Human arachnoid villi response to subarachnoid hemorrhage: possible relationship to chronic hydrocephalus

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Object. The origin of chronic communicating hydrocephalus following subarachnoid hemorrhage (SAH) is not well understood. Fibrosis of the arachnoid villi has been suggested as the cause for obstruction of cerebrospinal fluid (CSF) flow, but this is not well supported in the literature. The goal of this study was to determine the relationship between blood, inflammation, and cellular proliferation in arachnoid villi after SAH.

Methods. Arachnoid villi from 50 adult patients were sampled at autopsy. All specimens were subjected to a variety of histochemical and immunohistochemical stains. The 23 cases of SAH consisted of patients in whom an autopsy was performed 12 hours to 34 years post-SAH. Fifteen cases were identified as moderate-to-severe SAH, with varying degrees of hydrocephalus. In comparison with 27 age-matched non-SAH controls, the authors observed blood and inflammation within the arachnoid villi during the 1st week after SAH. Greater mitotic activity was also noted among arachnoid cap cells. The patient with chronic SAH presented with ventriculomegaly 2 months post-SAH and exhibited remarkable arachnoid cap cell accumulation.

Conclusions. The authors postulate that proliferation of arachnoidal cells, triggered by the inflammatory reaction or blood clotting products, could result in obstruction of CSF flow through arachnoid villi into the venous sinuses. This does not exclude the possibility that SAH causes generalized fibrosis in the subarachnoid space.

Key Words • inflammation • pathology • ventriculomegaly • arachnoid villi • hydrocephalus

In 1928, Bagley reported the development of hydrocephalus following injection of blood into the subarachnoid space. In 1956, Foltz and Ward published a description of 10 patients with intracerebral hemorrhages, half of them with intracranial aneurysms, in whom the diagnosis of communicating hydrocephalus was made using combined ventriculographic–pneumoencephalographic studies. Ellington and Margolis published a report in 1969 based on six patients who died only a few days after subarachnoid hemorrhage (SAH), in which they noted that the arachnoid villi were blocked by blood. Vassilouthis and Richardson observed delayed ventriculomegaly (> 2 weeks) in 7% of 210 patients with SAH. Others report the incidence of chronic ventricular enlargement post-SAH to be between 10% and 23%.

The precise origin of chronic communicating hydrocephalus following SAH is still uncertain. Kibler and coworkers suggested that blood causes fibrosis in the leptomeninges, which in turn leads to communicating hydrocephalus. This conclusion was based on only one personally studied case and two from the literature, one of which was described as an acute death in which marked proliferation of fibroblasts in the leptomeninges was observed. In a more recent publication, arachnoid granulations from 43 cases of SAH were analyzed, and the authors confirmed the earlier observation of erythrocytes blocking the arachnoid granulations. However, they did not find that arachnoid villus fibrosis was a significant sequela of SAH. Of significance is the absence of proper control cases in any of these studies.

We hypothesize that blood in the arachnoid villi causes cellular changes that could impair the flow of cerebrospinal fluid (CSF). The purpose of this study was to determine the relationship between blood, inflammation, and cellular proliferation in arachnoid villi after SAH, with reference to an extensive control group.

Materials and Methods

This study is a combination of retrospective and prospective analyses of arachnoid villi collected at autopsy from the superior sagittal sinus of 23 patients in whom SAH had been identified. The study method was in keeping with the ethical guidelines of our institution. Including controls, a total of 50 cases were studied by sampling arachnoid granulations, reviewing imaging studies, and correlating their clinical history. Computerized tomography scans were used to assign a Fisher grade. Because computerized tomography scans were not available for all patients, an additional semiquantitative grading system for SAH was used: minimal SAH was defined as patchy areas of blood on the brain surface; moderate SAH as thin blood collections in the basal cisterns, with no or only focal extension to the convexities of the brain; and severe SAH was defined as blood collections in the basal cisterns more than 5 mm thick, with wide extension over the convexities of the brain. A prospective collection of 27 autopsy cases with no history of a neurological disorder, SAH, intraparenchymal or intraventricular hemorrhages, or ventriculomegaly represented our control group.
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At autopsy, the brain and the dura over the convexity were removed together and fixed in 10% buffered formaldehyde within 48 hours. After approximately 2 weeks the brain was examined and multiple coronal sections were cut from the superior sagittal sinus at sites including the arachnoid granulations. Other sinuses (for example, transverse sinuses) were not available for examination. Sampling of arachnoid granulations from the cortical surface proved to be less rewarding for demonstrating morphological details. The tissues were dehydrated and embedded in paraffin. Tissue sections 6 μ thick were stained using histochemical methods including Martius scarlet blue, Perl’s iron, chloracacetate esterase (for neutrophils), reticulin, and hematoxylin and eosin. Tissues were also immunohistochemically labeled for leukocyte common antigen (Dako Corp., Carpinteria, CA), factor VIII (Dako Corp.), and Ki67 (MIB-1 antibody; Immunotech, Inc., Westbrook, ME) by using biotinylated secondary antibodies and peroxidase precipitation of diaminobenzidine.

