICP).

Intracranial pressure

The evidence that ICP is a predictor of outcome, together with the possibility to monitor ICP, has assured its central role in the treatment of head injury patients. Data from 429 patients in one study from Cambridge confirmed the findings, that high ICP (mean > 20 mmHg) and low CPP (< 55 mmHg) are strongly associated with fatal outcome, but excessive CPP (> 95 mmHg) also appeared to reduce the probability of obtaining a favourable outcome (Balestreri et al., 2006).

C. Outcome measure

The Glasgow Outcome Scale (GOS; Jennett and Bond, 1975) and the Modified Rankin Scale (van Swieten et al., 1988) are the most commonly used outcome measurement after acute brain injuries. The Glasgow Outcome Scale (GOS) was designed by Jennett and Bond as a companion to the GCS to describe the prolonged effects of head trauma, and it is still the most widely used outcome scale for the assessment of patients with traumatic brain injuries. Persisting disability after brain trauma usually comprises both mental and physical handicap. The mental handicap is often the more important in contributing to overall social disability. The GOS is less useful for minor head injury as it does not recognise subtle cognitive deficits, which may influence interpersonal relationships. The current recommendations are to use the GOS at six months to measure outcome after severe head injury.

Glasgow Outcome Scales (GOS and GOSE)

The GOS measures the ability of patients to take care of themselves. The scale is stratified into five outcomes; death, persistent vegetative state, severe disability, moderate disability and good recovery. Severe disability is defined as dependence for daily support. Moderate disability describes the person who is independent enough to travel by public transport and can work in a sheltered environment. Good recovery implies the resumption of normal life. In order to allow more sensitive measures of recovery, the GOS was extended to the Glasgow Outcome Scale-Extended (GOSE), which divides severe disability, moderate disability and good recovery categories into upper and lower divisions (Jennett et al., 1981; Table 5). Unfortunately, this led to an increase in inter-observer variability (Brooks et al., 1986). The refinement of the GOS using a structured interview questionnaire has led to 92% agreement

between observers using the GOS and 78% agreement between those using the GOSE (Wilson et al., 1998). This questionnaire is based on questions in five key areas: (1) independence at home, (2) independence outside the home, (3) employability, (4) ability to engage in premorbid social and leisure activities and (5) interpersonal relationships.

The scale reflects disability and handicap rather than impairment. It focuses on the way the injury has affected functioning in major areas of life, rather than on the particular deficits and symptoms caused by the injury. Disability is identified by a change from preinjury status as a result of the head injury. The best source of available information is used (Wilson et al., 1998). For statistical analysis outcome, it is often dichotomised into unfavourable (GOS 1-3, GOSE 1-4) and favourable (GOS 4-5, GOSE 5-8). We used the structured interview questionnaire of the GOSE to allow dispersion into eight categories.

Table 5. The extended Glasgow Outcome Scale (GOSE) and the Glasgow Outcome Scale (GOS)

GOSE	GOS	Description
1	1	Dead
2	2	Vegetative state
3	3	Lower severe disability completely dependent on others
4	3	Upper severe disability dependent on others for some activities
5	4	Lower moderate disability unable to return to work or participate in social activities
6	4	Upper moderate disability return to work at reduced capacity, reduced participation in social activities
7	5	Lower good recovery good recovery with minor social or mental deficits
8	5	Upper good recovery

King´s Outcome Scale for Childhood Head Injury (KOSCHI)

In the original paper from 1975, Jennett and Bond made particular mention of the special developmental considerations in the assessment of outcome in children, especially if the assessment is deferred for a year or more. Although the GOS has become a standard outcome scale in adult TBI, an equivalent scale for use in children has been lacking. Crouchman and

co-workers developed a specific paediatric adaptation of the original GOS for children aged 2-16 years (Crouchman et al., 2001).

Mini-Mental State Examination (MMSE)

The Mini-Mental State Examination is probably the most widely used rating scale for the simple assessment of cognitive function (Folstein et al., 1975). The MMSE requires vocal responses to questions covering orientation, memory and attention. Furthermore, the ability to name and follow verbal and written commands, as well as writing a sentence and copying a polygon is tested. It is recommended for screening after stroke and as a part of the American Heart Association Stroke Outcome Classification (Kelly-Hayes et al., 1998). As in many scales, patients with aphasia may be misclassified.

Barthel index

The Barthel index measures functional independence in personal care and mobility. It was initially developed to monitor performance in chronic patients before and after treatment and to indicate the amount of nursing care needed (Mahoney and Barthel, 1965). It is now widely used to describe the patients' capacity to perform activities of daily living (ADL). A patient scoring the maximum is continent, can eat, dress, bath, get out of bed and chairs, walk a block and ascend and descend stairs independently. This does not mean that he or she has the capacity to live alone. Full credit is not given for an activity if the patient needs even minimal help and/or supervision.

National Institute of Health Stroke Scale (NIHSS)

In 1989, Brott and co-workers designed a neurological examination scale for use in acute stroke therapy trials. The inter-rater reliability and test-retest reliability were high. The validity was assessed by comparing the scale scores by the size of the lesion and with the patients' clinical outcome, as determined at three months (Brott et al., 1989). The NIHSS is now one of the most frequently used impairment scales in stroke intervention trials and is used increasingly at emergency departments and in hospital settings. It is also recommended for the classification of neurological impairment in the American Heart Association Stroke

Outcome Classification (Kelly-Hayes et al., 1998). The NIHSS contains 15 items including level of consciousness, eye movement, visual field deficit, coordination, language (aphasia), speech (dysarthria), neglect and motor and sensory involvement. The scale was criticised for not measuring distal limb strength and an extra item regarding this function was attached and used in some trials of thrombolysis, but it did not obtain general acceptance. We used the extended version, including distal limb strength, as this was the predominant form when we started the study.

D. Biochemical markers of brain damage

The study of organ-specific proteins in body fluids has a long history. Myocardial infarction is one of the classical examples of tissue damage, that can be monitored by organ-specific proteins. Analyses of serum markers such as troponin T and creatine kinase (CK) are routine for patients with chest pain, and the high sensitivity enables myocardial infarction to be excluded.

The concept of "brain-specific proteins" is used for substances found in high concentrations in the central nervous system (CNS) and in low or negligible concentrations in other organs. The molecules are often specific for different cell types (neurons, glia) or subcellular components (axons or myelin). The proteins of neurofilament, Neuron Specific Enolase (NSE) and tau are the most established markers of neuronal damage, while Glial Fibrillary Acidic Protein (GFAP) and S100B are the most established for glial cell injury. Stroke and brain trauma cause acute brain injury and the general destruction of brain cells and "spill over" of different components to the CSF. The concentrations of both glial and neuronal proteins increase in the CSF, but at different points in time after the injury. Chronic brain diseases may cause different processes such as the degeneration of neurons and reactive gliosis, which can be reflected by markers in the CSF. Increased levels of brain-specific proteins are seen not only in the CSF, but also in serum (Missler et al., 1999). The proteins pass into the systemic circulation, probably directly through a disturbed blood brain barrier (BBB), or indirectly by release into the CSF followed by absorption to the circulation (Weller et al., 1992). The CSF concentrations of the brain damage markers generally exceed those in serum. So, due to sensitivity problems, analytical methods were first developed for the CSF, but, to be useful in clinical practice, a marker measurable in serum is preferable. Not only brain-specific proteins but also biochemical markers of stress and systemic inflammatory

response may be used as markers of brain damage. Factors such as hyperglycemia and leukocytosis have been related to poor outcome after SAH and TBI (Dorhout Mees et al., 2003, Yoshimoto et al., 2001, Rovlias and Kotsou, 2004). However, these phenomena are naturally not specific for brain injuries. Also, they may promote secondary brain injuries and there is consequently a direct connection to interventions. By contrast, brain-specific markers can reflect the situation in the brain, irrespective of other simultaneous processes.

Glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAP) is found almost exclusively in glial cells in the central nervous system and was first isolated by Eng and co-workers. GFAP builds up the glial intermediate filament that is a major cytoskeletal structure of astrocytes. Following brain injury and in some chronic diseases, astrocytes become reactive and respond in a typical manner termed astrogliosis. The cells proliferate, hypertrophy and form an abundance of intermediate filaments. While reactive gliosis is unspecific, the severity and time sequences vary in different diseases. Multiple sclerosis (MS; Eng et al., 1971) and Alexander's disease (Brenner et al., 2001) are distinguished by intense gliosis. After acute central nervous system injuries such as trauma or stroke, astrocyte disintegration is followed by the leakage of GFAP into the CSF.

In 1979, increased CSF-GFAP levels were observed after acute intracerebral haemorrhage (Hayakawa et al., 1979). A sensitive enzyme-linked immunosorbent assay (ELISA) for CSF was developed by Rosengren and co-workers (Rosengren et al., 1992; Rosengren et al., 1994). Numerous reports have documented the usefulness of CSF-GFAP as an indicator of CNS pathology, both in acute cell disintegration and in astrogliosis. After acute CNS injury, a temporary increase in GFAP in the CSF is observed from day 1-2, and it then normalises within a week or two. The levels are related to the extent of the injury and are pronounced after large cerebral infarctions (Aurell et al., 1991) and in patient with herpes encephalitis (Studahl et al., 2000). On the other hand, only modestly increased CSF levels are seen secondary to astrogliosis in MS (Malmeström et al., 2003), normal pressure hydrocephalus (Tullberg et al., 1998) and dementia (Wallin et al., 1996).

An early report on measurements of GFAP in human blood came from Missler and co-workers in 1999 (Missler et al., 1999). They showed that GFAP was released into the blood soon after severe traumatic brain injury. Some years later, the finding was confirmed in larger

studies of patients with traumatic brain injuries (Vos et al., 2004, Pelinka et al., 2004a). To date only a few clinical studies describing serum GFAP in patients with stroke have been published. Hermann and co-workers reported the delayed release of GFAP into serum in patients with ischemic stroke. Concentrations reached their maximum between days 2 and 4 (Herrmann et al., 2000). This delay probably reflects the gradual leakage of GFAP from necrotic glial cells. On the other hand, acute intracranial haemorrhage may cause a more sudden disruption of the BBB and the rapid destruction of astroglial cells, resulting in the earlier appearance of GFAP in serum. This hypothesis was supported by the findings of Foerch and co-workers who observed a rapid increase in serum GFAP levels in patients with haemorrhagic stroke, in contrast to those with ischemic stroke (Foerch et al., 2006).

Neurofilament protein

Neurofilaments are intermediate filaments of neurons and one of the major components of the neuronal cytoskeleton, responsible for the strength of the soma and for maintaining the calibre of axons. Neurofilaments are particularly abundant in large myelinated axons. The three neurofilament subunits, the light (NFL), medium (NFM) and heavy (NFH), assemble into a filamentous structure running the length of axons. NFL is the essential component of the neurofilament core.

Rosengren and co-workers developed a sandwich ELISA based on in-house antibodies for the detection of NFL in the CSF (Rosengren et al., 1996). CSF-NFL is increased when axons are injured. After cerebral infarctions substantially increased levels are seen late in the course, reaching a maximum several weeks after the infarction and normalisation after a period of months. The late increase is probably due to the release of NFL from the subsequent damage to the pyramidal tract after the infarction. Measurements of NFL can be used in chronic diseases as well. Modestly increased levels are observed in patients with upper motor neuron damage due to amyotrophic lateral sclerosis (ALS; Rosengren et al., 1996) and in patients with the impairment of subcortical myelinated axons due to normal pressure hydrocephalus (Tullberg et al., 1998). Sensitive NFL ELISAs using monoclonal antibodies (Norgren et al., 2003) and commercially available reagents have subsequently been described (van Geel et al., 2005). Moreover, the phosphoform of NFH (pNFH; Petzold et al., 2003) can be measured in the CSF. Although sensitive serum assays for NFL are not yet available it is

possible to analyse pNFH in serum (Shaw et al., 2005). The utility of this assay in the clinical setting remains to be studied.

S100

In 1965, Moore isolated a protein from bovine brain that was soluble in 100% ammonium sulphate (Moore 1965). The S100 protein turned out to be a group of Ca^{2+} binding proteins with different actions and distributions. They are involved in a variety of cellular processes, such as cell cycle regulation, cell growth and differentiation, and appear to have both intracellular and extracellular effects. In the brain, S100 is found predominantly in glial cells with higher concentrations in white matter and lower concentrations in grey matter. The proteins forms homo or heterodimers. Two classifications based on the different monomers have been used. The older classification divides the monomers into either α or β. The dimers were termed S100a0 (αα), S100a (αβ) and S100b (ββ) (Isobe et al., 1983). The discovery of the clustered organisation of S100 genes on human chromosome 1 provided a logical basis for a new classification. Different α monomers were numbered and called S100A1-S100A9. The former β monomer (localised to chromosome 21) was called S100B and the dimers were called S100BB, S100A1B, S100A1A1 and so on. A large variety of tissues have been shown to express members of the S100 family. The S100B monomer was first considered to be brain specific. However, it has also been found in other tissues such as adipocytes, chondrocytes and melanocytes. The S100A1 monomer is most abundant in cells outside the nervous system in skeletal muscle, heart and kidney. In the brain, the S100 proteins are composed mainly of the monomers S100A1 and S100B that form the two dimers, S100A1B and S100BB (Schäfer and Heizmann, 1996).

