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The role of cortisol and cortisol dynamics in patients after aneurysmal subarachnoid hemorrhage

vorgelegt von / submitted by
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Table of contents

Abbreviations

11β-HSD	11β-hydroxysteroid dehydrogenase
ACTH	adrenocorticotropic hormone
AI	adrenal insufficiency
ALQI	Aachen Life Quality Inventory
aSAH	aneurysmatic subarachnoid hemorrhage
AUC	area under the curve
BDI	Beck Depression Inventory
CAR	cortisol awakening response
CBG	corticosteroid binding globulin
CCT	cranial computed tomography
CFSC	calculated free serum cortisol
CRF	corticotropin-releasing factor
FCT	Fisher CT scale
GCS	Glasgow Coma Scale
GH	growth hormone
GHD	growth hormone deficiency
GHI	GH-insufficient
GOS	Glasgow Outcome scale
HH	Hunt & Hess
HPA	hypothalamo-pituitary-adrenal
ICU	intensive care unit
IES	Impact of Event Scale
IL-6	interleukin-6
ITT	insulin tolerance test
LHRH	luteinizing hormone releasing hormone
MCS-36	mental component score of SF-36
NHP	Nottingham Health Profile
PCS-36	physical component score of SF-36
PSQ	Prenatal Stress Questionnaire
PTSD	posttraumatic stress disorder

QoL	quality of life
QoL-AGHDA	Quality of Life Assessment of Growth Hormone Deficiency in Adults
SAH	subarachnoid hemorrhage
SD	standard deviation
SF-36	Short Form-36 questionnaire
TBI	traumatic brain injury
TCD	transcranial Doppler
TRH	thyroid releasing hormone

Index of tables and figures

Tables

Figures

1 Introduction

Spontaneous subarachnoid hemorrhage (SAH) is a rare disease that has been in the scope of research for decades. It occurs with a prevalence of 6-7 per 100.000 patients per year and, with a predilection age of 40-60 years, affects patients in the middle of their lives. Although only about 5% of all strokes are caused by a SAH (van Gijn, Kerr, & Rinkel, 2007) it plays an essential role because of the young age of the affected patients. Consequently in the past years the research interest in SAH has remained high (fig. 1).

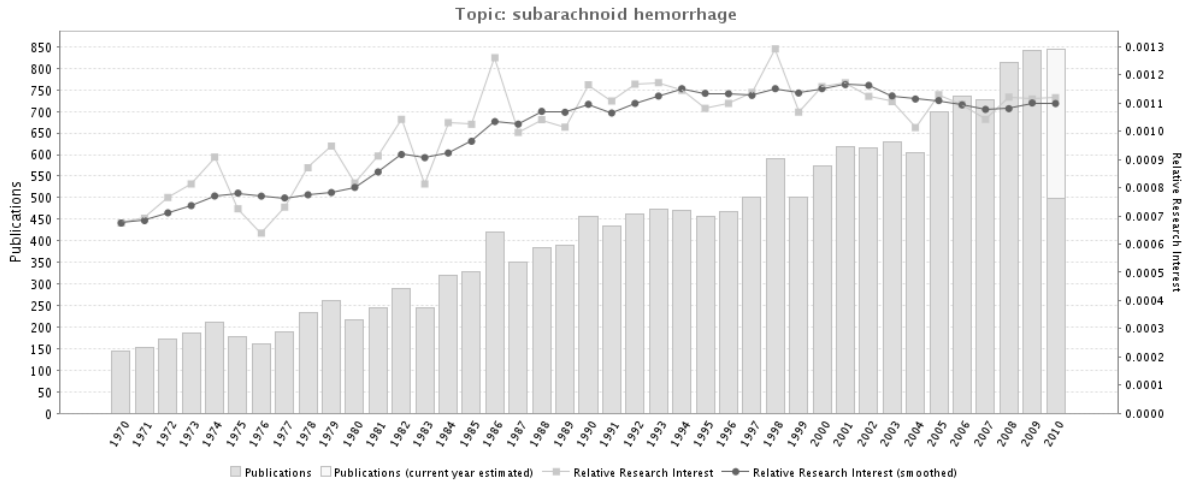


Figure 1: PubMed-listed publications with the topic subarachnoid hemorrhage since the 1970s (source: http://www.gopubmed.com, query date June 24th, 2010)

SAH is usually caused by the rupture of an aneurysm (in about 85% of all cases, aneurysmatic SAH, aSAH) (van Gijn et al., 2007). Mortality is high: around 50% of the patients die within the first 28 days after the bleeding, including the 10-15% of the patients that do not even arrive at the hospital alive. Those who survive are oftentimes physically impaired and may suffer from psychiatric symptoms such as depression. This is not surprising, as the experience of a life-threatening illness is a critical life event that may lead to posttraumatic stress syndrome, to depression or to general adjustment disorders. In addition physical disabilities that restrict the patient's abilities for work, social and leisure activities may contribute to a decrease of quality of life. In fact a study from Powell and colleagues, published in 2004, revealed a high proportion of intrusive thoughts and avoidance behavior, leading in 3/49 SAH

patients to the diagnosis of a full-blown posttraumatic stress disorder (PTSD) and of depressive symptoms in 16% of the patients.

Additionally SAH seems to be the cause of endocrine disturbances in some of the patients. Recently hormonal deficiencies have been in the scope of research leading to some interesting results regarding possible secondary adrenal dysfunction and other disorders of the hypothalamo-pituitary axis in the chronic phase after the SAH (i.e. more than one year after the acute event). Hemorrhagic damage, which may occur as the consequence of the rupture of an aneurysm, almost always located at the circle of Willis, may well affect hypothalamo-pituitary structures involved in the secretion and feedback regulation of hormones. In fact, decades ago, in the 1960ies and 70ies two studies explored cortisol secretion after SAH by measuring the diurnal rhythm of cortisol secretion with the standard methods back then (Jenkins, Buckell, Carter, & Westlake, 1969; Osterman, 1975). Unfortunately at that time the interest in the results of these studies was low and no further studies were performed. Only when two SAH patients were included in a study concerning endocrine failure after traumatic brain injury (TBI), revealing hormonal deficits in both of them (Kelly et al., 2000), the scientific community became aware of this topic again. Four years later four work groups published their study results of endocrine dysfunction after SAH – gained on a total of 120 patients (Aimaretti et al., 2004; Brandt, Säveland, Valdemarsson, Sjöholm, & Reinstrup, 2004; Dimopoulou et al., 2004; Kreitschmann-Andermahr et al., 2004). The results were striking: about 37-55% of the patients suffered from hormone deficiencies of some sort. Therefore, although being a rare disease, SAH as a cause of endocrine failure gained more attention in medical care.

Unfortunately none of those recent studies explored the potential connection between hypocortisolism and psychiatric outcome after SAH, despite the strong base of publications suggesting abnormal cortisol secretion patterns in patients suffering from depression, PTSD, fatigue or chronic fibromyalgia syndrome - symptoms that are often reported by SAH patients. It may be assumed that the possible connection between clinical variables of SAH, the following neuroendocrine and also the psychiatric sequelae of the trauma are not fully understood. Especially the coincidence of psychiatric symptoms and hypocortisolism in SAH patients still has to

be clarified as well as possible disturbances of the diurnal rhythm of cortisol secretion and feedback sensitivity. The documentation of a connection between endocrine failure and psychiatric symptoms in SAH patients could be a first step towards deeper insights in the psychiatric symptoms after SAH and towards a better adjusted treatment for those patients.

2 Objective and outline of the thesis

Objective of this thesis is to set cortisol secretion (serum cortisol as a standard marker in medical routine as well as diurnal salivary cortisol and the cortisol awakening response as biometric tools of stress research and psychoneuroendocrinology) and hypothalamo-pituitary-adrenal (HPA)-feedback regulation of SAH patients in relation with psychometric variables. Additionally not only patients in the chronic phase after SAH but also those in the acute phase are examined.

After an introduction and outline in this chapter, the next section will provide the theoretical background for this thesis. First the development of aneurysms and of subarachnoid hemorrhage deriving from the rupture of these malformations is described, followed by a description of the clinical course, possible complications and treatment modalities of aneurysmal SAH and the possible sequels of the illness. Second possible mechanisms of endocrine failure after SAH are introduced.

The following three chapters present the findings of three studies that form the base of this thesis. They cover psychosocial states in the chronic phase after SAH in respect to endocrine alterations (chapter 4), serum cortisol dynamics in the acute phase (chapter 5) and salivary cortisol dynamics in the chronic phase after SAH (chapter 6). Abbreviations are introduced in the chapters 4-6 anew, so the chapters can easily be read separately. In the chapters 7 and 8, the findings of all three studies are summarized and discussed in synopsis. The thesis finishes with a short outlook on future research options.

3 Theoretical background

3.1 Aneurysmal subarachnoid hemorrhage: a massive trauma to the brain

A subarachnoid hemorrhage is a rather infrequent form of stroke (1-7% of all strokes) and denotes a bleeding into the space between the arachnoid membrane and the pia mater. Most SAHs (around 85%) are caused by the rupture of an aneurysm. In this thesis, only patients suffering from aneurysmal SAH are investigated.

Etiology and epidemiology

In Germany, aneurysmal SAH occurs with an incidence of 6-7 per 100 000 inhabitants per year (Kolominsky-Rabas et al., 1998). Being between 45-65 years of age, the patients are younger than the usual stroke patients and are affected in the midst of their lives. The mortality rate of SAH lies at about 50% in the first 28 days and rises to up to 60% in the first three months after the bleeding (Kolominsky-Rabas et al., 1998). 10 to 15% of the patients die before reaching the hospital (van Gijn et al., 2007).

Cerebral aneurysms are pouch-like dilatations of the wall of arterial blood vessels. They occur mainly at the circle of Willis (cerebral arterial circle), a circle of arteries that lies near the base of the skull. Most aneurysms develop in the course of one's life, only few are congenital. Risk factors are autosomal dominant polycystic kidney disease, a familial predisposition, atherosclerosis and hypertension. Next to the rare genetically determined illnesses, also some less well defined genetic factors for aneurysm formation are postulated. Women are affected approximately twice as often as men (Rinkel, Djibuti, Algra, & van Gijn, 1998). The risk of rupture depends on the characteristics of the aneurysm: size and location play a role and also symptomatic aneurysms (causing neurological symptoms such as third cranial nerve palsy) have a higher risk of rupture (Rinkel et al., 1998). Additional patient-dependent factors are hypertension, high alcohol intake and, in some studies, the use of contraceptives (Lindner, Bor, & Rinkel, 2010).

Course

For the affected patients spontaneous SAH occurs mostly “like a bolt out of the blue”. When the aneurysm ruptures, intracranial pressure rises and, at the same time, cerebral perfusion drops. A sudden headache sets in that exceeds any other headache in intensity and develops within seconds or minutes. Other symptoms include nausea, vomiting, meningism, photophobia and loss of consciousness (Suarez, Tarr, & Selman, 2006) as well as focal neurological signs. The magnitude of the symptoms depends on the degree of the bleeding, the amount and functional relevance of the destroyed brain tissue and the rise of intracranial pressure. Only in around 25% of all patients warning signs in form of mild to severe headaches may occur (warning leaks) (Suarez et al., 2006).

Diagnosis

Patients presenting the above mentioned symptoms should always be tested for SAH. To confirm the clinical suspicion, cranial computed tomography (CCT) imaging, lumbar puncture and cerebral angiography are common means of diagnosis. The initial test for SAH should be a noncontrast CT (Bederson, 1996). When performed within the first 24 hours after the bleeding, the SAH can be visualized in 95% of all patients. Based upon the initial CT a score can be derived which marks the severity of the bleeding (Fisher CT-score, Fisher, Kistler, & Davis, 1980). In some cases the location of the ruptured aneurysm can be estimated based upon the initial CT (Karttunen, Jartti, Ukkola, Sajanti, & Haapea, 2003). In those cases where a SAH is clinically suspected, but the CT scan is negative, a lumbar puncture is performed. If results indicate xhanthochromia (Dupont, Wijdicks, Manno, & Rabinstein, 2008), angiography is performed. Neurological scales such as the Hunt & Hess Scale (Hunt & Hess, 1968) are used as a grading system to classify the severity of SAH based upon clinical criteria.

Treatment

As re-bleeding is a potentially fatal complication of SAH, fast and effective occlusion of the ruptured aneurysm is the first priority. There are basically two different

treatment modalities that are applied depending on the type and location of the aneurysm and on the neurological state of the patient: neurosurgical clipping and endovascular coiling. Clipping means that a craniotomy is performed, the vessel carrying the aneurysm is located and exposed and a clip is placed around the aneurysm's neck. Coiling is an endovascular treatment where a catheter is placed in the femoral artery and advanced to the aneurysm. The aneurysm is then filled with one or more detachable platinum coils with a strong form memory. As blood clots around the coil, the aneurysm is inactivated (for a comparison of both methods see Molyneux et al., 2005). The choice of occlusion method is made by a multidisciplinary team consisting of neurosurgeons and neuroradiologists. Both methods should be performed within the first 72 hours after the bleeding if possible. After the surgical or endovascular procedure is performed the patient is monitored on the intensive care unit. Usually a CCT is performed to detect possible complications such as a re-bleeding and daily transcranial Doppler (TCD) is performed to detect vasospasm.

Post-interventional course

Severe complications of SAH are cerebral vasospasm and delayed ischemic neurologic deficits Vasospasm is a narrowing of cerebral blood vessels whose etiology is complex and not fully understood. It leads to a restricted blood flow that, in turn, may cause ischemic lesions. However, recent evidence indicates that events other than arterial narrowing such as early brain injury and cortical spreading depression also contribute to delayed ischemia and thus to overall mortality and morbidity (Pluta et al., 2009).

To prevent vasospasm the calcium antagonist Nimodipin (Nimotop®) is administered (for an overview see Keyrouz & Diringer, 2007). Additionally daily TCD is performed to measure the mean cerebral blood flow. At a blood flow velocity of more than 120 centimeters per second means are taken to elevate the arterial blood pressure (preventive "Triple-H-therapy" (Al-Tamimi, Orsi, Quinn, Homer-Vanniasinkam, & Ross, 2010). Another complication is hydrocephalus which has to be treated with a shunt in about 10-15% of all SAH patients (Komotar et al., 2009). Other complications in the wake of SAH include re-bleeding, brain edema, cerebral salt wasting syndrome and more.

Of the patients who survive the SAH, nearly 80% experience residual symptoms of some type one year after the SAH (Molyneux et al., 2005). 5 years after the SAH still around 73% of the patients report residual symptoms (modified ranking scale, Molyneux et al., 2009). Independent from neurologic status, however, health related quality of life may be diminished, indicating that there may be other sequels of SAH than a loss of neurologic functionality. For example Hütter et al. reported the quality of life (QoL) data of 116 patients (Aachen Life Quality Inventory, ALQI) broken down into the scales activation, mobility, housework, social contact, family relations, free-time activities, ambulation, communication, autonomy and cognition (Hütter, Kreitschmann-Andermahr, & Gilsbach, 2001). The authors state a connection between patient age, initial neurologic state on hospital admission and bleeding pattern to health related quality of life. Meyer et al. used other instruments to measure QoL in 113 patients (Short-Form 36, EuroQoL and a visual analogue scale) (Meyer et al., 2010). Besides the relevance of SAH severity they showed an impact of functional disability, depression, a lower level of education and the lack of a stable partnership on QoL. In general, there is a body of evidence that QoL after SAH is diminished in a vast number of patients and that QoL may even be reduced in patients who do not suffer from neurological deficits (Hop, Rinkel, Algra, & van Gijn, 2001; Katati et al., 2007; Kim, Haney, & van Ginhoven, 2005). Connected to a reduction of QoL are findings of depression, posttraumatic stress disorder, fatigue and impaired sleep. Especially depression has been thoroughly investigated in SAH patients. Depending on method and cohort, the prevalence of depression after SAH ranges from 5% to 50% and seems to be a stable finding even 18 months after the SAH (Powell, Kitchen, Heslin, & Greenwood, 2004). On the other hand, a full blown PTSD is less often seen (Powell et al., 2004), although intrusion and avoidance per se seem to occur after SAH. In those patients who develop PTSD, however, the diagnosis is a stable finding (Noble et al., 2008). Fatigue affects around a third of SAH patients (Noble et al., 2008), was labeled as “post aneurysmal SAH fatigue” by Brandt et al. and consists of general exhaustion, lack of initiative and increased sleep demand (Brandt et al., 2004). Connected to fatigue are sleep disturbances, manifesting in insomnia, daytime sleepiness, impaired sleep quality and efficiency,

which emerge in about 40% of all SAH patients (Noble et al., 2008; Schuiling, Rinkel, Walchenbach, & de Weerd, 2005).

