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Time-courses of plasma IL-6 and HMGB-1 reflect initial severity of clinical presentation but do not predict poor neurologic outcome following subarachnoid hemorrhage

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ABSTRACT

Objective: Patients with aneurysmal subarachnoid hemorrhage (aSAH) experience high mortality and morbidity. Neuroinflammation causes brain damage expansion after aSAH. Due to the complexity of the inflammatory response multiple biomarkers are needed to evaluate its' progression. We studied inflammatory process after aSAH by measuring two inflammatory biomarkers, interleukin-6 (IL-6) and high-mobility group box 1 (HMGB1) at simultaneous time-points after aSAH.

Methods: In this prospective population-based study, IL-6 and HMGB1 were measured in aSAH patients ($n = 47$) for up to five days. Plasma concentrations of IL-6 and HMGB1 were measured at 0, 12 and 24 h after hospital admission, and thereafter daily for up to five days or until the patient was transferred from the intensive care unit (ICU). The patients' neurological outcomes were evaluated with the modified Rankin Scale at six months after aSAH.

Results: A high IL-6 level during the first day after aSAH was associated with a severe initial clinical presentation ($p = 0.002$) and infection during follow-up ($p = 0.031$). The HMGB1 level did not associate with these parameters. There was no correlation between IL-6 and HMGB1 levels at any time point during the follow-up. The concentrations of IL-6 and HMGB1 were not associated with neurological outcome.

Conclusions: High initial IL-6 values seem to reflect the intensity of the inflammatory response but not the brain damage per se. An early inflammatory response might even be beneficial since although elevated IL-6 levels were observed in patients with a more severe initial clinical presentation, they were not associated with neurological outcome. The lack of correlation between IL-6 and HMGB1 questions the role of macrophages in the process of the secretion of these inflammatory markers after aSAH, instead pointing to the activation of alternative pro-inflammatory pathways.

1. Introduction

Despite recent advances in the management of aneurysmal subarachnoid hemorrhage (aSAH), it remains a devastating disease with a mortality approaching 50% and fewer than 60% of the survivors achieving functional independence [1]. The brain injury developing after aSAH occurs in multiple phases. There is evidence suggesting that after the primary insult, an additional brain injury is evoked by early brain injury (EBI) and delayed cerebral ischemia (DCI). There are reports that the development of DCI doubles the risk of poor outcome in aSAH [2,3].

Early brain injury refers to the acute effects of blood in the subarachnoid space and the transient global ischemia caused by acute elevation in the intracranial pressure. Delayed cerebral ischemia is a multifactorial phenomenon involving angiographic vasospasm, microcirculatory vasoconstriction, microvascular thrombosis, cortical spreading depolarization and blood-brain barrier dysfunction. It is also thought that processes activated during EBI contribute to the development of DCI [2,4,5].

Various biomarkers have been examined in aSAH patients but thus far, there are no clinically reliable biomarkers for predicting DCI or

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prognosis [6]. Recent findings from our group support the hypothesis that UCH-L1 could be useful in predicting patient outcome after aSAH although no association between UCH-L1 and DCI was found [7]. Nevertheless, there is accumulating evidence that inflammation contributes to the development of DCI [2,8–10]. Knowledge about the neural control mechanisms of inflammation is accumulating rapidly [11]. A number of cytokines and proinflammatory markers have been associated with a poor outcome in aSAH [6]. Interleukin-6 (IL-6) and high-mobility group box 1 (HMGB1) are two important inflammatory biomarkers; their peripheral blood concentrations have been associated with a poor outcome in aSAH [12–14].

It has been postulated that the inflammatory response displays biphasic features in EBI and DCI [2,15]. The biphasic response refers to the double-edged effects of the inflammatory response which can be detrimental at some phase of the disease but alleviating at another. The biphasic properties of multiple molecular pathways have been proposed to contribute to the secondary brain injury also in other types of acute strokes [16,17]. The putative biphasic effects of the inflammatory response highlight the need for a better understanding of the mechanisms and timescale of the response in aSAH. The development of reliable biomarkers acting as surrogates for the response could help to achieve this goal.

The aim of the present study was to analyze the role of two biomarkers in a group of patients with recent aSAH. We evaluated whether there was any correlation between IL-6 and HMGB1 levels. By comparing the concentrations of these two inflammatory biomarkers at the same time points, we wanted to determine whether the changes in their concentrations could offer more specific information about the inflammatory pathways and possible mechanisms leading to the neural injury developing after aSAH. In addition, we assessed their association with neurological outcome and selected clinical conditions.

