Early Diagnosis of Delayed Cerebral Ischemia: Possible Relevance for Inflammatory Biomarkers in Routine Clinical Practice?

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BACKGROUND: **Delayed cerebral ischemia (DCI) after subarachnoid hemorrhage (SAH) is one of the main causes of neurologic deterioration. However, it frequently evades timely detection. Early identification and effective reversal may improve the clinical outcome. In this prospective study, we evaluate several serum inflammatory markers after aneurysmal SAH with regard to the occurrence of DCI.**

METHODS: **On days 1, 4, 7, 10, and 14 after SAH, leucocyte count, C-reactive protein, interleukin 6, E-selectin, matrix metallopeptidase 9, intercellular adhesion molecule 1, and leukemia inhibitory factor were assessed in patients' serum samples. Using a Cox regression model (SPSS 21.0), associations of baseline parameters, maximum and delta (maximum minus baseline) values with occurrence of DCI were evaluated.**

RESULTS: **Considering the assessed parameters, leucocyte count (high baseline and delta values) matches most closely with occurrence of DCI. Although baseline levels of C-reactive protein are also associated with occurrence of DCI, neither maximum (only on a borderline level) nor delta levels do so.**

CONCLUSIONS: **Our data analysis identified leucocyte count as the parameter most likely associated with occurrence of DCI. However, because of its lack of specificity leucocyte count, it cannot be used as a biomarker. As hypothesized earlier, the results indicate a possible involvement of the inflammatory reaction after aneurysmal SAH in the pathomechanism of DCI.**

BACKGROUND

Aneurysmal subarachnoid hemorrhage (aSAH) accounts for 3%–5% of all strokes[1] and long-term consequences of this disease are severe. Mostly younger people are affected by aSAH and only around 30% of the survivors regain their ability to work. Especially if psychological sequelae or more subtle parameters of functional outcome such as memory skills and executive function are assessed, limitations in their performance and quality of life are detected in most patients after aSAH.[2]

For decades, cerebral vasospasm (CVS) has been considered to be the major source of a poor outcome. The pathophysiologic approach was characterized by the mechanical narrowing of the vessel, resulting in ischemia and poor outcome. Recently, the focus of perception has switched from the macroscopically visible vasospasm to a multifactorial pathophysiology possibly consisting of microvasospasm, microthrombosis, inflammatory alterations, cortical spreading depression, and various sequelae of early brain injury.[3] This paradigm shift is based on the failure of clazosentan, an endothelin 1 antagonist, in improving functional outcome after aSAH despite its success in reducing CVS verified by angiography as well as the fact that neurologic deterioration and angiographic CVS do not always occur simultaneously.[4] The uncertainty concerning the pathophysiology of this delayed neurologic

Key words
- Delayed cerebral ischemia
- Inflammation
- Subarachnoid hemorrhage
- Vasospasm

Abbreviations and Acronyms
aSAH: Aneurysmal subarachnoid hemorrhage
CRP: C-reactive protein
CVS: Cerebral vasospasm
DCI: Delayed cerebral ischemia
ICAM-1: Intercellular adhesion molecule 1
IL-6: Interleukin 6
LIF: Leukemia inhibitory factor
WFNS: World Federation of Neurological Societies

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deterioration is reflected by confusing terminologies used to describe similar phenomena, such as delayed cerebral ischemia (DCI), delayed ischemic neurologic deficit, symptomatic vasospasm, or secondary cerebral ischemia. The development of delayed neurologic deterioration after aSAH is often associated with a proinflammatory response, which is initiated early after aSAH in early brain injury.[5-7] Various biomarkers have been proposed as predictors for DCI, but none has proved its usefulness for clinical purposes. This situation may be partly a result of the differences in study design, especially in the definition of the complication formerly known as vasospasm dependent on a surrogate parameter, such as angiographically confirmed vasospasm, neurologic deterioration, increase of flow velocity in Doppler ultrasonography, and so on. Here, we evaluate multiple inflammatory parameters in patients with aSAH regarding the occurrence of DCI, defined as secondary neurologic worsening based on increase in modified National Institutes of Health Stroke Scale $\geq$2 points and exclusion of other causes, such as infection, seizure, and metabolic or electrolyte disturbances with improvement after induced hypertension or occurrence of new cerebral ischemia or perfusion deficit confirmed by CCT or magnetic resonance imaging (in case of consciousness or sedation or persistent neurologic deficit).

