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# Association of early inflammatory parameters after subarachnoid hemorrhage with functional outcome: A prospective cohort study

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ABSTRACT

*Objective:* Early brain injury after aneurysmal subarachnoid hemorrhage (aSAH) comprises a pronounced neuroinflammatory reaction. Nevertheless, its relevance for functional outcome and its role as outcome predictor remains uncertain. We evaluated the relationship of various early inflammatory parameters regarding functional outcome according to the modified Rankin Scale score (mRS) at discharge (primary objective) and six months after aSAH.
*Patients:* A total of 81 patients (63% female) with a mean age of 53.8 ± 13.2 years were included.
*Methods:* At admission clinical data and various inflammatory parameters in serum and – wherever applicable – cerebrospinal fluid (CSF) of patients after aSAH were assessed. Outcome was evaluated according to dichotomized mRS at discharge and six months after aSAH (unfavorable outcome: mRS 3–6). Univariate and thereafter multivariate logistic regression analyses were performed using SAS 9.2.
*Results:* Elevated levels of interleukin 6 (IL-6) and leukemia inhibitory factor (LIF) in serum and CSF were related to unfavorable outcome at discharge ($p < 0.05$; univariate analyses). IL-6 remains the only parameter relevant for outcome applying a multivariate model including the relevant baseline characteristics. Six months after aSAH no significant correlation was found regarding the outcome, most likely due to the high drop-out rate (27%). A pronounced rise of LIF serum and CSF levels after aSAH was observed.
*Conclusion:* Higher early IL-6 serum levels after aSAH are associated with poor outcome at discharge. In addition, involvement of LIF in the early inflammatory reaction after aSAH has been demonstrated.


## 1. Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) remains a severe condition with a case fatality rate of >50% [1]. Half of the patients are younger than 55 years [2]. The outcome after aSAH remains detrimental with respect to individual fate, patient's family disposition and socio-economic burden [3,4]. Currently research efforts focus on the initial damage directly caused by aneurysm rupture [5,6].

At an early stage neuroinflammation is a common reaction after any brain injury. After aSAH a multitude of inflammatory reactions can be observed. For some of the inflammatory parameters prognostic values have been reported [7–10]. However, the inflammatory system is complex and frequently responds unspecifically. Therefore, results are difficult to interpret. Even the role of interleukin 6 (IL-6), one the most extensively investigated cytokines, seems to be ambivalent (neuroprotective or proinflammatory) and time-dependent [11,12]. After aSAH, a rise of IL-6 levels has been shown for samples from various compartments, such as serum, cerebral spinal fluid (CSF) as well as cerebral extracellular fluid generated via cerebral microdialysis [13–16]. There is evidence that – similarly to ischemic stroke [17] – higher IL-6 levels may be associated with poor outcome even if measured systemically [16]. Yet there is a controversy whether IL-6 reflects a detrimental or rather a protective reaction [13]. Additionally, the rise of IL-6 is a very unspecific reaction, especially if assessed systemically. As neuroinflammation is a highly cross-linked process, analysis of a single parameter with regard to outcome does not reflect the actual pathophysiology. Therefore, a wide range of parameters needs to

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be analyzed to identify determining factors, or a combination of factors, which may allow an assessment of prognosis. Thus, we aim to analyze the prognostic value of a spectrum of inflammatory biomarkers including two members of the IL-6-type family (IL-6 and leukemia inhibitory factor—LIF) at a very early stage of disease before treatment. Further we will correlate the aforementioned biomarkers with functional outcome. LIF has not yet been investigated in the context of aSAH. It has been related to self-renewal and neurogenesis related to injury-induced inflammation [18,19]. However it also exerts neuroendocrinological properties [20]. Due to its linkage to the IL-6-superfamily, LIF may also be involved in the very early triggered, complex neuroimmunological reaction after SAH. Therefore, the primary objective of our study is defined as influence of early inflammatory biomarkers (including IL-6 and LIF) on poor outcome at discharge. The secondary objective is the correlation with outcome six months after aSAH.

## 2. Methods

The prospective cohort study was approved by the local ethics committee (Ethikkommission an der Medizinischen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn, Germany; EK 199/08), written informed consent according to the Declaration of Helsinki was obtained from patients or legal representatives. Within a 21-month period (02/2009–11/2010) 109 consecutive patients with aneurysmal subarachnoid hemorrhage were screened for eligibility. Patients were not eligible if they were younger than 18 years, enrolled in other clinical trials, admitted more than 12 h after onset or if informed consent could not be obtained. Thus, nine patients had to be excluded due to delayed admission and four patients due to participation in another clinical trial, 15 patients declined participation, resulting in 81 study participants. We archived demographic, clinical, laboratory and radiological data within an anonymized file history. Diagnosis of aSAH consisted of typical clinical course (i.e. sudden severe headache, lowered level of consciousness, meningism), CT-scan pattern and confirmation by cerebral angiography. At admission, demographic data, severity scores of aSAH (WFNS, Hunt/Hess score, Fisher grade) and time-span to clinical ictus were assessed and the occurrence of systemic inflammatory reaction syndrome (SIRS) was determined by means of standardized criteria [21]. Treatment of patients was carried out according to standardized guidelines [22]. Serum parameters were assessed directly after admission (in line with routine diagnostic). Cerebral spinal fluid (CSF) was analyzed at day 1 after admission. CSF only was examined in patients with clinical necessity for external ventricular drainage ($n = 46$).

