Subarachnoid haemorrhage – clinical and epidemiological studies

Cecilia Lindgren
Omslagsbild: Etna volcano eruption symbolises the acute bleeding of a cerebral aneurysm and it’s disastrous effects.
Elektronisk version tillgänglig på http://umu.diva-portal.org/

Umeå, Sweden 2014
To Janne, Pierre, Mathias, Mikael and Strix

It always seems impossible until it’s done

- Nelson Mandela -
ABSTRACT

Background: Subarachnoid haemorrhage (SAH) is a severe stroke that in 85% of all cases is caused by the rupture of a cerebral aneurysm. The median age at onset is 50-55 years and the overall mortality is approximately 45%.

Sufficient cortisol levels are important for survival. After SAH hypothalamic/pituitary blood flow may be hampered this could result in inadequate secretion of cortisol. SAH is also associated with a substantial inflammatory response. Asymmetric dimethyl arginine (ADMA), an endogenous inhibitor of nitric oxide synthase, mediates vasoconstriction and increased ADMA levels may be involved in inflammation and endothelial dysfunction. Continuous electroencephalogram (EEG) monitoring can be used to detect non-convulsive seizures, leading to ischemic insults in sedated SAH patients. Elevated ADMA levels are risk factors for vascular diseases. Vascular disease has been linked to stress, inflammation and endothelial dysfunction. SAH possesses all those clinical features and theoretically SAH could thus induce vascular disease.

Aims: 1. Assess cortisol levels after SAH, and evaluate associations between cortisol and clinical parameters. 2. Assess ADMA levels and arginine/ADMA ratios after SAH and evaluate associations between ADMA levels and arginine/ADMA ratios with severity of disease, co-morbidities, sex, age and clinical parameters. 3. Investigate occurrence of subclinical seizures in sedated SAH patients. 4. Evaluate if patients that survive a SAH ≥ one year have an increased risk of vascular causes of death compared to a normal population.

Results: Continuous infusion of sedative drugs was the strongest predictor for a low (<200 nmol/L) serum cortisol. The odds ratio for a sedated patient to have a serum cortisol < 200 nmol/L was 18.0 times higher compared to an un-sedated patient (p < 0.001). Compared to admission values, 0-48 hours after SAH, CRP increased significantly already in the time-interval 49-72 hours (p<0.05), peaked in the time-interval 97-120 hours after SAH and thereafter decreased. ADMA started to increase in the time-interval 97-120 hours (p<0.05). ADMA and CRP levels were significantly higher, and arginine/ADMA ratios were significantly lower in patients with a more severe condition (p<0.05). Epileptic seizure activity, in sedated SAH patients, was recorded in 2/28 (7.1%) patients during 5/5468 (0.09%) hours of continuous EEG monitoring. Cerebrovascular disease was significantly more common as a cause of death in patients that had survived a SAH ≥ one year, compared to the population from the same area (p<0.0001).

Conclusions: Continuous infusion of sedative drugs was associated with low (<200 nmol/L) cortisol levels. ADMA increased significantly after SAH, after CRP had peaked, indicating that endothelial dysfunction, with ADMA as a marker, is induced by a systemic inflammation. Patients with a more severe condition had significantly higher ADMA and CRP levels, and significantly lower arginine/ADMA ratio. Continuous sedation in sedated SAH patients seems to be beneficial in protecting from subclinical seizures. Cerebrovascular causes of death are more common in SAH survivors.

Keywords: Subarachnoid haemorrhage, ADMA, Cortisol, EEG, inflammation, epidemiology, endothelium, arginine,
ORIGINAL PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I  Cecilia Lindgren, Per Dahlqvist, Peter Lindvall, Leif Nilsson, Lars-Owe Koskinen, Silvana Naredi.
Cortisol levels are influenced by sedation in the acute phase after subarachnoid haemorrhage.

II  Cecilia Lindgren, Magnus Hultin, Lars-Owe D Koskinen, Peter Lindvall, Ljubisa Borota, Silvana Naredi.
ADMA levels and arginine/ADMA ratios reflect severity of disease and extent of inflammation after subarachnoid hemorrhage.
Neurocrit Care 2014 Jan 10. [Epub ahead of print]

III  Cecilia Lindgren, Erik Nordh, Silvana Naredi, Magnus Olivecrona.
Low frequency of Non-Convulsive Seizures and Non-Convulsive Status Epilepticus Detected in Poor Grade Subarachnoid Haemorrhage Patients.

IV  Cecilia Lindgren, Magnus Hultin, Lars-Owe D Koskinen, Stefan Söderberg, Ludvig Edvardsson, Silvana Naredi.
Long-term survivors after subarachnoid haemorrhage have an increased risk of death due to cerebrovascular causes.
Manuscript.

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<tr>
<td>11-β-HSD</td>
<td>11-beta-hydroxysteroid-dehydrogenases</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic Hormone</td>
</tr>
<tr>
<td>ADMA</td>
<td>Asymmetric dimethylarginine</td>
</tr>
<tr>
<td>ALI</td>
<td>Acute lung injury</td>
</tr>
<tr>
<td>BOXes:</td>
<td>Bilirubin oxidized fragments</td>
</tr>
<tr>
<td>Bpm</td>
<td>Beats per minute</td>
</tr>
<tr>
<td>CBG</td>
<td>Corticosteroid-binding globulin</td>
</tr>
<tr>
<td>eEEG</td>
<td>continues Electroencephalography</td>
</tr>
<tr>
<td>eFC</td>
<td>calculated Free cortisol</td>
</tr>
<tr>
<td>CIRCI</td>
<td>Critical illness related corticosteroid insufficiency</td>
</tr>
<tr>
<td>CPP</td>
<td>Cerebral perfusion pressure</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin-Releasing Hormone</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>CTA</td>
<td>Computed tomography angiography</td>
</tr>
<tr>
<td>DDAH</td>
<td>dimethylarginine dimethylaminohydrolase</td>
</tr>
<tr>
<td>DIND</td>
<td>Delayed neurologic ischemic deficit.</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td>DSA</td>
<td>Digital subtraction angiography</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiography</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalography</td>
</tr>
<tr>
<td>EtCO₂</td>
<td>End-tidal carbon dioxide</td>
</tr>
<tr>
<td>EVD</td>
<td>External ventricular drainage</td>
</tr>
<tr>
<td>GCS</td>
<td>Glasgow Coma Scale</td>
</tr>
<tr>
<td>GOS</td>
<td>Glasgow outcome Scale</td>
</tr>
<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>H&amp;H</td>
<td>Hunt &amp; Hess</td>
</tr>
<tr>
<td>HDL</td>
<td>high-density lipoproteins</td>
</tr>
<tr>
<td>HPA-axis</td>
<td>hypothalamic-pituitary-adrenal axis</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>HT</td>
<td>Hypertension</td>
</tr>
<tr>
<td>ICH</td>
<td>Intracerebral hemorrhage</td>
</tr>
<tr>
<td>ICP</td>
<td>Intracranial pressure</td>
</tr>
<tr>
<td>ICU</td>
<td>Intensive care unit</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>IL-10</td>
<td>Interleukin-10</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin-1beta</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>IL-8</td>
<td>Interleukin-8</td>
</tr>
<tr>
<td>ISAT</td>
<td>International Subarachnoid Aneurysm Trial</td>
</tr>
<tr>
<td>kPa</td>
<td>Kilopascal</td>
</tr>
<tr>
<td>m</td>
<td>Minutes</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>MCA</td>
<td>Middle Cerebral Artery</td>
</tr>
<tr>
<td>MONICA</td>
<td>Multinational Monitoring of Trends and Determinants in Cardiovascular Disease</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>NCSE</td>
<td>Non-convulsive status epilepticus</td>
</tr>
<tr>
<td>NCSZ</td>
<td>Non-convulsive seizures</td>
</tr>
<tr>
<td>NHDU</td>
<td>Neurosurgical High Dependency Unit</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>ns</td>
<td>non-significant</td>
</tr>
<tr>
<td>p</td>
<td>p-value</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>partial pressure of carbon dioxide</td>
</tr>
<tr>
<td>PaO₂</td>
<td>partial pressure of oxygen</td>
</tr>
<tr>
<td>PIN</td>
<td>personal identity number</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>s</td>
<td>Seconds</td>
</tr>
<tr>
<td>SaC</td>
<td>Saliva cortisol</td>
</tr>
<tr>
<td>SAH</td>
<td>Subarachnoid haemorrhage</td>
</tr>
<tr>
<td>SC</td>
<td>Serum cortisol</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SIRS</td>
<td>Systemic Inflammatory Response Syndrome</td>
</tr>
<tr>
<td>SOFA</td>
<td>Sequential organ failure assessment score</td>
</tr>
<tr>
<td>TCD</td>
<td>Transcranial Doppler</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor Necrosis Factor-alpha</td>
</tr>
<tr>
<td>UUH</td>
<td>Umeå University Hospital</td>
</tr>
<tr>
<td>WFNS</td>
<td>WFNS World Federation of Neurological Surgeons Committee scale</td>
</tr>
</tbody>
</table>
INTRODUCTION

Subarachnoid haemorrhage (SAH) is a form of stroke characterized by extravasation of blood into the subarachnoid space. The SAH is, in 80% of all cases, caused by the rupture of an intracranial arterial aneurysm. The cerebral aneurysms develop during the course of life and are not congenital, indicating that SAH is more of a chronic disease (van Gijn et al. 2007, Rinkel et al. 2011). Risk factors for SAH are hypertension, smoking, age > 50 years, a family history with > 2 relatives with SAH, excessive use of alcohol, and cocaine abuse (Feigin et al. 2005). SAH is also associated with some uncommon diseases such as polycystic kidney disease, Ehlers-Danlos syndrome, and Marfans syndrome (Caranci et al. 2013). Increasing evidence points towards inflammatory processes as key factors in the development of cerebral aneurysms (Chalouhi et al. 2013).

SAH represent around 5% of all strokes, but due to the relative low age at onset (median age ranging between 50-60 years), the loss of life years is similar to ischemic or haemorrhagic stroke (van Gijn et al. 2007, Rinkel et al. 2011). During the last few decades the mortality has declined, and now nearly 65% of SAH patients survive the acute phase (Rinkel et al. 2011). According to long-term follow-up figures from the International Subarachnoid Aneurysm Trial (ISAT), 80% of the surviving patients were independent five years after the SAH (Molyneux et al. 2009). However, even if the SAH survivors are classified as independent, cognitive dysfunction is common, and different studies have reported a frequency between 60-75% for impairment of memory, executive function, and language (Rinkel et al. 2011). The diagnosis of SAH is suspected by the typical history from the patient as, ‘the worst headache of my life’; however, half of the patients become unresponsive at the onset of the SAH (Bederson et al. 2009, van Gijn et al. 2001). All patients with SAH should be transferred to a neurosurgical clinic as fast as possible to treat the cause of the bleeding (Vespa et al. 2011). The diagnosis of SAH is confirmed by computer tomography (CT) scan, and the cause of the SAH by CT angiography (CTA) or digital subtraction angiography (DSA) (Bederson et al. 2009, Diringer et al. 2011, Steiner et al. 2013). The cerebral aneurysms can be secured either by a neurosurgical operation with clipping of the aneurysm, or by interventional radiology with insertion of platinum coils (Bederson et al. 2009, Diringer et al. 2011).

Sympathetic nervous activation and SAH

After the cerebral aneurysm has been secured, the SAH patient should be taken care of at a neurosurgical intensive care unit because of a high frequency of severe systemic complications (Bederson et al. 2009, Vespa et al. 2011). SAH starts a massive activation of the sympathetic nervous system with release of catecholamines (Naredi et al. 2000). This sympathetic activation can cause arrhythmias,
Introduction


Inflammation and SAH

SAH initiates an inflammatory activation with release of cytokines, and a rise in C-reactive protein (CRP) (Naredi et al. 2006, Macmillan et al. 2002, Vespa et al. 2004, Carr et al. 2013). Systemic inflammatory response syndrome (SIRS) is found in 63% of the SAH patients in the acute phase (Yoshimoto et al. 2001, Tam et al. 2010, Lu et al. 2009). C-reactive protein (CRP) is a highly sensitive inflammatory marker that has been correlated to outcome in SAH patients in several studies, and to delayed cerebral ischemia and infarction with a weaker association as well (Jeon et al. 2012, Juvela et al. 2012).

