**Circulatory Dipeptidyl Peptidase 3 (cDPP3) is a Potential Biomarker for Early Detection of Secondary Brain Injury after Aneurysmal Subarachnoid Hemorrhage**

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**Abstract**

**Introduction**

Delayed cerebral ischemia (DCI) is a common complication after aneurysmal subarachnoid hemorrhage (aSAH) that can culminate in secondary brain damage. Although it remains one of the main preventable causes of aSAH-related morbidity, there is still a lack of prognostic criteria for identification of patients at risk of developing DCI. Because elevated circulatory levels of the enzyme dipeptidyl peptidase 3 (cDPP3) were recently identified as a potential biomarker for outcome prediction in critically ill patients, we evaluated the time-course of changes in cDPP3 levels after aSAH.

**Materials and Methods**

cDPP3 levels were quantified in serum obtained from 96 confirmed aSAH patients during the early (EP: d1-4), critical (CP: d5-8, d9-12, d13-15) and late (LP: d16-21) phase after aSAH onset. Associations between cDPP3 levels and demographic or clinical parameters were evaluated. The relations between cDPP3 levels and DCI, DCI-related infarctions and long-term clinical outcomes were examined by receiver operating characteristics (ROC) curve analysis and multivariate logistic regression.

**Results**

Significantly higher cDPP3 levels during CP (d5-8, d9-12, d13-15) were observed in patients with poor clinical (p<0.001 to p=0.033) or radiological (p=0.012 to p=0.039) status on admission, DCI (p<0.001 to p=0.001), DCI-related infarctions (p=0.002 to p=0.007), and poorer long-term outcome (p=0.007 to p=0.019). ROC curve analysis indicated that higher cDPP3 levels on d5-8 are predictive for a poor clinical outcome (area under the curve=0.677, p=0.007). In multivariate analysis, there was an independent association between cDPP3 levels on d5-8 and development of DCI-related infarctions (p=0.038).

**Conclusion**

Our results provide first evidence that cDPP3 could serve as a promising biomarker for early diagnosis of DCI-related infarctions in poor grade aSAH patients.

**Introduction**

Aneurysmal subarachnoid hemorrhage (aSAH) due to aneurysm rupture in the brain and extravasation of blood into the subarachnoid space accounts for only about 5-7% of all strokes but often occurs at a relatively young age [1]. In addition, aSAH is characterized by very high mortality and morbidity due to its complex pathophysiology with early and delayed mechanisms of brain damage. In the first 24-72 hours after aSAH onset, increased intracranial pressure, decreased cerebral perfusion, and transient or persistent cerebral ischemia result in early brain injury (EBI) [2]. EBI remains the main cause of death in aSAH patients [3,4] and is thought to trigger a number of pathophysiological processes that can culminate in secondary brain damage [5]. The clinical manifestation of these processes is delayed cerebral ischemia (DCI), a complication that typically occurs on day 4-14 after aSAH onset, is often but not always correlated with angiographic vasospasm and may progress to cerebral infarction [5]. DCI results from a complex interplay of systemic and cerebral inflammation, oxidative stress, and endothelial or neuronal apoptosis that is still poorly understood [6]. As a consequence, treatment decisions are still primarily guided by clinical grading, which may be subjective and confounded by the poor initial condition of most DCI candidates, the requirement for sedation and a number of possible complications [7]. In light of these circumstances, objective and reliable biomarkers for the prediction of DCI or its diagnosis in poor grade aSAH patients are urgently needed to guide therapeutic decision making [7].

The enzyme dipeptidyl peptidase 3 (DPP3) was recently identified as a potential biomarker for prediction of organ failure and short-term outcome in certain emergency settings [8–10]. DPP3 is an ubiquitous, predominantly cytosolic zinc metallopeptidase that cleaves dipeptides from the N-terminus of oligopeptides with 3 to 10 amino acid residues. Although the exact physiological role and full range of substrates remain poorly defined, DPP3 has been shown to cleave a number of small bioactive peptides like angiotensins (Ang II, III and IV), enkephalins and endorphins [11–13]. In addition, *in vitro* studies showed that cytosolic DPP3 is released from dying cells [14] and recent development of improved assays to specifically detect and quantify the enzyme in human blood revealed that circulatory DPP3 (cDPP3) levels are elevated in critically ill patients [15]. Subsequent studies in patients suffering from cardiogenic shock, severe burns and / or sepsis indicated that high cDPP3 levels on admission to the intensive care unit (ICU) are associated with disease severity and increased short-term mortality and that dynamic changes of cDPP3 levels during the first 24h are significantly associated with clinical outcome [8–10]. In addition, injection of purified DPP3 produced acute myocardial depression and impaired kidney function in healthy mice, while DPP3 inhibition by the specific antibody Procizumab normalized cardiac and kidney function and reduced oxidative and inflammatory damage in an acute heart failure mouse model [9]. Cardiac and renal dysfunction are common complications during the acute phase after aSAH and associated with increased mortality, DCI and poor clinical outcomes [16–20], suggesting that EBI or subsequent events could also be associated with release of DPP3 into the circulation. In the present pilot study, we determined the temporal profile of cDPP3 levels in a cohort of confirmed aSAH patients and analyzed its relation to DCI, DCI-related infarction and clinical outcome.

**Material and Methods**

**Patient population and demographics**

The present observational study is based on 96 confirmed aSAH patients who were treated at our neurointensive care unit (NICU) from April 2014 to July 2018 and met the following criteria: (1) age > 18 years, (2) not pregnant, (3) no compromise in hepatic or renal function (total bilirubin >51.3 µmol/L, plasma creatinine ≥22.1 µmol/L) at admission, (4) presence of an offending aneurysm, (5) no previous aSAH or recurrent bleeding and (6) no re-treatment for aneurysms.