2. Methods

Following the approval of the institutional ethics committee, we conducted a prospective, observational, single-center clinical study in Tampere University Hospital (Tampere, Finland). The hospital is one of five tertiary referral centers in Finland serving a population of approximately 1 million inhabitants and thus providing care for all patients suffering from subarachnoid hemorrhage in the area.

The study cohort consisted of 61 consecutive aSAH patients admitted to our center from March 2013 to December 2013. Written informed consent was obtained from each patient or from their next of kin. The time of the onset of symptoms associated with aSAH was registered from the patient records. We excluded patients with an unknown time of onset of symptoms as well as patients whose samples for the IL-6 and HMGB1 assays were not collected within the first 24 h after the onset of symptoms. All patients were treated according to standardized in-house guidelines. In total, 47 patients were considered eligible and were thus included in the study.

The severity of the initial clinical presentation was evaluated according to the World Federation of Neurological Surgeons (WFNS) and Hunt & Hess grading scales. The extent of the primary hemorrhage on the CT scan was graded with Fisher scale. WFNS and Hunt & Hess were dichotomized into non-severe (WFNS 1–3/Hunt & Hess 1–3) or severe (WFNS 4–5 / Hunt & Hess 4–5). The Fisher grade was dichotomized into non-severe (Fisher 1–2) or severe (Fisher 3–4). The neurological outcome was evaluated with the modified Rankin Scale (mRS) six months after aSAH based on a structured interview performed by telephone or during an outpatient clinic visit. mRS was dichotomized into a favorable outcome (mRS 0–2) or an unfavorable outcome (mRS 3–6). Detailed categorizations of WFNS, Hunt & Hess, Fisher and mRS are presented in a Supplementary table. Additionally, we assessed the incidence of infection, which was defined as the need for antimicrobial medication during the follow-up period.

Plasma concentrations of IL-6 and HMGB1 were measured in the samples collected at 0, 12 and 24 h after the admission, and thereafter at every 24 h for up to five days or until the patient was transferred from the ICU. Before the statistical analysis, the IL-6 and HMGB1 measurements were divided into consecutive 24 h intervals starting from the onset of symptoms. If IL-6 and HMGB1 were measured more than once per interval, the mean concentration was used. In a subgroup of 22 patients who had up to five days' follow-up, we also checked if the patient had been treated for DCI. Treatment of DCI was initiated based on the clinical evaluation.

Blood samples were collected into EDTA-containing tubes from an arterial cannula that had been routinely inserted for invasive blood pressure monitoring as well as for blood sampling. After collection, the sample was immediately delivered to the laboratory where it was centrifuged for 10 min at 2000*g* (room temperature). After centrifugation, the plasma was collected and kept at - 70 °C. IL-6 and HMBG1

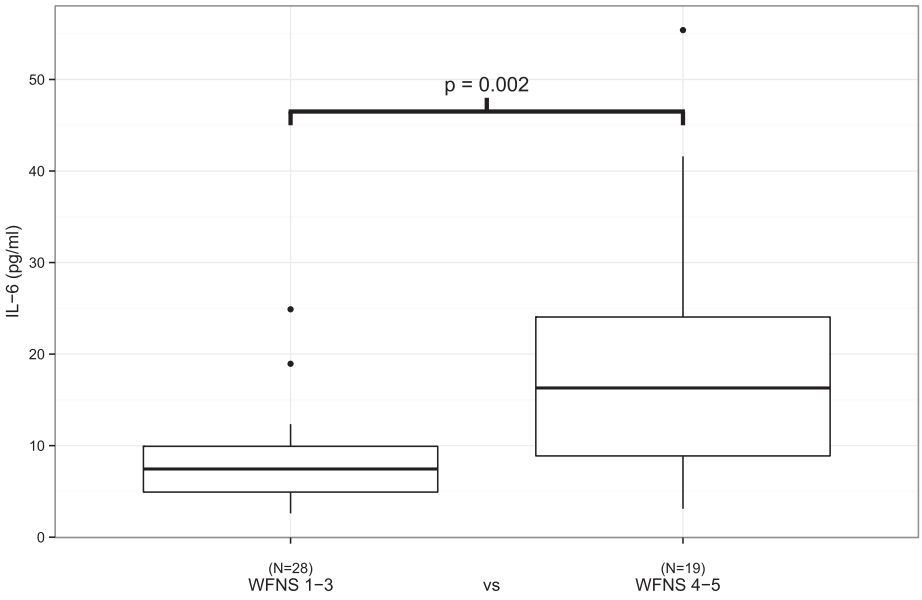

Fig. 1. IL6 levels measured within the first 24 h after aSAH in relation to the initial clinical presentation. Patients with more severe clinical presentations had significantly higher IL-6 levels ($p = 0.002$). Black circles represent outliers.