Direct Observation of the Human Cerebral Microcirculation During Aneurysm Surgery Reveals Increased Arteriolar Contractility

Frederik A. Pennings, MD; Gerrit J. Bouma, MD, PhD; Can Ince, PhD

Background and Purpose—The effects of aneurysmal subarachnoid hemorrhage on morphology and function of the cerebral microcirculation are poorly defined, partly due to the lack of suitable techniques to visualize the microvessels in vivo. We used orthogonal polarization spectral (OPS) imaging on the brain cortex during aneurysm surgery to directly observe the small cortical blood vessels and quantify their responses to hypocapnia.

Methods—In 16 patients undergoing aneurysm surgery, the diameter changes of small cortical vessels (15 to 180 μm) were observed using OPS imaging. Ten patients were operated on early (within 48 hours after bleeding) and 6 underwent late surgery. Immediately after dura opening, the response to hyperventilation of arterioles and venules was observed with OPS imaging under sevoflurane anesthesia.

Results—In patients operated on early, layers of subarachnoid blood were clearly visible. In this group, hyperventilation resulted in a 39±15% decrease in arteriolar diameter with a “bead-string” constriction pattern occurring in 60% of patients. In late surgery and in controls, no subarachnoid blood was seen. The arteriolar diameter decrease with hyperventilation was 17±20% in patients undergoing late surgery and 7±7% in controls. Venules were not affected by hyperventilation in any of the groups studied.

Conclusions—OPS imaging allows direct in vivo observation of the cerebral microcirculation enabling us, for the first time, to visually observe and quantify microvascular reactivity in the human brain. The present study demonstrates increased contractile responses of the cerebral arterioles in the presence of subarachnoid blood, suggesting increased microvascular tonus with possibly greater susceptibility to ischemia. (Stroke. 2004;35:1284-1288.)

Key Words: diagnostic imaging ■ microcirculation ■ neurosurgery ■ subarachnoid hemorrhage ■ vasospasm ■ vasospasm, intracranial

Cerebral ischemia frequently occurs following subarachnoid hemorrhage (SAH) and is a major cause of morbidity and mortality. It is generally accepted that the release of blood in the subarachnoid space contributes to the development of vasospasm and neurological deficits. To date, however, the mechanism of delayed cerebral ischemia after SAH remains poorly understood. Efforts to elucidate these mechanisms have focused on vasospasm of the large conducting cerebral arteries, but it is clear that this type of vasospasm cannot fully account for the occurrence of delayed ischemic neurological deficits. Clinical symptoms of cerebral ischemia (ie, decreased cerebral blood flow and oxygen consumption) can occur without evidence of angiographic vasospasm. On the other hand, angiographic vasospasm is often found in patients without clinical signs of cerebral ischemia. An explanation for these observations could be that the cerebral microcirculation and its regulatory mechanisms are directly affected by SAH. Nonetheless, the role of the cerebral microcirculation in the pathophysiology of delayed cerebral ischemia following SAH is obscure. Several animal studies have addressed the role of microcirculatory dysfunction during SAH, and arteriolar constriction was generally observed. Thus far, however, these results and insights have not been confirmed in humans.

Until recently, in vivo observation and quantitative functional assessment of the human cerebral microcirculation were limited by the absence of appropriate investigational techniques. We introduced orthogonal polarization spectral (OPS) imaging to the study of the human brain microcirculation, and validated this technique by comparison of OPS imaging to the gold standard of nail fold capillaroscopy. In the present study, we applied OPS imaging during aneurysm surgery to test the hypothesis that the presence of subarachnoid blood affects the contractile properties of the human cerebral microcirculation during the early course of SAH.

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TABLE 1. Summary of the Study Population’s Characteristics

<table>
<thead>
<tr>
<th>Sex (M:F)</th>
<th>SAH, Early Surgery (n=10, &lt;48 hours)</th>
<th>SAH, Late Surgery (n=6, range 2–7 wks)</th>
<th>Controls (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4:6</td>
<td>3:3</td>
<td>2:2</td>
</tr>
<tr>
<td>Age</td>
<td>54±10</td>
<td>62±11</td>
<td>44±8</td>
</tr>
<tr>
<td></td>
<td>Fisher grade*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2</td>
<td>n=7</td>
<td>n=6‡</td>
<td>NA</td>
</tr>
<tr>
<td>3–4</td>
<td>n=3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fisher grade†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2</td>
<td>n=3</td>
<td>n=6‡</td>
<td>NA</td>
</tr>
<tr>
<td>3–4</td>
<td>n=7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aneurysm Localization:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA:1, MCA:2, Acom:5, Pcom:2</td>
<td>MCA:1, Acom:2, Pcom:3</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