Demographic data obtained on admission or during in-hospital treatment included age, gender, aneurysm location, pre-existing diabetes, smoking habits, 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), ventilation, ventilation time, and long-term clinical outcome after 12 months according to the extended Glasgow outcome scale (GOS-E). Outcome was assessed by an independent investigator based on a telephone interview, clinical investigation in the outpatient clinic or clinical status compiled from the medical reports. The study protocol was approved by the local ethics committee (EK 062/14) and written consent for study inclusion was obtained from all patients or their authorized representatives. The trial has been registered at ClinicalTrials.gov (NCT02142166) as part of a large scale prospective data collection. Demographic and clinical data were recovered from the comprehensive databases of our local hospital information systems (MEDICO V22.10, Siemens AG, and IntelliSpace Critical Care, and Anesthesia (ICCA), VF01.02, Philips).

**Standard treatment procedure**

Upon admission to the NICU, aneurysms were treated by endovascular coiling or surgical clipping and prophylactic enteral nimodipine was applied as tolerated [21]. Thereafter, patients were continuously monitored for the following signs of deterioration. In awake patients, the occurrence of DCI was diagnosed according to the criteria for clinical deterioration defined by Vergouwen [22], namely a decrease in Glasgow Coma Scale ≥2 for ≥1 h or the appearance of new focal neurological deficits. In sedated or comatose patients, invasive neuromonitoring was performed according to recent consensus recommendations [23] 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 0.3 μl/min with standard perfusion fluid as described previously [24]. DCI in unconscious patients was defined as a metabolic (lactate / pyruvate ratio ≥ 40) or oxygenation (ptiO2 < 10 mmHg) crisis or the appearance of a new computed tomography (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, perfusion CT and / or cerebral angiography were used to verify relevant hypoperfusion and vasoconstriction before endovascular rescue treatment was performed by transluminal balloon-angioplasty or intra-arterial spasmolysis after multidisciplinary discussion as described in more detail elsewhere [24,25]. DCI-related infarctions were defined as cerebral infarctions during ongoing DCI and confirmed by CT. Nutrition was administered according to the guidelines of the European Society for Clinical Nutrition and Metabolism (ESPEN) for ICU patients [26].

**Sample collection and measurement of circulatory DPP3 levels (cDPP3)**

Blood samples from aSAH patients were collected in regular intervals (initially daily, every two days after day 4) as part of a larger scale prospective biomarker study. They were centrifuged immediately after removal and serum was pipetted into suitable polypropylene cryotubes (VWR International GmbH, Darmstadt, Germany). All receptacles were permanently deep-frozen at -80 °C (Ultra-low Temperature Freezer, Panasonic Biomedical, Etten-Leur, Netherlands). Protection from light exposure during transport and storage was ensured and only interrupted during analysis.

Human serum samples were measured using the DPP3 luminescence immunoassay (DPP3-LIA) [15]. Briefly, twenty microliters of samples or calibrators were pipetted into antibody-coated microtiter plates. After adding anti-DPP3 tracer antibody, the microtiter plates were incubated for 3 h at room temperature and 600 rpm (Titramax 101, Heidolph Instruments GmbH & CO. KG). Unbound tracer was removed by washing 4 times (350 μL per well). Remaining chemiluminescence was measured for 1s per well by use of the Centro LB 960 microtiter plate luminometer (Berthold Technologies GmbH & Co. KG). The concentration of DPP3 was determined with a 6-point calibration curve [0 (def. 0.01) - 200 ng/mL]. Calibrators were run in quadruplicate and samples were run in duplicate.

**Time-course analyses**

Because sampling was not performed on a daily basis and based on the typical time-course for development of EBI and DCI, comparisons were made between measurements obtained in pre-defined time intervals during the early (EP: d1-4), critical (CP: d5-8, d9-12, d13-15) and late (LP: d16-21) phase after aSAH onset. The number of patients for which measurements in the different intervals were available are summarized in **Tab. 1-3**. If more than one measurement was available for a given patient in a given interval, all measurements in this interval were used to calculate a mean value for this patient. For analysis, patients were stratified into groups according to gender, age (≤52 years vs. >52 years, based on the median value), 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), 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**

Quantitative, normally distributed data are presented as mean values ± standard deviation (SD), while non-parametric data are summarized by median values [1. quartile – 3. quartile]. In the case of categorical variables, data are given as numbers and percentages. After normality testing via the Shapiro-Wilk test, continuous normally distributed data were compared using T-tests, while the Mann-Whitney U test was used for non-parametric data. Nominal data was tested between groups using the Fisher´s exact test and in case of multinomial data with a Chi2 test. Correlation analysis was carried out using Spearman’s correlation coefficient. Risk factor selection was performed in two steps. We started with univariate logistic regression models of the potential association of age, gender, clinical grade, aneurysm location, modified Fisher grade, and type of treatment modalities (coiling or clipping) on the occurrence of DCI-related infarcts. This step was used as a model building process, based on the decision rule, that factors showing a p-value of less or equal to 0.1 are used in the corresponding multivariate logistic regression model. In this last step, factors were assessed as significant, if the corresponding p-value fell below the 5% margin. We reported our results by p-values, odds ratios and corresponding 95% confidence intervals (Cl). The predictive/diagnostic value of cDPP3 was assessed using a receiver operating characteristics (ROC) and area under the curve (AUC) analysis with their respective standard errors (SE**).** In addition, cDPP3 values between different periods during the critical phase (CP) were compared by a repeated measures analysis of variance with a Greenhouse-Geisser correction for sphericity.

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).

**2. Results**

**2.1. Participants**

Ninety-six patients with a mean age of 54±12 years met the criteria for inclusion in the present study. Their demographic data are summarized in **Table 1**. In total, 48 of the 96 patients (50%) developed DCI (median: day 7 after SAH onset), which progressed to infarction in 10 patients (10/96, 10%). The infarctions were diagnosed between day 1 and 15 (median: day 11) after aSAH onset.

