**Changes in Endogenous Daytime Melatonin Levels after Aneurysmal Subarachnoid Hemorrhage – Preliminary Findings from an Observational Cohort Study.**

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**Running title:**

Endogenous Melatonin Levels after Aneurysmal SAH

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This trial has been registered at ClinicalTrials.gov (NCT02142166) as part of a larger scale prospective data collection (Date of registration 20/05/2014).

**Abstract**

**Introduction**

Aneurysmal subarachnoid hemorrhage (aSAH) is associated with early and delayed brain injury due to several underlying and interrelated processes, which include inflammation, oxidative stress, endothelial, and neuronal apoptosis. Treatment with melatonin, a cytoprotective neurohormone with anti-inflammatory, anti-oxidant and anti-apoptotic effects, has been shown to attenuate early brain injury (EBI) and to prevent delayed cerebral vasospasm in experimental aSAH models. Less is known about the role of endogenous melatonin for aSAH outcome and how its production is altered by the pathophysiological cascades initiated during EBI. In the present observational study, we analyzed changes in melatonin levels during the first three weeks after aSAH.

**Materials and Methods**

Daytime (from 11:00 am to 05:00 pm) melatonin levels were measured by enzyme-linked immunosorbent assay (ELISA) in serum samples obtained from 30 patients on the day of aSAH onset (d0) and in five pre-defined time intervals during the early (d1-4), critical (d5-8, d9-12, d13-15) and late (d16-21) phase. Perioperative daytime melatonin levels determined in 30 patients who underwent elective open aortic surgery served as a control for the acute effects of surgical treatment on melatonin homeostasis.

**Results**

There was no difference between serum melatonin levels measured in the control patients and on the day of aSAH onset (p=0.664). However, aSAH was associated with a sustained up-regulation that started during the critical phase (d9-12) and progressed to the late phase (d16-21), during which almost 80% of the patients reached daytime melatonin levels above 5 pg/ml. In addition, subgroup analyses revealed higher melatonin levels on d5-8 in patients with a poor clinical status on admission (p=0.031), patients with anterior communicating artery aneurysms (p=0.040) and patients without an external ventricular drain (p=0.018), possibly pointing to a role of hypothalamic dysfunction.

**Conclusion**

Our observations in a small cohort of patients provide first evidence for a delayed up-regulation of circulatory daytime melatonin levels after aSAH and a role of aneurysm location for higher levels during the critical phase. These findings are discussed in terms of previous results about stress-induced melatonin production and the role of hypothalamic and brainstem involvement for melatonin levels after aSAH.

**Introduction**

Aneurysmal subarachnoid hemorrhage (aSAH) is a devastating form of stroke caused by sudden rupture of an intracranial aneurysm and extravasation of blood into the subarachnoid space. Increased intracranial pressure, decreased cerebral perfusion, transient or persistent cerebral ischemia, cytotoxic edema and blood-brain-barrier (BBB) breakdown during the first 24-72 hours after ictus result in early brain injury (EBI), which is the main cause of mortality in aSAH patients [1,2]. About one third of patients who survive the initial bleed suffer from delayed cerebral ischemia (DCI), a major complication that usually develops 4-14 days after aSAH onset and contributes to mortality and morbidity [3]. Originally attributed to angiographic vasospasm, DCI is now recognized to result from several underlying and interrelated processes, which include systemic, vascular and cerebral inflammation, oxidative stress, and endothelial and neuronal apoptosis [4].

Melatonin, a methoxyindole found in all organisms with aerobic respiration, is a well-known sleep-inducing agent and regulator of the biological clock. In mammals, circulatory melatonin is mainly produced by the pineal gland and secreted following a circadian cycle, with little release during the day and peak levels 4-5 hours after darkness onset [5]. The circadian rhythm of melatonin secretion is generated by a pacemaker located in the suprachiasmatic nuclei of the hypothalamus [6] and synchronized to the light-dark cycle by information received through the retinohypothalamic tract [7,8]. Apart from its chronobiotic function, melatonin is increasingly recognized as a cytoprotective agent with potent anti-inflammatory, anti-oxidant, and anti-apoptotic effects. For example, cumulative evidence demonstrates that administration of exogenous melatonin is highly effective in preventing the damage inflicted by ischemia and reperfusion of the central nervous system (CNS) [9,10], the cardiovascular system [11,12] and other tissues [13,14]. In addition, pinealectomy increases the infarct size and worsens the outcome in rats subjected to transient cerebral ischemia [15–17], suggesting that endogenous melatonin is also protective against ischemia/reperfusion damage. More recently, melatonin treatment has been shown to inhibit aSAH-induced lipid peroxidation, inflammation and apoptosis [18,19], to attenuate EBI [20–22] and to reduce or prevent delayed cerebral vasospasm [18,23,24] in experimental aSAH models. Less is known about the role of endogenous melatonin for aSAH outcome and how its production is altered by the pathophysiological cascades initiated during aSAH onset. In the present, observational pilot study on a group of human aSAH patients, we analyzed serum melatonin levels at different times after aSAH onset, and examined their relation to patient characteristics, aneurysm location, DCI and clinical outcome after 12 months.

**Material and Methods**

**Patient population and demographics**

The present study is based on serum samples, obtained as part of a larger scale prospective data collection (ClinicalTrials.gov registration: NCT02142166, Date of registration 20/05/2014), from 30 patients with confirmed aSAH who were treated at our neurointensive care unit between 2015 and 2017 and met the following criteria: (1) age > 18 years, (2) not pregnant, (3) no hepatic or renal compromise (total bilirubin >51.3 µmol/L, plasma creatinine ≥22.1 µmol/L), (4) presence of a ruptured aneurysm, and (5) no previous aSAH or recurrent bleeding. Thirty non-aSAH patients who underwent elective open aortic surgery served as a control group. Demographic data (**Tab. 1**) included age, gender, body mass index (BMI), comorbidities (smoking, diabetes), aneurysm location and affected vessel, treatment modality, clinical severity on admission according to the Hunt & Hess (HH) grading scale, radiological severity on admission according to the modified Fisher grading scale (mFS) and clinical outcome after 12 months according to the extended Glasgow outcome scale (GOS-E). Outcome was assessed by an independent investigator based on clinical investigation in the outpatient clinic or the clinical status compiled from the medical reports. The study protocol was approved by the local ethics committee (RWTH Aachen University, Aachen, Germany, ID: EK 062/14) and written consent for study inclusion was obtained from all patients or their authorized representatives.