A systemic elevation in pro-inflammatory cytokines such as interleukin 1, interleukin-6 and tumour necrosis factor-alpha (IL-1, IL-6 and TNF-α) has been reported in the acute phase after SAH (Naredi et al. 2006, Sercombe et al. 2002). An elevation of interleukin-8 (IL-8), also known as neutrophil chemotactic factor, is reported in serum and cerebrospinal fluid (CSF) (Naredi et al. 2006, Sercombe et al. 2002). The anti-inflammatory interleukin-10 (IL-10), also known as human cytokine synthesis inhibitory factor, is also reported as systemically elevated after SAH (Sercombe et al. 2002). The action and interaction of cytokines in the acute phase of SAH are not in detail studied (Sercombe et al. 2002, Mellergard et al. 2011). An overview of cytokines and their actions is given in Table 1. The inflammatory reaction indicated by cytokine release can promote coagulation leading to microvascular thrombosis and compromised cerebral circulation (Sercombe et al. 2002). Systemic inflammatory activation causes capillary leakage, which can lead to lower blood pressure and further compromised cerebral circulation (Hamrahian et al. 2004, Lu et al. 2009).
Introduction

Table 1. Major systemic actions of the cytokines measured

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Action</th>
</tr>
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<tbody>
<tr>
<td>IL-1β</td>
<td>Acts generally together with TNF-α and IL-6</td>
</tr>
<tr>
<td></td>
<td>Promotes inflammation</td>
</tr>
<tr>
<td></td>
<td>Induces fever</td>
</tr>
<tr>
<td></td>
<td>Activates T-cells and macrophages</td>
</tr>
<tr>
<td></td>
<td>Involved in the pathogenesis of septic shock</td>
</tr>
<tr>
<td></td>
<td>Involved in promotion of arteriosclerosis</td>
</tr>
<tr>
<td>IL-6</td>
<td>Acts generally together with TNF-α and IL-1</td>
</tr>
<tr>
<td></td>
<td>Induces CRP synthesis in the liver</td>
</tr>
<tr>
<td></td>
<td>Induces fever</td>
</tr>
<tr>
<td></td>
<td>Involved in the pathogenesis of septic shock</td>
</tr>
<tr>
<td>IL-8</td>
<td>Neutrophil chemotactic factor</td>
</tr>
<tr>
<td></td>
<td>Induces chemotaxis</td>
</tr>
<tr>
<td>IL-10</td>
<td>Human cytokine synthesis inhibitory factor</td>
</tr>
<tr>
<td></td>
<td>Reduces synthesis of pro-inflammatory cytokines</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Acts generally together with IL-1 and IL-6</td>
</tr>
<tr>
<td></td>
<td>Stimulates acute phase response</td>
</tr>
<tr>
<td></td>
<td>Attracts neutrophils</td>
</tr>
<tr>
<td></td>
<td>Stimulates phagocytosis</td>
</tr>
</tbody>
</table>

Adrenal insufficiency and SAH

Activation of the hypothalamic-pituitary-adrenal (HPA) axis is an important part of the stress response that interacts with the inflammatory and autoimmune nervous responses (Fig 1) (Marik et al. 2009). Critical illness-related corticosteroid insufficiency (CIRCI) might be suspected in hypotensive SAH patients who respond poorly to fluids and vasopressor support (Marik et al. 2009). Endocrine dysfunction, and particularly cortisol insufficiency, is reported as common in long-term survivors of aneurysmal SAH, and has been reported in up to 50% of SAH patients (Dimopoulou et al. 2004). The endocrine dysfunction seen in the convalescence period after SAH could be due to events occurring in the acute phase. Different explanations have been proposed, such as possibilities of hampered blood flow in the acute phase due to high intracranial pressure, cerebral vasospasm, and direct damage to pituitary circulation after intervention, especially in patients with aneurysm in the anterior cerebral artery (Schneider et al. 2007, Weant et al. 2008).
Introduction

However, only a few studies have investigated the cortisol response in the acute phase, and the results have been diverse (Zetterling et al. 2011, Bendel et al. 2008, Poll et al. 2010).

![Diagram of the hypothalamic-pituitary-adrenal axis (HPA axis).]

**Fig 1.** Activation of the hypothalamic-pituitary-adrenal axis (HPA axis).

The stress response mediated by the HPA axis results in an increased secretion of CRH from the hypothalamus. CRH stimulates the production of ACTH by the anterior pituitary gland, causing the adrenal cortex to produce cortisol. The released cortisol exerts a negative feedback. CRH= Corticotropin-releasing hormone, ACTH= Adrenocorticotropic hormone

Treatment with corticosteroids may have negative effects in the critically ill neurological patient. Excess levels of cortisol may disturb the repair processes after brain lesions and may also exacerbate secondary brain injuries. A correct diagnosis of cortisol deficiency, if present, is important to avoid unnecessary administration of glucocorticoid (Britt et al. 2006, Sorrels et al. 2009).

Under normal conditions, only 10% of the cortisol in plasma is free and biologically active, 75% is bound to corticosteroid-binding globulin (CBG) and 15% is bound to albumin. In critical illness, the CBG and albumin levels decrease due to capillary leakage (Hamrahian et al. 2004). This gives insecurity in the interpretation of the cortisol activity since serum cortisol measures the total cortisol (Bendel et al. 2008). The free cortisol can be estimated by analysing CBG at the same time as cortisol, and calculating the free cortisol by using Coolens formula (Coolens et al. 1987).

Saliva cortisol (SaC) correlates well with calculated free cortisol (cFC) (Arafah et al. 2007). Saliva cortisol could therefore be an alternative method to use for estimation of the free and biologically active cortisol in the critically ill patient.
Introduction

Asymmetric dimethylarginine (ADMA) and SAH

A feared complication after SAH is delayed ischemia. (Vergouwen et al. 2010). Delayed ischemic events have been associated with cerebral vasospasm, reported to be present in 30-70% of all SAH patients (Bederson et al. 2009, Wagner et al. 2013). Cerebral vasospasm refers to an arterial narrowing of the cerebral vessels that usually occurs between 3-7 days after the SAH (Dumont et al. 2003). Cerebral vasospasm might lead to cerebral ischemia and infarction in the affected area of the brain. The pathogenesis of the cerebral vasospasm may involve lack of nitric oxide (NO), the most competent vasodilator of the cerebral vessels. NO production is inhibited by ADMA (Fig 2) (Boger et al. 2006).

ADMA is a competitive inhibitor of nitric oxide synthase (NOS) and has, as such, the ability to reduce the availability of NO, and may consequently induce vasoconstriction, (Fig 2) (Pluta et al. 2007). The haemoglobin in the blood in the subarachnoid space is metabolized to bilirubin-oxidized fragments (BOXes) that increase ADMA (Pluta et al. 2005). A greater amount of blood in the subarachnoid space has been associated with an increased risk of cerebral vasospasm (Fisher et al. 1980, Lee et al. 2013). Increased platelet aggregation and leucocyte adhesion to the endothelium can be caused by reduced access to NO. Endothelial dysfunction and inflammation may thus be associated with increased ADMA levels (Boger et al. 2003). Diseases involving vessel dysfunction such as kidney failure, heart failure, pre-eclampsia, and stroke are associated with increased ADMA levels (Boger 2006). Intima media thickening of the carotid artery has also been reported associated with increased ADMA levels (Nanayakkara et al. 2006, Kielsten et al. 2006, Siroen et al. 2006). Decreased arginine/ADMA ratio in the acute phase after SAH has, in one study, been associated with poor outcome (Staalso et al. 2013).

Fig 2. ADMA effect on NOS.

NOS use arginine as a substrate to produce NO. ADMA is formed from arginine and is an endogenous competitive inhibitor of NOS. The inhibition of NOS by ADMA leads to decreased production of NO from arginine. ADMA = Asymmetric dimethylarginine, NOS = Nitric oxide synthase, NO=Nitric oxide
Introduction

Epileptic seizures and SAH

Epileptic seizures have been reported to occur in 1-26% of patients with SAH and are more frequent in patients with severe disease (Choi et al. 2009, Claassen et al. 2004, Claassen et al. 2013). Routine anticonvulsant prophylaxis is controversial and has been associated with adverse outcome (Rosengart et al. 2007, Choi et al. 2009). Continuous electroencephalography (cEEG) offers the opportunity to evaluate non-convulsive epileptic seizures (NCSZ) and non-convulsive status epilepticus (NCSE), conditions associated with increased mortality (Claassen et al. 2006). The recommendation from The Neurocritical Care Society is that cEEG monitoring should be considered in patients with poor-grade SAH (Diringer et al. 2011).

Causes of death and SAH

SAH patients have reduced life expectancy compared to sex- and age-matched controls) even though the risk of re-bleeding after SAH during the first five years after the SAH is only 1-2% (Wermer et al. 2005, Pyysalo et al. 2013, Rinkel et al 2011, Molyneux et al 2009, Korja et al. 2013). Risk factors for SAH are hypertension and smoking (Feigin et al. 2005). This indicates that SAH patients may have pre-existing risk factors for cardiovascular diseases.

Increased ADMA has been detected in a variety of cardiovascular diseases, indicating that elevated ADMA levels are linked to cardiovascular disease (Boger et al. 2006). In critically ill patients, ADMA has been used as a marker of severity of disease as well as a predictor of outcome (Brinkman et al. 2014, Koch et al. 2013). ADMA has been reported as elevated after SAH (Rodling-Wahlström et al. 2012). This indicates that SAH may initiate endothelial dysfunction, which could lead to an increased risk of cardiovascular complications beyond the acute phase of the SAH.
AIMS OF THE THESIS

- To assess the frequency of CIRCI after SAH, and to evaluate the associations between cortisol levels and sedation, circulatory failure, gender, age, severity of disease, endovascular/surgical treatment, and outcome

- To evaluate ADMA levels and arginine/ADMA ratios after SAH, and to evaluate associations between ADMA levels and arginine/ADMA ratios with severity of disease, co-morbidities, sex, age, systemic cardiovascular complications, impaired cerebral circulation, inflammatory response and outcome

- To investigate the frequency of subclinical seizures in sedated and mechanically ventilated SAH patients

- To evaluate if long-term survivors after SAH have an increased risk of cardiovascular causes of death compared to the general population
PATIENTS & METHODS

Study Design

Paper I and Paper II are based on the same prospectively designed observational study, divided into two parts. Part one (Paper I) evaluated the adrenal response over time after SAH and the associations between subnormal cortisol levels and different clinical parameters: sedation, circulatory failure, gender, age, severity of disease, and endovascular/surgical treatment. Part two (Paper II) evaluated the levels of ADMA and the arginine/ADMA ratios as markers of endothelial dysfunction over time after aneurysmal SAH, as well as associations between ADMA, arginine/ADMA ratios and severity of disease, co-morbidities, sex, age, systemic cardiovascular complications, impaired cerebral circulation, and inflammatory response. Part one was designed because of the debate on the occurrence of adrenal insufficiency in critically ill patients and the reports of a high frequency of adrenal insufficiency in the chronic phase after SAH (Marik et al 2009, Dimopoulou et al 2004). Part two was a continuation of the results from a pilot study on ADMA levels after SAH (Rodling-Wahlström et al. 2012). This pilot study showed that the increase in ADMA levels after SAH was not significant until day six after admission, indicating that the increase in ADMA was related to the time period where cerebral vasospasm usually occurs. The possibility of detecting a difference in ADMA between patients with or without signs of impaired cerebral circulation was hypothesized. At the Neurosurgical Department at Umeå University Hospital (UUH), 50-80 patients/year were treated for aneurysmal SAH before the study started. A total study period of 18 months for inclusion in the acute phase, with a follow-up after approximately one year was decided upon (total study period of 2.5 years). This study period would include a substantial number of patients, and the treatment approaches could be kept without considerable changes.

Paper III is a retrospective study including patients with aneurysmal SAH who had been monitored with cEEG. The rationale for this study was to investigate the frequency of NCSZ and NCSE in sedated SAH patients.

Paper IV is a retrospective register study of patients with a diagnosis of SAH from 1986 to 2006 that had died during the period, but had survived the SAH ≥ one year. The study cohort was compared to individuals in a Swedish health project at admission, the Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) from northern Sweden (Eriksson et al 2011). Causes of death in the study cohort and in the general population for the same geographical area were obtained from The National Board of Health and Welfare Sweden.

