Department of clinical science, Neurosciences, 2025

Prediction of outcome in Subarachnoid Haemorrhage using biomarkers

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ISBN: 978-91-8070-805-0 (print)
ISBN: 978-91-8070-806-7 (pdf)
ISSN: 0346-6612
Umeå University Medical Dissertations New Series no. 2383
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Printed by: Scandinavian Print Group, Skarpnäck, 2025

"Why do we fall Bruce?
So that we can learn to pick ourselves up"
-Thomas Wayne, from Batman begins

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Abstract

Background: Aneurysmal subarachnoid haemorrhage (SAH) is a complex form of stroke affecting approximately 10 per 100,000 individuals in Western populations. In addition to the damage caused by the initial haemorrhage, secondary complications such as rebleeding, hydrocephalus and delayed cerebral ischemia may further contribute to the overall extent of brain injury. Long-term outcomes range widely, from death to full recovery. Predicting functional outcomes during the acute phase of the disease remains challenging, and currently, no blood-based biomarkers are routinely used in clinical practice.

Aim: This thesis investigates whether the biochemical biomarkers myo-inositol (MI), neurofilament light chain (NFL), and S100-beta (S100B) measured in venous blood are associated with secondary complications and long-term functional outcomes. The focus is also on characterizing their trajectories during the acute phase of SAH. The main hypothesis is that levels of the biomarkers are associated with functional outcome 12 months after the haemorrhage.

Method: This work is based on four studies; all derived from a single observational cohort. The cohort included patients aged 18 years or older who were treated for SAH at Umeå University Hospital between 2014 and 2018. Serum samples were collected at multiple time points during hospitalization. Demographic and clinical data were recorded, including neurological status upon admission according to the World Federation of Neurosurgical Societies and Hunt and Hess scores, the amount of subarachnoid blood on the initial CT scan as classified by the Fisher grade, the presence of delayed cerebral ischemia and angiographic vasospasm, as well as the treatment modality used for aneurysm occlusion. Functional status was assessed one year after disease onset using the Glasgow Outcome Scale Extended and the modified Rankin Scale. Outcomes were dichotomized into favourable (Glasgow Outcome Scale 5-8, modified Rankin Scale 0-3) and unfavourable (Glasgow Outcome Scale 1-4, modified Rankin Scale 4-6) groups. In one study, health-related quality of life was also evaluated using the EuroQoL 5-Dimension Index. Biomarker levels were compared between groups, and multivariable logistic regression was applied to assess their predictive value for long-term outcomes.

Results: For MI, levels at admission did not correlate with World Federation of Neurosurgical Societies score, nor did they differ between favourable and unfavourable outcome groups. However, by day seven, significant differences in MI levels emerged between outcome groups, and the change in MI levels over this period also differed significantly. For NFL, levels were significantly associated with both World Federation of Neurosurgical Societies score at admission and outcome group, with differences observed at admission and throughout follow-up. S100B values obtained during the first days after SAH onset correlated significantly with both modified Rankin Scale and EuroQoL 5-Dimension Index with peak S100B levels showing the strongest association with functional outcome. All three biomarkers were significant predictors of long-term functional outcome in univariate analyses. When combined with established predictors, these biomarkers improved the performance of multivariable regression models.

Conclusions: The biomarkers MI, NFL, and S100B are associated with long-term functional outcomes in patients with SAH. Incorporating them into predictive models may provide a valuable tool for outcome prognostication. However, external validation in larger cohorts is required before these biomarkers can be considered for routine clinical implementation. The main hypothesis of this thesis has therefore to be accepted.

Keywords: Subarachnoid haemorrhage, Glasgow outcome scale extended, Modified Rankin scale, Myo-Inositol, Neurofilament-Light chain, S100-Beta, Vasospasm, Delayed cerebral ischemia

Abbreviations

The following abbreviations are used in the text:

ANOVA, Analysis of variance
AUC, Area under the curve
CBF, Cerebral blood flow
CI, Confidence interval
CPP, Cerebral perfusion pressure
CSD, Cortical spreading depolarization
CSF, Cerebrospinal fluid
CT, Computed tomography
CTA, Computed tomography angiography
CTP, Computed tomography perfusion
DCI, Delayed cerebral ischemia
DSA, Digital subtraction angiography
EBI, Early brain injury
EEG, Electroencephalography
EVD, External ventricular drainage
GOS, Glasow outcome scale
GOSE, Glasgow outcome scale extended
ICH, Intracerebral hematoma
ICP, Intracranial pressure
ITP3, Inositol tri-phosphate
MCA, Middle cerebral artery
MI, Myo-inositol
MRI, Magnet resonance imaging
mRS, Modified Rankin scale

MTT, Mean transit time

NFL, Neurofilament light-chain

OR, Odds ratio

QoL, Quality of life

ROC, Receiver operating characteristics

S100B, S100-Beta

SAH, Subarachnoid haemorrhage

SD, Standard deviation

SIMOA, Single molecule array

TBI, Traumatic brain injury

TCD, Transcranial doppler sonography

VAS, Visual analogue scale

WFNS, World federation of neurosurgical societies scale

Publications and manuscripts

I. **Serum Levels of Myo-inositol Predicts Clinical Outcome 1 Year After Aneurysmal Subarachnoid Hemorrhage.**
Johansson C, Koskinen LD, Sjoberg RL, Lindvall P.
Neurosurgery 2022; **91**(5): 790-8.

II. **Serum neurofilament light as a predictor of outcome in subarachnoid haemorrhage.**
Johansson C, Aineskog H, Koskinen LD, Gunnarsson A, Lindvall P.
Acta Neurochir (Wien) 2023; **165**(10): 2793-800.

III. **Serum S100B correlates with health-related quality of life and functional outcome in patients at 1 year after aneurysmal subarachnoid haemorrhage.**
Aineskog H, Johansson C, Nilsson R, Koskinen LD, Lindvall P.
Acta Neurochir (Wien) 2022; **164**(8): 2209-18.

IV. **Neurofilament-light chain, myo-inositol and S100-beta strengthens a multivariable regression model for predicting long-term clinical outcome in patients suffering from subarachnoid haemorrhage.**
Johansson C, Aineskog H, Koskinen LD, Sjöberg RL, Lindvall P.
Manuscript, submitted for publication

Author’s contribution

For Papers 1, 2, and 4, the author of this thesis served as the first author and was responsible for data collection, study design, statistical analyses, and drafting the main manuscripts. In Paper 3, the author served as the second author, contributing to data collection, discussions regarding study design and statistical analyses, as well as proofreading the manuscript, which was primarily written by the first author.

Use of artificial intelligence

Artificial intelligence was used exclusively for language refinement and grammatical correction of the thesis manuscript. It was not employed in study design, statistical analyses, data interpretation, or for generating original text.

Populärvetenskaplig sammanfattning på svenska

Subaraknoidalblödning (SAB) är en allvarlig form av stroke som drabbar ungefär 10 personer per 100 000 invånare varje år i västvärlden. I de flesta fall orsakas sjukdomen av att ett pulsåderbråck, ett så kallat aneurysm, brister i ett av hjärnans blodkärl. Då läcker blod ut i det utrymme som omger hjärnan. I en mindre andel av fallen beror blödningen på andra typer av kärlmissbildningar, och i omkring en tiondel av fallen hittar man ingen tydlig blödningskälla. Den här avhandlingen handlar enbart om aneurysmorsakad SAB.

Symtomen vid insjuknande kommer ofta plötsligt och kan vara mycket dramatiska. Det vanligaste tecknet är en plötslig och svår huvudvärk, ofta beskriven som den värsta huvudvärk man någonsin upplevt. Andra symtom kan vara nackstelhet, ljuskänslighet, förvirring, förlamning, medvetslöshet eller i värsta fall hjärtstillestånd.

Prognosen vid SAB varierar kraftigt. En del patienter avlider direkt, andra får bestående funktionsnedsättningar, medan vissa återhämtar sig helt. För läkare är det därför en stor utmaning att tidigt kunna avgöra hur en patient kommer att klara sig på lång sikt.

I denna avhandling har vi undersökt tre olika ämnen i blodet – så kallade **biomarkörer** – som skulle kunna hjälpa till att förutsäga patientens långsiktiga återhämtning redan under den akuta sjukdomsfasen. Ett år efter insjuknandet bedömdes patienternas funktion och livskvalitet via telefonintervjuer.

De tre första studierna fokuserade på varsin biomarkör:

- **Myo-inositol (MI)**
- **Neurofilament light chain (NFL)**
- **S100-beta (S100B)**

I den fjärde studien undersöktes om en kombination av dessa tre biomarkörer kunde ge ännu bättre prognostisk information än någon enskild markör.

Resultaten visade följande:

- MI-nivåer som uppmättes från dag sju och framåt var kopplade till långsiktig funktion, medan de initiala nivåerna inte var kopplade till hur allvarligt sjuk patienten var vid insjuknandet.

- NFL var förhöjt redan tidigt hos patienter med svår sjukdom och höll sig högre under hela den akuta fasen hos dem med sämre långtidsutfall.

- S100B var associerat både med långtidsutfall och med patienternas upplevda livskvalitet.

- När biomarkörerna kombinerades med redan kända riskfaktorer förbättrades möjligheten att förutsäga utfallet.

Sammanfattningsvis visar avhandlingen att MI, NFL och S100B kan vara värdefulla biomarkörer för att bedöma prognosen efter en SAB. Innan dessa blodprover kan börja användas i daglig sjukvård behövs dock fler och större studier som bekräftar resultaten.

Prelude

Margret, a 62-year-old woman, suddenly collapses in the presence of her husband. He immediately calls an ambulance, and she is transported to the nearest emergency department. Her medical history is notable for hypertension, and she quit smoking 15 years ago. Otherwise, she is healthy.

On examination, the emergency physician finds that Margret produces only inappropriate vocalizations, does not open her eyes in response to painful stimulation, but localizes the source of pain. Her Glasgow Coma Scale score is therefore 9, corresponding to a world federation of neurosurgical societies (WFNS) grade of 4. A computed tomography (CT) scan reveals a large subarachnoida haemorrhage (SAH), classified as Fisher grade 4, with dilated ventricles consistent with hydrocephalus (Figure 1). CT angiography (CTA) shows an aneurysm of the anterior communicating artery (Figure 1,2) which is confirmed by digital subtraction angiography (DSA) (Figure 2).

Margret is intubated and transferred to the nearest neurosurgical center. She immediately undergoes placement of an external ventricular drainage (EVD) and subsequent endovascular occlusion of the aneurysm in the interventional radiology suite. She is then admitted to the neurointensive care unit.

On day three after the bleed, sedation is reduced. Margret opens her eyes but does not establish eye contact or move her extremities. Her husband becomes distressed, recalling that Margret had always expressed that she would not want to live a life without independence in basic daily activities, preferring death to what she described as becoming a “vegetable.” He now asks about her prognosis and states that he would like to discontinue treatment if the likelihood of her regaining independence is low.

How should the treating neurosurgeon respond? What are Margret’s realistic chances of returning to an independent life?

The answers to these questions are complicated by the considerable variability in outcomes following subarachnoid haemorrhage. Long-term outcomes can range from death or severe, permanent disability to full recovery, allowing for an independent and productive life for decades. Gaining an early understanding of a patient's clinical trajectory—beyond what is immediately evident to the naked eye—is therefore crucial. Such insight not only improves prognostication but can also guide clinical interventions aimed at altering the course of disease when possible.

The exploration of biological markers that reflect these clinical trajectories in patients like Margret may represent one of the most promising avenues toward addressing this challenge. The present thesis can thus be viewed as a step toward developing such solutions.

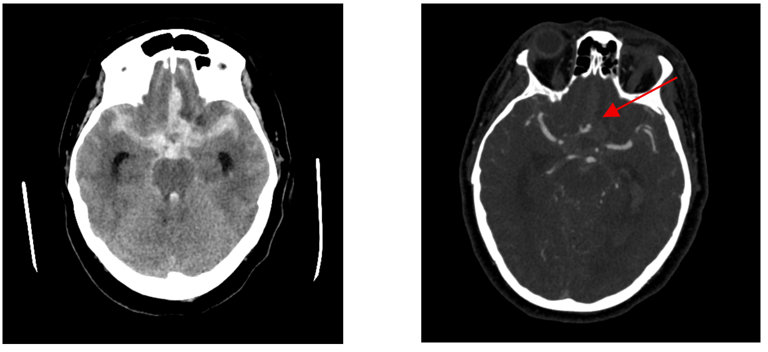

Figure 1. CT scan showing SAH (left) and a CTA showing an aneurysm on the anterior communicating artery (right).

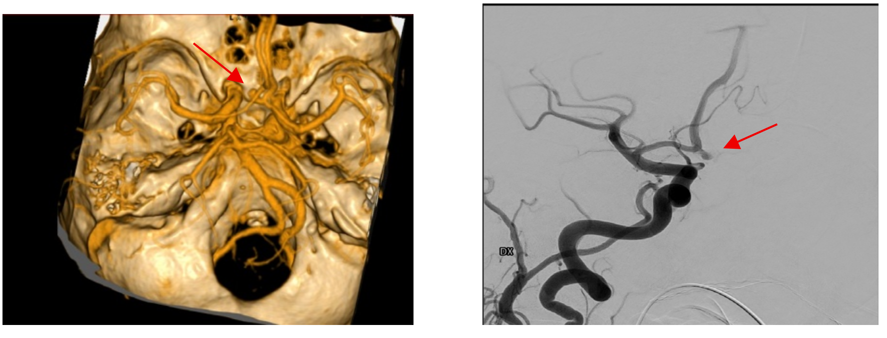

Figure 2. 3D reconstruction of the CTA (left) and DSA (right) showing the aneurysm on the anterior communicating artery.

Historical introduction

The first documented cases of SAH were most likely those reported by Dioni in 1718. In 1761, Morgagni described an unruptured intracranial aneurysm discovered at autopsy, and Biumi was probably the first to report an unruptured aneurysm that subsequently led to SAH in 1778.[1] It was not until Quincke developed the lumbar puncture that SAH could be detected in living patients.[2] The first to correlate the clinical symptoms of SAH with ruptured aneurysms were Collier and Symmonds in the early 1930s.[3-5] In 1927, Moniz invented cerebral angiography and, in 1933, successfully diagnosed an intracranial aneurysm using this technique.[6,7] This innovation paved the way for surgical intervention, with the first successful surgical treatment of an aneurysm performed by Dott in 1931, followed by the pioneering work of Dandy in the operative management of intracranial aneurysms.[8,9]

The largest epidemiological study on the natural course of SAH was conducted in Finland in the 1970s. It reported an initial haemorrhage mortality rate as high as 43%. If left untreated, the one-year mortality rate due to recurrent haemorrhage was 37.7%, with most recurrences occurring within four weeks of the initial bleed.[1]

The introduction of computed tomography (CT) by Hounsfield in 1971 represented a major breakthrough in the diagnosis of intracranial diseases.[10] Later, the development of endovascular coil embolization provided a less invasive treatment option for aneurysmal occlusion.[11]

In addition to the initial haemorrhage, SAH patients may experience a range of secondary complications that can lead to death or severe morbidity, as discussed later in this thesis. Despite substantial advances in diagnostic and therapeutic strategies, SAH remains a condition associated with high mortality and morbidity, underscoring the need for continued research and innovation to reduce its overall disease burden.

