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Vascular

High concentrations of procoagulant microparticles in the cerebrospinal fluid and peripheral blood of patients with acute basal ganglia hemorrhage are associated with poor outcome

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Abstract

Background: Apoptosis plays an important role in further brain injury after intracerebral hemorrhage (ICH). Procoagulant microparticles (MPs) are shed from the plasma membrane of apoptotic cells. The objective of this study was to determine plasma and cerebrospinal fluid (CSF) levels of MPs in patients with spontaneous ICH and to correlate MP levels with Glasgow Coma Scale (GCS) scores, ICH volumes, presence of intraventricular hemorrhage (IVH), and survival rate.
Methods: Ten patients with suspicion of subarachnoid hemorrhage and 36 patients with spontaneous basal ganglia hemorrhage were included. Plasma and CSF samples were collected. Circulating MPs were obtained by double centrifugation and captured with annexinV. Their procoagulant potential was measured with a prothrombinase assay.
Results: Plasma or CSF MP levels in the ICH group were significantly higher than those in the control group (8.2 ± 3.0 vs 3.2 ± 1.7 nmol/L phosphatidylserine [PS] equivalent; $P < .001$ or 9.8 ± 3.7 vs 1.4 ± 0.6 nmol/L PS equivalent; $P < .001$). The MP levels were highly associated with GCS scores, ICH volumes, presence of IVH, and survival rate (all $P < .05$) in ICH. A receiver operating characteristic curve identified CSF and plasma MP cutoff levels that predicted 1-week mortality of patients with the high sensitivity and specificity values. Areas under curves (AUCs) of GCS scores and ICH volumes were larger than those of CSF and plasma MP levels, but only the difference between AUC of GCS scores and that of plasma MPs levels reached statistical significance ($P < .05$).
Conclusions: High levels of procoagulant MPs are present in the CSF and peripheral blood of patients with ICH and may contribute to the pathogenesis of ICH. The levels of CSF and plasma MPs after spontaneous onset of ICH seem to correlate with clinical outcome in these patients. Taking clinical complexity into account, only plasma MP levels can be served as useful clinical markers for evaluating the prognosis of ICH.

Keywords: Microparticles; Apoptosis; Intracerebral hemorrhage; Intraventricular hemorrhage; Pathogenesis

1. Introduction

Spontaneous intracerebral hemorrhage (ICH) accounts for 10% to 15% of all strokes and exposes patients to high rates of mortality and poor functional outcome [36]. The mortality of patients with ICH is associated with Glasgow Coma Scale (GCS) score, age, and findings on radiographs, among which, GCS score, and ICH volume, and presence of intraventricular hemorrhage (IVH) have usually been the

Abbreviations: AUC, areas under curve; CI, confidence interval; CSF, cerebrospinal fluid; CT, computed tomography; GCS, Glasgow Coma Scale; HBSS, Hank's balanced salt solution; ICH, intracerebral hemorrhage; ICU, intensive care unit; IVH, intraventricular hemorrhage; MP, microparticle; PS, phosphatidylserine; ROC curve, receiver operating characteristic curve; TBS, tris-buffered saline; TF, tissue factor.

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most robust outcome predictors [3,5,13,37,38,40]. Apoptosis is a process of cell death that occurs during ICH [7,9,28,35]. Apoptosis is associated with the shedding of submicron fragments from the plasma membrane termed *microparticles* (MPs) [2,14,15,27,30,42]. Microparticles are generally considered bilayered membranes with an antigen distribution that may be representative of the cell membrane they stem from, and they can be enriched in procoagulant aminophospholipids such as phosphatidylserine (PS) at their exoplasmic leaflet. The consequence of the accessibility of PS at the extracellular membrane, an early event in the time-course of cell apoptosis, can be monitored using dye-labeled annexin-V, a 36 kDa, Ca^{2+}-dependent PS probe. Microparticles are reliable markers of cell damage detectable in biologic fluids, whereas the cells at their origin remain sequestered in tissues or are promptly submitted to phagocytotic clearance. In vitro, the degree of apoptosis is correlated to the amount of MPs shed in the supernatant by cultured cells in which a death program has been induced [2]. We assumed that MP levels in the cerebrospinal fluid (CSF) and peripheral blood could be increased and be indicators for secondary tissue destruction and be predictors for clinical outcome in ICH patients.