A summary of the study designs is given in Table 2, and more details on the studies are in Tables 3 and 4.
<table>
<thead>
<tr>
<th></th>
<th>Problem Approached</th>
<th>Design &amp; Setting</th>
<th>Number of patients</th>
<th>Study Period</th>
<th>Scoring systems used for evaluation</th>
<th>Laboratory assays and other methods used</th>
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<tbody>
<tr>
<td>I</td>
<td>Cortisol insufficiency Evaluation of cortisol levels over time; acute phase and follow-up. Association between cortisol and clinical parameters.</td>
<td>Observational prospective clinical study</td>
<td>50</td>
<td>Mar 2008-Jul 2009</td>
<td>H&amp;H Fisher SOFA GOS</td>
<td>SC, SaC, cFC, CBG</td>
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<tr>
<td>II</td>
<td>Endothelial dysfunction Evaluation of ADMA and arginine/ADMA ratio over time; acute phase and follow-up. Association between ADMA, arginine/ADMA ratio and clinical parameters.</td>
<td>Observational prospective clinical study</td>
<td>56</td>
<td>Mar 2008-Sept 2009</td>
<td>H&amp;H Fischer GCS SOFA GOS</td>
<td>ADMA, arginine, CRP IL-1β, IL-6, IL-8, IL-10, TNF-α, troponin, ECG, TCD, CT, CTA, DSA</td>
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<tr>
<td>III</td>
<td>Frequency of NCSZ and NCSE Evaluation of cEEG in sedated SAH patients.</td>
<td>Observational retrospective clinical study</td>
<td>27</td>
<td>Jan 2008 - Jun 2010</td>
<td>H&amp;H WFNS GOS</td>
<td>Initial 16-lead EEG, followed by continuous 4 lead EEG</td>
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<tr>
<td>IV</td>
<td>Cardiovascular causes of death after SAH Evaluation of causes of death and co-morbidities at the time of SAH.</td>
<td>Retrospective epidemiological cohort study</td>
<td>162</td>
<td>1986 - 2006</td>
<td>causes of death and co-morbidities compared to the general population and a health survey</td>
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</table>

Table 2. Summary of the study designs in the thesis
H&H= Hunt and Hess, SOFA= sequential organ failure assessment score, SC= serum cortisol, SaC= saliva cortisol, cFC= calculated free cortisol GOS= Glasgow outcome scale, CBG= corticosteroid-binding globulin, ADMA= Asymmetric dimethylarginine, CRP= C-reactive protein IL-1β= interleukin-1β, IL-6= interleukin-6, IL-8= interleukin-8, IL-10= interleukin-10, TNF-α= Tumor Necrosis Factor-alpha, ECG= Electrocardiography, TCD= Transcranial doppler, CT= Computed tomography, CTA= Computed tomography angiography, DSA= Digital subtraction angiography, NCSZ= non-convulsive seizures, NCSE= non-convulsive status epilepticus, cEEG= continues electroencephalography, EEG= electroencephalography, WFNS: World federation of neurological scale
Study Design- Paper I

Serum and saliva cortisol was followed over time in the acute phase after SAH, and was also obtained at a follow-up visit approximately one year after the SAH (Table 3). The effect of sedation, circulatory failure, gender, age, severity of disease, and endovascular/surgical treatment on cortisol levels was studied in the acute phase. For evaluation of the effect of sedation, a cortisol sample obtained when the patient had a continuous infusion of sedative drugs was categorized as a ‘sedated sample’, and a cortisol sample obtained in a patient without continuous infusions of sedative drugs at the time of sampling was categorized as an ‘unsedated sample’. Cardiovascular failure was defined as a Sequential Organ Failure Assessment score (SOFA) ≥ 3.

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<th>01</th>
<th>0-48 h</th>
<th>49-96 h</th>
<th>97-144 h</th>
<th>145-192 h</th>
<th>193-240h</th>
<th>Follow-up</th>
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Table 3. Study Design Paper I: Blood sampling and scoring

1The start of the symptoms of the SAH that brought the patient to hospital
SC= serum cortisol, SaC= saliva cortisol, CBG =Corticosteroid binding globulin, cFC= calculated free cortisol, H&H=Hunt & Hess SOFA=Sequential Organ Failure Assessments, GOS=Glasgow outcome scale
Patients and Methods

At the follow up visit serum and saliva cortisol were obtained and a careful medical and pharmaceutical history were taken. The patients were scored according to the Glasgow Outcome Scale (GOS).

Study design- Paper II

The primary aim was to follow ADMA levels and arginine/ADMA ratios over time in the acute phase after SAH and to investigate eventual associations between ADMA levels and arginine/ADMA ratios and severity of disease, co-morbidities, sex, age, systemic cardiovascular complications, impaired cerebral circulation, and inflammatory response. The secondary aim was to correlate ADMA levels and arginine/ADMA ratios to outcome (Table 4).

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Table 4. Study Design-Blood sampling, clinical tests and scoring Paper II.

1 The start of the symptoms of the SAH that brought the patient to hospital

ADMA= Asymmetric dimethylarginine, Arg.= arginine, CRP= C-reactive protein, Trop.=troponin, GCS= Glasgow Outcome Scale, H&H= Hunt and Hess, SOFA= sequential organ failure assessment score, ECG= electroencephalography IL-1β= interleukin-1beta, IL-6= interleukin-6, IL-8= interleukin-8, IL-10= interleukin-10, TNF-α= Tumor Necrosis Factor-alpha, GOS= Glasgow outcome scale,
 Patients and Methods

All CT, CTA, and DSA images were re-evaluated by a specialist in neuroradiology, blinded for ADMA levels and arginine/ADMA ratios. Definition of impaired cerebral circulation was the presence of any of the following signs: Focal neurological impairment or a decrease of ≥2 points on the Glasgow Coma Scale (GCS) without any other explanation, TCD verified mean flow velocity >120 cm/second in MCA, CT-verified suspected ischemia or angiographic vasospasm detected with DSA /CTA. This definition was based on a definition proposed by a multidisciplinary research group led by Vergouwen et al. in 2010 (Vergouwen et al. 2010).

ADMA, arginine/ADMA at admission and peak ADMA, and nadir arginine/ADMA ratio during the acute phase 0-240 h after SAH were associated with clinical and physiological parameters including gender, age, Hunt & Hess score, Fisher grade, hypertension, troponin, ECG, and respiratory-, circulatory-, and renal failure scored with SOFA.

At the follow up visit, ADMA and arginine were obtained and a careful medical and pharmaceutical history was taken. The patients were scored according to GOS.

Study design- Paper III

This is a clinical observational study with retrospective analysis of data. Sedated SAH patients with aneurysmal SAH in need of neuro-intensive care and monitored with cEEG were included. A specialist in clinical neurophysiology with more than10 years of EEG reading experience evaluated all EEG recordings. The aim of this study was to investigate the occurrence of clinical and electroencephalographic subclinical seizures in patients with SAH treated in the ICU.

Study design- Paper IV

This is a retrospective epidemiological register study. All medical records of patients treated at the neurosurgical department at UUH under a diagnosis of SAH from 1986-2006 were retrieved and examined. The intention was to find the patients that had died during the study period, yet who initially had survived their SAH ≥ 1 year. To come as close as possible to including only patients with a SAH caused by the rupture of cerebral aneurysms, the SAH had to, according to the medical records, be verified by CT or analysis of cerebrospinal fluid from a lumbar puncture.

- 1846 patients with a diagnosis of SAH according to ICD-9 (430-438) or ICD-10 (I60-I60.9) were treated at the neurosurgical department of UUH from 1986-2006.
Patients and Methods

The computerized medical records system at the UUH is linked to the Swedish personal identity number (PIN) register, so it was therefore possible to find out through the Swedish national population registry, available at the Swedish tax agency (Skatteverket, Stockholm), if the patients were deceased or alive in 2006.

- **596** patients had deceased up through 2006.
  1250/1846 (68%) patients with a diagnosis of SAH 1986-2006, treated at the neurosurgical department, were still alive in 2006 and 596/1846 (32%) had deceased during the period. From the list of the 596 deceased patients, those who had died < one year after the SAH were excluded.
- **288** patients had died < one year after the SAH.
  288/596 (48%) patients that had died during the period had survived their SAH < one year. That left 308/596 (52%) that had died during the period, yet had survived ≥ one year. The medical records of those 308 ≥ one-year survivors were then retrieved and examined.
- **81** medical records could not be found or contained insufficient data.
  81/308 (26%) patients were excluded due to missing data. That left 227 medical records to examine.
- **65** patients were excluded due to other proven causes of SAH.
  65/227 (29%) patients had another proven cause of SAH according to the medical records: perimesencephalic bleeding, SAH caused by arteriovenous malformations, intracerebral haematomas not caused by cerebral aneurysms, and traumatic SAH.
- **162** SAH patients that had died from 1986-2006 had survived their SAH ≥ one year; they formed the study cohort.

A requisition of the death certificates from the 162 patients in the study cohort was made from the National Board of Health and Welfare Sweden (Socialstyrelsen, Stockholm). The median year of death in the study population was 2001. In order to compare causes of death to a general population, all causes of death in the general population in 2001 from the same northern counties as the study cohort were retrieved from the open database, available at the open website of the National Board of Health and Welfare, Sweden (www.socialstyrelsen.se). For comparison of co-morbidities in the study cohort at the time of the SAH to a general population, answers in a questionnaire from the MONICA health project regarding self-reported data on cerebro- and cardiovascular diseases, diabetes mellitus, and tobacco use were used (Appendix). The median year for the SAH in the study cohort was 1992 for all patients (1992 in female patients, and 1993 in male patients). The closest MONICA health project, from 1994, was chosen for comparison of co-morbidities in the study cohort to a general population from approximately the same area.
Patients and Methods

Patients

Patients with SAH treated at the neurosurgical department of UUH were included in all four papers.

Papers I, II, and III included patients with verified aneurysmal SAH. In Paper IV, the intention was to include patients with aneurysmal SAH.

Papers I and II included patients ≥ 18 years of age that had arrived to UUH ≤ 48 hours after the start of the symptoms that brought the patient to hospital.

In Papers I and II, the exclusion criteria included: pregnant or lactating woman, and earlier SAH or previous intracranial surgery/treatment. In Paper I, patients with corticosteroid therapy, and known adrenal insufficiency were additionally excluded.

In Paper I, 50 patients were included. In Paper II, 56 patients were included, all the 50 from Paper I and six additional patients. Part one of the prospective observational study that Papers I and II were based on was terminated after the inclusion of 50 patients; part two continued further inclusion of patients all through the planned study period of 18 months.

Paper III included patients > 15 years of age, in need of controlled ventilation/sedation ≥ 48 hours, and with cEEG monitoring available. In total, 28 patients were included in this study, 19 of those patients were also included in study I and II.

Paper IV included patients ≥ 18 years of age with a SAH diagnosis based on ICD-9 (430-438) or ICD-10 (I60.0-I60.9) registration in the medical records. The SAH diagnosis had to be verified either by CT or analysis of cerebrospinal fluid after lumbar puncture. Patients with verified SAH due to other causes than a cerebral aneurysm were excluded. In total, 162 patients formed the study cohort.

Treatment

Patients in Papers I, II, and III were treated in accordance with a local protocol for SAH. This local protocol essentially follows the recommendations given by the Neurocritical Care Society’s Multidisciplinary Consensus Conference and the American Heart Association’s (AHA) guidelines (Benderson et al 209, Diringer et al 2011). The treatment protocol emphasizes maintenance of normovolemia by infusion with mainly albumin. Glucose, sodium, and albumin are kept within normal limits. Intravenous nimodipine (Nimotop®, Bayer) 0.2 mg/mL is administered for prevention of delayed neurologic ischemic deficit (DIND). The target for cerebral perfusion pressure (CPP) is ≥ 70 mmHg. The first choice for cardiovascular support is dobutamine. Norepinephrine and phenylephrine are used, but primarily during anaesthesia and postoperatively. Patients in need of assisted ventilation are normoventilated (PaCO₂ 4.5-5.5 kPa), with a target PaO₂ > 12 kPa. For sedation, continuous infusions of propofol and/or midazolam are used. The sedation level is adjusted so that the patients can cooperate with the ventilator and...
are able to cough. Thiopental is used for treatment of elevated intracranial pressure (ICP). For analgesia, paracetamol, ketobemidone, or fentanyl are used. Enteral feeding is started as early as possible. The cerebral aneurysms are usually treated within 24 h after admittance by surgical or endovascular intervention. Surgical evacuation of significant intracranial hematomas is performed. Hydrocephalus is treated with external ventricular drainage (EVD).

**Monitoring**

Repeated neurological examination for assessment of level of consciousness, focal impairment, and pupil reaction are performed at least every eight hours. ICP was monitored with an intraparenchymal pressure-measuring device (Codman MicroSensor™, Johnson & Johnson Professional Inc., USA) or by EVD. Calibration of the Codman microsensor was made according to the manufacturer’s instructions. The external meatus was used as zero reference level for the EVD. Usually the EVD’s were open for drainage at the level of 20 mmHg. Transcranial Doppler (TCD) (EZ Dop ® (DWL Elektronische system GmbH, Singen, Germany) was used for measurement of mean flow velocity in the middle cerebral artery (MCA). TCD was performed at suspicion of cerebral vasospasm. Repeated CT scans were used for detection of re-bleeding, hematomas, oedema, or hydrocephalus. CTA was, in the majority of cases, used for detection of the aneurysms. DSA was used for detection and treatment of the aneurysm, and for diagnosis and treatment of cerebral vasospasm.

Invasive systemic blood pressure was measured continuously with the zero reference at heart level. CPP was given as mean arterial pressure minus intracranial pressure (MAP-ICP). Physiological parameters: ICP, CPP, invasive systemic blood pressure, oxygen saturation, and end-tidal carbon dioxide (EtCO₂) were continuously monitored (Marquette, GE Medical Systems AB, Stockholm, Sweden) and stored by a computerized system (PICIS, Inc. Wakefield, MA, USA).

Electrocardiography (ECG) was continuously monitored, and a 12-lead ECG was taken daily during the first five days after admission.