The outcome after 12 months was favorable (GOS-E5-8) in 65 (68%), and unfavorable (GOS-E1-4) in 25 (26%) patients. Poor clinical (p=0.003) and radiological (p<0.001) status on admission, and the occurrence of DCI (p=0.002) were significantly associated with GOS-E1-4. Six patients were lost to follow-up at 12-months. Fifty patients (52.1%) required ventilation for a median of 22 days [12-32]. Twenty-four of the patients with an unfavorable outcome (24/25, 96%) and 21 patients with a favorable outcome (21/65, 32.3%) required ventilation (p<0.0001). Ventilation time did not significantly differ between the groups (p=0.161). Eight patients suffered from stunned myocardium related to induced arterial hypertension during DCI treatment. In these cases, induced hypertension was terminated.

**2.2. Temporal profile of circulatory DPP3 (cDPP3) levels after aSAH**

**Figure 1** shows median cDPP3 levels measured during the early (d1-4), critical (d5-8, d9-12, d13-15) and late (d16-21) phase after aSAH onset. There was little change in cDPP3 levels, with median values that increased slightly from 8.6 (6.6-10.6) ng/ml during the early to a maximum of 10.2 (7.8-14.8) ng/ml during the critical (d9-12) and 11.0 (8.0-14.5) ng/ml during the late phase. These values are within the range previously observed in healthy subjects (mean of 16.6±9.8 ng/ml) [15], suggesting that the initial insult was either not associated with an increase in cDPP3 levels or that patients who survived were capable of normalizing their levels within the first 24 hours.

**2.3. Association with clinical variables**

A comparison of the results between different subgroups of patients is provided in **Fig. 2-4** and **Table 2**-**3**. There was no association between cDPP3 levels during any of the time-points examined and gender, smoking or pre-existing diabetes (**Table 2-3**, **Suppl. Fig. 1**). There was also no difference when comparing patients with regard to aneurysm location, except that median cDPP3 levels during the late phase were higher in patients with aneurysms located in the anterior vs. posterior (12.3 ng/ml *vs.* 9.0 ng/ml, p=0.015) circulation (**Table 2**, **Suppl. Fig. 1**). To evaluate the association between the aneurysm localization and cDPP3 in the late phase in a multifactorial manner, multivariate analysis were conducted for certain factors, Hunt & Hess, DCI, DCI-related infarcts, and aneurysm location. Aneurysm location was not an associated predictor for the elevated cDPP3 in the late phase (p=0.491).

In addition, median cDPP3 levels on day 5-8 were significantly higher when the aneurysm was secured by surgical clipping as compared to endovascular coiling (10.3 ng/ml *vs.* 9.0 ng/ml, p=0.008), which could reflect the more invasive nature of the former treatment (**Table 3**, **Suppl. Fig. 1**)

**2.3.1. Relation between cDPP3 levels and aSAH severity**

**Figure 2A** compares the time course of cDPP3 levels in patients with a good vs. poor clinical status on admission according to the Hunt & Hess (HH) grading scale. Significant differences between groups are indicated by arrows and are shown in more detail in **Fig. 2B** and **Table 2 & 3**. Patients with a poor clinical status on admission (HH4-5) exhibited significantly higher cDPP3 levels throughout the whole observation period (see also **Table 2** & **3**). For example, the median cDPP3 level on day 5-8 was 8.9 (7.2-10.5) ng/ml in patients with a good clinical status compared to 11.6 (9.5-16.8) ng/ml in patients with a poor clinical status (p<0.001). Additionally, there was a highly significant correlation (Spearman r=0.369, p<0.001) between cDPP3 levels on day 5-8 and HH score (**Suppl. Table 1**). Although cDPP3 levels in patients with a poor radiological status on admission (mFS3-4) tended to be higher as well (**Fig. 2C**), the differences were much less evident and only significant during parts of the critical phase (CPd5-8: p=0.039, CPd9-12: p=0.012) (**Fig. 2D**, **Tab. 3**). In addition, there was no correlation between mFS scores and cDPP3 levels during any of the time-points examined (**Suppl. Table 1**).

**2.3.2. Relation between cDPP3 levels, DCI and DCI-related infarctions**

**Fig. 3A** & **B** compare cDPP3 levels in patients with or without DCI. There was little difference between the two groups during the early phase and cDPP3 levels in patients without DCI showed almost no changes over time. In contrast, cDPP3 levels in patients that developed DCI increased to significantly higher levels on day 5-8 (10.5 ng/ml *vs.* 8.8 ng/ml in patients without DCI, p=0.001) and 9-12 (12.7 ng/ml *vs.* 8.9 ng/ml in patients without DCI, p<0.001) after aSAH onset and remained significantly elevated during the remaining critical (12.2 ng/ml *vs.* 8.4 ng/ml in patients without DCI, p<0.001) and the late (12.2 ng/ml *vs.* 8.4 ng/ml in patients without DCI, p=0.010) phase (**Fig. 3A**&**B**). A more pronounced difference was observed when comparing patients with DCI only and patients that developed a DCI-related infarction (**Fig. 3C** & **D**). Thus, while patients with DCI only showed little changes in cDPP3 levels over time, patients with DCI-related infarction exhibited a steep increase during the critical phase, which peaked on day 13-15 after aSAH onset and declined again during the late phase. As a consequence, median cDPP3 levels in patients with DCI-related infarction were significantly higher on day 5-8 (12.4 ng/ml *vs.* 10.3 ng/ml in patients with DCI only, p=0.007), day 9-12 (20.2 ng/ml *vs.* 11.5 ng/ml in patients with DCI only, p=0.002), day 13-15 (25.3 ng/ml *vs.* 11.6 ng/ml in patients with DCI only, p=0.006) and day 16-21 (15.5 ng/ml *vs.* 11.6 ng/ml in patients with DCI only, p=0.019). It follows that the elevation of cDPP3 levels in patients with DCI was mainly due to the subgroup which suffered from DCI-related infarctions.