Commercial kits detect the S100B subunit, thus including both the S100A1B and S100BB dimers in the measured concentration. When used in the diagnosis of brain damage, this measurement is often inconsistently referred to and has been called S100, S100B, S100b or S100β. In this thesis, we use the name S100B for the results of commercial measurement of the S100 proteins, containing at least one B subunit, meaning both the S100A1B and the S100BB dimers. (The B monomer is clearly expressed as a subunit, when used.)

S100B is an established marker of brain injury after traumatic brain injury (Raabe et al., 1999a, Woertgen et al., 1999, Rothoerl et al., 2000, Ingebrigtsen et al., 2000). Elevated serum S100B levels are not necessarily associated with neuroglial damage but may instead reflect

the ongoing failure of the blood brain barrier (Kanner et al., 2003, Kapural et al., 2002). However, the brain specificity has been questioned and Anderson and co-workers concluded that trauma, even in the absence of head trauma, results in high serum concentrations of S100B. Among their trauma patients, serum S100B levels were highest after bone fractures and thoracic contusions without fractures, but also burns and minor soft-tissue damage caused increased S100B levels (Anderson et al., 2001a). Increased serum levels secondary to the release of S100B from traumatised extra-cerebral tissues seemed probable.

An assay measuring the A1-subunit in S100A1B has been developed (Fujirebio Diagnostics AB, Sweden). To date only a few previous studies have measured S100A1B and/or S100BB separately (Anderson et al., 2001a, Anderson et al., 2001b, Anderson et al., 2003, Nygren de Boussard et al., 2004, Nygren de Boussard et al., 2005).

AIMS OF THE STUDY

The main objectives were to study the clinical findings and long-term outcome after subarachnoid haemorrhage and severe traumatic brain injury in relation to levels of brain damage markers in serum and CSF. The specific aims were to:

Investigate whether the concentrations of serum GFAP were increased after aneurysmal subarachnoid haemorrhage and whether the concentrations were associated with clinical findings in the acute phase and after one year (Paper I).

Study the relationship between CSF-NFL and acute brain damage and long-term outcome after subarachnoid haemorrhage (Paper II).

Analyse GFAP in serum after severe traumatic brain injury and relate the concentrations to long-term outcome (Paper III).

Compare S100BB and S100A1B with S100B in relation to outcome after severe traumatic brain injury (Paper IV).

PATIENTS AND METHODS

A. Aneurysmal subarachnoid haemorrhage

Inclusion

All patients with an aneurysmal subarachnoid haemorrhage admitted to the NICU at Sahlgrenska University Hospital between October 2000 and December 2002 were considered for inclusion. Given that some patients may experience "warning headache" resulting from early bleeding, we defined the calendar day of the most severe symptoms before arrival as day 0. To be included in the study, the first serum sample had to be obtained at the latest on day 2 and the aneurysmal origin of the haemorrhage had to be proven by intra-arterial angiography. LP was performed in a subgroup of the study participants and, in addition to approval from the patient, the neurosurgeon in charge of patient care had to approve the LP prior to its performance.

Treatment

All the patients were treated according to well-established routines at the NICU, including the IV administration of tranexamic acid (Cyclokapron®) and nimodipine (Nimotop®). Higher dosages than standard of nimodipine, prostacyclin (Flolan®), as well as intravascular volume expansion and induced hypertension, were used in patients with vascular spasm. In most cases, transcranial Doppler sonography (TCD) was used for vasospasm detection. The indication for EVD was clinical and individual. In addition to the first diagnostic computed tomography (CT), subsequent CT scans were performed whenever clinically indicated. The aneurysm responsible for the haemorrhage was treated with neurosurgical clipping or by endovascular coiling. The choice of treatment strategy was based on clinical grounds or, in a few cases, after inclusion in the International Subarachnoid Aneurysm Trial (ISAT). Sahlgrenska University Hospital participated in this multicentre randomised clinical trial between 1997 and May 2002. ISAT compared neurosurgical clipping with endovascular coiling in patients considered suitable for either treatment (Molyneux et al., 2002).

Sampling, examinations and categorisation of clinical data

The diagnostic CT scan was performed at the "first" hospital and was re-examined by one neuroradiologist and categorised according to the Fisher scale. The definition of "focal lesion" was used if an ischemic lesion and/or intraparenchymal haemorrhage was seen. The condition of the patients at the initial medical consultation was graded retrospectively according to the WFNS, based on records from the local hospitals in the catchment area. The WFNS combines information about the level of consciousness and major focal deficits (aphasia and/or hemiparesis) on two different axes. We used major focal deficits as the most important axis for conscious patients. We also applied this axis separately as a clinical sign of focal brain injury.

Venous blood samples for GFAP were obtained as soon as possible after admission to the NICU and then every morning on days 1, 2, 3, 4, 6, 8 and once in the period between days 10-15. On the day of the last serum sampling (day 10-15), a neurological examination was performed on all patients and LP was performed in a subgroup of the patients, Table 6. Neurological status was graded according to NIHSS and WFNS. Aneurysm ruptures during intervention, re-bleeding and ischemic events (irrespective of the cause) during the sampling period were classified as secondary events. Bleeds were confirmed from CT scans and/or description from the neurosurgeon or radiologist performing the clipping or coiling, respectively. Ischemic events were confirmed with repeated CT or in a few cases clinically day on 10-15.

One year after the aSAH, outcome was categorised according to the GOSE, using the structured interview by Wilson and co-workers (Wilson et al., 1998). ADL function was assessed by the Barthel index and the MMSE was used for cognitive screening. Neurological examinations were performed and graded using the NIHSS and the presence of major focal deficits (hemiparesis/aphasia) was registered. In addition to these clinical data, magnetic resonance imaging (MRI) of the brain was performed.

Table 6. Summary of the study plan. Cross in grey squares indicates the day of the activity. Empty grey square indicates alternative day.

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 6	Day 8	Day 10-15	Day 365
Haemorrhage	X								
Inclusion in study	X								
Serum sampling	X	X	X	X	X	X	X	X	
Initial CT	X								
LP								X	
Major focal deficit	X							X	X
WFNS	X							X	
NIHSS								X	X
MMSE									X
Barthel									X
GOSE									X
MRI									X

The neurologist (K N) responsible for examinations, interviews and the categorisation of clinical data was blinded to the results of the biochemical markers.

B. Severe traumatic brain injury

Inclusion

Patients with severe TBI were included consecutively at the NICU at Sahlgrenska University Hospital between October 2000 and December 2002. The trauma was defined as severe if the following criteria were all fulfilled:

1) Reaction Level Scale (RLS) $\geq$ 4, corresponding to a score sum of $\leq$ 8 on the Glasgow Coma Scale (GCS), (Starmark et al., 1988, Jennett and Bond, 1975)
2) A therapeutic indication to monitor intracranial pressure (ICP)
3) Need for ventilator treatment

The calendar day of the trauma was defined as day 0. To be included in the study, the first serum sample had to be obtained on day 2 at the latest.

Treatment

Airway control, optimising circulation and primary estimation of the injuries were performed in the ambulance and at the emergency departments at the various hospitals in the region. On arrival at Sahlgrenska University Hospital, the patients were fitted with a ventricular catheter for intracranial pressure monitoring (and opportunity for therapeutic CSF drainage). When indicated, space-occupying lesions were removed surgically. At the NICU, patients were treated according to a standardised protocol, the "Lund concept", designed to maintain an ICP of < 20 mmHg.

Sampling, examinations and categorisation of clinical data

The condition of the patients in the ambulance and at the initial medical consultation was graded retrospectively. The initial CT was reviewed retrospectively by one neuroradiologist (blinded to clinical and laboratory data) and classified according to Marshall I-IV. Venous blood samples for GFAP, S100B, S100A1B and S100BB were obtained as soon as possible after admission to the NICU and then every morning on days 1, 2, 3, 4, 5, 6, 8 and once in the period between days 11 and 14 (Table 7).

One year after the TBI, outcome was categorised according to the GOS. We used the structured interviews by Wilson and co-workers (Wilson et al., 1998). The outcome for children was also compared with the KOSCHI category definitions for childhood head injury (Crouchman et al., 2001).

Table 7. Summary of the study plan. Cross in grey square indicates the day of the activity. Empty grey square indicates alternative day.

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 6	Day 8	Day 11-14	Day 365
Trauma	X								
Inclusion in study	X								
Serum sampling	X	X	X	X	X	X	X	X	X
Initial CT	X								
GOSE/GOS									X

C. Chemical analyses and statistics

Reference levels for brain damage markers

To determine GFAP reference levels, serum samples from 218 healthy individuals (mean age 46 years, range 18-80) were analysed. The levels did not correlate with age. The reference level was set at < 0.15 μg/ L (95 percentile).

Laboratory NFL reference levels were based on CSF-NFL determination from 138 healthy control individuals aged 18-83 years. Values were regarded as normal if the level of CSF-NFL was < 250 ng/L for patients aged < 60 years, < 380 ng/L for patients aged 60-70 years and < 750 ng/L for patients aged 70-80 years.

According to the manufacturer's specifications (Fujirebio Diagnostics AB, Göteborg, Sweden), the reference levels used for S100B, S100A1B and S100BB were ≤ 0.09 μg/L, ≤ 0.06 μg/L and ≤ 0.02 μg/L, respectively.

Analysis of serum GFAP

Serum GFAP was measured using a sandwich ELISA described by Rosengren and co-workers (Rosengren et al., 1994). The method was slightly modified. In short, the assays were run in microtest plates using hen anti-GFAP IgG as the capturing antibody. Duplicate samples of serum were incubated with phosphate-buffered saline in each well. Duplicate samples of reference GFAP were incubated in phosphate-buffered saline supplemented with 50% normal horse serum. Rabbit anti-GFAP IgG was used as the detecting antibody. Bound rabbit IgG was detected by the binding of peroxidase-conjugated donkey anti-rabbit IgG. The colour reaction was developed using *o*-phenylenediamine and Perhydrol and the optical density was measured at 490 nm. The concentrations of GFAP were interpolated from the standard curve. Interassay precision was determined by duplicate analyses of two CSF samples and one serum sample on 71 different days. The precision was close to 11% in the region of 1 and 0.25μg/L, but higher at levels of 0.1μg/L (22%). Mean intra-assay precision was determined as 15.3% by using the same sample run in four duplicates on 14 different days. The linearity of the assay was controlled by serial dilutions of three patient's samples with very high levels of GFAP (3.7, 6.2 and 15.8 μg/L) in phosphate-buffered saline supplemented with 50% normal horse serum. The dilution curves were close to linear (correlation coefficients 1.00, 0.99 and 0.99 respectively). The recovery of the assay as determined by spiking serum from 14 normal

controls with reference GFAP at 2.0, 1.0 and 0.5 μg/L was 54 ± 8% (SD), 52 ± 8% and 53 ± 8% respectively.

Analysis of CSF-NFL

CSF-NFL was measured using a sandwich ELISA as previously described (Rosengren et al., 1996). Briefly, microtest plates were incubated with capturing antibody (hen anti-NFL IgG). Samples or reference NFL were incubated in duplicate. Rabbit anti-NFL IgG was used as the detecting antibody. The plates were subsequently incubated with peroxidase-conjugated donkey anti-rabbit IgG. The colour reaction of o-phenylenediamine and H_2O_2 was developed and the optical density was measured at 490 nm. The concentrations of NFL in the samples were interpolated from the standard curve. The standard curve ranged from 125 to 16,000 ng/L. The sensitivity of the assay was 125 ng/L.

Analysis of serum S100A1B, S100BB and S100B

The serum concentrations were measured using three different ELISA methods (Fujirebio Diagnostics AB, Göteborg, Sweden). Samples were processed according to the manufacturer's specifications.