**EEG**

For cEEG the NicoletOne® equipment (VIASYS Healthcare Inc., USA) was used. The availability of EEG monitoring equipment and staff that could start the monitoring, decided how many patients could be included in the study. Initially a routine 16-lead surface EEG was made with electrodes placed according to the international 10-20 system. For continuous recording, five subcutaneous electrodes were used. Four active electrodes in positions F3/P3 and F4/P4, and one reference electrode were used (Fig 3). The bedside cEEG was displayed as a continuously scrolling two-channel display of F3/P3 and F4/P4 (Fig 4). Notch filtering was not used in order to permit the staff to detect poor quality electrode connection and increased impedance at the active electrodes. The staff was taught to write down
Patients and Methods

text comments on the recorded cEEG curve, not only regarding suspected epileptic seizures, but also about patient care.

![Fig 3. Schematic view over the placement of the EEG needle electrodes.]

![Fig 4. Continues two-channel display of F3/P3 and F4/P4 (with permission from Lars-Johan Liedholm and Mattias Schindele)]

After six hours, locally stored cEEG activity was transferred to a central server at the same time as the next six hours of recording was started. Evaluations of the recorded cEEG files were done offline.
Patients and Methods

The EEG criteria for epileptic seizure activity included:

1. Presence of uni- or bilateral rhythmic activity distinguishable from the background activity, with or without high frequency components, or with crescent-shaped appearance and short time for cessation/abolition, eventually preceded by a sudden and temporary drop in EEG amplitude.
2. Pseudo-rhythmic spikes or spike-wave activity.

Laboratory analysis

Total serum and saliva cortisol were obtained every second morning between 04.00 and 09.00 am. Blood sampling for serum cortisol is a standard laboratory procedure at the accredited laboratory at UUH. The blood sample was obtained according to the normal routine procedures at the intensive care unit (ICU) or at the Neurosurgical High Dependency Unit (NHDU). Serum cortisol was analysed with an immunoassay method, (Roche Elecsys reagents on a Modular E170 analyser).

Fig 5. Saliva test tube with cotton swab
(Sarstedt, Salivette, Orion Diagnostica, Finland)

Saliva samples were obtained using a test-tube containing a cotton swab (Sarstedt, Salivette, Orion Diagnostica, Finland) (Fig 5). The saliva sample was always obtained before breakfast. Patients who were awake and could understand instructions were asked to chew on the cotton swab until soaked with saliva. In sedated patients the cotton swab was placed in the buccal cavity until considered as sufficiently soaked. The soaked cotton swabs were thereafter placed in the test tubes and immediately taken down to the laboratory. At the laboratory, saliva was expressed out of the cotton swab into a collection vial by centrifugation for 10 minutes and thereafter analysed by Spectria Cortisol Ria (Orion Diagnostica, Finland), according to the manufacturer’s instructions.

CBG was obtained every second day as a research sample. The blood sample was immediately centrifuged at 3000 rpm for 15 minutes and stored in −70° until transported to the accredited laboratory at Sahlgrenska University Hospital.
Patients and Methods

(Gothenburg, Sweden) where it was analysed with a radioimmunoassay method (Biosource; Lifescreen, Watford, Herts, UK).

Determination of cFC from SC and CBG was done using Coolens’ equation (Coolens et al 1987).

\[
cFC = \sqrt{(z^2 + 0.0122 \times SC)} - z
\]

\[
z = 0.0167 + 0.182(CBG - SC)
\]

cFC = calculated free cortisol
SC = serum cortisol
CBG = corticosteroid binding globulin

ADMA and arginine in serum were obtained as research samples every morning between 08:00 and 11:00 am. Blood samples were immediately centrifuged at 3000 rpm for 15 minutes, instantly frozen, and stored at -70°C until analysed. ADMA and arginine concentrations were measured by high-performance liquid chromatography (HPLC). As previously described, ortho-phthalaldehyde was the agent used for fluorescence determination (Rodling-Wahlström et al. 2012). Fluorescence detection was performed using the CMA/280 (CMA Microdialysis AB, Solna, Sweden) with excitation 330-365 nm, and emission 440-530 nm. Integration of area under the curve for each peak was done using Chromeleon Software (Dionex Cooperation, Sunnyvale, CA, USA) (Fig 6).

An EDTA test tube was used for blood sampling of cytokines (IL-1β, IL-6, IL-8, IL-10, and TNF-α). Blood samples were immediately centrifuged at 3000 rpm for 15 minutes, instantly frozen, and stored at -70°C until analysed with the Bio-Plex cytokine assay (Bio-Rad Laboratories Inc., Hercules, CA, USA). The analyses were done according to manufacturer’s instructions.

CRP was obtained every morning at the ICU as well as when considered clinically necessary at the NHDU. The blood sample was obtained according to normal routine procedures at the ICU or NHDU. Creatinine and troponin were also obtained according to hospital standard procedures. Blood gases were analysed at the ICU and NHDU. CRP, creatinine, and troponin were analysed using standard laboratory procedures at the accredited laboratory at UUH.
Patients and Methods

Fig 6. Determination of ADMA and arginine levels
Typical curve for standard sample. Fluorescence detection of ADMA and arginine was used with excitation at 330-365 nm and emission at 440-530 nm. Integration of area under the curve for each peak was done to determine concentration in sample.

Epidemiological aspects

Registers used included:

- International Classification of Diseases (ICD), Ninth and Tenth Revisions (ICD-9, ICD-10)
- The medical record system at the neurosurgical department, UUH; Computerized System Cross from 2002, paper records 1986-2002
- Swedish personal identity number (PIN) register
- Swedish national registry (Folkbokföring) available at the Swedish tax agency (Skatteverket, Stockholm)
- National Board of Health and Welfare (Socialstyrelsen, Stockholm); Open database on causes of death
- MONICA study in northern Sweden 1994 (www9.umu.se/phmed/medicin/monica)

In Paper IV, after requisition, the death certificates in the study cohort as identified by the Swedish personal identity numbers were obtained from the
Patients and Methods

National Board of Health and Welfare (Socialstyrelsen, Stockholm). Causes of death in the general population from the same geographical area as the study cohort were retrieved from the open database found on the website of the National Board of Health and Welfare (www.socialstyrelsen.se).

The northern Sweden MONICA study started in 1984 in two of the northernmost counties in Sweden, Västerbotten and Norrbotten. The aim of the MONICA study was to evaluate mortality and morbidity in cardiovascular diseases over time and relate them to risk factors. From 1986-2006, during the period when the study cohort was included, five population-based surveys took place: 1986, 1990, 1994, 1999, and 2004. The participation rate was acceptable (69-81%). The questions used from the MONICA survey for comparison between the study cohort and a general population are given in the Appendix.

Statistics

The statistical software package, Prism, version 5.0 (GraphPad Software, Inc, CA, USA), and the Statistical Package for the Social Sciences, SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) were used to perform the statistical analyses. The limit for statistical significance was set at p<0.05.

Specific statistical aspects- Paper I

In this paper, the primary aim was to assess the frequency of CIRCI, defined as a serum cortisol < 200 nmol/L, in the acute phase (0-240 hours) after SAH, and to evaluate the associations between cortisol levels and clinical parameters: sedation, circulatory failure, gender, age, severity of disease, and endovascular/surgical treatment.

To evaluate how measurement of SC, cFC and saliva cortisol levels change over time; the mixed effect model was used. To adjust for correlations within patients over time, a first-order autoregressive residual covariance structure with heterogeneous variances was used.

Bonferroni adjustment was used to account for multiple comparisons. The goodness of fit of the model was estimated by investigating the residuals and crucial deviations from the normal distribution.

Logistic regression models were used to evaluate how cardiovascular SOFA, morning SC, calculated FC, gender, Hunt & Hess, type of intervention, and sedation and age influence morning SC (< 200 nmol/L), cFC (<22 nmol/L), and cardiovascular SOFA (≥3), respectively.

To adjust for correlations within patients over time, the method of generalized estimating equation with an unstructured correlation structure was used. From a model including all main effects, a backward elimination approach with a p-value cut-off point of 0.1 was used.
Patients and Methods

The Wilcoxon Sign Rank test was used for comparison of sedated and unsedated samples over time. Fisher’s exact test was used for association between survivors/non-survivors.

Specific statistical aspects - Paper II

In order to avoid the effect of repeated measurements and differences in number of values in individual patients, only peak ADMA values and peak/nadir arginine/ADMA ratios were used, which gives only one value/patient. The non-parametric Mann-Whitney test was used for comparison of ADMA and arginine/ADMA ratios, as well as different clinical parameters. The non-parametric Wilcoxon signed rank test was used for comparison of laboratory parameters at different time intervals. All cytokine values, including those under detection range, were included in the statistical analysis.

Specific statistical aspects - Paper IV

The Mann-Whitney test was used for comparison between male and female patients in the SAH cohort, and Fisher’s exact test or the chi-square test for comparison between the study cohort and a general population.

SCORING

Glasgow Coma Scale (GCS) is used to describe the level of consciousness. The GCS scale is a combination of motor response, verbal response, and eye movement (Teasdale et al. 1974)

<table>
<thead>
<tr>
<th>Motor response</th>
<th>Verbal response</th>
<th>Eye movement</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Obeying commands</td>
<td>5 Orientation</td>
<td>4 Spontaneous eye opening</td>
</tr>
<tr>
<td>5 Localising response</td>
<td>4 Confused conversation</td>
<td>3 Open eye on command</td>
</tr>
<tr>
<td>4 Withdraw, flexion</td>
<td>3 Inappropriate speech</td>
<td>2 Open eye on painful stimuli</td>
</tr>
<tr>
<td>3 Abnormal flexion</td>
<td>2 Incomprehensible sound</td>
<td></td>
</tr>
<tr>
<td>2 Extensor posturing</td>
<td>1 No response</td>
<td></td>
</tr>
<tr>
<td>1 No response</td>
<td>1 No response</td>
<td>1 No response</td>
</tr>
</tbody>
</table>

The total sum of GCS score_ 3 - 15
Patients and Methods

**Hunt and Hess (H&H) score** is a grading system used to classify the severity of the SAH based on the patient's clinical condition (Hunt et al. 1968)

**Hunt and Hess score**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Asymptomatic or minimal headache, slight nuchal rigidity</td>
</tr>
<tr>
<td>II</td>
<td>Moderate to severe headache, nuchal rigidity, no neurological insufficiency (except cranial nerve palsy)</td>
</tr>
<tr>
<td>III</td>
<td>Drowsiness, confusion or mild focal deficit</td>
</tr>
<tr>
<td>IV</td>
<td>Stupor, moderate to severe hemiparesis, vegetative disturbance or early decerebrate rigidity</td>
</tr>
</tbody>
</table>

**World Federation of Neurosurgical Societies scale (WFNS scale)** is a grading scale created for SAH, that uses the GCS, and adds the presence or not of focal neurological deficits to estimate the severity of SAH (Drake et al. 1988).

**WFNS scale**

<table>
<thead>
<tr>
<th>Grade</th>
<th>GCS</th>
<th>Focal neurological deficit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>Absent</td>
</tr>
<tr>
<td>2</td>
<td>13 - 14</td>
<td>Absent</td>
</tr>
<tr>
<td>3</td>
<td>13 - 14</td>
<td>Present</td>
</tr>
<tr>
<td>4</td>
<td>7 - 12</td>
<td>Present or absent</td>
</tr>
<tr>
<td>5</td>
<td>&lt;7</td>
<td>Present or absent</td>
</tr>
</tbody>
</table>
**Patients and Methods**

*Fischer scale* correlates to the amount of blood on the first CT scan (Fisher et al. 1980)

<table>
<thead>
<tr>
<th>Group</th>
<th>Amount of blood on CT scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No subarachnoid blood detected on CT</td>
</tr>
<tr>
<td>2</td>
<td>Subarachnoid blood &lt;1 mm with no clots</td>
</tr>
<tr>
<td>3</td>
<td>Localised clots and/or subarachnoid blood &gt;1 mm</td>
</tr>
<tr>
<td>4</td>
<td>Intraventricular or intracerebral haemorrhage or both, Subarachnoid blood &gt; or ≤1 mm</td>
</tr>
</tbody>
</table>

*Glasgow Outcome scale* (GOS) describes the degree of disability after brain damage (Jennet et al. 1975)

<table>
<thead>
<tr>
<th>Glasgow Outcome Scale</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Death</td>
<td></td>
</tr>
<tr>
<td>2 Vegetative state, persistent</td>
<td></td>
</tr>
<tr>
<td>3 Severe disability</td>
<td></td>
</tr>
<tr>
<td>4 Moderate disability</td>
<td></td>
</tr>
<tr>
<td>5 Good recovery</td>
<td></td>
</tr>
</tbody>
</table>
**Patients and Methods**