**2.3.3. Relation between cDPP3 levels and long-term clinical outcome**

**Figure 4A** compares the time course of cDPP3 levels in patients with a favorable vs. unfavorable clinical outcome after 12 months. There was little change in cDPP3 levels determined in patients with favorable clinical outcomes, while the levels in patients with unfavorable outcomes increased to a peak value on day 9-12 after aSAH onset and decreased again towards the late phase. As a consequence, median cDPP3 levels in patients with an unfavorable outcome (GOS-E1-4) were significantly higher on day 5-8 (10.7 ng/ml *vs.* 8.9 ng/ml in patients with GOS-E5-8, p=0.007), day 9-12 (15.1 ng/ml *vs.* 9.7 ng/ml in patients with GOS-E5-8, p=0.008) and day 13-15 (11.9 ng/ml *vs.* 8.9 ng/ml in patients with GOS-E5-8, p=0.019), while there was no difference between the two groups during the early (p=0.512) or late (p=0.054) phase (**Fig. 4B**). In addition, there was a significant correlation of cDPP3 levels during the critical phase and the GOS-E (Spearman r≤-0.316, p≤0.003) score (for details see **Suppl.** **Table 1**), further supporting the assumption that there was an association between circulatory levels of the enzyme and clinical outcome.

**2.3.4. Predictive value of cDPP3 levels**

To further determine if cDPP3 levels measured on day 5-8 were related to outcome, occurrence of DCI or DCI-related infarctions in our group of patients, we performed receiver operating characteristics (ROC) curve analyses. As illustrated in **Fig. 5**, ROC analysis of cDPP3 levels showed a significant area under the curve for DCI (AUC=0.703±0.056, p=0.001, **Fig. 5A**), DCI-related infarctions (AUC=0.789±0.070, p=0.007, **Fig. 5B**) and unfavorable outcomes after 12 months according to the GOS-E score (AUC=0.677±0.058, p=0.007, **Fig. 5C**). In multivariate analysis after adjusting for clinical severity upon admission (Hunt & Hess), there was an independent association between cDPP3 levels on day 5-8 and the development of DCI-related infarctions (p=0.038, **Tab. 4**), suggesting that circulatory levels of the enzyme could be a promising diagnostic aSAH biomarker for identification of patients suffering from delayed brain damage.

**Discussion**

Elevated circulatory levels of the cytosolic enzyme DPP3 were recently identified as a potential biomarker for prediction of organ failure and short-term outcome in critically ill patients [15]. In the present pilot study, we examined for the first time temporal changes in circulatoryDPP3 (cDPP3) levels after aSAH and their relation to clinical outcome, DCI and DCI-related infarction.

**aSAH and cDPP3 levels**

On average, cDPP3 levels during the first three weeks after aSAH showed little changes over time and were well within the range previously observed in healthy subjects [15]. Lacking data on cDPP3 levels on the day of aSAH onset, these findings indicate that aSAH was either not associated with an early elevation of cDPP3 levels or that patients who survived the initial bleed were capable of normalizing their cDPP3 levels within the first 24 hours. The latter would be in line with previous studies in patients suffering from cardiogenic shock, severe burns and / or sepsis, which indicate that cDPP3 could be a marker for short-term (i.e. in-hospital) outcome in these patients. Thus, patients capable of normalizing their cDPP3 levels within 24 to 48 hours after admission to the ICU generally had a lower risk of mortality than patients with cDPP3 levels that remained high [8–10]. However, the more important finding of the present study is that cDPP3 levels during the critical phase were significantly higher in patients with a poor clinical outcome after 12 months, in patients that developed DCI and especially in patients that developed a DCI-related infarction. Based on the subgroup comparison between patients with DCI only and DCI-related infarction, the elevation of cDPP3 levels in patients with DCI was almost exclusively due to the latter subgroup, which would be consistent with the idea that DPP3 is released from dying cells. Given that there was a significant association between DCI occurrence and clinical outcome after 12 months, it seems also likely that the higher cDPP3 levels in patients with an unfavorable outcome were at least in part due to patients with DCI-related infarctions as well.

**cDPP3 as a potential aSAH biomarker**

Because clinical grading in aSAH patients is often confounded by their poor initial condition and the need for sedation, there is an urgent need of objective and reliable diagnostic and prognostic biomarkers [7,27]. Our findings show that patients suffering from DCI-related infarction exhibited a transient increase of cDPP3 levels during the critical phase that became significant on day 5-8 after aSAH onset and peaked on day 13-15. In addition, cDPP3 levels on day 5-8 were predictive for outcome and multivariate analysis showed an independent association between higher cDPP3 levels on day 5-8 and DCI-related infarction in our patients. What makes it difficult to appraise the exact temporal relationship between DCI-related infarcts and the observed changes in cDPP3 levels is the fact that cerebral infarctions can only be diagnosed retrospectively once an infarct is detected radiographically [7,27]. However, taking into account that DCI-related infarctions were diagnosed between day 4 and 15 (median: day 11) after aSAH onset and that patients with DCI only exhibited no clear increase of cDPP3 levels, it seems likely that the observed cDPP3 increase occurred secondary to the infarctions. If this was the case, cDPP3 measurements might have no predictive value for the occurrence of cerebral infarction after aSAH, but they could be a very useful diagnostic biomarker for early detection of DCI-related infarcts in sedated or comatose patients, thereby facilitating timely initiation of counter-measures like endovascular rescue treatments [24].

**Limitations and future directions**

Our study has several important limitations, some of which have already been addressed above. For example, cDPP3 levels were not available on the day of aSAH onset, precluding conclusions regarding the relation between EBI and cDPP3 levels on admission and their predictive value for early mortality. Moreover, cDPP3 levels were not analyzed on a daily basis, so that the exact temporal profile should be elucidated in future studies with more frequent sampling. Another important question that remains to be answered is, if complications like cardiac and renal dysfunction during the acute phase are related to higher cDPP3 levels, in which case the specific antibody Procizumab [9] could be a potential treatment option. We can also not exclude that complications related to induced arterial hypertension with noradrenaline during DCI treatment affected our results, even though noradrenaline administration was immediately terminated in these patients. Likewise, enzyme levels in the present study were only measured in serum samples, but DPP3 activity has also been demonstrated in the cerebrospinal fluid, where aSAH could conceivably be associated with much more pronounced changes. With regard to the data at hand, our findings indicate that cDPP3 could be a diagnostic aSAH biomarker for DCI-related infarctions, but further studies in e.g. animal models will be required to determine if DPP3 released during the infarction itself is responsible for the increase of cDPP3 levels. Also, due to the exploratory nature of the present study, we have made no attempts to correct for the increased error probability due to multiple statistical comparisons. Finally, even though the inclusion of almost 100 aSAH patients provided sufficient power for identification of associations between cDPP3 levels and DCI-related infarction, this is still a relatively small number of patients. As such, our findings and hypotheses will clearly have to be validated by additional, well-designed prospective human studies.