**Standard treatment procedure**

Aneurysms were secured by surgical clipping or endovascular coiling, prophylactic enteral nimodipine was applied as tolerated [25] and patients were continuously monitored for signs of deterioration as follows: In awake patients, DCI was diagnosed according to the criteria for clinical deterioration defined by Vergouwen [26], which comprise a decrease in Glasgow Coma Scale ≥2 for ≥1 h or the appearance of a new focal neurological deficit. In sedated or comatose patients, invasive neuromonitoring was performed according to recent consensus recommendations [27] and included measurement of brain tissue oxygen levels (ptiO2) with Neurovent PTO catheters (Raumedic AG, Helmbrechts, Germany) and cerebral metabolism within the affected territory with 71 high cut-off brain microdialysis catheters (µdialysis, Stockholm, Sweden). Microdialysis probes were perfused at 0.3μl/min with standard perfusion fluid containing 147 mmol/l NaCl, 2,7 mmol/l KCl, 1,2 mmol/l CaCl2 and 0,85 mmol/l MgCl2 (μdialysis, Stockholm, Sweden). Samples were collected at a minimum of 3-hour intervals and analyzed on site for glucose, lactate, pyruvate, glutamate and glycerol (Iscusflex, μdialysis, Stockholm, Sweden) or stored for later off-line analysis as described previously [28]. A metabolic (lactate / pyruvate ratio ≥ 40) or oxygenation (ptiO2 < 10 mmHg) crisis may trigger CT perfusion imaging. DCI in unconscious patients was defined as a new CT perfusion deficit if other causes like untreated hydrocephalus, rebleed, infection, electrolyte imbalance, seizures or infection could be ruled out. The first line of treatment for DCI consisted of induced euvolemic hypertension by intravenous noradrenaline infusion to raise the systolic arterial blood pressure to ≥ 180 mmHg. In cases without clinical and functional improvement, relevant hypoperfusion and vasoconstriction were verified by perfusion CT and / or cerebral angiography before endovascular rescue treatment was performed by transluminal balloon-angioplasty or intra-arterial spasmolysis after multidisciplinary discussion as described in more detail elsewhere [29]. Cerebral infarctions during ongoing DCI or diagnosed as a first sign of DCI were considered DCI-related infarctions. Nutrition was administered according to the guidelines of the European Society for Clinical Nutrition and Metabolism (ESPEN) for intensive care unit patients.

**Measurement of serum melatonin concentrations**

Melatonin levels in serum samples obtained during the day (from 11:00 am to 05:00 pm) were measured and directly compared with standard samples by enzyme-linked immunosorbent assay (ELISA) using the melatonin ELISA kit from IBL International GmbH, Berlin, Germany. Sample preparation and analysis was performed according to the recommendations of the manufacturer. Briefly, 500 µl of serum were applied to the C 18 RP extraction columns delivered with the kit. The samples were eluted with methanol, vacuum dried in an Eppendorf concentrator plus at 45°C overnight and diluted with 150 µM H2O. 50 µl of each sample and each standard (in duplicate) were transferred to the microtiter plate and mixed with biotinylated antigen. Following removal of unbound antigen by a washing step, alkaline phosphatase was added and bound biotinylated antigen was detected by measurement at 405 nm in a multiplate reader (BioTek Synergy 2).

**Analysis**

Based on the typical time-frame for development of complications like DCI, comparisons were made between measurements obtained on the day of aSAH onset (d0) and in pre-defined intervals during the early (d1-4), critical (d5-8, d9-12, d13-15) and late (d16-21) phase after hemorrhage. The number of patients for which measurements in the different intervals were available are summarized in **Tab. 2-3**. Note that if more than one measurement was available for a patient in a given interval, all measurements in this interval were used to calculate a mean value for this patient. For subgroup analyses, patients were stratified into groups according to sex, age (<59 years vs. ≥59 years based on the median age), BMI (<26 vs. ≥26 based on the median BMI), pre-existing diabetes, cigarette consumption (smoker vs. non-smoker), clinical severity on admission (good grade = HH1-3 vs. poor grade = HH4-5), radiological severity on admission (good grade = mFS1-2 vs. poor grade = mFS3-4), treatment modality (coiling vs. clipping), aneurysm location (anterior vs. posterior circulation), affected vessel (anterior communicating artery or other vessel), external ventricular drain (EVD) placement (EVD vs. no EVD), occurrence of DCI (DCI vs. no DCI) or DCI-related infarction (DCI only vs. DCI-related infarction) and clinical outcome after 12 months (favorable = GOS-E5-8 vs. unfavorable = GOS-E1-4).

**Statistical Methods**

For direct statistical comparison of left-censored data, we used the ordinal method, where the [Mann-Whitney U](https://www.socscistatistics.com/tests/mannwhitney/) test for nonparametric data is applied and all values below the lower limit of quantification are considered as tied [30]. To determine whether the proportion of data above or below a threshold was contingent upon the group classification, we used Fisher's exact test as a binary nonparametric method [30]. In both cases, data analysis was performed in an explorative manner without correction for multiple statistical comparisons to facilitate identification of trends that deserve further investigation. Unless noted otherwise, all values in the text and figures are expressed as median [1.quartile - 3.quartile]. Boxplots show median values, upper and lower quartiles (box), minimum and maximum values (whiskers) and individual data points (dots). All analyses were performed using IBM® SPSS® Statistics v22.0 (IBM, Chicago, Illinois, USA), Minitab v17 (Minitab Inc., Pennsylvania, USA) and Microsoft Excel 2010.

**Results**

**Participants**

A total of 30 consecutive aSAH patients with a mean age of 56 ± 12 years were included in this study. A summary of the patient characteristics is provided in **Tab. 1**. 17 (57%) of the patients experienced DCI, which was typically observed between days 3 and 12 (median: day 7) and progressed to infarction in six (35%) of the DCI patients. Clinical outcome after 12 months was favorable (GOS-E5-8) in 16 (53.3%), unfavorable (GOS-E1-4)in 10 (33.3%) and unknown in 4 (13.3%) patients and was not significantly associated with any of the demographic data routinely obtained on admission or during in-hospital treatment (**Tab. 1**). Six (20%) of the patients suffered from early seizures, but there was no association between the occurrence of seizures and in-hospital mortality (p=0.557) or clinical outcome (p=0.340) ), in line with multivariate analysis in a previous study [31]. However, aneurysms in the anterior circulation were more frequent (70%) and they tended to be associated with an unfavorable clinical outcome (p=0.087). Blood samples for quantification of serum melatonin level were available from 12 patients for the day of aSAH onset and 27-29 patients for all other time periods examined (**Tab. 2 & 3**). As a control for the acute effects of neurosurgical treatment on melatonin homeostasis, we also determined post-operative daytime melatonin levels in 30 non-aSAH patients with a mean age of 58 ± 12 years who underwent elective open aortic surgery. There were no significant differences between the control and aSAH patients with regard to age (p=0.837), gender (p=1.000) or body mass index (p=0.386).