Other studies investigated neuropsychological sequelae (for an overview see Al-Khindi, Macdonald, & Schweizer, 2010). Neuropsychological deficits after SAH may be transient but sometimes they persist even years after the event (Benke, Köylü, Delazer, Trinka, & Kemmler, 2005). Common neuropsychological deficits after SAH concern memory, executive functions and language. Deficits in verbal memory are reported most often (Bellebaum et al., 2004; Martinaud et al., 2009; Mayer et al., 2002; Powell, Kitchen, Heslin, & Greenwood, 2002; Powell et al., 2004). The rate to which extent executive functions are impaired fluctuates with the methods applied and estimations range between 3% and 76% (Al-Khindi et al., 2010). Additionally self-reported impairments and measured deficits do not seem to be correlated; especially a reduction of attention is reported more often than should be expected. Language is predominantly measured by word fluency tests and may in the chronic phase be impaired in around 76% (Mavaddat, Sahakian, Hutchinson, & Kirkpatrick, 1999).

As a consequence of the aforementioned disturbances it is not astounding that an estimated 40% of SAH survivors who were employed prior to the event are not able to return to work (Al-Khindi et al., 2010). Given the relatively young age of the patients this means a considerable loss of productive life-years.

3.2 The connection of the HPA axis and traumatic experiences such as SAH

Although there is a strong overlap of the symptoms frequently occurring after SAH and the clinical symptoms of hypopituitarism, up to the year 2000 the endocrine evaluation of SAH patients was not considered. After the publication of a study comprising the endocrinological data of two SAH patients (Kelly et al., 2000) a handful of studies investigated endocrine failure after SAH and could show that hypocortisolism is a possible sequel of SAH (for an overview see Schneider, Kreitschmann-Andermahr, Ghigo, Stalla, & Agha, 2007). Methods to detect endocrine failure differ widely in the published studies and, not surprisingly, the data on prevalence of hypocortisolism after SAH are heterogeneous. Especially the percentage of corticotroph deficiency after SAH differs between studies and seems to be strongly modulated by the used endocrine assessment method (especially basal hormone measurement, short synacthen test and insulin tolerance test (ITT)) and

possibly also the employed assay. Consequently, based upon the small amount and the heterogeneity of the studies, the underlying mechanisms and time courses of endocrine failure after SAH are not fully understood. In fact there are several mechanisms that may possibly account for a reduction of cortisol levels in these patients.

A) Disposition

Dispositional effects, may they be genetic or epigenetic, could result in low cortisol values before the SAH took place. Genetic determinants of hypocortisolism provide evidence only for gene polymorphisms of the glucocorticoid and mineralocorticoid receptor genes but do not refer to low cortisol levels as observed in SAH survivors. There is, however, a substantial amount of data that children of traumatized mothers may develop abnormal HPA-axis activity and reactivity. A possible indicator of prenatal stress of mothers is gestational length. Klingmann et al. could show that female patients suffering from fibromyalgia had lower free cortisol concentrations after awakening if they were born after a shorter gestational length, indicating a more stressful pregnancy (Klingmann et al., 2008). Concrete evidence on a link between stress in pregnancy and low cortisol levels in newborns was provided by Yehuda et al. who presented data on n=38 mothers who developed PTSD symptoms after being exposed to the world trade center attacks in pregnancy (Yehuda et al., 2005). Salivary cortisol samples were collected at awakening and at bedtime. As a result mothers with PTSD and also their babies exhibited lower cortisol levels than those without PTSD. This effect was especially strong in babies whose mothers were exposed to the attacks when in their third trimesters. Thus it can be assumed that prenatal factors may alter functions of the HPA-axis. For SAH patients this would mean that low cortisol values have been present before the SAH. However, low cortisol or its consequences are not known risk factors for SAH and this assumption awaits further evidence.

B) Corticotropin-releasing factor (CRF) deficiency

Another possible mechanism for low cortisol levels post SAH is an impaired hypothalamic CRF-secretion. Lesions of the paraventricular nucleus as a result of

SAH have been described by the British neuropathologist Crompton in 1963 (Crompton, 1963), who found hypothalamic lesions in 65 of 106 patients who died after SAH. The author remarked that hypothalamic microhaemorrhages were often seen that were “remarkably selective in their site, namely in the paraventricular and supra-optic nuclei, often rendering these nuclei prominent to the naked eye”. If CRF was low in SAH survivors, however, it can be assumed that those patients exhibit symptoms of atypical depression, such as hypersomnia, increased appetite and weight gain and lethargy. Therefore these symptoms should be explored in SAH patients (Gold & Chrousos, 2002).

C) Adrenocorticotropic hormone (ACTH) deficiency

A primary dysfunction of corticotropic cells as a result of pituitary lesions could also lead to a decrease of cortisol levels in the wake of SAH. If this was the case ACTH levels should be low in patients with hypocortisolism. In fact Kreitschmann-Andermahr et al. found a significant correlation of stimulated ACTH and stimulated cortisol in the ITT (Kreitschmann-Andermahr et al., 2004). In other studies ACTH was measured but oftentimes the relation to cortisol was not reported. The measurement of basal ACTH in SAH patients would therefore make sense.

D) Primary cortisol deficiency

In rodents, after a prolonged period of HPA-axis hyperactivity due to 19 consecutive days of chronic social stress, Reber et al. observed a drop of cortisol secretion in combination with a hypertrophy of the adrenals. Histological analyses showed that the hypertrophy was due to water retention, indicating a dysfunction of corticosterone synthesis after chronic strain (Reber et al., 2007). The findings suggest adrenal exhaustion after prolonged adrenal hyperactivity. In human subjects, a time course of adrenal failure after acute or chronic stress is not proven; it is assumed that hypocortisolism may occur after a prolonged period of hypercortisolism: As early as in 1993, Hellhammer and Wade suggested that chronic stress may first result in an elevation and later in a drop of cortisol levels (Hellhammer & Wade, 1993). If such a mechanism is relevant in SAH patients, low levels of total cortisol should occur in the chronic phase after the bleeding. Low levels of basal cortisol can indeed be found in

SAH survivors after at least one year. Whether this is due to traumatic lesions or due to a possible adrenal exhaustion after chronic stress remains unclear. Nevertheless, when total serum cortisol is low, a reduction of corticosteroid binding globulin (CBG) would be necessary to maintain a sufficient amount of free cortisol.

Hypocortisolism as a result of chronic stress was also addressed by Heim et al (Heim, Ehlert, & Hellhammer, 2000). The authors state that the stress-induced hypocortisolism may promote increased vulnerability to bodily disorders such as chronic pain or inflammation, explaining the correlation of hypocortisolism and stress-related disorders. The concept of vulnerability but not inevitably bodily disorders goes in line with the observation of healthy subjects with hypocortisolism.

As hypocortisolism may be a vulnerability factor for bodily disorders, it could be easily assumed that a downregulation of cortisol may be dysfunctional. However, hypocortisolism may have protective effects in itself that are suitable to adapt to a changing environment or to chronic bodily states (Fries, Hesse, Hellhammer, & Hellhammer, 2005). A major beneficial effect may be the minimization of allostatic load (McEwen, 1998) which has been associated with higher risk of mortality. In SAH patients, reduction of allostatic load may be beneficial, given that their physical state after SAH may already be impaired.

In sum, in SAH patients, a significant upregulation of cortisol in the first days after the bleeding as a normal reaction to physical and psychological stress can be assumed. A subsequent downregulation of cortisol levels as suggested by the aforementioned authors may thus be possible and even adaptive.

E) CBG deficiency

A fast and useful method to increase biologically active cortisol in emergency situations is the downregulation of CBG. Without its binding globulin, a higher percentage of free cortisol is available while the total amount of cortisol may stay the same. This, in turn, leads to a stronger negative feedback and a subsequent downregulation of cortisol secretion, resulting in low total serum cortisol and high

unbound cortisol levels. To verify such a mechanism in SAH-patients it would be useful to measure CBG concentrations in serum.

F) Metabolism

Another possible mechanism behind low cortisol values is a lower basal HPA axis activity due to a downregulation of 5α-reductase and/or 11β-hydroxysteroid dehydrogenase-2 (11β-HSD2). 11β-HSD2 is an enzyme found mainly in cerebellum, hippocampus, cortex and pituitary but also in the hypothalamus including the paraventricular nucleus. It catalyses the conversion of active glucocorticoids to inert 11-dehydrocorticosterone and cortisone, in this way regulating access of glucocorticoids to receptors (Seckl, 1997). For a summary of the metabolic pathway, see Figure 2.

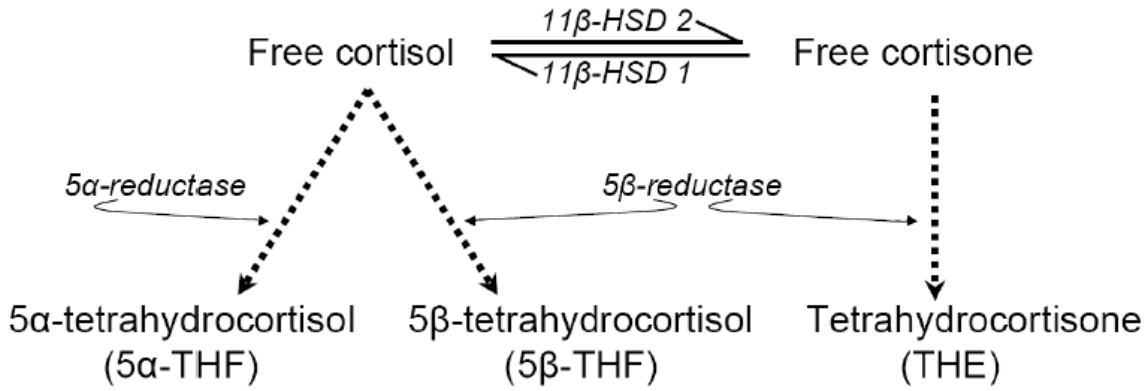


Figure 2: Summary of the metabolic pathway (Yehuda, Bierer, Andrew, Schmeidler, & Seckl, 2009)

Changes in these enzyme systems affect the half-life of cortisol, resulting in a change in HPA-axis regulation (Andrew, Phillips, & Walker, 1998). In holocaust survivors low 5α-reductase and 11β-HSD2 concentrations were found. A decrease of hepatic 5α-reductase leads to a decrease in cortisol breakdown, amplifying local glucocorticoid effects in the liver without elevating circulating cortisol. A decrease of both enzymes results in a lowered clearance of cortisol and a reduced drive of the HPA-axis (Yehuda et al., 2009).

In patients undergoing cardiac surgery a substantial shift from inactive cortisone to active serum cortisol could be shown in the acute and subacute phase after surgery

(Vogeser, Groetzner, Küpper, & Briegel, 2003; Vogeser, Zachoval, Felbinger, & Jacob, 2002). These changes, however, were unrelated to the typical serum cortisol increase found after surgery. The authors assume a downregulation of renal 11β-HSD-2 leading to a reduced metabolisation of cortisol as well as an upregulation of 11β-HSD-1 resulting in an enhanced restoration of active cortisol from circulating cortisone (Vogeser et al., 2002).

G) Inhibitory feedback

The last potential reason for reduced cortisol levels is enhanced inhibitory feedback. An upregulation of corticoid receptors would lead to a stronger negative feedback and therefore in reduced cortisol secretion. Evidence (Hellhammer: personal communication) suggests that a subgroup of patients exist which show both an enhanced hypothalamo-pituitary-adrenal axis (HPAA)-reactivity but lower basal cortisol levels, which is associated with an enhanced negative feedback. It seems that this phenomenon is associated with prenatal stress of the mother in the first trimester of pregnancy of the patient. A case has been published in 2007 (Buss et al., 2007).

3.3 The selfish brain of SAH survivors

In 1998 Peters introduced the concept of the “selfish brain”, a concept that is described in detail in a recent review (Peters, 2011). Originating from obesity research the selfish brain theory provides hints for understanding cortisol dysregulations in SAH patients. The base of the theory is the assumption that the brain covers its own energy supply with the highest priority when regulating energy fluxes in the body. Peters develops a supply chain model of glucose allocation which includes three methods of the brain to increase glucose flow to its own cells: 1) glucose transfer ‘on demand’ in cooperation with surrounding astrocytes, 2) inhibition of insulin secretion and thus limiting glucose storage in fat tissue and muscles and 3) increasing appetite and triggering eating behavior. For all this a sufficient availability of glucose is essential. This mechanism implies that once a traumatic lesion occurs; the brain tries to mobilize glucose by stimulation of the HPA axis as an emergency reaction. In severe traumatic brain injury the glucose demand of the brain is increased (Helbok et al., 2010). Additionally, physical trauma or chronic psychosocial

stress in the long run may contribute to an incompetence of the brain to maintain a steady and sufficient energy supply (Peters & Langemann, 2009). Therefore, an increase of blood glucose may be essential in coping with SAH, as high levels of free cortisol may be desirable. These two possible ways to increase free cortisol are 1) either activation of the HPA-axis which should result in high serum cortisol levels throughout the day and 2) the reduction of CBG which should lead to an increase of free cortisol with stable to low total cortisol values.

Elevated serum cortisol values can hardly be seen in chronic SAH survivors. On the contrary the studies published so far indicate that basal cortisol is low in a substantial amount of the patients except for the acute phase, where serum cortisol levels are high. Reduced CBG, however, could be found in a sample of 15 acutely brain-injured patients (nine following traumatic brain injury, six following SAH; Savaridas, Andrews, & Harris, 2004) but not in a cohort of SAH patients investigated between 0-7 months after SAH (Bendel et al., 2008).
Up to date in most studies basal serum cortisol values or dynamic tests were used to evaluate endocrine function in SAH survivors, but there are also two early studies from the 60s and 70s that cover cortisol dynamics and that have not been replicated with today's methods (Jenkins et al., 1969; Osterman, 1975). In both publications, of which one covers the acute phase and the other the chronic phase after SAH, alterations of the patients' diurnal cortisol rhythm were described. In summary there are early descriptions of disrupted diurnal cortisol rhythms as well as reports of low basal serum cortisol levels in SAH patients from the past 10 years.

In conclusion it can be assumed that there is evidence on low serum cortisol levels in a subgroup of SAH survivors and that diurnal cortisol rhythm may be abolished. To investigate the underlying mechanisms, the genesis of hypocortisolism in SAH patients has to be elucidated first.