Introduction to subarachnoid haemorrhage

Epidemiology and aetiology

Intracranial aneurysms are acquired rather than congenital, developing over the course of life and occurring in approximately 3% of the population, most commonly at arterial branching points.[12] SAH refers to bleeding into the space between the pia mater and the arachnoid mater and may occur spontaneously or following trauma. Spontaneous SAH accounts for about 5% of all strokes; in approximately 85% of cases, it is caused by a ruptured intracranial aneurysm, while about 10% occur without an identifiable bleeding source, and the remaining 5% arises from other vascular abnormalities such as arteriovenous malformation or fistulae. [1,12-15] This thesis focuses exclusively on spontaneous SAH of aneurysmal origin; therefore, the term SAH hereafter refers specifically to aneurysmal SAH. The prognosis for SAH is generally reported as approximately a 50% chance of surviving to an independent life, while the remaining 50% either die or survive with significant dependency.[16] The global incidence is approximately 10 per 100,000 person-years, with considerable regional variation. The highest incidence has been reported in Finland (19 per 100,000) and Japan (23 per 100,000). [17,18] Even within Sweden, incidence varies, with higher rates observed in the northern regions.[19] Overall, incidence has declined in recent decades, except for Japan. This trend has been attributed to improved hypertension management and declining smoking prevalence. [18] Incidence rises markedly after the age of 55.[17] Aneurysm formation is more common in women, although evidence regarding sex-specific differences in rupture risk is inconsistent. Some data suggest that rupture risk, and thus incidence, increases among women older than 55 years.[17,20] Regardless, the higher prevalence of aneurysms in women contributes to their greater risk of experiencing SAH.[21] With a mean age of onset around 55 years, SAH tends to affect younger patients compared with other types of strokes. Survivors frequently experience substantial long-term morbidity, leading to considerable socioeconomic consequences. [12,22,23]

Pathophysiology

When an intracranial aneurysm ruptures, blood enters the subarachnoid space under high pressure and may also extend into the parenchyma and subdural space.[13] This causes a rise in intracranial pressure (ICP), which reduces cerebral perfusion pressure (CPP) and thereby affects cerebral blood flow (CBF). Diminished CBF may result in ischemia, cerebral edema, and neuronal death, initiating a cascade of toxic events.[13,24-26] The clinical manifestations of SAH can be explained by these mechanisms. The sudden onset of headache and vomiting reflects increased ICP, while photophobia and nuchal rigidity arise from meningeal irritation caused by inflammation. Focal neurological deficits may result from reduced regional CBF or parenchymal hematoma. Approximately one in 14 patients with SAH experiences seizures at the time of aneurysm rupture. Lethargy and unconsciousness are typically consequences of critically reduced CBF due to elevated ICP and low CPP and the bleedings effect on structures critical for consiousness.[12,27-29] Blood clots within the subarachnoid space and ventricles can obstruct cerebrospinal fluid (CSF) circulation, leading to hydrocephalus and further increases in ICP.[26,29-31] Toxic injury caused by degradable haemoglobin products may contribute to secondary brain damage.[25] The brain injury that occurs immediately after the rupture and during the first days is commonly referred to as early brain injury (EBI) which is summarized in figure 3.[23,24,26]

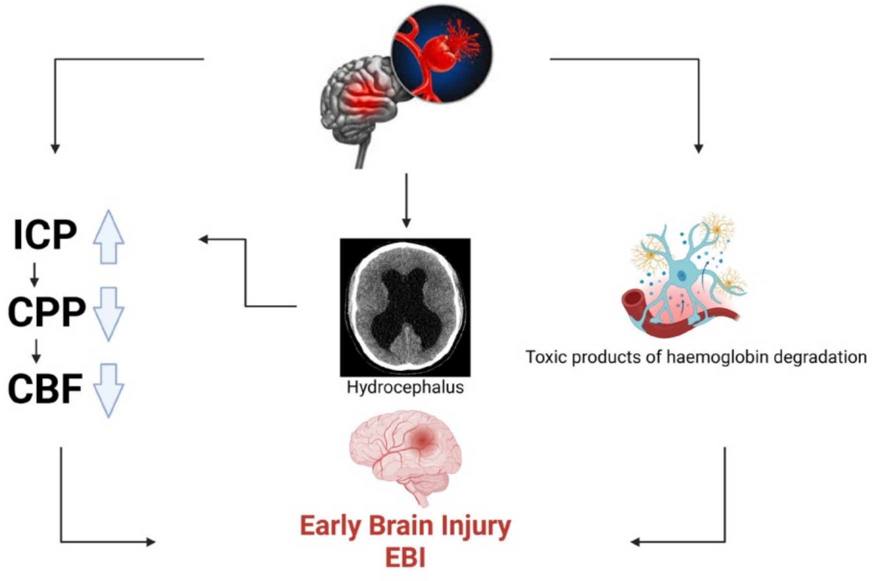

Figure 3. Summary of events leading to early brain injury. ICP = Intracranial pressure. CPP = Cerebral perfusion pressure. CBF = Cerebral blood flow. Created in BioRender. Johansson, C. (2025) https://BioRender.com/ben5wzt

Following EBI, a series of secondary processes may lead to delayed cerebral ischemia (DCI), defined as neurological deterioration (that cannot be attributed to any other cause, such as rebleeding, hydrocephalus or infection) occurring several days after the bleed that contributes to further brain injury.[23,30,32] DCI affects approximately 30% of SAH patients.[23,33,34] However, its definition has varied considerably across studies.[13,35-37] To address this, Vergouwen et al. proposed a standardized definition, suggesting that DCI should refer to clinically observed neurological deterioration, while angiographic vasospasm and cerebral infarctions detected by CT or magnet resonance imaging (MRI) should be considered distinct entities.[36] The following sections outline mechanisms thought to underlie DCI.

Angiographic vasospasm refers to vessel narrowing observed on DSA or CTA. First described in 1951, it was considered to be the cause of neurological decline and poor outcomes by causing hypoperfusion leading to cerebral infarctions.[38,39] Vasospasm can be experimentally induced by exposing vessels to CSF containing blood.[40] The risk of vasospasm correlates with the amount of subarachnoid blood visible on CT.[41,42] Historically, angiographic vasospasm was considered the primary cause of DCI.[39,43] Indeed, a correlation exists between vasospasm severity and cerebral infarctions.[44] However, vasospasm may occur without clinical deterioration, and DCI can occur in the absence of vasospasm.[41,44,45] Furthermore, reducing vasospasm does not consistently translate into improved functional outcomes.[43,46] Thus, although vasospasm likely contributes to DCI, it cannot fully account for it.

Microvascular vasospasm has been demonstrated in animal models of SAH.[47-51] Its precise mechanisms remain unclear but may involve nitric oxide depletion, hemoglobin degradation products, and pericyte dysfunction.[51-53] Microcirculatory occlusion can cause neuronal death even when overall CBF appears adequate, suggesting a role in DCI pathogenesis.[54] While these findings highlight a possible therapeutic target, evidence is currently limited to preclinical studies.[23,32]

Microthrombosis develops within the microvasculature as early as 48 hours after SAH.[55-57] It arises from dysregulated coagulation and vessel lumen narrowing, promoting thrombus formation in small vessels and leading to impaired capillary perfusion and ischemia.[48,51] Autopsy studies demonstrate associations between microthrombosis, DCI, and vasospasm.[58] However, clinical trials of platelet inhibitors have not improved outcomes, and vessels affected by microthrombi are too small for direct intervention.[59] Although microthrombosis appears central to DCI pathophysiology, its value as a therapeutic target remains uncertain.

Cortical spreading depolarization (CSD) involves disrupted neuronal ion homeostasis, causing sustained depolarization as cations flow into neurons, which prevents the generation of action potentials.[60] This depolarization propagates to adjacent neurons, suppressing action potential generation and thereby depressing neuronal signaling.[61] Restoration of homeostasis requires energy-intensive sodium-potassium pump activity.[62] Normally, the neurovascular unit responds with arteriolar dilation to increase regional CBF and meet metabolic demand.[63] In SAH, however, this coupling is impaired, producing paradoxical vasoconstriction and decreased CBF despite elevated energy requirements.[64,65] This mismatch may culminate in "terminal depolarization" and cell death, also termed spreading ischemia. Detection of CSD requires placement of subdural cortical electrodes. One study has confirmed that CSD occurs in SAH patients and is associated with tissue hypoxia via an inverse hemodynamic response.[65]

Inflammation also plays a role in DCI pathophysiology. Aneurysm rupture may cause increased levels of numerous inflammatory proteins linked to poor outcomes, vasospasm, and microthrombosis.[66-70] Vasospastic arteries are infiltrated by inflammatory cells and immunoglobulins, and experimental studies have shown these effects to be mitigated by ibuprofen.[71,72] Inflammation thus represents a potential therapeutic target. However, clinical trials of anti-inflammatory drugs in SAH have largely been unsuccessful[73-75] A pilot trial of 95 patients reported that methylprednisolone improved functional outcome, but findings remain preliminary. [76]

Although incompletely understood, DCI is a major contributor to cerebral infarction and is strongly associated with unfavourable outcomes and is summarized in figure 4.[77]

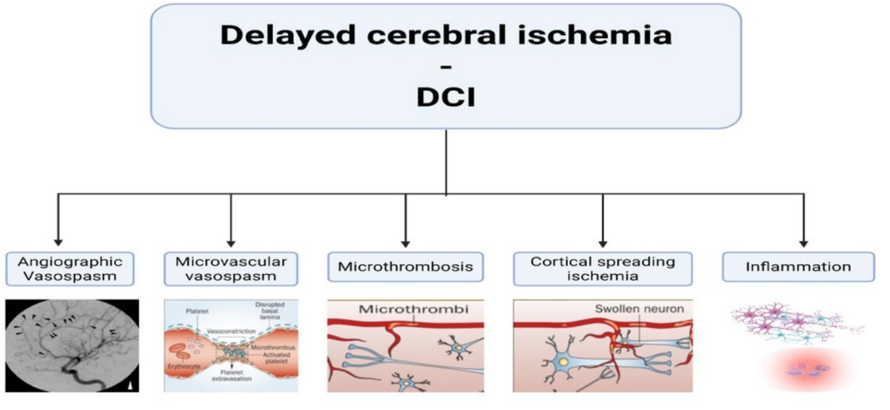

Figure 4. Summary of mechanisms proposed to contribute to delayed cerebral ischemia. Created in BioRender. Johansson, C. (2025) https://BioRender.com/7wlc0lg

Diagnosis

SAH

The classic presenting symptoms of SAH include sudden, severe headache, nuchal rigidity, and, in some cases, a decreased level of consciousness. Focal neurological deficits may also occur, particularly in the presence of an intracerebral haematoma (ICH). In its most severe form, SAH can present with cardiac arrest. The initial diagnostic step is a non-contrast CT scan. When performed within six hours of symptom onset, CT demonstrates a sensitivity of 98.7% and a specificity of 99.9%.[78] Beyond six hours, sensitivity decreases to below 90%. A negative CT scan followed by lumbar puncture with cerebral spinal fluid (CSF) spectrophotometry, has a sensitivity of 100%.[79] Lumbar puncture should therefore be considered despite a negative CT scan if the patient present with symptoms typical of SAH and the CT scan is performed >6 hours of onset of symptoms.

DCI

As previously noted, the term DCI has historically been defined heterogeneously and often used interchangeably with angiographic vasospasm.[13,35-37] The following definitions has been proposed to be used when designing studies: "The occurrence of focal neurological impairment (such as hemiparesis, aphasia, apraxia, hemianopia, or neglect), or a decrease of at least 2 points on the Glasgow Coma Scale (either on the total score or on one of its individual components [eye, motor on either side, verbal]). This should last for at least 1 hour, is not apparent immediately after aneurysm occlusion, and cannot be attributed to other causes by means of clinical assessment, CT or MRI scanning of the brain, and appropriate laboratory studies." Cerebral infarction is defined as: "The presence of cerebral infarction on CT or MRI scan of the brain within 6 weeks after SAH, or on the latest CT or MRI scan made before death within 6 weeks, or proven at autopsy, not present on the CT or MRI scan between 24 and 48 hours after early aneurysm occlusion, and not attributable to other causes such as surgical clipping or endovascular treatment. Hypodensities on CT imaging resulting from ventricular catheter or intraparenchymal hematoma should not be regarded as cerebral infarctions from DCI."[36] In this framework, angiographic vasospasm is regarded as a separate entity, though it is hypothesized to contribute to the pathophysiology of DCI. Detecting DCI in comatose patients, who cannot be fully assessed neurologically, remains particularly challenging. In such cases, several surrogate markers are applied, most of which assess vasospasm in large cerebral vessels.

Transcranial Doppler sonography (TCD) is a bedside technique used to evaluate flow velocity in major cerebral arteries, mainly the middle cerebral artery (MCA). Velocities between 120–200 cm/s are considered indicative of mild to moderate vasospasm, whereas velocities exceeding 200 cm/s suggest severe vasospasm.[80] TCD has a high negative predictive value for velocities below 120 cm/s and good predictive value for velocities above 200 cm/s. However, values within the 120–200 cm/s range should be interpreted with caution.[80] An alternative approach is to calculate the Lindegaard ratio, defined as the ratio of MCA to external carotid artery flow velocity. [81] A ratio between 3 and 6 suggests mild to moderate vasospasm, while a ratio greater than 6 indicates severe vasospasm.[37,81] TCD offers the advantages of being bedside-based and non-invasive, but it is operator dependent, affected by bone thickness, and limited to assessing flow selected large vessels.[32,37] Importantly, TCD reflects vasospasm rather DCI directly, and therefore should be interpreted alongside clinical evaluation and complementary diagnostic modalities.

The gold standard for detecting macrovascular vasospasm is DSA, although CTA demonstrates comparable sensitivity for larger vessels.[82,83] CTA is more accessible and non-invasive, but its accuracy may be compromised by artifacts from surgical clips or coils, and it has a tendency to overestimate the severity of vasospasm.[37]

CT perfusion (CTP) imaging provides parameters related to cerebral perfusion. One such parameter is mean transit time (MTT), defined as the mean time for blood to perfuse a given brain region, thereby reflecting both macro- and microvascular circulation and regional CBF.[84] CTP has primarily been applied in three contexts..[85] First, early CTP (within 72 hours of SAH onset) has been investigated for predicting the development of DCI, with promising results.[86] Second, CTP performed during suspected DCI has been used to detect angiographic vasospasm, showing strong correlation between MTT prolongation and vasospasm confirmed by DSA.[87] Third, CTP obtained at the time of suspected DCI demonstrated superiority over both CT and CTA for diagnosing DCI, though this was limited to non-comatose patients.[88] Additionally, prolonged MTT has been correlated with cerebral infarction and worse functional outcomes.[89] A limitation of CTP, however, is that parameter values and thresholds depend on the specific scanner and algorithm used. Consequently, absolute cutoffs reported across studies are not directly comparable, and no standardized thresholds have yet been established.[89]

Continuous electroencephalography (EEG) has also been investigated as a tool for predicting DCI. Its non-invasive nature and ability to monitor comatose patients make it an attractive modality. Studies have reported correlations between EEG patterns and the subsequent development of DCI.[90,91] Nevertheless, the high cost of equipment and software, along with the need for specialized neurophysiological expertise for interpretation, currently limit its widespread clinical use.[92]

Treatment

The primary objective in the treatment SAH is to *secure the aneurysm* and prevent rebleeding. This can be achieved either through craniotomy with microsurgical clip ligation or by employing various endovascular occlusion techniques.[93-95] The choice of approach depends on aneurysm size, location, and morphology. Craniotomy offers the added advantage of enabling evacuation of an ICH or subdural haematoma if present.[96,97] The International Subarachnoid Aneurysm Trial compared microsurgical clipping with endovascular treatment and found that endovascular therapy increased the likelihood of favourable functional outcome. However, the study was limited by selection bias, as one inclusion criterion required equipoise regarding the best treatment. The trial population included a disproportionately high number of patients with low WFNS grades and relatively few MCA aneurysms, in which microsurgical treatment is often superior.[94,95] Consequently, it is not appropriate to conclude that endovascular treatment is universally superior; rather, treatment decisions should be individualized.[96-98] Rebleeding risk is further reduced by controlling hypertension, with recommended systolic blood pressure thresholds of 180 mmHg or 160 mmHg.[96,97] Administration of the antifibrinolytic agent tranexamic acid has been shown to lower the risk of rebleeding before aneurysm securing, although no evidence supports an improvement in functional outcome.[99,100]

Acute *hydrocephalus* is a common complication of SAH, with an incidence of 20–50%.[101,102] Initial management typically involves EVD. While it has been suggested that EVD may increase rebleeding risk by lowering ICP and thereby raising the transmural pressure gradient across the aneurysm, evidence remains inconsistent.[103-105] One study demonstrated that early EVD placement was associated with better outcomes, highlighting the importance of prompt ICP management to limit EBI.[106] Approximately 22% of patients develop chronic hydrocephalus requiring permanent CSF diversion, typically via ventriculoperitoneal shunting. Risk factors for shunt dependency include age ≥65 years, microsurgical clipping, prolonged fever, infection, and prior EVD placement.[107]

Preventing DCI remains one of the greatest challenges in SAH management. Nimodipine, an L-type voltage-gated calcium channel antagonist, is the only pharmacologic agent proven to reduce the incidence of cerebral infarction and improve functional outcome.[108-110] Nimodipine administered perorally has not been shown to affect angiographic vasospasm.[110] In contrast, Clazosentan, an endothelin receptor antagonist, significantly reduced angiographic vasospasm but failed to improve clinical outcomes.[46] The precise mechanism of Nimodipine remains uncertain but is hypothesized to involve effects on microvascular spasm, CSD and microthrombosis.[23] When DCI develops despite Nimodipine, several rescue therapies are employed, though high-quality evidence is limited. Historically, induced hypertension was the first-line intervention, but a randomized controlled trial was terminated early due to increased adverse events without improvement in functional outcomes.[111,112] Milrinone, a phosphodiesterase inhibitor with both inotropic and cerebral vasodilatory effects, has shown promise in observational studies.[113-115] Retrospective studies suggest that intrathecal nicardipine (a calcium channel blocker) may reduce DCI incidence and improve outcomes.[116] Endovascular interventions, such as intra-arterial vasodilator infusion, are commonly used when medical therapy fails.[111] Meta-analyses show that while these interventions improve angiographic vasospasm, their effect on neurological recovery is less consistent.[117] Percutaneous intraluminal balloon angioplasty can be employed in proximal vessels when other therapies fail.[111,118] Observational studies have reported radiographic and clinical benefits, though the procedure carries a complication rate of approximately 6%.[119-121] In summary, Nimodipine remains the only treatment with strong evidence for preventing DCI in SAH patients. Other therapies show promise and are frequently used in clinical practice, but their efficacy requires further confirmation.[111,122]

Outcome assessment

Patient outcomes are influenced by physical and cognitive deficits as well as social limitations. Below is a brief introduction to the outcome measures used in this thesis.

Glasgow outcome scale extended (GOSE)

Jennett and Bond introduced the original Glasgow outcome scale (GOS) in 1975 as a tool to assess how physical and psychological sequelae of traumatic brain injury (TBI) affect major life domains.[123] It was innovative at the time because it considered not only neurological deficits but also psychological factors that influence daily life.[124] The original GOS comprised five categories, ranging from death to good recovery, with the three higher categories representing varying levels of consciousness and disability (Figure 5).[123] To increase sensitivity and better discriminate between degrees of disability in conscious survivors, the GOS was later expanded into an eight-point scale, the Glasgow outcome scale extended (GOSE).[125] Critics initially suggested that this modification could reduce interobserver reliability.[126,127] To address this, a structured interview format was developed, yielding weighted kappa values of 0.85 for GOSE and 0.89 for GOS, demonstrating strong interobserver agreement.[128] Both scales have since been widely applied to other forms of brain injury, including stroke, and shown to be reliable.[129] Scores are frequently dichotomized into favourable (GOS 4–5, GOSE 5–8) and unfavourable (GOS 1–3, GOSE 1–4) outcomes, with the threshold reflecting whether patients are considered functionally independent. In summary, GOSE is a structured, validated measure of functional outcome after brain injury, accounting for both physical and cognitive impairments.

