Cellular Microparticles as a Marker for Cerebral Vasospasm in Spontaneous Subarachnoid Hemorrhage

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Background and Purpose—Spontaneous subarachnoid hemorrhage (SAH) still has a high risk for poor outcome that is frequently attributable to symptomatic cerebral vasospasm (CVS). We hypothesize that cellular microparticles (MP) play a role in the pathogenesis of CVS and may serve as biomarkers for CVS.

Methods—In 20 consecutive SAH patients, endothelial, leukocyte, platelet, and erythrocyte MP were measured during 15 days after ictus. CVS was detected by transcranial Doppler sonography. Twenty matched volunteers served as healthy controls.

Results—Endothelial, leukocyte, and erythrocyte MP were elevated in SAH patients compared to healthy controls. CD105+ and CD62e+ endothelial MP were significantly higher in SAH patients with Doppler sonographic CVS. CD105+ endothelial MP were especially increased on the days of Doppler sonographic CVS onset. In patients experiencing cerebral infarction attributable to vasospasm, CD41+ platelet MP were elevated in addition to endothelial MP. CD41+ platelet MP were significantly higher in patients with any level of disability (modified Rankin Scale score ≥1) compared to those who made a full recovery (modified Rankin Scale score=0) on discharge from hospital.

Conclusion—Endothelial MP were elevated in patients with SAH. This elevation coincided with the occurrence of Doppler sonographic CVS and therefore could be a novel biomarker for CVS. Platelet MP might be involved in the pathogenesis of cerebral infarction attributable to vasospasm, resulting in neurological morbidity. (Stroke. 2010;41:2353-2357.)

Key Words: delayed cerebral ischemia • endothelial microparticles • subarachnoid hemorrhage

A neurymal subarachnoid hemorrhage (SAH) is still associated with high morbidity and mortality rates despite recent advances in neurosurgery and interventional radiology.1 Neurocritical care of patients with SAH focuses on the anticipation, prevention, and management of secondary complications with cerebral vasospasm (CVS) and subsequent cerebral infarction attributable to vasospasm (CIV) as a major cause of poor outcome.2 CVS is regarded as abnormal and prolonged smooth muscle contraction of cerebral arteries; inflammatory and calcium-dependent mechanisms have been implicated in its development.

Cellular microparticles (MP) are plasma particles of 0.05 to 1.0 μm in diameter bearing the surface antigens of their cells of origin and are formed by exocytic budding of cell membranes.3 They reflect cell stimulation or tissue degeneration and play important roles in blood coagulation as well as in inflammation in a variety of different disease entities.3 In ischemic stroke, levels of endothelial MP directly correlate with clinical disease severity and infarct volume and are markers of vascular pathology.4 We hypothesize that endothelial dysfunction has a central role for the induction of CVS and that cellular MP might be involved in this process. Therefore, 20 patients with SAH were prospectively followed-up to evaluate the implication of cellular MP in the pathogenesis of vasospasm and their potential as new biomarkers for CVS.

Patients and Methods

Study Population

Twenty consecutive patients admitted to the Department of Neurology of Innsbruck Medical University were included between November 2007 and January 2009. The study protocol was approved by the local Ethics Committee. Patients aged between 18 and 70 years meeting the following criteria were eligible: SAH confirmed by CT, ruptured aneurysm demonstrated by angiography, endovascular coil-possible, symptoms onset within 48 hours before screening, and written informed consent. Exclusion criteria were moderate to severe CVS at screening, known coagulopathies, treatment with platelet aggregation inhibitors or vitamin K antagonists, and severe concomitant diseases.

Transcranial Doppler sonography and blood sampling was performed daily from days 1 to 7 and every other day thereafter. Doppler sonographic CVS (dCVS) was defined as a mean blood flow velocity of ≥120 cm/sec in the middle cerebral artery.5 CIV was

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defined as new infarction on CT that was not detected on admission or the immediate postinterventional scan and was classified as vasospasm-related.