3.4 Questions arising from the aforementioned remarks

Following the possible reasons for hypocortisolism mentioned above, the following questions arise

a) Is there evidence for a disposition of SAH patients for hypocortisolism, determined by prenatal factors?

b) May CRF be reduced in hypocortisolemic SAH patients due to hypothalamic lesions as suggested by the work of Crompton?
c) Is ACTH deficiency linked to hypocortisolism in SAH patients?
d) Could adrenal exhaustion after chronic elevation of cortisol be responsible for low cortisol values in the chronic phase after SAH?
e) Is an accelerated metabolism of cortisol present in some SAH patients, resulting in a steep decline of the cortisol awakening response (CAR) after the peak?
f) Is CBG downregulated in an emergency reaction increase free cortisol after trauma, resulting in low basal cortisol levels and high salivary cortisol levels?
g) Is there increased negative feedback resulting in a stronger suppression of cortisol secretion?

In order to subsequently address these questions to further elucidate hypocortisolism in SAH survivors the following methods were used:

a) Disposition: Prenatal stress factors are assessed by a specifically designed questionnaire, the Prenatal Stress Questionnaire (PSQ) (chapter 6)
b) CRF deficiency: Reduced CRF can be measured indirectly via symptoms of atypical depression. This is done by Neuropattern- diagnostics (chapter 6)
c) ACTH deficiency: basal ACTH values are measured directly in serum. Additionally, stimulated ACTH values in the ITT are regarded (chapter 4)
d) Cortisol deficiency: Basal cortisol values are measured in serum, additionally stimulated cortisol values (ITT) are regarded (chapter 4 and 6)
e) Metabolism: An enhanced metabolism of cortisol should present an abrupt drop of the CAR with the disappearance of cortisol being quicker than the half life of cortisol. For this the CAR of hypocortisolemic patients is examined (chapter 6)
f) Acute downregulation of CBG: CBG can be measured directly or, alternatively, the relation of free to total cortisol may be examined (chapter 5)
g) Increased negative Feedback: To test feedback sensitivity the low-dose dexamethasone test is a useful method (chapter 6)

4 Quality of life (QoL) and psychiatric sequelae following aneurysmal subarachnoid haemorrhage (SAH): Does neuroendocrine dysfunction play a role?

Comment

In this study, pre-existing data was re-analyzed in respect to psychometric values. It can be shown that especially low basal cortisol values, but also low ACTH values in the ITT have a connection to a worse psychosocial outcome. This chapter addresses questions c and d: are ACTH and cortisol levels (adrenal exhaustion) low in chronic SAH patients?

4.1 Abstract

Objective: Patients who have sustained aneurysmal subarachnoid haemorrhage (SAH) oftentimes suffer of persisting impairments in quality of life (QoL) and psychological disturbances despite a good neurological outcome. In the light of the high prevalence of partial hypopituitarism in SAH survivors demonstrated in recent investigations, we wanted to elucidate whether neuroendocrine dysfunction has an impact on QoL and neurobehavioral symptoms in these patients.

Design / Patients: QoL, depression and psychological distress were assessed in 40 SAH survivors who had undergone endocrine function testing at least one year after the haemorrhage.

Measurements: QoL was assessed using the Nottingham Health Profile (NHP), Quality of Life Assessment of Growth Hormone Deficiency in Adults and Short Form-36 questionnaire (SF-36). The Beck Depression Inventory and the Impact of Event Scale were used to evaluate depression and symptoms of current subjective distress in response to the SAH as a stressful life event, respectively.

Results: In a stepwise multiple regression analysis, basal cortisol level was included as the first and oftentimes only predictor for several QoL domains assessing psychological aspects of well-being and depression whereas physical aspects of QoL were predicted primarily by neurological recovery from the SAH. Severe growth

hormone deficiency was the first predictor for the criterion NHP subscale *Energy* and highest stimulated adrenocorticotroph hormone level in the insulin tolerance test was the first predictor for disturbed sleep as assessed with the NHP subscale *Sleep*.
Conclusion: Our results provide preliminary data that neuroendocrine disturbances contribute to disturbed QoL, depression and sleeping disturbances in SAH patients.

4.2 Introduction

A large number of patients who survive an episode of aneurysmal subarachnoid haemorrhage (SAH) suffer of persistent impairments in their health-related quality of life (QoL), even when their neurological outcome has been classified as good according to conventional outcome measures such as the Glasgow Outcome scale (GOS) (Bellebaum et al., 2004; Hop, Rinkel, Algra, & van Gijn, 1998; Hütter et al., 2001; Jennett & Bond, 1975; Powell et al., 2004). Moreover, studies have reported an increased prevalence of depression (Hütter, Gilsbach, & Kreitschmann, 1995; Morris, Wilson, & Dunn, 2004), anxiety (Morris et al., 2004) and psychological distress (Berry, 1998; Powell et al., 2004) even among long-term SAH survivors.
The overlap between post-SAH symptoms such as lack of initiative, fatigue, loss of concentration, impaired QoL and psychiatric symptoms and those of patients with untreated partial or complete hypopituitarism (Arlt & Allolio, 2003; Vance, 1994; Wallymahmed, Foy, & MacFarlane, 1999) suggests that neuroendocrine dysfunction may contribute to the impairments of SAH patients. These considerations in addition to the close anatomical relationship between pituitary gland, hypothalamic structures and the arteries of the circle of Willis have spurred a number of recent studies assessing the prevalence of neuroendocrine dysfunction in patients who have had SAH. In fact, partial hypopituitarism has been diagnosed in 37.5 – 55% of SAH survivors (Aimaretti et al., 2005; Dimopoulou et al., 2004; Kreitschmann-Andermahr et al., 2004) investigated three months to more than one year after the acute event. Nevertheless, it is still unknown, whether hormone abnormalities contribute to the long-term sequelae of aneurysmal SAH. We therefore sought to evaluate the role of neuroendocrine dysfunction on QoL and the prevalence of psychiatric sequelae in patients with a good neurological outcome following SAH. The endocrinological results of this patient group have already been published (Kreitschmann-Andermahr et al., 2004).

4.3 Methods

Study population

QoL, depression and psychological traumatization were studied in a sample of 40 (14 male, 26 female) patients who had sustained aneurysmal SAH at least one year prior to the investigation and who exhibited a predominantly good neurological result at 12-month follow-up. The inclusion criteria, detailed demographic and clinical characteristics of the patient sample as well as the endocrinological work-up and results are reported elsewhere (Kreitschmann-Andermahr et al., 2004). A short overview of the descriptive data is given here: Mean age at the time of QoL, depression, psychological distress assessment and pituitary function testing was 43.8 years, ranging from 26-59 years. Patients were tested on average 27.3 months after the bleeding (SD = 15, range 12-66). Thirty-eight patients were treated by surgical aneurysm clipping, the aneurysm in one patient was occluded by means of coil embolization and the aneurysm in one patient was clipped after unsuccessful coiling. The clinical grades at hospital admission according to the grading system of Hunt and Hess (Hunt & Hess, 1968) were grade 1: n = 10, grade 2: n = 12, grade 3: n = 13, grade 4: n = 5. Three patients had a Fisher CT-score (Fisher et al., 1980) of 0, four a grade of 1, six a grade of 2 and 24 a grade of 3, respectively (rated on a scale of 0-3; initial CCT scan unavailable for grading in three patients). At the time of testing, patients exhibited the following GOS-grades: GOS III: n = 4, GOS IV: n = 19, GOS V: n = 17. Severe growth hormone deficiency (GHD) and secondary hypocortisolism were diagnosed by peak GH levels of < 3µg/l and by peak cortisol levels of < 500 nmol/l in the insulin tolerance test (ITT), respectively. Diagnosis of secondary hypothyroidism and/or hypogonadism were based on basal hormone levels and the thyroid releasing hormone (TRH)-luteinizing hormone releasing hormone (LHRH)-test. The following hormone deficiencies had been observed: isolated corticotroph deficiency in 13/40 patients, isolated severe GHD in 5/40 patients, severe GHD plus corticotroph deficiency in 3/40 patients, isolated thyrotroph deficiency once. All patients had given informed consent and the entire investigation had been approved by the local ethics committee of the University of Technology (RWTH) Aachen.

Questionnaires

Questionnaires were given to the patients at the time of the first endocrinological function test; they were to be completed at home and returned at the second visit. The instruments were chosen on the basis of their psychometric qualities and their previous use in studies of psychosocial outcome in patients with neurological and/or pituitary disease. QoL was measured with the Short Form (SF)-36 (Bullinger & Kirchberger, 1998), Nottingham Health Profile (NHP) (Hunt, McKenna, McEwen, Williams, & Papp, 1981) and Quality of Life Assessment of Growth Hormone Deficiency in Adults (QoL-AGHDA) (McKenna et al., 1999). Depression was evaluated by means of the Beck Depression Inventory (BDI) (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961), whereas the Impact of Event Scale (IES) (Horowitz, Wilner, & Alvarez, 1979) was used to assess the psychological response to a stressful life event.

A short description of the individual questionnaires is given below:

Short Form (SF)-36

The SF-36 is a widely used 36-item self-administered questionnaire which measures the following 8 health-related domains: physical functioning, role limitations because of physical health problems, bodily pain, social functioning, general mental health, role limitations because of emotional problems, vitality and general health perceptions (Bullinger & Kirchberger, 1998). The 8 subscales can be combined into two summary measures: physical function (PCS-36) and mental health (MCS-36). A higher score is associated with a better QoL. The SF-36 has been used to assess QoL in SAH patients (Deane, Pigott, & Dearing, 1996; Hop et al., 1998; Schuiling et al., 2005) as well as in patients with neuroendocrine dysfunction such as severe GHD (McMillan, Bradley, Gibney, Healy, et al., 2003; McMillan, Bradley, Gibney, Russell-Jones, & Sönksen, 2003). Reference values are published for healthy subjects and different patient groups.

Nottingham Health Profile (NHP)

The NHP is frequently used in patients with pituitary disease and evaluates subjective functional status with 38 yes/no statements in six domains: physical mobility, pain, sleep, energy, emotional reactions, and social isolation (Hunt et al.,

1981). Subscale scores are calculated as a weight mean of the associated items and are expressed as a value between 0 and 100. A higher score signifies a worse quality of life.

Quality of Life Assessment of Growth Hormone Deficiency in Adults (QoL-AGHDA)

The QoL-AGHDA was developed specifically to assess QoL in adult patients with GHD (McKenna et al., 1999). It consists of 25 yes/no items which were constructed based on in-depth interviews of adult patients with GHD. The sum score consists of the number of 'yes' answers. A higher score is associated with a worse QoL.

The Beck Depression Inventory (BDI)

The BDI is a commonly used 21-item self-rating questionnaire designed to assess depressive symptoms in adolescents and adults (Beck et al., 1961). The sum score of the 21 items is a measure of the present severity of depressive illness. It has been used in a number of studies to measure depression after SAH (Hütter et al., 1995; Hütter, Kreitschmann-Andermahr, & Gilsbach, 1998; Powell et al., 2004) and traumatic brain injury (TBI) (Seel & Kreutzer, 2003).

The Impact of Event Scale (IES)

The IES is a broadly applicable self-report measure designed to evaluate current subjective distress for any specific life event (Horowitz et al., 1979). It consists of 15 items, 7 of which measure intrusive symptoms (intrusive thoughts, nightmares, intrusive feelings and imagery), 8 tap avoidance symptoms (numbing of responsiveness, avoidance of feelings, situations, ideas), and combined, provide a total subjective stress score. All items of the IES are anchored to a specific stressor (Horowitz et al., 1979). Respondents are asked to rate the items on a 4-point scale according to how often each has occurred in the past 7 days. For the total sum score it is suggested that the cut-off point is 26, above which a moderate or severe impact is indicated. A score below 8 is subclinical, and 9-25 indicates a mild to moderate stress response (Horowitz et al., 1979).

Hormone measures and statistical methods

The following hormone measures were set into relation to the QoL- and psychiatric instruments: basal (9:00 am) and highest stimulated cortisol level (in ITT), basal (9:00 am) and highest stimulated ACTH level (in ITT), basal (9:00 am) and highest stimulated GH level (in ITT), area under the curve (AUC) for cortisol, ACTH and GH (in ITT), presence of any hormone deficit, corticotroph insufficiency and GHD (the latter three defined by the respective cut-off values in the endocrinological function tests). Moreover, clinical variables and gradings known to affect neurological outcome (Kassell et al., 1990) and thought to possibly affect QoL were extracted from the patients records and were also used for analysis of psychometric data: clinical grade on admission to the hospital according to the grading system of Hunt & Hess, severity of the bleeding on the initial cranial computed tomogram (CCT) rated using the Fisher-CT-Score, neurological grade at the time of psychometric assessment (GOS-late), patient age at time of testing and sex.

SPSS for Windows version 11.0 (SPSS Inc., Chicago, IL) was used to perform data analysis. Descriptive statistics of interval-scaled data were expressed as mean ± standard deviation (SD) unless otherwise mentioned. Bivariate correlations were calculated using Pearson correlation coefficients in case of data ranking on an interval scale. For ordinally scaled variables Spearman rank-order correlation coefficients were used. SF-36-scores are age- and gender-dependent, therefore for each scale a multiple linear regression analysis was calculated with age and sex as predictors. The standardized residuals of these regressions analyses were used for all calculations pertaining to SF-36-scores. In regard of the many tests performed, a conservative $p<0.01$ was considered to be statistically significant. Independent variables affecting QoL, depression and coping problems were explored by stepwise linear regression, adjusted R^2 was calculated to examine the shared variance of the model. To test for group differences, Student's t-tests were calculated. Before calculation, data were visually inspected for outliers (in case of group differences and for the analyses of relationships scatterplots were used).

4.4 Results

Table 4-1 gives the descriptive data of the psychometric instruments and the number of patients who completed the questionnaires without missing values. There was no

statistically significant difference between patients above and below the cut-offs in the endocrinological function tests with regard to missing values.

Table 4-1: Descriptive data of the psychometric inventories

		N	mean	sd
QoL	QoL-AGHDA	38	9.03	6.60
	NHP scale 1 - mobility	33	13.76	18.91
	NHP scale 2 - pain	35	12.40	25.04
	NHP scale 3 - sleep	35	20.78	27.89
	NHP scale 4 - social isolation	34	19.96	25.80
	NHP scale 5 - emotional reaction	35	27.69	28.68
	NHP scale 6 - energy	35	43.63	38.88
	PCS-36	35	49.68	10.83
	MCS-36	35	44.23	12.85
depression	BDI	40	8.33	5.85
coping	IES avoidance	35	8.00	8.15
	IES intrusion	35	7.40	8.35
	IES total score	35	15.40	14.57

The descriptive data demonstrate that predominantly psychosocial aspects of QoL were reduced in the study population. Only 11.4% of the 35 participants who completed the questionnaire rated their physical QoL as impaired (PCS-36 at least 2 SD below population mean), but 25.7% of these patients estimated their psychosocial QoL (MCS-36 at least 2 SD below population mean) to be significantly diminished. Mild to moderate depressive symptoms (BDI score 11-17) were observed in 27.5% of all patients, whereas a clinically relevant depression (BDI score > 17) was diagnosed in a further 10%. 12 of 35 patients (34%) exhibited signs of psychosocial traumatization according to the IES.

Basal cortisol level correlated with p<0.01 with the following QoL scales: NHP energy (r=-.44), NHP social isolation (r = -.50) and QoL-AGHDA (r = -.55). SF-36 PCS correlated only with neurological outcome (GOS) at the time of testing (r=.52, p<.01). Depression as measured with BDI correlated significantly with basal cortisol value (r = -.56, p<0.01) and neurological status after SAH (GOS late, r = -.44, p<0.01) In a stepwise multiple regression analysis with the hormone measures and clinical variables delineated above as independent variables and the different psychometric scales and subscales as dependent variables, basal cortisol level was included as the first and oftentimes only predictor for several psychological QoL domains and depression, whereas physical aspects of QoL were predicted primarily by bodily recovery from the SAH (GOS). Interestingly, severe GHD was first predictor for the NHP-Subscale *Energy* (beta = .40, p = .01) and stimulated ACTH level in ITT the first predictor for the NHP subscale *Sleep* (beta = -.43, p = .01). Table 2 gives an overview of the clinical predictors included in the regression equation for the various questionnaires. When performing group comparisons between patients with and without corticotroph deficiency based on the results of ITT (n = 16 vs n = 22) no significant differences with regard to QoL, depression and coping problems were found (t-tests, n.s.). This also held true when comparing patients with any hormone deficit (n = 22) based on the results of endocrinological function testing to those SAH patients with normal hormone test results.