Modified Rankin scale (mRS)

John Rankin first described the Rankin Scale in 1957 as a five-point tool to evaluate functional outcome in stroke patients.[130-132] In the 1980s, Farrell modified the scale by adding two categories, including death, resulting in the current 0–6 format.[133] The interobserver reliability of the mRS was first examined in 1988, showing a modified kappa value of 0.91,[134] later confirmed by a meta-analysis of 10 studies.[135] The scale correlates well with objective measures such as infarct volume.[136] Structured interviews have been shown to further improve reliability.[135,137] As with GOSE, the mRS is commonly dichotomized into favourable and unfavourable outcomes, with scores of 0–2 or 0–3 typically classified as favourable. Compared with GOSE, the mRS is more frequently used in studies of SAH.[138] However, GOSE provides greater emphasis on social and behavioral outcomes, which may be particularly relevant in SAH compared to other stroke subtypes.[137,139] Both mRS and GOSE are widely applied as outcome measures in SAH research.[140]

Euro quality of life 5-dimensions (EuroQoL 5D)

In 1998, the EuroQoL research group introduced the EQ-5D, a generic instrument for assessing health-related QoL.[141] Its primary purpose is to enable comparisons of health-related QoL across diseases, treatment modalities, and populations. The EQ-5D questionnaire includes five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Respondents rate each dimension on either three levels (EQ-5D-3L: no problems, some problems, extreme problems) or five levels (EQ-5D-5L).[142] Each set of responses generates a five-digit code (e.g., 11232). Alongside the questionnaire, respondents rate their overall health using a visual analog scale (VAS) ranging from 0 (worst imaginable health) to 100 (best imaginable health). Country- and region-specific value sets have been established to reflect population-level perceptions of health states, allowing conversion of individual responses into an index score (EQ-5D index). For example, the response 11232 may correspond to an index score of 0.65 in a given region. Higher scores indicate better perceived health. These values allow for comparisons between outcome groups or treatments.[143] It is important to note that the EQ-5D index is derived from population-based health valuations, whereas the EQ-VAS reflects the individual's self-reported health. While the EQ-5D questionnaire may be completed by a proxy, the VAS must be completed by the patient directly.[143] Unlike the mRS and GOSE, which focus on functional outcomes, the EQ-5D emphasizes patient-perceived health-related QoL.

GOS			GOSE			mRS		
1	Death	Non-survival.	1	Death	Non-survival.	6	Death	Non-survival.
2	Vegetative	No awareness of self or environment.	2	Vegetative	No awareness of self or environment.	5	Severe disability	Bedridden, incontinent, and requiring constant nursing care and attention.
3	Severe disability	Conscious but disabled. Dependent on daily support.	3	Lower severe disability	Dependent, needs frequent assistance in basic activities in daily life.	4	Moderately severe disability	Unable to walk without assistance and unable to attend to own bodily needs without assistance.
			4	Upper severe disability	Dependent, needs infrequent assistance in basic activities in daily life, or help with activities outside the home.			
4	Moderate disability	Disabled but independent. Able to work in a sheltered setting.	5	Lower moderate disability	Independent, but cannot return to one or more life roles.	3	Moderate disability	Requiring some help, but able to walk without assistance.
5	Good recovery	Resumption of normal life despite minor deficits.	6	Upper moderate disability	Some disability exists, but able to return to one or more life roles.	2	Slight disability	Unable to carry out all previous activities, but able to look after own affairs without assistance.
			7	Lower good recovery	Able to return to previous life roles, but with symptoms that affect daily life.	1	No significant disability despite symptoms.	Able to carry out all usual duties and activities.
			8	Upper good recovery	Fully recovered or may have minor symptoms not affecting daily life.	0	No symptoms at all.	Full recovery.

Figure 5. Comparison of different functional outcome scales. Red cells represent unfavourable outcomes; green cells represent favourable outcomes. GOS = Glasgow Outcome Scale, GOSE = Glasgow Outcome Scale Extended, mRS = Modified Rankin Scale

Factors affecting outcome in SAH

As described, long-term outcomes after SAH range from death to full recovery.[13] Numerous studies have investigated factors influencing both short-term (<12 months) and long-term (≥12 months) outcomes..[140] Among demographic factors, increasing *age* is consistently associated with unfavourable outcomes, particularly beyond 60 years.[77,144-147] *Hypertension* is the most frequently studied pre-existing condition linked to poor outcome, although findings are not entirely consistent.[77,140,144,145,147] *Initial disease severity* is another major determinant. Poor neurological status at presentation, as measured by the WFNS or HH scales, is among the most commonly reported predictors of unfavourable outcome.[77,140,144-147] Historically, the *amount of subarachnoid blood* on initial CT, graded according to the *Fisher scale (*initially created to predict angiographic vasospasm), was considered predictive of outcome.[13,147,148] However, advances in CT imaging have increased sensitivity for detecting blood, resulting in a greater proportion of patients receiving higher Fisher grades (3–4) and reducing the scale's discriminatory power. Recent studies question the Fisher grade's role as a predictor of long-term outcome.[140,145,146,149] *Aneurysm location* in the posterior circulation is sometimes associated with unfavourable outcomes, though evidence is inconsistent.[140,145,147] Large *aneurysm size* (>10 mm) has been linked to worse outcomes,[145,147] whereas the presence of *multiple aneurysms* may predict short-term but not long-term unfavourable outcomes.[140] Predictive models using admission factors, such as SAHIT (age, WFNS, hypertension) and SAFIRE (age, WFNS, Fisher grade, aneurysm size), have shown promise in forecasting long-term functional outcomes.[145,147] However, these models primarily reflect EBI and do not account for secondary events such as delayed DCI or rebleeding.

The risk of aneurysmal *rebleeding* is highest within the first 24 hours and is strongly associated with increased mortality and unfavourable outcomes but is uncommon once the aneurysm is secured.[1,77,150-152]

Evidence also demonstrates that *DCI* is a major predictor of poor outcome.[34,77] A recent study reported a fivefold increase in the odds of unfavourable outcome in patients with DCI-related cerebral infarctions, with infarct volume correlating with outcome severity.[153]

Several scales assess *patient frailty* by incorporating age and various preexisting conditions. A meta-analysis demonstrated that frailty is associated with unfavourable outcomes in patients with SAH.[154]

Background to the present study

Biomarkers and prognostication

As previously mentioned, patients suffering from SAH may experience complete recovery, death, or varying degrees of neurological deficits that can have minor or major impacts on daily life. Accurate prognostication in patients with a specific disease serves several important purposes. In addition to preparing patients and their families for what to expect, it helps identify individuals at higher risk of poor outcomes, thereby guiding research efforts toward developing targeted therapies that may improve prognosis and overall patient care. For SAH, the patient's clinical condition at disease onset only partially reflects the extent of brain injury caused by the initial haemorrhage, as reversible factors such as hydrocephalus may contribute to a worsened condition upon hospital admission. Although a more severe initial clinical presentation is strongly associated with unfavourable outcomes, only about 50–60% of patients presenting with a WFNS score of 3–4 experience an unfavourable outcome, and nearly 30% of those presenting with a WFNS grade of 2 also have unfavourable outcomes.[96,155] This highlights the significant contribution of secondary injuries to the total extent of brain damage following SAH.

While the injury caused by the initial bleed can only be mitigated through risk reduction and prevention of aneurysm formation and rupture, the secondary injuries that develop thereafter are the primary targets of medical intervention. Laboratory biomarkers that can help distinguish between the damage caused by the initial bleed and that resulting from secondary events are therefore highly desirable for several reasons:

1. **Pathophysiological insight:** Biomarkers associated with indicators of brain injury—such as functional outcome, cerebral infarctions, or bleeding volume—may provide valuable insight into the underlying pathophysiological mechanisms.

2. **Prognostication:** Such biomarkers could aid in predicting functional outcomes, which is essential for patient and family counseling as well as clinical decision-making.
3. **Therapeutic potential:** Biomarkers capable of detecting processes that threaten to cause additional brain injury could facilitate early intervention, thereby reducing total brain damage and improving functional outcomes.

Although numerous biomarkers have been investigated in relation to SAH and DCI, few have been adopted for routine clinical use.[96] Several studies have explored associations between biomarkers, outcomes, and potential pathophysiological mechanisms in SAH.

For instance, elevated sympathetic activity—reflected by increased catecholamine levels in CSF and higher cardiac troponin concentrations—has been linked to poor outcomes in SAH patients.[156,157] The role of inflammation in SAH pathophysiology has been demonstrated through associations between certain cytokine levels and both functional outcome and DCI.[158] General markers of brain injury, such as neuron-specific enolase, glial fibrillary acidic protein, and ubiquitin carboxy-terminal hydrolase L1, have also shown promising prognostic potential in SAH.[159-162]

This thesis focuses on three specific biomarkers. The rationale for selecting each biomarker and the potential scientific implications are discussed in the following sections.

Myo-inositol (MI)

In 2015, Sjöberg et al. conducted a metabolomics study examining the dynamics of 96 metabolites during the first seven days after SAH. Seventeen metabolites changed significantly between days 1–3 and day 7, among which MI was the only one to show a positive correlation with favourable one-year outcomes.[163] Inositol is a carbocyclic sugar polyalcohol that can be converted into nine stereoisomers, with MI and D-chiro-inositol being the most clinically relevant.[164] Approximately 99.8% of dietary MI is absorbed in the intestine.[165] Endogenous MI production occurs primarily in the kidney from glucose, inositol phosphate, and phosphoinositides, with additional production observed in brain, liver, and testis in animal studies.[166,167] Serum MI levels are regulated and eliminated by the kidney.[168] Physiologically, MI contributes to ion channel regulation, phosphate homeostasis, metabolic flux, transcription, mRNA export and translation, insulin signaling, embryonic development, and stress response, and is a key component of membrane phosphatidylinositols.[169] MI concentrations are higher in CSF than plasma (138 ± 50 μM vs. 28 ± 12 μM), underscoring its role in the brain.[170] Normal MI plasma levels are approximately 32.5 μM, with a reference range of 26–43 μM.[171] Genetic alterations affecting MI metabolism have been linked to bipolar disorder, ischemic stroke, carotid plaques, and spina bifida.[172-176] A metabolite offers the advantage of reflecting underlying cellular processes. The association between dynamic changes in MI levels, disease severity, and clinical outcome in patients with SAH has thus far only been investigated in a hypothesis-generating, semi-quantitative study. We aimed to study MI to quantitatively assess MI levels to determine whether previous findings can be reproduced. If the results can be replicated, MI could be established as a biomarker with the potential to predict outcomes in patients with SAH. Furthermore, MI may contribute to a deeper understanding of the underlying pathophysiological mechanisms and potentially facilitate the development of therapeutic interventions aimed at improving clinical outcomes.

Neurofilament light (NFL)

Neurofilaments are a family of five proteins (heavy, medium, light, α-internexin, and peripherin) present in central and peripheral neurons, forming the cytoskeleton and contributing to axonal growth, stability, mitochondrial integrity, and microtubule transport.[177-179] NFL is primarily localized in axons but also functions in dendrites and synapses.[180] Serum NFL concentrations increase with age (~2–3% per year between 18–70 years), necessitating age-adjusted reference intervals.[181-183] Mutations in neurofilament genes are associated with neurological disorders, including amyotrophic lateral sclerosis, Charcot–Marie–Tooth disease, autosomal recessive spastic ataxia of Charlevoix–Saguenay, and giant axonal neuropathy.[184] Neurofilaments are released from neurons following axonal injury or neuroinflammation.[185,186] Baseline concentrations are low in CSF (1–10 ng/mL) and even lower in blood (10–100 pg/mL).[187,188] One large American study reported an age-adjusted mean serum NFL value of 16.76 pg/mL (95% CI, 14.45–19.07).[189] While CSF measurement methods have existed for some time, reliable plasma quantification, such as via single molecule array (SIMOA), became available only in 2015.[190-192] CSF and plasma NFL levels correlate, though the strength of correlation varies by underlying condition.[190,193-195] Increased levels of NFL was seen as response to EVD placement, showing peak levels at one month post-procedure with normalization by 6–12 months, although early peaks may have been missed.[193] NFL levels peaked around 12 days post-trauma in TBI patients.[195] An association between CSF NFL and functional outcome in SAH was first demonstrated in 2006.[196] The introduction of high-sensitivity analyses like SIMOA and the possibility to quantify NFL in serum or plasma opened up for a more practical use of NFL as a biomarker, and several studies have explored its potential.[197-200] The advent of SIMOA has facilitated serum/plasma measurements, and subsequent studies have linked plasma NFL to both short-term (1 month) and long-term (6 months) functional outcomes.[197-199] We aim to evaluate the temporal dynamics of serum NFL and its potential associations with functional outcome and clinical parameters, including the WFNS score and Fisher grade, by quantifying NFL levels using a high-sensitivity ELISA assay. Furthermore, we seek to compare CSF and serum NFL levels to assess whether serum concentrations accurately reflect intracerebral processes. If serum NFL levels are shown to be associated with long-term functional outcomes in SAH and to correlate with CSF levels, this would strengthen its validity and utility as a biomarker in future research and potential clinical applications.

S100-Beta (S100B)

S100 was first isolated in 1965 from bovine brain, named for its solubility in saturated ammonium sulfate.[201] The S100 protein family exists primarily as homodimers, with heterodimers also present.[202] In the CNS, the S100BB homodimer and S100AB heterodimer are most abundant; their combined concentration is referred to as S100B.[203,204] Intracellularly, S100B regulates Ca^{2+} homeostasis, second messenger signaling, cell differentiation, and apoptosis inhibition.[205-207] Extracellularly, it promotes neurogenesis, plasticity, and memory-related processes.[208-212] S100B is also expressed in adipocytes, Langerhans cells, epithelial cells, cardiac and smooth muscle myocytes, chondrocytes, and melanocytes, and is used as a biomarker in malignant melanoma.[203,213] CSF concentrations are approximately 100-fold higher than serum levels.[214] Serum S100B levels in healthy individuals range from 0.02 to 0.15 µg/ml.[215] Following brain injury, S100B is rapidly released, peaking within six hours, and may also be upregulated intracellularly.[216-219] Its half-life is 60–120 minutes, and it is primarily cleared renally, with mild to moderate kidney dysfunction having minimal effect on serum levels.[220-223] S100B has been extensively studied in TBI and has been shown in SAH patients to correlate with functional outcome and initial WFNS score.[224-229] While S100B does not consistently predict vasospasm, one study demonstrated that vasospasm accompanied by rising S100B levels was associated with worse outcomes than vasospasm alone.[224,226,228] Levels also correlate with cerebral infarction.[224,228] Overall, S100B appears to be a reliable biomarker for functional outcome in SAH, although optimal timing of measurement remains unclear, and its association with $EQ\text{-}5D_{index}$ has not yet been investigated. Determining the optimal timing of measurement would be of great practical value for implementation of S100B in clinical routine.

Aims of the study

- To investigate whether MI, NFL and S100B are associated with long-term functional outcomes in patients suffering from SAH.
- To evaluate whether the findings regarding the association between MI and functional outcome after SAH could be replicated in a hypothesis-driven study.[163]
- To investigate the temporal dynamics of MI and NFL during the acute phase of SAH, and to examine their associations with clinical factors such as WFNS score, Fisher grade, DCI, vasospasm, and functional outcome.
- To assess the correlation between serum and CSF levels of MI and NFL.
- To determine whether S100B is associated with health-related quality of life as measured by the EQ-5D instrument.
- To identify the optimal timepoint for S100B measurement in predicting functional outcome after SAH.
- To evaluate whether incorporating MI, S100B, and NFL into established predictors improves the performance of multivariable regression models for outcome prediction.

Materials and Methods

Ethical considerations

This study was approved by the Regional Ethics Committee at Umeå University (2013/366-31) and conducted in accordance with the ethical standards of the Declaration of Helsinki. Written informed consent was obtained from all participants. For patients who were unable to provide consent due to their clinical condition, consent was obtained from a next of kin acting as a proxy.

Study population

A total of 147 patients aged ≥18 years who were treated for spontaneous SAH at Umeå University Hospital between 2014 and 2018 were screened for inclusion. Patients with non-aneurysmal SAH, missing follow-up data, or missing blood samples for biomarker analysis were excluded. For Study 3, patients presenting ≥48 hours after symptom onset were also excluded.

Diagnosis

The diagnosis of SAH was established by CT imaging, and the source of bleeding was determined by CTA and/or DSA. Of the included patients, only one had a Fisher grade 1 and was diagnosed with lumbar puncture with pathological CSF spectroscopy. Vasospasm was defined as vessel narrowing described by a radiologist on any CTA or DSA performed during hospitalization. TCD was performed regularly when a trained examiner was available, and flow velocities ≥120 cm/s were considered pathological. DCI was defined as any neurological deterioration not attributable to other causes such as rebleeding, hydrocephalus, sodium imbalance, or infection, as determined by the treating physician.

Treatment

Aneurysms were treated with either microsurgical clipping or endovascular occlusion, depending on aneurysm morphology and location. The treatment strategy was determined by a vascular neurosurgeon in consultation with an endovascular interventionist. All patients subsequently received standardized neuro–intensive care according to a local protocol for a minimum of 10 days. This protocol included continuous intravenous nimodipine infusion (Nimotop®, Bayer, Leverkusen, Germany; 0.2 mg/ml at 1–15 ml/hour) as prophylaxis against DCI, in addition to optimized management of sodium and fluid balance, temperature, blood pressure, and, in sedated patients, ICP and CPP. Patients with hydrocephalus received an EVD. In sedated patients, ICP was measured via the EVD, with treatment targets of ICP ≤20 mmHg and CPP ≥70 mmHg.