Results

The age of the 27 control patients (nine women and 18 men) ranged from 20 to 85 years (mean age 60.2 ± 3.7 years). The causes of death in the control group included myocardial infarction (nine patients), ruptured abdominal aortic aneurysm (two), drug overdose (three), unknown (three), pneumonia, pulmonary embolus, and trauma (two each), and burns, lung cancer, asphyxia, and liver failure (one each). In the study group, the eight patients with minimal SAH (age range 26–72 years, mean 40.8 ± 4.6 years) had minor cerebral trauma or small hemorrhages secondary to coagulopathy. In these individuals the time between SAH and death varied from 12 hours to 9 days. The age of the patients with moderate-to-severe SAH ranged from 42 to 89 years (mean 62.5 ± 4.4 years; six women and nine men). The causes of SAH in this group included 10 saccular aneurysms, three cerebral injuries, one hypertension, and one intracerebral hemorrhage secondary to congophilic angiopathy. Table 1 displays the demographic information and associated neuropathological changes in this group.

Between 11 and 15 arachnoid villi were seen in sections obtained at autopsy in both control and SAH cases. The Martius scarlet blue stain proved to be the most revealing for anatomical details. The nomenclature used for components of the arachnoid villus in the following discussion is based on the study by Kida, et al.11 The dura stained a deep blue-purple, whereas the central core of arachnoid villi stained pale blue. Arachnoid cap cells were stained red to pink. The central core was full of open channels that are presumed to communicate directly from the subarachnoid space to the subcapsular space of the villus, but not to the lumen of the venous sinus (Fig. 1.1).

Arachnoid villi from the 27 control cases contained a negligible quantity of erythrocytes, no inflammatory cells, and no proliferating cells. Factor VIII–like immunoreactivity of endothelial cells was consistently detected along the sinus lumen. Contrary to the assertion made by Kida, et al.,11 we observed absence of factor VIII immunoreactivity in a random pattern rather than specifically over the arachnoid cap cells. The usual thickness of the arachnoid cap was three to five cells, depending on the orientation of the section. The autopsy materials obtained in the eight patients with minimal SAH did not exhibit any differences from those acquired in the control group.

Arachnoid villi from patients with moderate-to-severe SAH differed notably from controls. Table 2 shows a synopsis of the observations in patients from the study group. In acute cases, erythrocytes were located in the central core and subdural compartment around the periphery (Fig. 1.2). After 2 to 3 days, erythrocytes accumulated more around the periphery, and erythrocyte degeneration with loss of staining was noted. Hemosiderin-containing macrophages and iron deposits were observed by 3 days at the earliest and prominently by 1 week post-SAH. Infiltration of inflammatory cells, both neutrophils (Fig. 1.3) and lymphocytes (Fig. 1.4), was observed. The neutrophils were present acutely and decreased in quantity after 10 days, whereas the lymphocytes were more persistent, but had disappeared by 2 months.

Cell cycle activity among the arachnoid cap cells was detected in six cases (Cases 5, 6, 10–13) by immunoreactivity to Ki-67 (Fig. 1.5) or the presence of mitotic figures (Fig. 1.6). Villi with blood and/or hemosiderin exhibited mitotic activity more often in four cases (Cases 10–13). Arachnoid cap cell collections 10 days post-SAH were focally thicker. This accumulation was more pronounced 2 months post-SAH (Case 14), on the order of 10 to 15 cell layers (Fig. 1.7), and immunoreactivity for epithelial membrane antigen highlighted the arachnoid cell accumulation (Fig. 1.8). In the same patient, neovascular channels were observed among the arachnoid cap cell collections (Fig. 1.7). The patient in Case 15 had suffered an SAH 34 years prior to death and exhibited a moderate degree of hydrocephalus. There was only mild accumulation of the arachnoid cap cells but a significant number of vascular channels within the villus cores and caps. A better appreciation of the altered distribution of vascular endothelia was achieved using factor VIII immunohistochemical