Sequential Organ Failure Assessment (SOFA) scores the function of six different organs. The most abnormal value from the preceding 24 hours is used (Vincent et al 1996)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respiration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{PaO}_2/\text{FiO}_2$ kPa</td>
<td>40.0 - 53</td>
<td>26.7 - 39.9</td>
<td>13.4 - 26.6</td>
<td>0.0 - 13.3</td>
</tr>
<tr>
<td><strong>Haematology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets $\times 10^9/L$</td>
<td>101 - 150</td>
<td>51 - 100</td>
<td>21 - 50</td>
<td>0 - 20</td>
</tr>
<tr>
<td><strong>Hepatic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilirubin $\mu$mol/L</td>
<td>20 - 32</td>
<td>33 - 101</td>
<td>102 - 204</td>
<td>&gt; 205</td>
</tr>
<tr>
<td><strong>Cardiovascular</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypotension:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mm Hg</td>
<td>MAP &lt; 70</td>
<td>Dopamine 0.1 - 4.9</td>
<td>Dopamine 5 - 14.9</td>
<td>Dopamine &gt; 15</td>
</tr>
<tr>
<td>$\mu$g/kg/min</td>
<td></td>
<td>Dobutamine &gt; 0.1</td>
<td>Epinephrine &lt; 0.1</td>
<td>Epinephrine &gt; 0.1</td>
</tr>
<tr>
<td>Renal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine $\mu$mol/</td>
<td>110 - 170</td>
<td>171 - 299</td>
<td>300 - 440</td>
<td>&gt; 441</td>
</tr>
<tr>
<td>Diuresis mL/day</td>
<td></td>
<td>201 - 499</td>
<td>300 - 440</td>
<td>&gt; 441</td>
</tr>
<tr>
<td><strong>Central Nervous system</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCS</td>
<td>13 - 14</td>
<td>10 - 12</td>
<td>6 - 9</td>
<td>3 - 6</td>
</tr>
</tbody>
</table>
Results

RESULTS

Patients

For Paper I, 89 patients were eligible; for paper II, 95 patients. In all, 39 patients with aneurysmal SAH were excluded:

- 24 patients, due to lack of research nurses at the time of admission
- 6 patients, due to late arrival (≥ 48h after the SAH)
- 5 patients, due to earlier SAH or earlier intracranial surgery
- 3 patients, due to two that were admitted to UUH from another neurosurgical department only for neurointervention, and one patient sent from UUH to another neurosurgical department for surgery
- 1 patient declined participation in the study

In Paper I, two patients were excluded due to hydrocortisone treatment and previous radiotherapy to the head (these patients were included in Paper II). Since the inclusion in Paper I ended one month earlier than inclusion in Paper II, an additional four patients were included in Paper II. In total, 50 patients were included in Paper I and 56 patients in Paper II.

In Paper III, 28 patients with aneurysmal SAH, treated at the ICU, sedated, and with cEEG monitoring, were retrospectively included. Of these patients, 19/28 (68%) were also included in Papers I and II.

In Paper IV, 162 SAH patients treated at UUH between 1986 and 2006 who became deceased during the period after having survived the SAH ≥ one year were included. None of the patients were included in any of the other papers.

Results- Paper I

The primary aim was to assess the frequency of CIRCI in the acute phase (0-240 h) after SAH. CIRCI was defined as morning SC < 200 nmol/L. The lower limit for a cFC was set at < 22 nmol/L, with the lower limit for morning SaC at < 7.7 nmol/L. The percentage of patients with cortisol values under the reference limit in the acute phase (0-240 h) is given in Fig 7.

There was no significant difference found between different time intervals in the acute phase in SC, cFC or in SaC. However although not significant the lowest mean values were detected at 49-96 h after the SAH (Table 5).

The primary aim was also to evaluate associations between cortisol levels and different clinical parameters.
Results

Fig 7. Percentage of patients under reference limit for morning SC (<200 nmol/L), calculated FC (<22 nmol/L) and saC (<7.7 nmol/L) during the acute phase (0-240 h) after SAH.
SC= morning serum cortisol, cFC= calculated free cortisol, SaC= Saliva cortisol

<table>
<thead>
<tr>
<th>Time-interval after SAH</th>
<th>Morning SC mean±SD in nmol/L</th>
<th>cFC mean±SD in nmol/L</th>
<th>SaC mean±SD in nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-48 h</td>
<td>571±352</td>
<td>64.4±65.4</td>
<td>37.1±42.7</td>
</tr>
<tr>
<td>n</td>
<td>34</td>
<td>33</td>
<td>12</td>
</tr>
<tr>
<td>49-96 h</td>
<td>458±316</td>
<td>48.1±52.0</td>
<td>25.6±20.9</td>
</tr>
<tr>
<td>n</td>
<td>46</td>
<td>46</td>
<td>23</td>
</tr>
<tr>
<td>97-144 h</td>
<td>584±268</td>
<td>51.9±38.8</td>
<td>34.5±27.6</td>
</tr>
<tr>
<td>n</td>
<td>46</td>
<td>46</td>
<td>24</td>
</tr>
<tr>
<td>145-192 h</td>
<td>617±293</td>
<td>55.0±46.7</td>
<td>33.2±24.6</td>
</tr>
<tr>
<td>n</td>
<td>42</td>
<td>41</td>
<td>21</td>
</tr>
<tr>
<td>193-240 h</td>
<td>593±256</td>
<td>45.1±33.0</td>
<td>31.9±28.3</td>
</tr>
<tr>
<td>n</td>
<td>36</td>
<td>35</td>
<td>21</td>
</tr>
</tbody>
</table>

Table 5. Mean morning SC, cFC and SaC in the acute phase (0-240 h) after SAH.
Time-interval with the lowest mean value of morning SC, cFC and SaC in grey boxes.
SC= serum cortisol, SaC= saliva cortisol, cFC= calculated free cortisol, n = number of values

A significant impact on cortisol levels was found when the effect of continuous infusion of sedative drugs was evaluated. Continuous intravenous sedation at the time of blood sampling was the strongest predictor for a morning SC < 200 nmol/L and for a cFC < 22 nmol/L. The odds of having a morning SC < 200 nmol/L was 18 times higher in samples obtained in a patient during continuous infusion of sedative drugs compared to samples obtained in a patient without continuous infusion of sedative drugs (95% Confidence interval 4.2-85.0, p < 0.001). The odds of having a cFC < 22 nmol/L was 2.4 times higher in a patient with continuous infusion of sedative drugs compared to a patient without continuous infusion of sedative drugs (95% Confidence interval 1.2-4.7, p<0.05).
**Results**

The odds of having a morning SC < 200 nmol/L was 4.0 times higher in a patient with a cardiovascular SOFA score ≥ 3 (indicating severe cardiovascular failure) compared to a patient with a SOFA < 3 (95% Confidence interval 1.2-13.7, p < 0.05). But a cardiovascular SOFA ≥ 3 was only registered in patients with continuous infusion of sedative agents.

No patient > 62 years of age had a morning SC of < 200 nmol/L. The odds for having a morning SC > 200 nmol/L increased by 1.07 for every year added (95% confidence interval 1.02–1.12, p < 0.05).

Saliva samples were difficult to obtain primarily due to dry mouth. SaC could only be analysed in 101 samples compared to 204 samples for SC and 201 for cFC. Due to the limited numbers of values, SaC could not be included in the multivariate analysis.

A secondary aim was to evaluate whether an association could be found between cortisol levels in the acute phase and outcome. Mortality at 28 days or unfavorable outcome (GOS 1-3) was not significantly associated with morning cortisol levels SC < 200 nmol/L, or cFC < 22 nmol/L in the acute phase.

**Results- Paper II**

The primary aim was to evaluate ADMA levels and arginine/ADMA ratio in the acute phase (0-240 h) after SAH. The results showed that ADMA levels in SAH patients at admission were significantly higher (0.43± 0.14 µmol/L, n=56) compared to sex- and age-matched healthy controls (0.32±0.13 µmol/L, n=112), p < 0.0001. Compared to admission values 0-48 h after SAH, ADMA levels were significantly increased at 97-240 h after SAH. The arginine/ADMA ratio and inflammatory markers (CRP, IL-1β, IL-6, IL-8, IL-10 and TNF-α) were also followed, and were significantly elevated at different time intervals after SAH compared to admission values (Table 6, Table 7).

The primary aim was also to evaluate associations between ADMA levels and arginine/ADMA ratios in the acute phase (0-240 h) after SAH with different parameters such as gender, age, Fischer grade, H&H score, hypertension troponin, ECG, and organ failure.

ADMA levels and arginine/ADMA ratio at admission (0-48 h) after SAH in comparison to different parameters studied are given in Table 8.

Peak/nadir ADMA levels and peak /nadir arginine/ADMA ratios during the acute phase (0-240 h) after SAH in comparison to different parameters studied are given in Table 9.

In 31/56 (55%) patients, one or more signs of impaired cerebral circulation could be found in the acute phase (0-240 h) after the SAH (Fig 8). However, no significant difference in peak ADMA levels or nadir arginine/ADMA ratios could be found between patients with or without any sign of impaired cerebral circulation (Fig 9).
### Results

<table>
<thead>
<tr>
<th>Time-interval after SAH</th>
<th>ADMA μmol/L</th>
<th>arginine/ADMA ratio</th>
<th>CRP mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-48 h Admission n</td>
<td>0.43±0.14</td>
<td>13.2±5.5</td>
<td>11±10</td>
</tr>
<tr>
<td>49-72 h n</td>
<td>0.39±0.17</td>
<td>14.0±5.0*</td>
<td>90±61*</td>
</tr>
<tr>
<td>73-96 h n</td>
<td>0.44±0.15</td>
<td>14.6±5.5</td>
<td>122±90*</td>
</tr>
<tr>
<td>97-120 h n</td>
<td>0.50±0.16*</td>
<td>14.7±4.7</td>
<td>120±97*</td>
</tr>
<tr>
<td>121-144 h n</td>
<td>0.58±0.21*</td>
<td>15.5±6.5</td>
<td>85±97*</td>
</tr>
<tr>
<td>145-168 h n</td>
<td>0.56±0.19*</td>
<td>15.3±5.0*</td>
<td>69±62*</td>
</tr>
<tr>
<td>169-192 h n</td>
<td>0.61±0.20**</td>
<td>14.7±4.3*</td>
<td>54±48*</td>
</tr>
<tr>
<td>193-216 h n</td>
<td>0.62±0.19**</td>
<td>14.9±4.3*</td>
<td>50±52*</td>
</tr>
<tr>
<td>217-240 h n</td>
<td>0.69±0.20**</td>
<td>14.5±4.4*</td>
<td>32±21*</td>
</tr>
</tbody>
</table>

Table 6. ADMA, arginine/ADMA ratio and CRP at different time intervals in the acute phase (0-240 h) after SAH. ADMA= Asymmetric dimethylarginine, n = numbers of samples, CRP= C-reactive protein Grey box=Significant difference compared to admission value 0-48 h after SAH *significant increase. # significant decrease

<table>
<thead>
<tr>
<th>Time-interval after SAH</th>
<th>IL-1β pg/ml</th>
<th>IL-6 pg/ml</th>
<th>IL-8 pg/ml</th>
<th>IL-10 pg/ml</th>
<th>TNF-α pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-48 h Admission n</td>
<td>0.5±1.2</td>
<td>62.0±72.1</td>
<td>14.6±13.8</td>
<td>3.4±4.2</td>
<td>5.9±10.2</td>
</tr>
<tr>
<td>49-96 h n</td>
<td>0.8±1.8</td>
<td>75.2±116</td>
<td>15.3±18.0</td>
<td>2.9±3.5</td>
<td>7.4±11.7</td>
</tr>
<tr>
<td>97-144 h n</td>
<td>0.5±1.1</td>
<td>28.6±116#</td>
<td>15.6±11.9</td>
<td>4.0±6.3</td>
<td>6.1±7.8</td>
</tr>
<tr>
<td>145-192 h n</td>
<td>0.4±0.9</td>
<td>17.2±18.2#</td>
<td>13.8±10.6</td>
<td>3.7±4.5</td>
<td>5.9±6.2</td>
</tr>
<tr>
<td>193-240 h n</td>
<td>0.7±2.1</td>
<td>24.7±40.0#</td>
<td>13.2±11.3</td>
<td>4.3±5.1</td>
<td>7.3±11.9</td>
</tr>
</tbody>
</table>

Table 7. IL-1β, IL-6, IL-8, IL-10, and TNF-α at different time intervals in the acute phase (0-240 h) after SAH. IL-1β= interleukin-1beta, IL-6= interleukin-6, IL-8= interleukin-8, IL-10= interleukin-10, TNF-α= Tumor Necrosis Factor-alpha, n = numbers of samples. Grey box=significant difference compared to admission value 0-48 h after SAH. # significant decrease
## Results