**Acute effects of aSAH on daytime serum melatonin levels**

**Fig. 1** and **2A** show individual and average daytime (11:00 am to 05:00 pm) serum melatonin levels determined after elective open aortic surgery (Ctrl) or aSAH onset (d0) respectively, and in different time periods during the early (d1-4), critical (d5-8, d9-12, d13-15) and late (d16-21) phase after hemorrhage. To provide an accurate representation of the information contained in the data, values below the lower limit of quantification (LLOQ: 3 pg/ml) have been blanked out, and the proportion of censored data is indicated below the boxplots in **Fig. 2A**. Based on the upper limits for daytime and 24 hour peak levels previously observed in healthy subjects with a similar age (for details see discussion), we also determined the proportion of patients with melatonin levels above 5 pg/ml (**Fig. 2B**) and 15 pg/ml (**Fig. 2C**) respectively. There was no difference (p=0.664) between median melatonin levels measured on the day of aSAH onset (d0: 3.8 [<3.0-10.1] pg/ml) and after elective open aortic surgery (Ctrl: 4.0 [<3.0-6.7] pg/ml), with roughly 40% of patients in both groups having values below the LLOQ (**Fig. 2A**). Another 40% of patients from both groups exceeded the limit of 5 pg/ml (**Fig. 2B**), while serum melatonin levels above 15 pg/ml were observed in ~3% (1/30) of the non-aSAH patients and ~17% (2/12) of the aSAH patients (**Fig. 2C**).

**Sustained effects of aSAH on daytime melatonin levels during the early, critical and late phase after aSAH**

During the first eight days after aSAH onset, median melatonin levels decreased from 3.8 pg/ml to values below 3 pg/ml on day 1-4 [<3.0-7.3 pg/ml] and day 5-8 [<3.0-4.2] (**Fig. 1, 2A, Tab. 2**). This was paralleled by a progressive decline in the fraction of patients with melatonin levels above 5 pg/ml (**Fig. 2B**), while the proportion of patients with levels below 3 pg/ml increased to a peak value of >70% on day 5-8 (**Fig. 2A**). Thereafter, melatonin levels increased again to 5.1 [<3.0-8.8] pg/ml on day 9-12 and 7.7 [<3.0-9.9] pg/ml on day 13-15 after aSAH onset (**Fig. 2A**), which was mainly due to an increase in the proportion of individual values above 5 pg/ml to ~52% on day 9-12 and ~71% on day 13-15 (**Fig. 2B**). These changes continued during the late phase (d16-21), when median melatonin levels reached their peak value of 9.2 [5.6-11.0] pg/ml, the fraction of individual values above 5 pg/ml reached almost 80% and that of individual values below 3 pg/ml decreased to <15%.

**Subgroup analyses**

The results from subgroup analyses are summarized in **Tab. 2** & **3**. There was no evident association between serum melatonin levels at the different times after aSAH onset and body mass index (BMI), smoking, diabetes, treatment modality (clipping or coiling) or radiological severity on admission according to the modified Fisher scale. Median values on the day of aSAH onset were higher in female patients (5.6 [3.6-13.0] pg/ml *vs.* <3.0 [<3.0-<3.0] pg/ml in male patients, p=0.044), but there was no difference between the two subgroups during any of the later time points examined (**Tab. 2** & **3**).

**Relation to clinical status on admission**

**Fig. 3** compares changes in serum melatonin levels in patients stratified according to their clinical status on admission. Median values during the first eight days after aSAH onset tended to be higher in patients with a poor clinical status (HH4-5) (**Fig. 3A**), and this was mainly due to a higher proportion of individual values above 15 pg/ml in this subgroup (**Fig. 3C**). The difference reached statistical significance on day 5-8, when median melatonin levels amounted to 4.2 [<3.0-15.6] pg/ml in patients with a poor clinical status compared to <3.0 [<3.0-<3.0] pg/ml in patients with a good clinical status on admission according to the Hunt and Hess grading scale (p=0.031) (**Table 2**).

**Relation to aneurysm location and EVD placement**

Because melatonin release from the pituitary gland is controlled by the suprachiasmatic nuclei in the hypothalamus, it was of particular interest to analyze the role of aneurysm location. **Fig. 4** provides a comparison of serum melatonin levels in patients stratified with regard to aneurysms in anterior vs. posterior circulation. Melatonin levels on day 5-8 were significantly higher in patients with aneurysms in the anterior circulation (<3.0 [<3.0-7.9] pg/ml *vs.* <3.0 [<3.0-<3.0] pg/ml in patients with aneurysms in the posterior circulation, p=0.031). In addition, during the first eight days after hemorrhage, melatonin levels above 5 pg/ml (**Fig. 4B**) and 15 pg/ml (**Fig. 4C**) were (almost) exclusively observed in patients with aneurysms in the anterior circulation, although there were no statistically significant differences. Similar results were obtained when patients were instead stratified with regard to aneurysms in the anterior communicating artery (Acom), which is part of the anterior circulation and gives rise to vessels that supply the relevant parts of the hypothalamus. Thus, as illustrated in **Fig. 5A** and **Tab. 2**, melatonin levels on day 5-8 were significantly higher (p=0.040) in patients with Acom aneurysms (<3.0 [<3.0-15.5] pg/ml) as compared to patients with non-Acom aneurysms (<3.0 [<3.0-<3.0] pg/ml). In addition, significantly more patients from the former subgroup showed individual values that exceeded both 5 pg/ml (6/14 [43%] vs. 1/15 [7%] in patients with non-Acom aneurysms, p=0.035, **Fig. 5B**) and 15 pg/ml (5/14 [36%] vs. 0/15 [0%] in patients with non-Acom aneurysms, p=0.017, **Fig. 5C**).

Another potentially interesting result of the subgroup analyses is shown in **Suppl. Fig. 1**, which compares patients stratified according to the placement of an external ventricular drain (EVD). Although there were only 3 patients without an EVD, these patients exhibited by far the highest melatonin levels on the day of aSAH onset (55.6 [30-58.5] pg/ml *vs.* <3.0 [<3.0-5.6] pg/ml in patients with EVD, p=0.069). Moreover, even though melatonin levels in these patients decreased during the first eight days as well, median values were still significantly higher during day 1-4 (14.2 [9.1-20.7] pg/ml *vs.* <3.0 [<3.0-5.2] pg/ml in patients with EVD, p=0.028) and day 5-8 (5.3 [4.7-10.4] pg/ml *vs.* <3.0 [<3.0-<3.0] pg/ml in patients with EVD) (**Suppl.** **Fig. 1A**, **Tab. 2**), and two of the three patients in this subgroup had individual values above >5 pg/ml (**Suppl.** **Fig. 1B**) and/or >15 pg/ml (**Suppl.** **Fig. 1C**).