**Conclusion**

Taken together, our results provide first evidence that cDPP3 could be a promising biomarker for the early diagnosis of DCI-related infarctions in poor grade aSAH patients. These findings and their clinical value should be validated and extended by additional well-designed human and animal studies.

**Authors’ contributions**

Conceived, designed and performed the experiments: CT WA CS. First Drafting of the manuscript and illustrations: FN WA. Data acquisition: FN MV MW WA. Analysis and interpretation of data: FN CS WA. Critical review of the manuscript: AH TM MV HC CS GM. The final manuscript was critically revised and approved by all authors.

**Conflict of interest**

There are no conflicts of interest to report.

**Role of the funding source**

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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**

Ang angiotensin

aSAH aneurysmal subarachnoid hemorrhage

cDPP3 circulatory dipeptidyl peptidase 3

CNS central nervous system

DCI delayed cerebral ischemia

DPP3 dipeptidyl peptidase 3

EBI early brain injury

GOS-E Glasgow outcome scale - extended

HH Hunt and Hess grading scale

ICU intensive care unit

IQR interquartile range

mFS modified Fisher scale

NICU neurointensive care unit

ptiO2 brain tissue oxygen level

SD standard deviation

SE standard error

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**Figures**



**Figure 1.** Temporal profile of circulatory DPP3 (cDPP3) levels after aSAH.

Shown are median [1.quartile – 3.quartile] cDPP3 levels determined during the early (EP: d1-4), critical (CP: d5-8, d9-12, d13-15) and late (LP: d16-21) phase after aSAH.Using repeated measures ANOVA, there were no significant differences between the different periods during the critical phase (CP). F(1.939, 112.487)=2.077 , p=0.132.



**Figure 2.** Comparison of cDPP3 levels in predefined subgroups based on clinical and radiological severity on admission.

(**A**) Temporal profile of median [1.quartile – 3.quartile] cDPP3 levels in patients with a good (open circles) or poor (closed circles) clinical state on admission according to the Hunt & Hess grading scale (HH). Significant differences between the groups are indicated by arrows. (**B**) Boxplots illustrating significant differences between the groups in panel A. (**C**) Temporal profile of median [1.quartile – 3.quartile] cDPP3 levels in patients with a good (open circles) or poor (closed circles) radiological state on admission according to the modified Fisher scale (mFS). Significant differences between the groups are indicated by arrows. (**D**) Boxplots illustrating significant differences and trends between the groups shown in panel C. EP, early phase; CP, critical phase; LP, late phase.

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**Figure 3.** Comparison of cDPP3 levels in patients with or without DCI and DCI-related infarctions.

(**A**) Temporal profile of median [1.quartile – 3.quartile] cDPP3 levels in patients without (open circles) or with (closed circles) DCI. Significant differences between the groups are indicated by arrows. (**B**) Boxplots illustrating significant differences between the groups in panel A. (**C**) Temporal profile of median [1.quartile – 3.quartile] cDPP3 levels in patients with DCI only (open circles) or DCI-related infarction (closed circles). Significant differences between the groups are indicated by arrows. (**D**) Boxplots illustrating significant differences between the groups shown in panel C. EP, early phase; CP, critical phase; LP, late phase.



**Figure 4.** Comparison of cDPP3 levels in patients stratified according to clinical outcome.

(**A**) Temporal profile of median [1.quartile – 3.quartile] cDPP3 levels in patients with a favorable (open circles) or unfavorable (closed circles) clinical outcome after 12 months according to the extended Glasgow outcome scale (GOS-E). Arrows indicate significant differences between the groups. (**B**) Boxplots illustrating significant differences between the groups in panel A. EP, early phase; CP, critical phase; LP, late phase.

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**Figure 5.** Receiver operating characteristic (ROC) curve analysis for cDPP3 levels on day 5-8.

Shown are ROC curve analyses for cDPP3 levels on day 5-8 with regard to (**A**) DCI, (**B**) DCI-related infarctions and (**C**) unfavorable clinical outcomes after 12 months according to the extended Glasgow outcome scale (GOS-E1-4). AUC, area under the curve.

