Stress-Induced Cardiomyopathy - Clinical and Experimental Studies

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Abstract

Background: Stress-induced cardiomyopathy (SIC) is an acute cardiac condition with akinesia in the left ventricle (LV) that can be severe. A stress-trigger, physical or emotional, is usually identified preceding onset of symptoms and catecholamine overstimulation is involved in the pathogenesis of SIC. The akinesia seen in SIC is reversible and the short term prognosis is therefore considered to be very good. However, recent data suggest that the long term prognosis is equivalent to patients suffering from myocardial infarction. Physical triggers and critical illness are the major triggers of SIC and especially patients with subarachnoid hemorrhage (SAH) frequently develop SIC. Patients with SIC after SAH have an increased risk of secondary cerebral infarction and have a worse short-term prognosis.

Aim: The aim was to evaluate if the biomarkers of myocardial injury (hsTNT) and cardiac dysfunction (NT-proBNP) could be used for identification of patients with SIC after SAH and if patients with increased levels of hsTnT and NTproBNP had an increased risk of poor long-term prognosis (Paper I, II). In an experimental animal model of SIC, the aim was to evaluate cardioprotective properties of different anesthetics (Paper III, IV).

Methods: The first study (Paper I) was retrospective. Data was collected from all patients admitted to the NICU, Sahlgrenska University Hospital, during almost five years. Patients with an echocardiography performed and the biomarkers hsTnT or NTproBNP were obtained were included in the analysis. The second study (Paper II) was prospective. All consecutive patients admitted to the NICU, Sahlgrenska University Hospital, during two years were enrolled in the study. hsTnT and NTproBNP were taken on admission and the three following days and clinical data were obtained. Follow-up was performed one year after onset of symptoms. In Paper III and IV, SIC was induced with an intraperitoneal bolus of isoprenaline in Sprague Dawley rats. Different anesthetics were applied prior to induction of SIC. Vital parameters were measured and small animal echocardiography was performed. A proteomic analysis was performed for assessment of cardioprotective pathways.

Results: Patients with SIC after SAH could be identified with the cardiac biomarkers hsTnT and NTproBNP (Paper I). Increased levels of hsTnT were independently associated with a higher risk of poor long-term outcome when adjusted for age, neurological status on admission and cerebral infarction. Increased levels of hsTnT and NTproBNP was associated with a higher risk of delayed cerebral infarction (Paper II). In the experimental studies, isoflurane had a cardioprotective dose-response effect while propofol and ketamine were not cardioprotective. The cardioprotective mechanism was not mediated through anesthesia per se, by reducing myocardial oxygen demand or by activating the mKatp-channels described in anesthetic preconditioning. In a proteomic analysis, we found that isoflurane attenuated virtually all the pathogenic pathways induced in SIC. Isoflurane seem to act by competitive inhibition the intracellular beta-receptor signalling pathway.

Conclusion: Patients with increased levels of hsTnT or NTproBNP have a higher risk of delayed cerebral infarction and poor long-term prognosis. These patients should be examined with echocardiography for detection of SIC and cardiac output should be monitored to optimize hemodynamics, ensuring cerebral perfusion. Although many aspects are to be considered, isoflurane sedation might be beneficial in patients suffering from SAH.

Keywords: stress-induced cardiomyopathy, tako-tsubo, subarachnoid hemorrhage, isoflurane, outcome, cerebral infarction, hsTnT, NTproBNP, proteomics, bioinformatics

List of papers

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

I. J. Oras, C. Grivans, K. Dalla, E. Omerovic, B. Rydenhag, S-E. Ricksten, H. Seeman-Lodding. High-Sensitive Troponin T and N-Terminal Pro B-Type Natriuretic Peptide for Early Detection of Stress-Induced Cardiomyopathy in Patients with Subarachnoid Hemorrhage. *Neurocrit Care 2015 Oct; 23(2):233-242.*

II. J. Oras, C. Grivans, A. Bartley, B. Rydenhag, S-E. Ricksten, H. Seeman-Lod ding. Elevated high-sensitive troponin T on admission is an indicator of poor long-term outcome in patients with subarachnoid haemorrhage: a prospective observational study. *Submitted manuscript*

III. B. Redfors, J. Oras, Y. Shao, H. Seeman-Lodding, S-E. Ricksten, E. Omero vic. Cardioprotective effects of isoflurane in a rat model of stress-induced cardiomyopathy (takotsubo). *Int J Cardiol 2014 Oct 20;176(3):815-21.*

IV. J. Oras, B. Redfors, A. Ali, H. Seeman-Lodding, E.Omerovic, S-E. Ricksten. Mechanisms of isoflurane-induced cardioprotection in an experimental model of stress-induced cardiomyopathy. *Manuscript*

Populärvetenskaplig sammanfattning, *Summary in Swedish*

Stressutlöst hjärtsvikt (Stress-induced cardiomyopathy, SIC) är en allvarlig akut hjärtsjukdom med symtom som påminner om akut hjärtinfarkt, patienten insjuknar med exempelvis bröstsmärta, andnöd och sjukdomskänsla. SIC orsakas dock inte av en förträngning i hjärtats kranskärl, vilket är fallet vid hjärtinfarkt, utan av psykisk eller fysisk stress. Ett kraftigt stresspåslag gör normalt att hjärtat pumpar bättre, men om stresspåslaget är tillräcklig kraftigt kan man få en paradoxal reaktion med nedsatt rörlighet i vissa delar av hjärtat. Detta gör att blodet inte pumpas runt tillräckligt bra i kroppen. Om man klarar den akuta fasen av SIC är korttidsprognosen god och hjärtat återhämtar sin funktion inom några dagar till veckor efter insjuknande. Nya data visar dock att långtidsprognosen är densamma som vid akut hjärtinfarkt. Mer än 90% av patienterna med SIC är kvinnor som är äldre än 50 år. Vi vet inte exakt vad som orsakar SIC och det finns idag ingen bra behandling för SIC.

Subarachnoidalblödning (SAH) är en allvarlig typ av hjärnblödning som beror på att en kärlmissbildning i hjärnan brister. SAH drabbar relativt unga personer, medelåldern vid insjuknande är ca 50 år. Dödligheten är hög, ca 40% av patienterna dör av sjukdomen och en tredjedel av de som överlever blir så skadade att de behöver hjälp att klara sitt dagliga liv. SAH är en ovanlig typ av stroke och står bara för 5% av totala antalet stroke-patienter. Eftersom unga drabbas och dödligheten är så hög har dock SAH-patientgruppen lika många förlorade levnadsår som patientgruppen med vanlig stroke, orsakad av en propp i hjärnans blodkärl.

Fysisk stress, till exempel allvarlig sjukdom, är den vanligaste utlösande orsaken till SIC. Patienter med neurologiska skador och SAH utvecklar ofta SIC. Syftet med den första, kliniskt inriktade delen av avhandlingen var att utvärdera om man med ett blodprov kan mäta biomarkörer från hjärtat för att hitta patienter med SIC efter SAH. Dessutom studerades vad utvecklingen av SIC tidigt efter ankomst till sjukhuset betyder för den neurologiska långtidsprognosen efter SAH. I den andra, experimentella delen av av-

handlingen, utvärderades den möjliga hjärtskyddande effekten av olika narkosmedel i en djurmodell av SIC, samt eventuella verkningsmekanismer bakom en sådan skyddande effekt.

I delarbete I visade vi att det går att hitta patienter som utvecklar SIC efter SAH genom att ta ett enkelt blodprov direkt då patienten kommer till sjukhuset. I delarbete II visade vi att patienter med hjärtskada efter SAH har en sämre långtidsprognos och ökad risk för ytterligare hjärnskador i efterförloppet av blödningen. Vi anser att de patienter som utvecklar hjärtkomplikationer efter SAH kräver ett snabbt omhändertagande med extra övervakning och åtgärder för att ge dem bästa möjliga förutsättningar.

I delarbete III visade vi i en djurmodell av SIC att det går att förebygga SIC genom att söva djur med en viss narkosgas (isofluran). I delarbete IV jämfördes de hjärtskyddande effekterna av isofluran i djurmodellen av SIC med propofol, vilket är det vanligaste sömnmedlet som används idag. I det försöker såg vi att isofluran var hjärtskyddande medan propofol inte hade någon hjärtskyddande effekt. Den hjärtskyddande effekten beror alltså inte på att djuren blir sövda, utan verkar vara en direkt effekt av isofluran. I en vävnadsanalys av hjärtan som användes i försöket såg vi att det aktiverades en mängd skadliga system i hjärtcellen, bland annat aktiverades flera inflammatoriska system. Denna aktivering hämmades påtagligt med isofluran men inte av propofol. Det verkar som om isoflurane dämpar de skadliga effekterna av de stresshormoner som orsakar SIC.

Sammanfattningsvis har avhandlingen visat att hjärtskada vid SAH medför en ökad risk för ytterligare hjärnskador och försämrad neurologisk långtidsprognos. Med ett enkelt blodprov kan vi nu dock hitta dessa patienter mycket tidigare och snabbt sätta in nödvändiga behandlingsåtgärder. I en djurmodell har vi hittat en lovande behandling mot SIC vilken skulle kunna prövas på patienter.

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Abbreviations

ABG	Arterial blood gas
ANOVA	Analysis of variance
APC	Anesthetic preconditioning
ATP	Adenosine triphosphate
cAMP	Cyclic adenosine monophosphate
CBN	Contraction band necrosis
CI	Cerebral infarction
CI	Confidence interval
CNS	Central nervous system
CO	Cardiac output
CPP	Cerebral perfusion pressure
CSF	Cerebrospinal fluid
DCI	Delayed cerebral ischemia
EBI	Early brain injury
ECG	Electrocardiogram
ECMO	Extracorporeal membrane oxygenation
EF	Ejection fraction
ET-1	Endothelin 1
GOSE	Glasgow outcome scale extended
GPRC	G-protein coupled receptor
hsTnT	High sensitive troponin T

I/R	Ischemia/reperfusion
i.p.	Intraperitoneally
ICP	Intracranial pressure
ICU	Intensive care unit
LV	Left ventricle
LXR/RXR	Liver X Receptor/Retinoid X Receptor
MAC	Minimal alveolar concentration
MAP	Mean arterial blood pressure
MVO_2	Myocardial oxygen demand
NF-kappaB	Nuclear factor kappa beta
NICU	Neuro intensive care unit
NTproBNP	N-terminal pro B-natriuretic peptide
OR	Odds ratio
PI3K/Akt	Phosphatidylinositol-3-Kinase/Protein Kinase B
SAH	Subarachnoid hemorrhage
SERCA	Sarcoplasmic/endoplasmic reticulum calcium ATPase
SIC	Stress-induced cardiomyopathy
SVR	System vascular resistance
TCD	Transcranial Doppler
WFNS	World federation of neurosurgeons classification of subarachnoid hemorrhage

Introduction

Stress-induced cardiomyopathy

Clinical features

Stress-induced cardiomyopathy (SIC) is an acute cardiac syndrome with sudden onset of hypokinesia of the left ventricle (LV). The typical patient presents with symptoms mimicking acute myocardial infarction (Table 1); chest-pain, dyspnea, ECG-changes and troponin release. However, in SIC, a culprit coronary stenosis/occlusion is not seen. In most patients, an emotional or physical stress-trigger, can be identified, preceding the onset of symptoms. Further examination with echocardiography reveals regional hypokinesia of the LV, which can be profound and severe (akinesia). In most cases, the apical portion of the ventricle is affected but midventricular and basal (reversed, atypical) variants are seen. This hypokinesia is associated with hyperkinesia in the non-hypokinetic portions of the heart i.e. a hypokinetic apex is associated with hyperkinesia in the basal portion of the ventricle. If the patient survives the acute phase of SIC, the hypokinesia in SIC is generally reversible and resolve within a couple of days to weeks[1-5]. (Figure 1)

Epidemiology

SIC typically afflicts post-menopausal women, representing approximately 90% of SIC patients[3,4,6]. SIC is identified in 2-3% of patients undergoing acute angiography[7]. In Sweden, approximately 1000 patients are affected each year. Although the short term prognosis in SIC is considered to be excellent[3], data suggest that the long-term prognosis is poor with a one-year mortality of ~10% which is equivalent to that of acute coronary syndromes[4,7-9].

Terminology and definition

The LV contraction pattern in typical SIC, with hypokinesia in the apical- and hyperkinesia in the basal portions of the LV, has given rise to the name Tako-tsubo cardiomyopathy because the appearance is similar to a Japanese octopus trap, named Tako-tsubo[10]. Other names of SIC are “apical ballooning syndrome” , “broken heart syndrome” and ”Tako-tsubo syndrome”. LV hypokinesia in SIC does not have to be localized to the apical portion of the left ventricle; in the largest cohort described so far, 80% had apical

hypokinesia, 15% had midventricular hypokinesia and basal or focal variants were seen in 5% of SIC-patients[9]. SIC is a new diagnosis; the first case-reports describing SIC are only 20 years old[11], the first proposal of a new diagnosis was written in 2004[12] and during the last decade, SIC is being increasingly recognized. There is still no consensus about the nomenclature or diagnostic criteria for SIC[13]. However, all proposed diagnostic criteria have a common definition of acute onset of transient akinesia/hypokinesia of the LV not caused by ischemia[13], which is the most important characteristic of SIC. SIC is now a generally accepted diagnosis and the knowledge about SIC is rapidly increasing. In this thesis, we refer to SIC according to the Gothenburg criteria[13]. The term Tako-tsubo is used occasionally in this thesis to be congruent with previous papers and references.

Subarachnoid hemorrhage

Clinical features

Subarachnoid hemorrhage (SAH) is a cerebrovascular emergency, characterized by extravasation of blood in the subarachnoid space. Most cases of SAH are caused by rupture of an aneurysm in one of the cerebral vessels. Patients present with an acute onset of headache, usually described as "thunderclap headache", nausea, neck pain and photophobia. Neurological symptoms vary; most patients develop impaired consciousness of any degree, from mild drowsiness to lethargy and coma. Most common focal neurological deficits are cranial nerve dysfunction, e.g. diplopia, but severe symptoms such as hemiplegia are not unusual. Neurological status upon admission is important as it correlates with both short- and long-term prognosis and several grading systems of severity of SAH are based on the neurological status upon admission[14,15]. SAH is a rather uncommon cause of stroke and accounts for ~5% of the total number of strokes[16,17]. The worldwide incidence is approximately 10 in 100.000 persons per year with a lower incidence in the Western World (approx. 6/100.000). SAH is more common in women than in men and most important risk factors are smoking and hypertension. Although mortality rates have declined during the last years, overall mortality in SAH is high; 40-50% of SAH patients die from the bleeding, in whom 10% die before receiving medical attention and 25% within the first 24 hours. Of survivors, about one third are disabled and requires some degree of assistance in daily life [18]. The average age at onset of SAH is 50 years which is low when compared to patients with ischemic stroke. Although SAH-patients constitute a small proportion of the total number of stroke-patients, they have the same total number of loss-of life-years as patients with ischemic stroke or intracranial hemorrhage [17,19].