At the end of hospitalization and 6 months thereafter, outcome was evaluated by modified Rankin Scale and Glasgow Outcome Scale. A detailed description of the local standard of care has been published recently.² Twenty age- and gender-matched healthy volunteers were recruited from hospital workers and relatives of the study investigators (mean age, 52.2 years; range, 33–68 years). All data were analyzed on an intention-to-treat basis.

**MP Analysis**

MP were analyzed by a modified protocol according to Combes et al.¹ In plasma after centrifugation. Single blood samples were drawn from healthy controls. Plasma samples were double-labeled with 2 to 7 μL monoclonal antibodies (CD31-PE, CD41-PeCy5, CD54-PeCy5, CD62e-PE, CD106-PeCy5 [all Becton Dickinson], CD105-PE [Abcam], and CD45-PerCP and CD235a-PE [both R&D Systems]) for 15 minutes at room temperature. Subsequently, 10 μL of 1:10 FITC-conjugated Annexin V (Becton Dickinson) followed by 450 μL of annexin-binding buffer was added. Analysis was performed by FACSscan flow cytometer (Becton Dickinson).

Instrument settings were adjusted by running 0.8-μm, 1-μm, 1.0-μm, and 3-μm latex beads (Sigma). Gates were positioned to include particles <1.0 μm in size. For quantification of MP, TruCount tubes (Becton Dickinson) were used according to the manufacturer’s protocol.

**Statistical Methods**

For data reduction, the mean values of MP in each patient over the course of the hospital stay were calculated. MP levels were compared between patient groups by Wilcoxon rank-sum test. The course of logarithmically transformed levels of CD105⁺ and CD62e⁺ MP was modeled by generalized estimation equations with days after SAH and presence of dCVS as factors, including important covariates (age, gender, white blood cell count, C-reactive protein, and body temperature). For correlation analyses, Spearman rho was calculated.

**Results**

Demographic and clinical data are listed in Table 1. Patients had dCVS onset between days 1 and 13 (1 patient on day 1, 1 patient on day 2, 4 patients on day 4, 3 patients on day 6, 1 patient on day 11, and 1 patient on day 13).

Mean values of MP are shown in Table 2. Correlation analyses of MP levels and the following variables did not yield relevant associations: red blood cell count, hemoglobin, white blood cell count, platelets, C-reactive protein, heart rate, body temperature, Therapeutic Intervention Scoring System-28, Simplified Acute Physiology Score, mean arterial blood pressure, heart rate, and central venous pressure.

**MP Levels in Patients With dCVS and CIV**

In patients with dCVS (n=11), significantly higher CD105⁺/A⁺ and CD62e⁺/A⁺ endothelial microparticle (EMP) levels were observed (data not shown). In patients with CIV (n=7), the increase of CD105⁺/A⁺ EMP was even higher (P<0.01; Figure A). In addition to EMP, CD41⁺/A⁺ platelet microparticles (PMP) were significantly higher in patients with CIV (P<0.05; Figure B).

To verify the association between presence of dCVS and elevated levels of CD105⁺ EMP, generalized estimation equations models were calculated with day after SAH and presence of dCVS as factors, including important covariates to control for systemic infection. These models showed statistically highly significant effects of both factors and their interaction (Figure C). For CD62e⁺ EMP, the same analysis yielded significant effects for days after SAH and the interaction with presence of dCVS but not for dCVS alone (data not shown).

In all patients with dCVS, CD105⁺/A⁺ EMP increased on the days of dCVS onset compared to day 1 (Figure D; P<0.01). Clinical and laboratory data (age, gender, red blood cell count, hemoglobin, white blood cell count, platelets, C-reactive protein, heart rate, body temperature, Therapeutic Intervention Scoring System-28, Simplified Acute Physiology Score, mean arterial blood pressure, heart rate, central venous pressure) did not show significant associations with presence of dCVS.
Association of Clinical Data, Outcome, and MP Levels

The mean MP levels over the hospital stay did not show significant associations with WFNS (World Federation of Neurosurgical Societies) grade, Fisher score, parenchymal hemorrhage, or hydrocephalus. In patients with any level of disability (modified Rankin Scale ∼1), significantly higher levels of overall MP as well as PMP could be observed compared to those who had made a complete recovery (modified Rankin Scale ∼0) on discharge (mean SEM; A, 1840.6 ± 122.2 vs 1390.3 ± 235.5; CD41+/A⁺: 1418.9 ± 95.0 vs 992.2 ± 221.4; both P < 0.05). At 6-month follow-up, no significant associations with outcome could be found.