Statistics

Standard mathematical operations were used for descriptive purposes. Statistical analyses were performed using non-parametric tests. Spearman's rank correlation test was used for correlations. For comparisons between two groups, Fisher's exact test was used for dichotomous variables, the Mantel-Haenzel Exact Chi 2 test was used for ordered categorical variables and the Mann-Whitney U-test was used for continuous variables (Papers I-IV). Van Elteren's test was used for comparisons of continuous variables between favourable and unfavourable outcome, adjusting for neurosurgery after TBI (Papers III and IV) and after aSAH (Paper II). Logistic regression analysis was performed for each independent variable to predict the outcome. The area under ROC curve (c-statistics) was calculated for descriptions of goodness of predictors (Papers I and IV). Multiple logistic regression analyses were used to assess the independent contribution of s-GFAP in predicting the outcome after aSAH (Paper

I) and after TBI (Paper III; stepwise regression analysis). Multiple logistic regression analysis was also used to adjust for neurosurgery after aSAH (Paper I). Odds ratios (OR) were used to describe the probability of unfavourable outcome after TBI (Paper III). All the tests were two-tailed and were conducted at the 5% significance level. Because of the very large variation in the concentrations of the biochemical markers, some figures were presented on a logarithmic scale.

RESULTS

A. Aneurysmal subarachnoid haemorrhage

We reviewed 199 consecutive patients referred to the NICU due to subarachnoid haemorrhage. Aneurysm was not proven in 53 patients (no aneurysm in 36 cases, angiography not performed in 17 cases). The first serum sample was not taken according to the time window in the inclusion criteria in 22 cases (delay of referral, n=18, and failure of routines, n=4). Informed consent was not possible in eight cases. As a result, 116 patients (81 women and 35 men) with aneurysmal SAH were eligible for the study. One woman with multiple aneurysm and two bleeds (1 ½ years apart) was included twice in the study. Two patients were included even if an intra-arterial angiography was not performed. One had a known aneurysm and the angiography was not repeated, while the other underwent urgent surgery and the aneurysm was identified by the neurosurgeon. The characteristics of the 116 patients are shown in Table 8. Early treatment of the aneurysm was preferred and only five patients were treated after day 2. Endovascular coiling was performed in 90 patients and neurosurgical clipping in 24 patients. Two patients were managed conservatively. External ventricular drainage was frequently inserted (n=72) and revision was necessary in 21 cases. In addition to the aneurysm operation, haematomas were evacuated (n=3) and decompressive craniectomy had to be performed (n=5) after clipping or coiling. In all, only 35 patients did not undergo any neurosurgery (coils alone or conservative treatment).

We tried to perform a complete follow-up after one year and mean as well as median time was 12 months (range 11-14 months). Patients who were not able to visit the outpatient clinic for practical reasons were visited at their nursing home. Only one patient was lost to follow-up. Outcome was assessed by face-to-face interviews and clinical examinations in all but three patients, who did not reside in the region (one by telephone interview and two from medical records). The outcome was favourable (GOSE 5-8) for 79 patients and unfavourable (GOSE 1-4) for 36. Twelve patients died within the first month and another six within one year.

Table 8. Characteristics of all 116 patients with aSAH included in the serum GFAP study. Characteristics of the subgroup of patients (n=48) in whom LP was performed in parenthesis.

	Total number	Number in the LP subgroup
Women (one woman with two aSAHs 1½ years apart included twice)	81	(35)
Men	35	(13)
Age (years)		
Median	55	(59)
Range	20-81	(26-75)
Initial CT (Fisher scale; subarachnoid blood)		
Grade 1 (none)	1	(0)
Grade 2 (diffuse only)	0	(0)
Grade 3 (clot or thick layer)	27	(10)
Grade 4 (diffuse or none, with cerebral or ventricular blood)	85	(38)
Missing data	3	(0)
WFNS, initial assessment		
WFNS I	57	(23)
WFNS II	27	(13)
WFNS III	8	(2)
WFNS IV	11	(7)
WFNS V	13	(3)
Numbers of aneurysms		
One aneurysm	86	(40)
Two aneurysms	22	(4)
Multiple (3-6)	8	(4)
Target aneurysm size		
≤ 5 mm	51	(20)
6-10 mm	46	(25)
≥ 11 mm	17*	(3)
Not possible to define target aneurysm size	2	(0)
Location of target aneurysm		
Anterior circulation	95	(40)
Posterior circulation	21	(8)
External ventricular drainage	72	(32)
Treatment		
Endovascular coiling	90	(35)
Neurosurgical clipping	22	(12)
Clipping and endovascular coiling	2	(0)
Conservative	2	(1)
Outcome		
GOSE 1 (=dead)	18	(3)
GOSE 2 (=vegetative state)	0	(0)
GOSE 3 (=lower severe disability)	17	(7)
GOSE 4 (=upper severe disability)	1	(0)
GOSE 5 (=lower moderate disability)	22	(12)
GOSE 6 (=upper moderate disability)	26	(13)
GOSE 7 (=lower good recovery)	6	(3)
GOSE 8 (=upper good recovery)	25	(10)
Data missing (GOSE > 1)	1	(0)

* In one case as determined by the surgeon alone

Serum samples were drawn from all 116 patients. The results of the serum GFAP study (Paper I) are presented below. In 48 of the 116 patients, LP was possible to perform on day 10-15 for CSF-NFL analysis. (LP was contraindicated in 31 cases, informed consent not possible in 19 cases and CSF was not available due to technical problems in six cases. Eight patients were discharged and four patients died before the day of the LP.) The results of the CSF-NFL study (Paper II) are also presented below.

Serum GFAP and aneurysmal subarachnoid haemorrhage (Paper I)

The time from the bleed to the first sample in each series varied between three and 62 h. The first sample was typically taken on day 1. The individual s-GFAP series included a mean of seven samples. Maximum s-GFAP was seen in the first few days (median on day 2, range day 0-15). The temporal release patterns, as well as the maximum levels, showed a huge inter-individual variation. The maximum s-GFAP was in the range of 0.03 to 34.43 μg/L (median 0.33 μg/L, mean 1.13 μg/L) and the majority (n=81) of the patients had maximum s-GFAP above the normal reference level (0.15 μg/ L). All the patients with completely normal s-GFAP series (n=35) had initial CT scans without focal lesions. They were all treated with the endovascular technique and thirteen of them had EVD.

Relationship with focal brain injury

Maximum s-GFAP correlated with status on arrival as graded by WFNS and with the initial CT findings according to Fisher (r=0.37, 0.34 respectively, p<0.001). Furthermore, maximum s-GFAP was increased in patients with "focal brain lesions" (parenchymal haematomas or infarctions; n=20) on the initial CT compared with patients without such lesions (p<0.001).

Patients with major focal deficits on arrival (n=14), at day 10-15 (n=21) or at one year (n=23) had increased maximum s-GFAP compared with patients without deficits, Figure 1. Major focal deficits categorised according to WFNS (hemiparesis and/or aphasia) are a somewhat crude description of neurological deficits. NIHSS provides more details, but on the other hand NIHSS on arrival at the first hospital was not possible to estimate from medical records. On day 10-15, one neurologist performed a standardised neurological examination, but patients sedated in a ventilator (n=25) had to be excluded from categorisation according to WFNS. (Another ten patients were not categorised due to early discharge or death.) NIHSS was not appropriate in sedated or spontaneously unconscious patients (n=25+7). Maximum s-

GFAP correlated with status as graded by WFNS and NIHSS on day 10-15 (r=0.47, p<0.001, and r=0.47, p<0.001 respectively). At one year, the conditions for examination were optimal and NIHSS scoring was possible for all patients. Maximum s-GFAP correlated with NIHSS (r = 0.50, p<0.001).
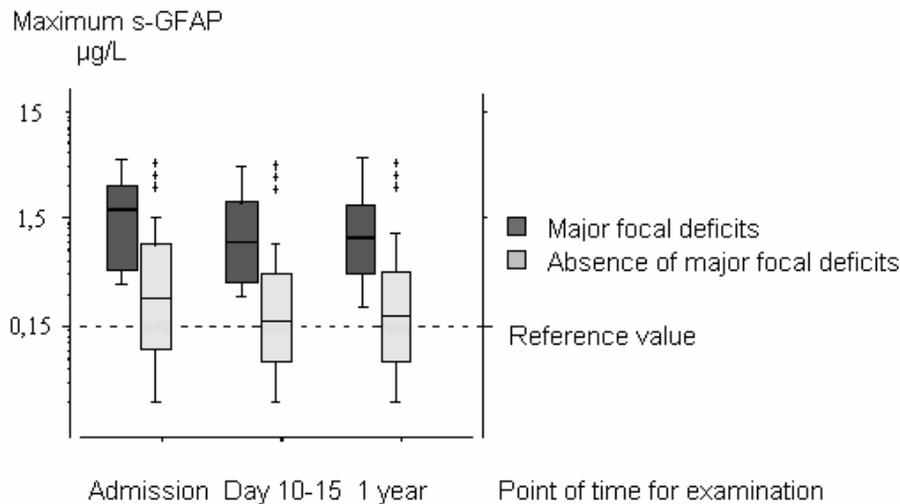

Figure 1. *Maximum s-GFAP (sampling period day 0-15) in the patient group with major focal deficits (hemiparesis/aphasia) compared with patients without focal deficits. Neurological examination performed at three different points in time. Box plots show the 10th, 25th, 50th, 75th and 90th percentiles. Outliers excluded.*
[+++] *p<0.001, Mann-Whitney U-test.*

CT was not repeated as part of the study, but in most cases it was repeated on clinical indications. To validate the relationship between the initial CT and serum GFAP, we compared serum GFAP with the "final CT" during the sampling period and confirmed increased levels for patients with "focal brain lesions" (p<0.001). The patient group with radiological signs of ischemia or previous intracerebral haemorrhage (n=56) at one year had higher maximum s-GFAP concentrations in the acute phase, compared with those without these findings (p<0.001, MRI in 88 cases and CT in six cases).

Relationship with secondary events

Secondary neurological events were seen in 39 patients (four ruptures during embolisation and three ruptures during neurosurgery, three re-bleedings and 29 ischemic events) during the sampling period. The patient group with secondary events reached maximum levels later in the series (median day 3 versus day 1) and maximum levels were increased, compared with patients without secondary events (p<0.001 in both comparisons), Figure 2. Calculating an s-GFAP fraction (last/first value) also produced a significant increase (p<0.01) in the patient group with secondary events. Furthermore, all patients (n=11) with s-GFAP maximum after day 4 had a secondary event.
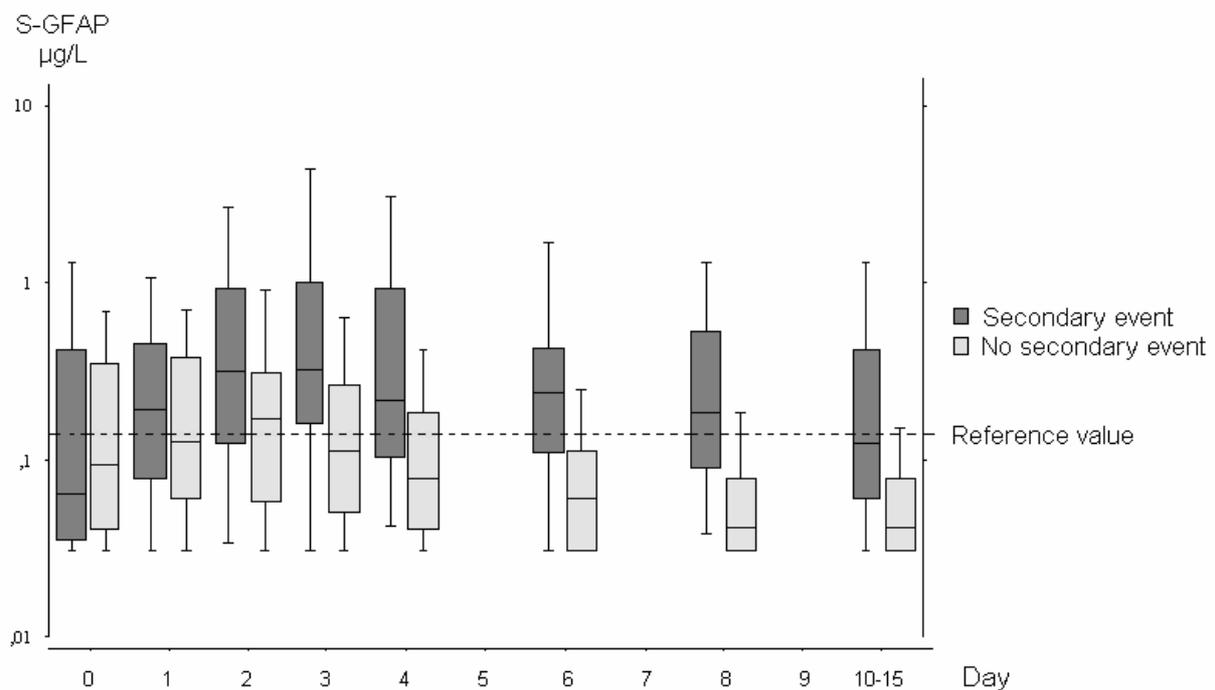
Figure 2. *Temporal course of s-GFAP in patients with and without secondary events respectively. Each box plot displays the 10th, 25th, 50th, 75th and 90th percentile. Outliers are excluded. The group without secondary events comprised 77 patients. The number of samples was 28 on day 0 and 67-76/day on the other days. The group with secondary events comprised 39 patients. The number of samples was 12 on day 0 and 31-39/day on the other days.*