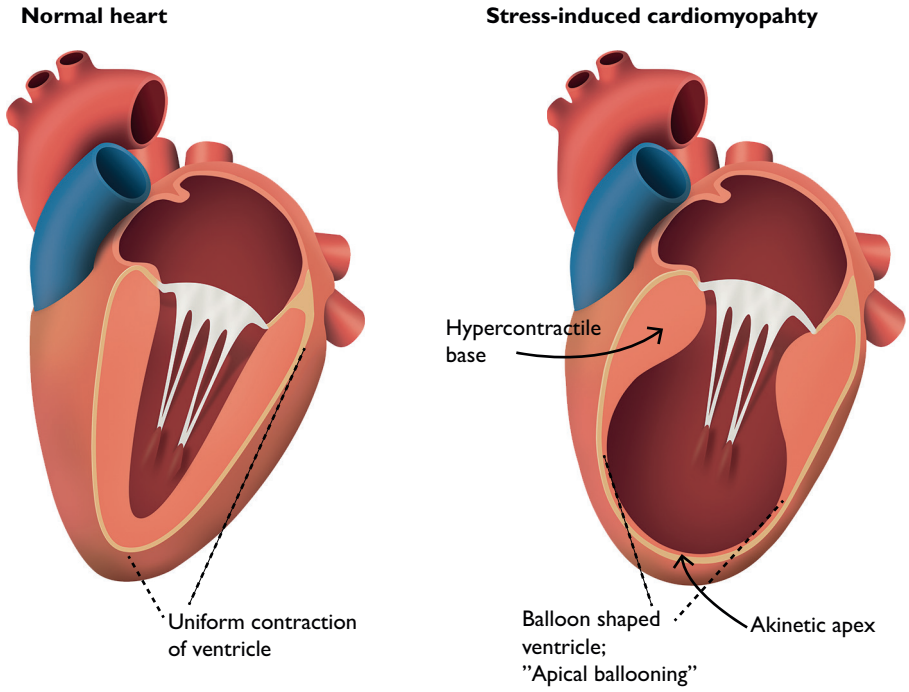


Figure 1. Morphological characteristics of typical stress-induced cardiomyopathy or Tako-tsubo syndrome. The apex is akinetic and the base is hypercontractile. This results in a balloon shaped pattern called apical ballooning.

Table 1. Clinical characteristics of stress-induced cardiomyopathy vs ischemic heart disease

Stress induced cardiomyopathy	Ischemic heart disease
Chest pain	Chest pain
Dyspnea	Dyspnea
ECG-changes	ECG-changes
Increased troponins	Increased troponins
Angiography without culprit lesion	Angiography with culprit lesion
Hypokinesia not following coronary artery anatomy	Hypokinesia in area of myocardial ischemia
Regress of hypokinesia in days to weeks	Possible regress of hypokinesia but permanent damage is common
Usually a stress-trigger	No special trigger
Women are overrepresented	Males are overrepresented

Neurosurgical treatment

The most feared complication following SAH is re-bleeding of the aneurysm, which carries a mortality of 50%. It is agreed that aneurysms are to be treated as soon as possible after detection to reduce this risk[20,21]. The goal of the treatments is to isolate the aneurysm from the cerebral circulation. This is achieved by two treatment options: endovascular treatment with coiling or open surgery[16,17]. Endovascular treatment is the first choice of treatment if the aneurysm is approachable with this technique, otherwise open surgery is preferred[16,17]. About two-thirds of aneurysms are treated by endovascular technique and one-third by open surgery. There are virtually no differences in short- and long term outcome between the two treatment strategies [22,23]. With endovascular treatment, the operator guides a flexible catheter from the femoral artery to the cerebral circulation and further to the aneurysm. Once in correct position, a thin platinum wire is inserted into the aneurysm sac and the wire wraps around itself to form a coil. The coils block the cerebral blood flow from reaching the aneurysm and over time, a thrombus is formed inside the aneurysm, permanently eliminating risk of aneurysm rupture. With open surgery, a craniotomy is performed and the cerebral arteries, which lie deep in the brain, are reached by retracting brain tissue. Once at the aneurysm, a clip is placed at the base of the aneurysm, permanently isolating the aneurysm from cerebral circulation.

Acute hydrocephalus is common after SAH. In SAH, blood is spread throughout the subarachnoid space and reaches the ventricular system containing cerebrospinal fluid (CSF). The blood can cause a mechanical obstruction of intraventricular CSF-flow in the aqueduct between the third and fourth ventricle or a blockade or reuptake of CSF by the arachnoid villi. This causes hydrocephalus with subsequent drowsiness and coma. Acute hydrocephalus in SAH is treated by an external ventricular drainage[16,17].

Brain damage after subarachnoid hemorrhage

Brain damage from SAH is complex and consists mainly of two components; early brain injury (EBI) and delayed cerebral ischemia (DCI) [24,25]. When the aneurysm ruptures, blood enters the subarachnoid space and possibly also into cerebral parenchyma and ventricles. This leads to an explosive increase in intracranial pressure (ICP) [26-28]. As a response to the sudden rise in ICP, the Cushing reflex is initiated which increases the blood pressure. This increase in blood pressure increases ICP even further [27]. Ultimately, ICP reaches the level of the patient´s blood pressure within one minute from start of the bleeding. ICP then successively decreases during minutes to normal levels unless parenchymal hematoma or CSF obstruction is present [26-28]. Secondary to the rise in ICP, cerebral perfusion pressure (CPP) drops resulting in cerebral hypoperfusion[26-29]. This leads to unconsciousness at ictus which is seen in a majority of SAH patients[25]. The rise

in ICP and decreased cerebral blood flow might be beneficial as this promotes aneurysm hemostasis,[26] but gives rise to ischemic damage. In some patients, a prolonged period of decreased cerebral blood flow, i.e. intracranial circulatory arrest is seen,[26,28,29] which is followed by a poor neurological admission status and a worse prognosis[30,31]. EBI could further be aggravated by intraparenkymal hemorrhage, cerebral edema, cerebral inflammation, microcirculatory disturbances including abnormal autoregulation and injured blood-brain-barrier[24,25]. (Figure 2)

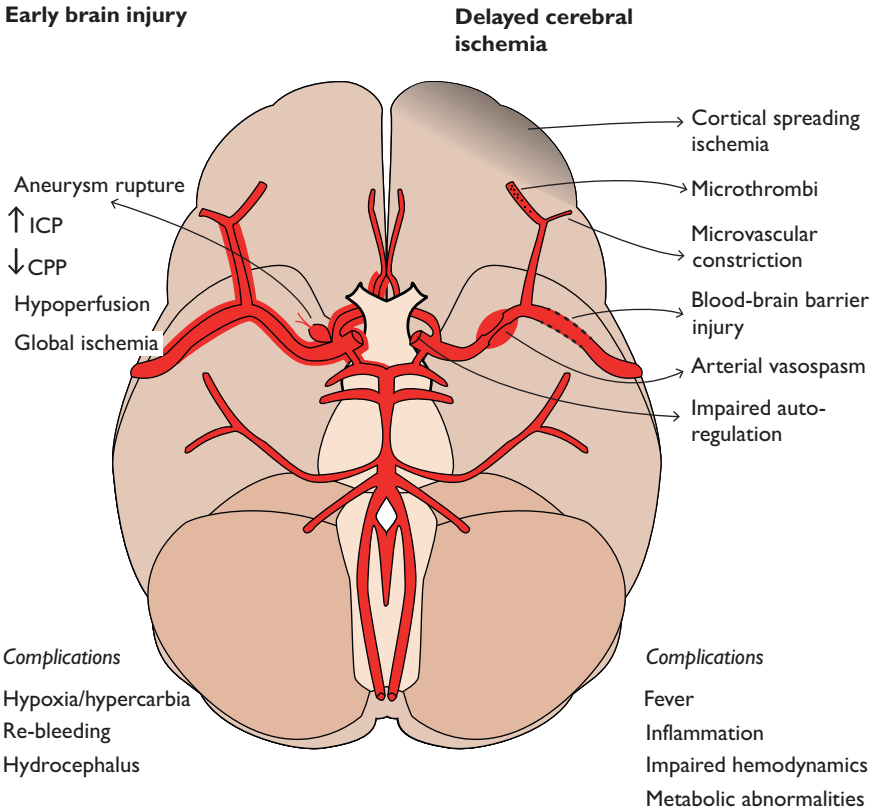


Figure 2. Mechanisms contributing to cerebral damage after subarachnoid hemorrhage. In early brain injury, the major cerebral damage is due to hypoperfusion and global ischemia after the sudden and extensive rise in intracranial pressure (ICP) with decreased cerebral perfusion pressure (CPP.) Although the pathogenesis of delayed cerebral ischemia (DCI) is multifactorial and complex, arterial vasospasm is considered to be one of the major component of DCI. Injury is further aggravated by systemic complications such as inflammatory response and cardiac dysfunction.

DCI is a term used in a broad sense and refers to cerebral ischemic events later than 48-72 hours after the bleeding[24,25,32]. Clinical symptoms vary from minor focal neurological deficits to massive cerebral infarction. Traditionally, DCI has been attributed to cerebral vasospasm but it has been shown that other mechanisms are involved and the pathogenesis is multifactorial[24]. Angiographic vasospasm represents a paradoxical narrowing of major cerebral vessels, which can be identified during angiography. The main trigger of angiographic vasospasm is the blood surrounding cerebral arteries[33,34]. Hemolysis of red blood cells initiates an inflammatory processes and endothelial injury which promotes production of the vasoconstrictor endothelin-1 (ET-1) and reduces production of the vasodilator nitric oxide[35]. Although there is a correlation between angiographic vasospasm and DCI[36], this alone cannot fully explain the pathogenesis of DCI[24]. Other interacting mechanisms are microcirculatory disturbances in the form of microthrombus formation[37] and microvascular constriction[38] due to e.g. endothelial injury and release of ET-1. Cortical spreading ischemia is syndrome with spreading depolarization in the cortex with neuronal swelling. This is normally followed by a physiological vasodilation and hyperemia but in SAH an inverse response, with arterial vasoconstriction and cortical hypoperfusion, is seen[39]. Other contributing mechanisms are inflammation, disruption of blood-brain barrier and systemic complications such as fever, hyponatremia and cardiopulmonary complications [24]. DCI is an exclusion diagnosis and the current diagnostic criteria are focal neurological deficits or decreased mental consciousness without other cause[32]. Diagnosis can be supported by angiographic vasospasm or increased flow velocities, usually detected by transcranial Doppler (TCD). If DCI is detected, all possible measures must be taken to avoid development of a permanent cerebral infarction. There is currently no specific or causal treatment for DCI. Induced hypertension and fluid boluses with evaluation of improvement in clinical status are recommended. The patient's vital functions also have to be optimized[40]. All patients with SAH are treated with the calcium-blocker nimodipine, which is the only drug shown to be effective to reduce DCI and improve outcome after SAH[41]. (Figure 2)

Cardiac complications after subarachnoid hemorrhage

Clinical features

A majority of patients with SAH suffer from any type of cardiac dysfunction. The importance of these cardiac events varies from subtle, subclinical electrocardiographic changes to severe heart failure (Table 2). Cardiac dysfunction in SAH has been known for a long time; the first article presenting increased blood pressure and arrhythmias after SAH was published in 1903 and during the last decade, knowledge about cardiac

dysfunction after SAH has grown (Table 2). SIC in SAH was firstly described in 1994[42]; a couple of years after the first case reports describing the Tako-tsubo syndrome. At first, this was considered to be a phenomenon separate from Tako-tsubo and in the first proposed diagnostic criteria of Tako-tsubo, SAH was an exclusion criterion[13] but is now an accepted trigger of SIC[43]. The pathogenesis of SIC after SAH is discussed below.

Epidemiology

Arrhythmias[44] and ECG changes[45-47] are common in SAH patients and hypertension is seen in almost all admitted patients [48]. Cardiac injury, diagnosed as increased serum levels of troponin, is reported in 20% to 50% of patients with SAH[46,49-52]. The big difference in incidence between the studies are most likely due to different inclusion criteria, timing of troponin sampling in relation to onset of symptoms, different cut-off levels and different laboratory techniques. Merging data from the two largest studies and a meta-analysis, with a total inclusion of 1295 SAH patients, demonstrates that the incidence of cardiac injury in SAH is 34% [46,49,50]. In prospective, consecutive studies, the incidence of SIC in SAH was described to be 8-28%[46,53-56]. The three largest consecutive studies, with a total inclusion of 1189 patients [46,53,56], had a total incidence of 15%. The female overrepresentation seen in patients with primary SIC is not as clear in SAH patients with SIC. However, data are inconclusive as gender distribution is not reported in many papers[54,56-58].

Table 2. Expressions of cardiac complications after SAH

Clinical finding	Frequency	First publication (year)
Increased blood pressure	90%	1903
Arrhythmias	5-100%	1903
Neurogenic pulmonary edema	10-30%	1908
ECG changes	50%	1947
Cardiac damage (autopsy)	25%	1964
Increased troponins	20-40%	1994
Regional hypokinesia (SIC)	10-25%	1994
Heart failure from SIC (EF<40%)	10-15%	1995

SIC; Stress-Induced Cardiomyopathy, EF; Ejection Fraction

Clinical consequences of cardiac dysfunction after SAH

Patients with cardiac dysfunction after SAH have worse prognosis when compared to patients without cardiac dysfunction. A recent large multicenter study showed that SAH patients with SIC had an increased risk of DCI and a higher risk of poor 3-month outcome[46], which has also been shown in previous smaller studies[55,59,60]. Several studies and a meta-analysis have reported that troponin release, as a marker of cardiac damage, is associated with poor short-term outcome [49,51]. Also ECG abnormalities, such as ST-segment depression, have shown a correlation with poor outcome[61]. The causal link between cardiac dysfunction and poor outcome is not clear and there is no information on whether specific treatment of cardiac complications can improve outcome[46,49,51,62]. The long-term effects of cardiac dysfunction after SAH are sparsely studied. The few studies available are either retrospective or have small sample size and the results are contradictory[60,63,64].