Table 4-2: Regression analyses with psychometric instruments

instrument	included predictors	adj. R^2	confidence interval 95%	
			lower limit	upper limit
QoL-AGHDA	basal cortisol level	.27	-0.050	-0.015
NHP mobility	AUC ACTH	.23	-1.082	-0.118
	basal cortisol		-0.115	-0.004
NHP pain	GOS-late	.23	-31.258	-6.911
NHP sleep	stimulated ACTH level	.16	-1.441	-0.183
NHP social isolation	basal cortisol level	.42	-0.195	-0.059
	age at testing		-2.453	-0.521
	sex		-36.080	-3.694
NHP emotional reaction	basal cortisol level	.14	-.194	-.019
NHP energy	GHD	.30	8.24	67.8
	basal cortisol level		-0.242	-.03
MCS-36	basal cortisol level	.13	0.00	0.007
PCS-36	GOS-late	.26	0.303	1.247
BDI	basal cortisol level	.30	-0.044	-0.015

4.5 Discussion

The results of our questionnaires demonstrate an increased prevalence of depression and insufficient coping as well as reduced QoL in SAH survivors with a good neurological outcome, which is comparable to earlier results from our own group (Hütter et al., 1998, 2001) and further data published in the literature (Berry, 1998; Hop et al., 1998; Powell et al., 2004).

The most striking result was the association between basal cortisol, AUC of ACTH and the QoL scores as well as the predictive value of low basal cortisol levels for a wide range of psychological aspects of QoL as well as depression whereas physical aspects of QoL were predicted primarily by neurological recovery from the SAH (compare table 2). Even when keeping the relatively small sample size in mind, the

relation between hormonal measures of cortisol output and QoL scales mainly reflecting psychosocial well-being were quite apparent. This was only seen for basal morning cortisol levels and ACTH AUC in ITT, but not for stimulated cortisol levels in the ITT. A possible explanation may be that these patients have subtle dysregulations of the hypothalamus-pituitary-adrenal (HPA) axis under basal conditions and put out a lower total amount of cortisol in stress situations but are capable of mounting a sufficient cortisol peak under pharmacological stimulation.

An association between low cortisol output (low basal morning cortisol, reduced free 24-h urinary cortisol and/or diminished salivary cortisol), depression and reduced QoL has also been reported in a number of stress-related neuropsychiatric disorders such as fibromyalgia or chronic fatigue syndrome (for an overview see Fries et al., 2005). In these patients, a disturbed HPA axis has been suggested, but the background of inadequate cortisol availability is, however, not fully understood (Fries et al., 2005). In SAH patients, the haemorrhage itself is possibly the cause for neuroendocrine dysfunction. Neuropathological evidence demonstrates a high degree of hypothalamic damage in casualties of aneurysmal SAH, with emphasis on selective and oftentimes bilateral haemorrhages into the supraoptic and paraventricular nuclei of the hypothalamus (Crompton, 1963), with the latter of the two being a point of origin for corticotropin releasing hormone - secreting neurons which project to the anterior pituitary (Reichlin, 1998).

In our SAH patients we found a significant correlation between depression measured with the BDI and low basal cortisol. This was surprising at first glance, because some authors suggest that HPA overdrive and hypernoradrenergic function is associated with melancholic depression (Tse & Bond, 2004). However, it must be kept in mind that major depression is a symptom-based diagnosis that probably consists of several entities with different pathophysiological and neuroendocrine patterns (Halbreich, 2006). For instance, there is growing evidence that hypoactivity of the HPA axis with low cortisol levels is present in patients with atypical features of depression and that many patients with depression exhibit normal endocrine responses (Antonijevic, 2006; Gold & Chrousos, 2002). The BDI has been developed for depressive symptoms in general, so a possible explanation for the negative correlation between BDI and cortisol levels may be the lack of specificity of the used instrument with its symptom-based approach to diagnosis of depression.

In a recent investigation (Schuiling et al., 2005) severe problems with sleep were found in 28/83 (34%) SAH patients and QoL was considerably reduced in patients with severe sleep problems. As hormones of the HPA axis, specifically cortisol and GH play important roles in sleep modulation (Buckley, 2005; Van Cauter et al., 2004), and, in untreated Addison's patients, cortisol plays a positive, permissive role in REM sleep regulation and may help to consolidate sleep (García-Borreguero et al., 2000), one can postulate, in view of our findings, that disturbances of ACTH and cortisol secretion may also play a role in the pathogenesis of sleep disorders in SAH patients. This issue should be investigated in future studies.

One further interesting finding of the present study was the inclusion of GHD as the first predictor in a stepwise regression with the NHP subscale *Energy* as criterion. Lack of physical energy is one key feature of adult-onset GHD of pituitary origin and in a placebo-controlled trial of treatment response to recombinant GH, the NHP dimensions of *Energy* and *Emotions* responded most to treatment in patients with adult-onset GHD (Burman et al., 1995). Furthermore a recent study concerning the psychosocial outcome of patients after traumatic brain injury (TBI) has suggested, that GH-insufficient (GHI) and GH-deficient patients have higher levels of fatigue measured by SF-36 subscale energy (also termed vitality) in contrast to GH-sufficient TBI-patients 6-9 months after the trauma (Kelly et al., 2006). In this context, it is noteworthy that the QoL-AGHDA as a supposedly disease-specific inventory did not show any association with severe GHD in our study. These findings must, however, be regarded as preliminary because of the small sample size and the even smaller size of the patient subgroup with GHD.

It must be kept in mind that impairments in QoL after aneurysmal SAH are influenced by a wide range of factors pertaining among others to the severity of the initial haemorrhage, patient age and functional outcome (Bellebaum et al., 2004; Hütter et al., 2001). In addition, our exploratory study now provides preliminary evidence that also neuroendocrine dysregulations, especially a relative hypocortisolemic state and GHD might contribute to impairments in QoL, fatigue, sleep disturbances and psychiatric morbidity in SAH survivors. Besides aiming to replicate our finding in larger patient groups it needs to be established whether physiological hormone replacement improves these symptoms in SAH patients.

5 Cortisol dynamics in the acute phase of aneurysmal subarachnoid hemorrhage – associations with disease severity and outcome

Comment

In this study SAH patients in the acute phase are examined. The question addressed here is whether an acute downregulation of CBG occurs, which, in turn leads to higher levels of free cortisol and to lower levels of total cortisol. Therefore, multiple CBG measures were performed in the acute and subacute phase of SAH.

5.1 Abstract

The purpose of this study was to assess cortisol dynamics in the acute phase after aneurysmal subarachnoid hemorrhage (SAH) and to set the parameters of cortisol release in relation to severity of illness and outcome. In 22 consecutive patients with aneurysmal SAH, cortisol, corticosteroid binding globulin (CBG), interleukin-6 (IL-6) and adrenocorticotrophic hormone (ACTH) were measured immediately after hospital admission (t_0), 7 days (t_1) and at least 14 days later (t_2). Additionally, diurnal profiles of cortisol secretion were assessed at t_1 and t_2 and area under the curve (AUC) was computed for calculated free serum cortisol (CFSC). In this study, normal diurnal CFSC profiles were associated with a significantly shorter intensive care unit (ICU)-stay, less complications and a more favourable outcome than abnormal diurnal profiles. AUC and 8 a.m. cortisol were not related to clinical course or outcome. It is concluded that cortisol secretion patterns are associated with severity and outcome of SAH. For an appraisal of the hypothalamo-pituitary-adrenal-axis in SAH-patients, single cortisol measurements are insufficient.

5.2 Introduction

Activation of the HPAA is an important mechanism in the response to stressful events and critical illness. Not surprisingly, a vast number of studies has given evidence of excessive HPAA activation in patients in acute critical illness (for an overview see Arafah, 2006). Nevertheless, recently it has been argued that critically ill patients, especially those with sepsis, may have relative adrenal insufficiency despite cortisol values within or above the given reference range (for an overview see Thomas & Fraser, 2007).

While sepsis-induced relative hypocortisolism may be largely attributable to primary adrenal insufficiency, there is concern that traumatic brain injury (TBI) and aneurysmal SAH may also compromise the HPAA on the hypothalamo-pituitary level, with the potential consequence of secondary adrenal insufficiency (AI) (Cooper & Stewart, 2007). Within the last years SAH has been identified as a major and formerly underestimated cause of hypopituitarism as a long-term complication of the bleeding. Adrenocorticotrophic hormone (ACTH) insufficiency and other neuroendocrine disturbances have been confirmed in 47.5 % of 102 SAH survivors investigated between 5 months and several years after the acute event (for an overview see Schneider et al., 2007) and, very recently, in a retrospective study, AI was identified in a high proportion of SAH patients who were nonresponsive to vasopressor therapy and received cosyntropin for the evaluation of AI within 14 days of the hemorrhage (Weant, Sasaki-Adams, Dziedzic, & Ewend, 2008). Another study on the HPAA in the acute phase of aneurysmal subarachnoid hemorrhage (SAH) identified elevated total and free serum cortisol values on the first day after SAH as compared to a control group of patients undergoing elective surgery for an unruptured aneurysm (Bendel et al., 2008), but argued since SAH severity did not affect cortisol concentrations, that this may indicate relative adrenal insufficiency in those patients with a more severe bleeding.

However, in patients with severe brain injury such as SAH, the pattern of cortisol secretion and regulation is still poorly understood. One study, addressing cortisol dynamics by sampling a morning and an evening cortisol and corticosteroid binding globulin (CBG) values in 15 patients after TBI (n = 9) and SAH (n = 6) showed that the total morning cortisol in the majority of these patients was similar to the reference

range in normal individuals, but that CBG was significantly decreased, leading to an increased free cortisol level (Savaridas et al., 2004).

It was the aim of the present study to broaden and augment the knowledge of cortisol dynamics in the acute phase of aneurysmal SAH as a building stone for establishing a tool for the assessment of AI in these patients. Therefore, we investigated the circadian rhythm of cortisol and CBG, as well as ACTH and set parameters of cortisol release in relation to clinical events and complications. As IL-6 constitutes a potentially important factor of extra-ACTH cortisol release, we additionally assessed this parameter (Bornstein & Chrousos, 1999).

5.3 Patients and Methods

Patients

All patients admitted for aneurysmal SAH between October 2005 and August 2006 to the Department of Neurosurgery, RWTH Aachen University Hospital Aachen were screened for eligibility for this study. Exclusion criteria were pregnancy, glucocorticoid medication on admission to hospital or during treatment, prior pituitary insufficiency as well as age below 18 and over 70 years. 22 patients could be included in this prospective observational study (14 females, 8 males; mean age 47.2 years, range 25 – 69 years). The study was approved by the local ethics committee of the University of Technology, RWTH Aachen and was carried out according to the Declaration of Helsinki. Written informed consent was given in all cases. In patients too ill to give consent at the time of ICU admission consent by proxy was obtained.

No patient received dopamine, ketoconazole or etomidate. 15 patients received norepinephrine for blood pressure maintenance or prophylactic elevation of blood pressure in case of dopplersonographically proven elevated blood flow velocities of intracranial vessels (induced hypertension, see Kreitschmann-Andermahr & Gilsbach, 1996). Antibiotic drugs for treatment of bacterial infection were administered to 15 patients during their stay on the ICU.

Table 5-1 shows the patient characteristics with sex, age, aneurysm location, clinical status on admission to hospital according to the grading system of Hunt & Hess (HH) (Hunt & Hess, 1968), the amount of subarachnoid blood visualised by computed tomography (CT) according to the Fisher scale (Fisher et al., 1980) and outcome at

the time of hospital discharge as measured by Glasgow Outcome Scale (GOS) (Jennett & Bond, 1975). 7 days after the bleeding (t_1) and at least 14 days after the bleeding (t_2), Glasgow Coma Scale (GCS, Teasdale & Jennett, 1974) was recorded for each patient.

The symptomatic aneurysm could be identified in all patients by angiography and was treated in all patients. In addition to the symptomatic aneurysm in 6 patients multiple aneurysms were present; none of those aneurysms was treated within the study period. 15 patients underwent microsurgery and 7 patients endovascular therapy with coil embolisation, the choice of occlusion method was interdisciplinary decided upon and based on aneurysm characteristics and patient's preference. In all cases, the ruptured aneurysm was occluded within 48 hours after the initial SAH.

The duration of ICU stay in this cohort was 6-37 days (mean 19 days). All patients received intensive care management by experienced neurointensivists. Nimodipine was administered intravenously for 14 days (2mg/hour) and a daily transcranial ultrasound (TCD) for the detection of cerebral vasospasm was performed. Cerebral vasospasm was defined as a TCD mean cerebral blood flow in the middle cerebral and/or the internal carotid artery >120 cm/s with a Lindegaard index >3 (Lindegaard, Nornes, Bakke, Sorteberg, & Nakstad, 1988); vasospasm was diagnosed in 11 patients. At t_1, 6 patients were analgosedated. Of them, 5 were fully ventilated and one additional patient received assisted ventilation but was not analgosedated. At t_2, 3 patients were analgosedated. Of them, one was fully ventilated and two received assisted ventilation. Above this, 2 patients were ventilated without analgosedation (one patient assisted ventilation, one patient full ventilation). Patients who received assisted ventilation are classified as ‘ventilated’ in the following calculations. The neurosurgical ICU is fitted with large daylight windows. Additional lighting is dimmed over night, and disturbances are kept to a minimum. In all patients who do not require parenteral nutrition regular meal times are observed. This means that external time cues exist which help patients to keep in synchrony with normal circadian rhythms.

Table 5-1: Patient characteristics

Pat-No	Sex	Age	Hunt & Hess Grade	Fisher CT Score	GOS	Aneurysm Location	CFSC Rhythm	
							t_1	t_2
1	f	44,9	4	3	3	MCA	missing	missing
2	f	46,7	5	3	5	ACoA	regular	regular
3	f	48,7	4	3	3	ACoA	flat	abnormal
4	f	47,1	4	3	4	PICA	abnormal	regular
5	f	69,1	2	2	4	MCA	flat	abnormal
6	m	49,1	4	3	3	ACoA	abnormal	abnormal
7	f	51,6	3	2	4	MCA	abnormal	abnormal
8	m	52,0	1	2	5	ACoA	regular	regular
9	f	39,9	5	3	5	A1	abnormal	regular
10	m	49,8	4	3	2	MCA	abnormal	abnormal
11	f	33,4	5	3	5	PCoA	regular	regular
12	m	31,7	1	2	5	ACoA	regular	regular
13	f	53,5	1	2	5	AChorA	abnormal	abnormal
14	f	55,6	2	1	4	PICA	abnormal	abnormal
15	f	26,8	2	2	5	PICA	abnormal	abnormal
16	f	54,0	3	2	4	ACoA	abnormal	abnormal
17	m	48,1	2	1	5	ACoA	regular	abnormal
18	m	59,1	5	3	4	ACoA	abnormal	abnormal
19	f	43,7	4	3	5	MCA	flat	missing
20	m	25,1	3	3	5	ACoA	regular	regular
21	f	69,5	3	3	5	BAS	flat	regular
22	m	38,2	1	2	5	ACoA	flat	regular

Hunt and Hess grade: 1 = asymptomatic or minimal headache and slight nuchal rigidity; 2 = moderate to severe headache, nuchal rigidity, and no neurological deficit other than cranial nerve palsy; 3=drowsiness, confusion, or mild focal deficit; 4 = stupor, moderate to severe hemiparesis, possibly early decerebrate rigidity, and vegetative disturbances; and 5 = deep coma, decerebrate rigidity, moribund appearance.
Fisher CT score: 1 = no blood detected; 2 = a diffuse disposition or thin layer with all vertical layers of blood (interhemispheric fissure, insular cistern, ambient cistern) less than 1 mm thick; 3 = localized clots and/or vertical layers of blood 1 mm or greater in thickness and 4 = diffuse or no subarachnoid blood, but with intracerebral or intraventricular blood.
GOS grade: 1 = death; 2 = persistent vegetative state; 3 = severe disability (patient depends upon others for daily support due to mental or physical disability or both); 4 = moderate disability (disabled but independent), and 5 = good recovery.
Aneurysm location: MCA = middle cerebral artery, ACoA = anterior communicating artery, PICA = posterior inferior cerebellar artery, VA = vertebral artery, A1 anterior cerebral artery, PCoA = posterior communicating artery, AChorA = anterior choroidal artery, ICA = internal carotid artery, BA = basilar artery.