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**TABLE 1**

Characteristics of 15 patients with moderate-to-severe SAH*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs)</th>
<th>Fisher Grade</th>
<th>Sex</th>
<th>SAH</th>
<th>IVH</th>
<th>Hydrocephalus</th>
<th>Cause of SAH</th>
<th>Timing of SAH</th>
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<tbody>
<tr>
<td>1</td>
<td>61, F</td>
<td>sev</td>
<td>NA</td>
<td>mod</td>
<td>no</td>
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<tr>
<td>2</td>
<td>56, M</td>
<td>2</td>
<td>mod</td>
<td>no</td>
<td>mod</td>
<td>trauma</td>
<td></td>
<td>&lt;12 hrs</td>
</tr>
<tr>
<td>3</td>
<td>68, F</td>
<td>sev</td>
<td>sev</td>
<td>mod</td>
<td>mod</td>
<td>hypertension</td>
<td>&lt;12 hrs</td>
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</tr>
<tr>
<td>4</td>
<td>78, M</td>
<td>2</td>
<td>mod</td>
<td>mod</td>
<td>mod</td>
<td>trauma</td>
<td>24 hrs</td>
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</tr>
<tr>
<td>5</td>
<td>89, F</td>
<td>NA</td>
<td>sev</td>
<td>no</td>
<td>mod</td>
<td>congophilic angioptathy</td>
<td>24–48 hrs</td>
<td></td>
</tr>
</tbody>
</table>

* ACoA = anterior communicating artery; An = aneurysm; BA = basilar artery; ICA = internal carotid artery; IVH = intraventricular hemorrhage; MCA = middle cerebral artery; min = minimal; mod = moderate; NA = not available; PCoA = posterior communicating artery; sev = severe; un = unknown.

† This patient had two aneurysms. One had bled 7 years previously and the other bled 48 hours prior to death, causing massive intracerebral hemorrhage that compressed and distorted the ventricles.
Fig. 1. Photomicrographs of stained sections of arachnoid villi obtained at autopsy. 1: Normal arachnoid villus illustrating arachnoid cap cells stained red (arrow), central core with channels stained pale blue (asterisk), and surrounding dura stained dark purple-blue. Martius scarlet blue, original magnification ×100. 2: Section from patient with acute SAH (<24 hours) showing numerous erythrocytes (red) in the central core of the arachnoid villus. Martius scarlet blue, original magnification ×65. 3: Red-stained neutrophils (arrows) located in the apical portion of an arachnoid villus 10 days post-SAH. Chloracetate esterase, original magnification ×385. 4: Leukocyte common antigen immunoreactive lymphocytes (brown) located among the erythrocytes in the subcapsular space of an arachnoid villus 2 days post-SAH. Immunoreactive labeling, original magnification ×680. 5: Section showing Ki-67 immunoreactive arachnoid cell nuclei (arrows) 72 hours post-SAH. MIB-1 antibody labeling, original magnification ×510. 6: Mitotic figure among arachnoid cells 7 days post-SAH. H & E, original magnification ×495. 7: Arachnoid villus 2 months post-SAH, with marked accumulation of arachnoid cap cells (arrow). Martius scarlet blue, original magnification ×65. 8: Same site as illustrated in panel 7, showing immunoreactivity for epithelial membrane antigen (brown). Original magnification ×335.
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studies. No evidence of fibrosis was noted; specifically, the architecture of the villi remained generally intact and channels in the central core remained patent.