Follow up

Patients were followed approximately one year after disease onset. A trained research nurse conducted structured interviews and assessed outcomes using the GOSE, mRS, and EQ-5D scales. Outcomes were dichotomized as unfavourable (GOSE 1–4, mRS 4–6) or favourable (GOSE 5–8, mRS 0–3).

Blood and CSF sampling

Venous blood samples were obtained upon admission (day 0) and repeatedly thereafter, with a goal of at least two samples per week. In patients with an EVD, CSF samples were collected simultaneously. All samples were centrifuged, fractionated, and stored at −80°C by a trained research nurse. The availability of research staff determined the frequency and timing of collection. Due to variability in sampling times for MI and NFL, samples were categorized into three temporal epochs: early (days 0–2), mid (days 3–6), and late (days 7–18). If multiple samples were collected within the same epoch for a given patient, the highest value was used for analysis. Daily venous blood sampling was also performed for routine clinical care.

Sample analysis

Study 1

Samples were transported on dry ice to the Swedish University of Agricultural Sciences in Umeå for blinded analysis. MI levels in serum, and in CSF when available, were quantified using a gas chromatography/mass spectroscopy method (see Supplemental Materials for details). Results are reported in µM.

Study 2

Samples were transported on dry ice to UmanDiagnostics® (Umeå, Sweden). In addition to serum, paired CSF samples from 20 patients (early and late time points, n=40) were analyzed. NFL was quantified using the UmanDiagnostics® NF-light™ enzyme-linked immunosorbent assay. Detailed methods are provided in the Supplemental Materials.

Study 3

Serum S100B was measured at the accredited clinical laboratory at Umeå University Hospital using a sandwich biotinylated monoclonal antibody and ruthenium complex–based electrochemiluminescence assay (ECLIA, Elecsys S100, Cobas 801, Roche Diagnostics Scandinavia). This assay became routine during the study period. For patients enrolled before its introduction, frozen research samples were thawed and analyzed retrospectively.

Statistical analysis

Analyses were performed using SPSS® (IBM®, version 28) or R (v4.4.2). All tests were two-tailed unless otherwise specified, with significance set at $P < .05$. Confidence intervals (CI) are presented at 95-percent range. Odds ratio (OR) are presented for logistic regression analyses. Standard deviation (SD) are presented for mean value distributions. Continuous variables were compared using the independent t-test (for parametric data distribution) or Mann–Whitney U test (for non-parametric data distribution). Categorical variables were compared with the χ^2 test. Correlations were examined using Spearman's rank correlation unless otherwise noted. Variables were log-transformed if deemed necessary. Study-specific analyses are described below.

Study 1

Temporal dynamics of MI were examined using two approaches: (1) linear regression for each patient, with the slope coefficient (B-value) as a marker of change over time; and (2) the ratio of late-to-early epoch MI levels (MI Ratio). Both MI-Ratio and B-value were compared between outcome groups using the Mann-Whitney U test. Serum and CSF levels of MI were compared using Spearman's rank correlation analysis. Associations between early MI and baseline parameters were evaluated with Spearman's rank correlation. Overall MI levels were compared between the three different epochs using the Mann-Whitney U test with Bonferroni corrected p-values. Outcome groups were compared using the Mann–Whitney U test for each epoch with Bonferroni corrected p-values. Logistic regression (univariate and multivariable) was performed with favourable outcome as the dependent variable. Receiver operation characteristic (ROC) analysis assessed the predictive capacity of MI levels on day 7. A subgroup analysis was performed using the Mann–Whitney U test to compare the MI-ratio between outcome groups among patients presenting with a favourable neurological condition upon admission (WFNS grades 1–3) and those presenting with a more severe neurological condition (WFNS grades 4–5).

Study 2

NFL values were correlated with clinical and outcome parameters using Spearman's rank test. Serum and CSF NFL levels were compared using Pearson's correlation. ANCOVA tested differences across treatment modalities. Repeated-measures mixed analysis of variance analysis (ANOVA) examined within-patient changes. NFL values were dichotomized into normal/high using reference ranges from Hviid et al.[182] The χ^2 test compared dichotomized NFL values between outcome groups. Late-epoch NFL, which correlated most strongly with GOSE, was selected for regression modeling. Values were log-transformed for interpretation. Logistic regression (univariate and multivariable) was performed, and ROC analysis assessed predictive ability.

Study 3

Associations between S100B and outcomes (mRS, EQ-5D index, EQ-VAS) were assessed with Spearman's correlation. Kruskal–Wallis testing compared S100B between favourable and unfavourable outcome groups on each day. ROC analysis determined predictive potential and optimal cut-off values. Logistic regression (univariate and multivariable) used unfavourable outcome as the dependent variable. The multivariable model incorporated clinically relevant predictors and S100B (dichotomized at the ROC-derived cut-off at 0.4 μg/l). Model fit was evaluated with Nagelkerke's R^2.

Study 4

Biomarker values with the strongest predictive capacity in prior analyses were selected: late-epoch MI and NFL (day 7–18) and peak S100B during hospitalization. Group comparisons were performed with the Mann–Whitney U test. NFL values were log-transformed (ln_NFL) for regression models. Missing data were multiply imputed (100 imputations per model). Logistic regression (univariate and multivariable with Firth's correction) was conducted with unfavourable outcome as the dependent variable. Model 1 included established predictors only; Models 2–4 sequentially added MI, ln_NFL, and S100B. Nagelkerke's R^2, area under the curve (AUC), and Akaike information criterion (AIC) were used to evaluate model performance.

Results

Study population

A total of 147 patients were assessed for inclusion. Eight patients declined participation. Nineteen patients had no, or non-aneurysmal source of bleeding. Thirty-two patients arrived when research staff were unavailable and therefore lacked frozen venous samples for later analysis. This resulted in a total of 88 patients included in Studies 1, 2, and 4 (Figure 6 left).

Since S100B analysis had been implemented as an in-hospital routine, some patients who could not have MI or NFL analyzed due to unavailability of research staff still had S100B levels measured. Seventeen patients arrived >48 hours of symptom onset. Eighteen patients lacked S100B-samples. Fourteen patients had no, or non-aneurysmal source of bleeding and 1 patient was excluded since the bleeding came from a previously treated aneurysm. This resulted in 89 patients included in study 3 (Figure 6 right).

Baseline characteristics for study 1,2 and 4 are summarized in Table 1, and for study 3 in Table 5. Outcome parameters are summarized in Table 3 for study 1,2 and 4 and in Table 6 for study 3. Characteristics of the excluded patients are summarized in Table 7.

In univariate binary regression analyses of baseline parameters from the cohort used in study 1,2 and 4 with favourable outcome as the dependent variable, age, WFNS score, and Fisher grade were statistically significant (Table 2).

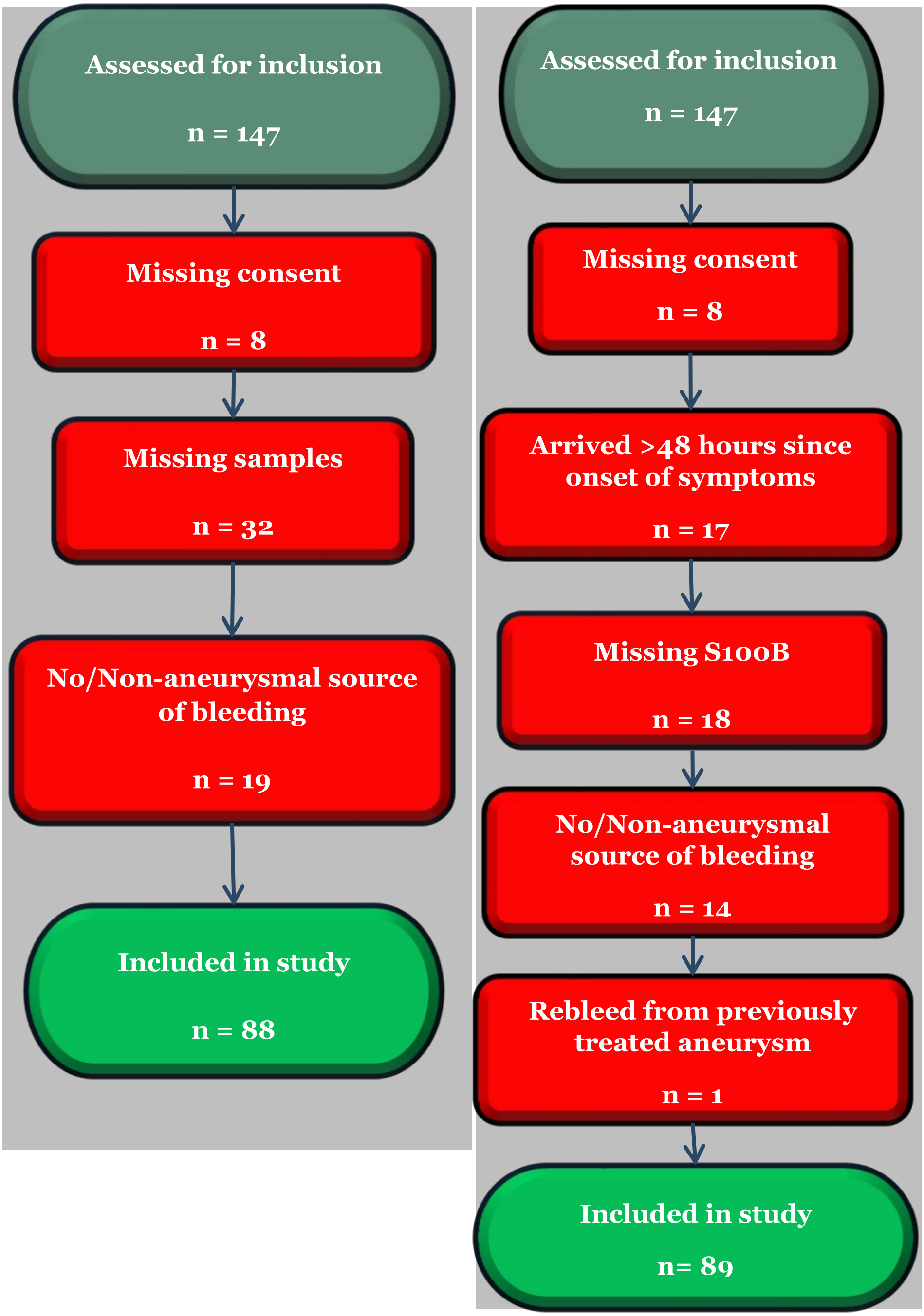

Figure 6. Flowchart for cohort selection in study 1,2 and 4 (left) and 3 (right).

Characteristics		**Outcome measured as dichotomized GOSE**			
		Total	Favourable	Unfavourable	p-value
Age (years)	Mean (±SD)	59±13	56±13	66±11	**.001**
Hypertension	Yes (%)	38 (43)	27 (44)	11 (42)	.915
Current smoking	Yes (%)	33 (38)	25 (40)	8 (31)	.398
Gender	Female (%)	66 (75)	43 (69)	23 (89)	.059
Multiple aneurysms	Yes (%)	16 (18)	8 (13)	8 (31)	**.047**
Aneurysm location	Posterior (%)	14(16)	8(13)	6(23)	.234
Aneurysm size (mm)	Mean(±SD)	6.8±3.8	6.5±3.6	7.5±4.3	.22
Fisher grade	(%)				**.025**
	1	1 (1)	1 (1)	0 (0)	
	2	5 (6)	5 (8)	0 (0)	
	3	21 (24)	19 (31)	2 (8)	
	4	61 (69)	37 (60)	24 (92)	
WFNS scale score	(%)				**<.0001**
	1	34 (41)	56	2 (8)	
	2	14 (20)	23	3 (12)	
	3	4 (6)	7	1 (4)	
	4	5 (16)	8	9 (35)	
	5	4 (17)	7	11 (42)	

Table 1. Baseline characteristics for the population used in study 1,2 and 4. GOSE = Glasgow outcome scale extended, WFNS = World federation of neurosurgical societies

Variable	**OR**	**CI**	**p-value**
Age	0.94	0.89-0.98	**.004**
WFNS	0.37	0.25-0.56	<**.001**
Fisher grade	0.15	0.03-0.65	**.011**
Multiple aneurysms	0.33	0.11-1.0	.054
Smoking	1.5	0.57-4.0	.40
Hypertension	1.0	0.42-2.7	.92
Aneurysm size	0.94	0.84-1.1	.30
Gender (Male)	3.4	0.91-12	.07

Table 2. Univariate binary logistic regression analyses for baseline variables using favourable outcome as dependent variable. WFNS = World federation of neurosurgical societies

Characteristics		**Outcome measured as dichotomized GOSE**			
		Total	Favourable	Unfavourable	p-value
GOSE	No. (%)				
	1	12 (14)	0 (0)	12 (46)	
	2	2 (2)	0 (0)	2 (8)	
	3	5 (6)	0 (0)	5 (19)	
	4	7 (8)	0 (0)	7 (27)	
	5	7 (8)	7 (11)	0 (0)	
	6	17 (19)	17 (27)	0 (0)	
	7	15 (17)	15 (24)	0 (0)	
	8	23 (26)	23 (37)	0 (0)	
Treatment modality	No. (%)				.092
	Endovascular	36 (41)	24 (39)	12(46)	
	Surgical	48 (55)	37 (60)	11 (42)	
	Combined	2 (2)	1 (2)	1 (4)	
	No treatment	2 (2)	0 (0)	2 (8)	
DCI	Yes (%)	9 (10)	6 (6)	3 (12)	.793
TCD ≥120cm/s	Yes (%)	27 (31)	21 (34)	6 (23)	.316
Angiographic Vasospasm	Yes (%)	27 (31)	20 (32)	7 (27)	.621
EVD	Yes (%)	43 (49)	23 (37)	20 (77)	**<.0001**
Shunt dependency	Yes (%)	28 (32)	15 (24)	13 (50)	**.018**

Table 3. Outcome parameters for the population in study 1,2 and 4. GOSE = Glasgow outcome scale extended, DCI = Delayed cerebral ischemia, TCD = Transcranial doppler sonography, EVD = External ventricular drainage

Variable	**OR**	**CI**	**p-value**
Treatment modality	0.78	0.39-1.6	.50
DCI	0.82	0.19-3.6	.79
TCD ≥120cm/s	1.7	0.60-4.9	.32
Angiographic vasospasm	1.3	0.47-3.6	.62
EVD	0.18	0.06-0.50	**.001**
Shunt dependency	0.30	0.11-0.78	**.014**

Table 4. Univariate binary logistic regression analyses for treatment modality and adverse events using favourable outcome as dependent variable.

Characteristics		**Outcome measured as dichotomized mRS**			
		Total	Favourable	Unfavourable	p-value
Age (years)	Mean (±SD)	59±13	57±12	65±12	**.006**
Hypertension	Yes (%)	39 (44)	26 (41)	13 (52)	.480
Current smoking	Yes (%)	33 (38)	24 (38)	13 (52)	.087
Gender	Female (%)	64 (72)	45 (70)	19 (76)	.794
Multiple aneurysms	Yes (%)	20 (22)	10 (16)	10 (40)	**.0022**
Aneurysm location	Posterior (%)	14(16)	8(13)	6(24)	.345
Aneurysm size (mm)	Mean(±SD)	7.0±4.7	6.4±4.2	8.3±5.5	.101
Fisher grade	(%)				**<.001**
	1	0(0)	0 (0)	0 (0)	
	2	1 (1)	1 (2)	0 (0)	
	3	22 (26)	22 (34)	0 (0)	
	4	66 (73)	41 (64)	25 (100)	
HH scale score	(%)				**<.001**
	1	9 (10)	8 (13)	1 (4)	
	2	29 (33)	29 (45)	0 (0)	
	3	17 (19)	15 (23)	2 (8)	
	4	16 (18)	7 (11)	9 (36)	
	5	7 (8)	0 (0)	7 (28)	
	Missing	11(12)	5 (8)	6 (24)	

Table 5. Baseline characteristics for the cohort in study 3. mRS = Modified Rankin scale, HH = Hunt and Hess scale

Characteristics		**Outcome measured as dichotomized mRS**			
		Total	Favourable	Unfavourable	p-value
mRS	No. (%)				
	0	33 (37)	33 (52)	0	
	1	15 (17)	15 (23)	0	
	2	7 (8)	7 (11)	0	
	3	9 (10)	9 (14)	0	
	4	10 (11)	0	10 (40)	
	5	3 (3)	0	3 (12)	
	6	12 (14)	0	12 (48)	
Treatment modality	No. (%)				**.011**
	Endovascular	34 (38)	22 (34)	12 (48)	
	Surgical	48 (54)	40 (63)	8 (32)	
	Combined	2 (2)	1 (2)	1 (4)	
	No treatment	5 (6)	1 (2)	4 (16)	
DCI	Yes (%)	9 (10)	7 (11)	2 (8)	.68
TCD ≥120cm/s	Yes (%)	29 (33)	23 (36)	6 (24)	.28
Angiographic Vasospasm	Yes (%)	27 (30)	19 (30)	8 (32)	.831
EVD	Yes (%)	48 (54)	29 (45)	19 (76)	**.009**
Shunt dependency	Yes (%)	28(32)	18 (28)	10 (40)	.176