Relationship with outcome

Patients with an unfavourable outcome (GOSE 1-4) had increased maximum s-GFAP levels compared with patients with a favourable outcome (GOSE 5-8; p<0.001). Maximum s-GFAP correlated with overall outcome assessed after one year and categorised according to GOSE, Figure 3.
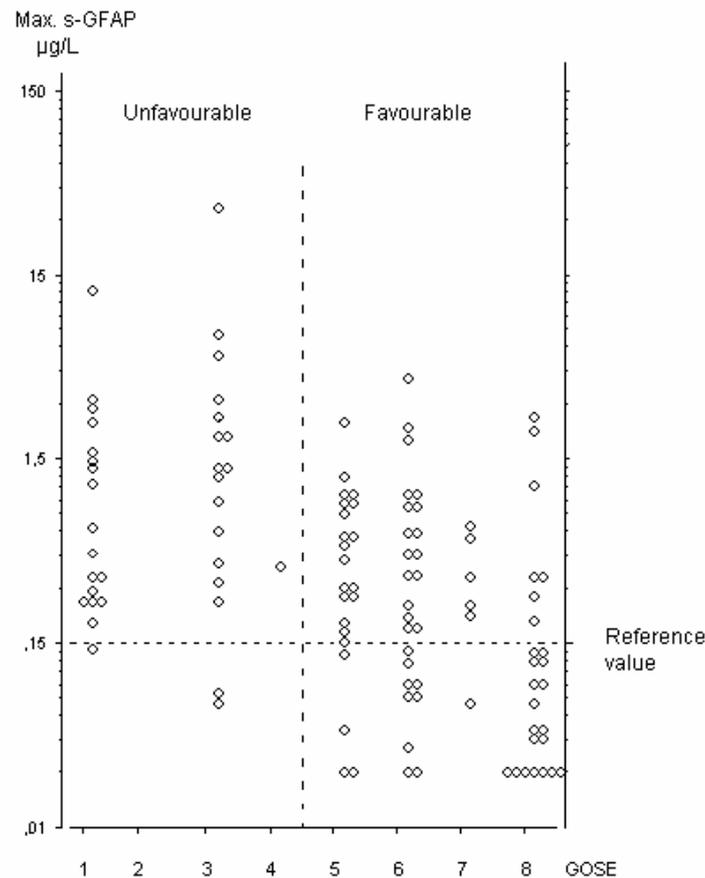
Figure 3. *Maximum s-GFAP in 115 patients after aSAH in relation to long-term outcome (GOSE) shown as a scattergram (r=-0.48, p <0.001, Spearman's test).*

Patients undergoing any form of neurosurgery had increased maximum s-GFAP levels compared with those who did not undergo neurosurgery (p<0.001). However, maximum s-GFAP was an independent predictor of outcome when adjusting for neurosurgery (multiple logistic regression analysis). In addition to this global outcome assessment (GOSE), the results of the neurological examination (NIHSS), estimations of functional independence (Barthel index) and the cognitive screening (MMSE) at one year all correlated with maximum s-GFAP (r =0.50, -0.39, -0.34, respectively and p<0.001, <0.001, <0.01 respectively). As

expected, the GOSE was associated with the NIHSS, Barthel index and MMSE respectively, Figure 4.

Figure 4. *Overall outcome assessed as GOSE in relation to neurological status (NIHSS), cognitive function (MMSE) and personal care/mobility (Barthel index) respectively. (NIHSS ≥ 1p, MMSE ≤ 27p and Barthel < 100p were regarded as pathological.)*

Prognostic information
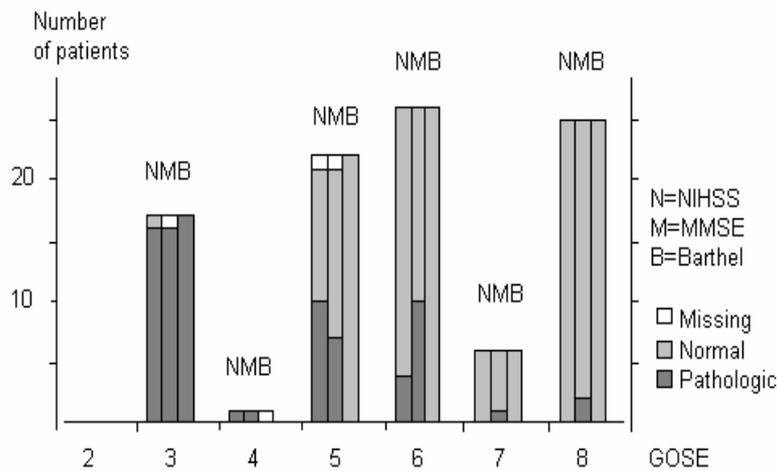
Only three of 35 patients with a normal s-GFAP series had an unfavourable outcome. When scrutinising these three cases, one suffered a severe vasospasm with major ischemic lesions (day 9) and finally herniation on day 11. Serum samples were taken according to protocol (day 8 and 11), but the last sample was taken after pupil dilation, when brain circulation was likely to have ceased, and it may be too late to detect a brain infarction. Finally, the two remaining patients with an unfavourable outcome turned out to have normal pressure hydrocephalus when investigated at the one-year control. A normal s-GFAP series in these two cases during the acute phase seems reasonable. Including all the patients in the study and using the normal reference level (<0.15 μg/L) for maximum s-GFAP as the cut-off point for predicting dichotomised outcome results in a negative predictive value (npv) of 91%. Excluding the patients with a normal s-GFAP series but other explanations of unfavourable outcome (n=3) produces an npv of 100%. A normal s-GFAP series therefore appears to have potential when it comes to the early prediction of favourable outcome. Repeated sampling to obtain a normal s-GFAP series/maximum s-GFAP value is impractical. Consequently, we also calculated the predictive values for each sampling day. The highest negative predictive value was observed on the day including most samples (day 3, n=113) with an npv of 86% and a

relatively low ppv, 45%. (The sensitivity was 79% and the specificity was 58%). To make a clinical comparison, both neurological (WFNS) and radiological (Fisher scale) findings on arrival were related to dichotomised outcome (favourable/unfavourable) at one year (p= 0.004, p= 0.016, univariate logistic regression analysis). However, s-GFAP on day 3 was at least as good at predicting the long-term outcome as WFNS or Fisher scale (c-statistics 0.72, 0.68 and 0.61 respectively) and also independently predicted outcome after adjustment for age, clinical (WFNS) and radiological (Fisher scale) findings (multiple logistic regression analysis). The combination of the predictors (age, WFNS, Fisher grade, s-GFAP day 3) improved the prediction only slightly compared with s-GFAP alone (c- statistics 0.77 and c-statistics 0.72, respectively).

CSF-NFL and aneurysmal subarachnoid haemorrhage (Paper II)

Lumbar puncture was performed once in the period of day 10-15 (median day 11) in 48 patients. CSF-NFL concentrations were above the normal limits in all but two patients and showed a huge variation in the range of 350 and 62,190 ng/L (median 9,035 ng/L). (Concentrations were regarded as normal if they were < 250 ng/L for patients aged < 60 years, < 380 ng/L for patients aged 60-70 years and < 750 ng/L for patients aged > 70 years.) The association between CSF-NFL and neurological findings on the day of the lumbar puncture was strong. CSF-NFL correlated with WFNS (r=0.65, p<0.001), as well as NIHSS (r=0.73, p<0.001). NIHSS was inappropriate in 11 cases and WFNS was inappropriate in nine cases. (Nine patients were sedated in a ventilator and two had impaired consciousness.)

Patients affected by secondary events (n=14, re-bleeding or ischemia) had elevated CSF-NFL levels compared with those without secondary events (n=34, p<0.01).

Even if relatively few patients were treated with neurosurgical clipping, external ventricular drainage was frequently inserted and the majority of the patients (n=37) therefore experienced some kind of neurosurgical intervention (clips, EVD, evacuation of haematomas). This joint neurosurgical intervention group had higher CSF-NFL levels than the "non-surgical" group (p<0.01).

At one year, outcome was assessed and categorised as favourable (GOSE 5-8) in 38 cases and unfavourable (GOSE 1-4) in 10 cases. Only two patients had normal CSF-NFL and the outcome was excellent for both of them (GOSE 8). CSF-NFL correlated significantly with the GOSE, Figure 5. CSF-NFL was increased in the patient group with an unfavourable

outcome compared with those with a favourable outcome even after adjustment for neurosurgery (Van Elteren's test, p<0.01).
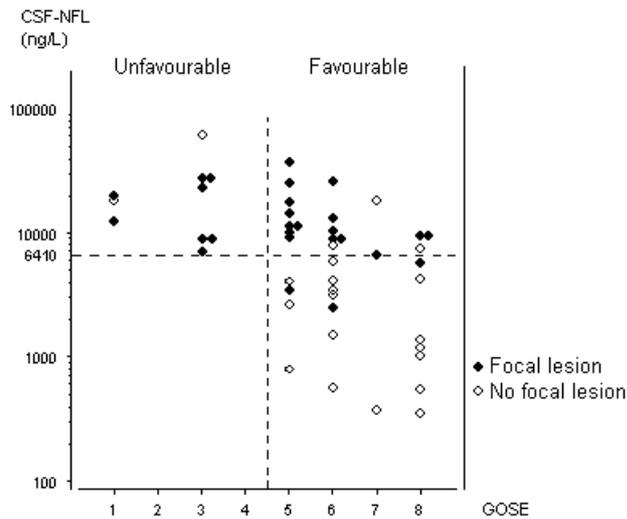
Figure 5. CSF-NFL 10-15 days after aSAH in relation to long-term outcome (GOSE) in 48 patients, shown as a scattergram. CSF-NFL correlates with GOSE (r=-0.56, p<0.001, Spearman's test). Radiological findings at one year depicted as the presence or absence of focal lesions. (Final CT shown for three non-survivors and for one patient (GOSE 5, lesion) not followed at one year.)

All the patients with an unfavourable outcome had CSF-NFL levels of > 6,410 ng/L (range 6,410-62,190 ng/L and median 19,250 ng/L), while patients with a favourable outcome had CSF-NFL levels ranging from 350 to 37,600 ng/L with a median of 6,320 ng/L. Patients with parenchymatous lesions induced by ischemia or bleeding (n=25), visible at follow-up neuroradiology at one year, had increased CSF-levels of NFL compared with those without lesions (n=19, p < 0.001), Figure 5.

In the patient group in whom LP was allowed, CSF-NFL concentrations correlated with the s-GFAP concentrations (taken from Paper I), Figure 6.

Figure 6. Maximum S-GFAP in relation to CSF-NFL in 48 patients with aSAH (r=0.49, p<0.001. Spearman's test) Outcome after one year dichotomised into favourable or unfavourable.

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B. Severe traumatic brain injury

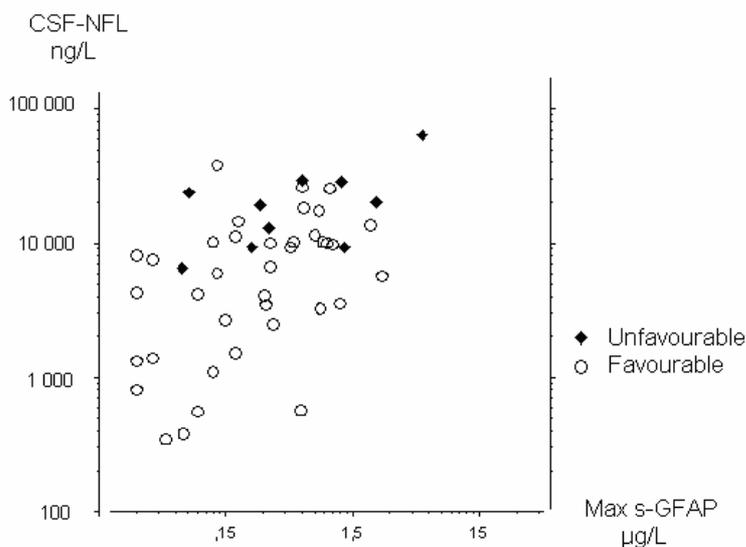During the inclusion period, 222 patients with traumatic brain injury were treated at the NICU. Due to the strict criteria for severe traumatic brain injury, only 73 patients were considered for inclusion in the study. Eleven were not eligible (foreign citizen (n=4), lack of informed consent (n=4) and delay of referral to the NICU (n=3)). In three cases, the routines for sampling failed. Finally, 59 patients with a predominance of younger men were included in the study. The patients' characteristics are shown in Table 9.