**Tables**

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

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Patient characteristic** | **n (%)****Median****[1q-3q]** | **missing data** | **Favorable outcome (GOS-E5-8)****Median****[1q-3q]** | **Unfavorable outcome (GOS-E1-4)** **Median****[1q-3q]** | **p-value** |
| **All patients** | 96 (100%) | 6 (6%) | 65 (68%) | 25 (26%) |  |
| **Gender** |  |  |  |  | 0.456 |
|  Female | 71 (74%) | 3 (50%) | 50 (77%) | 18 (72%) |  |
|  Male | 25 (26%) | 3 (50%) | 15 (23%) | 7 (28%) |  |
| **Age** |  |  |  |  | 0.513 |
|  ≤52 years | 48 (50%) | 2 (33%) | 34 (52%) | 12 (48%) |  |
|  >52 years | 48 (50%) | 4 (67%) | 31 (48%) | 13 (52%) |  |
| **Arterial hypertension** |  |  |  |  | 0.215 |
|  No | 61(63.5%) | 5 (83.3%) | 43 (66.2%) | 13 (52%) |  |
|  Yes | 35 (36.5%) | 1 (16.7%) | 22 (33.8%) | 12 (48%) |  |
| **Smoker** |  |  |  |  | 0.304 |
|  No | 73 (76%) | 3 (50%) | 52 (80%) | 18 (72%) |  |
|  Yes | 22 (23%) | 2 (33%) | 13 (20%) | 7 (28%) |  |
|  n/a | 1 (1%) | 1 (17%) | 0 (0%) | 0 (0%) |  |
| **Diabetes** |  |  |  |  | 0.083 |
|  No | 90 (94%) | 5 (83%) | 63 (97%) | 22 (88%) |  |
|  Yes | 6 (6%) | 1 (17%) | 2 (3%) | 3 (12%) |  |
| **Aneurysm location** |  |  |  |  | 0.328 |
|  Anterior circulation  | 68 (71%) | 3 (50%) | 44 (68%) | 21 (84%) |  |
|  Posterior circulation  | 27 (28%) | 3 (50%) | 21 (32%) | 3 (12%) |  |
|  Both | 1 (1%) | 0 (0%) | 0 (0%) | 1 (4%) |  |
| **Treatment modality** |  |  |  |  | 0.078 |
|  Clipping | 39 (41%) | 2 (33%) | 22 (34%) | 15 (60%) |  |
|  Coiling | 55 (57%) | 4 (67%) | 41 (63%) | 10 (40%) |  |
|  Both | 2 (2%) | 0 (0%) | 2 (3%) | 0 (0%) |  |
| **Hunt and Hess grade** |  |  |  |  | **0.003** |
|  Good grade (HH1-3) | 75 (78%) | 6 (100%) | 57 (88%) | 12 (48%) |  |
|  Poor grade (HH4-5) | 21 (22%) | 0 (0%) | 8 (12%) | 13 (52%) |  |
| **Modified fisher scale** |  |  |  |  | **<0.001** |
|  Good grade (mFS1-2) | 41 (43%) | 1 (17%) | 37 (57%) | 3 (12%) |  |
|  Poor grade (mFS3-4) | 55 (57%) | 5 (83%) | 28 (43%) | 22 (88%) |  |
| **DCI** |  |  |  |  | **0.002** |
|  no DCI | 48 (50%) | 2 (33%) | 40 (61%) | 6 (24%) |  |
|  DCI | 48 (50%) | 4 (67%) | 25 (38%) | 19 (76%) |  |
| **DCI-related infarction** |  |  |  |  | 0.162 |
|  DCI only | 38 (79%) | 3 (50%) | 22 (34%) | 13 (52%) |  |
|  DCI-related infarction | 10 (21%) | 1 (17%) | 3 (5%) | 6 (24%) |  |
| **Ventilation** |  |  |  |  | **<0.0001** |
|  No | 46 (47.9%) | 1 (16.7%) | 44 (67.7%) | 1 (4%) |  |
|  Yes | 50 (52.1%) | 5 (83.3%) | 21 (32.3%) | 24 (96%) |  |
| **Ventilation time, days** | 22 [12-32] | - | 20 [6-26] | 25[ 14-37] | 0.161 |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

 Abbreviations: DCI, delayed cerebral ischemia; GOS-E, Glasgow outcome scale - extended; HH, Hunt and Hess grading scale; mFS, modified Fischer scale. 1q, first quartile; 3q, third quartile.

**Table 2.** Subgroup comparison of circulatory DPP3 levels during the early and late phase after aSAH.

|  |  |  |
| --- | --- | --- |
|  | **Early phase (EPd1-4)** | **Late phase (LPd16-21)** |
|  | **n** | **Median****(1.q-3.q)** | **p-value** | **n** | **Median****(1.q-3.q)** | **p-value** |
| **All patientsa** | 83 | 8.6 (6.6-10.6) | 68 | 11.0 (8.0-14.5) |
| **Gender** |  |  | 0.569 |  |  | 0.304 |
| Male | 20 | 7.9 (6.4-9.7) | 18 | 12.5 (8.5-17.1) |
| Female | 63 | 8.8 (6.7-11.1) | 50 | 10.7 (7.9-14.0) |
| **Age** |  |  | 0.258 |  |  | 0.480 |
| ≤ 52 years | 43 | 7.7 (6.4-10.4) | 32 | 10.5 (7.9-13.5) |
| > 52 years | 40 | 9.1 (6.9-12.1) | 36 | 11.6 (8.5-15.7) |
| **Arterial hypertension** |  |  | 0.235 |  |  | 0.717 |
| No | 50 | 8.8 (6.7-10.6) | 45 | 11.1 (8.2-13.9) |
| Yes | 33 | 8.6 (6.9-12.1) | 23 | 11.6 (7.1-18.0) |
| **Smoking** |  |  | 0.373 |  |  | 0.622 |
| No | 65 | 8.6 (6.6-10.5) | 53 | 10.7 (7.8-15.5) |
| Yes | 17 | 9.2 (6.9-12.9) | 14 | 11.7 (9.6-12.5) |
| **Diabetes** |  |  | 0.559 |  |  | 0.634 |
| No | 78 | 8.6 (6.7-10.6) | 62 | 11.0 (8.1-14.1) |
| Yes | 5 | 6.9 (6.5-9.2) | 6 | 9.9 (6.7-16.7) |
| **Aneurysm location** |  |  | 0.709 |  |  | **0.015** |
| Anterior circulation | 57 | 8.6 (6.5-10.5) | 46 | 12.3 (8.8-16.0) |
| Posterior circulation | 25 | 8.7 (6.9-12.6) | 21 | 9.0 (7.2-11.4) |
| **Treatment** |  |  | 0.731 |  |  | 0.457 |
| Clipping | 34 | 8.2 (6.8-10.5) | 26 | 11.4 (8.8-13.6) |
| Coiling | 47 | 8.7 (6.7-10.6) | 41 | 10.6 (7.5-15.7) |
| **Hunt & Hess grade** |  |  | **0.036** |  |  | **0.009** |
| Good (HH1-3) | 64 | 7.5 (6.4-10.4) | 52 | 10.3 (7.5-14.0) |
| Poor (HH4-5) | 19 | 10.0 (8.3-12.1) | 16 | 13.1 (11.1-19.7) |
| **Mod. Fisher scale** |  |  | 0.081 |  |  | 0.620 |
| Good (mFS1-2) | 35 | 7.4 (6.2-10.3) | 25 | 9.6 (8.0-15.6) |
| Poor (mFS3-4) | 48 | 9.2 (6.9-11.8) | 43 | 11.4 (8.3-14.0) |
| **DCI** |  |  | 0.379 |  |  | **0.010** |
| no DCI | 42 | 7.6 (6.2-10.6) | 25 | 8.4 (6.8-11.8) |
| DCI | 41 | 9.0 (6.9-10.6) | 43 | 12.2 (9.3-15.7) |
| **DCI-rel infarction** |  |  | 0.473 |  |  | **0.019** |
| DCI only | 32 | 8.6 (6.8-10.8) | 34 | 11.6 (8.6-14.1) |
| DCI-rel. infarction | 9 | 9.5 (8.0-10.0) | 9 | 15.5 (12.2-20.1) |
| **Outcome** |  |  | 0.512 |  |  | 0.054 |
| Favorable (GOS-E5-8) | 56 | 8.2 (6.5-10.5) | 43 | 9.5 (7.7-14.1) |
| Unfavorable(GOS-E1-4) | 23 | 8.8 (6.8-11.0) | 20 | 12.3 (10.0-16.2) |