Occurrence of delayed ischemic neurological deficit (DIND) was assessed during the entire clinical course. DIND was defined as secondarily neurological impairment (increase on modified NIHSS $\geq$2 points) with improvement after induced hypertension or verification of cerebral vasospasm by angiography (in case of lacking clinical improvement or persistent impairment of consciousness or sedation).

Samples were analyzed at the Department of Clinical Chemistry and Clinical Pharmacology, University of Bonn, either in line with routine diagnostics (leucocyte count [G/l], determination of C-reactive protein [mg/l], interleukin-6 in serum and CSF samples [pg/ml]). E-selectin (a cell adhesion molecule expressed on endothelial cells, also known as endothelial-leukocyte adhesion molecule 1 (ELAM-1) [ng/ml]), matrix metallopeptidase 9 (MMP-9 [ng/ml]), intercellular adhesion molecule 1 (ICAM-1 [ng/ml]) and leukemia inhibitory factor (LIF [pg/ml]) in serum and CSF samples were assessed by means of enzyme-linked immunofluorescence assays (ELISA; all assays purchased by IBL International GmbH, Hamburg, Germany). Samples were centrifuged for 10 min at 2000 $\times$ *g*.

At discharge and at six months follow-up, clinical outcome was assessed by an independent investigator applying the modified Rankin Scale score (mRS). The primary endpoint of this study was defined as identifying a relationship between initially assessed inflammatory parameters and outcome at discharge and – as secondary endpoint – six months after aSAH. Prior to the study, a power analysis had been performed using data for early ICAM-1 serum levels after aSAH related to outcome [23]. Sample size was calculated with $n = 64$ ($n = 32$ for each group) according to an effect size based on differences between the means of early ICAM-1 levels (power: 0.95, effect size: 1.36; alpha: 0.05). Due to potential lack of data a total sample size of at least 120% of the calculated value ($n = 77$) was pursued.

For statistical analysis, outcome according to the modified Rankin Scale score (mRS: 0–2 = favorable, mRS: 3–6 = unfavorable) and clinical severity score according to WFNS (mild: 1–3; severe: 4, 5) as well as blood load according to Fisher scale (mild: 1, 2; severe: 3, 4) were dichotomized. Not normally distributed serum and CSF parameters were logarithmized. Statistical analyses were performed in two steps: At first a univariate analysis was performed using the logistic regression model (binary logit model optimized with Fisher's scoring). In a second step all variables with a $p$-value < 0.1 were included in the stepwise regression model (forward, backward and stepwise logistic regression model) for adjusted analysis. Multicollinearity and interactions of the identified variables were taken into account but did not show relevance. Additionally, logarithmized inflammatory parameters were compared via independent $t$-test. To detect a feasible cut-off value for unfavorable outcome receiver-operating characteristic (ROC) curves were processed. The cut-off value was defined as the value that maximizes the sum of sensitivity and specificity. Additionally, the likelihood ratio (LR) is given. A $p$-value less than 0.05 was regarded as statistically significant and a $p$-level of less than 0.1 was regarded as trend. Statistical analyses were performed using IBM SPSS 21.0 software and SAS 9.2 for regression modeling. Graphs were acquired using GraphPad Prism 6. Six months after aSAH 27% of the cohort was lost to follow-up.

## 3. Results

### 3.1. Baseline characteristics and outcome

Within a 21-month period 109 patients with aSAH were assessed for eligibility. Nine patients had to be excluded due to delayed admission (>12 h after aSAH), four patients due to participation in another study and 15 patients declined participation. The median patient age was 53.8 $\pm$ 13.2 (range: 29–87) years with 63% ($n = 51$) females. Baseline characteristics of the patients enrolled are shown in Table 1. Patients were dichotomized according to mRS with favorable outcome (largely independent lifestyle) defined as mRS from 0 to 2 and unfavorable outcome from 3 to 6. During inpatient stay, 13 (16%) patients died. After discharge, no additional case of death was assessed. Yet, a total of 22 (28%) patients were lost to follow-up six months after aSAH (see Fig. 1). DIND was defined as secondarily neurological impairment with improvement due to induced hypertension, or in case of refractory neurological impairment, verification of delayed vasospasm via angiography. This occurred in 22.2% ($n = 18$) of the cases.

Correlations of baseline characteristics with outcome at discharge are shown in Table 2. Favorable outcome was observed in 48% ($n = 39$) (see Fig. 1). Prognostic value of the classic clinical severity scores WFNS ($p < 0.001$) and Fisher score ($p = 0.005$) could be confirmed. The factor of patient's age ($p = 0.066$) and occurrence of