In the ambulance, 35 of the patients were assessed as being unconscious (RLS ≥ 4) and 17 as conscious (RLS ≤ 3) (missing data in 7 patients). On arrival at the primary hospital, the physician on call assessed 45 patients as being unconscious and 14 as conscious. Among the unconscious patients 12 were assessed as RLS 7-8. In the ambulance systolic blood pressure was clearly registered as < 90 mmHg in one case. At arrival to hospital one or both pupils were unreactive in 15 cases (normal in 15 and description vague or missing in 29 cases). Twenty-one patients were admitted directly to Sahlgrenska University Hospital and 38 were transferred from other hospitals. The initial CT of the brain was normal in one case and pathological in the remaining 58 cases. Midline shifts of > 5 mm were seen in 21 cases. If possible, patients with contemporary life-threatening traumata to other organs were treated at other intensive care units due to routines at the hospital. However, even at a "neuro" intensive care unit, only 13 patients were regarded as having an "isolated" brain injury. Brain injury in combination with fracture (especially skull fracture) was most common. ICP monitoring was performed either by a ventricular catheter (n=57) or by a parenchymal ICP device (Codman Express®; n=2). ICP was continuously recorded and was at least once above 25 mm Hg in 39 patients (CPP below 60 mm Hg at least once in 45 patients). All the patients were treated in a ventilator for at least four days. Therapeutic neurosurgery (besides ICP device), in particular the evacuation of haematomas and decompressive craniectomy, was indicated in 34 cases (day 0-9). On average, patients left the NICU after 14 days, 31 patients were transferred to ICUs at local hospitals and the others to general wards. Three patients died during the first four days and another eight within six weeks after the trauma. All these deaths could be attributed to the brain injury, although systemic complications and respiratory failure may have contributed (one 81-year-old woman had severe heart failure, one alcoholic had liver failure and coagulopathy and finally one man with dens fracture had respiratory problems).

Table 9. Characteristics of 59 patients with severe traumatic brain injury

	Number
Men	44
Women	15
Age (years)	
Mean	39
Median	37
Range	8-81
Cause of trauma	
Road traffic accident	30
Fall	10
Miscellaneous	14
Assault	5
Type of trauma	
Isolated brain injury	13
Brain injury and fractures (including skull fractures)	28
Brain injury and fractures and trauma to internal organs	17
Brain injury and trauma to internal organs	1
Initial CT	
Type I (normal)	1
Type II (lesions, present cisterns, midline shift 0-5 mm)	20
Type III (lesions, cisterns compressed, midline shift 0-5 mm)	17
Type IV (midline shift > 5mm)	21
Neurosurgery	
Evacuation of haematomas	17
Epidural (n=3)	
Subdural (n=9)	
Intracerebral (n=2)	
Multiple localisation (n=3)	
Evacuation of haematomas and decompressive craniectomy	12
Decompressive craniectomy	3
Revision of skull fracture	2
Outcome	
GOS 1 = Dead	11
GOS 2 = Vegetative state	1
GOS 3 = Severe disability	16
GOS 4 = Moderate disability	20
GOS 5 = Good recovery	11

The outcome of the 48 survivors was scored using face-to-face interviews in 45 cases, by telephone in two cases and from medical records in one case. A favourable outcome (GOS 4-5) was seen in 31 patients and an unfavourable outcome (GOS 1-3) in 28 patients. Among the included patients five were children aged 8-16 years. Four of them had a favourable and one an unfavourable outcome.

Age and initial CT findings (Marshall) were related to dichotomised outcome (p<0.05 and p<0.01 respectively). Since the description of the initial pupillary response was vague or missing in many reports (29/59) we refrained from using this information in the statistical analyses. The exact RLS score was not documented in all cases, but the patient group in which the severity was regarded as critical (RLS 7-8), did not have a significantly different outcome (dichotomised) from the remainder (RLS 4-6, p=0.4). There was no significant difference in maximum ICP levels or minimal CPP levels between the patient group with an unfavourable outcome compared with those with a favourable outcome. Nor was dichotomised outcome significantly related to whether or not the patients were already unconscious in the ambulance, whether or not they had multiple trauma, or whether or not further neurosurgical interventions (in addition to the ICP device) were necessary.

Serum GFAP after severe traumatic brain injury (Paper III)

Maximum s-GFAP was increased in all but one of the patients. The highest levels were seen during the first few days (median day 1) and they then decreased gradually. On day 6, the levels had normalised in 50% of the samples. Maximum ICP correlated with maximum s-GFAP (r=0.36, p<0.01). Patients with a midline shift of > 5 mm on the initial CT had increased maximum s-GFAP compared with patients without a midline shift (p < 0.05). There was no significant difference in maximum GFAP levels between the patient group requiring further neurosurgery, compared with those only operated upon with an ICP device (p=0.1). Nor was there a significant difference in patients with additional trauma compared with those with an "isolated" brain injury (p=0.6). Patients with an unfavourable outcome (n=28; GOS 1-3) had significantly (p<0.001) higher maximum s-GFAP compared with patients with a favourable outcome. Their maximum s-GFAP ranged from 0.38 to 49.58 μg/L and all the patients with s-GFAP of > 15.04 μg/L died (n=5). Patients with a favourable outcome (n=31, GOS 4-5) had maximum s-GFAP below 6.98 μg/L (Figure 7). Not only the maximum value but also the values on days 1, 2, 3, 4, 6, 8 and 11-14 were significantly increased in the patient group with an unfavourable outcome compared with the group with a favourable outcome. CT findings, age and maximum s-GFAP (log transformed) correlated significantly with dichotomised outcome and were therefore included in a forward stepwise multiple logistic regression analysis. S-GFAP was found to be the strongest predictor of dichotomised outcome.
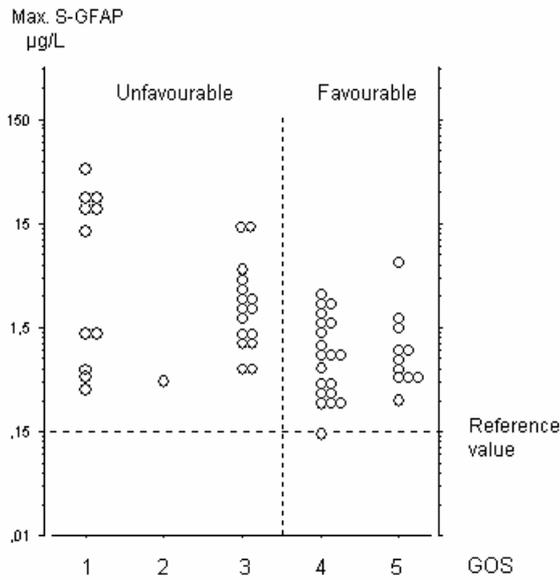
Figure 7. *Maximum s-GFAP in 59 patients after severe traumatic brain injury in relation to long-term outcome (GOS), shown as a scattergram. (r=-0.39, p<0.01, Spearman's correlation).*

Serum S100A1B and S100BB after severe traumatic brain injury (Paper IV)

Maximum serum S100B, S100A1B and S100BB were increased in all but a few cases. The three markers S100B, S100A1B and S100BB, followed the same temporal course with an early maximum (median day 1), followed by gradually decreasing values. On day 3 concentrations were normalised in approximately 50% of the samples, Figure 8. The concentrations of S100A1B exceeded those of S100BB. The sum of S100A1B and S100BB values did not fully correspond to that of S100B (80% or less).
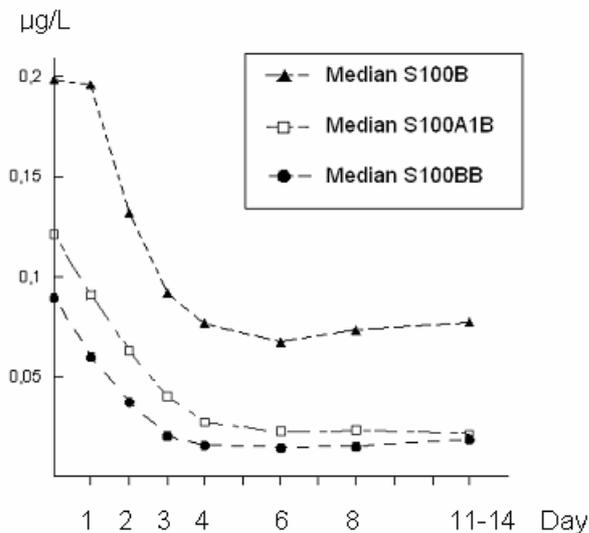

Figure 8. *Median serum concentrations of S100B, S100A1B and S100BB in relation to day after severe traumatic brain injury in 59 patients. The number of samples was 17 on day 0, 35 on day 11-14 and 44-55/day on the other days.*

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Maximum ICP correlated with maximum S100B, S100A1B and S100BB (r= 0.38, 0.34, 0.37, p<0.01, <0.05 and <0.01, respectively). Patients with an initial CT with a midline shift of > 5 mm had increased maximum S100B, S100A1B and S100BB levels compared with patients without a midline shift (p<0.001, p<0.01 and p<0.001 respectively). Patients requiring further neurosurgery (n=34) had increased maximum S100B, S100A1B and S100BB levels compared with those only operated upon with an ICP device (p<0.05 in all three comparisons). None of the three markers was significantly increased in patients with additional trauma compared with those with an "isolated" brain injury.

Patients with an unfavourable outcome (n=28; GOS 1-3) had significantly increased maximum levels of all three markers respectively, compared with patients with a favourable outcome (n=31, GOS 4-5), Figure 9.
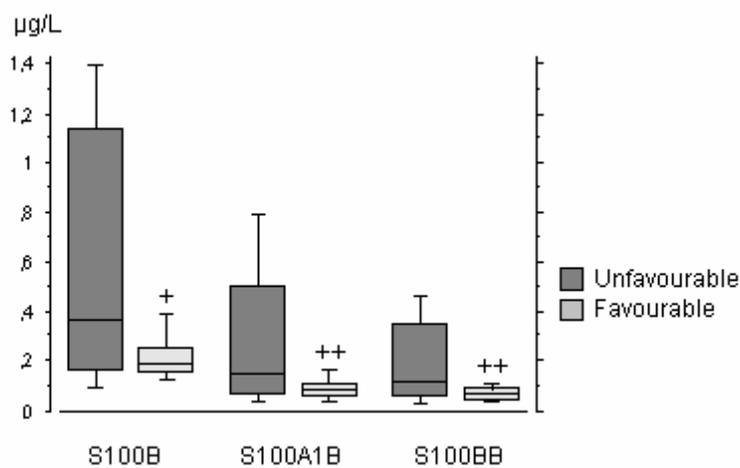

Figure 9. Maximum concentration of S100B, S100A1B and S100BB after severe traumatic brain injury in relation to outcome. Each box plot displays the 10th, 25th, 50th, 75th and 90th percentile. Outliers are excluded.
+ = p<0.05
++ = p<0.01, Mann-Whitney U-test.

The differences in serum concentrations between favourable and unfavourable outcome remained significant regardless of whether or not further neurosurgery was performed (Van Elteren test). Not only the maximum concentration but also the concentrations on days 0 and 1 respectively were increased in the patient group with an unfavourable outcome compared with those with a favourable outcome. (Number of samples day 0=17 and day 1=46.)

Maximum serum concentrations of all three S100 parameters correlated significantly with maximum s-GFAP (from Paper III; r=0.71 (S100B), r=0.81 (S100A1B) and r=0.61 (S100A1B), p<0.001 in all three comparisons).

Logistic regression analyses were performed for the three S100 markers and s-GFAP (from Paper III) to predict the long-term outcome. The area under Receiver Operating Characteristic (ROC) curve was calculated for description of goodness of predictors, Figure 10. There was no significant difference between s-GFAP and the S100 parameters.
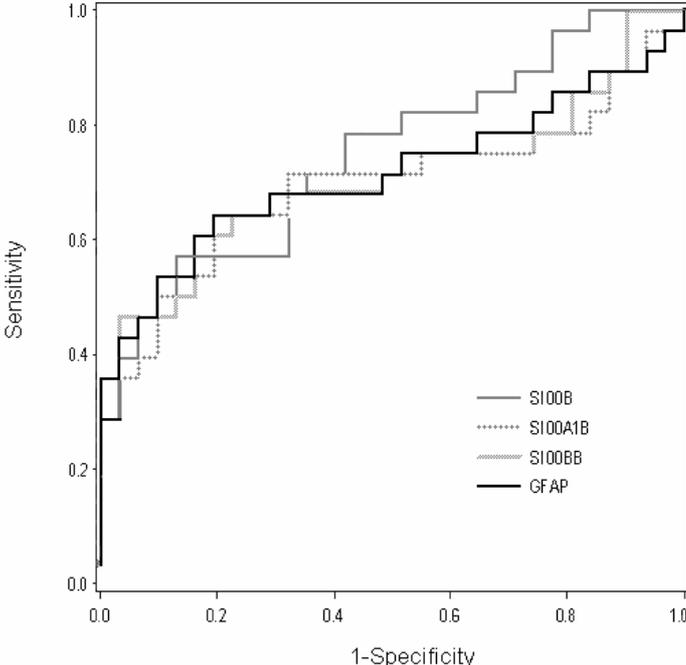

Figure 10. ROC curves for S100B, S100A1B, S100BB and GFAP concentrations in serum for unfavourable outcome at one year after severe traumatic brain injury. The area under curve (c-statistics) was 0.70 (S100B), 0.71 (S100A1B), 0.71 (S100BB) and 0.75 (GFAP).