Abbreviations: DCI, delayed cerebral ischemia; GOS-E, Glasgow outcome scale - extended; HH, Hunt and Hess grading scale; mFS, modified Fischer scale; 1q, first quartile; 3q, third quartile. a total number of patients from which samples were available

**Table 3.** Subgroup comparison of circulatory DPP3 levels during the critical phase after aSAH.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Critical phase (CPd5-8)** | **Critical phase (CPd9-12)** | **Critical phase (CPd13-15)** |
|  | **n** | **Median****(1.q-3.q)** | **p-value** | **n** | **Median****(1.q-3.q)** | **p-value** | **n** | **Median****(1.q-3.q)** | **p-value** |
| **All patientsa** | 89 | 9.2 (7.4-11.4) | 92 | 10.2 (7.8-14.8) | 66 | 9.2 (7.4-13.0) |
| **Gender** |  |  | 0.670 |  |  | 0.527 |  |  | 0.151 |
| Male | 23 | 8.9 (7.9-10.6) | 25 | 10.7 (8.7-13.2) | 18 | 10.4 (8.8-12.6) |
| Female | 66 | 9.3 (7.3-11.9) | 67 | 9.7 (7.6-15.3) | 49 | 8.9 (6.9-13.3) |
| **Age** |  |  | 0.498 |  |  | 0.431 |  |  | 0.784 |
| ≤52 years | 45 | 9.1 (7.3-11.0) | 48 | 9.7 (7.9-13.4) | 39 | 9.0 (7.3-12.4) |
| >52 years | 44 | 9.2 (7.9-12.3) | 44 | 10.6 (7.7-15.5) | 27 | 9.4 (7.8-14.3) |
| **Arterial hypertension** |  |  | 0.351 |  |  | 0.187 |  |  | 0.057 |
| No | 54 | 9.0 (6.9-10.9) | 59 | 8.9 (7.4-12.4) | 46 | 8.8 (6.9-12.0) |
| Yes | 35 | 9.1 (8.2-13.7) | 34 | 10.8 (9.3-16.6) | 20 | 9.5 (8.3-21.4) |
| **Smoking** |  |  | 0.965 |  |  | 0.684 |  |  | 0.188 |
| No | 68 | 9.1 (7.3-11.1) | 69 | 10.2 (7.7-15.1) | 46 | 8.9 (6.9-12.2) |
| Yes | 20 | 9.2 (7.9-12.2) | 22 | 10.3 (8.3-14.6) | 19 | 10.1 (8.5-14.0) |
| **Diabetes** |  |  | 0.533 |  |  | 0.994 |  |  | 0.591 |
| No | 84 | 9.2 (7.4-11.5) | 86 | 10.2 (7.8-15.3) | 62 | 9.2 (7.3-12.5) |
| Yes | 5 | 9.1 (6.8-9.2) | 6 | 11.7 (9.6-12.6) | 4 | 11.7 (8.4-14.9) |
| **Aneurysm location** |  |  | 0.125 |  |  | 0.280 |  |  | 0.082 |
| Anterior circulation | 62 | 9.4 (7.7-13.5) | 67 | 10.5 (8.0-16.0) | 48 | 9.7 (8.4-13.2) |
| Posterior circulation | 26 | 9.1 (7.1-10.1) | 24 | 8.9 (7.6-11.8) | 17 | 8.3 (5.8-10.4) |
| **Treatment** |  |  | **0.008** |  |  | 0.072 |  |  | 0.134 |
| Clipping | 35 | 10.3 (8.8-13.7) | 38 | 11.7 (8.8-16.6) | 27 | 11.1 (8.4-14.8) |
| Coiling | 52 | 9.0 (7.1-10.3) | 52 | 9.5 (7.6-12.9) | 37 | 8.9 (6.9-12.2) |
| **Hunt & Hess grade** |  |  | **<0.001** |  |  | **0.016** |  |  | **0.033** |
| Good (HH1-3) | 69 | 8.9 (7.2-10.5) | 73 | 9.6 (7.7-13.2) | 52 | 8.8 (7.0-12.2) |
| Poor (HH4-5) | 20 | 11.6 (9.5-16.8) | 19 | 12.6 (9.9-19.0) | 14 | 12.0 (8.9-15.3) |
| **Mod. Fisher scale** |  |  | **0.039** |  |  | **0.012** |  |  | 0.192 |
| Good (mFS1-2) | 38 | 9.0 (6.8-10.2) | 41 | 9.0 (7.8-11.5) | 27 | 8.8 (7.0-11.1) |
| Poor (mFS3-4) | 51 | 9.3 (8.0-13.9) | 51 | 11.5 (7.9-18.2) | 39 | 9.7 (8.1-14.8) |
| **DCI** |  |  | **0.001** |  |  | **<0.001** |  |  | **<0.001** |
| no DCI | 46 | 8.8 (6.9-9.5) | 47 | 8.9 (6.9-11.2) | 34 | 8.4 (6.8-9.4) |
| DCI | 43 | 10.5 (8.4-14.0) | 46 | 12.7 (9.1-18.3) | 32 | 12.2 (9.3-15.9) |
| **DCI-rel. infarction** |  |  | **0.007** |  |  | **0.002** |  |  | **0.006** |
| DCI only | 35 | 10.3 (8.0-13.7) | 37 | 11.5 (8.7-16.5) | 28 | 11.6 (8.8-14.9) |
| DCI-rel. infarction | 8 | 12.4 (10.2-19.8) | 9 | 20.2 (13.2-20.5) | 4 | 25.3 (21.6-26.1) |
| **Outcome** |  |  | **0.007** |  |  | **0.008** |  |  | **0.019** |
| Favorable (GOS-E5-8) | 61 | 8.9 (7.0-10.3) | 62 | 9.7 (7.5-12.8) | 45 | 8.9 (7.0-11.1) |
| Unfavorable(GOS-E1-4) | 24 | 10.7 (8.5-14.5) | 25 | 15.1 (9.5-20.5) | 20 | 11.9 (8.4-15.9) |