**Relation to long-term clinical outcome and DCI**

A comparison of melatonin levels in patients stratified according to the clinical outcome after 12 months is provided in **Fig. 6**. On the day of aSAH onset, median melatonin levels (**Fig. 6A**) and the fraction of individual values above 5 pg/ml (**Fig. 6B**) tended to be higher in patients with an unfavorable outcome. In contrast, during the early and most of the critical phase, the proportion of patients with melatonin levels above 5 pg/ml was larger in the subgroup with a good clinical outcome (**Fig. 6B**). This difference was no longer observed during the end of the critical or the late phase, where both groups showed a comparable increase in serum melatonin levels (**Fig. 6**). Similar observations were made when comparing patients with and without DCI (**Suppl. Fig. 2**) or DCI-related infarctions (**Suppl. Fig. 3**), as melatonin levels during early and part of the critical phase tended to be higher in patients without DCI and/or DCI-related infarction respectively, while these differences subsided after day 9 (**Tab. 2** & **3**).

**Discussion**

Melatonin is a neurohormone and cytoprotective agent with potent anti-inflammatory, anti-oxidant, and anti-apoptotic effects that has been shown to attenuate EBI and to prevent cerebral vasospasm in experimental aSAH models [18–24]. To our knowledge, this is the first study on changes in endogenous circulatory melatonin levels after aSAH in human patients. Although statistical analysis was complicated by the small number of patients and left-censoring of the data below the lower limit of quantification (LLOQ: 3 pg/ml), there are several interesting aspects of our findings that encourage further studies.

**Evidence for a delayed up-regulation of serum melatonin levels after aSAH**

When comparing our results with previous findings, it is important to take into account the advanced age of our patients, as there appears to be an almost linear negative relationship between endogenous melatonin levels and age [32]. Based on a number of previous studies in healthy subjects of a similar age, average daytime melatonin levels should be in the order of 2-3 pg/ml, with an upper limit of approximately 5 pg/ml during the day and 15 pg/ml during the night [33–35]. While on the day of aSAH onset, roughly 40% of the patients in our study had serum melatonin levels below 3 pg/ml, another 40% exceeded the expected threshold of 5 pg/ml. Given that the situation was similar in patients who underwent elective open aortic surgery, this observation can most likely be attributed to a non-specific elevation of melatonin levels due to general anesthesia and / or surgery, which have both been shown to transiently increase daytime melatonin levels [36,37]. Consistent with this assumption, the proportion of patients with supra-physiological melatonin levels decreased between day 1-8, but this was followed by a sustained increase, so that almost 80% of aSAH patients exhibited daytime melatonin levels above 5 pg/ml during the late phase (i.e. day 16-21). Considering the neuroprotective action of exogenous melatonin in experimental aSAH models [18–24] and given that the late up-regulation was observed in all subgroups of aSAH patients, it could be speculated to reflect an endogenous mechanism by which the brain protects itself from injury. Stress-induced melatonin production has previously been observed after ischemia/reperfusion of the pancreas in rats [38] and in human CSF after traumatic brain injury [39]. However, it is also possible that some delayed process, such as the development of neuroinflammation, might have altered synchronization of melatonin release to the light-dark cycle by e.g. impairing the light-induced suppression of melatonin release mediated via the retinohypothalamic tract [6–8].

**Possible role of hypothalamic dysfunction**

The most prominent difference observed in the subgroup analyses was that melatonin levels during parts of the critical phase were significantly higher in patients with aneurysms located in the anterior *vs.* posterior circulation and especially in patients with Acom *vs.* non-Acom aneurysms. In particular, on day 5-8 after aSAH onset, more than 35% of the patients with Acom aneurysms exhibited daytime melatonin levels above 15 pg/ml, which corresponds to the nocturnal peak level previously observed in healthy subjects of comparable age, suggesting that this finding cannot be accounted for by a phase shift due to e.g. impairments in the circadian rhytm. Because Acom-derived arteries supply parts of the hypothalamus [40] and signs of transient hypothalamic dysfunction are significantly more frequent in patients with Acom aneurysms [41], these findings could instead point to a role of hypothalamic dysfunction with excessive melatonin release. In support of this assumption, CSF melatonin levels during different CNS pathologies have previously been shown to depend on brainstem and hypothalamic involvement, and particularly high levels of the hormone were found in the CSF of patients with hypothalamic syndrome after traumatic SAH [42,43]. In the present study, the majority of patients with aneurysms in the anterior circulation had Acom aneurysms (14/21=67%), which could then at least partly account for higher melatonin levels the former subgroup as well. Some degree of brainstem and hypothalamic involvement could possibly also explain why patients with a poor clinical status on admission exhibited higher melatonin levels during the first eight days after aSAH onset. However, another possible explanation for the apparent association between aneurysm location and serum melatonin levels during early and critical phase could be that the anterior circulation is responsible for at least 80% of the brains blood supply [44], so that more extensive cerebral ischemia in patients with aneurysms in the anterior circulation might have resulted in a more pronounced, stress-induced melatonin production.

Interestingly, we also observed much higher early and critical melatonin levels in the three patients without an EVD, which could be speculated to reflect excessive melatonin release due to subclinically elevated intracranial pressure and mild compression of the brainstem and / or hypothalamus. Alternatively, lower levels in the remaining patients could reflect a loss of endogenous melatonin with the CSF removed via the ventricular drain. In any case, the early elevation appeared to be distinct from the delayed up-regulation and transient in nature, as it was restricted to the first eight days after aSAH onset during which melatonin levels generally tended to decrease. With regard to the underlying mechanisms, different scenarios could be envisioned. For example, cell death in patients with brain injury is often preceded by overexcitation and excitotoxicity in the affected regions, which could result in a transient increase of melatonin release. Alternatively, some of the other pathophysiological cascades initiated during EBI could (transiently) impair neuronal transmission via the retinohypothalamic tract, thereby reducing light-induced suppression of daytime melatonin secretion during the critical phase. In any case, if future studies could confirm that there is a relation between increased circulatory melatonin and hypothalamic dysfunction, serum melatonin levels could possibly serve as an easily accessible biomarker for a hypothalamic involvement after aSAH.

**Protective effects of endogenous melatonin**

Our findings provide no clear-cut evidence for a protective role of endogenous melatonin after aSAH, but there was a tendency for patients with a good clinical outcome and for patients that developed no DCI to exhibit higher daytime melatonin levels during the critical phase. Considering the beneficial effects of exogenous melatonin in experimental aSAH models, it seems reasonable to assume that higher endogenous melatonin levels could also contribute to a favorable outcome and reduce the incidence of DCI during the critical phase. However, as discussed in the preceding section, our results and previous findings also indicate that higher melatonin levels during the critical phase might be related to hypothalamic dysfunction, which could overshadow potential beneficial effects of the hormone and complicate their identification. Moreover, even though a number of studies reported that exogenous administration of the neurohormone ameliorates aSAH-induced cerebral vasospasm [18,23,24], there is also evidence that melatonin can directly constrict cerebral arteries [45] and reduce cerebral blood flow in regions supplied by circle of Willis and the basilar arteries [46].