Methods

In all patients cortisol, CBG, ACTH and IL-6 were assessed at three different time points: (t_0) at admission to the hospital, at 8:00 a.m. on the seventh day after the bleeding (t_1, mean 7.5 days, SD 0.9), and at 8:00 a.m. at least 14 days (mean 17.1, SD 4.7) after the bleeding (t_2).
All blood samples were collected via an existing indwelling central or peripheral line.
Furthermore, at t_1 and t_2 diurnal cortisol profiles were assessed.
For the assessment of these diurnal cortisol profiles, serum cortisol and CBG measurements were performed at t_1 and t_2 at 4 time points each (8, 12, 16, 20h). To calculate the amount of free cortisol in plasma (calculated free serum cortisol, CFSC), we used Coolens' equation (Coolens, Van Baelen, & Heyns, 1987).
In order to gain an overview about the course of cortisol secretion, the circadian variation of CFSC was rated as a normal or abnormal: Normal diurnal cycles included regular cycles with clearly raised morning values and a consistent decline over the day, resulting in the lowest value in the evening ("regular" rhythm). CFSC-profiles were also labelled as normal when they exhibited only a minimal diurnal variation (flat cycles, Stone et al., 2001). Flat cycles presumably occur in at least 10% of the healthy population, connections to health status are not evident (Stone et al., 2001).

Cortisol slopes which did not follow a normal pattern, i.e. with a reversed slope or profiles that followed a strong zigzag-pattern were classified as abnormal. Rating was performed only if there were at least three measurements throughout the day. Allocation to the different profiles was done by visual inspection by an investigator blinded to the clinical variables of the patients. All curves could clearly be allocated to one of the three types (regular, flat, abnormal) of secretion pattern. In addition to visual inspection of the slope, the area under the curve (AUC, here calculated in respect to ground (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003)) was calculated for CFSC.

In order to classify the course of the illness, a complication index which ranged from 0 to 2 was created. This clustering of complications was performed due to the small sample size and in order to avoid multiple comparisons, which would have led to an increased type-1 error. An uneventful clinical course without any relevant medical complications was rated as 0 (n=10). Mild to moderate complications such as pneumonia not requiring reintubation and which could easily be treated with antibiotics were rated as 1 (n=7) while severe complications such as rebleeding, sepsis or severe pneumonia requiring reintubation were rated as 2 (n=5). The complication index was rated independently by two of the authors (IKA and MR).

Analytes and assays

All analyses were carried out in the central laboratory of the University Hospital Aachen. Reference values for the analytes in the normal population as provided by the laboratory are listed in parentheses behind the respective assays.

Specific quantitative detection of cortisol in serum was carried out using an automated immunometric chemiluminescence assay (ADVIA Centaur, Bayer Health Care Diagnostics, Fernwald, Germany; reference value: 171-536 nmol/l). Serum CBG concentrations were determined by quantitative ^{125}I-CBG radioimmunoassay (RIA) (ZenTech, Angleur, Belgium, reference value men: 33.3-61.3 mg/l, reference value women: 21.8-82.6 mg/l). Serum IL-6 was measured by chemiluminescent sequential immunometric assay (Immulite 1000, DPC Biermann, Bad Nauheim, Germany, reference value: <15 ng/l). Due to technical reasons the method of ACTH determination had to be changed twice during the study period. Until January 2006 the assay from Nichols Institute Diagnostics, San Clemente, USA (reference value:

2.0-11.5 pmol/l) was used; from January to May 2006 Diasorin, Saluggia (Vercelli), Italy (reference value: 0.9-6.1 pmol/l). Since May 2006 ACTH was determined via E170, Roche GmbH, Basel, Switzerland (reference value: 1.5-14.7 pmol/l).

Statistical analysis

SPSS 17.0 for Windows (SPSS Inc.) was used for statistical analysis. Due to the sample size of n=22, nonparametric statistics were calculated. To control for possible confounding effects of norepinephrine administration, Mann-Whitney U-tests with the dependent variables CFSC (08:00, 12:00, 16:00, 20:00), AUC, and CFSC morning/evening ratio were calculated. U-tests were also calculated in order to examine possible group differences between normal vs. abnormal diurnal cortisol profiles in respect to clinical parameters and to compare patients of both treatment modalities (coiling/clipping). To test whether ventilation and sedation have an influence on the pattern of diurnal cortisol secretion, a chi^2-test (Monte Carlo method used due to small cell sizes) was performed with the variables “sedated and ventilated: yes/no” and “normal rhythm yes/no”. In order to analyze changes over time in the total amount of daily secreted cortisol (AUC), Wilcoxon signed-rank tests were calculated. Kruskal-Wallis-H-tests were calculated in order to explore possible group differences between the different ACTH gradings (below, in and above reference range) and 08:00 CFSC values at t_1 and t_2. This analysis for the exploration of the relationship between cortisol and ACTH release was performed owing to the change of ACTH assays during the study period, which rendered the calculation of correlation coefficients impossible. Spearman rank-order-correlations were calculated to analyze relationships between single CFSC levels and ordinally scaled clinical variables, including IL-6 as a possible marker for ACTH independent cortisol secretion. In all tests, p values of <0.05 were considered statistically significant.

5.4 Results

Basal hormone values

Basal serum cortisol concentration at t_0 (admission) ranged between 189 and 857 (mean 536.05), at t_1 (08:00) between 261 and 1189 (mean 570.36), and at t_2 (08:00) between 151 and 1123 nmol/l (mean 430.50) (table 2). The mean cortisol values at t_2 were significantly lower than the 08:00 values at t_1 (Wilcoxon signed-rank test p=.013).

Cortisol values were above the reference range (08:00 samples) provided by the laboratory for healthy individuals at t_1 in 13 patients and at t_2 in 4 patients. The concentration was below the normal reference range at t_1 in no patient and at t_2 in 1 patient (table 5-2). CBG concentrations were slightly below the reference range in one patient at t_1 (16:00 measurement: 28.4 mg/l, reference range: 33,3-61,3) and another patient at t_2 (08:00 measurement: 30.5 mg/l, reference range: 33.3-61.3), all other measures at t_1 and t_2 were within the reference range. CBG was measured together with cortisol four times a day, for better clarity and comparability of the data, statistics in table 2 are given for 08:00 values only. Additionally, CFSC at 08:00 is given in table 2. ACTH concentration was above the respective reference range for healthy individuals at t_0 in 1 patient, at t_1 in 4 patients, and at t_2 in 2 patients. ACTH was measured below the reference range at t_0 in 6 patients, at t_1 in 6 patients, and at t_2 in 2 patients. Kruskal-Wallis-H-tests showed an association between 08:00 ACTH-classifications (above, within or below reference range) in respect to 08:00 CFSC values at t_1 (p=0.038), but not at t_2 (p=0.091). This means that high CFSC values in SAH patients were not consistently associated with high ACTH values. Correlation analysis between IL-6 and CFSC levels was not statistically significant at any point of time. The correlation of CFSC and serum cortisol for all measurements was statistically significant (Spearman correlation, r=.954, p<.0001).

Table 5-2: Concentrations of cortisol and IL-6

	t_0	t_1	t_2
Cortisol nmol/l (08:00 a.m.)			
mean (SD)	536.05 (203.48)	570.36 (225.61)	430.50 (217.21)
range	189 - 857	261 - 1189	151 - 1123
below/within/above reference range	0/12/9	0/9/13	1/17/4
CFSC ng/ml (08:00 a.m.)			
mean (SD)	-	16.01 (9.84)	10.62 (7.82)
range	-	4.2 – 41.8	1.7 – 39.6
CBG mg/l (08:00 a.m.)			
mean (SD)	-	44.90 (5.84)	43.90 (8.98)
range	-	37.0-58.6	25.0-56.6
below/within/above reference range	-	0/21/0	1/20/0
IL-6 ng/l (08:00 a.m.)			
mean (SD)	32.24 (49.21)	27.71 (44.98)	15.22 (19.68)
range	3 - 180	3 - 210	2 - 82
within/above reference range	10/11	14/8	16/6

t0: hospital admission, t1: 7 days after the haemorrhage, t2: 14-21 days after the haemorrhage. Reference values: cortisol: 171-536 nmol/l. CBG women: 21.8-82.6, men: 33.3-61.3 mg/l. IL-6: 0-15 ng/l

Diurnal cortisol rhythm

Serum cortisol profiles could be obtained in 21 patients at t_1 and 20 patients at t_2 and were each allocated to the normal or abnormal different secretion patterns as

described above (see table 5-3). Flat profiles, which were allocated to the normal rhythms exhibited a standard deviation (SD) of the respective CFSC patterns of < 2 ng/ml (in all cycles, intrasubject SD ranged between 0.66 and 25.13 ng/ml). In 1 patient, both diurnal profiles were missing due to technical reasons. Of the 11 patients with a normal profile at t_1 (6 regular, 5 flat), 3 switched to abnormal patterns at t_2. 7 patients still had normal patterns at t_2, 1 was missing. Of the 10 patients with an abnormal profile at t_1, 8 still had an abnormal profile at t_2, and 2 patients now exhibited a normal (regular) CFSC profile (table 5-1). At t_1 and t_2 patients that were sedated and ventilated exhibited abnormal diurnal profiles significantly more often than patients able to breathe on their own (p=.012 and p=.001, respectively).

Table 5-3: Allocation to the different patterns of diurnal cortisol secretion

		t_1 n (%)	t_2 n (%)
normal	regular	6 (27.3 %)	9 (40.9 %)
	flat	5 (22.7 %)	0 (0.0 %)
abnormal	reversed / undefined	10 (45.5 %)	11 (50.0 %)
missing		1 (4.5 %)	2 (9.1%)

t1: 7 days after the haemorrhage, t2: 14-21 days after the haemorrhage

Total amount of daily CFSC as assessed by AUC ranged at t_1 between 49.08 and 339.49 (mean: 135.89) and at t_2 between 31.55 and 191.62 (mean: 95.03). In contrast to the 8:00 a.m. cortisol value, the mean AUC was not significantly higher at t_1 than at t_2 (Wilcoxon signed-rank test p=.062).

Mann-Whitney U-tests showed that patients with a normal diurnal profile of CFSC at t_1 had a shorter ICU-stay (p=.038), a lower complication index (p=.043), a more favourable GCS at t_1 (p=.043) and a more favourable outcome (GOS, p=.051) than patients with an abnormal diurnal profile. Neither Hunt & Hess score (p=.468) nor Fisher CT score (p=.863) differed between both groups. At t_2, the appropriate GCS (p=.016) as well as GOS (p=.012) differed significantly between both groups. Group differences did not reach statistical significance for ICU-stay (p=.182), complication index (p=.112), Hunt & Hess Score (p=.766) and Fisher CT score (p=.175).

CFSC-values at 8:00 and at 20:00 alone were not associated with ICU days, complication index, GOS, Fisher CT or Hunt & Hess score at t_1. Only morning CFSC and GCS exhibited a small correlation (r=.439, p=.046, Spearman correlations) At t_2, however, evening-values correlated with the number of days spent on ICU (r=.646, p=.005), complication index (r=.544, p=.020), GOS (r=-.668, p=.002) and GCS at that day (r=-.559, p=.016, all Spearman correlations), indicating a connection between elevated evening values and longer ICU stay, higher complication index, a less favourable outcome and a worse level of consciousness at that day. Fisher CT score and Hunt & Hess score were not associated with morning or evening values of CFSC at t_2. The total amount of CFSC, defined as AUC, was not associated with any of the above-mentioned variables at both points of time.

Noteworthily, a separate analysis of vasospasm in relation to AUC revealed a reduced amount of CFSF in the group of patients with vasospasm at t_2 (p=.019, U-Test) but no group differences in respect to other CFSC-related parameters such as evening value, morning value or secretion pattern. There was no significant difference in cortisol values between patients with and without norepinephrine medication (U-test, n.s., data not shown). Treatment condition was neither significantly associated with the dependent variables vasospasm, GCS, complication index or outcome (GOS), nor with different markers of cortisol secretion (AUC, 08:00 CFSC, 20:00 CFSC, normal diurnal rhythm vs. abnormal rhythm) at t_1 or t_2 (U-test, n.s., data not shown).

5.5 Discussion

This investigation was performed to prospectively examine cortisol dynamics in patients with aneurysmal SAH in the acute phase of the illness. The analysis of the basal hormones post-admission and at day 7 and ≥ 14 revealed the following insights: Cortisol values at all three time points showed a wide variability. At t_1 8:00 a.m. serum cortisol was significantly higher than at t_2. At t_1, this parameter was, however, not associated with clinical parameters of disease severity or outcome, resembling the findings of Bendel et al. who assessed serum free and total cortisol concentrations in 30 patients shortly after SAH and found them unaffected by the severity of SAH (Bendel et al., 2008). Furthermore, 8:00 a.m. CFSC and total CFSC output (AUC) exhibited a significant correlation only at t_1 but not at t_2. This weak association may be due to the distorted diurnal variation of CFSC in some patients,

leading to the fact that the 08:00 am value is not the highest value throughout the day, or to the oftentimes strong variation of CFSC throughout the day including high peaks, possibly leading to imprecise estimations of the total amount of cortisol released. In the latter case, variables less sensitive to abrupt variation may be more useful, such as measurements of cortisol in 24h-urine. A reason for the missing association between AUC and clinical variables may be that not the total amount of cortisol is important for the stress response in the acute phase after aneurysmal SAH, but a normal pattern of cortisol secretion. In our study, a normal diurnal profile of CFSC at t_1 was connected to a less severe illness and a more favourable outcome, at t_2 to a more favourable outcome only. This may be due to abnormally raised evening values; 2-3 weeks after the bleeding elevated CFSC values in the evening were connected to a more severe illness and a poorer outcome.

There was, moreover, no consistent relationship between ACTH values in respect to reference ranges and 08:00 CFSC levels or a significant association between IL-6 and CSFC. In the traditional view of HPA regulatory mechanisms, an adaptation to stress would be suggested by parallel increases in ACTH and cortisol levels according to the degree of stress (Groeneveld, Beishuizen, & Molenaar, 2008). In our SAH cohort, this was only observed at t_1, although this result must be viewed with caution due to the limitation of the twice changed ACTH assay. All the same, Bendel also reported a dissociation of ACTH and cortisol levels on most points of time in the author's cohort. However, it is important to keep in mind that a separation of ACTH and cortisol secretion has clinical relevance. This concept must be integrated into the appraisal of the HPA axis during acute illness, since there is evidence that cortisol release in acute illness is also promoted by ACTH-independent mechanisms such as neurones, neuropeptides, cytokines and gonadal steroids, which regulate HPA axis function at the pituitary or adrenal level (for an overview see Bornstein, Engeland, Ehrhart-Bornstein, & Herman, 2008). However, in this study, also no association between IL-6 as a possible marker for extra-ACTH cortisol secretion and CFSC levels was observed.