Pathogenesis of stress-induced cardiomyopathy

The role of stress in stress-induced cardiomyopathy

The pathogenesis of SIC is not fully understood. The first proposed hypotheses suggested microvascular dysfunction, coronary artery spasm[11] or a dissolved coronary artery thrombus[65]. However, the LV hypokinesia in the ventricle during SIC usually extends beyond the area supplied by one single coronary artery. These hypotheses required a simultaneous engagement of all coronary arteries or an abnormal coronary artery anatomy and are now considered to be less credible[4,66]. It is now generally accepted that sympathetic activation, with excess of circulating and locally released epinephrine and norepinephrine with overstimulation of beta-receptors is most likely involved in the pathogenesis of SIC[1]. This is supported by the stress-trigger identified in patients with SIC, higher levels of circulating norepinephrine in patients with SIC[1,56,58,67] and case-reports series of patients with an iatrogenic induction of SIC with beta-agonists[68,69]. It is also supported by animal-models in which SIC is induced with beta-stimulants[70-72]. SIC is also seen in a number of hyperadrenergic states, such as pheochromocytoma[73], SAH[42,53,54], brain death[74] and occasionally in sepsis[75], which strengthens this hypothesis.

Beta-receptor stimulation and catecholamine cardiotoxicity

Catecolamine stimulation of beta-receptors is supposed to have a central role in SIC pathogenesis. Catecholamine cardiotoxicity is well-described with the first studies origi-

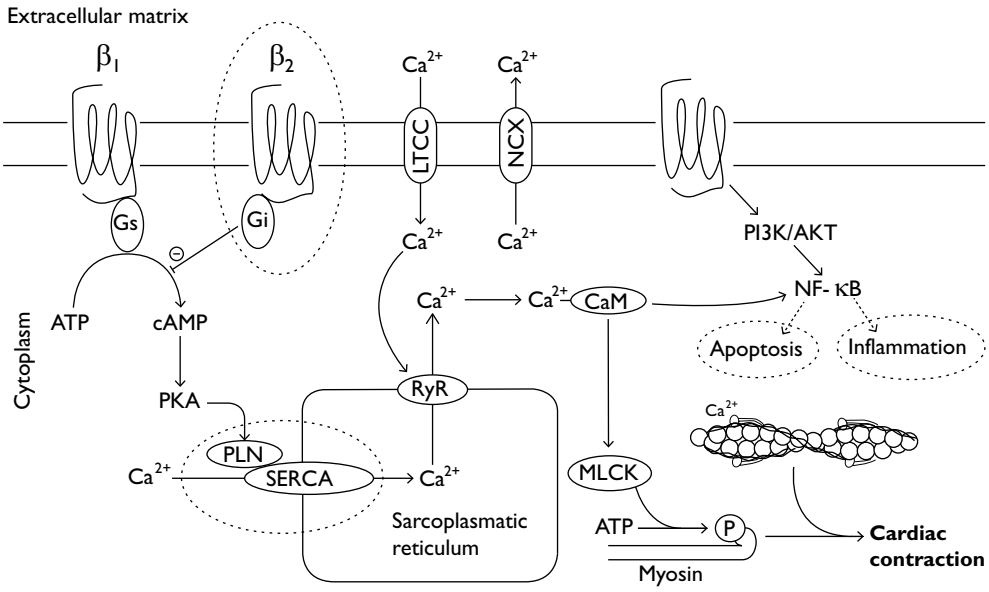


Figure 3. Schematic figure of beta-stimulatory effects in the cardiomyocyte and the pathway to cardiac contraction. In cardiac contraction, the ryanodine receptor (RyR) is activated by increased calcium concentrations, initiated by the L-type calcium channel (LTCC). Calcium in the cytoplasm forms a calcium-calmodulin complex which activates myosine light chain kinase (MLCK). MLCK participates in phosphorylation of myosine-heads which binds to actin-filaments resulting in muscular contraction. Beta-stimulation increases formation of adenosintriphospate (ATP) to cyclic adenosinmonophosphate (cAMP). cAMP activates protein kinase A (PKA) which phosporylates phospholamban (PLN). PLN normally inhibits the sarcoplasmatic/endoplasmatic calcium reticulum calcium ATPase (SERCA) but phosphorylation of PLN decreases this inhibition. SERCA pumps calcium from the cytoplasm to the sarcoplasmatic reticulum (SR) and is considered to be the main determinant of cardiac contraction. A faster influx of calcium to the SR shortens the time for the heart to relax (diastole) and the higher concentration of calcium in the SR the more calcium is pumped out to the cytoplasm by RyR, thus resulting in a higher force of contraction. The natrium-calcium exchanger (NCX) is not parcitipating in cardiac contraction but maintains the intracellular calcium-homeostasis. Inhibitory G-proteins (Gi) are described to be activated in SIC. It is suggested that this activation decreases cAMP-levels and thus reduces cardiac contraction. The PLN/SERCA ratio is shown to be affected in SIC, resulting in reduced contraction. Both inflammation and apoptosis, which are shown to be present in SIC-tissue, can be activated by both beta-stimulation and the calcium-calmodulin complex(Ca-CaM). Dashed lines; described pathogenic mechanism of SIC. PI3K/AKT; Phosphatidylinositol-3-Kinase and Protein Kinase B, NF-KB; Nuclear Factor-Kappa Beta.

nating from the 60´s[76]. Excessive beta-stimulation leads to cardiomyocyte apoptosis[77,78], cardiac inflammation[79,80] and cause a patchy, diffuse and myocardial damage referred to as contraction band necrosis[81] (CBN). In CBN, the cardiomyocytes are fully contracted, resulting in sarcolemma rupture and cell death. CBN has a typical histopathological

finding with hypercontracted fibrils and a band formation of hypercontracted sarcomeres[82]. CBN is caused by intracellular calcium overload[81]; the excessive beta-stimulation activates intracellular signaling systems leading to excessive calcium release and influx to the cytoplasm. Ultimately, the intracellular calcium homeostasis cannot be maintained leading to hypercontracture and cell-death[81,83]. Patients with primary SIC show a mild increase in troponin levels, most likely due to catecholamine-induced cardiac damage[8,9]. Beta-recceptor antagonists are shown to be protective in development of cardiac injury. In a randomized study on patients with traumatic brain injury, treatment with beta-blocker lowered levels of cardiac enzymes[84]. SAH patients with pre-admission treatment with beta-blockers are less likely to develop SIC.[59,85] However, the protective role of beta-blockers is not seen in observational studies in patients with primary SIC[5,86,87].

Beta-receptor signaling is complex and several intracellular systems are involved potentially leading do cardiac damage or cardiac protection. Different beta-receptors are coupled to different G-protein coupled receptors (GPCR), which can stimulate or depress the contractile system. Beta-stimulation can also activate other pathways such as the IP3K/Akt pathway[88,89] which activates NF- kappaB, eventually resulting in inflammation and apoptosis[80,90-92] (Figure 3).

Catecholamine surge in SAH-patients

The pathogenesis of SIC after SAH share many features with primary SIC and is supposed to be overlapping or even the same[43,93]. SAH-patients suffer from sympathetic overstimulation in conjunction with the bleeding. Naredi et al showed an increased sympathetic activity by measuring norepinephrine spillover in SAH-patients compared to controls[94]. In animal experiments, increased levels of circulating epinephrine and norepinephrine are seen following induction of SAH, which are also closely correlated with troponin levels[95]. The cause of sympathetic hyperactivity in SAH is not fully known. Animal experiments have shown that an explosive rise in ICP, which is seen in SAH[26-28], and not a gradual increase, is essential for development of sympathetic hyperactivity[96,97]. The link between increased ICP and sympathetic hyperactivity is not fully understood. Suggestions include mechanical distortion or ischemia of the vasomotor center in the medulla[96], ischemia of the insular cortex[98] and an imbalance between excitatory and inhibitory pathways in the CNS[99]. The sympathetic hyperactivity leads to locally released norepinephrine from sympathetic nerves and epinephrine from the adrenal medulla. This could be a physiological phenomena, e.g. the Cushing reflex, promoting cerebral circulation. However, excessive stimulation, or stimulation in vulnerable patients may leads to cardiac damage or SIC. (Figure 4)

In SAH-patients, there is a close correlation between myocardial damage and development of SIC[51,57,59,100]. The causality of cardiac damage and development of SIC after SAH is uncertain, as not all patients with myocardial damage develop SIC[57,100]. In an autopsy study of patients with SIC after brain damage, there was a poor correlation between areas of LV hypokinesia and areas of cardiac necrosis in the left venricle[74].

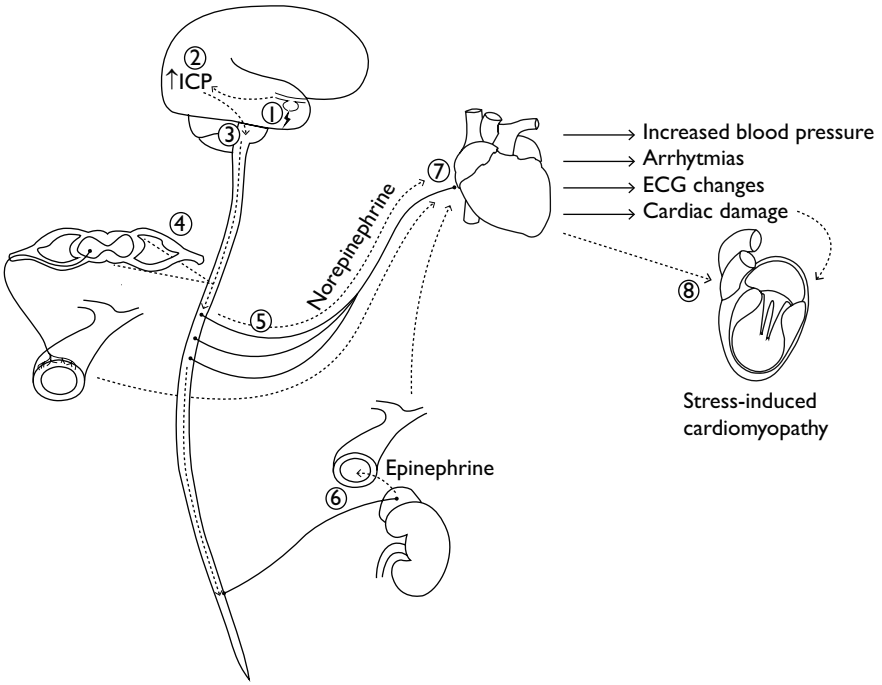


Figure 4. Mechanism of catecholamine-release and cardiac complications after subarachnoid hemorrhage. An intracerebral aneurysm ruptures leading to blood extravasation in the subarachnoid space (1). This leads to an explosive rise in ICP (2) activating the vasomotor center in the medulla (3). This is most likely due to ischemia in the vasomotor centre. Sympathetic nerves in the spinal cord and sympathetic chain are activated resulting in constriction of blood vessels in skin and muscles (4) and sympathetic nerve stimulation of the heart (5). Norepinephrine is the major transmittor in sympathetic nerve activation. Signals are transmitted to the adrenal gland releasing catecholamines, mainly epinephrine, to the blood stream (6), constricting vessels and activating beta-recepors in the heart (7). The sympathetic activation of the heart results in arrhytmias, ECG-pathologies and possible cardiac damage and SIC.

Suggested pathophysiological mechanisms of SIC

Although there is evidence for an involvement of stress, catecholamines and beta-receptor stimulation in development of SIC, the link to the development of LV hypokinesia is not understood and several hypotheses have been presented. One of the most cited theories suggests that excessive beta-stimulation with adrenaline activates beta-2-adrenoceptors, which activates inhibitory G-proteins, resulting in reduced cardiac contraction by decreased cAMP-formation[71]. As studies have shown that beta-2-adrenoceptors are most abundant in apex of the ventricle, this theory has gained a lot of supporters as this easy can explain the over representation of apical hypokinesia. However, this study has later been contradicted by others[66]. A biomechanical hypothesis suggests that the inotropic action of catecholamine stimulation leads to a hyper-contractive left ventricle with near obliteration of the ventricle lumen during systole. This leads to aortic outlet obstruction with subsequent stretch, overload and relative ischemia in apical cardiomyocytes[66,101]. One study have shown disturbances in the proteins regulating calcium-fluxes directly involved in cardiac contraction (SERCA/phospholamban)[102]. Cardiomyocyte apoptosis is activated by beta-stimulation and can cause reduced cardiomyocyte contration[78]. Local cardiac inflammation and intracellular lipid droplets are found in SIC-tissue which might contribute to LV akinesia[72,103,104]. None of the theories have actually presented a hypothesis that can explain all the characteristics of SIC. Most likely, pathogenesis of SIC is complex with an involvement of several mechanisms. (Figure 3)

Aim

The aims of this thesis were:

To evaluate whether the cardiac biomarkers, high-sensitive troponin T (hsTnT) and N-terminal pro B-type natriuretic peptide (NTproBNP), are useful biomarkers for early detection of stress-induced cardiomyopathy after subarachnoid hemorrhage (Paper I).

To evaluate whether hsTnT and NTproBNP, are associated with poor one-year neurological outcome and cerebral infarction due to delayed cerebral ischemia after subarachnoid hemorrhage (Paper II).

To study the differential cardioprotective effects of the inhalation anesthetic isoflurane and the intravenous anesthetics pentobarbital and ketamine, in an experimental model of stress-induced cardiomyopathy (Paper III)

To study the differential cardioprotective effects of isoflurane and the intravenous anesthetic propofol, in an experimental model of stress-induced cardiomyopathy (Paper IV).

To study the cardioprotective intracellular pathways activated by isoflurane in a global proteomic analysis in an experimental model of stress-induced cardiomyopathy (Paper IV).