METHODS

Patient Enrollment

This prospective 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 was obtained from all patients or their legal representatives. One hundred and nine consecutive patients with a proved aSAH were screened for eligibility within a period of 21 months. Patients were not eligible if they were younger than 18 years, enrolled in other clinical trials, admitted more than 12 hours after onset, or if informed consent could not be obtained. In total, 89 patients were included. At admission demographic data, severity score of aSAH according to World Federation of Neurosurgical Societies (WFNS) classification and time span to clinical ictus were assessed. Treatment of patients was carried out according to standardized guidelines.[8] Occurrence of DCI was assessed throughout the clinical course of treatment. DCI was defined as secondary neurologic worsening based on increase in modified National Institutes of Health Stroke Scale $\geq$2 points; exclusion of other relevant known causes such as infection, seizure, and metabolic, or electrolyte disturbances; and neurologic improvement in terms of level of consciousness or degree of muscular strength after induced hypertension or occurrence of new cerebral ischemia or perfusion deficit confirmed by C computed tomography or magnetic resonance imaging (in case of consciousness or sedation or persistent neurologic deficit). Seizures were excluded only if observed. However, subclinical seizures were not diagnosed with the help of electric activity monitoring.

Serum Sample Processing

Serum parameters were assessed directly after admission and on days 1, 4, 7, 10, and 14 after aSAH (always in the morning in line with routine diagnostic). Serum samples of patients were centrifuged for 10 minutes at 2000*g* before processing and analyzed at the Department of Clinical Chemistry and Clinical Pharmacology, University of Bonn, either as a part of the routine diagnostics (leucocyte count [g/L], determination of C-reactive protein [CRP] [mg/L], and interleukin 6 [IL-6] [pg/mL]) or for this study by means of enzyme-linked immunofluorescence assays for determination of E-selectin (a cell adhesion molecule expressed on endothelial cells, also known as endothelial leucocyte adhesion molecule 1 [ng/mL]), matrix metallopeptidase 9 (ng/mL), intercellular adhesion molecule 1 (ICAM-1 [ng/mL]), and leukemia inhibitory factor (LIF [pg/mL]) in serum samples (all assays purchased from IBL International GmbH, Hamburg, Germany).

Statistical Analysis

For statistical analysis, clinical severity score according to WFNS scale (mild, 1–3; severe, 4 and 5) and Fisher scale (mild, 1 and 2; severe, 3 and 4) were dichotomized. Because of the explorative character of the present study and weak previous knowledge, reasonable sample size calculations could not be performed before study onset. However, a sample size (patients with DCI event) of at least 19 was aimed at, as recommended by Dochtermann and Jenkins,[9] to enable performance of multivariate analyses with a moderate number of parameters. Percentages are shown rounded to the nearest whole numbers. Categorical variables were compared via a χ^2 test. When expected values in any of the cells of a contingency table were <5, a 1-tailed Fisher exact test was used. An independent *t* test was applied for metric values. *P* values <0.05 were considered significant.

Cox regression analyses were performed to evaluate the association of inflammatory serum parameters on occurrence of DCI. Therefore, baseline serum parameters (acquired on day 0), maximum values during inpatient treatment, and the differences of maximum and baseline values (referred to as delta values) were considered. A backward stepwise analysis was carried out to keep models as parsimonious as possible. To adjust for the small sample size and possible low power, parameters with a significance of $P < 0.2$ were regarded as relevant for Cox regression analysis and retained in the model. Regarded as potential confounders, dichotomized WFNS scale score, age, and sex were always included in the model during the stepwise procedure. *P* values <0.05 were considered significant associations. Statistical analysis was performed using SPSS version 21.0 (IBM Corp., Armonk, New York, USA).

RESULTS

Of the 89 patients initially screened, 24 (27%) developed DCI (as defined in the Methods section). Baseline data of these patients are presented in **Table 1**. Comparison of patients with and without occurrence of DCI showed no difference in sex ratio ($P = 0.237$, χ^2 test), age ($P = 0.108$, independent *t* test), aneurysm treatment ($P = 0.342$, χ^2 test) and grading according to dichotomized Fisher score (grade 1 and 2, mild; grade 3 and 4, severe; $P = 0.205$, Fisher exact test). However, there was a significant difference in grading of severity (according to dichotomized WFNS score) and occurrence of DCI with lower grading in the