**Limitations**

Apart from the small number of patients and left-censoring of the data below the LLOQ, interpretation of our findings is complicated by the fact that melatonin levels can show considerable inter-individual variation, which might have affected the results of our subgroup analyses. In addition, serum melatonin levels in the non-aSAH patients were only available for a single time-point, precluding comparison with the temporal profile of changes after aSAH. However, previous findings indicate that the increase of serum melatonin due to anesthesia/surgery is transient in nature and followed by a return towards normal levels [36,37], which is similar to what we observed on day 1-8 after aSAH onset. Furthermore, even if a control group with similar time-elapsed serum sampling would have been available, comparisons would have been complicated by the fact that aSAH patients are typically examined on a regular basis, which may by itself result in significant disruptions of the sleep/wake cycle. In this context, it is interesting that we observed a sustained increase of serum melatonin levels in aSAH patients, even though regular examinations would be expected to trigger (light-induced) reductions of melatonin release. In any case, given the delayed increase of serum melatonin levels in aSAH patients, an additional shortcoming of the present study is that serum samples were only available for the first three weeks after the ictus, precluding assessment of the trend of melatonin levels following the apparent peak during the late phase. Another important consideration is that we only measured circulatory melatonin levels, and that there is evidence that CNS levels of the hormone, as measured in the cerebrospinal fluid (CSF), can be much higher [47]. Although melatonin readily passes the BBB, the exact origins of CSF melatonin, as well as the role of serum *vs.* CSF levels of the hormone for its central effects are still unclear [48]. For example, endogenous CSF but not serum melatonin levels have been shown to increase following TBI [39], demonstrating that circulatory concentrations of the hormone do not necessarily reflect those in CSF. It is also worth noting that our study was purely observational and aimed at identifying trends that deserve further investigation, so that we made no correction for the increased error probability due to multiple statistical comparisons. Finally, a role of transient or permanent hypothalamic injury for changes in serum melatonin levels was not confirmed by e.g. radiological examination with MRI/DTI and remains speculative. With this in mind and taking into account that many other factors may have influenced serum melatonin levels, further studies with more patients and in experimental aSAH models will clearly be required to confirm our findings and to determine if there are associations and/or causal relationships between endogenous serum melatonin levels and outcome, DCI, and/or hypothalamic dysfunction.

**Conclusion**

Based on the observations of our hypothesis-generating pilot study, there may be a delayed up-regulation of circulatory daytime melatonin levels after aSAH and a role of aneurysm location for transient elevations during the critical phase. Further human and experimental studies will be required to verify our findings and to analyze their relation to CSF melatonin levels, clinical outcome and other factors.

**Authors’ contributions**

Conceived, designed and performed the experiments: FN, MiW, WA. Data acquisition: FN, MiW, MV, WA. Analysis and interpretation of data: FN, GAS, WA. First drafting of the manuscript and illustrations: FN, WA. Critical review of the manuscript: FN, WA, MiW, MV, KK, MaW, AH, HC, HSS, GAS.

**Conflict of interest**

The authors report no conflicts of interest concerning the materials or methods used in this study or the findings specified in this paper.

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**Ethical statement**

The trial was conducted in accordance with the recommendations of the ethics committee of the Medical Faculty of the RWTH Aachen University. (EK 062/14).

**Abbreviations**

1.q 1.quartile

3.q 3.quartile

Acom anterior communicating artery

BBB blood-brain-barrier

CNS central nervous system

CSF cerebrospinal fluid

DCI delayed cerebral ischemia

EBI early brain injury

ELISA enzyme-linked immunosorbent assay

EVD external ventricular drain

GOS-E Glasgow outcome scale - extended

HH Hunt and Hess grading scale

LLOQ lower limit of quantification

mFS modified Fisher scale

ptiO2 brain tissue oxygen level

SAH subarachnoid hemorrhage

SEM standard error of the mean

TBI traumatic brain injury

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**Figure Legends**

**Figure 1. Temporal profile of daytime serum melatonin levels after aSAH.**

****

Shown are median [1.q-3.q] serum melatonin levels (Mel) determined on the day of aSAH onset (d0) and during the indicated time periods thereafter. Dotted line indicates the lower limit of quantification (LLOQ: 3 pg/ml).

**Figure 2. Comparison of serum melatonin levels measured in aSAH and non-aSAH patients.**



(**A**) Boxplots and individual serum melatonin levels determined between 11 p.m. and 5 a.m. in non-aSAH patients (Ctrl, grey circles) and in aSAH patients (open circles) at the indicated time-points after aSAH onset (d0). The dotted line indicates the lower limit of quantification (LLOQ, 3 pg/ml). The proportion of patients below the LLOQ is indicated below. (**B**) Percentage of patients with daytime serum melatonin levels above 5 pg/ml. Grey circle and line indicate the proportion in the control group. (**C**) Percentage of patients with daytime serum melatonin above 15 pg/ml. Grey circle and line indicate the proportion in non-aSAH patients. p-Values in A & B indicate trends and significant differences compared to d0.

**Figure 3. Comparison of serum melatonin levels in aSAH patients stratified according to clinical status on admission.**



(**A**) Boxplots and individual serum melatonin levels determined between 11 p.m. and 5 a.m. in aSAH patients with a good (HH1-3, filled circles) or poor (HH4-5, open circles) clinical status on admission at the indicated time-points after aSAH onset. The dotted line indicates the lower limit of quantification (LLOQ, 3 pg/ml). The proportion of patients below the LLOQ is indicated below (**B**) Percentage of patients in both groups with daytime melatonin levels above 5 pg/ml. Grey circle and line indicate the proportion in non-aSAH patients. (**C**) Percentage of patients in both groups with daytime melatonin above 15 pg/ml. Grey circle and line indicate the proportion in non-aSAH patients.

**Figure 4. Comparison of serum melatonin levels in aSAH patients stratified according to aneurysm location.**



(**A**) Boxplots and individual serum melatonin levels determined between 11 p.m. and 5 a.m. in aSAH patients with aneurysms in the posterior (open circles) or anterior (filled circles) circulation at the indicated time-points after aSAH onset. The dotted line indicates the lower limit of quantification (LLOQ, 3 pg/ml). The proportion of patients below the LLOQ is indicated below (**B**) Percentage of patients in both groups with daytime melatonin levels above 5 pg/ml. Grey circle and line indicate the proportion in non-aSAH patients. (**C**) Percentage of patients in both groups with daytime melatonin above 15 pg/ml. Grey circle and line indicate the proportion in non-aSAH patients.