Materials and Methods

Materials

Patients, Paper I

This study was approved by the Gothenburg Regional Ethics Committee for Human Research. Medical records of all patients admitted to the intensive care unit (ICU) or the neuro-intensive care unit (NICU) at the Sahlgrenska University Hospital, Gothenburg from January 2010 to August 2014 with the diagnosis subarachnoid hemorrhage were reviewed. Inclusion criteria were echocardiography performed within 72 hours after onset of symptoms and available blood samples for cardiac damage, high sensitive troponin T (hsTnT), or cardiac dysfunction, N-terminal pro natriuretic peptide (NT-proBNP). One-hundred and twelve patients were included in whom 25 patients fulfilled criteria for SIC.

Patients, Paper II

This study was approved by the Gothenburg Regional Ethics Committee for Human Research. All patients admitted to the NICU at Sahlgrenska University Hospital, Gothenburg with suspected or verified subarachnoid hemorrhage were enrolled between 1 january 2012 and 31 december 2013. Patients with SAH-diagnosis not confirmed, long time to admission (>72hours after onset of symptoms), benign bleeding and poor prognosis upon arrival (no intervention done) was excluded. A total of 143 patients were included of whom 126 fulfilled one-year follow-up.

Animals, Paper III and IV

The studies were approved by the Gothenburg Regional Ethics Committee for Animal Experiments,.

All animals studied were 10 weeks old, male, Sprague Dawley rats. All animal work was performed in accordance with the NIH guidelines for the use of experimental animals. Animals were housed in a temperature-controlled facility with a 12-h light/dark cycle and had free access to food and water. In Paper III, 90 animals were studied and in Paper IV, 75 animals were studied.

Methods

Study protocol, Paper I

Clinical, hemodynamic, echocardiographic and laboratory data was retrieved from medical journals. SIC was defined according to the Gothenburg criteria for SIC[13].

Study protocol, Paper II

In all included patients, blood samples for measurement of hsTnT and NTproBNP were taken on admission and the following three days. Follow-up was performed according to the Glascow Outcome Scale Extended (GOSE) scale, primarily by telephone calls to the patient and secondarily by letters and telephone calls to the patient´s next of kin. GOSE ≤ 4 was defined as poor outcome corresponding to a dependent living in every-day life, vegetative state or dead. Cerebral infarction (CI) due to delayed cerebral ischemia (DCI) was defined according to Vergouwen et al[32]. Clinical, hemodynamic and laboratory data were recorded during the ICU-stay.

Biomarker analysis (Paper I and II)

Biomarkers were analyzed with immunoassay technique; hsTnT was analyzed with the Roche high-sensitive troponin T assay and NTproBNP was analyzed with the Elecsys assay (Roche). Analyzes were performed at the Laboratory of Clinical Chemistry at Sahlgrenska University Hospital during the study period.

Common features of the experimental studies (Paper III and IV)

In an experimental rat model, a bolus dose of isoprenaline was injected intraperitoneally at a dose of 50 mg/kg. This induces a SIC-like condition with LV apical akinesia and basal hyperkinesia within 90 minutes after injection[70] (Figure 6). Cardiac function was evaluated in cine-loops acquired by an ECG-gated acquisition technique in parasternal long axis view. Primary outcome was the degree of LV akinesia and expressed as % akinesia of total LV endocardial length (Figure 5). Other cardiac variables such as stroke volume and ejection fraction were extrapolated from the parasternal long axis, as this is the most reproducible view in small animals[105]. Echocardiographic evaluation blinded for treatment was performed after the experimental series were completed. In invasive experiments, animals were anesthetized and prepared with arterial cannulation of the right carotid artery, along with tracheotomy and mechanical ventilation. Heart rate, blood pressure and arterial blood gases (ABG´s) were obtained throughout the experi-

ment. Echocardiography was performed at 90 minutes after bolus dose of isoprenaline. In non-invasive experiments, animals were anesthetized and the bolus dose of isoprenaline was given. Animals were observed for 90 minutes followed by echocardiographic examination. Anesthesia was maintained from induction until echocardiography was performed in both invasive and non-invasive experiments. (Figure 5)

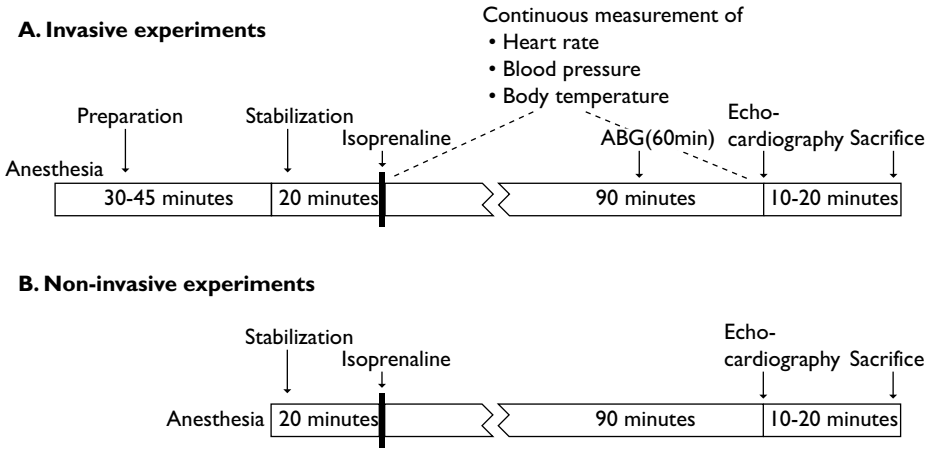


Figure 5. Schematic figure over invasive and non-invasive experiments. In brief, animals were prepared with an arterial cannula, trachetomised and mechanically ventilated. This was followed by a period of stabilization. An arterial blood gas (ABG) was obtained during this period. A bolus dose of isoprenaline, 50mg/kg, was injected intraperitoneally. Vital parameters were monitored and a second ABG was obtained after 60 minutes. Echocardiography was perfomed after 90 minutes. Anesthesia was maintained from induction until echocardiography was performed in both invase and non-invasive experiments. After echocardiography, animals were sacrified and the heart was collected for tissue analysis.

Study protocol, Paper III

This study was performed in two settings. The first setting was non-invasive; animals were randomized to either no anesthesia (n=12) or anesthesia with ketamine (n=12), pentobarbital (n=12) or isoflurane (n=12). One additional group was pretreated with the ATP-dependent potassium channel blocker, glyburide, before isoflurane anesthesia. SIC was induced with isoprenaline, followed by echocardiographic examination after 90 minutes. The second setting was invasive; all animals were anesthetized with ketamine + midazolam, and prepared with tracheotomy, carotid artery cannulation and mechanical ventilation. Animals were randomized to inhalation with air (n=12), isoflurane 0.75% (equivalent to 0.5MAC) (n=12) or 1.5% (equivalent to 1MAC) (n=12)[106]. SIC was induced with isoprenaline followed by echocardiographic examination after 90 minutes.

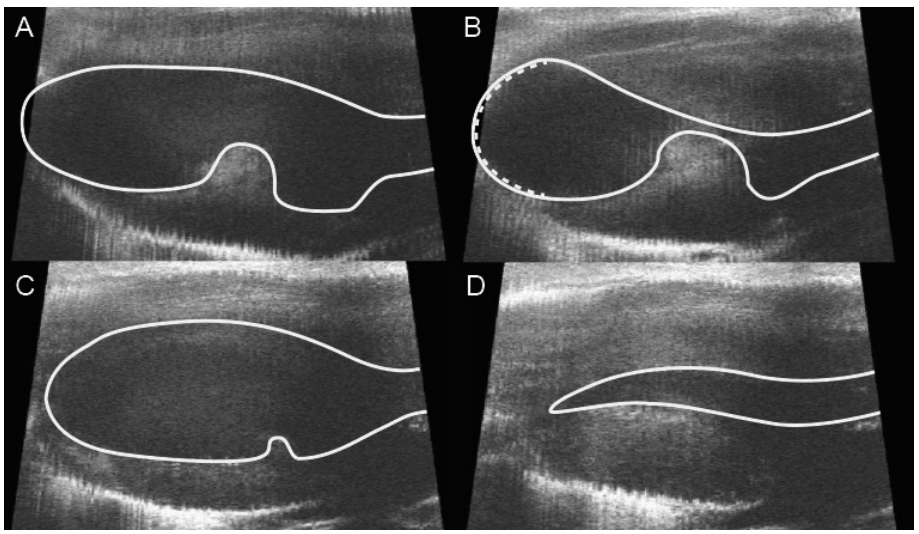


Figure 6. Echocardiography in a rat with SIC in diastole (A) and systole (B) and a normal/isoflurane-treated rat in diastole (C) and systole (D). Subendocardium is marked with the white line. The dashed white line in B represents area of akinesia. Degree of akinesia in the left ventricle (LV) was calculated by dividing length of akinesia in the LV with total length of the LV.

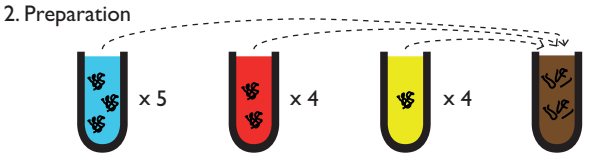
Study protocol, Paper IV

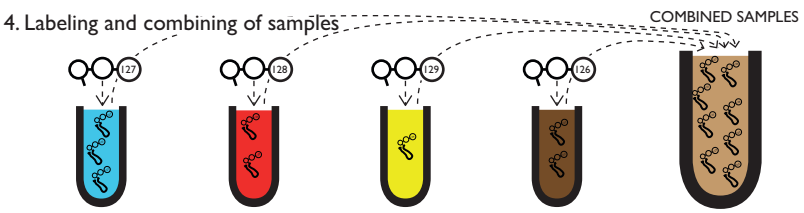
This study was performed in two different settings. The first setting was invasive; animals were randomized to anesthesia with ketamine + midazolam (n=15), propofol (n=15) or isoflurane (n=15). In the propofol group, the animals were induced with propofol, intraperitoneally (i.p.), a venous cannula was inserted and a propofol infusion was started corresponding to 0.6 MAC equivalents (360mg/kg)[107,108]. In the isoflurane-group, animals inhaled 0.9% isoflurane, equivalent to 0.6 MAC[106], after induction of anesthesia in an induction-chamber. In the ketamine + midazolam group, anesthesia was induced by ketamine 100 mg/kg + midazolam 10mg/kg, intra-peritoneally (i.p.) and maintained with iterated i.p. doses of ketamine 50mg/kg + midazolam 5mg/kg, if necessary. Depth of anesthesia was not fine-tuned in this group. Local anesthetics were applied before preparation. If necessary, the anesthetic dose was increased, as needed, in each animal if adequate anesthesia was not achieved. This was defined as a loss of hindlimb withdrawal to toe-pinch stimulation, hypertension (systolic pressure > 180 mmHg) or lack of limb movements during surgery. SIC was induced and echocardiography was performed after 90 minutes. The second setting was non-invasive; animals were induced with propofol i.p. and randomized to inhalation with no isoflurane (n=10) or inhalation with isoflurane (n=20) 1.0%. Animals inhaling isoflurane were randomized to receive either standard doses of isoprenaline (50mg/kg) (n=10) or high dose isoprenaline (100mg/kg) (n=10), while the group inhaling air, received 50 mg/kg isoprenaline ip. SIC was induced with isoprenaline and echocardiography was performed after 90 minutes.

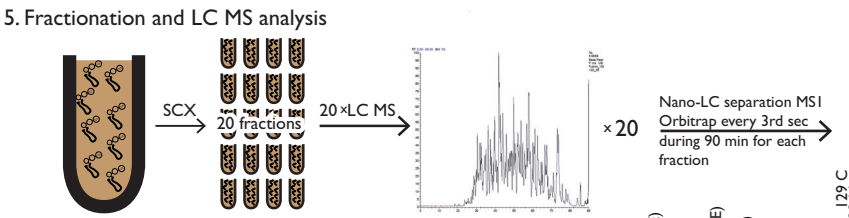
Proteomic analysis

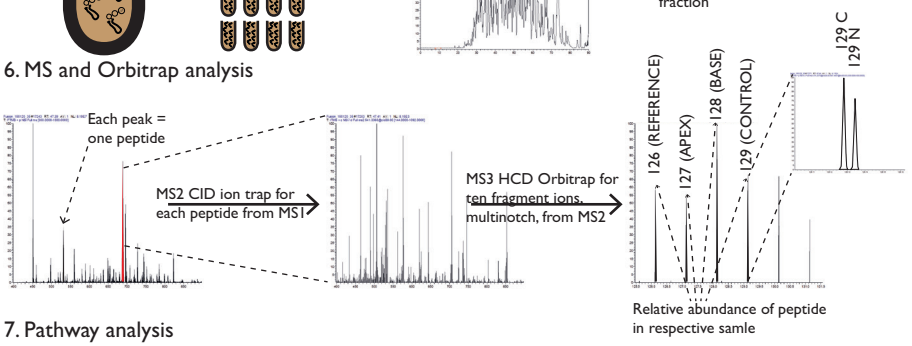
For determination of possible cardioprotective intracellular pathways activated by isoflurane, we used a global discovery proteomic analysis. In this method, basically, proteins in tissue samples are degraded to peptides and each tissue sample is marked with a specific label. The samples are mixed and analysed in a mass-spectrometer. The label can identify which peptide is that is eminating from a certain sample. By this mean, relative concentration of peptides in a sample is obtained. By identification of specific peptides, the peptide date is converted "backwards" to the corresponding protein. Thus, the relative concentration of all proteins found by the mass-spectrometer is identified in each samples. In addition to the exploration of single proteins, we identified biological pathways that are up- or downregulated, using a bioinformatics software (Figure 7 figure+text, Supplementary Matererial in Paper IV).