<table>
<thead>
<tr>
<th>Parameter studied</th>
<th>Significant difference in ADMA µmol/L</th>
<th>Significant difference in arginine/ADMA ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender M/F</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Age years&lt;60/≥60</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Fisher grade 2/3/4</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>H&amp;H 1/2/3/5</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Hypertension no/yes</td>
<td>0.39±0.10/ 0.50±0.18</td>
<td>NO</td>
</tr>
<tr>
<td>Troponin &lt;0.01/≥ 0.01µg/L</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>ECG no remarks/remarks</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>SOFA Cardiovascular 0-2/3-4</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>SOFA Respiratory 0-2/3-4</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>SOFA Renal 0-2/3-4</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

Table 8. ADMA and arginine/ADMA ratio ratios at admission 0-48 h after SAH. Comparison and significant differences between parameters studied

1median age = 60 years, H&H= Hunt & Hess, SOFA= Sequential Organ Failure Assessment, na= not applicable; Grey box=significant difference
### Results

<table>
<thead>
<tr>
<th>Parameter studied</th>
<th>Significant difference in ADMA µmol/L</th>
<th>Significant difference in arginine/ADMA ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak</td>
<td>Nadir</td>
</tr>
<tr>
<td>Gender M/F</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Age years(^1) &lt;60/≥60</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Fisher grade 2-3/4</td>
<td>0.61±0.20/0.78±0.22</td>
<td>NO</td>
</tr>
<tr>
<td>H&amp;H 1-2/3-5</td>
<td>0.65±0.14/0.83±0.23</td>
<td>NO</td>
</tr>
<tr>
<td>Hypertension no/yes</td>
<td>0.73±0.23/0.83±0.18</td>
<td>0.30±0.1/0.37±0.1</td>
</tr>
<tr>
<td>Troponin &lt;0.01/≥ 0.01µg/L</td>
<td>NO</td>
<td>no</td>
</tr>
<tr>
<td>ECG no remarks/remarks</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>SOFA Cardiovascular 0-2/3-4</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>SOFA(^7) Respiratory 0-2/3-4</td>
<td>0.66±0.16/0.82±0.23</td>
<td>NO</td>
</tr>
<tr>
<td>SOFA(^7) Renal 0-2/3-4</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

Table 9. Peak/nadir levels of ADMA and arginine/ADMA ratios during the acute phase (0-240 h) after SAH. Comparison and significant differences between parameters studied.\(^1\)median age = 60 years. Peak value= the highest value observed in every patient. Nadir value=the lowest value observed in every patient. H&H= Hunt & Hess, SOFA= Sequential Organ Failure Assessment, na= not applicable. Grey box=significant difference
Results

Fig 8. Percentage of patients with pathological findings in relation to patients examined. The patients can have more than one examination and one patient can have more than one pathological finding. Numbers given inside the bars = numbers of patients. DSA= Digital subtraction angiography, CTA= Computed tomography angiography, CT=computed tomography, TCD= Transcranial Doppler, GCS= Glasgow Coma Scale, Total = Patients with one or more signs of impaired cerebral circulation.

Fig 9. Peak ADMA levels and nadir arginine/ADMA ratio in patients with or without signs of impaired cerebral circulation. Mean ± SD during the acute phase after SAH (0-240 h). White bars = Peak ADMA levels and nadir arginine/ADMA ratio in patients without signs of impaired circulation. Grey bars = Peak ADMA levels and nadir arginine/ADMA ratio in patients with signs of impaired circulation.
Results

When patients were divided according to their clinical condition at admission, in patients with a severe clinical condition (H&H 3-5), and patients with a less severe clinical condition (H&H 1-2), significant differences were found in ADMA, arginine/ADMA ratios, CRP, and other inflammatory markers (Fig 10, Fig 11).

Fig 10. ADMA, arginine/ADMA ratio and CRP levels between patients with a severe clinical condition (H&H 3-5), and patients with a less severe clinical condition (H&H 1-2).

Mean ± SD during the acute phase after SAH (0-240 h). White bars H&H 1-2, Grey bars H&H 3-5, * = Significant difference
Results

Fig 11. IL-1β, IL-6, IL-8, IL-10 and TNF-α levels between patients with severe clinical condition (H&H 3-5) and patients with less severe clinical condition (H&H 1-2). Mean ± SD during the acute phase of SAH (0-240 h). White bars H&H 1-2. Grey bars H&H 3-5. Figures inside the bars are number of samples. *= Significant difference
ADMA increased significantly after SAH, with the increase in ADMA starting after the pro-inflammatory markers (CRP, IL-6) had peaked (Fig 12).

![Graph showing CRP, IL-6, and ADMA levels](image)

**Fig 12.** CRP, IL-6, and ADMA levels in the acute phase (0-240 h) after SAH. Mean values of all obtained values.

Follow-up was performed at median 13 (6 ± 24) months after the SAH. ADMA levels at follow-up (0.44±0.16 µmol/L, n=35) were not significantly different compared to admission values (0.41±0.15 µmol/L, n=35). At the follow-up, outcome was assessed according to GOS, where GOS 1-3 (n=15) was considered as unfavorable outcome, and GOS 4-5 (n= 41) as favourable outcome. There was no significant difference in peak ADMA levels in the acute phase between patients with unfavorable (0.76±0.22 µmol/L, n=15) or favourable (0.72±0.20 µmol/L, n=41) outcome. Further, there was no significant difference in nadir ADMA levels in the acute phase between patients with unfavorable (0.29±0.08 µmol/L, n=15) or favourable arginine/ADMA ratios in the acute phase (0-240 h) after SAH between patients with unfavourable /favourable outcome, however, peak arginine/ADMA ratio was significantly elevated in patients with favourable outcome (Fig 13).
Results

Fig 13. Peak and nadir arginine ADMA ratio in relation to outcome measured as GOS. Mean±SD during the acute phase (0-240 h) after SAH. White bars = Patients with unfavourable outcome (GOS 1-3) n=15. Grey bars = Patients with favourable outcome (GOS 4-5) n=41. *=significant differences

Results - Paper III

The aim was to assess the frequency of NCSZ and NCSE in sedated SAH patients, treated at the ICU at UUH. An EEG was continuously recorded during a total of 5500 h in 28 patients with aneurysmal SAH, median 190 h (68-475 h) per patient. Epileptic activity on the cEEG was found in 2/28 (7%) patients. One patient had a four-minute-long NCSZ on day eight after the SAH, and one patient had a 20 minutes long NCSE on day three after the SAH. Both patients were sedated with midazolam, fentanyl, and thiopental in continuous infusions when the epileptic activity was detected. Neither any clinical seizures nor any interictal epileptiform activity on the cEEG was observed in any of the 28 patients included.

Results - Paper IV

The aim of this study was to evaluate if SAH patients have an increased incidence of cardiovascular causes of death compared to the general population in the same geographical area. In connection with SAH, an inflammatory activation and elevation of ADMA is observed that might initiate development of vascular endothelial dysfunction leading to cardiovascular diseases. From 1986 to 2006, according to medical records, 1,846 patients were treated at the neurosurgical department at UUH with a diagnosis of SAH. A total of 596 patients deceased during the period; 288 had deceased < one year after the SAH. In 81 patients, medical records could not be found or the data found was incomplete; 65 patients had a proven non-aneurysmal cause of the SAH. A total 162 patients, 89 women,
and 73 men had survived their SAH ≥ one year; these patients formed the study cohort.

The median year of death in the study cohort was 2001, therefore the causes of death in the study cohort was compared to the causes of death for 2001 in the general population from the same geographical area. There was a significant difference between the two groups compared; a significantly larger part of the SAH cohort in both gender and all ages died from cerebrovascular diseases, as compared to the general population. No differences in cardiovascular causes of death were seen (Fig 14, Fig 15).

The median year of the SAH was 1993 for men and 1992 for women. The MONICA health project from 1994 was the closest to the median year of the SAH in the study cohort, and was therefore used. Compared to the controls, there was a significantly higher incidence of hypertension in the study cohort in both male and female patients ≤ 64 years of age. Tobacco use was also significantly more common in the study cohort (Fig 16).
Results

Fig 15. Causes of death in the SAH cohort compared to the general population from the same geographical area in 2001, divided in age and sex. *=significant difference
Fig 16. Co-morbidities at the time of SAH compared to the MONICA health project 1994 dived in sex and age. AMI=acute myocardial infarction, Stroke=Stroke, DM=Diabetes Mellitus, HT=Hypertension. *=significant difference
DISCUSSION

Discussion- Paper I

The main finding in this study was that continuous infusion with sedative drugs significantly decreased morning SC and cFC. Therefore, the conclusion drawn was that sedation should be considered whenever cortisol values are evaluated in critically ill SAH patients. This effect of sedation on cortisol levels could be a direct effect on the HPA axis, with reduced Adrenocorticotropic hormone (ACTH) release due to the sedative drugs given; however, this needs to be further investigated (Locatelli et al. 2010).

Hypopituitarism is described in long-term survivors of aneurysmal SAH. In a study by Dimopoulou et al, 47% of the patients had endocrine failure 12-24 months after SAH (Dimopoulou et al. 2004). Other studies have shown diverse results, with a frequency from 17% to 55% of endocrine disturbances after SAH (Tanriverdi et al. 2007, Kreitschmann et al. 2004, Lammert et al. 2012).

The HPA axis can be affected by a SAH from a ruptured cerebral aneurysm. At the time of the SAH, a reduction of blood flow to the pituitary gland can occur and also later the HPA axis can be disturbed by impaired cerebral circulation (Kelly et al. 2000, Bendel et al. 2008, Kreitschmann et al. 2005). However, few studies have been performed during the acute phase of SAH (Zetterling et al. 2011, Bendel et al. 2008, Poll et al. 2010). These studies have shown elevated cortisol levels in the early acute phase and no correlation between severity of disease and cortisol levels (Bendel et al. 2008, Poll et al. 2010). In a study by Zetterling et al, higher serum cortisol was associated with global cerebral oedema and suppressed ACTH with brain ischemia (Zetterling et al. 2011).

Etomidate, not used in Sweden, is well known for its properties to inhibit the HPA axis (Cuthbertson et al. 2009). Other sedative drugs and opioids have been described as inhibiting the HPA axis, and subsequently the cortisol production at different stages (Ambrogio et al. 2008). Long-term use of midazolam has been reported to reduce concentrations of ACTH (Mistraletti et al. 2005). Opiates such as morphine have been reported to attenuate corticotropin-releasing hormone (CRH) and induce ACTH with cortisol release (Colameco et al. 2008). Adrenal insufficiency has been found in patients that have been treated during longer periods of time with high doses of morphine (Ambrogio et al. 2010). In this study, termination of sedative drugs in continuous infusion caused an increase in cortisol levels. This finding is in accordance with a study investigating wake-up tests and cortisol levels, where an increase in cortisol levels was found in conjunction with interruption of sedation (Skoglund et al. 2012).

Depending on how cortisol was analysed (SC, cFC, SaC), 45-60 % of the patients in Paper I had, at any time during the acute phase (0-240 h) after the SAH, cortisol levels under the lower reference limit defined in this study. The lowest
mean values, although not significant, of morning SC, cFC and SaC were found in the time-interval 49-96 h after SAH. This may indicate a transient exhaustion of the HPA axis, or a shortage of substrate for cortisol synthesis, after excessive release of cortisol immediately after SAH (Venkataraman et al. 2007, Poll et al. 2010, Bendel et al. 2008).

There is no storage of cortisol in the adrenal gland, and therefore an increase in cortisol, as a response to ACTH stimulation is mainly due to de novo synthesis of cortisol in the adrenal cortex. The substrate for cortisol synthesis is cholesterol, preferably high-density lipoproteins (HDL) (Marik et al. 2009). In critically ill patients, HDL levels have been reported as decreased, and the magnitude of this hypocholesterolaemia seems to reflect severity of inflammation (Kruger et al. 2009).

Under normal conditions, only 10 % of the cortisol in plasma is free and active, 75 % is bound to CBG, and 15 % is bound to albumin (Hamrahian et al. 2004). In this study, the lowest levels of CBG were found early after SAH, in the time-interval 0-96 h. This result could be due to capillary leakage. Serum cortisol samples taken in this early interval after SAH may therefore overestimate a true adrenal insufficiency, and repeated cortisol samples should be taken before substitution with hydrocortisone is considered.

Older age was associated with significantly higher cortisol values in the acute phase, no patient > 62 years had a morning SC < 200 nmol/L. This effect of age on cortisol in this study was earlier described and explained as a reduced glucocorticoid feedback in older individuals (Wilkinson et al. 2001).

Since low cortisol values could indicate adrenal insufficiency, it could have been plausible that low cortisol levels during the acute phase would have influenced outcome. However, unfavourable outcome was not significantly associated with cortisol levels under the lower reference level for the acute phase in this study.

Discussion - Paper II

The ADMA level in the SAH cohort was increased at admission in comparison to controls from a MONICA health project. Patients with pharmacologically treated hypertension had significantly higher ADMA levels compared to patients without known hypertension. This is in accordance with other studies, since ADMA has been reported to be associated with a variety of cardiovascular diseases (Sibal et al. 2010, Boger et al. 2009).