Serum GFAP concentrations after TBI compared with those after aSAH (from Papers III and I)

The concentrations in the TBI group were generally high and the maximum was almost always reached at an early stage. In the patient group with aSAH, many patients had normal maximum concentrations and also a favourable outcome (n=32). Increases after aSAH were often related to focal lesions, occurring immediately or later on as a secondary event.

When considering all the patients, a normal maximum value (<0.15μg/L) had a high npv (92%) but was rare after TBI (n=1). Using such a high cut-of level as 7μg/L results in 100% ppv for unfavourable outcome. However, relatively few patients (n=11) reached these concentrations, especially in the aSAH group (3 of the 11 patients). Accordingly many patients with increased maximum concentrations still had a favourable outcome, Figure 11.
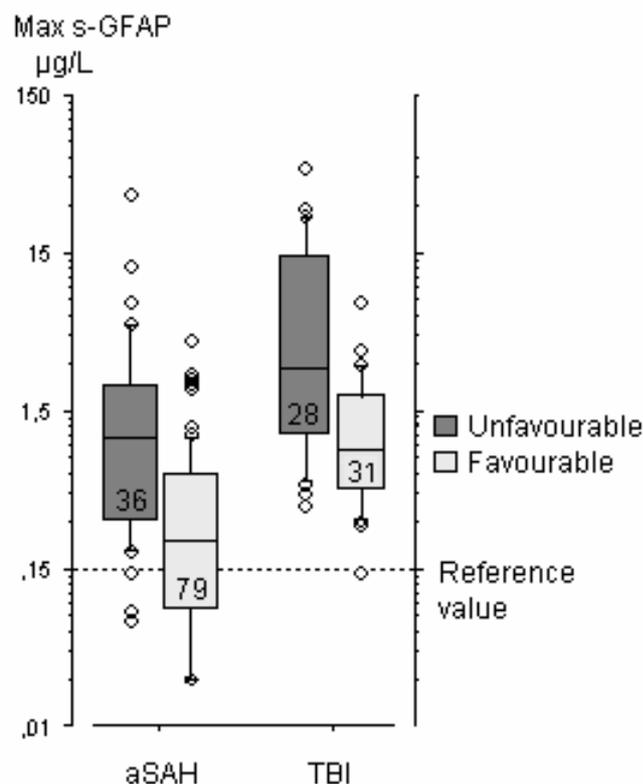

Figure 11. Maximum s-GFAP for patients with unfavourable and favourable outcomes after aSAH and TBI. Box plots show the 10th, 25th, 50th, 75th and 90th percentiles. Outliers are plotted as points. Number of patients as a figure in the boxes.

DISCUSSION

These studies focus on subarachnoid haemorrhage and traumatic brain injury in relation to biochemical brain damage markers. The main findings were the observed association of the markers with brain injury severity in the acute phase and the relationship with the long-term outcome.

The aim of the present work was to perform a study from a neurologist's point of view, with a detailed collection of clinical data and neurological examinations as the "gold-standard", making comparisons with laboratory data reliable. We chose to include patients with aSAH and severe TBI at a NICU to facilitate the inclusion of one patient group with very severe brain injuries and one with varying degrees of severity. Only on rare occasions are these patients treated at other hospitals in the region and the groups dominate among patients at an NICU.

The patient group with aSAH could be characterised in different ways and at different points in time. It was often possible to use both radiological and clinical data to describe the same phenomenon. This was an advantage, as the relationship between s-GFAP and different characteristics of the patients could be tested in alternative ways, thereby reinforcing the validity of the results. The conditions were quite different for the patient group with severe TBI. These patients were all unconscious secondary to a trauma, but they had few other common characteristics. The trauma was diversified and in some cases unclear. Neurological examination was hampered by treatment including sedation and mechanical ventilation. This relative lack of clinical reference points in the acute phase was an obstacle and consequently interest focused on outcome.

Study design and performance

When the studies were instigated, serum GFAP had not previously been evaluated in patients with aSAH. Concentrations of s-GFAP were shown to increase very soon after traumatic brain injury but with a delay after ischemic stroke (Missler et al., 1999, Herrmann et al., 2000). Serial serum sampling therefore appeared to be an important part of the study design. At the time, serum S100B was already an established marker of glial cell injury after TBI, but

the specificity had been questioned. Since we had the opportunity to analyse the S100A1B and S100BB dimers separately, this was a logical complement to the GFAP analysis.

Previous research had shown that CSF-NFL was a sensitive marker of axonal injury, but the marker was unproven in patients with SAH. We knew that most patients with aSAH were discharged from the university hospital within two weeks and that the increase in NFL was delayed for many days. We therefore had to compromise and decided that LP was to be performed on day 10-15, even if a few days later might have been more optimal. Since LP was presumed to be contraindicated in the majority of the patients with traumatic brain injury, we refrained from LP in this patient group.

Patients with SAH present as acute medical emergencies and they are frequently admitted to a general hospital before being transferred to the nearest available neurosurgical unit. The very first neurological status was therefore categorised retrospectively based on medical records. There may have been misclassification of the presence of major focal deficits, since the acute circumstances could have influenced the performance and reliability of the neurological examination. A great deal of effort was put into scrutinising records from ambulances, the first hospital and supplementary anamnesis. In spite of this, the information remained incomplete in some cases when patients never regained the ability to communicate further details. However, the initial status of all the patients before possible deterioration and intervention was presumed to be more informative than their status on arrival at the NICU. Aneurysm ruptures during intervention, re-bleeding and ischemic events (irrespective of the cause) throughout the sampling period were classified as secondary events. Bleeds were confirmed with CT scans and/or description from the neurosurgeon or radiologist performing the clipping or coiling respectively. Ischemic events were confirmed with repeated CT or in a few cases clinically on day 10-15. In contrast to secondary events, the results from MRI at one year were categorised from the records alone and regardless of symptoms. Dichotomisation into focal brain lesions (signs of ischemia or previous intracerebral haemorrhage) or not was used. In most cases, the relationship with the aSAH was obvious, but in some cases small ischemic lesions of undefined age and cause could be identified. In others, the lesions were probably secondary to the aSAH, but the relationship was not "proven" clinically or radiologically during the acute phase. This way of regarding any ischemic lesion as a potential consequence of the disease was used to avoid subjective judgement. In all probability, some of the lesions were not related at all to the aSAH and consequently the stated association between s-GFAP and MRI findings was as least as good as presented in the result section.

The present study did not aim to compare different treatment strategies. Categorisations based on treatment were in fact created with the hope of describing concentrations of the biochemical markers even in situations when "neurosurgical contribution" was excluded. Naturally, neurosurgical interventions were necessary more frequently in patients with more severe diseases.

The LP study was based on the inclusion of patients with aSAH positive to perform the procedure and after the approval of neurosurgeon in charge of the patient care on the day in question. In this way patients with contraindications to LP were excluded. The inclusion was naturally biased depending on different factors, primarily the patients' attitude but also minor differences between the neurosurgeons and in some cases due to logistical problems (early discharge).

Traumatic brain injuries are graded (mild, moderate or severe) on the basis of level of consciousness (GCS). For patients with severe TBI the exact score (3-8) on the GCS is not always easy to obtain (trauma to the face). The effects of alcohol, drugs and seizures may also influence the result. Emergency intubation and drug administration often prevent re-examination, which may have improved the quality of the grading. The initial clinical status had to be based on retrospective analyses of ambulance and medical reports. We wanted to study a homogeneous patient group with unmistakable severe TBI, i.e. those requiring attention at a specialised NICU for more than just observation or postoperative care. To select these patients, we added the need for ventilator treatment and an indication to monitor ICP to our inclusion criteria for severe TBI. Needless to say, this inclusion was dependent on a "correct" assessment that the patient was unconscious due to the trauma and the judgement of the neurosurgeon to monitor ICP. Also, patients with isolated large lesions "cured" by the evacuation of the haematoma were not included since they had no need for EVD. To better describe the patients selected for the present study and to allow a neuroradiologist to classify the CT scans while blinded to clinical data, we categorised all the CT scans on the basis of impact on midline shift and compression of cisterns. We therefore used the Marshall type I-IV but excluded the originally described types V and VI.

Very early and frequent sampling was not easy to assess due to routines on the ward. Instead, we gave priority to the complete inclusion of a relatively large number of patients. This was enabled by the inclusion period (day 0-2). The approach with daily or almost daily sampling for more than a week made it possible to approximate the temporal course of the markers and their capacity to detect neurological complications.

The case fatality ratio after severe TBI and aSAH does not change considerably after the first few weeks. In contrast, functional outcome and quality of life may change gradually over a long period. In 2001, Hop and co-workers demonstrated improvements even up to 18 months post SAH (Hop et al., 2001) and King and co-workers recently reported a significant improvement between three and 12 months after TBI (King et al., 2005). However, the majority of patients probably reach their final outcome category within six months, with very little change after one year. A very late follow-up carries the risk of drop-outs and other causes of a changed functional status than the bleed or trauma. We chose the time for assessment as one year for both patients with aSAH and patients with TBI. Since we wanted to include the patients' opinion on astheno-emotional symptoms after SAH, we used the structured interview of the eight-grade GOSE as the primary outcome measure. In contrast to most other studies, neurological status was analysed in detail and categorised according to NIHSS. In spite of this, the number of scales and examinations had to be limited for the patients to cope. The structured interview of the eight-grade GOSE was also used for the adults, but not for the children, in the TBI study. Based on these circumstances and the fact that the five-grade GOS was used in the TCDB and subsequent reports of severe TBI, we chose to present the results in that form.

Outcome is often used as a "retrospective estimation/confirmation" of the severity of a traumatic brain injury. Length of unconsciousness or length of amnesia cannot be used to assess brain injury severity in these severe cases treated with anaesthetisation. Moreover the lack of well-established "clinical indexes" to grade the severity of the "most severe brain injuries" complicates the evaluation of biochemical markers. Outcome, CT, ICP and GCS all have their shortcomings. However, outcome is what matters for the patients, making the comparison important, but not ideal, both for its own value and as an instrument to estimate brain injury severity.

No single measure fully describes all the dimensions of recovery and disability and it should also be remembered that biochemical markers probably mainly reflect the number of injured cells. Clinical outcome is a functional index of brain damage, which depends not only on the extent of the lesion but also on its location and various other factors. Unsatisfactory physical, social and emotional recovery may be a direct consequence of brain damage, but it may also be influenced by many other factors, some of which may be individual and not easy to measure. Since the ideal biochemical marker reflects the number of injured brain cells and nothing more, an absolute correlation with outcome cannot be expected.

General results

Inclusion in the studies was consecutive, with no breaks for holidays or festivals, and only a few more patients could hypothetically have been included (aSAH; n=4, TBI; n=3). Like other naturalistic clinical studies we lack data from patients with a markedly poor prognosis who were not referred for treatment at the NICU (and those in whom angiography was cancelled). We excluded patients who were not living in Sweden to make follow-up feasible (four foreign citizens were not included). Only two included patients had/were affected by severe non-neurological progressive diseases that made outcome classification doubtful. Addiction and subsequent social problems appeared to be more common in the TBI group than in other groups of patients. However, one year after the trauma/haemorrhage, it was possible to obtain information from all the survivors but one. Even so, these efforts to obtain a complete patient series do not guarantee a representative study.

Large studies often compare different treatment strategies and the inclusion criteria and outcome measurements vary. In addition, advances in clinical practice makes comparisons with historical data unreliable. ISAT, one of the largest clinical studies of aSAH, only included patients suitable for either neurosurgical clipping or endovascular coiling (Molyneux et al., 2002). In conformity with the present study, ISAT ran for approximately the same period of time, used WFNS to describe clinical grade at admission and assessed outcome after one year. However, outcome was categorised according to the Modified Rankin Scale and not according to GOSE. Dichotomisation into dead or dependent versus independent is generally used for both scales, making a crude comparison possible. In our small study, 31% were dead or dependent at one year. In ISAT, the corresponding figures were 24% for patients treated with coiling and 31% for patients treated with clipping. As mentioned before, we lack data from patients with a markedly poor prognosis who were not referred for treatment. An even higher percentage of these patients was probably excluded from randomisation in ISAT. In ISAT, only a minority of the screened patients were actually enrolled in the trial and the percentage of patients graded as poor (WFNS IV-V) was only 4.4% (Molyneux et al., 2002). In the large study (3,567 patients) by Rosen and co-workers (Rosen and Macdonald, 2004), the percentage of patients graded as poor was 22%, a figure very close to the 21% observed in the present study. So, as far as we can understand, our patient population was representative of an aSAH cohort at a neurosurgical department, making the laboratory results applicable to such groups.