Figure 7. Brief description of the proteomic method. Tissue samples were taken from apex of the propofol-group (blue), isoflurane-group (red) and control-group (yellow) (1). The tissue samples were homogenised and a reference pool containing equal amounts of all samples was produced, representing the mean of all samples (2). Equal amounts of each sample and the reference pool were trypsin-digested using filter-aided sample preparation (FASP) (3). The peptides were subjected to isobaric mass tagging reagent TMT® with a unique tag for each sample and the reference. After labelling, samples were combined, resulting in two 10-plexed sets, both including the reference pool (4). The peptides were fractionated by strong cation exchange (SCX) chromatography and analysed by nano-liquid chromatography (LC) on-line, coupled to an Orbitrap Fusion Tribrid mass spectrometer. Peptides were analysed in a data-dependent multi-notch mode, in high-resolution mass spectrometer (MS) 1 mode. (5) Selection for MS2-fragmentation was performed by collision-induced dissociation (CID) with detection in the ion trap for peptide sequence information and ten synchronous peptide precursors selected, e.g., multi-notch, for MS3-fragmentation by high-energy collisional dissociation (HCD) with Orbitrap detection for relative quantification. MS-raw data for each TMT-set were merged during the database search for protein identification and relative quantification. Since the tags had an isobaric chemical structure, the peptide labelled with different tags, was indistinguishable during chromatographic separations and in MS mode. Each tag contained a characteristic so-called 'reporter ion' with a unique set of C13 or N15, which was detectable upon fragmentation. The ratios of these reporter ion intensities in MS3 spectra were used for quantitation. Only peptides unique to the specific protein were considered for quantitation. (6) Fold-change calculation between groups and statistical analysis were performed using Welch´s t-test, together with pathway analysis using Ingenuity Pathway Analysis to determine the biological relationships, mechanisms, functions, and pathways relevant to the identified proteins. (7)



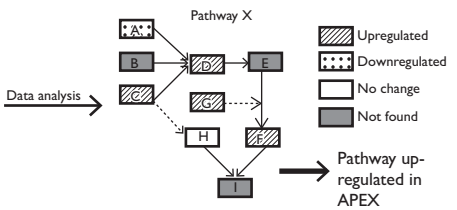






7. Pathway analysis

Protein	Fold change Propofol/Isoflurane	p-value
A	0.6	0.01
C	1.7	0.04
D	2.0	0.01
G	1.3	0.03
F	1.6	0.01
H	1.1	0.93



Statistics

Continuous variables were checked for normal distribution with inspection of histogram distribution and Shapiro–Wilks test. Fisher's exact test was used for comparing incidences between two groups with binary variables (All papers). Student's T-test was used to compare means of continuous normally distributed and Mann–Whitney U test was used to compare medians of non-normally distributed variables between the two groups (all papers). ANOVA, followed by by Fisher´s least significance difference post-hoc test was used to compare between-group differences for normally distributed variables and Kruskal-Wallis test followed by Mann-Whitney test was used for non-normally distributed variables (Paper III, IV). Jonckheere-Terpstra test for trend was used to test a dose-response effect of isoflurane (Paper I). For determination how variables evolved over time, linear mixed models were used (all papers). In this context, in Paper I, variables had to be logarithmed to fulfil normally distribution criteria and in Paper II, a generalized linear mixed model with gamma regression was used to handle this problem. Uni- and multivariable linear regression was used to determine most important variables associated with a continuous dependent variables (Paper I, III, IV). The dependent variable was logarithmed if necessary to fulfil normal distribution criteria (Paper I). All linear models were verified with scatterplot of residuals/predictive values and normal distribution of residuals. Uni- and multivariable logistic regression were used to determine the most important variables associated with a binary dependent variable (Paper II). Hosmer-Lemeshow goodness-of-fit was used to verify the logistic regression models. All data included in the multivariable models were checked for multi-collinearity and that data was balanced. In all multivariable models, we were cautious not to include more variables than the data-set allowed; our guideline was to include approximately one predictor per 10 observations in linear regression and one predictor per 10 observations with a positive outcome in the dependent variable in logistic regression. ROC curves were used to determine sensitivity, specificity and cut-off levels for continuous variables to predict/detect a clinical condition (Paper I, II).

Results

Paper I

One-hundred and twelve patients were included in whom 25 patients fulfilled criteria for SIC. Patients with SIC had higher peak levels of hsTnT and NTproBNP (Figure 8). Serial measurements of hsTnT and NTproBNP were obtained in 96% and 87% of the patients respectively. hsTnT had peak levels on day 1 while NTproBNP had peak levels on day 2 after onset of symptoms. This pattern was similar in both patients with SIC and without SIC (Figure 9). hsTnT on day 1 and NTproBNP on day 2 and day 3 after onset of symptoms as well as systolic blood pressure on admission had a high sensitivity and specificity to detect SIC. These variables were superior to high grade SAH, ECG abnormalities and signs of heart failure on chest x-ray. In a multivariate model, SIC and high grade SAH, were independently associated with peak levels of hsTnT. SIC was the only variable independently associated with NTproBNP.

Peak levels of hsTnT and NTproBNP closely correlated to each other (unpublished data, Figure 10). In a multivariable logistic regression model of variables associated with SIC, we found that high grade SAH, female sex and hsTnT were independent predictors of SIC. NTproBNP and hsTnT were highly correlated and could not be included in the same analysis. Twenty-one patients presented with an echocardiographic hyperdynamic left ventricular contraction pattern, with an EF>70%, with or without an intracavitary pressure gradient were identified.

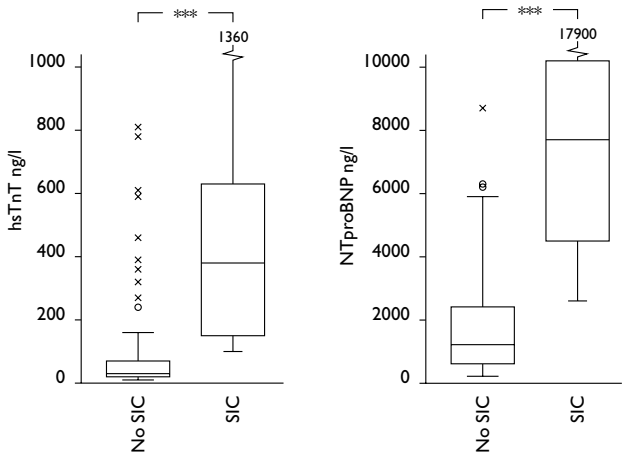


Figure 8. Peak levels of hsTnT (left) and NTproBNP (right) in patients with SIC and without SIC. SIC; Stress-induced cardiomyopathy. *** p<0.001

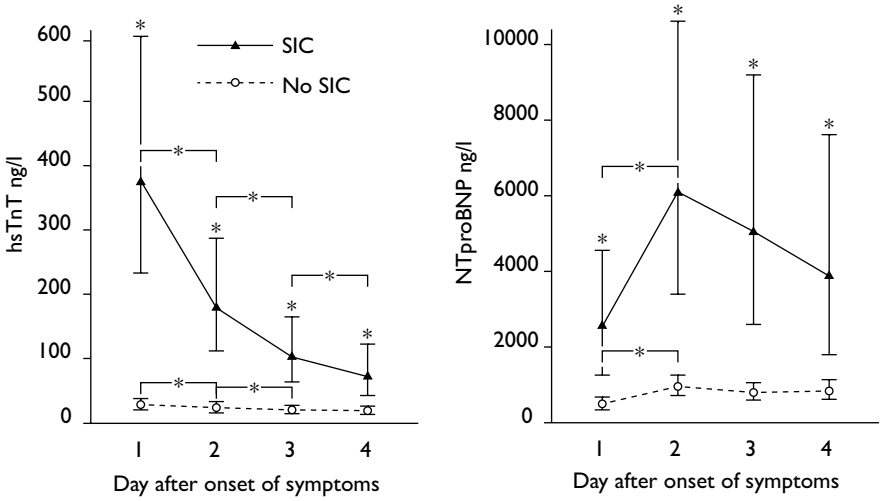


Figure 9. Levels of hsTnT (left) and NTproBNP (right) over time in patients with or without SIC. Levels of hsTnT and NTproBNP were higher in SIC patients at all days. hsTnT had its peak on day 1 after onset of symptoms with a daily decline in both groups. NTproBNP had peak levels at day 2 after onset of symptoms in both groups, the difference between day 2, 3 and 4 were not significant in any of the groups. Day 1 refers to first 24 hours after onset of symptoms. SIC; Stress-induced cardiomyopathy. * p<0.05

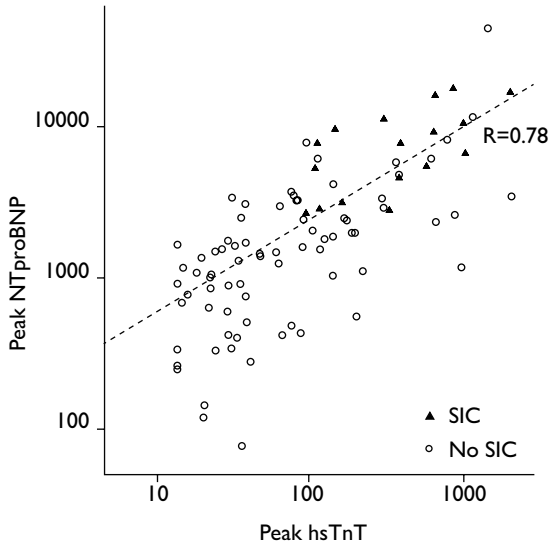


Figure 10. Peak levels of hsTnT and NTproBNP in patients with SIC and without SIC. There was a positive correlation between peak levels of hsTnT and NTproBNP. SIC; Stess-induced cardiomyopathy

Paper II

A total of 41 patients had poor long-term outcome and 18 patients had cerebral infarction due to delayed cerebral ischemia (DCI). Patients with poor long-term outcome were more likely to have a high grade SAH (WFNS grade 4-5), cerebral infarction and modified Fischer grade 4. They also had a higher mean age, higher heart rate and higher dose of given norepinephrine. Patients with cerebral infarction were more likely to have increased flow velocities, detected with transcranial Doppler (TCD) and a higher heart rate and dose of given norepinephrine. Levels of hsTnT and NTproBNP were higher in patients with poor long-term outcome and cerebral infarction (Figure 11). hsTnT peaked on admission, followed by a daily decline. NTproBNP had the lowest levels on admission followed by increased levels the following days (Figure 12). In a multivariable model, cerebral infarction of any cause, age, high grade SAH and hsTnT were all independently associated with poor outcome (Table 3). NTproBNP was not independently associated with poor outcome in the multivariable model. Increased flow velocities and hsTnT as well as NTproBNP were independently associated with cerebral infarction due to DCI. We also found that hsTnT had peak levels when taken as close as within six hours after onset of symptoms in 88% of patients (unpublished data). ECG pathologies, defined as ST-elevations or negative T-waves was associated with poor outcome but not taken consecutively in all patients and therefore not included in the published data (unpublished data).

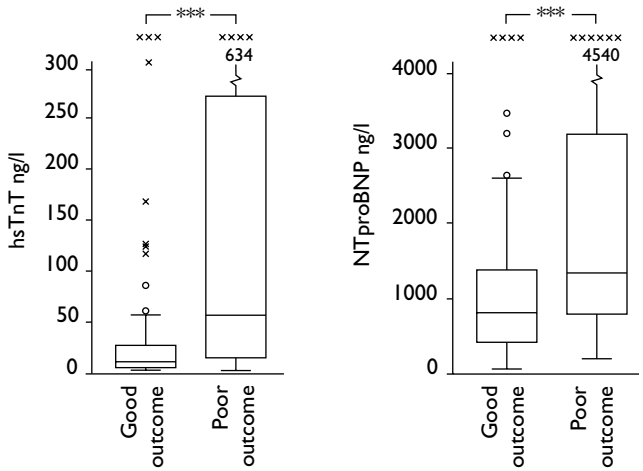


Figure 11. Peak levels of hsTnT (left) and NTproBNP (right) in patients with good (GOSE≥5) and poor (GOSE≤4) 1-year outcome. Levels of hsTnT and NTproBNP were considerably higher in patients with poor outcome. ***p<0.001

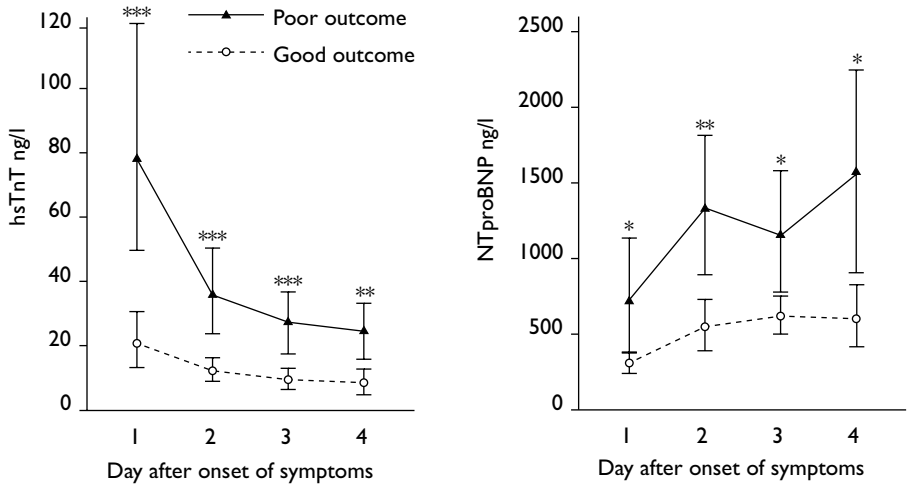


Figure 12. Levels of hsTnT (left) and NTproBNP (right) over time. Levels of hsTnT had its peak on day 1 after onset of symptoms followed by a daily decline both in patients with good (GOSE≥5) and poor (GOSE≤4) 1-year outcome. NTproBNP had its lowest levels on day 1 after onset of symptoms followed by increased levels the following days. Both hsTnT and NTproBNP levels were higher in in patients with poor outcome. Day 1 refers to first 24 hours after onset of symptoms. *p<0.05, **p<0.01, ***p<0.001

Table 3. Multivariable regression models, one-year poor outcome (GOSE≤4)

	Variable	OR	95% CI	p-value	Sig model change
Model 1	Cerebral infarction	11.43	4.15 - 31.5	<0.001	
	WFNS grade 4-5	6.57	2.39 - 18.04	<0.001	
	Age	1.05	1.01 - 1.10	0.009	
Model 2	Cerebral infarction	11.43	3.99 - 32.57	<0.001	<0.001
	WFNS grade 4-5	3.58	1.21 - 10.67	0.022	
	Age	1.06	1.01 - 1.10	0.013	
	TnT peak, per 100ng/l	1.59	1.10 - 2.29	0.013	
Model 3	Cerebral infarction	9.74	3.48 - 27.25	<0.001	0.056
	WFNS 4-5	5.72	2.06 - 15.87	0.001	
	Age	1.05	1.01 - 1.09	0.021	
	NTproBNP peak, per 1000ng/l	1.10	0.97 - 1.24	0.140	

GOSE; Glasgow Outcome Scale Extended, WFNS; World Federation of Neurosurgeons grading scale of subarachnoid hemorrhage, OR; Odds Ratio, CI; Confidence Interval

Paper III

In the first setting, animals anesthetized with pentobarbital and isoflurane had significant lower degree of LV apical akinesia than animals with no anesthesia (control). Degree of akinesia was lowest in isoflurane- anesthetized animals (Figure 13). There was no difference between ketamine anesthesia and control. There was no difference between isoflurane-anesthetized animals with or without pretreatment with glyburide. In the second setting, animals receiving isoflurane had a lower degree of LV apical akinesia than animals ventilated with air only. Animals receiving isoflurane 1.0 MAC had lower degree of akinesia than animals receiving isoflurane 0.5MAC; this difference was not significant but a test for trend was positive indicating a dose-response effect of isoflurane (Figure 14). This was accompanied by a higher LV ejection fraction, stroke volume and cardiac output. Animals receiving isoflurane had also lower blood pressure (Figure 15), body temperature, pH, pCO2, lactate levels and base excess at 90 minutes. In a multivariable regression model with degree of LV akinesia as the dependent variable, adding determinants of cardiac oxygen demand (body temperature, pO2, pressure-rate product) did not provide a significant better model than inclusion of treatment group only.