*Clinical grade according to Hunt and Hess classification.19
†Amount of blood on CT according to Fisher et al.20
SAH indicates subarachnoid hemorrhage; CA, carotid artery; MCA, middle cerebral artery; Acom, anterior communicating artery; Pcom, posterior communicating artery.

Patients and Methods

Patient Population

The Medical Ethics Committee of the Academic Medical Center of the University of Amsterdam approved this study, and written informed consent was obtained from each patient. In 16 patients undergoing aneurysm surgery, the diameter changes of small cortical vessels (range 15–180 μm) were observed using OPS imaging. Ten patients were operated within 48 hours after the initial bleeding (group A, acute surgical group) and 6 underwent surgery 2 to 7 weeks after the SAH (group B, late surgical group). Four non-SAH patients served as controls (group C), two of whom had a small skull base meningioma, one who had a cavernous hemangioma of the brain stem, and one who had a small deep-seated glioma. Further details of the study population are provided in Table 1.

Orthogonal Polarization Spectral Imaging (OPS) Technique

OPS imaging is a new method for imaging the microcirculation using reflected light that allows imaging of the microcirculation noninvasively through mucus membranes and on the surface of solid organs.11 In OPS imaging, the tissue is illuminated with linearly polarized light and imaged through a polarizer oriented orthogonal to the plane of the illuminating light. Only depolarized photons scattered in the tissue contribute to the image. The optical response of OPS imaging is linear and can be used for reflection spectrophotometry over the wide range of optical density typically achieved by transmission spectrophotometry. Thus, OPS imaging produces high-contrast microvascular images that appear as in transillumination. The technology can be implemented in a small optical probe, providing a convenient method for intravital microscopy on the brain surface.

For imaging of the cerebral surface a Cytoscan-EH back focus OPS imaging device (Cytometrics) was used. OPS imaging uses green polarized light of 550 nm for the visualization of the microcirculation at a depth of approximately 500 μm. The green light is absorbed by the erythrocytes that appear black. An orthogonal polarizer placed in front of a charge-coupled device camera filters the reflected light, thus allowing the microcirculation to be observed. Total magnification of the microcirculation on the computer monitor was 195× using a 2.5× lens. To provide a sterile barrier between the brain surface and the device a sterilized plastic cap was placed over the tip of the probe followed by a plastic drape, covering the rest of the instrument as described elsewhere.12 The probe was held stable in place on the cortical surface without pressure using an adapted Layla retractor arm.

Image Analysis

After dura opening but prior to arachnoid dissection, the response to hyperventilation of arterioles and venules was observed with OPS imaging. Fields were selected that included at least 2 arterioles and 2 venules. Arterioles were identified as high-flow vessels in which the direction of flow is from larger diameter vessels to smaller diameter vessels, while the reverse was true for venules. Furthermore, arterioles could be distinguished from venules by the difference in erythrocyte flow velocity, which was substantially higher in arterioles than in venules.

At least 2 arterioles and 2 venules were taken from each captured image sequence to measure the blood vessel diameter. The diameter of blood vessels was determined using image processing software specifically designed for analysis of the microcirculation (CapImage). The diameter of microvessels was determined by drawing a perpendicular line from one side of the luminal vessel wall to the other at 3 separate locations. After hyperventilation, this procedure was repeated for the same 3 measurement points and the percent diameter change was calculated using the formula: Δd% = (dbefore − dafter)/dbefore × 100. The quality of the images allowed accurate and objective measurement of diameters with a resolution of 5 μm.11 Furthermore, morphological changes of the vessel wall resulting from hyperventilation were characterized. Data are expressed as mean±SD. Comparisons of means before and after hyperventilation were made using an independent samples Student’s t test. Statistical significance was defined as P<0.05.