Using eight categories to assess outcome (GOSE) resulted in an uneven distribution. The very low frequency of GOSE 2 (vegetative state) was expected. The low frequency of GOSE 4 probably reflected the fact that very few patients had the capacity to take care of themselves for eight but not 24 hours a day (definition of GOSE 4). Surprisingly few patients were classified as GOSE 7. The majority of patients with a favourable outcome tended to regard their situation as normal (GOSE 8) or else they had mental symptoms of a severity that still impaired work and leisure activities (GOSE 6). The results might have been different if outcome had been assessed at an earlier stage, as astheno-emotional symptoms are known to decline.

As expected, the patient group in whom LP was performed had a better outcome than the remaining patients in the study. This selection bias was due to contraindications for LP. As a result, we do not have the CSF data from patients with expansive haematomas and infarctions.

Despite the clear definition of a severe traumatic brain injury based on the GCS, these patients are not a homogeneous group and comparisons between different centres and different points in time may be doubtful. In the TCDB from the 1980s, 43% had a favourable outcome and, in the European Brain Injury Consortium survey of head injuries from the 1990s, the number was 40% (Murray et al., 1999). As examples of more contemporary studies, Raabe and co-workers included 84 patients (median age 39 years) and 58% had a favourable outcome (Raabe et al., 1999a). Rothoerl and co-workers studied 44 patients (mean age 35 years) and 51% had a favourable outcome (Rothoerl et al., 2000). Eventually, Vos and co-workers included 85 patients (median age 32 years) and 52% had a favourable outcome (Vos et al., 2004). In the present study, we used additional inclusion criteria apart from level of consciousness (ICP device, ventilator treatment). Needless to say, we are unable to make exact comparisons, but the inclusion of patients with a median age of 37 years and a favourable outcome in 31 of 59 patients (52%) as observed in the present study appears reasonable. Accordingly the results are most probably representative of a patient group with severe traumatic brain injury at a neurosurgical department.

The principal strength of the studies was the consecutive inclusion and almost complete follow-up at the same point in time. With a few exceptions all the interviews and examinations were performed by one and the same neurologist. The sparse sampling at the end of the series and the lack of standardised CT controls in the acute phase might appear to be a limitation in the design. However, the aim of the study was not to obtain information about the development of the laboratory parameters in the individual cases. Needless to say,

while looking at the results, these additional efforts could have produced valuable information in this area as well. The principal shortcomings in performance were the relatively few serum samples that were drawn before neurosurgery in both patient groups. The objective was to draw the very first sample as soon as possible after arrival at the NICU, but in many cases urgent neurosurgical interventions had first priority. In 30% of the cases the first sample could theoretically have been taken on the calendar day before the actual sampling. Once started, the series were almost always complete and fewer than 5% of 1,149 samples were missing. The frequency of incomplete and contradictory information in ambulance and medical reports regarding the initial level of consciousness and pupillary reactivity after severe TBI was high. Accordingly, a comparison between the exact RLS score or pupillary reactivity and outcome was not feasible, but it was possible to distinguish the patients with RLS 7-8 (=GCS 3-4, regarded as critical traumatic brain injuries). The aim of the study was not to evaluate whether biochemical markers were able to predict outcome more reliably than information from clinical or radiological scales. We used these scales to describe the patient population and to demonstrate the relationship between the markers and clinical or radiological findings. Statistical analyses were performed to produce an approximate comparison with some of the most well-known predictors.

Remarks on the result for markers in the patient group with aneurysmal subarachnoid haemorrhage

Serum GFAP increases with a delay after cerebral infarction and probably more directly after haemorrhage. We expected the release pattern to be even more complex in patients with aSAH. The brain injury due to the initial haemorrhage could be accompanied by intracerebral haematoma, re-bleeding, complications during the treatment and delayed ischemic events. All these factors may contribute to the increase in serum GFAP, as a mixture or isolated, depending on the severity and interval between the events in relation to sampling. However, the sparse sampling in relation to the high frequency of events early in the course made it impossible to distinguish the initial haemorrhage from the other events in terms of laboratory findings. Accordingly, we focused on the brain injury at different points in time, irrespective of the exact cause. Taken together, our data indicate that there is an association between serum GFAP and focal brain injury, assessed by neurological or radiological examinations. The results can be repeated at different points in time and are in agreement with the few

previous studies of s-GFAP and stroke. As mentioned before, Herrmann and co-workers studied 32 stroke patients and reported not only a delay in the increase in serum but also an association between GFAP and neurological status (NIHSS; Herrmann et al., 2000). In the study by Foerch and co-workers, serum GFAP was determined at hospital admission in stroke patients admitted within six hours. GFAP was detectable in 81% (34 of 42) of patients with intracranial haemorrhage, but only in 5% (5 of 93) with ischemic stroke. For the patients with intracranial haemorrhage, both haematoma-volume and NIHSS correlated with serum GFAP. Their hypothesis was that serum GFAP should be measurable only in hemorrhagic and not ischemic stroke at such an early point in time (Foerch et al., 2006). In contrast, we repeated serum sampling with the aim of detecting an increase, also occurring during the first days after an ischemic event. Recently Vos and co-workers presented a study of 67 patients with SAH and serum GFAP, S100B and NSE taken on arrival at hospital. Interestingly, they showed that patients with intracerebral blood visible on CT had significantly higher levels of GFAP compared with those in whom the location of blood was restricted to other locations (Vos et al., 2006). This is in accordance with our observations in which patients with parenchymal haemorrhages (n=19) had increased first s-GFAP compared with the others (p<0.001, not shown) and the s-GFAP in the first sample was above the normal reference level in all but one of the patients with a parenchymal haemorrhage (not shown). To summarise, our findings are generally in line with those of other stroke studies, but this is the first study with repeated measurements of serum GFAP in patients with aSAH. Serial sampling thus enabled us to show the association between serum GFAP and focal brain injuries, irrespective of hemorrhagic or ischemic genesis.

CSF-NFL concentrations are known to reach a maximum several days after parenchymal damage and to remain at this increased level for many weeks (Rosengren et al., 1996, Studahl et al., 2000). The concentration 10-15 days after SAH could be regarded as a "sum of the brain injuries" during the period from the initial bleed to the LP. Most SAH studies of biochemical markers in CSF are based on ventricular CSF from drainage, which is accordingly confined to patients with a need for EVD and during that period of time. We managed to perform LP in 48 cases at a predetermined point in time. An association between CSF-NFL and focal brain injury based on clinical or radiological data was observed. There are no previous studies of neurofilament light chain (NFL) from lumbar CSF in patients with aSAH in relation to clinical findings. Petzold and co-workers have recently reported increased levels of the phosphoform of neurofilament heavy chain (NFH), in ventricular CSF after SAH. Their study included 17 patients and CSF-NFH levels were found to correlate with the

severity of the injury categorised as WFNS (Petzold et al., 2006a). Inclusion bias was unavoidable insofar as all the patients had clinical indications for EVD, but repeated measurements were possible and the results corroborate ours with support for axonal damage after SAH. Concentrations between ventricular and lumbar CSF can differ. Tullberg and co-workers investigated patients with normal pressure hydrocephalus and described higher levels of GFAP in ventricular CSF than lumbar CSF but no difference with regard to NFL (Tullberg et al., 1998).

However, not all patients had evidence of focal brain injury (clinical or radiological), but they still had increased CSF-NFL. One hypothesis is that the levels were increased due to diffuse injury. The diffuse axonal injury following global ischemia after cardiac arrest is well established. CSF-NFL increased after cardiac arrest and levels above 10,000 ng/L were associated with a poor outcome in 90% of cases (Rosén et al., 2004). Unlike patients with cardiac arrest, an NFL level above 10,000 ng/L in combination with a good outcome was not uncommon after aSAH, but these patients also had "focal brain lesions" (at the control after one year, n=12 of 13). It is well known that outcome after stroke depends not only on the volume of the lesion but also on the localisation. Petzold emphasised that axonal injury in SAH is often is too diffuse to be detected by standard brain imaging techniques (Petzold et al., 2005). In the present study, 17 patients with radiology (MRI or CT at one year) "excluding" focal lesions had increased NFL levels (16 of them below 10,000 ng/L), which may be an indication of diffuse axonal injury in these cases. (All but one had a normal neurological status according to the NIHSS at one year, nine underwent surgery and eight did not). This study cannot answer the question of etiology or not even correctly detect diffuse axonal injury, but it seems reasonable to assume that focal brain injury is as least as important as diffuse axonal injury for outcome after aSAH.

Serum GFAP has not been evaluated in patients with cardiac arrest, but S100B is known to increase in serum after cardiac arrest (Rosén et al., 1998). When scrutinising our patients in whom both CSF-NFL and serum GFAP were available, we found that 19 had no evidence of focal lesion at radiology after one year. As mentioned before, 17 of them had increased CSF-NFL, but only seven had increased maximum s-GFAP. This difference is probably mainly due to the fact that CSF-NFL is a more sensitive marker than serum GFAP. Hypothetically, other factors can also be discussed. It is possible to miss the serum GFAP peak due to sparse sampling, which is less relevant for CSF-NFL. Different neuropathological courses with NFL corresponding to axonal injury and GFAP to glial cell injury may influence the result. However, no comparisons can be made between neuronal and glial injury using these data,

but it can be hypothesised that not only axons but also astrocytes are affected by diffuse brain injury in aSAH.

Patients requiring any kind of neurosurgical intervention (clips, external ventricular drainage, evacuation of haematomas, decompressive craniectomy) had increased maximum s-GFAP and CSF-NFL levels, compared with the "non-surgical group" (endovascular coiling as the only procedure). This was supposedly due to a more severe clinical picture, but the surgical trauma may have contributed to some part of the difference. To evaluate the contribution of the actual neurosurgery, a study of biochemical markers after the elective clipping of unruptured aneurysms would have had to be performed. However, the disease was "proven" to be the cause of the increase in many cases, and it was probably the dominat cause in the remaining cases. In the "non-surgical group", nine patients had increased CSF-NFL levels. Consequently, the increase was not due to surgery. Analogously, in 37 cases, serum GFAP was increased in samples taken without any relation to neurosurgery. (In 13 cases, there were increases in non-operated patients and in 24 cases there was an increase in samples taken before neurosurgery.) We also noted that external ventricular drainage could be inserted without any increase in s-GFAP (<0.15 μg/L, n=13) and clips operation could be performed with a s-GFAP as low as 0.22 μg/L. The relationship between concentrations of the markers (s-GFAP and CSF-NFL) and dichotomised outcome remained significant when adjusting for neurosurgery (multiple logistic regression analysis and Van Elteren's test respectively).

Outcome after aSAH depends not only on the initial bleed but also on secondary events, which was the rationale for repeated serum sampling and one reason for difficulties in early outcome prediction. Patients with secondary events (aneurysm ruptures during intervention, re-bleedings or ischemic events) had increased s-GFAP and reached maximum levels later than patients without secondary events. A more frequent evaluation of clinical status and standardised radiology in relation to serum sampling "covering" the whole period would have been advantageous when it came to evaluating the full potential of s-GFAP in this respect. Such a procedure would also have made it possible to follow the development of the individual cases using laboratory methods. Petzold and co-authors suggested that CSF-GFAP identified secondary brain damage in SAH (Petzold et al., 2006b). They measured repeated CSF-GFAP from nine patients with external ventricular drainage and demonstrated a rapid wash-out in survivors and secondary peaks in non-survivors. Again, a serum marker is preferable. Back in 1997, Wiesmann demonstrated an association between higher plasma concentrations of S100B in the first week after SAH and worse outcome at six months (Wiesmann et al., 1997). Recently Stranjalis and co-workers showed an association between

secondary neurological deterioration and S100B measured daily after SAH (Stranjalis et al., 2007). Their study included 52 patients but only 34 had a proven aneurysm. In agreement with us they concluded that more frequent serum sampling and clinical evaluation might offer reliable information that may influence management decisions. The brain specificity of S100B has been questioned. Increased levels in patients without head injury have been seen after trauma (Anderson et al., 2001a) but also after severe medical diseases requiring ventilator treatment (Routsi et al., 2006). The practical implication of trauma-induced increase, is probably negligible in the aSAH group, even if the surgical trauma to the skull bone might hypothetically contribute to serum increase. It has been hypothesised that the increase seen in intensive care situations could be related to tissue hypoperfusion, which is probably relatively uncommon in the aSAH group. However from this point of view a specific brain damage marker such as GFAP is preferable to S100B.