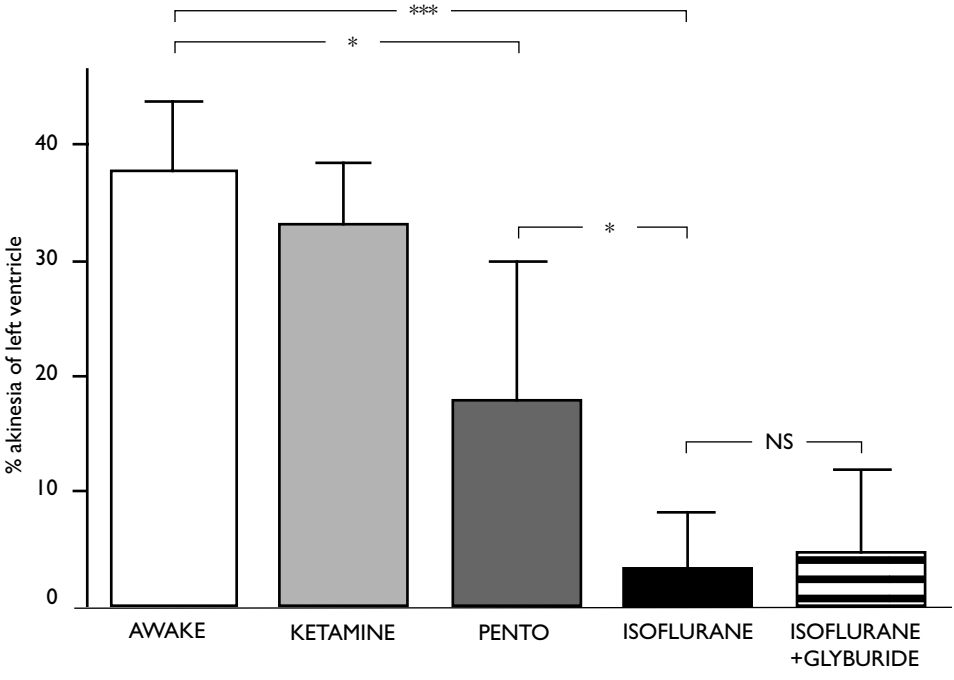


Figure 13. Degree of left ventricular akinesia in animals anesthetized with ketamine, pentothal (PENTO), isoflurane and without anesthetics (AWAKE). There was no difference between awake animals or animals anesthetized with ketamine. Degree of akinesia was lower in animals anesthetized with pentothal or isoflurane and levels were lowest in the isoflurane-group. Pretreatment with glyburid did not affect degree of akinesia in isoflurane anesthetized animals.

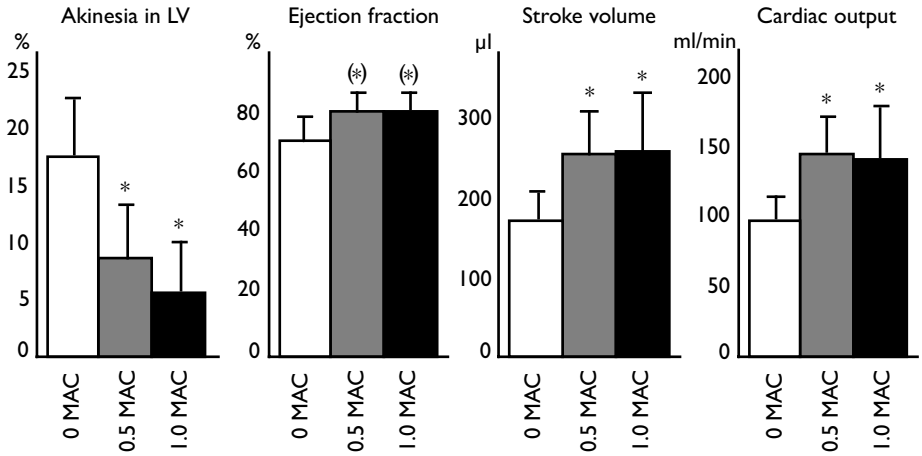


Figure 14. Degree of akinesia, ejection fraction, stroke volume and cardiac output in animals anesthetized with ketamine + midazolam only (0 MAC), ketamine + midazolam *and* 0.5 MAC isoflurane (0.5 MAC) or ketamine + midazolam *and* isoflurane 1.0 MAC (1.0 MAC). Degree of akinesia was lower in 0.5 MAC and 1.0 MAC-groups with a dose response of isoflurane. This was followed by an higher ejection fraction, stroke volume and cardiac output.

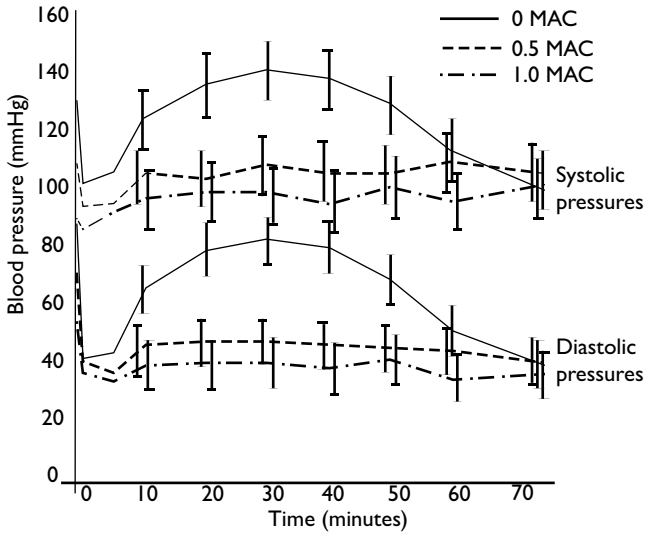


Figure 15. Blood pressure over time in animals anesthetized with ketamine + midazolam only (0 MAC) or ketamine + midazolam *and* isoflurane 0.5 MAC or 1.0 MAC. Blood pressure was higher in the 0 MAC group between 20 minutes to 60 minutes. Akinesia starts to develop 30 minutes after isoprenaline bolus, at the same time-point blood pressure starts to drop in the 0 MAC group.

Paper IV

Animals anesthetized with isoflurane had a significantly lower degree of LV akinesia than animals anesthetized with ketamine + midazolam or propofol (Figure 16). At baseline, pH and lactate were higher and pCO2 was lower in isoflurane animals, but they were all within the normal range. Blood pressure was higher and heart rate lower in ketamine + midazolam animals but heart rate, blood pressure and body temperature did not differ significantly between groups over time in the experiment. At 60 minutes, lactate levels was the only variable differing between groups, being higher in the isoflurane group compared to the ketamine-midazolam group. In a multivariable model, isoflurane concentration was the only variable independently associated with degree of LV akinesia. There was a significant negative correlation between the degree of LV akinesia and the inhaled isoflurane concentration (r=0.57) but not between degree of LV akinesia and propofol MAC-equivalents (r=0.26) (Figure 18). In setting two, isoflurane-anesthetized animals, receiving high dose isoprenaline (100mg/kg) had a significantly higher degree of LV akinesia compared to isoflurane-anesthetized animals receiving normal dose of isoprenaline (50mg/kg). There was no difference between animals receiving propofol + normal dose of isoprenaline (50mg/kg) and isoflurane + high dose of isoprenaline (100mg/kg) (Figure 17). In the proteomic analysis, multiple pathways were upregulated in propofol-anesthetised animals compared to the control group. A number of pathways involved in inflammation (e.g., acute phase signaling, IL-12 signaling, production of nitric oxide and reactive oxygen species), coagulation, lipid metabolism (LXR/RXR) and clatrine-mediated endocytosis signalling had higher activity. All these pathways had a lower activity in the isoflurane when compared to the propofol group. Comparing the isoflurane group with the control group, there was no activation of inflammation, the LXR/RXR pathway was upregulated as well as one pathway of coagulation system and caveolar-mediated endocytosis (Table 4).

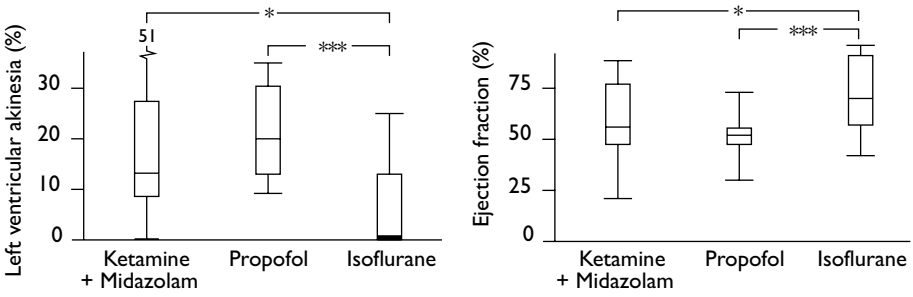


Figure 16. The degree of LV apical akinesia (left) and LV ejection fraction (right) 90 minutes after intra-peritoneal isoprenaline injection in groups receiving isoflurane, propofol or ketamine-midazolam anesthesia, The degree of LV apical akinesia was lower and LV ejection fraction was higher in the isoflurane group compared to both the propofol and ketamine + midazolam groups, while there were no differences between the propofol and the ketamine + midazolam groups. * $p < 0.05$, ** $p < 0.001$.

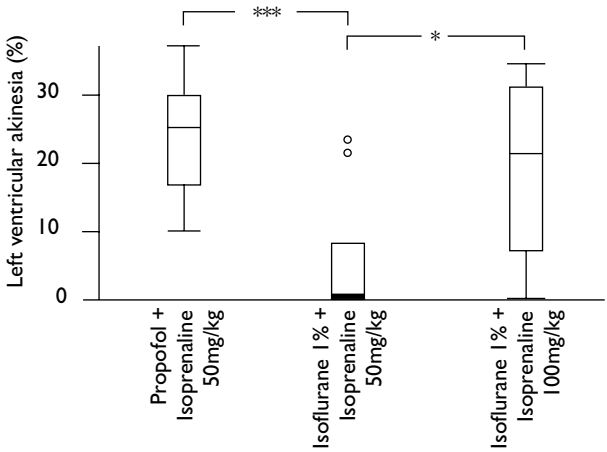


Figure 17. The degree of left venticular akinesia in groups receiving either propofol only + isoprenaline 50 mg/kg, isoflurane 1% + isoprenaline 50 mg/kg or isoflurane 1% + isoprenaline 100 mg/kg. Doubling the dose of isoprenaline in isoflurane anesthetized animals resulted in higher degree of LV akinesia. * $p < 0.05$, ** $p < 0.001$.

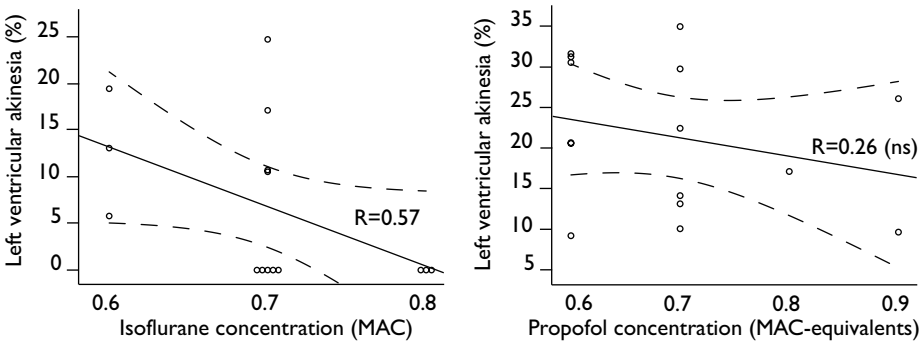


Figure 18. The dose-dependent effects of isoflurane (left) and propofol (right) on the extent of LV akinesia. The correlation was significant for isoflurane (p=0.027) but not for propofol (p=0.535).

Table 4. Important pathways identified with higher or lower activity in respective group

Category	Pathway	Propofol vs control	Isoflurane vs propofol	Isoflurane vs control
Inflamamtion	Acute Phase Response Signaling	↑↑↑	↓↓↓	-
	IL-12 Signaling and Production in Macrophages	↑↑↑	↓↓↓	-
	Production of NO and ROS in Macrophages	↑↑↑	↓↓↓	-
	Atherosclerosis Signaling	↑↑↑	↓↓↓	-
Coagulation	Coagulation System	↑↑↑	↓↓↓	↑
	Intrinsic Prothrombin Pathway	↑	↓	-
	Extrinsic Prothrombin Pathway	↑	↓↓	-
Endocytosis	Clathrin-mediated Endocytosis	↑↑↑	↓↓↓	-
	Caveolar-mediated Endocytosis	-	-	↑
Lipid metabolism	LXR/RXR Activation	↑↑↑	↓↓↓	↑↑
Other	FXR/RXR Activation	↑↑↑	↓↓↓	-
	Actin Cytoskeleton Signaling	↑	-	-
	PPAR/RXR Activation	-	↓↓	-
	Lipid Antigen Presentation by CD1	-	↓	-

IL-12; Interleukin 12, LXR/RXR; Liver X receptor/Retinoid X receptor, FXR/RXR; Farsenoid X receptor/Retinoid X receptor, PPAR/RXR; Peroxisome proliferator-activated receptor/Retinoid X receptor, CD1; cluster of differentation 1, ↑ $p < 0.05$; ↑↑ $p < 0.01$; ↑↑↑ $p < 0.001$, ↑ $p < 0.05$; ↑ $p < 0.01$; ↑ $p < 0.001$

Discussion

Methodological considerations

Ethical issues

The studies were approved by the regional ethical board. In paper I, which was a retrospective study, informed consent was not obtained. However, all patients were anonymised. In Paper II, all patients or their next-of-kin were asked for consent at time of follow-up.