**Figure 5. Comparison of serum melatonin levels in aSAH patients stratified according to the affected vessel.**



(**A**) Boxplots and individual serum melatonin levels determined between 11 p.m. and 5 a.m. in aSAH patients with Acom aneurysm (Acom, filled circles) or other aneurysms (other, open circles) at the indicated time-points after aSAH onset. The dotted line indicates the lower limit of quantification (LLOQ, 3 pg/ml). The proportion of patients below the LLOQ is indicated below (**B**) Percentage of patients in both groups with daytime melatonin levels above 5 pg/ml. Grey circle and line indicate the proportion in non-aSAH patients. (**C**) Percentage of patients in both groups with daytime melatonin above 15 pg/ml. Grey circle and line indicate the proportion in non-aSAH patients.

**Figure 6. Comparison of serum melatonin levels in aSAH patients stratified according to the clinical outcome after 12 months.**



(**A**) Boxplots and individual serum melatonin levels determined between 11 p.m. and 5 a.m. in SAH patients with a favorable (GOS-E5-8, open circles) or unfavorable (GOS-E1-4, filled circles) clinical outcome at the indicated time-points after aSAH onset. The dotted line indicates the lower limit of quantification (LLOQ, 3 pg/ml). The proportion of patients below the LLOQ is indicated below. Note that four patients were lost to follow-up. (**B**) Percentage of patients in both groups with daytime melatonin levels above 5 pg/ml. Grey circle and line indicate the proportion in non-aSAH patients. (**C**) Percentage of patients in both groups with daytime melatonin above 15 pg/ml. Grey circle and line indicate the proportion in non-aSAH patients.

**Tables**

**Table 1.** Patient characteristics and their relation to clinical outcome after 12 months.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Patient characteristic** | **n (%)** | **missing data** | **Favorable outcome (GOS-E5-8)** | **Unfavorable outcome (GOS-E1-4)** | **p-value** |
| **All patients** | 30 (100%) | 4 (13.3%) | 16 (53.3%) | 10 (33.3%) |  |
| **Sex** |  |  |  |  | 1.000 |
| Female | 22 (73%) | 2 (50%) | 12 (75%) | 8 (80%) |  |
| Male | 8 (27%) | 2 (50%) | 4 (25%) | 2 (20%) |  |
| **Age** |  |  |  |  | 0.756 |
| < 59 years | 14 (47%) | 2 (50%) | 7 (44%) | 5 (50%) |  |
| ≥ 59 years | 16 (53%) | 2 (50%) | 9 (56%) | 5 (50%) |  |
| Median [q1-q3]a | 59 [50-64] | 58 [49-63] | 59 [51-62] | 57 [47-71] | 0.958 |
| **Body mass index (BMI)** |  |  |  |  | 0.688 |
| BMI < 26 | 14 (47%) | 2 (50%) | 9 (56%) | 3 (30%) |  |
| BMI ≥ 26 | 16 (53%) | 2 (50%) | 7 (44%) | 7 (70%) |  |
| Median [q1-q3]a | 26 [22-28] | 26 [24-27] | 25 [22-26] | 27 [25-31] | 0.094 |
| **Smoker** |  |  |  |  | 0.340 |
| No | 23 (77%) | 2 (50%) | 14 (87.5%) | 7 (70%) |  |
| Yes | 7 (23%) | 2 (50%) | 2 (12.5%) | 3 (30%) |  |
| **Diabetes** |  |  |  |  | 1.000 |
| No | 28 (93%) | 4 (100%) | 15 (94%) | 9 (90%) |  |
| Yes | 2 (7%) | 0 (0%) | 1 (6%) | 1 (10%) |  |
| **Aneurysm location** |  |  |  |  | 0.087 |
| Anterior circulation | 21 (70%) | 4 (100%) | 8 (50%) | 9 (90%) |  |
| Posterior circulation | 9 (30%) | 0 (0%) | 8 (50%) | 1 (10%) |  |
| **Affected vessel** |  |  |  |  | 1.000 |
| ACOMb | 14 (47%) | 4 (100%) | 6 (37.5%) | 4 (40%) |  |
| Other | 16 (53%) | 0 (0%) | 10 (62.5%) | 6 (60%) |  |
| **External ventricular drain (EVD)** |  |  |  |  | 1.000 |
| No | 3 (10%) | 0 (0%) | 2 (12.5%) | 1 (10%) |  |
| Yes | 27 (90%) | 4 (100%) | 14 (87.5%) | 9 (90%) |  |
| **Hunt and Hess (HH) grade** |  |  |  |  | 0.339 |
| Good grade (HH1-3) | 20 (67%) | 4 (100%) | 11 (69%) | 5 (50%) |  |
| Poor grade (HH4-5) | 10 (33%) | 0 (0%) | 5 (31%) | 5 (50%) |  |
| **Modified Fisher (mFS) scale** |  |  |  |  | 0.190 |
| Good grade (mFS1-2) | 7 (23%) | 0 (0%) | 6 (37%) | 1 (10%) |  |
| Poor grade (mFS3-4) | 23 (77%) | 4 (100%) | 10 (63%) | 9 (90%) |  |
| **Treatment modality** |  |  |  |  | 0.234 |
| Clipping | 11 (37%) | 2 (50%) | 4 (25%) | 5 (50%) |  |
| Coiling | 19 (63%) | 2 (50%) | 12 (75%) | 5 (50%) |  |
| **Delayed cerebral ischemia (DCI)** |  |  |  |  | 0.248 |
| no DCI | 13 (43%) | 1 (25%) | 9 (56%) | 3 (30%) |  |
| DCI | 17 (57%) | 3 (75%) | 7 (44%) | 7 (70%) |  |
| **DCI-related infarction** |  |  |  |  | 0.592 |
| DCI only | 11/17 (65%) | 3/3 (100%) | 5/7 (71%) | 3/7 (43%) |  |
| DCI-related infarction | 6/17 (35%) | 0/3 (0%) | 2/7 (29%) | 4/7 (57%) |  |