Abbreviations: DCI, delayed cerebral ischemia; GOS-E, Glasgow outcome scale - extended; HH, Hunt and Hess grading scale; mFS, modified Fischer scale; 1q, first quartile; 3q, third quartile. a total number of patients from which samples were available

**Table 4.** Results from univariate and multivariate logistic regression regarding the occurrence of DCI-related infarcts.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **n (%) or Median (1.q-3.q)** | **Univariate logistic regression** | **Multivariate logistic regression** |
|  | **No infarcts** | **DCI-rel. inf.** | **OR** | **95% CI** | **p-value** | **OR** | **95% CI** | **p-value** |
| Total, n=96 | 86 | 10 |  |  |  |  |  |  |
| **Gender**Female : Male | 64 : 22(74% : 26%) | 7 : 3(70% : 30%) | 1.247 | 0.296-5.244 | 0.764 |  |  |  |
| **Age**≤52 yrs : >52 yrs | 45 : 41(52% : 48%) | 3 : 7(30% : 70%) | 0.390 | 0.095-1.611 | 0.193 |  |  |  |
| **Art. hypertension**Yes : No | 30 : 56(35% : 65.%) | 5 : 5(50% : 50%) | 0.536 | 0.144-1.998 | 0.353 |  |  |  |
| **Smoking**Yes : No | 21 : 64 a(25% : 75%) | 1 : 9(10% : 90%) | 2.908 | 0.348-24.315 | 0.325 |  |  |  |
| **Diabetes**Yes : No | 6 : 80(7% : 93%) | 0 : 10(0% : 100%) | - | - | 0.999 |  |  |  |
| **Aneurysm location**Anterior : Posterior | 60 : 25 b(71% : 29%) | 8 : 2(80% : 20%) | 0.567 | 0.113-2.857 | 0.492 |  |  |  |
| **Treatment**Clipping : Coiling | 32 : 52 c(38% : 62%) | 7 : 3(70% : 30%) | 0.259 | 0.062-1.073 | 0.062 |  |  |  |
| **Hunt & Hess grade**HH1-3 : HH4-5 | 70 : 16(81% : 19%) | 5 : 5(50% : 50%) | 4.375 | 1.130-16.933 | **0.033** | 4.732 | 0.934-23.973 | 0.060 |
| **Mod. Fisher scale**mFS1-2 : mFS3-4 | 39 : 47(45% : 55%) | 2 : 8(20% : 80%) | 0.301 | 0.060-1.502 | 0.143 |  |  |  |
| **cDPP3 [ng/ml]**d5-8 (n=89) | 9.02(7.3-11.0) | 12.4(10.2-19.8) | 1.174 | 1.045-1.320 | **0.007** | 1.145 | 1.007-1.302 | **0.038** |

Abbreviations: Art. Hypertension, arterial hypertension; CI, confidence intervals; DCI, delayed cerebral ischemia; HH, Hunt and Hess grading scale; mFS, modified Fischer scale; OR, odds ratio.

a one patient for which information was unavailable was excluded

b one patient with aneurysms in anterior and posterior circulation was excluded

c two patients treated by clipping and coiling were excluded



**Suppl. Figure 1.** cDPP3 and subgroup comparisons.

Shown are subgroup comparisons for patients stratified according to (**A**) gender, (**B**) age, (**C**) smoking habits (**D**) pre-existing diabetes, (**E**) aneurysm location and (**F**) treatment modality. Significant differences between groups are indicated by arrows (for details see Tab. 2 & 3).

**Suppl. Table 1.** Results from correlation analysis.

|  |  |  |
| --- | --- | --- |
|  | **Spearman r** | **p-value** |
| **GOS-E vs cDPP3** |  |  |
| Day 1-4 | -0.117 | 0.303 |
| Day 5-8 | -0.316 | **0.003** |
| Day 9-12 | -0.335 | **0.002** |
| Day 13-15 | -0.396 | **0.001** |
| Day 16-21 | -0.388 | **0.002** |
| **HH vs cDPP3** |  |  |
| Day 1-4 | 0.175 | 0.114 |
| Day 5-8 | 0.369 | **<0.001** |
| Day 9-12 | 0.192 | 0.067 |
| Day 13-15 | 0.268 | **0.029** |
| Day 16-21 | 0.285 | **0.019** |
| **mFS vs cDPP3** |  |  |
| Day 1-4 | 0.179 | 0.106 |
| Day 5-8 | 0.165 | 0.122 |
| Day 9-12 | 0.194 | 0.064 |
| Day 13-15 | 0.200 | 0.107 |
| Day 16-21 | 0.021 | 0.863 |

Abbreviations: cDPP3, circulatory DPP3; GOS-E, Glasgow outcome scale - extended; HH, Hunt and Hess grading scale; mFS, modified Fischer scale.