Anesthesia and Hyperventilation

Anesthesia was induced with thiopental (5 mg/kg) or propofol (2 mg/kg) and fentanyl. Intubation was facilitated with 0.5 mg/kg rocuronium. Anesthesia was maintained with 0.4% sevoflurane/O2 air-mixture with an inspired oxygen fraction (FiO2) of 0.4. A central venous line and a radial artery catheter were inserted for continuous hemodynamic monitoring. Mannitol was administered before opening of the dura. Ventilation was adjusted to maintain a PaCO2 close to 35 mm Hg. A reduction in PaCO2 for 5 minutes was obtained by increasing the ventilator rate or end-tidal stroke volume. The mean arterial pressure (MAP) was kept constant during hyperventilation.

Results

Morphology Under Resting Conditions in Controls and SAH

In the normal brain cortex (group C) the various components of the cerebral microcirculation could easily be distinguished.
The arteriolar vessel walls were regularly shaped and showed pulsations synchronous with cardiac rhythm. The average diameter of arterioles was 53 ± 39 μm (range 6 to 109 μm). Due to the very high erythrocyte flow, neither red blood cell (RBC) velocity nor flow patterns could be reliably determined. The venular vessel wall was regularly shaped and not pulsating. The average diameter of venules was 51 ± 48 μm (range 40 to 141 μm). The flow pattern was laminar, but, again, RBC velocity values could not be obtained in a reproducible manner.

Typical for group A (acute surgery) under resting conditions was the presence of large amounts of subarachnoid blood (\textsuperscript{*}) causing the arterial wall to appear illuminated (arrow), arterioles (A) and venules (V) can be distinguished from each other. After hyperventilation, a decrease in arteriolar diameter is observed with a bead-string–like constriction pattern (B). Note that the caliber of the venules is unaffected.

Reactivity to CO\textsubscript{2} in Controls and SAH

The mean \textit{paCO\textsubscript{2}} and MAP prior to hyperventilation were 36 ± 1 mm Hg and 78 ± 8 mm Hg, respectively. Following 5 minutes of hyperventilation, \textit{paCO\textsubscript{2}}, values declined to 27 ± 2 mm Hg with MAP remaining stable at 76 ± 9 mm Hg.

In group B (late surgery) the microcirculatory characteristics resembled that of controls. Subarachnoid blood was absent, except in 1 case, where a layer of subarachnoid blood was still visible (Figure 3). The average diameter of arterioles (61 ± 18 μm, range 26 to 153 μm) of this group did not significantly differ from group A or controls. Venular diameter measured 69 ± 40 μm (range 16 to 138 μm).

The arteriolar vessel walls were regularly shaped and showed pulsations synchronous with cardiac rhythm. The average arteriolar baseline diameter was 51 ± 22 μm (range 20 to 180 μm). The morphology and flow pattern of venules did not differ from controls. The average venular diameter was 50 ± 36 μm (range 16 to 152 μm).

In group C (controls), the arterioles showed a diffuse constrictive response pattern to hyperventilation with a 7.7% decrease in diameter, whereas venules did not show a diameter change. In group A (acute surgery), hyperventilation resulted in a vasoconstriction of arterioles with a 39 ± 15% decrease in arteriolar diameter. The observed constriction pattern of venules did not differ from controls. The average venular diameter was 50 ± 36 μm (range 16 to 152 μm).

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patterns were multifocal ("bead-string," Figure 1B) in 4 patients, diffuse (4 patients), or both (2 patients). There was no clear relationship between the amount of blood on CT (Fisher grade) and the degree of vasoconstriction to hyperventilation.

Although RBC velocity did not change during hyperventilation in the majority of acute surgical patients, a severe reduction in RBC velocity could be observed in 1 case. In group B (late surgery), the diameter decrease of the arterioles was 17±20%. This response was significantly lower than the response in group A (P=0.024), and only a diffuse constriction pattern was observed. The constriction patterns and diameter changes to hyperventilation are summarized in Table 2 and Figure 4.

### Discussion
This study employing OPS imaging is to our knowledge the first one reporting direct visualization of the responses of the human cerebral microcirculation to hyperventilation. We were able to observe and quantify alterations in microvascular diameters, and found that arterioles responded to hyperventilation by contraction where venules were largely unaffected. Our main finding in this study is that SAH was associated with increased contractility of arterioles in patients undergoing early aneurysm surgery as indicated by enhanced vasoconstriction in response to hyperventilation.