Table 6. Outcome parameters for the population in study 3. mRS = Modified Rankin Scale, DCI = Delayed cerebral ischemia, TCD = Transcranial doppler sonography, EVD = External ventricular drainage

Characteristics		Outcome measured as dichotomized GOSE			
		Total	Favourable	Unfavourable	p-value
Age (years)	Mean (SD)	58(16)	56 (11)	66 (10)	**.005**
Hypertension	Yes (%)	26 (44)	14 (40)	11 (69)	.057
Current smoking	Yes (%)	20 (34)	11 (31)	5 (31)	.99
Gender	Female (%)	39 (66)	25 (71)	9 (56)	.286
Multiple aneurysms	Yes (%)	3 (5)	2 (6)	1 (6)	.841
Aneurysm location	Posterior (%)	4 (7)	1 (3)	2 (13)	.073
Aneurysm size (mm)	Mean (SD)	8.8(6.6)	6.1 (4.2)	14 (7.3)	**<.001**
Fisher grade	(%)				**.003**
	1	4 (7)	3 (9)	0 (0)	
	2	2 (3)	2 (6)	0 (0)	
	3	23 (39)	20 (57)	2 (13)	
	4	27 (46)	10 (29)	13 (81)	
	Missing	3 (5)	0 (0)	1 (6)	
WFNS scale score	(%)				**<.001**
	1	30 (51)	26 (74)	2 (13)	
	2	7 (12)	5 (14)	1 (6)	
	3	3 (5)	0 (0)	2 (13)	
	4	8 (14)	3 (9)	5 (32)	
	5	9 (15)	1 (3)	5 (32)	
	Missing	2 (3)	0 (0)	1 (6)	
GOSE	No. (%)				
	1	9 (15)		9 (56)	
	2	1 (2)		1 (6)	
	3	0 (0)		0	
	4	6 (10)		6 (38)	
	5	0 (0)	0 (0)		
	6	7 (12)	7 (20)		
	7	13 (22)	13 (37)		
	8	15 (25)	15 (43)		
	Missing	8 (14)			
Treatment modality	No. (%)				.835
	Endovascular	6(10)	1 (3)	1 (6)	
	Surgical	23 (39)	15 (43)	6 (38)	
	Combined	1 (2)	1 (3)	0 (0)	
	No treatment	29 (49)	18 (52)	9 (56)	
TCD ≥120 cm/s	Yes (%)	5 (9)	4 (11)	2 (13)	.159
Angiographic Vasospasm		7 (12)	5 (14)	2 (13)	.863
DCI		2 (3)	2 (6)	0(0)	.329
EVD		17 (30)	7 (20)	8 (50	**.029**
Shunt dependency		6 (10)	2 (6)	4 (25)	**.027**

Table 7. Characteristics of excluded patients. WFNS = World federation of neurosurgical societies, GOSE = Glasgow outcome scale extended, DCI = Delayed cerebral ischemia, EVD = External ventricular drain

Outcome

Twelve patients had died at the one-year follow-up, corresponding to a mortality rate of 14%. Twenty-six patients (30%) had an unfavourable outcome, defined as a GOSE score of 1–4 in studies 1, 2, and 4, while 25 patients (28%) had an unfavourable outcome, defined as an mRS score of 3–6 in Study 3. When dichotomized into favourable and unfavourable outcome groups, the mRS and GOSE demonstrated comparable classification (Table 8). Mean EQ-5D$_{index}$ in the favourable outcome group (GOSE 5-8) was 0.77 (SD 0.25) and 0.13 (SD 0.23) in the unfavourable outcome group. Both the GOSE (ρ = 0.84, p < .001) and the mRS (ρ = −0.85, p < .001) were significantly correlated with the EQ-5D$_{index}$ (Figure 7).

Scale	**Favourable outcome**	**Unfavourable outcome**
Modified Rankin Scale	65	23
Glasgow Outcome Scale Extended	62	26

Table 8. Distribution of dichotomized outcome according to different outcome scales. Favourable outcome = Modified Rankin Scale 0-3, Glasgow outcome scale extended 5-8. Unfavourable outcome = Modified Rankin Scale 4-6, Glasgow Outcome Scale Extended 1-4.

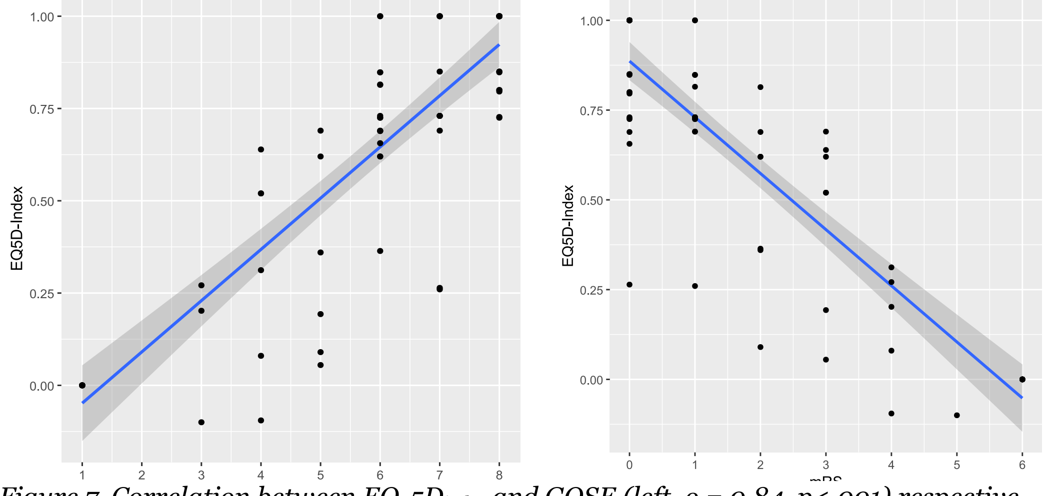

Figure 7. Correlation between EQ-5D$_{index}$ and GOSE (left, ρ = 0.84, p<.001) respective mRS (right, ρ = -0.85, p<.001). EQ5D-Index = EuroQoL 5D Index. GOSE = Glasgow Outcome Scale Extended. mRS = Modified Rankin Scale.

Study 1

The mean MI level at admission was 23.8 μM (SD 12.6) for the entire cohort. Among baseline factors, early MI levels demonstrated a significant correlation only with age ($\rho = 0.32$, $p = .003$). A significant correlation between CSF and serum MI levels was also observed ($\rho = 0.26$, $p = .002$) (Figure 8). Negative correlations between MI levels and time were found for the entire cohort ($\rho = -0.37$, $p < .001$), as well as within the favourable ($\rho = -0.24$, $p < .001$) and unfavourable ($\rho = -0.61$, $p < .001$) outcome groups (Figure 9). Distribution of sample frequency can be seen in Table 11.

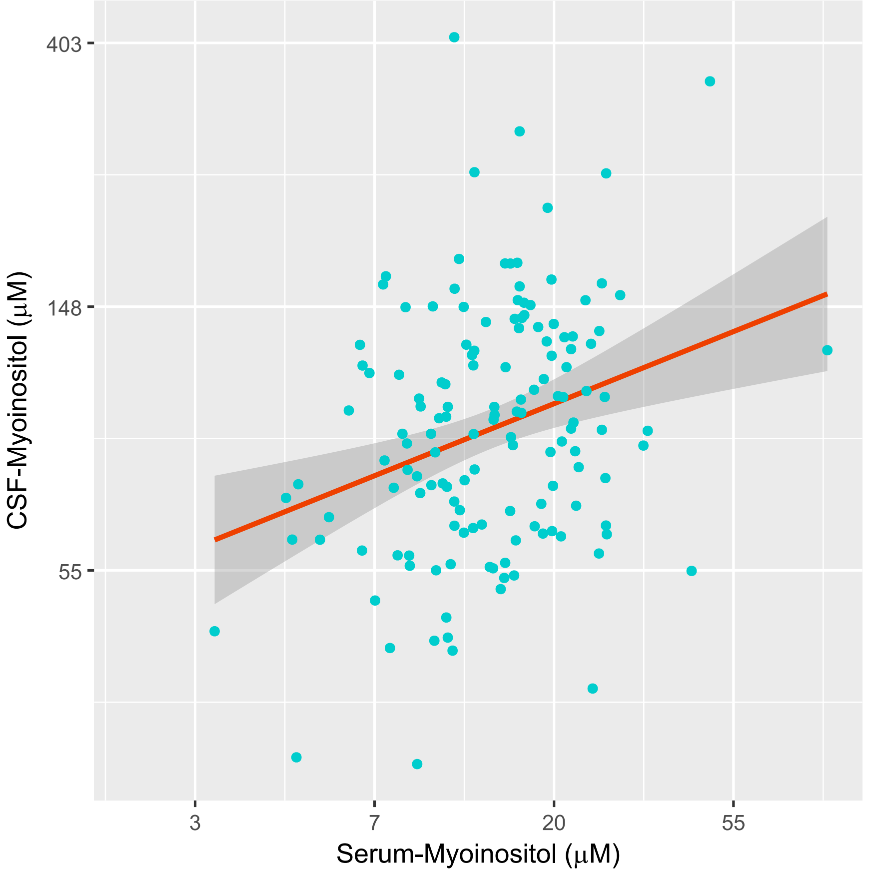

Figure 8. Relation between Myo-Inositol levels in serum and cerebrospinal fluid (CSF). Scales are logarithmic. ρ =0.26, p=.002.

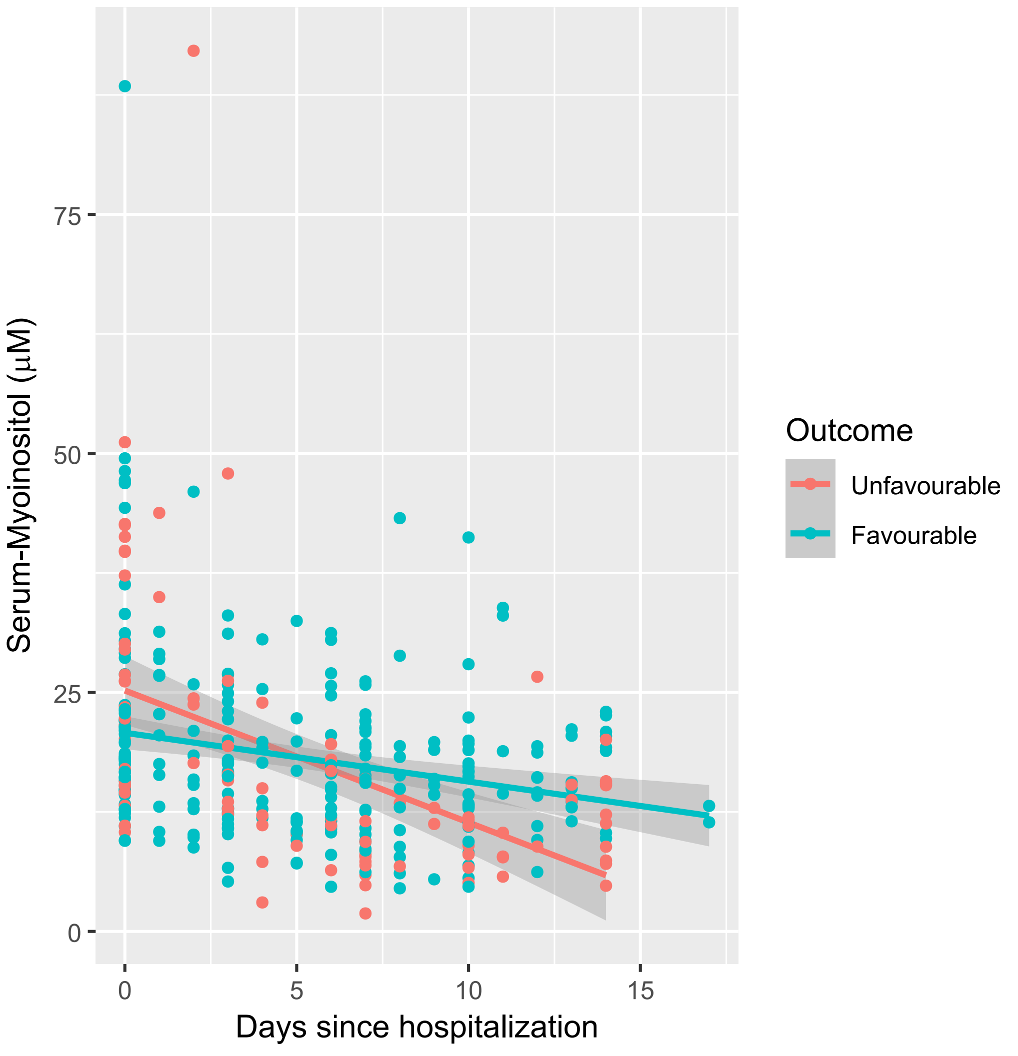

Figure 9. Relation between serum levels of MI and time since hospitalization for unfavourable (ρ =-0.61, p<.001) and favourable (ρ =-0.24, p<.001) outcome groups.

When comparing median MI levels across the early, mid, and late epochs, significantly lower values were observed in the late epoch compared to the early epoch ($p<.001$) and mid-epoch compared to early epoch ($p<.001$) but no difference between late and mid epoch ($p=.168$). A statistically significant difference in MI levels between outcome groups was evident during the late epoch ($p< .001$), but not in the earlier epochs (Figure 10).

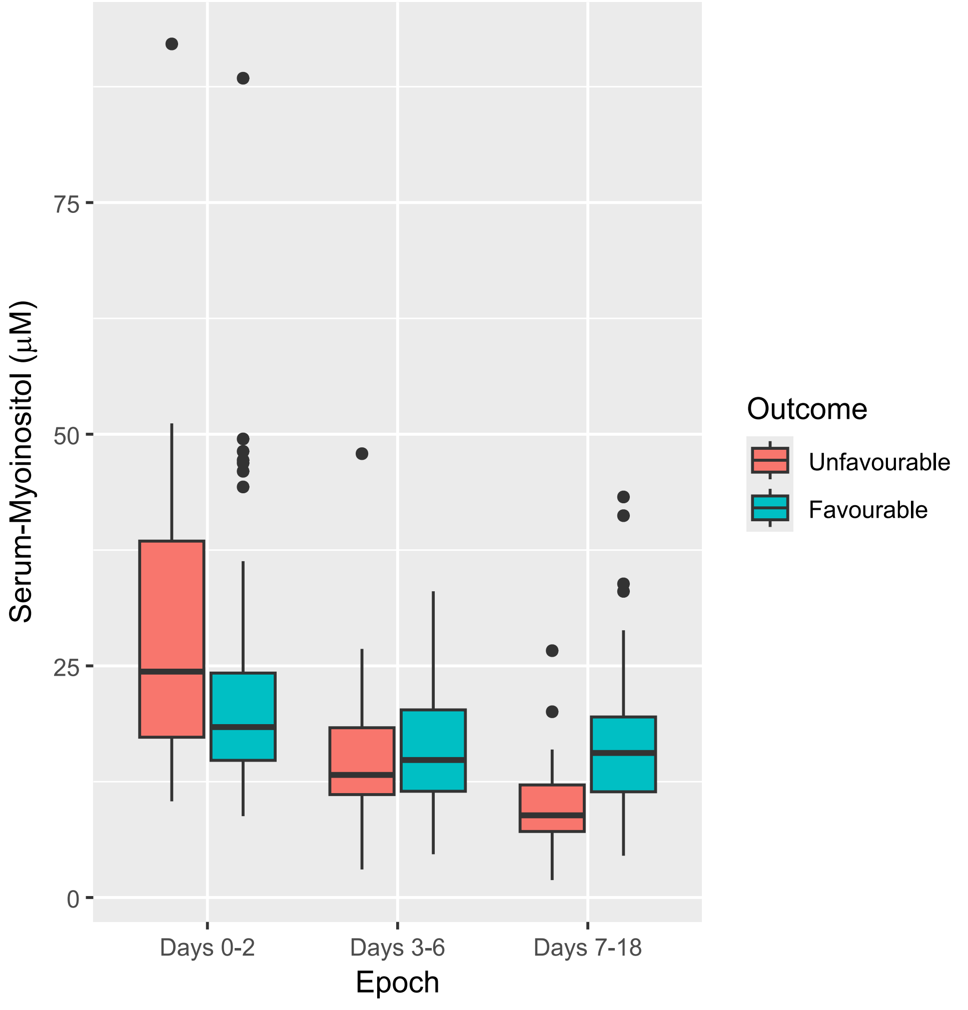

Figure 10. Boxplot showing the distribution of serum Myo-Inositol levels among different epochs.

Both the B-value and MI ratio were significantly correlated with dichotomized GOSE (ρ = 0.40 and 0.48, respectively; $p < .001$ for both) (Figure 11).

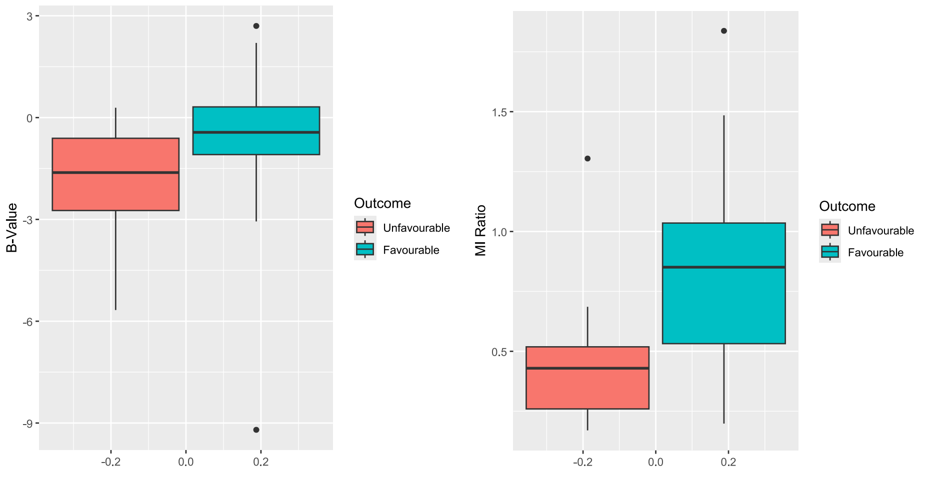

Figure 11. Differences in B-value (individual linear regression coefficient) and MI-ratio (late/early epoch values) between outcome groups.