a 1.quartile - 3.quartile; b anterior communicating artery

**Table 2.** Subgroup comparison of serum melatonin levels during days 0-8 after SAH.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **aSAH onset (d0)** | | | **Early phase (d1-4)** | | | **Critical phase 1 (d5-8)** | | |
|  | **n** | **Median**  **[1.q-3.q]**c | **p-value** | **n** | **Median**  **[1.q-3.q]**c | **p-value** | **n** | **Median**  **[1.q-3.q]**c | **p-value** |
| **All patientsa** | 12 | 3.8 [<3.0-10.1] | | 29 | <3.0 [<3.0-7.3] | | 29 | <3.0 [<3.0-4.2] | |
| **Sex** |  |  | **0.044** |  |  | 1.000 |  |  | 0.518 |
| Male | 3 | <3.0 [<3.0-<3.0] | | 8 | <3.0 [<3.0-5.5] | | 7 | <3.0 [<3.0-<3.0] | |
| Female | 9 | 5.6 [3.6-13.0] | | 21 | <3.0 [<3.0-6.8] | | 22 | <3.0 [<3.0-5.0] | |
| **Age** |  |  | 0.113 |  |  | 0.442 |  |  | 0.076 |
| < 59 years | 7 | 3.6 [<3.0-4.8] | | 14 | <3.0 [<3.0-7.6] | | 13 | <3.0 [<3.0-<3.0] | |
| ≥ 59 years | 5 | 9.2 [<3.0-55.6] | | 15 | <3.0 [<3.0-5.2] | | 16 | <3.0 [<3.0-15.4] | |
| **Body mass index (BMI)** |  | 0.618 | |  | 0.582 | |  | 0.506 | |
| BMI < 26 | 6 | <3.0 [<3.0-10.6] | | 14 | 3.3 [<3.0-5.3] | | 14 | <3.0 [<3.0-<3.0] | |
| BMI ≥ 26 | 6 | 4.8 [<3.0-8.3] | | 15 | <3.0 [<3.0-5.4] | | 15 | <3.0 [<3.0-4.8] | |
| **Smoking** |  |  | 0.379 |  |  | 0.615 |  |  | 0.365 |
| No | 8 | <3.0 [<3.0-6.2] | | 22 | <3.0 [<3.0-5.0] | | 22 | <3.0 [<3.0-<3.0] | |
| Yes | 4 | 7.4 [4.7-20.8] | | 7 | <3.0 [<3.0-15.4] | | 7 | <3.0 [<3.0-10.4] | |
| **Diabetes** |  |  | - |  |  | 0.220 |  |  | 0.585 |
| No | 12 | 3.8 [<3.0-10.1] | | 27 | 3.6 [<3.0-7.7] | | 27 | <3.0 [<3.0-3.1] | |
| Yes | 0 | - | | 2 | <3.0 [<3.0-<3.0] | | 2 | 3.7 [<3.0-4.6] | |
| **Aneurysm location** |  |  | 0.504 |  |  | 0.915 |  |  | **0.031** |
| Anterior circulation | 10 | 4.6 [<3.0-12.0] | | 21 | <3.0 [<3.0-6.8] | | 20 | <3.0 [<3.0-7.9] | |
| Posterior circulation | 2 | 3.1 [<3.0-3.5] | | 8 | <3.0 [<3.0-5.2] | | 9 | <3.0 [<3.0-<3.0] | |
| **Affected vessel** |  | 0.740 | |  | 0.632 | |  | **0.040** | |
| ACOMb | 6 | 3.9 [<3.0-8.3] | | 14 | <3.0 [<3.0-10.8] | | 14 | <3.0 [<3.0-15.5] | |
| Other | 6 | 3.8 [<3.0-10.7] | | 15 | <3.0 [<3.0-4.8] | | 15 | <3.0 [<3.0-<3.0] | |
| **External ventricular drain (EVD)** |  | 0.069 | |  | **0.028** | |  | **0.018** | |
| No | 3 | 55.6 [30-58.5] | | 3 | 14.2 [9.1-20.7] | | 3 | 5.3 [4.7-10.4] | |
| Yes | 9 | <3.0 [<3.0-5.6] | | 26 | <3.0 [<3.0-5.2] | | 26 | <3.0 [<3.0-<3.0] | |
| **Hunt & Hess (HH) grade** |  |  | 0.319 |  |  | 0.920 |  |  | **0.031** |
| Good (HH1-3) | 6 | 3.0 [<3.0-5.2] | | 19 | <3.0 [<3.0-5.2] | | 20 | <3.0 [<3.0-<3.0] | |
| Poor (HH4-5) | 6 | 8.3 [<3.0-44.9] | | 10 | <3.0 [<3.0-11.7] | | 9 | 4.2 [<3.0-15.6] | |
| **Mod. Fisher (mFS) scale** |  |  | 0.881 |  |  | 0.889 |  |  | 0.897 |
| Good (mFS1-2) | 1 | 4.0 | | 7 | 3.6 [<3.0-5.0] | | 7 | <3.0 [<3.0-3.8] | |
| Poor (mFS3-4) | 11 | 3.6 [<3.0-11.1] | | 22 | <3.0 [<3.0-6.4] | | 22 | <3.0 [<3.0-3.7] | |
| **Treatment modality** |  |  | 0.207 |  |  | 0.900 |  |  | 0.415 |
| Clipping | 5 | 9.2 [3.6-13.0] | | 10 | 3.1 [<3.0-4.8] | | 10 | <3.0 [<3.0-5.0] | |
| Coiling | 7 | <3.0 [<3.0-4.8] | | 19 | <3.0 [<3.0-6.1] | | 19 | <3.0 [<3.0-<3.0] | |
| **Delayed cerebral ischemia (DCI)** |  |  | 0.245 |  |  | 0.829 |  |  | 0.736 |
| no DCI | 6 | 6.6 [3.7-12.0] | | 13 | <3.0 [<3.0-6.8] | | 12 | <3.0 [<3.0-5.3] | |
| DCI | 6 | <3.0 [<3.0-4.7] | | 16 | <3.0 [<3.0-5.0] | | 17 | <3.0 [<3.0-<3.0] | |
| **DCI-related infarction** |  |  | - |  |  | 0.339 |  |  | 0.345 |
| DCI only | 6 | <3.0 [<3.0-4.7] | | 10 | 3.1 [<3.0-10.4] | | 11 | <3.0 [<3.0-<3.0] | |
| DCI-related infarction | 0 |  | | 6 | <3.0 [<3.0-3.9] | | 6 | <3.0 [<3.0-43.2] | |
| **Outcome** |  |  | 0.066 |  |  | 0.669 |  |  | 0.484 |
| Favorable (GOS-E5-8) | 6 | <3.0 [<3.0-3.9] | | 15 | <3.0 [<3.0-5.2] | | 15 | <3.0 [<3.0-5.3] | |
| Unfavorable (GOS-E1-4) | 3 | 13 [9.3-37.2] | | 10 | <3.0 [<3.0-4.4] | | 10 | <3.0 [<3.0-<3.0] | |

a total number of patients from which samples were available; b anterior communicating artery; c 1.quartile – 3.quartile