It is conceivable that cortisol secretion in patients after aneurysmal SAH is influenced by central dysregulation that may be caused by decreased hypothalamo-pituitary perfusion due to vasospasm, irritation of the pituitary stalk due to subarachnoid blood or hypothalamo-pituitary hemorrhage. Indeed, one neuropathological investigation

has confirmed a high incidence of hypothalamic lesions in patients who died shortly after aneurysmal SAH (Crompton, 1963). Of note, in our investigation, we observed that patients with cerebral vasospasm as assessed by TCD – which may indicate hypothalamic hypoperfusion - exhibited a significantly lower amount of total CFSC (AUC) 14 to 21 days after the hemorrhage. Additionally, since SAH constitutes a trauma to the brain, it can be assumed that the disturbance of cortisol profiles reflects an alteration of central cortisol secretion and not an alteration of cortisol clearance.

Diurnal cortisol output in the acute phase may be influenced by sleeping patterns (Lasikiewicz, Hendrickx, Talbot, & Dye, 2008). The assessment of sleep-wake cycles in patients on an ICU requires close monitoring and is usually not performed. Therefore, in our study no data concerning sleep patterns was available in the investigated patients. However, as pointed out in the method section, external zeitgebers were available in the ICU setting to allow patients to keep up circadian rhythmicity. Patients that were sedated and ventilated exhibited abnormal diurnal cortisol profiles more often than patients without this intervention. Whether this is due to the lack of a normal sleeping pattern, or if this is just a marker for disease severity, remains to be clarified.

In our study, the diurnal cortisol profile was abolished in about half of the SAH patients 7 days and also ≥ 14 days after the acute event. This results in a very important finding for clinical routine, namely that a random cortisol sample drawn sometime during the course of a day cannot be regarded as representative of the course or the appropriateness of daily cortisol secretion in patients shortly after SAH and therefore is of no avail in evaluating the HPAA-response. In our sample this also holds true for the common routine parameter, the 08:00 a.m. cortisol value. We, therefore, can not approve the use of one single basal or random cortisol sample that is still advocated by some authors as a marker to define relative AI in critically ill patients (Arafah, 2006; Cooper & Stewart, 2007) and has been used among others to define AI in patients with SAH in a recent study (Weant et al., 2008).

It has been proposed, that measured serum total cortisol levels in critically ill patients can be misleadingly lower than anticipated, due to decreased concentrations of CBG and albumin, resulting in the incorrect conclusion that adrenal function is impaired (Hamrahian, Oseni, & Arafah, 2004). This led us to investigate CFSC. However, in our sample, CBG was below the reference range in two of 154 samples only.

Additionally, correlation coefficients between serum cortisol and CFSC were high, a finding that is also reflected in the recently published study by Bendel et al. (Bendel et al., 2008). This suggests that total serum cortisol probably is a sufficient marker for the evaluation of cortisol status in patients shortly after SAH, but that a singular measurement of CBG may be useful to identify patients with severe CBG deficiency.

Despite the efforts to establish clear rules for the assignment of specific secretion patterns, our method contains a degree of subjectivity. In this cohort, however, all patterns were clearly recognizable; Flat cycles had a SD of < 2 ng/ml and normal cycles all were dichotomously falling throughout the day. Still, a study on clear rules to differentiate between normal and abnormal diurnal cycles is called for.

In summary, in our study we could not identify an association between morning or total cortisol output and markers of disease severity and outcome in patients shortly after SAH. We could, however, draw an important association between abolished diurnal profile of CFSC and elevated CFSC evening values and measures of disease severity and outcome, leading to the conclusion that other measures of cortisol output besides random cortisol values are needed to make an appraisal of the HPAA in these patients. Due to the small sample size, these findings must be regarded as preliminary. Further prospective investigations of the HPAA in the wake of SAH which include biochemical and clinical assessments as well as long-time follow-ups are necessary to identify hormonal risk factors for later adrenal insufficiency or impaired recovery after SAH and to improve diagnosis and treatment strategies for neuroendocrine dysfunction after SAH.

6 Salivary free cortisol in the chronic phase after aneurysmal subarachnoid hemorrhage – is stress the culprit?

Comment

In this study the remaining questions are addressed. A possible pre-existing predisposition for hypocortisolism in SAH patients is examined by means of the Prenatal Stress Questionnaire (PSQ) which assesses pre- peri and postnatal stress factors. Atypical depression as a marker of reduced CRF is assessed via Neuropattern™. Adrenal exhaustion is measured by serum cortisol values at 08:00 and inhibitory feedback via the low-dose dexamethasone suppression test. Metabolism of cortisol is covered indirectly via the examination of CAR-data, this topic will be addressed in the results section of this publication.

6.1 Abstract

Spontaneous aneurysmal subarachnoid hemorrhage (SAH) is a form of stroke which constitutes a severe trauma to the brain and often leads to serious long-term medical and psychosocial sequelae, which persist for years after the acute event. Recently, adrenocorticotrophic hormone deficiency has been identified as one possible consequence of the bleeding which is assumed to occur in around 20% of all survivors. Preliminary data suggest that a poor psychosocial outcome such as depression and diminished quality of life in SAH survivors are linked to alterations of cortisol secretion. Up to now, cortisol levels in SAH patients have been investigated with respect to basal values and in response to challenge tests, but not in regard to diurnal dynamics and feedback-regulation. In this study, basal serum cortisol and diurnal salivary free cortisol profiles were investigated in 31 patients in the chronic phase following SAH (more than one year after the acute event) as well as in 25 healthy controls. Additionally, low-dose dexamethasone suppression tests were performed and sensitivity to stress and stress resilience were measured with psychometric questionnaires. A higher salivary cortisol awakening response (CAR) was observed in SAH patients. Total serum cortisol levels were blunted, but only in patients with high levels of perceived stress. Dexamethasone suppression was not

significantly different among patients and controls. The results of this study suggest that total and free serum cortisol levels provide different information about the HPA-axis in patients after aneurysmal SAH. Enhanced free cortisol levels may reflect a meaningful biological coping mechanism in SAH patients.

6.2 Introduction

Aneurysmal subarachnoid hemorrhage (SAH) is defined as bleeding into the subarachnoid space, precipitated by the rupture of an aneurysm of the cerebral vessels which develops during the course of life (Rinkel et al., 1998). In Germany, it affects about 3-10 of 100.000 people each year (Kolominsky-Rabas et al., 1998), predominantly in the fourth to sixth decade. SAH accounts for about 5% of all strokes (van Gijn et al., 2007). Women are affected more often than men. Mortality rates in patients with ruptured aneurysms are high: around 50% of SAH patients die within the first month (Kolominsky-Rabas et al., 1998). Re-bleeding of the aneurysm is a possible and potentially fatal complication. To avoid this, the timely occlusion of the aneurysm via microsurgical methods (clipping) or endovascular coil embolization (coiling) is performed. Complications in the acute phase of the illness include vasospasm of cerebral vessels, hydrocephalus, seizures, electrolyte disturbances and other accompanying symptoms of acute severe diseases (Gilsbach, Poeck, & Piscol, 1993). Of the surviving patients, one third needs continuous support for activities of daily living, and even patients with a good neurological outcome often suffer from a poor quality of life (Kreitschmann-Andermahr et al., 2004).

Endocrine disturbances after SAH have been considered rare sequelae for a long time. During recent years however, a growing body of evidence suggests that a considerable amount of SAH patients suffer from partial hypopituitarism, especially adrenocorticotrophic hormone (ACTH) deficiency, even years after the bleeding. A meta-analysis published in 2007 (Schneider et al., 2007) revealed a prevalence of ACTH deficiency in about 20% of SAH patients investigated more than five months after the hemorrhage.

Another study, published by Kreitschmann-Andermahr et al. (2007), showed an association between low basal serum cortisol levels, poor quality of life, and psychiatric syndromes such as depression in SAH patients. In contrast to this finding, response measures of the hypothalamus-pituitary-adrenal axis (HPA) to hypoglycemia (insulin tolerance test; ITT) were not related to psychological well-

being in these patients. Thus, it can be assumed that the derangements of the HPA-axis in some SAH patients are subtle, so that the endocrine reaction to a strong pharmacological stimulus such as the ITT is preserved. This hypothesis is supported by a recent study of Klose et al. who found hypocortisolism as diagnosed by means of the ITT to be uncommon after aneurysmal SAH (Klose et al., 2010). Nevertheless, the course of diurnal cortical output and the feedback-sensitivity of the HPA-axis (Fries et al., 2005) may be disturbed, and, by this, contribute to the psychological symptoms of the patients. To a large extent, SAH patients demonstrate long-lasting depressive symptoms, fatigue, poor coping with the trauma of SAH as well as a reduction in quality of life (Brandt et al., 2004; Kreitschmann-Andermahr et al., 2007). Several possible mechanisms may account for the observed symptoms. Firstly, damage to the hypothalamo-pituitary area of the brain as a consequence of the hemorrhage may lead to a dysregulation of cortisol secretion. This, in turn, could lead to psychiatric symptoms that are linked to a dysfunctional HPA-axis. Given the observation of reduced serum cortisol in SAH patients, one may assume that a lack of free cortisol may result in a reduced energy supply for the brain (Fehm, Kern, & Peters, 2006), which may sustain the chronic psychosocial impairment in SAH patients, suffering from a diminished cortisol secretion throughout the day. Another mechanism refers to chronic stress as a result of the traumatic event and its social and work-related sequelae, which, in turn, could lead to the typical stress-related disorders in these patients. The latter explanation is the line of reasoning often employed to explain the frequent hormonal dysregulations, particularly hypocortisolism and altered feedback-reactivity in patients with stress-related disorders of other causes. A hypoactive HPA-axis is seen in patients suffering from burnout, chronic pain, atypical depression, chronic fatigue syndrome, or other illnesses (Fries et al., 2005; Heim et al., 2000). In some patients, fatigue and depressive symptoms may improve after administration of dexamethasone, prednisone or hydrocortisone (Bouwer, Claassen, Dinan, & Nemeroff, 2000; Dinan et al., 1997; Tops, van Peer, Wijers, & Korf, 2006). A hyperactive HPA-axis, on the other hand, is associated with major depression and general anxiety disorders (Gold & Chrousos, 2002).

In order to test our hypothesis of disturbed diurnal cortisol secretion and altered HPA-axis feedback sensitivity in SAH patients, we assessed the diurnal cortisol secretion

and the cortisol awakening response (CAR, Pruessner et al., 2003) of cortisol in saliva and performed a low-dose dexamethasone suppression test in SAH patients and age- and sex-matched healthy controls. Sensitivity to stress and stress resilience were measured with psychometric questionnaires (Neuropattern^{TM}, Hellhammer & Hellhammer, 2008).

6.3 Patients and methods

Patients

We investigated 31 patients in the chronic phase after SAH (at least 1 year after the SAH, mean 2.78 years, SD 1.24) as well as 25 age- and sex-matched healthy participants. SAH patients visiting the outpatient clinic of the Department of Neurosurgery, RWTH Aachen University Hospital Aachen were screened for eligibility for this study between April 2006 and September 2007. Simultaneously, controls were recruited via word-of-mouth recommendation of co-workers at the University Hospital Aachen and via the homepage of the University Hospital Aachen. Exclusion criteria for both groups were pregnancy, medication during the testing period known to affect cortisol levels, acute or chronic inflammatory diseases, glaucoma, osteoporosis, history of traumatic brain injury or brain tumours and age below 18 and over 65 years. Controls were excluded if a free clinical interview revealed symptoms of depression or other mental disorders. Additionally, SAH patients were excluded when there was any history of pituitary insufficiency prior to the SAH. Both groups were matched in respect to age (SAH: mean 50.83 years, controls: mean 49.80 years, t-test n.s.) and gender (SAH: 11 male, 20 female, controls: 13 male, 12 female, chi² test n.s.). The study was approved by the local ethics committee of the University of Technology, RWTH Aachen and was conducted according to the Declaration of Helsinki. Written informed consent was given in all cases.

In the SAH patients, Hunt & Hess grading (HH, Hunt & Hess, 1968) on hospital admission showed the following distribution: 1: Asymptomatic, mild headache, slight nuchal rigidity: n=8, 2: moderate to severe headache, nuchal rigidity, no neurologic deficit other than cranial nerve palsy n=11, 3: Drowsiness / confusion, mild focal neurologic deficit, n=7, 4: Stupor, moderate to severe hemiparesis, n=2, 5: Coma,

decerebrate rigidity, moribund appearance, n=3. The amount of subarachnoid blood visualized by computed tomography (CT) on admission according to the Fisher scale (FCT, Fisher et al., 1980) was grade 0: no blood detected in n=1, 1: diffuse deposition / thin vertical layers of blood <1 mm, n=4, 2: localized clots / layers > 1mm, n=10, 3: diffuse or no subarachnoid blood, with intracerebral or intraventricular clots n= 11. The ruptured aneurysm could be identified in all patients. Most commonly, the aneurysm was located at the anterior communicating artery (n=14) and at the middle cerebral artery (n=8). Occlusion of the aneurysm was performed microsurgically in n=26 patients and 5 patients underwent endovascular coil embolization. The patients stayed on the intensive care unit (ICU) for 6-16 days (mean 12.25 days, SD 4.79) and in hospital for 14-34 days (mean 23.26 days, SD 6.38). Cerebral vasospasm, defined as mean cerebral blood flow in the middle cerebral and/or the internal carotid artery >120 cm/s measured by transcranial Doppler sonography was detected in=15 patients. Hydrocephalus in the acute phase of SAH was present in n=9 patients. At the time of testing, Glasgow Outcome Scale rating was III (severe disability) in n=2 patients, IV (moderate disability) in n=5 patients and V (good outcome) in n=24 patients.

Assessments

Both patients and controls underwent the same procedure. Participants gave a blood sample at 8 a.m. to assess basal fasting cortisol levels. Subsequently, all participants received a study pack containing pre-labelled sampling material for saliva collection (standard reaction tubes), a capsule containing 0.25 µg dexamethasone and written instructions on saliva sampling. The study subjects were asked to perform saliva sampling at home on two consecutive work-free days directly after awakening, +30min, +45min and +60min after awakening (awakening response) as well as at 8 am, 11 am, 3 pm and 8 pm (diurnal pattern). On the following weekend or other work-free day, the participants were asked to complete the same sampling routine again after taking 0.25 µg dexamethasone the evening before sampling (11:00 pm). Saliva was collected by passive drool into the tube. Smoking, brushing teeth, eating and drinking were prohibited 30 minutes prior to sampling. Sports or any type of physical or psychological arousal should be noted, once the stressor was unavoidable.

Additionally, all patients completed the Neuropattern Diagnostic Kit (DAACRO GmbH, Trier, Germany). This diagnostic system refers to 23 endophenotypes of the stress response, which are ascertained by biological, psychological, and symptom measures. The questionnaires encompass items regarding physical reactions to stress, some psychological state- and trait-characteristics of the patient, pre- and postnatal influences, chronic stress, and depression. Additionally, the information gained from salivary cortisol measures and the dexamethasone suppression test is both included in the diagnostics.