In univariate binary logistic regression analysis, both the B-value and MI ratio were significant predictors. In multivariate regression models that included the significant variables from the univariate analysis (age, WFNS score, Fisher score) along with either the B-value or MI ratio, all variables except the Fisher score remained significant (Table 9).

Parameter	*OR*	*CI (95%)*	*p-value*
Univariate			
B-value	1.68	1.2-2.4	**.006**
MI-ratio	60	5.9-617	**<.001**
Multivariable			
B-value	1.5	1.0-2.3	**0.04**
WFNS	0.34	0.20-0.60	**<.001**
Age	0.90	0.84-0.98	**.008**
Fisher	0.30	0.041-2.1	0.23
Multivariable			
MI-ratio	21	1.2-380	**.036**
WFNS	0.34	0.18-0.63	**<.001**
Age	0.90	0.83-0.97	**.009**
Fisher	0.53	0.065-4.3	0.55

Table 9. Univariate and multivariable regression models using B-value and MI-ratio for predicting favourable outcome.

A ROC curve using late-epoch MI levels as a predictor yielded an AUC of 0.78 (95% CI, 0.68–0.89; p < .0001) for predicting favourable outcome (Figure 12).

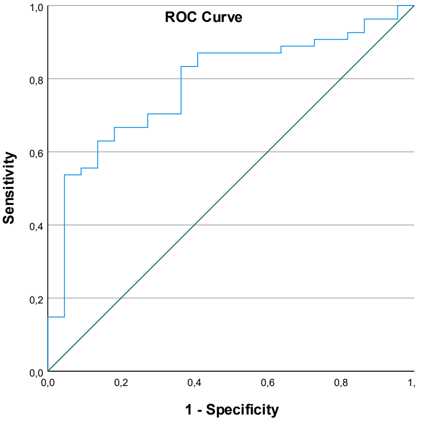

Figure 12. Discriminative properties of late epoch Myo-Inositol levels to predict favourable outcome.

In a subgroup analysis, patients presenting with a more favourable neurological condition upon admission (WFNS grades 1–3) showed a significant difference in MI-ratio between outcome groups (p = .019). This finding could not be replicated among patients with a poorer neurological condition upon admission (WFNS grades 4–5; p = .27, Table 10).

	Median MI-Ratio (Min-Max)			
	Total	Favourable	Unfavourable	p-value
WFNS 1-3 (n=58)	0.78 (0.24-1.8)	0.85 (0.24-1.8)	0.38 (0.26-0.62)	**.019**
WFNS 4-5 (n=29)	0.45 (0.17-1.3)	0.62 (0.2-1.2)	0.43 (0.17-1.3)	.269

Table 10. Subgroup analysis of patients' neurological status upon admission and the temporal changes in MI levels. MI = Myo-Inositol; MI-ratio = late epoch MI value / early epoch MI value; WFNS = World federation of neurosurgical societies.

Time (Day)	*n*		
	Total	*Favourable*	*Unfavourable*
0	85	60	25
1	15	13	2
2	15	11	4
3	39	29	10
4	14	8	6
5	15	13	2
6	27	21	6
7	37	25	12
8	14	12	2
9	9	7	2
10	32	24	8
11	8	4	4
12	11	9	2
13	10	8	2
14	18	8	10
15	0	0	0
16	0	0	0
17	2	2	0

Table 11. Frequency of venous samples taken on different days for Myo-Inositol and Neurofilament-light analysis.

Study 2

The distribution of sample frequency can be seen in table 6. Mean NFL levels upon admission were 39.6 pg/mL (SD 62.3) for the entire cohort. When comparing early serum NFL values with baseline factors, significant correlations were observed with age ($\rho = 0.274$, $p = .01$), WFNS score ($\rho = 0.508$, $p < .0001$), and Fisher grade ($\rho = 0.233$, $p = .03$). Serum and CSF levels of NFL were strongly correlated (Pearson's $r = 0.7$, $p < .0001$) (Figure 13).

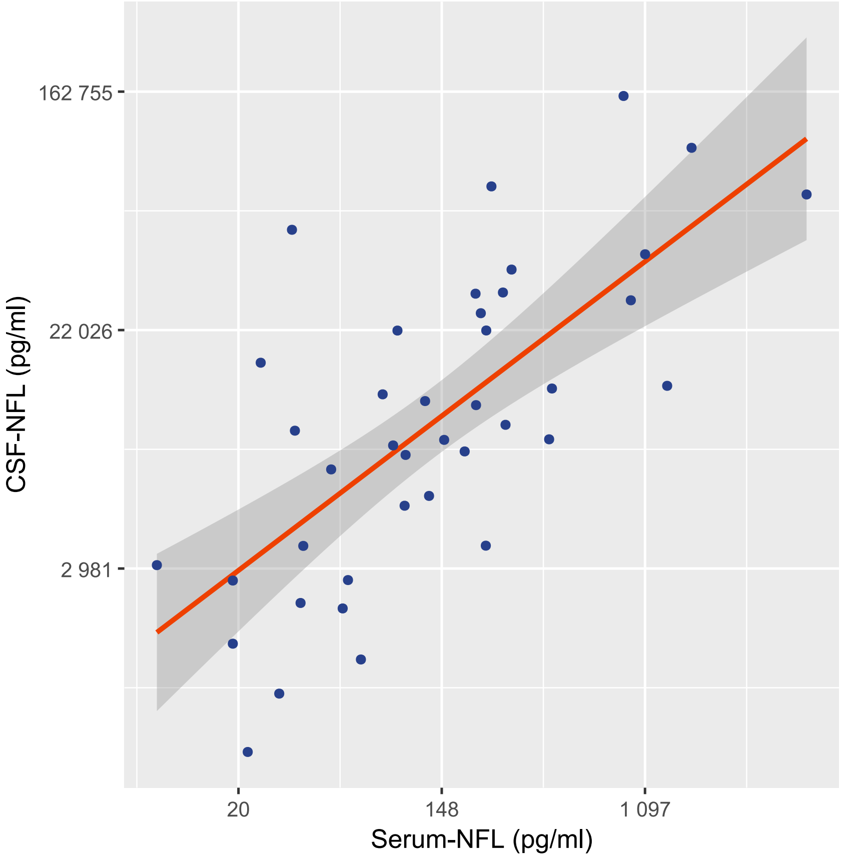

Figure 13. Relation between serum and cerebrospinal fluid (CSF) Neurofilament-light chain levels. Scales are logarithmic. Pearson's correlation coefficient = 0.7, p<.0001.

NFL levels increased across epochs. A mixed repeated-measures ANOVA revealed no significant interaction effect ($F(2, 126) = 2.123$, $p = 0.12$), but there were significant differences in NFL levels between outcome groups ($F(1, 63) = 21.76$, $p < .0001$, $\eta^2 = 0.201$) as well as across epochs ($F(1.81, 113.95) = 184.6$, $p < .0001$, $\eta^2 = 0.441$). Post-hoc Bonferroni tests indicated statistically significant differences in NFL levels between outcome groups in all epochs (Figure 14). Negative correlations between NFL and favourable outcome were observed in all epochs, strongest during the late epoch ($\rho = -0.529$, $p < 0.0001$). A higher proportion of the unfavourable outcome group had NFL levels above the reference range (80%) compared to the favourable outcome group (47%) in the early epoch ($p = 0.005$).[182] In the mid-epoch, only three patients (4%) had NFL values within the reference range, and in the late phase, all patients exceeded reference values.

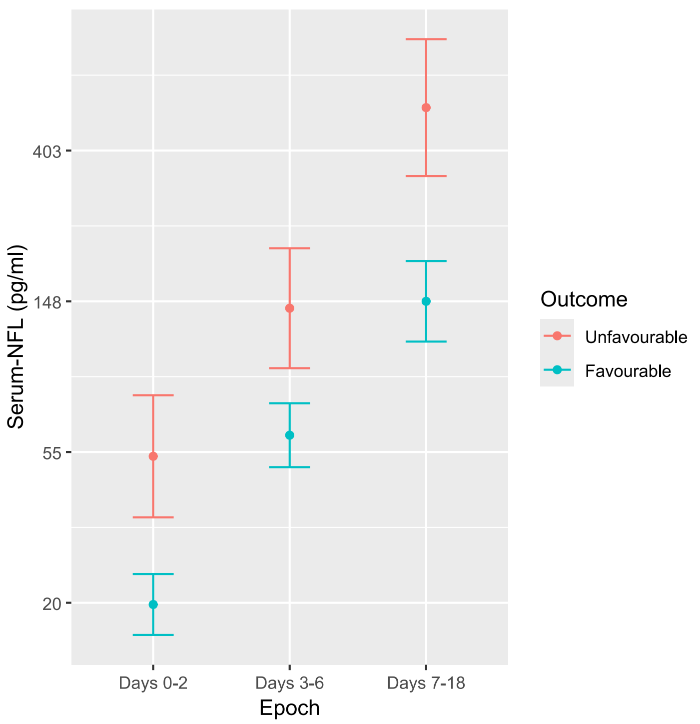

Figure 14. Mean levels of Neurofilament-light chain between outcome groups in different epochs. Bars represent 95-percent CI of the mean. The Y-axis utilizes a logarithmic scale to ease interpretation.

A ROC curve using late-epoch NFL values to predict unfavourable outcome was significant (AUC = 0.84, 95% CI, 0.75–0.94; p < .0001) (Figure 15). In a univariate regression model, late-epoch ln_NFL predicted favourable outcome with an OR of 0.33 (95% CI, 0.18–0.55; p < .0001). A multivariable regression model including late-epoch ln_NFL, WFNS score, Fisher grade, and age demonstrated significance for all variables except Fisher grade (Table 12).

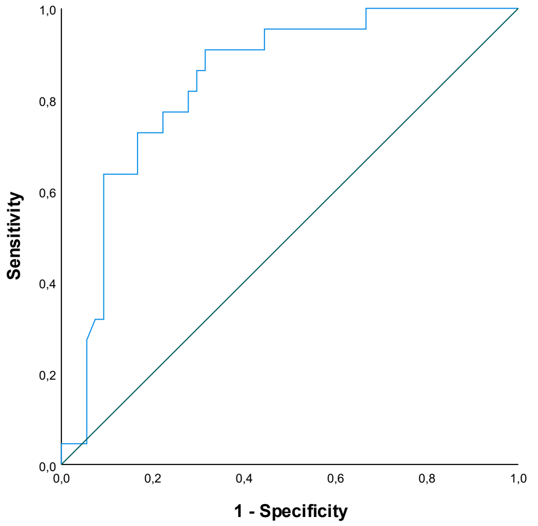

Figure 15. ROC curve using late epoch NFL levels to predict unfavourable outcome.

Parameter	*OR*	*CI (95%)*	*p-value*
Univariate			
ln_NFL (Late epoch)	0.33	0.18-0.56	**<.001**
Multivariable			
ln_NFL (Late epoch)	0.39	0.19-0.79	**.009**
WFNS	0.41	0.24-0.71	**.001**
Age	0.90	0.84-0.97	**.006**
Fisher	0.35	0.068-1.8	0.215

Table 12. Univariate and Multivariable binary logistic regression model using favourable outcome as dependent variable. NFL = Neurofilament light chain, WFNS = World federation of neurosurgical societies.

Study 3

The mean EQ-5D$_{index}$ was 0.58 ± 0.38, and the mean EQ-VAS was 72.1 ± 21.8 (Table 13). S100B levels at several time points were significantly correlated with the EQ-5D$_{index}$ and mRS, but not with EQ-VAS. The peak S100B value demonstrated the strongest negative correlation (Table 14).

EQ-5D outcome at 1-year follow-up	
Mobility, n (%)	
No problems	49(74)
Moderate problems	15(23)
Severe problems	2(3)
Self-care, n (%)	
No problems	53(80)
Moderate problems	8(12)
Severe problems	5(8)
Usual activities, n (%)	
No problems	44(67)
Moderate problems	14(21)
Severe problems	8(12)
Pain/discomfort, n (%)	
No problems	33(50)
Moderate problems	27(41)
Severe problems	6(9)
Anxiety/depression, n (%)	
No problems	35(53)
Moderate problems	30(45)
Severe problems	1(2)
EQ-5D index, mean ±SD	0.58 ±21.8
EQ-VAS, mean ±SD	72±21.8

Table 13. Summary of Euro QoL-5D answers at 1 year follow-up.

	D0	D1	D2	Mean D0-10	Peak	Dich Peak
mRS						
Rho	0.46*	0.46*	0.40**	0.53*	0.57*	0.62*
n	65	59	58	89	89	89
EQ-5D$_{index}$						
Rho	-0.39**	-0.46**	-0.25	-0.42*	-0.49*	-0.52*
n	57	52	49	76	78	78
EQ-VAS						
Rho	-0.05	-0.16	-0.08	-0.12	-0.19	-0.16
n	45	45	49	66	66	66

*Table 14. Spearman correlation between S100B levels at different times and outcome parameters. D=Day. Dich = dichotomized at 0.4 µg/l. *Significant at p≤.0001, **significant at p≤.001.*

S100B levels tended to peak during the first three days, and significant differences in median values between outcome groups were observed on days 0-5 and 7 (Figure 16, Table 15).

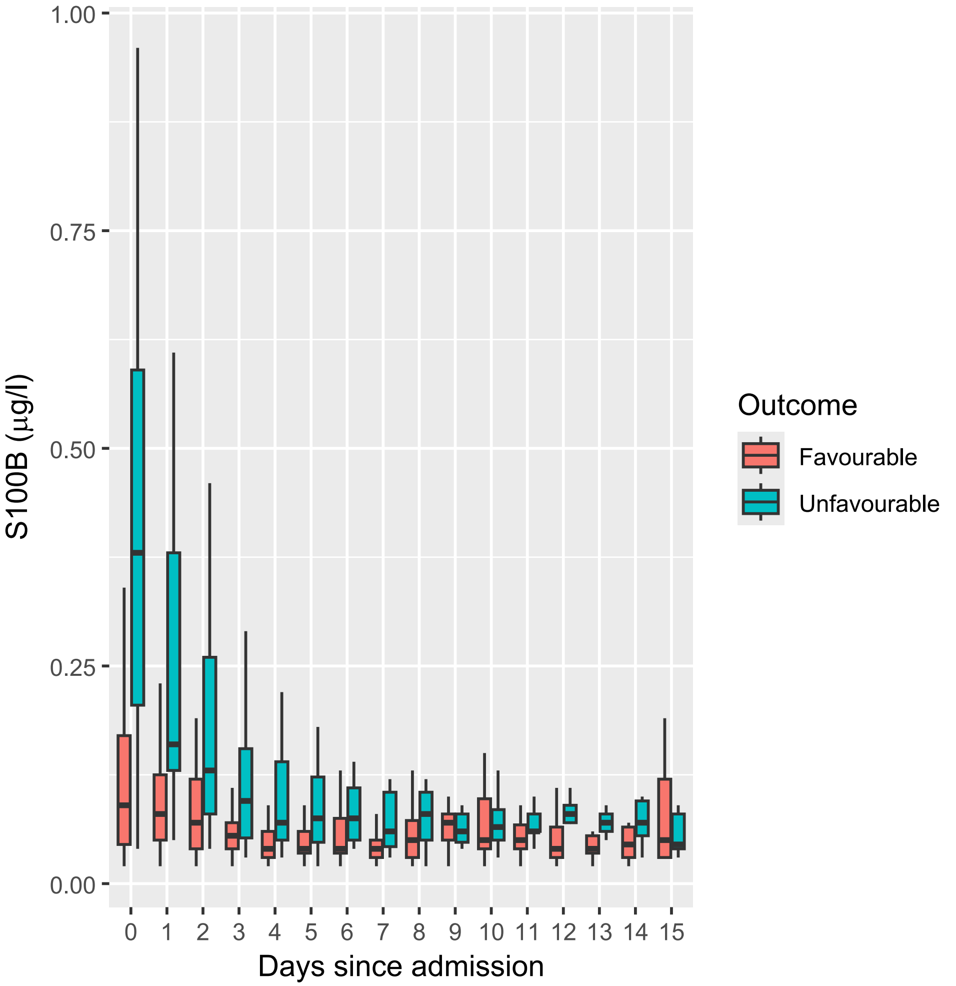

Figure 16. Boxplot showing daily distribution of S100B levels for the favourable and unfavourable outcome group.

Time (day)	Median S100B (µg/l)		n (favourable/unfavourable)		p-value
	Favourable	Unfavourable			
0	0.09	0.44	43	22	**<.001**
1	0.08	0.17	39	20	**<.001**
2	0.08	0.14	38	20	**.006**
3	0.06	0.10	41	17	**.004**
4	0.04	0.07	31	18	**.004**
5	0.04	0.08	33	15	**.029**
6	0.04	0.08	27	14	.085
7	0.04	0.06	30	14	**.021**
8	0.05	0.08	33	15	.069
9	0.07	0.06	21	12	.792
10	0.05	0.07	22	12	.598
11	0.13	0.06	2	3	.288
12	0.11	0.08	2	3	.065
13	0.10	0.08	2	3	.090
14	0.12	0.06	2	3	.193
15	0.16	0.09	2	3	1.00

Table 15. Median differences in S100B between outcome groups and frequency of sampling. Significant differences are bold.