ADMA levels in the SAH cohort started to increase significantly compared to admission values from the time interval 97-120 h after SAH, and continued to increase until the end of the acute phase (240 h after SAH). This delayed increase in ADMA levels in SAH patients has been reported earlier (Rodling-Wahlstrom et al. 2012).
ADMA is a marker of endothelial dysfunction and, as such, is associated with inflammation (Boger et al. 2009). SAH patients have an activated inflammatory response (Carr et al. 2013). In this study, an immediate and early increase in pro-inflammatory markers, (CRP, IL-6) was observed. This early increase in pro-inflammatory markers was followed by a decline at the same time as an increase in ADMA started.

The inflammatory pattern after SAH thus seems to be divided into two stages. First, an early stage 0-96 h after SAH, and dominated by an early rise and an early decline in CRP and IL-6 levels; the later second stage, starting between 97-120 h after SAH, is dominated by an increase in ADMA. This could indicate that an inflammatory response could initiate endothelial dysfunction, indicated by a rise in ADMA.

Patients with a more severe clinical condition (H&H 3-5) had a more pronounced inflammatory response, higher ADMA levels, and lower arginine/ADMA ratios in the acute phase (0-240 h after SAH) compared to patients in a less severe clinical condition (H&H 1-2).

In one study by Staalso et al, a low arginine/ADMA ratio was associated with poorer outcome (Staalso et al. 2013). In this study, unfavourable outcome was not significantly associated with the peak ADMA level or the nadir arginine/ADMA ratio during the acute phase. However, a higher peak arginine/ADMA ratio during the acute phase was significantly associated with favourable outcome, indicating that the levels of arginine and ADMA are of importance for outcome in SAH patients.

Cardiovascular failure measured with circulatory SOFA, troponin release, and ECG abnormalities had no impact on ADMA levels in the acute phase. The cardiovascular failure, troponin release, and ECG abnormalities seen after SAH are probably more influenced by the massive catecholamine release reported to occur after SAH (Naredi et al. 2000).

In this study, severe respiratory failure was associated with higher ADMA levels, and this result is in line with findings reported from animal studies of acute lung injury (Sharma et al. 2010). The higher ADMA levels could reflect a higher degree of inflammation in patients with respiratory failure.

DIND was defined according to the criteria proposed by Vergouwen et al (Vergouwen et al. 2010). Surprisingly, no difference in ADMA level or in nadir arginine/ADMA ratio was seen between patients with or without signs of impaired cerebral circulation in this study. This could be due to that only systemic samples were investigated. The systemic response seems to be more dependent on the clinical condition of the patient and the degree of systemic inflammation. In a small clinical study, ADMA levels in CSF were associated with vasospasm (Jung et al. 2007).
Discussion

**Discussion- Paper III**

Few studies have been performed on the subject of occurrence of NCEZ/NCSE in patients treated at neurointensive care units (Claassen et al. 2013). The published studies show different frequencies of seizures detected, from 1 to 36% (Dennis et al. 2002, Little et al. 2007, Claassen et al. 2004). This difference in frequency is partly due to diverse inclusion criteria. In this study, all sedated and mechanically ventilated SAH patients treated at the ICU were eligible for inclusion. The cEEG monitoring was only limited by the access of EEG monitors. The frequency of NCEZ/NCSE found was 7% (2/28 patients). This relatively low frequency could be due to different causes:

1. All patients were under continuous intravenous sedation with midazolam or propofol in the majority of hours recorded.
2. Wake-up tests were not used.
3. All patients received intravenous nimodipine (Nimotop®, Bayer), a calcium channel blocker that might have anti-epileptic properties (Hasan et al. 2013).
4. Only five electrodes were used for the cEEG monitoring so, even if not likely, NCEZ/NCSE could have been missed even though the possibility for digital off-line recombination of electrode montages was presumed to allow a sufficient quality of monitoring for detecting seizures.

Epileptic seizures are known to induce metabolic injuries indicated by the elevation of extracellular glutamate and lactate, and a decrease in glucose (Claassen et al. 2013, Vespa et al. 1998). This could worsen an injury in an already vulnerable brain. Even though the frequency of NCEZ/NCSE found in this study was low, it was discovered in sedated patients. Therefore the conclusion is that continuous EEG is an important monitoring tool in sedated SAH patients. (Bederson et al. 2009)

**Discussion- Paper IV**

The main result in this study was that a significantly higher risk of death due to cerebrovascular causes was seen in long-term survivors of SAH compared to a general population from the same geographical area in northern Sweden. The cerebrovascular causes of death were not only the result of sequelae due to the SAH or re-bleeding of the initial SAH, but SAH patients also died due to intracerebral haemorrhage or ischemic stroke, indicating a vulnerability of the cerebrovascular system after SAH. This vulnerability could due to development of an endothelial vascular dysfunction after SAH. The primary hypothesis put forward, namely that long-term survivors after SAH could, due to the development of endothelial vascular dysfunction, have an increased incidence of cardiovascular causes of death, had to be rejected. No increased incidence of cardiovascular causes of death was seen in this study, in contrast to other studies (Ronkainen et al. 2001, Huttunen et al. 2011).
In this study, at the time of the SAH, a significant difference in co-morbidities was found in the SAH cohort compared to individuals from the MONICA health project from 1994. The well known risk factors for SAH – hypertension and tobacco use – were significantly more common in both men and women in the SAH cohort compared to the individuals from the health project. But this difference was only found in younger patients (≤ 64 years). The occurrence of co-morbidities in older patients (> 64 years) did not differ between the SAH cohort and individuals from the MONICA health project.

The finding that long-term survivors of SAH have a significant increased risk of death from cerebrovascular causes indicates that SAH patients should have the same rigorous follow-up as other stroke patients.
LIMITATIONS

**Limitations - Paper I**
- Limited number of patients included
- The level of sedation was not standardised
- Only SAH patients were studied; the results, therefore, cannot be transferred to critically ill patients in general

**Limitations - Paper II**
- Limited number of patients included
- Only serum concentrations were obtained, which may limit the results concerning impaired cerebral circulation
- Problems with the arginine analyses at the end of the study may have affected the results

**Limitations - Paper III**
- Two channel, five-electrode registration was used, which theoretically can miss some seizure activity.
- Retrospective study
- Lack of monitoring capacity

**Limitations - Paper IV**
- 16% of the medical records were either lost to find or incomplete
- Control cohorts from the median year of SAH and death were used, respectively. This implies a risk that changes over time in co-morbidities, and causes of death can bias the results.
- Only 2/4 of the northern counties participated in the health survey.
CONCLUSIONS

Conclusions- Paper I

Serum cortisol and calculated free cortisol are affected by continuous infusion of sedative drugs. Serum cortisol < 200 nmol/L and calculated free cortisol < 22 nmol/L were significantly associated with the presence of continuous intravenous sedation at the time of blood sampling. Sedation needs to be considered when low cortisol values are found. Repeated measurements over time are important to diagnose an eventual adrenal insufficiency.

Conclusions- Paper II

ADMA, as a marker of endothelial dysfunction, increased significantly in the acute phase after SAH. The pro-inflammatory markers CRP and IL-6 increased early after SAH, and thereafter significantly decreased in the acute phase. The increase in ADMA was delayed and associated with decrease in inflammatory markers. This implies that endothelial dysfunction, with ADMA as a marker, is induced by a systemic inflammation.

ADMA levels and arginine/ADMA ratios in the systemic circulation were affected by the severity of disease and degree of inflammation.

Systemic ADMA levels during the acute phase were not significantly different between patients with or without signs of impaired cerebral circulation.

Neither ADMA levels nor nadir arginine/ADMA ratios in the acute phase were associated with outcome, but the peak arginine/ADMA ratio during the acute phase was significantly higher in patients with favourable outcome when compared to patients with unfavourable outcome.

Conclusions- Paper III

Even though the occurrence of NCSZ and NCSE were rare and only found in 2/28 (7%) patients, NCSZ and NCSE do occur even in sedated patients. It is therefore important that continuous EEG monitoring be performed in sedated SAH patients.

Conclusions- Paper IV

In long-term survivors (≥ one year) after SAH, cerebrovascular causes of death were significantly more common compared to the general population from the same geographical area. This implies that SAH survivors should have the same rigorous follow-up as other stroke patients.

The well-known risk factors of hypertension and tobacco use were more common in SAH patients ≤ 64 years of age compared to data from the MONICA health project.
FUTURE CONSIDERATIONS

Solving the puzzle of what happens in the acute phase after an aneurysmal SAH has just started. This thesis raises questions that need further investigations.

Future considerations- Paper I

The knowledge of the effects of sedative and analgesic drugs on complicated biological systems such as the effect on the HPA-axis is limited and needs to be further investigated.

No benefit has been shown from ‘substitution’ doses of hydrocortisone in SAH patients, and directly harmful effects have been reported with higher doses (Vespa et al 2011).

The CIRCI concept of recommending hydrocortisone treatment in critically ill patients with circulatory failure and low cortisol levels has recently been questioned (Boonen et al 2013). Thus the indication for hydrocortisone treatment is still unknown.

Even though not significant, the lowest cortisol values were found in the time-interval 48-96 h after the SAH. This could signify HPA axis exhaustion or a lack of substrate yet needs to be further investigated.

Future considerations- Paper II

In the acute phase after SAH, systemic ADMA levels seem to be triggered by severity of disease and degree of inflammation. In this study, neither serum ADMA levels nor arginine/ADMA ratio were associated with impaired cerebral circulation. Whether ADMA levels or arginine/ADMA ratio in CSF can be used as markers for impaired cerebral circulation needs to be further investigated.

Future considerations- Paper III

EEG monitoring should be used, not only for detection of seizures, but also in the future it may be possibly to detect ischemia. New devices for monitoring, for example intracortical EEG shows promising results in detecting seizures, predicting altered metabolic demands, and evaluating brain injuries. The use of cEEG in clinical practice has not yet been fully evaluated (Claassen et al 2013).

Future considerations- Paper IV

The increased mortality in cerebrovascular diseases found in long-term SAH survivors raises questions of how the care of SAH survivors should best be organized. These patients probably need a follow-up within stroke programs in neurological and medical departments.
Future Considerations

Prospective long-term epidemiological studies in SAH patients are needed, including a more detailed medical history, especially for known cerebral risk factors and better investigation of causes of death.

“Life is an unfoldment, and the further we travel the more truth we can comprehend. To understand the things that are at our door is the best preparation for understanding those that lie beyond”

Hypatia AD 350-370
ACKNOWLEDGEMENTS

First and foremost I wish to extend my deepest gratitude to my mentor, Silvana Naredi. Without her inexhaustible patience, this thesis would never have seen the light of day. She has been my guiding star on the path of scientific enlightenment, giving me the fortitude to manage any obstacle, and kept me focused on the task before me. Silvana is, for me, a wellspring of inspiration, a true muse and a companion that happily indulges in laughter.

In the absence of patients, a clinical study cannot be performed. I would like to thank those SAH patients that have participated, and shared their experience to deepen our medical knowledge.

I would also like to thank my co-supervisors and co-writers, Docent Magnus Hultin, for allowing me to borrow some of his chemical expertise, and Professor Lars-Owe Koskinen, whose valuable advice in neurosurgical science has been of great assistance.

My sincerest thanks go to my co-writers, for applying their invaluable knowledge and expert opinions, as well as providing new angles of approach to my studies. Per Dahlqvist, master of cortisol. Leif Nilstson, wizard of numbers. Erik Nordh for passionate EEG interpretation. Peter Lindvall, aneurysmal surgeon. Lujbisa Borota, peering into the skull. Ludvig Edwardson for his ICD summer. Magnus Olivecrona as a good discussion partner, Stefan Söderberg for epidemiological insight.

Thanks go to research nurses Kicki Nyman and Anna-Lena Östlund, who have been busy as bees collecting samples and data. X-Ray personnel in NUS; Carina Olofsson for alerting me when SAH patients were arriving. Pär Jonasson for interpreting X-Ray pictures and Jonas Bergdahl for advices. Thanks to IVA-NIVA- and C-Op 2’s personnel for your invaluable help and your careful treatment of patients. Mona Bäck, Elisabeth Eden, Kristina Stänge and Anna Chmielewska for introducing me to the art of Neuroanesthesia. Thank you to Ola Winsö and Niklas Lindberg for allowing me to take the time to produce this thesis, and to Ulla Greta Gidlund along with Britta Olofsson for scheduling it. Doctors at C-Op2; Siv Törnell, Tara Rauf, Svante Holmberg and Nezar Al Zaidi for your fantastic support during my work.

My sincerest thanks go to my research mates Camilla Brorsson and Marie Rodling-Wahlström, for encouragement and sound advice. My dear Neurosurgeons and colleagues Anaesthetists, none mentioned, none forgotten for always providing me with new challenges.

Thanks go to my father Sven Bergman for always believing in me.