Specimen handling and assays

Samples were immediately frozen in the participant's domestic freezer and, once the sampling had been completed, stored in a freezer of the central laboratory of RWTH Aachen University Hospital at -20°. After completion of the study protocol, all samples were shipped to the laboratory of the Department of Psychophysiology at Trier University and analyzed there. After thawing, samples were centrifuged at 2000g for 6 minutes, which resulted in a clear supernatant of low viscosity. 100ul of saliva were used for duplicate analysis (50µl per well). Cortisol levels were determined employing a competitive solid phase time-resolved fluorescence immunoassay with fluoromeric end point detection (DELFIA). The intra-assay coefficient of variation was between 4.0% and 6.7%, and the corresponding inter-assay coefficients of variation were between 7.1% -9.0%.
Blood samples were analyzed at the central laboratory of the University Hospital RWTH Aachen directly after sampling. Serum cortisol was detected via an automated immunometric chemiluminescence assay (ADVIA Centaur, Bayer Health Care Diagnostics, Fernwald, Germany, reference value: 171-536 nmol/l).

Statistical methods

For data analysis, SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL) was used. Descriptive statistics of interval-scaled data are expressed as mean and standard deviation (SD). To test for group differences of independent measures, Student's t-tests were calculated. For the comparison of cortisol secretion patterns, three-way analyses of variance with repeated measures were calculated separately

for diurnal cortisol pattern and awakening responses. Between-subjects factors were group (SAH patients/controls) and dexamethasone-administration (DEX, y/n), while the (repeated) within-subjects factor was time of sampling. Baseline measurements from the first two days were merged in order to gain a mean CAR and diurnal profile. In subjects who did not show an awakening response on one of the two days, we assumed that they were awake prior to sampling. Thus, we only used the profile with the normal CAR.

6.4 Results

Basal serum cortisol values were available in 54 subjects (31 patients and 23 controls); diurnal salivary cortisol profiles were available in all patients and controls and dexamethasone suppression test was done in all patients and in 23 controls.

In the SAH group, serum cortisol levels were slightly below controls (SAH: mean=401.39 nmol/l, SD=126.09, controls: mean=466.77 nmol/l, SD=144.44). The difference between both groups did not reach statistical significance (t-test p=.082, n.s.). However, in contrast SAH patients exhibited significantly higher salivary cortisol levels in the morning (CAR p=.028). This difference did not persist for the rest of the day: The diurnal secretion of salivary cortisol was not significantly raised in SAH patients (p=.136), but still showed a similar trend towards higher cortisol levels in the SAH group. After administration of dexamethasone, both the CAR and the diurnal profile showed significantly blunted cortisol levels in patients and controls (main effect p<.0001 for both measures). For the CAR, a statistically significant interaction between group (SAH/controls) and DEX (dexamethasone suppression yes/no) was observed (p=.014), with a relatively stronger suppression of cortisol in SAH patients. For diurnal profiles no such interaction was observed (p=.908).

With respect to stress load, SAH patients showed more symptoms of stress-related physical complaints than those in the control group. After Bonferroni-correction, six scales differed between both groups: Patients achieved higher scores in excitability, aggression, fatigue and lethargy, and lower scores in vigilance and cognitive performance. Based on the latter scales, a two-step cluster-analysis was performed to discriminate subgroups of patients with respect to stress load. The analysis revealed two clusters. The first cluster comprised n=12 patients with a high stress-load and a second cluster with the remaining n=19 patients, and all of the controls. These subjects had a lower stress-load. For further analyses, three subgroups were

compared with respect to the endocrine measures: SAH high stress, SAH low stress, and controls.

First, serum cortisol levels were compared between the three groups, which revealed significantly different serum cortisol values (p=0.021, ANOVA). Interestingly, a post-hoc test showed that only highly stressed SAH patients had significantly lower serum cortisol values than controls (p=0.018,Tukey-HSD), while no such difference was observed for the low stressed SAH patients (mean values: controls: 466.77, SD 144.44; SAH low stress: 442.37, SD 138.53; SAH high stress: 336.50, SD 66.82).

Secondly, free salivary cortisol levels (CAR) were higher in the SAH low stress group when compared to controls (main effect: p=0,022; post-hoc Tukey –HSD: p= 0,020). Salivary free cortisol levels of the high-stress SAH group were in between these two groups (see figure 3), not reaching a statistical significant difference.

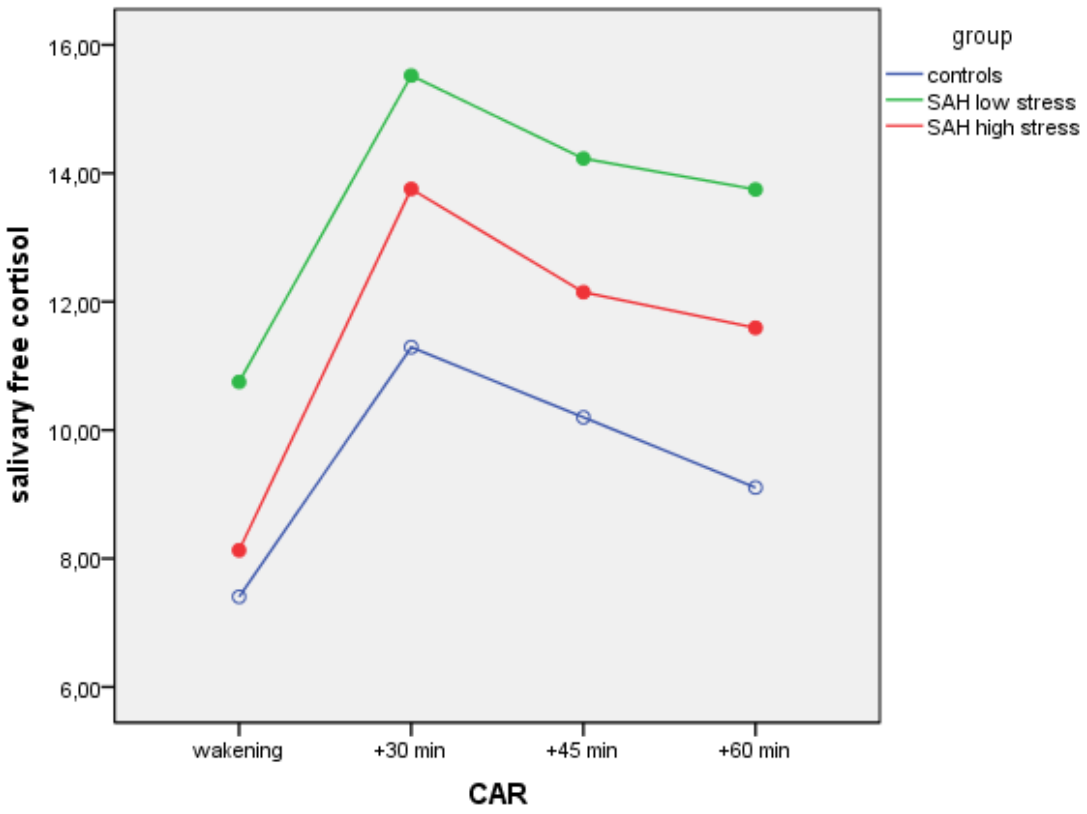


Figure 3: Free salivary cortisol levels (CAR) without dexamethasone administration in both patient groups (high vs. low stress) and in controls.

Thirdly, cortisol levels after dexamethasone showed a similar picture, with a higher CAR in the SAH low stressed group. However, group differences were not significant.

Diurnal salivary cortisol profiles did not differ between the three groups regardless of dexamethasone administration.

SAH patients with low and high stress did not differ with respect to Hunt and Hess grading scores, Fisher CT-score, and length of stay in the hospital.

6.5 Discussion

In accordance with previous research studies, we found a trend towards lower serum cortisol levels in patients in the chronic state after aneurysmal SAH, which reached significance in SAH-patients with a high stress load according to the Neuropattern^{TM}. This difference in cortisol levels was not associated with the clinical measures of severity of the acute hemorrhage, giving rise to the hypothesis that stress also plays a major role in blunting the cortisol acrophase in serum.

However, in the present study, the free cortisol levels were elevated in SAH-patients, reaching significance in the low-stressed group. This finding was unexpected and requires further investigation.

Differences in total and free cortisol levels are related to cortisol binding. In serum, cortisol is bound to corticosteroid-binding globulin (CBG), albumin and erythrocytes, leading to a fraction of about 10% unbound cortisol. In patients shortly after severy traumatic brain injury (TBI), free serum cortisol was found to be increased owing to a decline in plasma CBG (Savaridas et al., 2004), whereas in the acute phase after SAH (7-21 days after the acute bleeding), no such divergence was observed (Poll et al., 2010). In the chronic phase, i.e. months to years after the trauma, neither in TBI or SAH patients have CBG data ever been investigated. However, enhanced free and lower to normal total cortisol levels seem to be present in SAH patients, possibly pointing to low cortisol binding as a determinant of this effect.

Enhanced free cortisol levels may reflect a meaningful biological coping mechanism in SAH patients. The brain is the organ with the highest energy consumption, without having own energy stores. Thus, the brain initiates its own glucose supply via activation of the HPA (Fehm et al., 2006). A drop of CBG may help SAH patients to optimize the energy supply for the brain.

A downregulation of CBG was observed in acute and severely stressed animals (Garrel, 1996). In rats, chronic social stress leads to reduced levels of CBG (Spencer et al., 1996). Thus, it is reasonable to assume that the drop of CBG is more pronounced in highly stressed SAH patients, which would result in a different relation

between serum cortisol and salivary free cortisol than in low stressed patients (higher salivary free cortisol in relation to serum cortisol). As a result, such a downregulation of CBG could help to maintain a stable supply of free cortisol even in spite of reduced serum cortisol levels, which were observed in our group of highly stressed SAH patients.

A possible explanation of the paradox of reduced serum cortisol levels in patients who are under chronic stress is that continuous stress seems to initially result in elevated cortisol levels and later in a drop of cortisol with low levels in serum and saliva (Hellhammer & Wade, 1993). This phenomenon has been reported in a series of studies, and in most of these studies low cortisol levels were associated with fatigue, low vigilance, and excitability (Heim et al., 2000), as observed in this study. Recently, Fries et al. (2005) argued that a long lasting elevation of cortisol levels under chronic stress impairs physical and mental health. Thus, the organism may down regulate cortisol levels to protect cardiovascular and metabolic functioning. In other words, the drop in free and total cortisol levels may be a consequence of the severity and chronicity of stress. Consequently, one may expect that cortisol levels in the low stress SAH group may follow the same time course, and eventually reach the same end-point.

Although this study generated some unexpected findings, the data suggests that stress is an important mediator of HPA dysregulation in SAH patients. As observed in other stress related disorders, cortisol levels may follow a bi-phasic time course with an initial phase of elevated and a later phase of lowered glucocorticoid levels. Chronic stress seems to a relevant initiator and promoter of this time course. Our observation of a differential regulation of free and total cortisol levels suggests that CBG is a possible mediator of these effects. The results of this study indicate that total and free serum cortisol levels give different information about the HPA-axis in patients after aneurysmal SAH and give rise to the suspicion that total serum cortisol levels alone may not be adequate for diagnosing (secondary) adrenal insufficiency in this patient group.

Thus, further studies on the HPA-axis in SAH patients should monitor the time course and the stress load of the patients, as well as free and total cortisol levels and CBG measures.

7 Comprehensive Results

Basal serum cortisol in the chronic phase after SAH have been found to be lower than in controls, but only in a subgroup of SAH patients. In the third study, SAH patients with a high reactivity to stress had lower basal cortisol values than controls and SAH patients with low stress reactivity (mean values: controls: 466.77, SAH low stress: 442.37, SAH high stress: 336.50 nmol/l). Additionally, in the first study it could be shown that lower basal cortisol was associated with low QoL and higher scores of depression.

To provide a better overview, the results of the three studies are summarized according to the questions described in chapter 3.

7.1 Is there evidence for a prenatal disposition of hypocortisolemic SAH patients?

In the third study, data on prenatal stressors have been collected via the PSQ. Of the 31 SAH patients, n=9 answered items indicating prenatal stress (for example: reported financial distress or lack of social support, as well as biological data such as low birth weight and short gestational length). SAH patients did not report prenatal stress more often than controls (controls: n=5, chi^2, n.s.). SAH patients with or without prenatal stress did not differ in CAR or diurnal cortisol rhythm (GLM repeated measures). Because retrospective reports of prenatal stress may be vague and erroneous, the factor birth weight was analyzed in SAH patients (available in n=12 patients). As a result, birth weight was not related to CAR or diurnal salivary cortisol (GLM repeated measures, factors being birth weight high vs. low (split half) and cortisol values). Notably, only one SAH patient and two controls had a low birth weight (<2500g). A moderately low birth weight (<3200g) was seen in 6 patients and 6 controls). Gestational length was available in 5 patients only and was, therefore, not regarded. Based on these results it is unlikely that prenatal stress factors are the basis of hypocortisolism in our cohort of SAH patients.

7.2 Is CRF reduced in hypocortisolemic SAH patients due to hypothalamic lesions as suggested by the work of Crompton?

To test this hypothesis, an indirect measurement was performed. As low CRF is associated with atypical depression, the corresponding scale of the Neuropattern questionnaire was looked at (study 3). When regarding the scale “atypical depression”, the values of SAH patients did not differ from those of controls (mean controls=58.32, SAH=56.32, t-test, n.s.). Neither in SAH patients nor in controls were measures of atypical depression correlated with basal serum cortisol or salivary cortisol of the CAR (Pearson correlation coefficient, p=n.s.). Thus, a significant reduction of CRF as underlying mechanism of hypocortisolism in our cohort of SAH patients is not supported by the data.

7.3 Is ACTH deficiency linked to hypocortisolism in SAH patients?

In the first study, basal and stimulated (ITT) ACTH and cortisol were measured in n=40 patients in the chronic phase after SAH, whose endocrinological data were published elsewhere (Kreitschmann-Andermahr et al., 2004, the following data was not published). Stimulated maximal cortisol levels correlated statistically significant with stimulated maximal ACTH levels (r=0.50 p=0.001, Pearson correlation coefficients). Basal cortisol and ACTH values (ITT basal value) were not correlated (r=0.24, p=n.s.). Also, basal ACTH values did not differ between patients with insufficient stimulated cortisol (stimulated cortisol <500nmol/l) or a normal cortisol response. Stimulated ACTH, however, was higher in patients reaching stimulated cortisol values of >500nmol/l (stimulated ACTH=23.4 pmol/l in cortisol sufficient patients vs. ACTH=7.8 pmol/l, p=0.000, t-test). Based on the result that low basal cortisol was not connected to low basal ACTH, a low basal ACTH output may not be the cause of hypocortisolism in this cohort. However, there is at present not sufficient evidence to confirm or negate a link between ACTH deficiency and hypocortisolism in SAH survivors.

7.4 Could adrenal exhaustion after prolonged chronic stress be responsible for low cortisol values in the chronic phase after SAH?

Relatively low serum cortisol in the acute phase can be seen, but at what point of time after the SAH this phenomenon occurs first, can, of course, not be determined in

this setting but needs a prospective longitudinal study that covers a couple of months or even years after the SAH.

A single hint concerning a drop of cortisol after some time can be derived from the second study which covers the acute phase after SAH. In this study on 22 SAH patients from hospital admission (t_0) to up to 2-3 weeks after SAH (t_2), only one patient exhibited basal cortisol values below the appropriate reference range at t_2. This patient had higher cortisol levels later that day. In a short Synacthen test performed at this point of time, the patient showed high stimulated cortisol values of 1026 nmol/l. Unfortunately, after t_2 the patient did not proceed with the study and failed to make routine visits to our clinic, so the further functionality of this patient's cortisol secretion is unknown. It is interesting, however, that the low basal cortisol values occurred only at t_2 and not at t_0 and t_1, where high to normal cortisol values were measured.
As a result, our data do not sufficiently support the thesis of adrenal exhaustion in SAH patients, but also do not rebut it. As longitudinal data of our patients is lacking, no conclusions can be drawn.