Discussion

The pathogenesis of CVS is still incompletely understood. Although there is little doubt that intrathecal blood breakdown products are involved, the downstream effectors of smooth muscle cell constriction have not been fully characterized. One central mediator of vessel wall relaxation is nitric oxide. Experimental data suggest that CD105 (endoglin) regulates nitric oxide-dependent vasodilatation by inhibition of transforming growth factor-beta and subsequently reduced activation of endothelial nitric oxide synthase. Therefore, the shedding of CD105⁺ EMP observed in patients with dCVD could lead to impaired vasodilatation by nitric oxide, attributable to dysregulated transforming growth factor-beta signaling.

EMP released after apoptotic stimuli or attributable to cell activation, respectively, are phenotypically distinct. In the current study, primarily EMP with surface molecules suggestive for endothelial activation (ie, CD54, CD62e, and CD106) were upregulated. However, in SAH patients with dCVD and CVI, only CD105⁺ EMP, reflecting cell degeneration, remained a significant predictor after controlling for variables of systemic inflammation. Apoptosis of endothelial cells has been shown to occur in animal models of SAH during the initial phase of CVS, and inhibition of the apoptosis cascade resulted in neuroprotection. Clinical data on endothelial apoptosis is limited; however, high-dose systemic erythropoietin was effective in reducing the incidence of severe vasospasm and CIV in SAH patients. The increase of CD105⁺ EMP at dCVD onset observed in the current study might reflect endothelial damage by apoptosis in the early phase of CVS.

In patients with CIV, CD41⁺ PMP also were elevated, in addition to EMP. Although this difference was not high, this result deserves special attention because of the potential

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MP indicates microparticles; SAH, subarachnoid hemorrhage; SEM, standard error of the mean.
impact on the understanding of the development of CIV. In SAH, there is no clear bidirectional relationship between cerebral infarction and the presence of vasospasm. In fact, several recent studies show that levels of serological coagulation markers are elevated before the occurrence of CVS. Our data show an increase of CD41+ PMP in patients with CIV, supporting the concept that CIV could be a consequence of microthrombosis rather than of CVS. If procoagulant CD41+ PMP prove to have a pathophysiological role in SAH, then antagonization of CD41—a subunit of the platelet GPIIb/IIIa receptor—could be a novel treatment strategy protecting patients from CIV.

Absolute levels of EMP were low compared to PMP, erythrocyte MP, and overall MP. The higher values of erythrocyte MP and PMP might, in part, result from incomplete cellular depletion by centrifugation. However, this issue does not influence MP differences between groups and EMP, which were dramatically increased in SAH patients and compared to healthy controls. Logarithmically transformed data are shown. EMP and PMP differences were highly significant. It has to be kept in mind that MP could also be generated by systemic inflammatory response. The observed elevation of leukocyte and erythrocyte MP in SAH patients might be explained in this context. However, most SAH patients had more or less severe systemic inflammatory response (Table 1), and parameters indicative for infection were not different between patients with and without dCVS, making a systemic cause of the observed EMP and PMP differences unlikely.

**Conclusion**

In conclusion, this study shows that cellular microparticles are elevated in patients with SAH. In patients with dCVS, and especially in those with CIV, EMP were elevated. This elevation was seen in the early phase of dCVS onset and therefore could represent a reliable biomarker for CIV. New concepts of CVS and CIV pathophysiology might be derived from this study and potentially lead to the development of novel treatment strategies for this devastating neurological disease.

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**Disclosures**

None.

**References**


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