A ROC curve was generated using peak S100B, which exhibited the strongest negative correlation with mRS, to predict unfavourable outcome. The AUC was 0.90 (95% CI 0.83–0.97, p < .0001) (Figure 17).

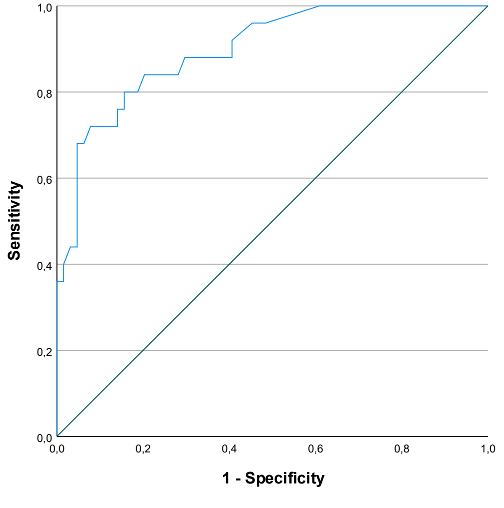

Figure 17. ROC curve describing the discriminative properties of Peak S100B value to predict unfavourable outcome (modified Rankin Scale 4-6). Area under the curve 0.90 (95% CI 0.83–0.97, p < .0001)

Univariate binary logistic regression models were constructed for variables considered relevant. The multivariable model was built using variables that were significant in univariate analysis, with the addition of aneurysm location, which was considered clinically relevant (Table 16).

	Variable	n	OR (95% CI)	R^2	p-value
Univariate					
	HH	78	7.1 (2.9-17)	0.56	**<.0001**
	Age	89	1.06 (1.0-1.1)	0.12	**0.01**
	Multiple Aneurysms	89	3.5 (1.2-10)	0.090	**0.02**
	Aneurysm location	89	2.2 (0.66-7.1)	0.019	0.20
	Fisher score	89	N/A	0.27	1.0
	Hypertension	89	1.6 (0.63-4.0)	0.011	0.333
	BMI	82	0.97 (0.89-1.1)	0.010	0.553
	Ongoing smoking	86	2.4 (0.90-6.5)	0.052	0.082
	Dich S100B	89	43 (10-180)	0.51	**<.0001**
	Peak S100B	89	1030 (42-25000)	0.56	**<.0001**
	Mean S100B D0-10	89	75000 (128-N/A)	0.43	**<.0001**
Multivariable		78		0.90	
	HH				0.06
	Age				0.08
	Multiple Aneurysms				0.08
	Aneurysm location				0.18
	Dichotomized S100B				**0.05**

Table 16. Univariate and multivariable binary logistic regression models for predicting unfavourable outcome (modified Rankin Scale 4-6). HH = Hunt and Hess, BMI = Body mass index.

Study 4

Significant differences between outcome groups were observed for all three selected biomarkers (Table 17).

Parameter		Favourable	Unfavourable	p-value
MI Day ≥7 (µM)	median (IQR)	15.1 (8.34)	7.95 (4.62)	**<.001**
NFL Day ≥7 (pg/ml)	median (IQR)	135 (152)	574 (1180)	**<.001**
Peak S100B (µg/l)	median (IQR)	0.10 (0.118)	0.49 (0.558)	**<.001**

Table 17. Comparison of the biomarkers used in the regression models. MI = Myo-Inositol, NFL = Neurofilament light chain

Among commonly recognized predictors, age, WFNS score, and Fisher grade were statistically significant in univariate binary regression models and were therefore selected as the base variables for Model 1. MI, NFL, and S100B were all statistically significant in univariate regression analyses (Table 18).

Parameter	*OR*	*CI (95%)*	*p-value*
Age	1.07	1.03-1.12	**.004**
WFNS	2.67	1.85-4.14	**<.001**
Fisher grade	6.74	1.98-42.9	**.011**
Multiple aneurysms	3.00	0.97-9.33	.054
Aneurysm location	2.02	0.60-6-57	.240
Aneurysm size	1.06	0.94-1.20	.301
Hypertension	0.95	0.37-2.39	.914
MI	0.79	0.68-0.89	**<.001**
ln_NFL	3.07	1.83-5.70	**<.001**
S100B	650.1	34.8-27174	**<.001**

Table 18. Univariate binary logistic regression model using unfavourable (GOSE 1-4) outcome as independent variable.

In Model 1, all variables except Fisher grade remained statistically significant. Model 2 incorporated MI into Model 1; in this model, age and WFNS score remained statistically significant, whereas MI approached significance with a p-value of 0.051. Model 3 added NFL to the variables from Model 2, resulting in all variables except MI remaining statistically significant. In Model 4, S100B was added to the variables from Model 3; in this model, only age and WFNS score remained statistically significant (Table 19).

Model 1				**Model 2**			
Nagelkerke R^2: 0.595 AUC: 0.91 (0.85-0.97)				Nagelkerke R^2: 0.642 AUC: 0.93 (0.87-0.98)			
Parameter	*OR*	*CI (95%)*	*p-value*	*Parameter*	*OR*	*CI (95%)*	*p-value*
Age	1.09	1.03-1.16	**.004**	Age	1.10	1.03-1.17	**.004**
WFNS	2.46	1.57-3.86	**<.001**	WFNS	2.08	1.29-3.36	**.003**
Fisher grade	3.00	0.64-14.04	0.164	Fisher grade	2.27	0.50-10.39	.292
				MI	0.85	0.72-1.00	.051
Model 3				**Model 4**			
Nagelkerke R^2: 0.719 AUC: 0.95 (0.90-0.99)				Nagelkerke R^2: 0.762 AUC: 0.96 (0.91-1.00)			
Parameter	*OR*	*CI (95%)*	*p-value*	*Parameter*	*OR*	*CI (95%)*	*p-value*
Age	1.09	1.02-1.17	**.007**	Age	1.09	1.02-1.17	**.012**
WFNS	1.84	1.09-3.13	**.023**	WFNS	2.02	1.09-3.74	**.026**
Fisher grade	2.41	0.53-10.85	.253	Fisher grade	1.73	0.29-10.17	.544
MI	0.83	0.69-1.01	.058	MI	0.84	0.68-1.03	.098
ln_NFL	2.46	1.24-4.89	**.010**	ln_NFL	1.73	0.78-3.91	.170
				S100B	18.97	0.12-3069	.257

Table 19. Multivariable binary logistic regression models for using unfavourable outcome (Glasgow outcome scale extended 1-4) as dependent variable. WFNS = World federation of neurosurgical societies, MI = Myo-Inositol, NFL = Neurofilament light-chain

With each variable added to Model 1, both the Nagelkerke R^2 and the AUC increased (Figures 18–20).

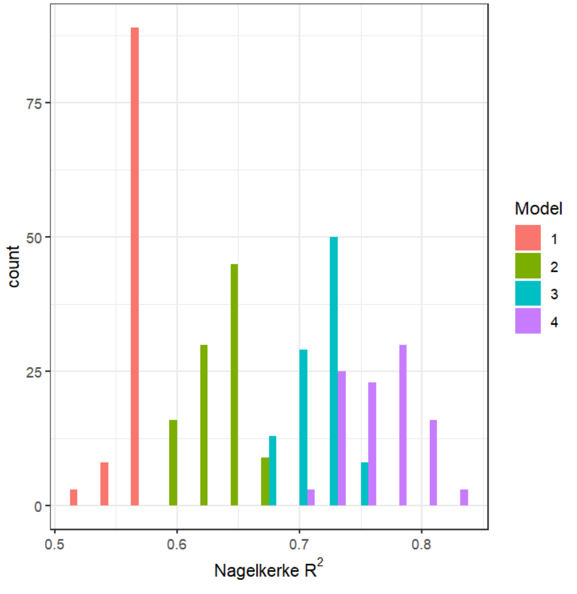

Figure 18. Histogram shows the distribution of the imputed models Nagelkerke R^2 values.

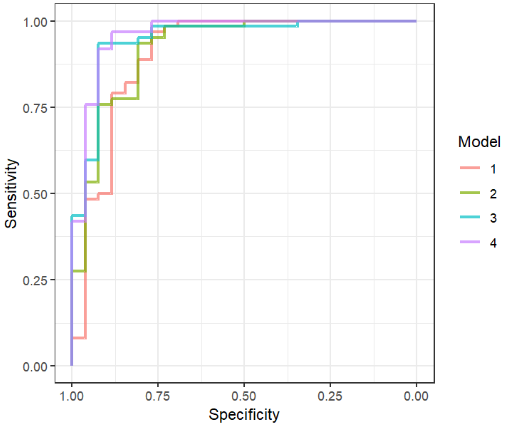

Figure 19. ROC curves for each model's performance in discriminating unfavourable outcome.

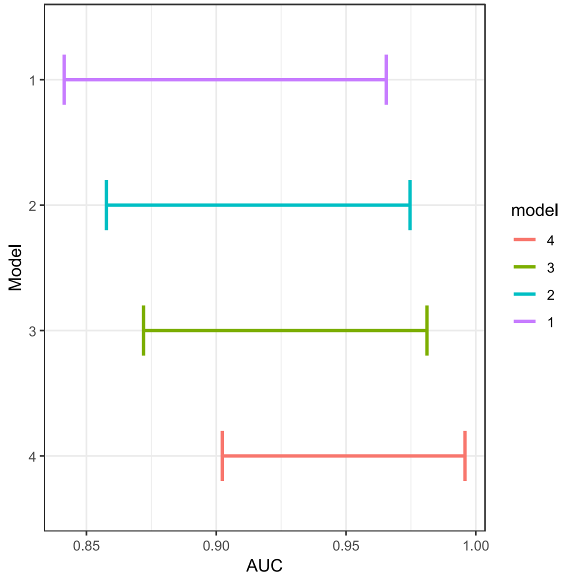

Figure 20. 95% confidence intervals of AUC of each model in ROC analysis.

The Akaike information criterion decreased for each variable added, reaching its lowest value in Model 4, suggesting that this model had the lowest prediction error (Figure 21).

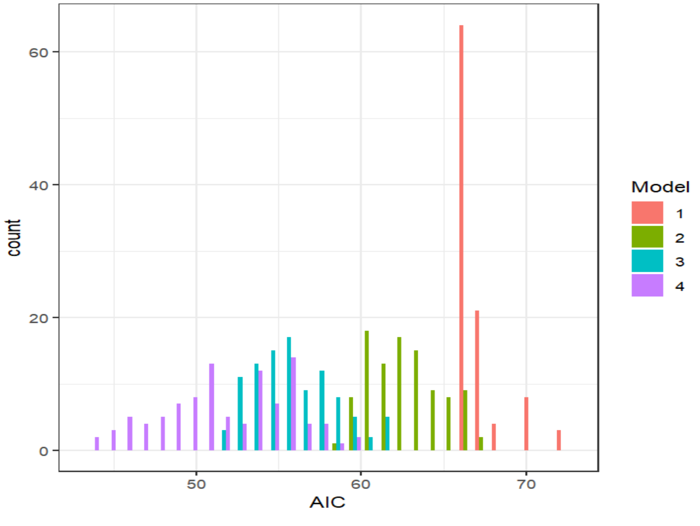

Figure 21. Histogram showing the distribution of the imputed models Akaike information criterion value. Lower value means less prediction error.

Discussion

The identification of biological markers associated with specific clinical trajectories is important for several reasons, including prognostication, guiding therapeutic interventions, and providing insight into underlying molecular mechanisms.

This thesis explored three such candidate biomarkers— MI, NFL, and S100B —and examined their associations with long-term functional outcomes in a cohort of patients with SAH.

Although the sample size was limited and the findings require replication in larger, independent cohorts, the results presented in this thesis are promising. Both the individual analyses of each biomarker and the combined multivariable models incorporating all three markers demonstrated predictive value, supporting the need for further investigation of these biomarkers in future studies.

Study 1

We successfully reproduced the previous findings regarding the association of MI and outcome in SAH in a hypothesis-driven study.[163]

MI levels at admission were in the lower range of previously reported values for the general population, suggesting that the initial haemorrhage has minimal impact on baseline MI levels.[170,171] There was no correlation between initial MI levels and clinical severity, as measured by WFNS score, indicating that MI levels are not affected by EBI. In the late epoch, MI levels were significantly different between outcome groups. Both late epoch MI levels and the rate of decline (measured as MI-ratio or B-value) were associated with functional outcome at one-year follow-up. MI levels declined over time in both outcome groups, but the decline was steeper in the unfavourable outcome group. These findings suggest that the decline in MI levels reflects metabolic processes occurring during the acute phase after SAH that contributes to total brain injury that takes the expression of functional deficits.

It is reasonable to assume that, among patients presenting with a more favourable neurological condition at disease onset but who ultimately experience an unfavourable outcome, secondary injuries such as DCI contribute more substantially to the overall extent of brain injury than EBI. In this subgroup (WFNS grades 1–3), the MI-ratio—representing the change in MI levels over time—was significantly lower in the unfavourable outcome group. Conversely, among patients presenting with severe neurological impairment at onset (WFNS grades 4–5), no significant difference in MI-ratio was observed between outcome groups, suggesting that EBI is the predominant contributor to total brain injury in this subgroup.

Potential mechanisms underlying the association between declining MI levels and functional outcome may involve inositol-tri-phosphate (ITP3), for which MI serves as both precursor and metabolite.[230] ITP3 mediates Ca^{2+} efflux from the endoplasmic reticulum, which can induce cell death.[231-233] Thus, MI levels could serve as a surrogate marker for ITP3 activity involved in Ca^{2+}-dependent cell death.

It is tempting to hypothesize that the decline in MI levels reflects mechanisms contributing to DCI. However, no association was found between MI levels and DCI or angiographic vasospasm. Angiographic vasospasm may occur without clinical deterioration and is not independently associated with functional outcome in the absence of such deterioration.[45] This suggests that the decline in MI levels may represent alternative mechanisms of brain tissue injury distinct from angiographic vasospasm. One proposed mechanism is CSD, which involves disrupted ion homeostasis which may be followed by microvascular vasoconstriction leading to ischemia in injured brain tissue.[234] Experimentally, nimodipine—a Ca^{2+} antagonist—has been shown to reverse the pathologic hemodynamic response associated with CSD and protect the brain, underscoring the importance of Ca^{2+} regulation.[64] The role of MI in Ca^{2+} metabolism provides a hypothetical link between CSD and the observed changes in MI levels. Detecting CSD requires placement of electrodes on the cortical surface, which poses both ethical and technical challenges due to its invasive nature and the need for specialized equipment.[65] To test the hypothesis that MI dynamics are associated with specific subtypes of DCI, such as CSD, future studies would require substantially larger patient cohorts and access to the advanced infrastructure necessary to monitor CSD. Consequently, such investigations were beyond the scope of this thesis.

We demonstrated a correlation between CSF and serum MI levels, indicating that serum levels reflect changes in brain MI. CSF MI levels alone were not associated with outcome, likely due to selection bias, as CSF was only obtained from patients with an EVD, representing a more severely affected subgroup.

In summary, a steeper decline in MI levels, as well as the MI level during the late phase, are associated with long-term functional outcomes in patients with SAH. The observed decline in MI levels may reflect pathophysiological processes contributing to the overall extent of brain injury and subsequent functional outcome. Identifying these mechanisms could enable MI to serve as a biomarker for the early detection of ongoing brain damage, thereby informing therapeutic interventions. Serum MI shows promise as a clinically relevant biomarker in the management of SAH and warrants further investigation in larger cohorts to validate its predictive value and clarify its underlying biological mechanisms.

Study 2

Previous studies of NFL in SAH patients have primarily been conducted in CSF due to analytical limitations.[196,235-237] We found that serum and CSF NFL levels were correlated, consistent with prior data.[198] This supports the use of NFL as a biomarker in all SAH patients, not just those with an EVD.

NFL values upon admission were higher in the study cohort than previously reported normative values, suggesting that NFL levels are influenced by the initial haemorrhage.[182,189]

As in previous studies, NFL levels differed significantly between outcome groups. [197,198,236] Significant differences were observed in all three temporal epochs. The early epoch differences, and correlations between early NFL levels and both clinical severity

(WFNS score) and radiological severity (Fisher grade), suggest that NFL reflects brain tissue damage from EBI. NFL levels peaked in the late epoch, consistent with prior reports.[197,198,200] The strongest correlation between NFL levels and dichotomized GOSE occurred in the late epoch, which is in accordance with previous reports.[198,237] This indicates that late NFL reflects total white matter damage from both EBI and DCI. No correlation was found between NFL and angiographic vasospasm or DCI, likely reflecting similar biases as discussed in Study 1. Thus, while we cannot confirm that DCI drives increased NFL levels, this possibility remains plausible.

Previous reports suggest that EVD placement affect NFL levels.[193] Most patients who needed an EVD received this urgently, before venous sampling could be done. Therefore, we cannot assess the effect of the tissue damage caused by the EVD has on NFL levels.

No significant differences in NFL levels were observed between endovascular and microsurgical treatment groups, indicating that treatment modality does not affect NFL's predictive capability.

Regression modeling using late epoch NFL demonstrated significance in univariate analysis but not in the multivariable model. Logarithmic transformation of NFL values restored significance in the multivariable model, reflecting that small absolute changes have minimal effect, while larger changes are predictive. ROC analysis demonstrated good predictive ability of late epoch NFL for unfavourable outcomes.