In the brain cortex of patients undergoing early aneurysm surgery, large amounts of subarachnoid blood were present in the perivascular space causing the arteriolar walls to appear thickened. Furthermore, decreased pulsatility of arterioles was observed as well as microvascular multifocal vasospasm in 20% of arterioles, suggesting increased vascular tone. Similar observations were described in a recent OPS imaging study by Uhl et al.14 The authors postulated that the varying thickness of the vessel wall in the presence of SAH could be explained by swelling of the endothelial layer. Another explanation could be that the vessel wall only appears thickened because of enhanced visibility of the vessel wall due to presence of erythrocytes on both sides of the vessel wall itself. Furthermore, they observed segmental vasospasm in 55% in patients with a SAH.

The presence of subarachnoid blood on OPS imaging was associated with a marked decrease in arteriolar diameter of 39% in response to hyperventilation, and with a characteristic bead-string–like constriction pattern in 60% of patients. A correlation between the amount of blood on CT (Fisher grade) and the degree of vasoconstriction could not be found, probably because of the small number of patients in each subgroup. In the absence of subarachnoid blood, a smaller vasocostrictive response of 17% and 7% was seen in the late surgical group and the controls group, respectively. In 1 patient of group B, a thick layer of subarachnoid blood was still visible on OPS imaging. In this patient, a decrease in arteriolar diameter of 56% after hyperventilation was observed. Deletion of this patient from the late surgical group would result in an average arteriolar constriction of only 9% after hyperventilation. Taken together, these findings suggest a relationship between the presence of subarachnoid blood and the arteriolar vasoconstrictive response to hyperventilation. The explanation for the increased arteriolar contractility, the occurrence of a bead-string constriction pattern of the cerebral arterioles, to changes in CO2 in the presence of subarachnoid blood is unclear as yet.

Normal values of arteriolar diameter that decrease in response to hyperventilation are unknown in humans. In animal studies, hyperventilation has been reported to lead to a 7% to 10% decrease under physiological conditions,15,16 These values are in good agreement with the decrease of 7% and 9% found in the controls and in the late surgical group, respectively.

The response of cerebral vessels to CO2 is thought to be mediated by the opening and closing of ATP sensitive potassium channels. With hyperventilation, a reduction in H+-ions in the perivascular environment is responsible for closing of the ATP-sensitive potassium channels and, consequently, constriction of smooth muscle cells.16 It is generally accepted that release of blood in the subarachnoid space contributes to the development of delayed cerebral ischemia.17 Although the exact pathophysiological mechanisms remain unclear, SAH probably inhibits potassium channels, which leads to depolarization of the vascular muscle. In addition, a reduction in vasodilatory response and an increase in vasoconstrictor response to vasoactive agents have been reported in human vessels after SAH.18 With hyperventilation, a potent dilator in the form of H+-ions is removed from the perivascular space, which further disturbs the balance between vasodilator and vasoconstrictor agents, and closure of potassium channels.

**Table 2. Type of Constriction Pattern in Surgical Groups**

<table>
<thead>
<tr>
<th>Constriction Pattern</th>
<th>SAH, Early Surgery (n=10)</th>
<th>SAH, Late Surgery (n=6)</th>
<th>Controls (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multifocal (&quot;Bead-string&quot;)</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diffuse</td>
<td>4</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Both</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

**Figure 4.** Graph showing the percent decrease of the arteriolar diameter in response to hyperventilation during early surgery (n=10), late surgery (n=6) and in controls (n=4). Data are expressed as means±SD (bars); *P<0.05.
In conclusion, the present study demonstrates increased contractility and altered morphology of the cerebral arterioles in the presence of SAH in vivo in humans. These findings suggest that microvascular tonus is increased following SAH, and one may speculate that this leads to greater susceptibility to vasospasm-induced ischemia. For obvious practical and ethical reasons however, we were not able to assess the vasodilatory responses to hypocapnia or normocapnia, and therefore, firm conclusions about the role of arteriolar disturbances in the pathophysiology of delayed cerebral ischemia cannot be drawn.

Nevertheless, our data implicate that future efforts aiming at preventing or reversing cerebral ischemia following SAH should be targeted at the cerebral microcirculation rather than large cerebral arteries alone. OPS imaging appears to be a useful tool in the conduct of such investigations.

Acknowledgments
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References
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