In this study, we were to investigate whether MP levels in both the CSF and peripheral blood could be enhanced and to evaluate whether MP levels were associated with GCS scores, ICH volumes, presence of IVH, and survival rate.

2. Patients and methods

2.1. Patient and control populations

Between February 2007 and August 2008, 38 consecutive spontaneous basal ganglia hemorrhage patients, who were admitted to the intensive care unit of The First Hangzhou Municipal People's Hospital (Hangzhou, China) and met the inclusion criteria, were enrolled into this study. *Intracerebral hemorrhage* was defined as intraparenchymal hemorrhage of the brain. Hypertension and diabetes mellitus were defined as previous diagnosis.

The inclusion criteria included the following: (1) ICH noted on brain computed tomographic (CT) scan on presentation to the emergency department; (2) patient age between 40 and 80 years; (3) admission time less than 6 hours; (4) no other previous systemic diseases including uremia, liver cirrhosis, malignancy, and chronic heart or lung disease, with the exceptions of diabetes mellitus and hypertension; (5) need and acceptance of surgical therapy; and (6) time to surgery less than 12 hours. Exclusion criteria included the following: (1) history of head trauma or previous stroke, (2) use of antiplatelet or anticoagulant medication, (3) history of arteriovenous malformation of the brain, and (4) history of ruptured cerebral aneurysm.

A control group consisted of 10 age and sex-matched patients admitted to the neurosurgery department for suspicion of subarachnoid hemorrhage and with normal results on brain magnetic resonance imaging scan and without vascular risk factors and in whom a lumbar puncture had to be performed. Lumbar puncture was negative in all cases.

Patients (or their relatives) and controls (or their relatives) gave informed consent to participate in the study, and this protocol was approved by the Ethics Committee of The First Hangzhou Municipal People's Hospital before implementation.

2.2. Initial clinical evaluation

The following data were collected when the patients were admitted to the emergency department: age, sex, GCS score, body temperature, heart rate, respiratory rate, systolic arterial pressure, and diastolic arterial pressure. Arterial pressures were measured noninvasively using a conventional blood pressure sphygmomanometer. Mean arterial pressure was calculated from the diastolic and systolic values (mean arterial pressure = diastolic arterial pressure + 1/3 [systolic arterial pressure − diastolic arterial pressure]).

2.3. Neuroradiologic data

All diagnoses were confirmed by brain CT scan examination. Intraventricular hemorrhage was determined by assessing brain CT scan for the presence of blood in the ventricles. The amount of IVH was graded as follows: grade 0, no IVH; grade 1, slight hemorrhage in the third or fourth ventricle or occipital horns; grade 2, moderate hemorrhage in the ventricles; and grade 3, severe hemorrhage packing the ventricular system [20,21]. The ICH volume was calculated according to the formula $A \times B \times C \times 0.5$, where A and B represent the largest perpendicular diameters through the hyperdense area on CT scan, and C represents the thickness of ICH (the number of 10-mm slices containing hemorrhage) [23]. *Hydrocephalus* was defined as the presence of dilated ventricles on brain CT scan. The hydrocephalus of ICH patients was assessed by 2 doctors. *Preoperative hemorrhage growth* was defined as an increase in the volume of intraparenchymal hemorrhage of more than 33% as measured by CT compared with the initial CT scan [4]. Postoperative rebleeding was identified when the postoperative CT volume was either greater than the preoperative volume or there was a less than 5 mL difference in the preoperative and postoperative CT blood volume measurements [33].

2.4. Intracerebral hemorrhage management

The treatments included surgical therapy, mechanical ventilation, blood pressure control, intravenous fluids, hyperosmolar agents, H_2 blockers, early nutritional support, and physical therapy. The decision to intubation and mechanical ventilation was based on the individuals' level of consciousness, ability to protect their airway, and arterial blood gas levels [11,12,39]. When clinical and radiologic examinations provide some estimation of elevation of intracranial pressure, the osmotherapy in the form of intravenous mannitol was administered [5]. The mean