7.5 Is an accelerated metabolism of cortisol present in some SAH patients, resulting in a steep decline of CAR after the peak?

Metabolism of cortisol was not addressed in the studies. Indirect measurements can be derived from the third study, where CAR was available. Based upon a half life of cortisol of around 90 minutes, expected cortisol values (CAR +60min) were computed in relation to the maximum value of the CAR. Those study subjects, who had +60min values below the expected amount regarding half-life, were classified as "fast metabolizer". In sum, SAH patients were not significantly more often classified as fast metabolizer than controls. Basal serum cortisol did not differ between fast and normal metabolizers. Moreover, metabolism rate was not connected to markers of disease severity (e.g. Fisher score, Hunt & Hess score, hospital days, GOS). It is considered unlikely that accelerated metabolism is the basis of hypocortisolism in SAH.

7.6 Is CBG downregulated as part of an emergency response action increase free cortisol after trauma, resulting in low basal cortisol levels and high salivary cortisol levels?

To test this hypothesis, CBG measurements were taken four times a day at one week and 2-3 weeks after SAH (study two). When regarding 08:00 values, only one patient showed reduced CBG values. This patient had, however, normal CBG values at the rest of the day (t_2, 2-3 weeks after SAH). Therefore, data from the second study do not support this assumption.

In study three, however, an unexpected finding occurred. CBG was not measured in this study but basal total serum cortisol was assessed as well as free salivary cortisol (CAR and diurnal profile) in patients and controls. Now, while basal serum cortisol was slightly reduced in all patients (n.s.) and statistically reduced in patients with high stress sensitivity, salivary cortisol in the CAR was significantly higher in patients than in controls. Additional tests showed that predominantly those patients accounted for the elevated CAR, that had a low stress sensitivity and that the high-stress patients (with low serum cortisol) had normal or only slightly elevated salivary cortisol in the CAR. Since salivary profiles and serum measures have been performed on different days, a direct relation between salivary and serum cortisol may not be calculated. Still, in sum there are hints that SAH patients may have a higher ratio of serum cortisol to salivary cortisol, indicating a higher amount of unbound cortisol in serum and, presumably, a drop in CBG. This hypothesis seems worthy to follow up.

7.7 Is there increased negative feedback, resulting in a stronger suppression of cortisol secretion?

In study three, the issue of negative feedback via the low-dose dexamethasone suppression test was addressed. In sum, SAH patients after dexamethasone administration did show a similar CAR as controls, while exhibiting an elevated CAR without dexamethasone. It seems that the negative feedback effect is similar, and that the drug effect masked CAR differences among groups. This interaction was not statistically significant. Also, when comparing controls to SAH patients in respect to high vs. low stress reactivity, an interaction of group and dexamethasone administration was not seen. Therefore, hyperactive negative feedback is unlikely the cause of hypocortisolism in this cohort of SAH patients.

8 Comprehensive discussion and perspective

From the three aforementioned studies some interesting conclusions could be derived. First, it could be shown that some SAH patients exhibit lower serum cortisol levels than controls but at the same time their salivary free cortisol after awakening is higher than in healthy controls. While a possible explanation for this may be lowered CBG levels, this was not seen in patients in the acute phase after SAH. Also, patients in the chronic phase after SAH do have a stable diurnal cortisol rhythm while there are disturbances in around 50% of all patients in the acute phase. Based upon the aforementioned results, containing probably very different patterns of cortisol dynamics in the acute / subacute and the chronic phase, it is assumed that in SAH patients endocrine changes occur over time and that a combination of adrenal exhaustion and a subsequent downregulation of CBG may be the most probable causes for hypocortisolism in the chronic phase in our cohorts of SAH patients.

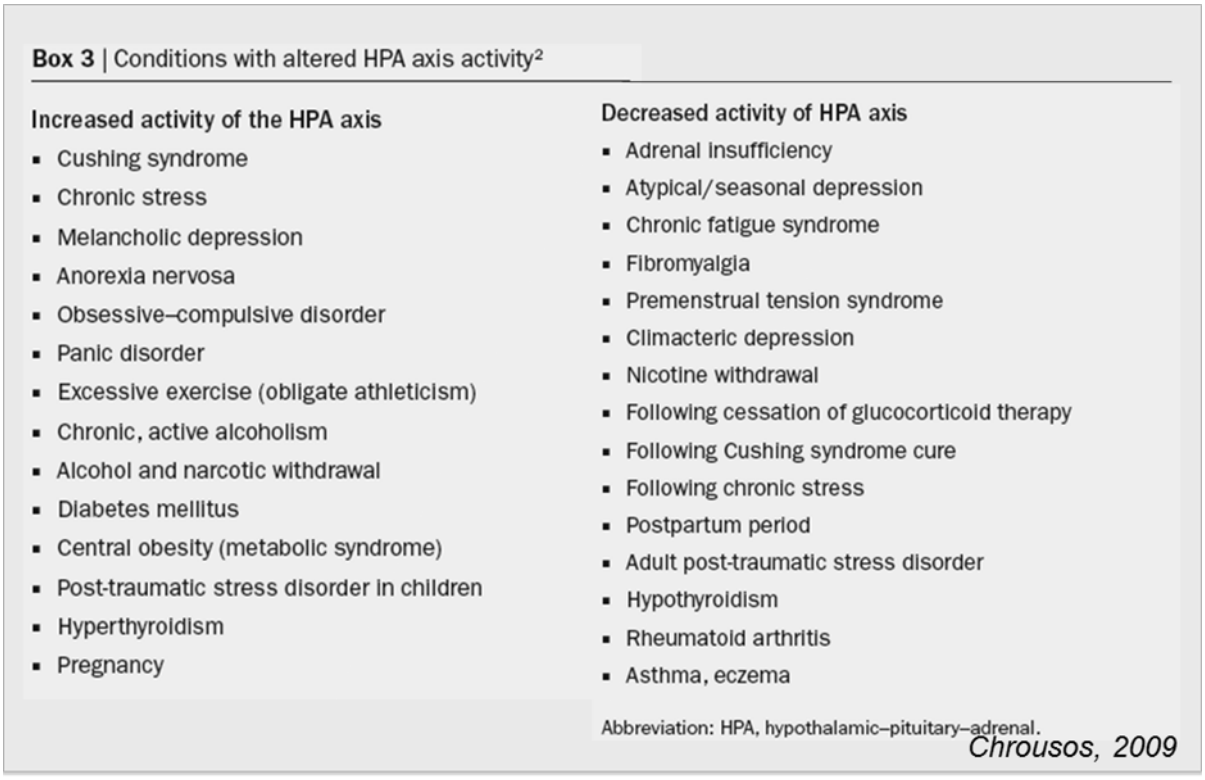
In general, SAH patients may be not so different from other patient cohorts suffering from hypocortisolism. In the first study low basal serum cortisol was linked to depression and a worsened QoL, a finding often reported in patients suffering from stress-related disorders. The mechanism of endocrine dysfunction in these patients is not fully understood. Since SAH is traumatic not only in physiological but also in psychological terms, a substantial number of patients may show similar pathologies as patients with stress-related disorders, with the SAH being the triggering event. After all, the experience of a life-threatening illness that comes without warning may be traumatic for patients and their relatives. Recently, Baisch and colleagues could show that over one third of all SAH patients suffer from PTSD and that the key factors of developing traumatization were post-ictal events including “realizing that their life could have/had changed, that they may have been left with long-term problems, that they could have died” (Baisch, Schenk, & Noble, 2011). So, are SAH patients just another cohort of PTSD-patients?

Another possible cause of hypocortisolism is the physiological trauma caused by the SAH. The hemorrhage itself may cause hypothalamic lesions as have been

described by Crompton (Crompton, 1963). As mentioned earlier, Crompton stated that “microhaemorrhages were often seen that were remarkably selective in their site and surprisingly localized, namely in the paraventricular and supra-optic nuclei, often rendering these nuclei prominent to the naked eye”. One can assume that such a cumulative appearance of microhemorrhages has its effects and result in a lowered or insufficient ACTH secretion. In our patients, ACTH values below the reference range were not seen (chapter 4), but since Crompton’s data was derived from patients who died after the SAH, hypothalamic lesions may be less prominent or rarer in the patients with a good outcome investigated in the reported studies. A lowered secretion of ACTH and cortisol due to minor hypothalamic lesions may, therefore, be subtle and not strong enough to be detected by comparison with reference ranges. In the third study a reduction of cortisol was only seen in comparison to a control group but not in respect to reference ranges, as ACTH was measured only in SAH patients but not in healthy controls, subtle dysregulations of ACTH were possibly missed.

A hypothesis that could not be validated through the designs of the three studies is adrenal exhaustion after a prolonged period of adrenal hyperactivity. After a continuous elevation of CBG, a compensatory downregulation of ACTH and cortisol was proposed by Hellhammer & Wade (Hellhammer & Wade, 1993). To maintain sufficient amounts of free cortisol, however, a reduction of CBG would be necessary. The design of all three studies was cross-sectional in nature, so it was not possible to investigate time courses. CBG was measured in the acute phase, only, so statements concerning the chronic phase cannot be made. Still, since in the chronic phase SAH patients exhibit higher levels of free cortisol in the CAR and moderately elevated levels of free cortisol throughout the day ($p=.085$, n.s.) a downregulation of CBG is likely. At this point the question arises whether SAH patients with low basal serum cortisol are truly hypocortisolemic. When keeping the third study in mind, hypocortisolism might be a misleading term for the endocrinological state of SAH patients with low serum cortisol. It must be kept in mind that biologically active, free cortisol might even be elevated and, due to a different ratio of free to total cortisol, this state may be masked as hypocortisolism. If SAH patients have sufficient amounts of free cortisol despite lowered serum cortisol, connections of lowered serum cortisol with psychosocial impairments have to be reconsidered. In the first

study reported, patients with a low basal serum cortisol had higher scores in questionnaires assessing QoL and depression. This was surprising at first glance, since some authors state a connection of hypercortisolism and melancholic depression (for an overview see figure 4).



Box 3 | Conditions with altered HPA axis activity[2]

Increased activity of the HPA axis

- Cushing syndrome
- Chronic stress
- Melancholic depression
- Anorexia nervosa
- Obsessive–compulsive disorder
- Panic disorder
- Excessive exercise (obligate athleticism)
- Chronic, active alcoholism
- Alcohol and narcotic withdrawal
- Diabetes mellitus
- Central obesity (metabolic syndrome)
- Post-traumatic stress disorder in children
- Hyperthyroidism
- Pregnancy

Decreased activity of HPA axis

- Adrenal insufficiency
- Atypical/seasonal depression
- Chronic fatigue syndrome
- Fibromyalgia
- Premenstrual tension syndrome
- Climacteric depression
- Nicotine withdrawal
- Following cessation of glucocorticoid therapy
- Following Cushing syndrome cure
- Following chronic stress
- Postpartum period
- Adult post-traumatic stress disorder
- Hypothyroidism
- Rheumatoid arthritis
- Asthma, eczema

Abbreviation: HPA, hypothalamic–pituitary–adrenal.

Chrousos, 2009

Figure 4: Conditions with altered HPA axis activity (Chrousos, 2009).

An elevation of free cortisol, however, could be a first step in explaining this paradoxon.

Independent from underlying mechanisms and consequences of endocrine dysregulation in SAH patients an important conclusion must be drawn for clinical routine. Based upon the assumption that SAH patients with low basal serum cortisol might present with unexpectedly high free cortisol, the usual clinical method of measuring only serum cortisol may lead to erroneous conclusions. This may also hold true for standardized stimulation tests such as the ITT. Patients with low serum cortisol or insufficient stimulated cortisol might have totally normal to elevated free cortisol levels and thus be incorrectly diagnosed with hypocortisolism. To gain a feeling for total and free cortisol levels in SAH patients it could be helpful to

implement CBG measures or salivary free cortisol measures in clinical routine. This issue is also addressed in the next chapter.

8.1 Perspective: what to test, when and how

In sum, to gain a complete understanding of who develops HPA dysregulations after SAH, when this happens and what implications lie within possibly lowered total and heightened free cortisol levels, a longitudinal study that covers several months or even years is inevitable. As could be seen from the second study, substantial variation of cortisol dynamics in the first days following the trauma occur. Still, longitudinal measurements would have to start in the acute or subacute phase after SAH to accurately assess the time course after SAH. An exact point of time for the first measurement is hard to define, as a substantial number of patients encounter complications that may influence cortisol dynamics on their own (e.g. sepsis), masking the direct consequences of the hemorrhage. This would suggest choosing a flexible time point for the first measurement, such as one or two days before hospital demission, when acute complications can be ruled out. The downside of this method is a weaker comparability of the first measurement when regarding the acute phase as a cross section. Additionally, in the second study one patient with normal basal cortisol values shortly after SAH and one week later showed lowered basal cortisol values at 2-3 weeks after the ictus, indicating that important changes in cortisol dynamics may occur as early as within the first three weeks. On the other side, cortisol dynamics were not stable at that point and cortisol values of this patient increased later that day. As significant changes in cortisol secretion in the first 2-3 weeks cannot be ruled out, weekly measurements until hospital discharge are encouraged. Since basal cortisol values may be misleading due to disturbed diurnal rhythms, other methods of cortisol diagnosis may be more appropriate. One possible method is cortisol detection in 24h-urine. With this method, independence from diurnal rhythm is given. Moreover, cortisol in urine is unbound, as in saliva. As patients are still in hospital, collection errors should be minimal. Therefore, cortisol detection in 24h-urine may be the method of choice in the acute phase until hospital demission (phase 1).

After hospital demission, further visits should be performed on a bi-monthly basis for the first year after SAH (phase 2). Follow-up visits after the first year may be performed in the frame of clinical routine. Since changes in endocrine status in the first year (Tanriverdi et al., 2007) and between 3 and 12 months (Aimaretti et al., 2005) have been described, it is highly probable that the first year is of substantial importance in the time course of cortisol dynamics after SAH. It is assumed that the endocrinological state is stable at 1 year after SAH. Up to now, however, no longitudinal data of SAH patients are available that exceed those 12 months, therefore, the only way to determine a stable phase after SAH is to check for endocrine abnormalities repeatedly.

Variables to be assessed should include cortisol in 24h-urine (phase 1), serum basal cortisol, ACTH and CBG measures as well as salivary cortisol at 08:00 (phase 2). Those measurements could easily be performed within the framework of routine visits, wherever possible. 24h-urine in non-hospitalized patients is error-prone and disliked by the patients, therefore of uncertain diagnostic relevance. Since former studies could show that a certain percentage of SAH survivors suffer from clinically relevant corticotroph deficiency, an ITT should be performed additionally to the basal hormone values. Due to the possibility of transient hypocortisolism in the first year (Aimaretti et al., 2005) and to the unknown long-term course of cortisol regulation in chronic SAH, a first ITT after 1 year and a second ITT after e.g. 3 years may be sensible. It could provide helpful to measure the cortisol response in serum and saliva simultaneously.

To check for possible psychiatric and psychosocial confounders, the use of questionnaires is strongly advised. They should include depression, PTSD and quality of life. Prenatal factors should also be assessed once.

As different possible time courses and underlying mechanisms are assumed, a significant number of SAH patients should be included (n>80). This restricts such a study to those centers of maximal care that treat lots of SAH patients each year. Alternatively, a multicenter design may be possible. In this case, however, a central measurement of the endocrine variables is advised due to the use of different assays and to the complex handling of saliva.

Unfortunately, so far no sensitive high-resolution imaging techniques exist, that would capture subtle hypothalamic or pituitary lesions as a consequence of SAH.

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Hiermit versichere ich, dass ich die vorliegende Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen oder Hilfsmittel verwendet habe.

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Dipl.-Psych. Eva Poll