In summary, NFL is a robust predictor of functional outcome in SAH patients. While this study cannot clarify the mechanisms linking NFL to outcome, NFL should be considered as a biomarker in future studies on outcome prediction. Mechanism-focused studies of DCI could further clarify the relationship between NFL levels and pathophysiologic processes leading to poor outcomes.

Study 3

Median S100B levels upon admission were higher than reference values, particularly in the unfavourable outcome group, suggesting that S100B is influenced by the initial haemorrhage (Figure 12).[215]

Serum S100B levels correlated with unfavourable outcome, measured as dichotomized mRS, consistent with previous reports.[224-226,228,238-242] We defined unfavourable outcome as mRS 4–6, though some studies use 3–6. A score of 3 indicates partial dependence; our group agreed that this still represents acceptable quality of life, and thus we included it in the favourable outcome category. Other studies have applied similar dichotomization.[243,244]

S100B was negatively correlated with EQ-5D$_{index}$, suggesting its utility as a predictor of both functional outcome and self-assessed quality of life. The mean EQ-5D$_{index}$ in our cohort was 0.58 ± 0.38, consistent with previous Swedish SAH cohorts.[245]

No correlation was observed between S100B and EQ-VAS. Since EQ-VAS requires independent completion, patients unable to respond may bias results, and the lack of correlation may reflect limited power or the insensitivity of S100B to differentiate among patients with relatively preserved function.

S100B differences were most pronounced in the early epoch, indicating its reflection of EBI. Previous studies have shown that mean S100B over 8 days is an independent outcome predictor.[241] We found that peak S100B correlated more strongly with functional outcome than mean values, likely due to its short half-life (60–120 minutes).[220,221] S100B wasn´t sampled daily in all patients in this cohort, and therefore peak values might have been missed in some patients. After significant brain injury, subsequent low S100B levels may underestimate cumulative damage; thus, peak values seem preferable for prognostication rather than mean value.

Study 4

Prognostic modeling in SAH has become increasingly common, often employing Bayesian networks or recursive partitioning to identify complex relationships and subgroups.[145,147,246] Each variable may be critical in specific contexts, although not necessarily for the population as a whole. Clinically practical predictors, including laboratory biomarkers, are particularly valuable in sedated patients. Large multicenter datasets are required, but preliminary studies are necessary to identify candidate predictors. Our findings suggest that MI, NFL, and S100B are promising candidates.

In regression modeling, biomarker values can be treated either as continuous variables or dichotomized based on a predefined cutoff value (as was done in Study 3). Such cutoffs may be determined using population-based normative values, comparisons with control groups (if available), or derived from the point of highest combined sensitivity and specificity in a ROC curve analysis. Following consultation with a statistician, it was determined that the biomarkers would provide the greatest amount of information when analyzed as continuous variables.

The time point at which each biomarker demonstrated the strongest predictive capability, as determined by ROC curve analyses in previous studies, was selected for inclusion in the regression analyses of this study. For MI, this decision may be subject to debate, as the relative decline (expressed as the MI ratio or B-value) showed a stronger association with outcome than the absolute MI concentration. However, calculating the MI ratio or B-value requires multiple measurements. Therefore, we opted to use the absolute MI level on day 7, which was itself significantly associated with outcome.

Although none of the biomarkers reached statistical significance in the final multivariable model, their inclusion increased the model's explanatory power (R^2) and demonstrated a trend toward improved AUC for predicting unfavourable outcomes (Figure 13), as well as a reduction in prediction error according to the AIC score (Figure 18). Given the relatively small sample size of our cohort for this type of analysis, accepting the null hypothesis that no difference exists in AUC scores among the models may represent a type II error resulting from insufficient statistical power.

Functional outcome after SAH reflects the total amount of permanent brain tissue damage, resulting from both EBI and DCI. Effective outcome prediction must therefore consider mechanisms affecting both processes. MI, S100B, and NFL are all associated with long-term outcome but display distinct temporal profiles, suggesting they reflect different mechanisms contributing to total brain damage. This study focused on their predictive utility rather than mechanistic pathways, which should be investigated in future studies to improve understanding of SAH pathophysiology and inform novel treatments.

Although causality between the investigated biomarkers and brain tissue damage cannot be established here, MI, S100B, and NFL are all associated with functional outcome. We propose their inclusion in future studies of acute-phase SAH for outcome prediction and mechanistic exploration.

Strengths and limitations

There are some design issues regarding this study that requires to be mentioned.

No power calculations were performed during the design of these studies, primarily because it was difficult to estimate the expected effect size (i.e., the difference in biomarker levels between outcome groups). This limitation stemmed from the scarcity of prior research on serum NFL and MI levels in patients with SAH.

We only included patients who were admitted to a tertiary care center. Patients who died at home or were in too poor neurological condition to be transported for tertiary care were not assessed. Consequently, our cohort contained a higher proportion of patients with favourable outcomes compared to other studies.[16] This can partly be explained by evolving treatment options, such as developed neurointensive care and development of endovascular techniques leading to improvement of outcome over time as previously reported.[16,247] However the mortality rate in this cohort does not accurately reflect the true mortality of patients suffering from SAH.

Due to limited availability of research staff, venous sampling was inconsistent across patients, resulting in variability in both the timing and number of samples obtained for each individual. To maintain statistical power, we divided the results into three epochs for most analyses. This approach may oversimplify the data and should be considered when interpreting the findings of this thesis. If this study were to be remade, ensuring resources for daily sampling would be of great use.

The cohort used in this thesis is part of a larger nationwide multicenter study. However, the collection of frozen serum samples was conducted exclusively at our center, which limited data availability. When the multicenter study was designed, variables related to secondary events—such as DCI and angiographic vasospasm—were not defined according to standardized or widely accepted criteria.[36] In our cohort, these variables were therefore largely based on the subjective judgment of the treating clinician or radiologist, resulting in vague and potentially inconsistent definitions. The presence of cerebral infarctions on follow-up CT scans was not assessed, as our team lacked a dedicated neuroradiologist to interpret the images. Consequently, we had no objective measure of brain injury and instead relied on the patient's functional status as an outcome indicator.

In contrast to previous findings, neither DCI nor angiographic vasospasm was related to functional outcome in our cohort.[34] We believe that the absence of such an association is primarily attributable to the variable definitions used, and thus, a genuine relationship between DCI/angiographic vasospasm and functional outcome should not be ruled out. For the same reason, we were unable to link biomarker levels to any specific secondary event that could contribute to unfavourable outcomes, and such relationship should not be dismissed nor confirmed. If these studies were to be repeated, we would apply standardized definitions for DCI and angiographic vasospasm and include an analysis of cerebral infarctions—preferably with volumetric assessment—on follow-up imaging.

The use of serum rather than CSF biomarkers strengthens external validity by avoiding the selection bias introduced by requiring an EVD.

Furthermore, the assessment of long-term rather than short-term outcomes represent an additional strength, as rehabilitation exerts a major influence on functional recovery.

General summary

The three biomarkers studied in this thesis were all associated with dichotomized functional outcomes in SAH patients.

When considering the implementation of biomarkers to aid in prognostication, several ethical aspects must be taken into account. Biomarkers could be particularly valuable in patients who are sedated and therefore difficult to assess neurologically. If a biomarker indicates a high likelihood of a favourable outcome, this information can provide reassurance to relatives. However, the interpretation of a biomarker suggesting a poor functional outcome presents a more complex ethical dilemma. Should treatment be discontinued based on such a result? If so, do we risk withdrawing care from a patient who might have otherwise achieved a favourable recovery?

These are routine challenges faced by clinicians. For a biomarker to be used as a decisive prognostic tool in clinical decision-making, it would need to demonstrate near-perfect specificity to ensure that no patient with the potential for recovery is denied treatment. However, achieving 100% specificity without compromising sensitivity is rarely possible. Therefore, biomarkers should not serve as the sole basis for clinical decisions.

In daily medical practice, prognostication involves the continuous integration of multiple clinical factors. We believe that biomarkers should be regarded as complementary tools—enhancing the accuracy of prognostication when used alongside other clinical parameters—rather than as standalone determinants, as might be implied by the multivariate models presented in this thesis.

We utilized three different scales to assess patient outcomes in this thesis. The decision to use the GOSE to assess functional outcomes in Studies 1, 2, and 4, and the mRS in Study 3, was made by the primary author. However, the two scales are largely comparable, and both demonstrated a similar ability to discriminate between outcome groups within our cohort.[140] While the mRS and GOSE evaluate patients' functional status—encompassing both cognitive and physical domains—the EQ-5D$_{index}$ measures patients' self-perceived health, which may arguably be of greater importance, as the concept of health is inherently subjective.[125,130-132,248] One could argue that GOSE and mRS should be analyzed as ordinal variables and that dichotomization oversimplifies the data. However, given the small number of patients within the categories representing unfavourable outcomes, meaningful statistical analysis using multiple categories was not feasible in this cohort. Moreover, dichotomization is a widely accepted approach in outcome assessment. This choice is further supported by the strong correlations observed between both mRS and GOSE with the EQ-$5D_{index}$, as well as by the differences in EQ-$5D_{index}$ scores between outcome groups. The mean EQ-$5D_{index}$ for the entire cohort was 0.58, while patients in the favourable outcome group had a mean value of 0.77. Although this represents a relatively good recovery, it remains somewhat lower than that of the general Swedish population, where individuals aged 50–59 have an average EQ-$5D_{index}$ of 0.83.[249] This suggests that subarachnoid haemorrhage continues to exert a measurable impact even among patients considered to have achieved a favourable outcome.

The gender distribution in our cohort was consistent with previously reported data.[21] Although the proportion of women was slightly higher in the unfavourable outcome group, this difference did not reach statistical significance. Given the limited sample size and the absence of significant gender differences between outcome groups, no further gender-stratified analyses were performed.

In the background section of this thesis, the potential roles of biomarkers were discussed. Beyond their use in prognostication, biomarkers may contribute to elucidating pathophysiological mechanisms, evaluating treatment effects, and even serving as therapeutic targets. In this thesis, the primary focus was on the temporal dynamics of each biomarker and their associations with functional outcomes. Mechanisms of injury and treatment-related effects were not the focus of the current work, and thus, these potential relationships remain unexplored.

The biomarkers investigated in this thesis possess distinct biochemical and physiological properties. While NFL and S100B are proteins predominantly found in neurons and astrocytes, MI is a carbohydrate present in most cell types. [166,167,177-179,203,213] Although S100B participates in various cellular processes, its levels rise rapidly following brain injury, suggesting that it primarily reflects direct tissue damage.[205-207] NFL demonstrates a similar pattern; however, its levels continue to increase throughout the acute phase of SAH. This sustained elevation may either indicate ongoing injury from secondary events or reflect the kinetic properties of NFL, including slow release and prolonged half-life, as reported in previous studies. [193,195] In contrast, MI appears to reflect metabolic processes associated with secondary injuries, as it shows no relationship with the initial WFNS score. Instead, the ratio between late and early MI values correlates with functional outcome. One could argue that S100B and NFL, both of which correlate with initial WFNS and Hunt and Hess scores, offer limited additional prognostic value since they largely represent EBI. However, both S100B and NFL remained statistically significant in multivariate regression models controlling for age and WFNS or HH scores, indicating that they contribute prognostic information beyond initial disease severity.

Future directions

Preventing secondary injury from DCI remains the primary challenge in SAH management. Nimodipine is the only drug with demonstrated benefit, though its mechanism remains unclear.[108-110] While prior research emphasized angiographic vasospasm as the cause of DCI, current evidence suggests a multifactorial pathophysiology, including CSD and microvascular spasm.[32,65,84] Laboratory biomarkers could complement or replace invasive monitoring, facilitating evaluation of new interventions targeting these mechanisms.

Although the mechanisms underlying the associations between each biomarker and the extent of brain injury cannot be established within the scope of this thesis, the findings support the notion that each of the biomarkers investigated herein represents a promising candidate for inclusion in future large-scale studies.

While this thesis demonstrated associations between MI, NFL, and S100B with functional outcome and self-reported quality of life, it also raises new questions regarding the underlying mechanisms. To test such hypotheses, large multicenter cohorts with frequent daily biomarker sampling and clearly defined criteria for DCI, angiographic vasospasm, and cerebral infarctions are warranted.[36] Furthermore, advanced tools for investigating proposed mechanisms of DCI—such as subdural electrodes for detecting cortical spreading depolarizations (CSD) or CT perfusion (CTP) imaging for assessing microvascular spasm—should be considered.[65,86] When designing large multicentre studies aimed at evaluating potential biomarkers, understanding underlying mechanisms, or assessing treatment effects, pilot studies that identify and validate candidate markers are of critical importance.

Multicenter studies offer advantages such as increased statistical power and opportunities for external validation. In recent years, complex statistical approaches, including Bayesian network models, have gained popularity for predicting outcomes in SAH patients.[145,147,246] Such models may help identify specific subgroups in which biomarkers could have prognostic value, even if they are not broadly applicable across all patient populations. However, these analyses require extensive datasets, underscoring the importance of selecting appropriate candidate biomarkers. We believe that this thesis presents three promising biomarkers suitable for inclusion in future large-scale studies.

Conclusions

- Serum levels of MI from day 7 onward, as well as steeper decline in MI levels are associated with long-term functional outcome reproducing previous findings in a hypothesis-driven observational study.[163]

- Among baseline factors, early MI levels are only associated with age, whereas early NFL levels are additionally associated with neurological severity according to the WFNS score and radiological severity according to the Fisher scale.

- NFL levels increase during the acute phase of SAH in both outcome groups, with the unfavourable outcome group exhibiting higher levels across all epochs.

- MI levels decline over time in both outcome groups, with a steeper decrease observed in the unfavourable outcome group.

- CSF and serum levels of both MI and NFL are correlated.

- S100B levels are correlated with both mRS and the EQ-5D at multiple time points, with peak S100B levels showing the strongest correlation.

- Incorporating MI, NFL, and S100B into regression models with established predictors increased the explanatory power of the models, although the biomarkers did not retain independent statistical significance.

- MI, NFL, and S100B represent promising candidate biomarkers for future studies involving large patient cohorts and multivariable regression models. Further research is needed to elucidate the mechanisms underlying their association with long-term outcomes in SAH patients.

Epilogue

The neurosurgical clinic where Margret was treated had taken an interest in the results of this thesis and therefore began measuring MI, NFL, and S100B levels in blood. At admission, Margret presented with a high WFNS score, indicating an increased risk of an unfavourable outcome. Another negative prognostic factor was the extent of subarachnoid blood on her initial CT scan, corresponding to Fisher grade 4. However, her initial S100B level was 0.10 µg/L, which also turned out to be her peak value. Her initial NFL level was 24 pg/mL, rising to 100 pg/mL by day 7. MI measured 20 µM at admission and decreased slightly to 18 µM on day 7.

Based on these findings, her husband was informed that despite her severe neurological condition and extensive SAH, the biomarker profile suggested a relatively low risk of surviving in a dependent state. Margret's treatment was continued, and she was extubated one week after the haemorrhage. At that point, she opened her eyes, established eye contact, and began moving her arms and legs, although she was not yet following commands. Her hydrocephalus resolved, and the EVD was successfully removed.

She was subsequently transferred to a specialized stroke rehabilitation clinic. One year later, Margret returned to the neurosurgical department for a follow-up DSA to confirm that the aneurysm had not revascularized. At that time, she demonstrated no motor deficits and was fully independent in her daily life. She had also resumed her work as a florist, although now part-time. She reported some memory difficulties compared to before the SAH and experienced mental fatigue that limited the extent of her activities. Nonetheless, Margret valued her quality of life and expressed gratitude that her treatment had not been discontinued.

Acknowledgements

First and foremost, I would like to express my deepest gratitude to my supervisor, **Associate Professor Peter Lindvall**, for giving me the opportunity to develop my scientific knowledge, for continuously encouraging me to move forward, and for guiding me along this academic journey.

My sincere thanks also go to my co-supervisors, **Professor Lars-Owe D. Koskinen** and **Professor Rickard L. Sjöberg**, for their invaluable input regarding academic writing, scientific reasoning, and statistical analyses.

To **Kristin Nyman**, the research nurse responsible for collecting venous samples and conducting follow-up evaluations—without your dedicated work, this thesis would not have been possible.

To my academic partner-in-crime, **Helena Aineskog**, for sharing both the challenges and the anxieties of research, and for your insightful discussions during the design of these studies.

To my fellow residents—**Gabriel, Klas, Amar, Axel, and Jacob**—thank you for sharing laughter, frustration, and long nights during the demanding years of neurosurgical training.

To **Sajedha Mahmood, Saeed Shahidi, Maria Eriksson, Balasim Najar, Pedram Tabatabaie**, **Hosam Shamma** and **Arne Roos**, the consultants who have generously shared their knowledge and experience—thank you for your support and mentorship throughout my training.

To all the **nurses, scrub nurses, anaesthesiologists**, and other colleagues who made my residency one of the most memorable times of my life—thank you for your teamwork and dedication.

To **Christoffer, Alexander N., Michel, Magnus, Micke**, and **Alexander H.**, thank you for taking me on adventures and reminding me of life beyond work when I needed it most.

To my brother **André** and his **Hannah**, and my sister **Rebecca** and her **Love**, thank you for always walking beside me through thick and thin.

To my beloved **Matilda**, for your unwavering support during the long months when this thesis came to life—for always lifting my spirits when I was discouraged, and for filling my life with joy and love.

Finally, and most importantly, to my parents, **Carolyn** and **Mats**—thank you for giving me the gift of life and for being there for me, always. You lifted me up when I could not do so myself, taught me right from wrong, and instilled in me the values that guide my life. Your endless love and support have made everything possible. I am proud to be your son.

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