The ethical issues of the conduction of animal experiments for scientific purposes, are complex and debatable. If conducting animal experiments, the potential scientific value has to be of such importance that it overcomes the potential suffering of the animals. Animal experiments should only be performed when relevant information cannot be obtained from experimental studies in humans or *in vitro* studies. Even though animal experiments might give extraordinary information in the pathogenesis of, or therapeutic mechanisms of a disease, most results obtained from animal studies are not applicable in clinical praxis and results from animal experiments and clinical trials are often discordant[109]. However, much of the advances in heart failure therapy are based on animal studies, including e.g. the use of angiotensin converting enzyme-inhibitors and beta-blockers[110,111]. We consider the experiments performed in this thesis not being possible to perform in humans or *in vitro*. When conducting animal experiments, from which this thesis is based, we practice the principle of 3R (replace, reduce, refine), the hierarchy of alternatives to animal testing. All animals were treated in according to the NIH guidelines for care and treatment. Experiments were on beforehand thoroughly designed to acquire as much data as possible. All animals were treated with greatest respect in handling and preparation, optimizing experimental results and avoiding unnecessary loss of animals.

Prospective and retrospective observational studies

The clinical studies in this thesis are observational studies by nature. A retrospective study (Paper I) have the advantage that it is easy to perform and data can be acquired from a long time period. Since SIC in SAH is not frequent, we needed a long time frame for inclusion of patients. In Paper I, we therefore collected data from a time period corresponding to almost five years. A multicenter study would have been more appropriate,

but is costly and are resource intense. The disadvantage with retrospective studies is that data are more likely to be biased; in this paper, e.g., patients examined with echocardiography had a more severe disease and were more hemodynamically unstable.

Biomarkers for cardiac function in ICU patients

The use of biomarkers for diagnostic purposes has the advantage of being easy to implement in clinical practice, in contrast to echocardiography which is time-consuming and operator dependent. hsTnT has a very high sensitivity and specificity for myocardial injury and NTproBNP has a high sensitivity and specificity for congestive heart failure[119]. Several studies have shown that levels of hsTnT and NTproBNP are increased in patients with primary SIC and SIC after SAH [9,112-114]. However, both hsTnT and NTproBNP are increased also in other conditions in the ICU, such as sepsis, respiratory failure, and acute renal failure[115-119]. Especially in conditions like SIRS and sepsis, NTproBNP increases to levels comparable with, or higher than, levels seen in heart failure. A highly increased NTproBNP is found to be discriminatory for septic shock vs non-septic shock with *higher* levels in the septic shock group[115,120]. The new high-sensitive troponin assays are also problematic since they are "too sensitive" and detectable levels are not uncommon in healthy individuals. No absolute cut-off level for the diagnosis of myocardial infarction (MI) exist and current recommendations suggest that "the higher the level, the greater the likelihood of MI" [119]. Although there are false positives among patients with increased hsTnT and chest pain, this is not be neglected, as they can suffer from other acute conditions e.g., pulmonary embolism, aortal dissection[119]. However, as SAH-patients seldom suffer from these non-cardiac conditions increasing hsTnT and NTproBNP on admission, hsTnT and NTproBNP could possibly be used to identify patients with SIC after SAH.

Global proteomic analysis

A global proteomic analysis gives information of the proteome in a tissue sample. This data can be further analyzed by bioinformatics analysis, revealing up- or downregulated pathways. As the pathogenesis of SIC in unknown, finding the cardioprotective mechanism(s) by isoflurane is like searching a needle in a hay-stack. A global proteomic apporach is especially useful in these cases, when the pathogenesis is not fully understood, as it is gives a global picture of the biological activity in a sample and does not need a carefully pre-defined hypothesis[121-123].

Clinical perspectives

One of the main findings in this thesis was that hsTnT and NTproBNP have an excellent negative predictive value for detection of SIC after SAH. We suggest the use of these biomarkers for screening of SIC after SAH, which might shorten time to diagnosis and treatment of SIC. As patients with SIC after SAH have a higher risk of poor outcome[55,59,60], this could potentially improve outcome in these patients.

The second clinical important finding was that increased hsTnT obtained close to admission to NICU, had an association with poor long-term outcome. This finding was significant when adjusting for cerebral infarction, neurological status on admission and age. The long-term outcome effects of cardiac complications after SAH, is barely studied and the previous results are inconclusive, most likely due to non-prospective, consecutive study designs[60,63,64]. These results tell us that cardiac damage, as measured by troponins, is an ominous clinical sign that should be taken in consideration in the medical care of the patient.

We also found that an increased heart rate and ECG pathologies were associated with poor outcome. These factors, together with increased troponins, are known to be associated with an increased sympathetic tone after SAH[95,124,125]. Exogenously administered catecholamines were also higher in patients with poor outcome. Thus, patients with poor outcome were most likely suffering from increased cardiac sympathetic activity and higher plasma catecholamine levels. An increased sympathetic tone could be due to a more severe SAH with a prolonged period of increased intracranial pressure at the time of bleeding, with subsequent global brain ischemia and more cerebral damage. However, as troponins were associated with poor outcome even when adjusting for neurological status on admission, this cannot fully explain this finding. In animal experiments it has been shown that catecholamines are shown to increase cerebral metabolism if the blood-brain barrier is damaged[126,127], which is seen after SAH[128,129]. Impaired autoregulation is frequently seen after SAH[130,131], which, in combination with compromised systemic hemodynamics might impair cerebral perfusion. Indeed, global and focal cerebral perfusion has been found to be decreased in patients with cardiac complications after SAH[132]. An increased cerebral metabolic demand, due to catecholamine surge and damaged blood-brain barrier, in combination with decreased cerebral perfusion, increases the risk of cerebral oxygen supply/demand mismatch (Figure 19). This could increase the risk of cerebral infarction due to DCI, which was found in the present study. However, troponins were also independently associated with poor outcome when adjusting for cerebral infarction. Based on this, we suggest the possibility of a diffuse cerebral damage caused by oxygen supply/demand mismatch not detectable on ordinary CT-scan in patients

with excessive sympathetic activity. There are also animal studies indicating a direct toxic effect of catecholamines on the CNS, although this subject is not widely studied[133].

Currently there is no accepted treatment of SIC. Recommendations are based on treatment of congestive heart failure which do not have scientific evidence and could be harmful[134-136]. Inotropes are debatable as this might "stress" the myocardium further. Based on the best available evidences, in patients with severe, primary SIC, an active, non-pharmacological support is suggested allowing the myocardium to recover itself[136]. However, in patients with secondary SIC due to critical illness, this might not be the best treatment as decreased cardiac output with or without hypotension contribute to multi-organ failure.

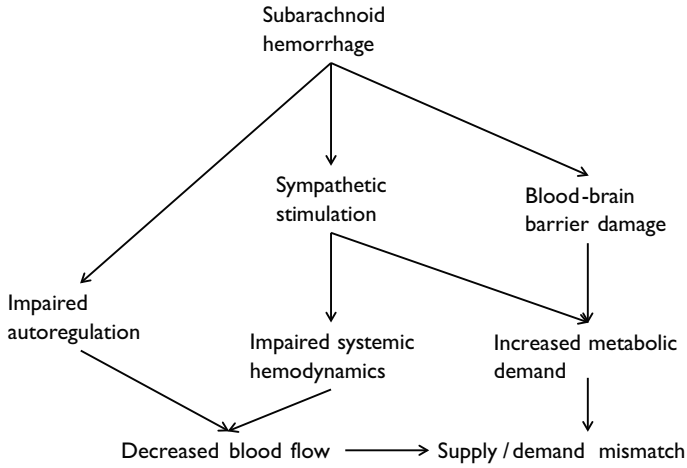


Figure 19. Suggested mechanism of sympathetic overstimulation contributing to cerebral damage. After subarachnoid hemorrhage, blood-brain barrier is often damaged which results in increased metabolic demand from catecholamines. Impaired autoregulation in combination with impaired systemic hemodynamics lead to decreased cerebral blood flow. An increased metabolic demand in combination with reduced blood flow increases the risk of cerebral damage.

Patients with cardiac complications after SAH are to be meticulously treated to optimize global hemodynamics and ensure cerebral perfusion. However, this should probably be achieved with the minimal use of inotropes and vasopressors. There are some important key-points regarding diagnosis and treatment of SIC in SAH-patients:

Analysis of Troponin T and NTproBNP. Should be performed as soon as possible after admission for rapid detection of cardiac complications. Increased levels of troponin T>50ng/l or NTproBNP >2500ng/l should be an indication for echocardiography and possibly cardiac output monitoring.

Monitoring cardiac output. To optimize system hemodynamics and avoid under- or overtreatment, cardiac output (CO) monitoring should be considered[40]. Some patients with primary SIC have a low system vascular resistence (SVR) and normal CO[8], thus cardiac output measuring is essensial in discriminating if hypotension caused by low SVR or CO. The PiCCO device is most likely preferable over Swan-Ganz catheter, as it can measure extravascular lung water and avoiding catheterisation of the heart.

Define the lowest acceptable MAP. Define the lowest acceptable MAP/CPP, according to the clinical situation[40]. Is the patient conscious? Are there new symptoms of neurological deterioration? Which is the lowest MAP needed to maintain diuresis?

Optimization of oxygenation and haemoglobin. To optimize oxygen delivery. Ensure adequate oxygenation. Traditionally, induced hemodilution was recommended in treatment of vasospasm but is no longer recommended. A haemoglobin level *over* 80-100g/l is recommended[40]. In patients with cardiac complications the upper threshold is probably beneficial.

If ICP is high, consider neurosurgical interventions to lower ICP. To obtain an acceptable CPP with a lower MAP, if ICP is high, neurosurgical intervention should be considered, e.g. ventricular drainage or evacuation of intracerebral hematoma.

Is there an **outflow obstruction** of the left ventricle, treat cautiously with plasma volume expansion (colloid) in combination with beta-blockers and phenylephrine[137].

Optimize fluid balance. Euvolemia should be maintained. Hypovolemia, fluid excess and pulmonary edema should be avoided[40]. If using PiCCO, fluid balance could be guided by cardiac output measurements and extravascular lung-water measurement[138]. Maintaining global end-diastolic volume index slightly above normal levels is recommended in one study[139].

Minimise the dose of vasodilators and sedatives. Use the lowest dose of sedatives cardio-depressants and vasodilators. Nimodipine is suggested to be lowered if having problems maintaining adequate MAP[40] but is controversial scince this is the only drug shown efficient in preventing cerebral infarction and improving outcome[41].

If an adequate MAP or CPP is not achieved; use **vasopressors** if system vascular resistance is low and use **inotropes** if cardiac output is compromised. **Milrinone** is probably superior to dobutamine as it increases cardiac output to a greater extent than dobutamine, in SIC after SAH [140]. In addition, milrinone might have a positive effect on cerebral vasospasm as it is efficient in the intra-arterial treatment of established vasospasm[141]. Furthermore, dobutamine is shown to induce SIC and might stress the myocardium further[68,69].

Vena-arterial ECMO. If cardiogenic shock is refractory, veno-arterial ECMO could be considered until the heart regain normal function. Intra-aortic balloon pump (IABP) is recommended in some papers[137] and is considered safe in SAH-patients[142]. However, case-reports suggest that hemodynamics might be further impaired by use of IABP[136].

Thrombosis prophylaxis. Are not to be forgotten. There is an increased risk of thrombosis formation in patients with hyperadrenergic states and patients with high levels of catecholamines with or without SIC are at higher risk of thrombosis formation[143,144]. In one study, almost 5% of patients with primary SIC had embolic events[8].

New perspectives in the pathogenesis of stress-induced cardimyopathy after SAH

We could demonstrate that cardiac troponins are released shortly after the onset of symptoms followed by a subsequent decline. The vast majority (~90%) of patients had peak levels of hsTnT on admission also when taken as close as six hours after onset of symptoms. This is a much faster increase in troponin than in myocardial infarction, where peak-levels are seen on day two or three after onset of symptoms[145]. This further strengthens the evidence that cardiac damage is a consequence of the sympathetic stimulation seen in conjunction with the bleeding. We also found that cardiac damage is highly associated with SIC. However, other co-factors are probably important in the development of SIC as not all patients with increased troponins developed SIC. Female sex seem to be a risk-factor; although troponin levels did not differ between genders, SIC was highly overrepresented in women (~90%) and in patients with a substantial troponin release (>89ng/l), 78% of women, while only 27% of men, developed SIC.

This was further supported by an unpublished multivariable analysis, in which both troponins and female sex were independent predictors of SIC. However, other un-identified factors seem to be important in development of SIC (Figure 20). We also found that some patients developed a hyperdynamic LV contraction pattern with high ejection fractions. A high ejection fraction in patients with SAH is described in one previous study[53] and suggests that some patients may develop a physiological response to the excessive beta-stimulation, while some patients develop a paradoxical LV hypokinesia (Figure 20). This is, to our knowledge, the first time SIC after SAH is described in a Scandinavian population. This is somewhat important as there is evidence for a genetic predisposition with different frequencies of SIC in different populations[8].