**Table 3.** Subgroup comparison of serum melatonin levels during days 9-21 after SAH.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Critical phase 2 (d9-12)** | | | **Critical phase 3 (d13-15)** | | | **Late phase (d16-21)** | | |
|  | **n** | **Median**  **[1.q-3.q]**c | **p-value** | **n** | **Median**  **[1.q-3.q]**c | **p-value** | **n** | **Median**  **[1.q-3.q]**c | **p-value** |
| **All patientsa** | 29 | 5.1 [<3.0-8.8] | | 28 | 7.7 [<3.0-9.9] | | 27 | 9.2 [5.6-11] | |
| **Sex** |  |  | 0.600 |  |  | 0.365 |  |  | 0.670 |
| Male | 7 | 3.3 [<3.0-7.0] | | 8 | 4.4 [<3.0-8.2] | | 8 | 7.9 [5.7-9.8] | |
| Female | 22 | 5.1 [<3.0-8.6] | | 20 | 7.8 [<3.0-11.0] | | 19 | 9.3 [5.3-11.0] | |
| **Age** |  |  | 0.621 |  |  | 0.906 |  |  | 0.114 |
| < 59 years | 13 | 5.2 [<3.0-8.1] | | 14 | 7.8 [<3.0-10.4] | | 14 | 9.9 [7.6-12.6] | |
| ≥ 59 years | 16 | 4.9 [<3.0-9.4] | | 14 | 6.3 [<3.0-9.0] | | 13 | 6.1 [<3.0-9.3] | |
| **Body mass index (BMI)** |  | 0.346 | |  | 0.102 | |  | 0.466 | |
| BMI < 26 | 14 | 4.8 [<3.0-6.1] | | 14 | 8.4 [6.7-12.1] | | 13 | 9.2 [6.1-11.9] | |
| BMI ≥ 26 | 15 | 5.1 [<3.0-9.3] | | 14 | 4.4 [<3.0-8.5] | | 14 | 8.8 [4.8-10.0] | |
| **Smoking** |  |  | 0.582 |  |  | 0.466 |  |  | 0.793 |
| No | 22 | 4.9 [<3.0-7.7] | | 21 | 7.8 [4.2-9.7] | | 21 | 9.2 [6.1-11.6] | |
| Yes | 7 | 5.1 [<3.0-9.3] | | 7 | <3.0 [<3.0-10.1] | | 6 | 7.5 [5.6-9.9] | |
| **Diabetes** |  |  | 0.376 |  |  | 0.586 |  |  | 0.071 |
| No | 27 | 5.1 [<3.0-8.4] | | 26 | 7.7 [<3.0-9.5] | | 25 | 9.2 [6.1-11.6] | |
| Yes | 2 | 7.2 [6.0-8.5] | | 2 | 8.7 [6.9-10.5] | | 2 | 3.3 [<3.0-3.9] | |
| **Aneurysm location** |  |  | 0.942 |  |  | 0.717 |  |  | 0.246 |
| Anterior circulation | 20 | 5.1 [<3.0-8.3] | | 20 | 7.0 [<3.0-11.0] | | 18 | 9.2 [6.2-11.7] | |
| Posterior circulation | 9 | 4.7 [<3.0-8.8] | | 8 | 8.2 [4.3-9.2] | | 9 | 8.4 [4.2-9.8] | |
| **Affected vessel** |  | 0.857 | |  | 0.624 | |  | 0.732 | |
| ACOMb | 13 | 5.1 [<3.0-8.8] | | 14 | 6.1 [<3.0-11.4] | | 14 | 7.0 [5.7-10.5] | |
| Other | 16 | 4.9 [<3.0-8.3] | | 14 | 7.8 [4.4-9.5] | | 15 | 9.2 [5.5-11.0] | |
| **External ventricular drain (EVD)** |  | 0.685 | |  | 0.521 | |  | 0.164 | |
| No | 3 | 5.3 [3.7-7.7] | | 3 | <3.0 [<3.0-9.1] | | 2 | 11.1 [11-11.5] | |
| Yes | 26 | 4.9 [<3.0-8.6] | | 25 | 7.8 [<3.0-9.7] | | 25 | 8.4 [5.5-10.1] | |
| **Hunt & Hess (HH) grade** |  |  | 0.706 |  |  | 0.903 |  |  | 0.571 |
| Good (HH1-3) | 19 | 5.1 [<3.0-8.4] | | 18 | 7.7 [<3.0-9.0] | | 18 | 7.9 [5.6-9.9] | |
| Poor (HH4-5) | 10 | 3.6 [<3.0-7.9] | | 10 | 6.2 [<3.0-10.4] | | 9 | 10.1 [6.5-11.6] | |
| **Mod. Fisher (mFS) scale** |  |  | 0.979 |  |  | 0.820 |  |  | 0.448 |
| Good (mFS1-2) | 7 | 4.4 [<3.0-9.2] | | 6 | 8.2 [3.5-11.4] | | 6 | 7.5 [3.3-9.4] | |
| Poor (mFS3-4) | 22 | 5.1 [<3.0-7.7] | | 22 | 7.0 [<3.0-9.5] | | 21 | 9.2 [5.8-11.6] | |
| **Treatment modality** |  |  | 0.392 |  |  | 0.707 |  |  | 0.381 |
| Clipping | 11 | 5.1 [<3.0-6.0] | | 9 | 7.8 [4.2-10.6] | | 9 | 9.3 [6.7-11.7] | |
| Coiling | 18 | 5.0 [<3.0-9.2] | | 19 | 7.6 [<3.0-9.4] | | 18 | 7.9 [4.7-10.0] | |
| **Delayed cerebral ischemia (DCI)** |  |  | 0.718 |  |  | 0.574 |  |  | 0.981 |
| no DCI | 13 | 5.1 [3.3-8.8] | | 13 | 7.8 [<3.0-8.7] | | 12 | 9.2 [5.5-10.3] | |
| DCI | 16 | 3.7 [<3.0-8.3] | | 15 | 7.6 [<3.0-11.6] | | 15 | 7.4 [5.8-11.0] | |
| **DCI-related infarction** |  |  | 0.056 |  |  | 0.454 |  |  | 0.902 |
| DCI only | 10 | 6.5 [<3.0-9.8] | | 10 | 4.8 [<3.0-39.2] | | 10 | 8.3 [5.6-10.3] | |
| DCI-related infarction | 6 | <3.0 [<3.0-<3.0] | | 5 | 9.7 [6.4-10.6] | | 5 | 6.7 [6.5-11.6] | |
| **Outcome** |  |  | 0.887 |  |  | 0.856 |  |  | 0.078 |
| Favorable (GOS-E5-8) | 15 | 5.3 [<3.0-9.1] | | 15 | 7.8 [<3.0-11] | | 15 | 7.4 [3.1-9.5] | |
| Unfavorable (GOS-E1-4) | 10 | 4.9 [<3.0-8.6] | | 9 | 6.4 [4.6-8.0] | | 9 | 10.4 [6.5-11.7] | |

a total number of patients from which samples were available; b anterior communicating artery; c 1.quartile – 3.quartile