Urinary F_2-Isoprostane Concentration as a Poor Prognostic Factor After Subarachnoid Hemorrhage.

Karol Wiśniewski[1], Michał Bieńkowski[2], Bartłomiej Tomasik[3,5], Marcin Braun[4,5], Ernest J. Bobeff[1], Paweł P. Liberski[2], Dariusz J. Jaskólski[1]

BACKGROUND: **The role of isoprostanes in cerebral vasospasm (CVS) following aneurysmal subarachnoid hemorrhage (aSAH) is controversial. Recent studies have suggested that the level of isoprostanes in cerebrospinal fluid could play a role in outcomes of patients with aSAH. We measured concentration of urinary F_2-isoprostanes (F_2-IsoPs), which is simple and noninvasive.**

METHODS: **A prospective analysis was performed of clinical data and urine samples of 20 patients with aSAH who underwent microsurgical clipping of the aneurysmal neck between May 2016 and January 2017. The role of F_2-IsoPs as a CVS biomarker was analyzed with regard to clinical conditions of patients. Outcome was assessed at discharge and 1-month and 4-month follow-up using the Glasgow Outcome Scale and modified Rankin Scale.**

RESULTS: **The concentration of urinary F_2-IsoPs was significantly greater in patients with aSAH than in healthy control subjects ($P < 0.001$). Additionally, increased F_2-IsoP levels on day 3 after aSAH were associated with development of CVS ($P = 0.015$) and worse neurologic performance after 1 month ($P = 0.042$) and 4 months ($P = 0.027$). The prognostic value of urinary F_2-IsoPs on day 3 in terms of CVS was found to be high (area under the curve 0.864, 95% confidence interval 0.691–1.000).**

CONCLUSIONS: **Urinary F_2-IsoPs may be used as a noninvasive prognostic biochemical marker in patients with aSAH. F_2-IsoP levels in urine may have significant implications in pathogenesis of CVS.**

INTRODUCTION

Stroke is the second leading cause of death and a leading cause of disability in adults. Subarachnoid hemorrhage (SAH) is most commonly caused by a rupture of a cerebral aneurysm. Aneurysmal subarachnoid hemorrhage (aSAH) accounts for <5% of all strokes.[1] It usually affects people approximately 50 years old, who until the occurrence of SAH were completely healthy and professionally active. Patient outcomes are poor with mortality rates of 45% and significant morbidity among survivors.[2] A major contributor to death and disability in survivors of aSAH is cerebral vasospasm (CVS).[3] The most dangerous as well as the least understood complication of aSAH is cerebrovascular spasm leading to delayed cerebral ischemia.[4,5] CVS is complex; it can be diagnosed angiographically and clinically. The narrowing of cerebral arteries visible on angiography occurs in 50%–70% of patients (radiographic vasospasm) and can lead to neurologic deterioration (delayed cerebral ischemia) secondary to focal ischemia in up to 50% of patients surviving SAH (clinical vasospasm).[6-9]

Vasospasm almost never occurs before 3 days after SAH, and the peak incidence has been observed between 5 and 7 days after aneurysm rupture. Typically, the risk period lasts 3–14 days. Vasospasm has been associated with blood in the subarachnoid space.[10,11] Nonetheless, studies of pathophysiology of CVS have

Key words
- Aneurysmal subarachnoid hemorrhage
- Cerebral vasospasm
- F_2-isoprostanes
- Reactive oxygen species

Abbreviations and Acronyms
aSAH: Aneurysmal subarachnoid hemorrhage
AUC: Area under the curve
CVS: Cerebral vasospasm
F_2-IsoP: F_2-isoprostane
GOS: Glasgow Outcome Scale
mRS: modified Rankin Scale
ROC: Receiver operating characteristic
ROS: Reactive oxygen species
SAH: Subarachnoid hemorrhage

From the [1]Department of Neurosurgery and Neurooncology, Medical University of Lodz, Barlicki University Hospital, Lodz; Departments of [2]Molecular Pathology and Neuropathology, [3]Biostatistics and Translational Medicine, and [4]Pathology, Medical University of Lodz, Lodz; and [5]Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland

To whom correspondence should be addressed: Karol Wiśniewski, M.D. [E-mail: karol.wns@gmail.com]

Karol Wiśniewski, Michał Bieńkowski, and Bartłomiej Tomasik are co–first authors.

Citation: World Neurosurg. (2017) 107:185-193.
http://dx.doi.org/10.1016/j.wneu.2017.07.145

Journal homepage: www.WORLDNEUROSURGERY.org

Available online: www.sciencedirect.com

reported ambiguous results. It is unclear whether the vasoconstrictive action is directly mediated by hemoglobin or hemoglobin-associated compounds. Extravasated blood, specifically, hemoglobin, is known to cause the release of reactive oxygen species (ROS), which take part in the peroxidation of membrane lipids of endothelial cells and the proliferation of smooth muscle cells, leading to CVS.[12,13] ROS, such as superoxide and hydroxyl radicals, are involved in the pathogenesis of various diseases, including ischemia/reperfusion injury, via damaging lipids, proteins, and nucleic acids.[14] Recently, F_2-isoprostanes (F_2-IsoPs) have been demonstrated to be the most specific markers of lipid peroxidation in vivo. F_2-IsoPs are compounds similar to prostaglandins, generated via nonenzymatic, free radical peroxidation of polyunsaturated fatty acids, in particular, arachidonic acid.[15] They are considered oxidative stress markers, as their concentration directly reflects the free radical content. The most extensively analyzed isoprostane, 8-iso-prostaglandin $F_{2\alpha}$, has been shown to cause vasoconstriction, platelet aggregation, and induction of DNA synthesis in smooth muscle cells.[16,17] According to an animal study, isoprostanes could cause CVS.[18]