Discussion

The route of passage of CSF through the arachnoid villi to reach the venous system is still a point of contention. Key and Retzius6 demonstrated the function of arachnoid villi in 1875, and the concept of valvular action was introduced by Cushing.1 Ultrastructural studies of arachnoid villi in dog6 and monkey4 demonstrated an intact, nonfenestrated layer of endothelial cells over the villi. The opposing views concerning villus function are active (closed) and passive (open) transport between the subarachnoid space and venous lumen. Passive flow of CSF relies on the existence of endothelium-covered channels, creating an open communication between the two spaces. The active transport mechanism is favored if a continuous endothelial cell layer with tight junctions surrounds the arachnoid villus. Pinocytotic transport of macromolecules is shown by the presence of cytoplasmic vacuoles.2,23 However, in human arachnoid villi a point of controversy persists concerning the investment of arachnoid villi with an endothelial cell layer.5,12,17 Using light and electron microscopy, Kida, et al.,11 concluded that the apical portion of arachnoid villi is not covered by the collagenous capsule derived from dura or by endothelium. Our study confirmed that endothelial investment, based on factor VIII immunoreactivity, is interrupted, although we observed random absence of immunoreactivity. These observations indicate that the arachnoid cap cells themselves might constitute the last barrier between subarachnoid space and venous lumen. If so, their alteration could increase resistance to CSF flow.

Clearly, in acute cases of SAH obstruction of CSF flow can be caused by clotted blood in the ventricular system. This is the most probable origin of hydrocephalus in our patients with intraventricular hemorrhage (Cases 1, 3, 4, 6, 8, 9, and 11). However, our observations clearly indicate an increase in arachnoid cap cell mitotic activity 3 to 10 days post-SAH, and the cytoarchitecture of the arachnoid villi was subsequently noted to be altered by the accumulation of arachnoid cap cells, with aggregation of small vascular channels. These modifications, which were not seen in any control or nonhydrocephalic patients, might result in chronic obstruction of CSF flow. A notable shortcoming of this report is the small number of patients with chronic hydrocephalus post-SAH. Unfortunately, this patient population does not often come to autopsy.

Our observation of arachnoid cap cell proliferation is supported by animal experiments. Watanabe20 studied arachnoid villi in dogs 24 hours to 90 days post-SAH, and found that the arachnoid villi exhibited an increase in cellularity beginning at 30 days. Alksne and Lovings6 also used the dog model and observed an increase in arachnoid cell processes by 2 weeks. Proliferation of arachnoid cap cells could potentially obstruct the flow of CSF because the density of intertwined processes would not allow free movement of the extracellular fluid.

The mechanism by which SAH triggers proliferation of arachnoid cap cells is uncertain. Elevated levels of thrombin released by the blood clotting cascade in the CSF of patients with SAH15 could trigger this proliferation. An in vitro study has demonstrated proliferation of leptomeningeal cells after application of thrombin.13 Transforming growth factor-β (TGFβ), which can be released by inflammatory cells, also induced proliferation in this experiment. Inflammatory cells were consistently observed in the arachnoid villi of our patients with SAH. If the trigger for proliferation involves the inflammatory cascade, there may be a role for pharmacological inhibition. The use of steroid drugs in the management of patients with SAH is not currently recommended.6 However, experimental work in rabbits demonstrated a reduction in the incidence of hydrocephalus after SAH by treatment with intramuscularly administered steroid agents.21

The original hypothesis of arachnoid granule fibrosis as a cause for obstruction of CSF was based on only three cases.10 That neuropathological report included one case in which the convexity leptomeninges were thickened and fused; the other two cases involved fibrosis of the leptomeninges at the base of the brain. Torvik, et al.,16 concluded from their 43 cases of SAH that fibrosis or scarring of the granulations could not account for the development of hydrocephalus. Similarly, we observed no evidence of fibrosis in the arachnoid villi of our SAH group. Nonetheless, we cannot exclude the likelihood that diffuse fibrosis in the broader subarachnoid compartment might occur after SAH and that this could impede CSF flow.

Conclusions

The cause of hydrocephalus after SAH varies and a distinction must be made between acute and chronic situations. In most cases acute hydrocephalus is likely a simple mechanical obstruction caused by clotted blood. Chronic development of hydrocephalus post-SAH differs. We postulate that arachnoid cap cell proliferation causes resistance to CSF flow through arachnoid villi beginning days after SAH. This proliferation may be triggered by thrombin or cytokines, which are present in CSF after SAH. Interruption of the inflammatory cascade might prove clinically beneficial in reducing the development of chronic communicating hydrocephalus. Further experimental work is needed to confirm this hypothesis and to clarify the role of diffuse collagenscarring in the subarachnoid space.

Acknowledgments

We thank Sharon Allen and Susan Janeczko for technical assistance.
References

Manuscript received October 14, 1998.
Accepted in final form March 12, 1999.
Dr. Del Bigio is supported by a scholarship from the Manitoba Health Research Council.
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