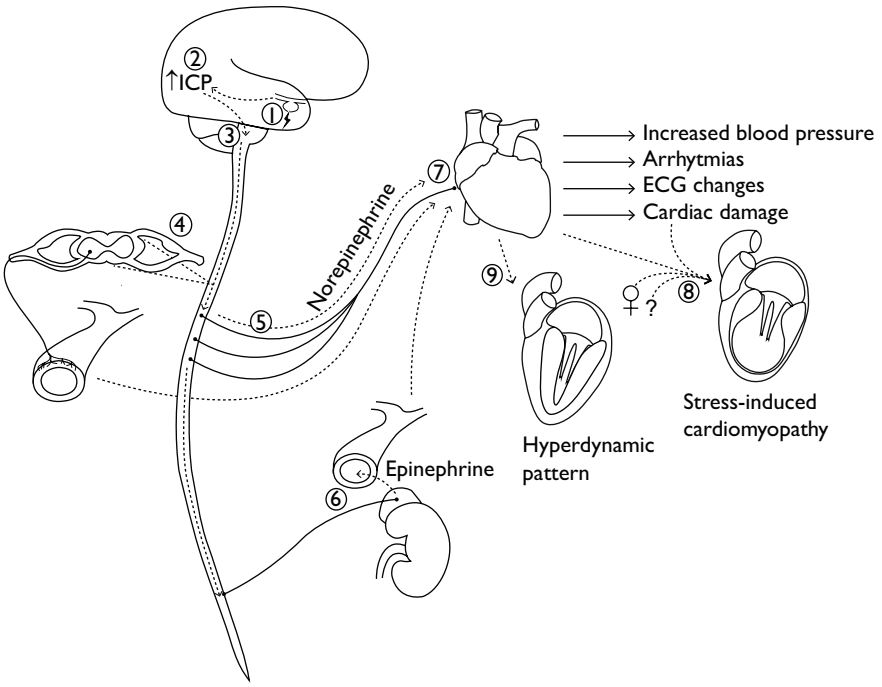


Figure 20. New perspectives in pathogenesis of stress-induced cardiomyopathy (SIC) after subarachnoid hemorrhage (SAH). Rupture of a cerebral aneurysm gives rise to a sudden rise in ICP which activates the sympathtic nervous system resulting in catecholamine stimulation of the heart (1-7, for details see Figure 4). ~ 20% of patients developed a left ventricular hyperdynamic pattern (9) suggesting that some patients develop a physiological response the the catecholamine stimulation and some patients develop SIC. We found a close correlation between cardiac damage and developement of SIC (8). However, not all patients with substantial cardiac damage developed SIC and other contributing factors seem to be important. Women were highly overrepresented in development of SIC but not all women with troponin release developed SIC. Other co-factors, that we did not identify in these studies, seem to be important in development of SIC.

New perspectives on cardioprotection in stress-induced cardiomyopathy

In the two experimental studies, we showed that isoflurane exerted a cardioprotective effect in the development of experimental SIC, a finding that we could reproduce in four different experimental settings. This should be considered as one of the main findings, as animal experiments, in general, have a low reproducibility[146,147]. In this context, our animal model is reproduced by two other research groups[148,149] and the cardioprotective effect of isoflurane has demonstrated by one other group (unpublished data).

As isoflurane decreases myocardial oxygen demand (MVO_2) by lowering the three major determinants of MVO_2: LV wall tension (arterial blood pressure), heart rate and contractility[150-152], one could argue that the cardioprotective effects of isoflurane are mediated by attenuation of the stress-induced increase in MVO_2. In the first experimental setting, in Paper III, animals had different doses of anesthetics and vital parameters differed between groups. A multivariable analysis indicated that it was unlikely that decreased MVO_2 was the mediator of the isoflurane-induced cardioprotection. In this experiment, ketamine was used, which increases MVO_2 and enhance cardiac sympathetic activity[153-155]. The myocardial effects of propofol, which was studied in Paper IV, are more similar to those of isoflurane, as it also decreases MVO_2.[156,157] Both isoflurane and propofol also suppress cardiac sympathetic nerve activity[153,158,159] and both agents are shown to be cardioprotective in ischemia/reperfusion[160-162]. In the first setting in Paper IV, the degree of LV akinesia was significantly lower in isoflurane-group compared to propofol when compared at equi-anesthetic doses. Our conclusion from these experiments is that reduced cardiac oxygen demand is not the mechanism of cardioprotection of isoflurane in SIC.

Isoflurane is known to be cardioprotective in ischemia and ischemia/reperfusion (I/R) injury. I/R have a pathophysiology that is similar to SIC, as cAMP and calcium overload is involved in the pathogenesis of reperfusion damage[101,163]. Isoflurane exerts a well described cardioprotective mechanism, mimicking ischemic preconditioning, also known as anesthetic preconditioning (APC) in I/R injury[164]. APC is mediated through mitochondrial K_{ATP}-channels and the effect is efficiently blocked by glyburide[165]. Interestingly, pretreatment with glyburide did *not* attenuate the cardioprotective effects of isoflurane in Paper III. However, isoflurane is described to have a number of other cardioprotective properties in I/R injury that may be active also in SIC, as these two conditions share several pathophysiological mechanisms. These include activation of inhibitory G-proteins[166], preservation of calcium-regulating proteins (SERCA/phospholamban)[167], improvement of cardiac microcirculation[168], attenuation of apoptosis induced by beta-stimulation[169] and attenuation of inflammation[170-172].

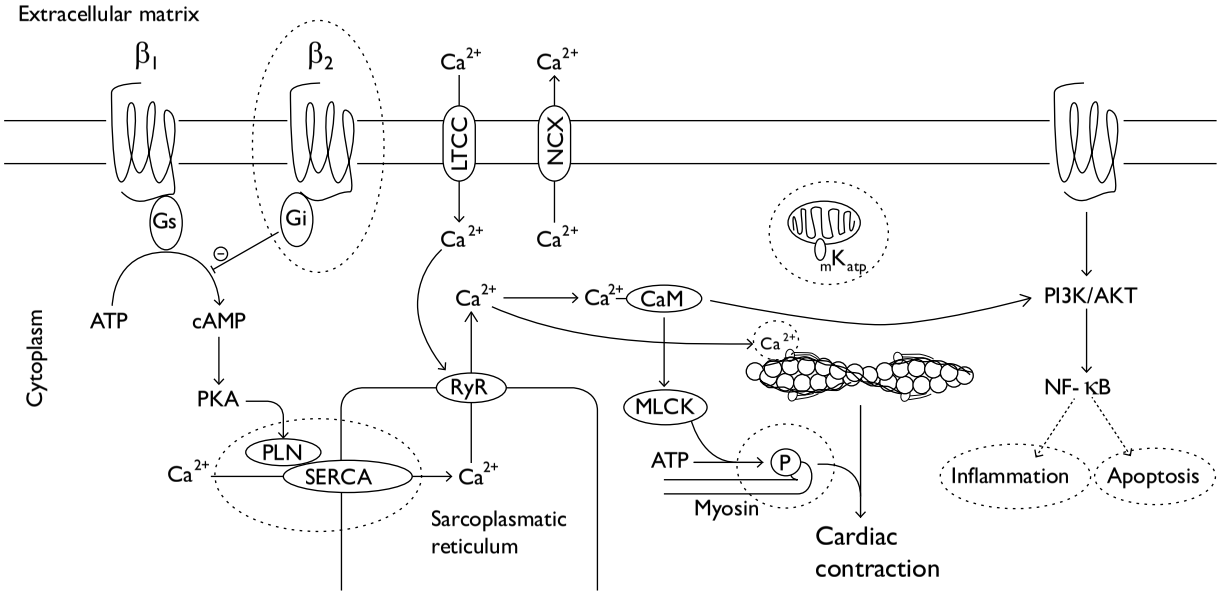


Figure 21. Described intracellular potential cardioprotective mechanisms of isoflurane. Anesthetic preconditioning (APC) is induced by isoflurane by activating mitocondrial Katp-channels. Isoflurane is shown to activate inhibitory G-proteins in anesthetic preconditioning (APC) and retain PLN/SERCA-ratio in ischemia/reperfusion (I/R). Isoflurane is shown to reduce cytoplamic calcium load in I/R. Isoflurane also decreases binding of myosin to actin-filaments and reduces calcium sensitivity in myofilaments. This leads to a reduced myocyte contraction, thus reducing the risk of hypercontracture and formation of contraction band necrosis (CBN). Isoflurane also has a primary effect in attenuating inflammation and apoptosis. This effect is shown to be induced through the PI3K/AKT pathway and NF-KB modulation. Dashed lines; potential cardioprotective mechanism of isoflurane. PLN; Phospholamban, SERCA; Sarcoplasmic/endoplasmic reticulum calcium ATPase, ATP; Adenosine triphosphate, cAMP; Cyclic adenosine monophosphate, PKA; Protein Kinase A, RyR; Ryanodine receptor, CaM; Calcium-calmodulin complex, MLCK; Myosine light chain kinase, LTCC; L-type calcium channel, NCX; Sodium calcium exchanger, Gs; Stimulatory G-proteins, Gi; Inhibitory G-proteins, PI3K/AKT; Phosphatidylinositol-3-Kinase/Protein Kinase B, NF-KB; Nuclear factor kappa beta

We used a global proteomic analysis with pathway analysis to identify potential cardioprotective pathways. We found that pathways of inflammation, cogulation, endocytosis and lipid metabolism were highly more active in tissue from animas anesthetised with propofol. The pathways of inflammation and coagulation were not active in isoflurane-anesthetised animals and the only pathway still upregulated was the LXR/RXR pathway. The LXR/RXR pathway is shown to induce lipid droplet formation which reduce calcium overload and CBN in I/R and could be considered as cardioprotective[173-175]. However, as almost all pathways were downregulated, with isoflurane, our conclusion from this analysis is that isoflurane acts as a general suppressor of the pathogenic cascade activated by the beta-receptor overstimulation. Interestingly, the two proteins S100-A8/A9 which was highly upregulated in tissue from propofol-anesthetised animals, and to a lesser degree in isoflurane-anesthetised animals, are activated in sepsis-induced cardiac dysfunction[176]. This suggest a possible link between SIC and sepsis-induced cardiac dysfunction.

To evaluate whether the cardioprotective effects of isoflurane is caused by a competitive inhibition of the beta-receptor signalling pathways, we induced SIC by a higher dose of isoprenaline. With a double dose of isoprenaline, isoflurane was not cardioprotective, as the degree of LV impairment did not differ from a control group not receiving isoflurane. We have also described a dose-response cardioprotective effect of isoflurane in SIC in two settings (Setting two, Paper III and Setting one, Paper IV). In summary, this suggests a competitive inhibition in the beta-receptor signalling induced by isoflurane. Isoflurane is shown not to affect the affinity of the beta-receptors[177] and, therefore, do not impair the response of the beta-receptor to isoprenaline. Beta-stimulatory vasodilation is attenuated by isoflurane, a mechanism that is downstream of cAMP-formation[178]. Studies have shown that isoflurane is a potent inhibitor of calcium influx to the cytoplasm and decreases calcium myofilament sensitivity[179-184], thus potentially reducing risk of calcium overload, hypercontracture and CBN. This is in contrast to propofol, which has been shown to have no influence on calcium release in the beta-stimulation signalling pathway[185-187], increases cytoplasmic calcium levels[188] and increases calcium myofilament sensitivity[187,189,190]. Indeed, studies with isoflurane have verified a cardiomyocyte protection from calcium overload with a reduced amount of contraction band necrosis[181,182]. From our results and these previous studies, we suggest that isoflurane act its cardioprotecive mechanism by reducing calcium influx or calcium myofilament sensitivity (Figure 21). However, further experiments are needed to verify this hypothesis.

Potential benefits of sedation with volatile anesthetics in ICU-patients

Isoflurane have potential benefits in patients suffering from hyperadrenergic conditions and appealing to use in ICU-patients. Isoflurane sedation in the ICU is safe and a non-costly equipment is commercially available that is easy to handle and can be managed by ICU nurses[191-193]. Wake up time is short and some studies have shown a shorter wake up time than with propofol[191,194]. The cardioprotective effect with isoflurane sedation in the ICU has also been described clinically. In patients undergoing coronary artery bypass surgery, randomized to sedation in the ICU with propofol or volatile anesthetics, troponin release over time tended to be lower in volatile anesthetic-groups[195-198]. Other positive effects are also described; a retrospective study has shown improved long-term mortality in patients with volatile anesthetic-sedation. Furthermore, animal studies have shown reduced inflammatory response and improved oxygenation in pigs with ARDS and isoflurane used per-operatively attenuate apoptosis[199-201]. Sedation with volatile anesthetics in the NICU is controversial as volatile anesthetics are potent vasodilators of cerebral vessels and might increase ICP and impair cerebral autoregulation[138,202]. Two studies have reported the effects of volatile anesthetics sedation in stoke; both studies reported that the method can be used but increased ICP and decreased MAP was found in some patients and was more pronounced with sevoflurane[203,204]. However, in patients with SAH, cerebral vasospasm, and *not* increased ICP, is the main problem. Isoflurane is alluring to use in SAH-patients, as it is a potent cerebral vasodilator[202], reduces cerebral metabolism[205,206], have cardioprotective properties[164] and is a potent calcium-inhibitor[179-182]. There is one study with a cross-over design in patients with SAH, which showed that cerebral blood flow was increased when sedated with isoflurane compared to propofol and there were no problems with increased ICP in that study[207]. Interestingly, isoflurane is also shown to attenuate vasoconstriction due to ET-1[208], which is one of the major components in cerebral vasospasm.

Conclusions

The mains findings of this thesis were:

Myocardial injury, as measured with the release of high-sensitive troponin-T (hsTnT), is common after subarachnoid hemorrhage and is independently associated with an increased risk for poor long-term neurological outcome and cerebral infarction.

Biomarkers for myocardial injury (hsTnT) and dysfunction (NTproBNP) are useful for early detection of stress-induced cardiomyopathy after subarachnoid hemorrhage and can be used for screening of this condition.

Cardiac damage is seen very close to onset of symptoms in patients with subarachnoid haemorrhage.

Stress-induced cardiomyopathy is seen after subarachnoid hemorrhage in a Scandinavian population and is more frequent in women.

In an animal model of stress-induced cardiomyopathy, isoflurane attenuates the degree of LV dysfunction, an effect not seen with propofol.

The cardioprotective effect of isoflurane in the development of SIC is not mediated by an opening of mitochondrial ATP-controlled potassium channels, as previous described in myocardial ischemia/reperfusion injury.

The cardioprotective mechanism of isoflurane in the development of SIC is not mediated by an attenuation of stress-induced increases in myocardial oxygen demand induced by excessive beta-stimulation.

The cardioprotective mechanism of isoflurane in development of SIC is likely mediated by a competitive inhibition of the beta-receptor signalling pathways, thus, suppressing up-regulated intracellular pathways activated by the overstimulation of beta-receptors.

Future perspectives

Patients developing cardiac complications after SAH have a poor prognosis. Isoflurane may have a cardioprotective potential in these patients and is a potent cerebral vasodilator that may be useful for prevention of cerebral vasospasm. Although there are many aspects to consider; isoflurane sedation in patients with SAH might be beneficial and could be tested in future clinical trials.

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