In aSAH, both a sudden generalized ischemic event (owing to increased intracranial pressure and decreased cerebral perfusion pressure) and vasospasm may lead to ischemia/reperfusion brain injury. Nevertheless, the connection between ROS and aSAH has not been investigated extensively thus far. There are few studies to date devoted to the pathophysiology of CVS. Most studies have focused on a comparison of the effectiveness of drugs and outcomes. Only oral nimodipine has a level Ia indication for treatment of CVS, whereas intra-arterial and intravenous administration of nimodipine is associated with a beneficial effect on cerebral blood flow if cerebral perfusion pressure is maintained.[3,19] We hypothesized that ROS production after aSAH resulting in increased levels of F_2-IsoPs may serve as a prognostic factor. Therefore, in this pilot study, we collected patient urine samples daily between the second and fifth day after aSAH and quantified the concentration of F_2-isoprostanes. Subsequently, the patients were followed for 4 months to assess the potential clinical value of this marker.

MATERIALS AND METHODS

Clinical Evaluation of Patients

The analyzed group consisted of 20 patients operated on for a burst cerebral aneurysm within 2 days after bleeding. On admission, the patients were informed of the procedural risks and benefits of both procedures and chose between microsurgery and endovascular intervention. All patients included in this study underwent aneurysm clipping between May 2016 and January 2017. The control group consisted of 7 healthy volunteers (4 women and 3 men in the fifth and sixth decades of life). Any prior relevant medical history of systemic disease, such as kidney disease, urinary infection, diabetes mellitus, neurodegenerative disease, or cardiovascular disease, had been ruled out in all participants before inclusion into the study. In all cases, preoperative chest x-ray, resting electrocardiogram, and routine laboratory tests were normal. SAH was confirmed on computed tomography, and the presence of an aneurysm was confirmed on computed tomography angiography or cerebral angiography. The condition of each patient was assessed according to the Hunt and Hess scale (only grade I—IV patients were included into the analysis; grade V patients were excluded), and the extent of hemorrhage was assessed on computed tomography according to the Fisher scale.[20,21] The suspicion of clinical vasospasm (also referred to as delayed ischemic neurologic deficit or symptomatic vasospasm) was raised in patients presenting with symptoms of confusion or decreased level of consciousness with or without focal neurologic deficits (speech or motor). Such cases were verified with angiography (to detect arterial narrowing) or transcranial Doppler (to detect increases of blood velocity of >120 cm/second in middle cerebral artery). In each case, CVS was diagnosed by 2 independent neurosurgeons and a radiologist. According to these criteria, CVS was diagnosed in 9 patients between 4 and 7 days after SAH (**Table 1**). Each patient underwent a thorough neurologic examination before discharge and 1 month and 4 months after discharge, including assessment using the Glasgow Outcome Scale (GOS) (at 4 months the modified Rankin Scale [mRS] was also employed).[22,23]

Specimen Collection

Serial urine samples (5—10 mL of morning midstream sample taken following at least 8 hours of fasting) were collected from each patient daily between the second and sixth day after aSAH. The samples were processed immediately: first, they were aliquoted, centrifuged for 3 minutes at 3500*g* to remove particulates, snap frozen in liquid nitrogen, and stored at −80°C until analysis. Single urine samples were collected from the volunteers in the control group and processed in the same way.

Detection of Free Form of F_2-IsoPs in Urine

Free form of F_2-IsoPs in urine was quantified using STAT-8-Isoprostane ELISA Kit (Cayman Chemical, Ann Arbor, Michigan, USA) according to the manufacturer's protocol. The samples were thawed and diluted 10-fold directly before analysis. In parallel, creatinine was quantified in the same samples using Creatinine (urinary) Colorimetric Assay Kit (Cayman Chemical) according to the manufacturer's protocol. A 10-fold dilution of samples was used. Plate readings were performed using Synergy 2 Multi-Mode Reader (BioTek Instruments, Inc., Winooski, Vermont, USA) and the dedicated software. The concentration of F_2-IsoPs was normalized per 1 mg of creatinine.

Statistical Analysis

All statistical analyses were performed using R version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria) with packages stats, car, pROC, glm2, and ggplot2.[24-28] Correlation between categorical variables was analyzed using Fisher exact test. Comparison of continuous variables between 2 groups was performed using *t* test, Welch *t* test (if homogeneity of variance criterion was not met, verified with Levene test) or Mann-Whitney-Wilcoxon test (if normality of distribution criterion was not met, verified with Shapiro-Wilk test); effect size was assessed using Cohen's *d*. Increased probability of type I errors as a result of multiple comparisons was controlled using Bonferroni correction ($\alpha' = \alpha$/number of comparisons). Analogously, correlation between numeric variables was analyzed using Pearson correlation or Kendall rank correlation. Receiver operating characteristic (ROC) curves were employed to depict the dependence between sensitivity and specificity of the analyzed markers in