Lastly I would like to thank my family; life without you would be nothingness, my husband Janne and my sons, Pierre and Mathias - for loving me as I am.
Grants

Funding: The Swedish Society of Medicine, the Faculty of Medicine at Umeå University, The Kempe Foundations and The Stroke Foundation of Northern Sweden supported this study financially.
Populärvetenskaplig sammanfattnings på svenska

Subarachnoidalblödning (SAH) är en allvarlig form av stroke som i 80 % av fallen är orsakad av ett brustet intrakraniellt aneurysm. Aneurysm är en försvagning av kärlväggen i en artär och utvecklas under livet och är mer av en kronisk sjukdom. Riskfaktorer är högt blodtryck, rökning, ålder > 50 år, en familjehistoria med mer än 2 nära släktingar med SAH, hög alkoholkonsumtion och vissa ärfliga sjukdomar såsom polycystisk njursjukdom. Ökande kunskapar har visat att inflammatoriska processer är en del av aneurysm utvecklingen.

SAH utgör ca 5 % av alla stroke, men då den drabbar vid en relativt låg ålder, medianåldern är 50-60 år är förlusten i levnadsår och kostnaden för rehabilitering i nivå med ischemisk stroke. Dödligheten är hög, 45 % av de som insjuknar dör första månaden. Av de som överlever kan ca 80 % återgå till hemmet men betydande störningar i kognitiva och minnesfunktioner förekommer i upp till 75 % av fallen.

Diagnosen SAH ställs med datortomografi (CT) och misstänks på den typiska anamnesen där patienten beskriver plötsligt i sättande huvudvärk som är ”den värsta huvudvärken i mitt liv”

För att konfirma orsaken till blödningen görs även CT angiografi och vissa fall angiografi då man även har möjlighet behandla aneurysmet med att packa det med tunn metallstrådar s.k. coils och därigenom förhindra re-blödning som är en fruktad komplikation efter SAH. Det andra sättet att behandla är kirurgiskt genom att sätta en klämma på basen av aneurysmet, clipsning. Kirurgisk behandling kan också vara nödvändig för att utrymma hematom efter blödningen.

Hälften av patienterna insjuknar med medvetande sänkning och/eller någon form av lokala neurologiska symptom. Ca 12 % dör innan de når sjukhus.

SAH skall behandlas på s.k. ”hög volym centra” dvs. > 60 fall/år. Norra regionen har en av de högsta incidenserna i världen tillsammans med Japan och Finland. Incidensen är 13 män och 24 kvinnor per 100 000 invånare och ca 90 patienter/år kommer till Neurokirurgiska kliniken NUS med SAH för att få vård.

Tillräckliga nivåer av kortisol är viktigt för överlevnaden. Vid SAH kan regleringen av produkten kortisol via hypotalamus-hypofys och binjurebark axeln vara rubbad. Kortisol kan påverka cirkulationen och ge hypertension, salt- och vätkebalansen kan också vara rubbad, något som är ofta ses vid SAH.

Efter 3-5 dagar kan blodförsörjningen i hjärnan minska till följd av blödningen och man kan se ischemiska områden och konstriktion av blodkärlen en s.k. vasospasm. Detta kan ytterligare försämrå patients neurologiska tillstånd.

SAH är förknippad med både en generell och cerebral inflammatorisk reaktion.

Asymmetriskt dimethylarginin (ADMA) är en kompetitiv hämmare av kväve oxid (NO) som är en potent dilaterare av blodkärl. ADMA är även en markör för kärlväggsskada. ADMA är förhöjt i samband med SAH.
Förhöjda nivåer av ADMA är en riskmarkör vid en rad kärl sjukdomar såsom hypertoni, hjärtinkompensation och carotisstenos. Kärlsjukdomar som sammankopplas med en kronisk inflammation i kärlväggen. Detta skulle kunna innebära att SAH startar en kronisk inflammation som leder till kärlskador och död i hjärt-kärlsjukdom.

Epileptiskaktivitet i hjärnan ökar hjärnans metabolism och kan ge skador. Kontinuerligt EEG kan övervaka hjärnan och varna för epileptisk aktivitet både den typ som går med synliga kramper och epileptisk aktivitet utan kramper så kallat non-konvulsiva kramper.

Avhandlingen bestär av 4 delstudier och målet är att belysa olika kliniska och fysiologiska skeenden i den akuta fasen 0-240 timmar efter SAH samt vid återbesök efter ca 1 år.

**Delstudie I:** Bedöma frekvensen av låga kortisolnivåer och sambandet mellan kortisol och olika fysiologiska parametrar såsom cirkulationssvikt, ålder, kön sjukdomens svårighetsgrad, intervention coil/clipsning, och påverkan av kontinuerlig infusion med sederande läkemedel i den akuta fasen. Samt att utvärdera det neurologiska utfallet.

**Delstudie II:** Att utvärdera ADMA och arginine/ADMA kvoten i den akuta fasen 0-240 timmar efter SAH samt att utvärdera sambandet mellan toppvärdet på ADMA samt dalvärdet på arginine/ADMA kvoten i förhållande till sjukdomens svårighetsgrad, co-morbiditet, kön, ålder, kardiovaskulära komplikationer, nedsatt cerebral cirkulation, inflammation och det neurologiska utfallet.

**Delstudie III:** Undersöka frekvensen av icke-konvulsiva epileptiska kramper hos sederade och mekaniskt ventilerade SAH patienter monitorerade med kontinuerligt EEG.

**Delstudie IV:** Att utvärdera dödsorsaken hos patienter som vårdatas för SAH under tiden 1986-2006 på NUS och överlevt sin SAH > 1 år och sedan avlidit under tidsperioden.

**Resultat**

**Delstudie I:** Kontinuerlig infusion av sederande läkemedel var den starkaste prediktionen för ett lågt serumkortisol. Oddskvoten, risken för en patient som är sederad att ha ett lågt (<200nmol/L) kortisol värde är 18ggr högre än för en patient utan sedation.

**Delstudie II:** Jämfört med inkomstvärdet steg CRP signifikant redan i tidsintervallet 49–72 timmar och nådde sin topp i tidsintervallet 97-120 timmar efter SAH, för att sedan minska. ADMA började stiga i tidsintervallet 97-120 timmar och fortsatte sedan att öka under mätperioden (0-240 timmar efter SAH). Toppvärdet på ADMA var signifikant högre och dalvärdet på arginine/ADMA kvoten var signifikant lägre hos patienter med ett mer allvarligt neurologiskt tillstånd vid ankomsten.
**Sammanfattning på svenska**

**Delstudie III:** Icke-konvulsiva kramper uppträdde hos 7 % (2/28) av patienterna under sammanlagt 0,09 % (5/5468) timmar.

**Delstudie IV:** Cerebrovaskulär sjukdom var betydligt vanligare som dödsorsak hos patienter som har haft en SAH och överlevt minst 1 år jämfört med en normal befolkning från samma geografiska område.

**Slutsatser**

**Delstudie I:** Låga kortisol nivåer; definierade som serum kortisol < 200 nmol/L och beräknat fritt kortisol < 22 nmol/L var associerat med kontinuerlig intravenös sedation. Hänsyn måste tas till om patienten är sederad när man ska tolka kortisol svaret. Då kortisol växlar över tid hos samma patient är upprepade mätningar över tiden är viktigt för att kunna diagnostisera eventuell binjurebarks insufficiens.

**Delstudie II:** ADMA som en markör för endoteldysfunktion ökar markant under den akuta fasen efter SAH. De pro-inflammatoriska markörena CRP och IL-6 ökade tidigt och hade efter en topp en snabb nedgång samtidigt som en ökning av ADMA kunde ses. Detta gör det troligt att ADMA som markör för endoteldysfunktion induceras av en systemisk inflammation. ADMA och arginine/ADMA kvoten påverkades av patientens sjukdomsgrad. Ingen påverkan kunde ses mellan patienter med eller utan påverkad cerebral cirkulation, om detta beror på att endast systemiskt prova togs får ytterligare studier svara på. Vid uppföljning efter ca 1 år såg man ingen skillnad i utfall hos de patienter som hade höga ADMA eller låga dalvärden på arginine/ADMA i akutfasen. dock hade de patienter som hade sämre utfall högre toppvärd på arginine/ADMA

**Delstudie III:** Även om frekvensen av non-konvulsiva epileptiska anfall var lågt och endast förekommer hos 7 % och uppträder även hos sederade patienter. Därför är det viktigt med kontinuerlig EEG övervakning även hos sederade patienter.

**Delstudie IV:** Patienter som genomgått en SAH och överlevt minst 1 år har en överdödlighet i cerebrovaskulära sjukdomar jämfört med en normalbefolkning från samma geografiska område. Patienter som insjuknar i SAH behandlas oftare med blodtrycks medicin – detta gäller endast för patienter i den yngre åldersgruppen dvs. patienter yngre än 65 år.
APPENDIX

Question used from MONICA population surveys 1994 (Original)

--- TOBAKSVANOR ---

8. Röker du cigarett för närvarande?
  - ja, regelbundet (1 cigarett eller mer per dag) ➞ gå till fråga 9
  - nej ➞ gå till fråga 12
  - ibland (mindre än en cigarett/dag) ➞ gå till fråga 10

9. Ungefär hur många cigaretter röker du i genomsnitt per dag? (Ange antal cigaretter/dag)

10. = järngallret ➞ gå till fråga 16
    = nej ➞ gå till fråga 18

12. Har du någon som rökt cigarett regelbundet tidigare?
  - ja, regelbundet ➞ gå till fråga 13
  - nej ➞ gå till fråga 18

18. Har du någon som rökt pipa?
  - ja, för närvarande regelbundet ➞ gå till fråga 19
  - nej ➞ gå till fråga 20
  - jag röker pipa ibland (mindre än 1 gång/dag) ➞ gå till fråga 19
  - tidigare, men inte nu ➞ gå till fråga 20

23. Har du någon som använt snus?
  - ja, snusade tidigare men inte nu ➞ gå till fråga 26
  - ja, snusar mindre än 2 doser per vecka ➞ gå till fråga 26
  - ja, snusar 2 - 4 doser per vecka ➞ gå till fråga 26
  - ja, snusar mer än 4 men mindre än 7 doser per vecka ➞ gå till fråga 26
  - nej ➞ gå till fråga 26

--- HÄLSA ---

28. Har du under de senaste 2 veckorna tagit läkemedel mot förhöjt blodtryck?
  - ja ➞ gå till fråga 26
  - nej ➞ gå till fråga 26
  - osäker om den medicin jag åter är mot högt blodtryck ➞ gå till fråga 26

47. Har du legat på sjukhus för saker hjärnfarkt (propp i hjärtat)?
  - ja,ange årtal 191_____1 och sjukhus...............
  - nej ➞ gå till fråga 26

50. Har du haft slagfall (hjärnblödning eller propp i hjärnan)?
  - ja, ange årtal 19_____1 och sjukhus...............
  - nej ➞ gå till fråga 26

51. Har du diabetes/söckersjuka?
  - ja ➞ gå till fråga 54
  - nej ➞ gå till fråga 54

52. Hur behandlar du din diabetes/söckersjuka?
  - insulin ➞ gå till fråga 54
  - tabletter ➞ gå till fråga 54
  - enbart kost ➞ gå till fråga 54
  - ingetdera ➞ gå till fråga 54
Appendix

Questions used from MONICA population surveys 1994

TOBACCO HABITS

8. Do you currently smoke cigarettes?
Yes, regularly (one cigarette or more per day) > go to Question 9
No > go to Question 12
Sometimes (less than one cigarette per day) > go to Question 12

9. Approximately how many cigarettes do you smoke on average per day? (Indicate the number of cigarettes per day)
> go to Question 16

12. Have you ever previously smoked cigarettes regularly? (Line partially missing / trans.)
Yes, regularly. > go to Question 13
No > go to Question 18

18. Have you ever smoked a pipe?
Yes, regularly, at present > go to Question 19
No > go to Question 20
I smoke a pipe sometimes (less than one time a day) > go to Question 19
Previously, but not now > go to Question 20

23. Have you ever used snuff?
Yes, used snuff previously, but not at present
Yes, use less than two tins of snuff a week
Yes, use between two and four tins of snuff a week
Yes, use more than four tins, but less than seven tins of snuff a week
Yes, use seven or more tins of snuff per week
No > go to Question 26

28. During the past two weeks, have you taken medicine for high blood pressure?
Ja
No
Not sure if the medicine that I am taking is for high blood pressure.
Appendix

47. **Have you ever been hospitalized for certain heart infarct (thrombosis)?**
   Yes, indicate the year 19 -- -- and the hospital ______
   No
   Don’t know whether it was a certain heart infarct.

50. **Have you ever had a stroke (bleeding in the brain or thrombosis in the brain)?**
   Yes, indicate the year 19 -- -- and the hospital ______
   No

51. **Do you have diabetes?**
   Yes
   No
   > go to Question 54

52. **How do you treat your diabetes?**
   Insulin
   Tablets
   Diet only
   None of these
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