In the present study a normal serum GFAP concentration on any sampling day had a relatively high negative predictive value. A normal s-GFAP series thus appears to have potential in the early prediction of a favourable outcome. On the other hand, a pathological s-GFAP does not forecast a poor outcome, as the positive predictive values were relatively low. Many patients with increased s-GFAP levels may therefore still have a favourable outcome. It is well known that outcome depends on a variety of factors apart from brain lesion size and location. As expected, neurological status (WFNS) and CT findings (Fisher scale) on arrival were related to outcome. However, s-GFAP on day 3 predicted long-term outcome at least as good as WFNS and Fisher scale and it also independently predicted outcome after adjustment for the other predictors.

We found a correlation not only between serum GFAP and GOSE but also between s-GFAP and NIHSS, MMSE and Barthel Index respectively. In most cases, adequate neurological, cognitive and ADL-functions are a prerequisite for a favourable outcome. As expected, the GOSE in turn was associated with NIHSS, MMSE and Barthel Index respectively. We suggest that these findings confirm the GOSE classification rather than adding so much new information.

The correlation between CSF-NFL and outcome was at least as good as the correlation between serum GFAP and outcome. Again, a comparison can be made with the study by Petzold and co-workers, who described increased levels of phosphorylated NFH from ventricular drainage as being associated with outcome after three months (Petzold et al., 2006a). The finding that both CSF-NFL and phosphorylated CSF-NFH are related to outcome supports the idea of irreversible axonal injury following SAH. Naturally, the clinical

usefulness of a marker in CSF is inferior to one in serum. Today, CSF-NFL may serve as an adjunct in unclear cases when LP is feasible and probably also when CSF from ventricular drainage can be obtained. The delayed and prolonged increase in NFL in CSF may be an advantage in some situations. LP can be performed after the acute phase when patients and physicians are prepared and the result of a single test represents the brain injury during a long period of time. After diffuse axonal injury, it is possible to speculate that CSF-NFL analysis may help to discriminate between organic psychiatric and depressive sequelae. Moreover, the opportunity for late sampling may be an advantage and a complement to serum markers taken in the acute phase. In the future, it is to be hoped that more sensitive techniques will be developed, making the serum determination of NFL practicable.

Remarks on the result for markers in the patient group with severe traumatic brain injury

Serum GFAP as well as S100A1B, S100BB and S100B displayed a convincing increase after the brain injury with an early maximum and decline in a few days. The temporal profile indicates that samples do not need to be taken urgently immediately after the trauma. This was especially true for GFAP, as concentrations were increased for many days and the difference between favourable and unfavourable outcome remained significant for samples taken even after the first few days. However, the limited sampling from the initial hours after the trauma leaves the question of the kinetics during the very early phase unanswered. In this context, the findings of Vos and co-workers supplement our data. They observed increased levels of GFAP and S100B in samples within a few hours after the trauma (median 2.5 h), but they did not repeat the sampling (Vos et al., 2004). In the present study, the patient group with an unfavourable outcome had increased serum concentrations of these markers compared with those with a favourable outcome. The association with outcome was in agreement with previous studies by Pelinka and co-workers and by Vos and co-workers (Vos et al., 2004, Pelinka et al., 2004 a). As in-house methods for serum GFAP analyses were used (as well as slightly different inclusion criteria), direct comparisons of the serum concentrations between these studies are not possible. Even if the patient group with an unfavourable outcome had increased concentrations of the serum makers compared with those with a favourable outcome, a relatively "low" serum concentration was no guarantee of a favourable outcome or survival for the individual patient. Naturally, the situation is complex and many factors other

than brain injury severity may contribute to the outcome. All the deaths (n=11) could be attributed to the brain injury, but it is difficult to determine whether there was any disproportion between the severity of the brain injury and the medical complications given in the death certificates and discharge notes. Nor will we know whether the serum concentrations would have been even higher if the first sample had been taken even earlier. As a result, from the present study, we will not know for sure whether the markers in fact failed to distinguish between the very severe brain injuries inconsistent with survival and the less severe injuries. We do know, however, that the five patients with the most extreme increases in s-GFAP concentrations (100 times normal levels) all died, and that the five patients with the highest levels of S100B, S100A1B and S100BB respectively died or had an unfavourable outcome (not shown). Both s-GFAP and S100B measure glial cell injury and the values were intercorrelated. Severe brain injuries probably affect both neurons and glia cells. Diffuse axonal injury (DAI) with shearing damage is difficult to detect non-invasively and is poorly defined as a clinical syndrome. In the present study, subacute MRI was performed on clinical indications in a few cases. One patient who did not regain consciousness after a violent car crash turned out to have extended axonal injuries in the brain stem. Concentrations of the serum markers (s-GFAP, S100B, S100A1B and S100BB) were relatively "low" in relation to outcome (GOS 2). Naturally a sensitive serum marker of axonal injury would have been of extraordinary interest in this case.

Unlike most other studies, we regarded all fractures, including skull fractures, as trauma to multiple "organs/tissues" (classified as "multiple trauma"), since serum S100B levels are known to increase after bone fractures in particular. As a result, only a few patients (n=13) were classified as having an "isolated" brain injury. Their maximum serum levels (of GFAP, S100B, S100A1B and S100BB) were not significantly changed compared with those regarded as "multiple trauma". Anderson and co-workers have previously shown that the extra-cerebral S100A1B/S100BB concentration ratio varies widely without any apparent association with the type of trauma or tissue damage (Anderson et al., 2001a). Furthermore, separate analyses of the dimers S100A1B and S100BB did not differentiate between S100B of cerebral or extra-cerebral origin after cardiac surgery (Anderson et al., 2001 b). The present study was not designed to answer the question of the extra-cerebral contribution of the different markers. To make that possible, trauma patients without brain injuries should have been included. The timing of the sampling critically affects the measured concentrations and potential extra-cerebral contribution. S100B has a half-life of approximately 25 minutes (Jönsson et al., 2000). After minor head injury, S100B declines rapidly during the first hours (Ingebrigtsen

and Romner, 1996, Ingebrigtsen et al., 1999). After severe traumatic brain injuries, S100B first returns to baseline within two to 10 days. Raabe and co-workers suggest that the continuous release of S100B is responsible for this prolonged increase beyond the time expected as a result of the half-life (Raabe et al., 2003). On the other hand, serum S100B concentrations appear to decrease within hours after isolated soft-tissue and bone trauma (Anderson et al., 2001a). In the present study, we do not have these very early samples, but in approximately 50% of the samples concentrations were still increased on day 3 for both S100A1B and S100BB, which suggest a cerebral origin.

Patients requiring therapeutic neurosurgery had significantly increased maximum levels of S100B, S100A1B and S100BB respectively, compared with those with only an ICP device (p<0.05). Likewise, the actual mean (and median) s-GFAP values in the group undergoing surgery were increased compared with the non-operated group, but the difference fell short of statistical significance (p=0.1). Neurosurgically operated patients (apart from the ICP device) probably had more severe TBI, thereby explaining the increase in the operated group. Since GFAP is brain specific, the more pronounced increase in S100B might indicate surgically induced trauma to tissues enclosing the brain, with an extra-cerebral contribution by S100B in the operated group.

The S100ββ dimer is regarded as the dominant isoform in the CNS (Isobe et al., 1983, Jensen et al., 1985). Accordingly, our hypothesis was that S100BB would be more closely associated with outcome than S100A1B or S100B. Instead, the results for S100BB, S100A1B and S100B appeared to be equally good. We also noted that the serum concentrations of S100BB were lower than those of S100A1B. This finding was in agreement with the findings relating to mild traumatic brain injuries (Nygren de Boussard et al., 2004). Furthermore, Anderson and co-workers reported a normal dominance of S100A1B in both serum and CSF. A proportional increase in the dimers (S100A1B and S100BB) was also seen in one patient with stroke and one patient with spinal damage (Anderson et al., 2003). In quoted studies, as well as in the present study, the S100A1B and S100BB concentrations were all analysed using the same ELISA method (Fujirebio Diagnostics AB, formerly CanAg Diagnostic, Sweden).

Theoretically, the sum of S100A1B and S100BB values should equal the concentration of S100B. However, this was not the case in the present study. The sum of S100A1B and S100BB was 80% or less than that of S100B. The concentration of S100A1B was higher than that of S100BB at all points in time. The low S100BB concentrations detected normally, as well as the discrepancy in sum concentrations, might be related to properties of the antibodies

or other methodological factors of the assay. Still the dominance of S100A1B in serum and CSF is somewhat contradictory to the fact that S100BB dominates in the brain.

The most important advantage of serum GFAP is probably the brain specificity (Missler et al., 1999, Pelinka et al., 2004b, Vos et al., 2004), making estimations of extracranial contribution superfluous. On the other hand, serum S100B is already an established biochemical marker and a huge amount of research in different settings is available for comparisons and sensitive S100B assays are easy to access. In the present study, maximum serum S100B, S100A1B and S100BB correlated with maximum s-GFAP respectively. A correlation between s-GFAP and S100B after traumatic brain injury has already been shown (Missler et al., 1999, Vos et al., 2004, Pelinka et al., 2004a). A study of traumatic brain injury ought to favour GFAP. However, in the present clinical setting, neither S100B, S100A1B, S100BB nor GFAP appeared to have a significant advantage over the others. The purpose of a biochemical marker of TBI is not only to be a laboratory measurement of brain injury severity but also to monitor secondary complications and therapeutic interventions. Some reports have shown a secondary excessive increase in S100B before any increase in ICP (Raabe et al., 1999b, Undén et al., 2004). This "early" response must be an advantage in monitoring. Raabe and co-workers have recently demonstrated the effectiveness of serum S-100B in detecting secondary neurological complications in a mixed patient population at a neurointensive care unit (Raabe et al., 2004). Results from similar studies including s-GFAP are needed.

REFLECTIONS AND CONCLUSIONS

As expected, many of the patients had very severe brain injuries, complications were frequent and outcome on an individual basis was poor in many cases. Clinical and radiological estimation of the consequences of the brain injury could be difficult, especially in the group with traumatic brain injuries.

The studies corroborate the hypothesis that the concentrations of the markers are related not only to brain injury severity but also to long-term outcome. S-GFAP was at least as good as a predictor of dichotomised outcome as the neurological or radiological scales used on arrival. Furthermore, s-GFAP appeared to have the potential to detect neurological complications after aSAH. However, from this study, we do not know the extent to which the markers add information that could be valuable in the care of the individual patient. An ideal marker should also guide the treatment and alert the clinician to complications early in the course. Although a laboratory parameter can never replace clinical or radiological examinations, support for the clinical assessment, would be welcome, particularly when the conditions for complete examinations are restricted. Like clinical scales, laboratory parameters can also be used when communicating about a patient or patient group. The information is easy to obtain and provides a quick and objective answer that is not subjected to inter-rater variability. Serum analyses reflecting the status of the "target organ" are routine in many other specialities. Until we have biochemical brain damage marker for daily use in medical practice further research is justified.

From this study we can conclude that:

GFAP levels in venous blood are related to focal brain injury and long-term outcome after aneurysmal subarachnoid haemorrhage. A normal concentration predicts a favourable outcome.

CSF-NFL is related to the severity of the brain injury and to the long-term outcome after aneurysmal subarachnoid haemorrhage.

Serum GFAP is increased after severe traumatic brain injury and concentrations are related to long-term outcome.

Serum S100A1B and S100BB are increased after severe traumatic brain injury and concentrations are related to long-term outcome. Both dimers appear to predict outcome approximately as well as S100B.

There was no significant difference between s-GFAP and S100B regarding predictions of long-term outcome after severe traumatic brain injury.

FUTURE PERSPECTIVES

This study was designed with the aim of improving our knowledge of some biochemical brain damage markers in the clinical setting. However, from the study we can also draw some conclusions about when to use or not to use the markers.

Today, we can use a normal s-GFAP series after aSAH to predict a favourable long-term outcome. If LP is appropriate CSF-NFL after aSAH also serves this purpose, but in clinical practice we will probably not make much use of this opportunity. We are hoping that sensitive methods to analyse NFL in serum will be developed in the future.

After severe TBI, an extraordinary increase in s-GFAP (100 times normal) appeared to predict unfavourable outcome, but this finding cannot be used without further confirmation. From this study we are not able to suggest the use of s-GFAP, S100A1B or S100BB instead of the established marker, S100B, in outcome prediction after severe TBI.

The clinician looks forward to a marker for the rapid detection of complications, evaluation of therapy and monitoring the clinical course. Even if s-GFAP appeared to detect serious neurological complications, we are not able to make full use of this information at present. We do not now the precise time perspectives between the events and the increase, or how sensitive the marker is. Further studies designed to answer these questions are needed.

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