Arachnoid cysts are intraarachnoid sacs containing CSF-like fluid that do not communicate with the ventricular system. They account for approximately 1% of all intracranial masses, with 50 to 60% occurring in the middle cranial fossa. Most arise as developmental anomalies. A small number are associated with neoplasms or can occur as complications following leptomenigoitits, hemorrhage, or surgery. They also occur within the spinal canal, with cysts or diverticula located subdurally or in the epidural space. Spinal arachnoid cysts are commonly located dorsal to the cord in the thoracic region. Intradural spinal arachnoid cysts are caused by a congenital deficiency within the arachnoid or are the result of adhesions due to previous infection or trauma.

Ultrastructural examination has shown that the cyst wall is formed from a splitting of the arachnoid membrane, with an inner and outer leaflet surrounding the cyst cavity. The origin of arachnoid cysts is still a matter of debate. Despite the numerous scientific theories, none has been proven yet. In summary, there are four proposed theories regarding the cause of these cysts: 1) a ball-valve mechanism; 2) an osmotic gradient between the intra- and extracystic medium; 3) primary malformation of the arachnoid membrane or cerebral lobe agenesis; and 4) fluid hypersecretion by the lining cells of the cyst wall. The cause of cyst enlargement is also debatable, although there is strong controversial evidence supporting the last two theories rather than the former. Brain water homeostasis and its regulatory pathways are weakly understood at the molecular level. In this brief report the authors attempt to add new insights into the pathogenesis of arachnoid cysts by considering aquaporin expression in the cyst wall and discuss possible future research directions and molecular targets.

**Key Words** • arachnoid cyst • hydrocephalus • aquaporin

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**Abbreviations used in this paper:** AQP = aquaporin; CSF = cerebrospinal fluid; NPA = asparagine-proline-alanine.
secretion theory, we tested surgical specimens of normal arachnoid membrane, arachnoid cysts, and arachnoid villi for the expression of AQP1, the second member of the AQP family. We have previously studied APQ1 and found it abundantly expressed in the choroid plexus (Fig. 2),

choroid plexus tumors (Fig. 3) associated with hypersecretive hydrocephalus, and cystic hemangioblastomas. The aim of this preliminary investigation was to describe the anatomical distribution of AQP1 in arachnoidal specimens in an attempt to find new data for the pathophysiological interpretation of arachnoid cyst formation and development.

**Materials and Methods**

We analyzed six specimens obtained during neurosurgical procedures: two normal arachnoid layers, two specimens of arachnoid cysts, and two arachnoid villi. Tissue specimens were fixed in 10% formaldehyde/0.9% NaCl and embedded in paraffin wax after 24 hours. Part of each specimen was then stained with H & E for diagnostic control. Coronal sections (5 μm thick) were collected on polylysine-coated slides and immunostained with a monoclonal antibody against the intracellular C-terminal AQP1 epitope (clone ab9566 1/22, dilution 1:100, Abcam). Immunohistochemical stainings were performed using a sensitive polymer-based detection system (Envision Plus, DakoCytomation). Heat-induced antigen retrieval was performed by incubating the slides for 40 minutes at 98˚C in a water bath and using 7.0 pH citrate buffer. All immunostainings were performed on an automated immunostainer (Dako Autostainer, DakoCytomation).

**Results**

No AQP1 immunoreactivity was found in the specimen of the normal arachnoid layer, in the arachnoid villus, or in the cells lining the cystic wall (Fig. 4 and 5).

**Discussion**

Congenital arachnoid cysts are developmental anomalies and contain clear CSF-like fluid. Arachnoid cysts most likely originate from a minor aberration in the development of the arachnoid that leads to splitting or duplication of the membrane. It has also been postulated that the cyst develops from a defect in condensation of the mesenchyme or from abnormalities of CSF flow. The association of other developmental abnormalities of the brain, such as heterotopias, lent support to this developmental theory. In the 208 reported cases of arachnoid cysts analyzed by Rengachary and colleagues, they found that the structural features of the arachnoid cyst wall that distinguish it from the normal arachnoid membrane were as follows: 1) splitting of the arachnoid membrane at the margin of the cyst; 2) a thick layer of collagen...
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in the cyst wall; 3) the absence of traversing trabecular processes within the cyst; and 4) the presence of hyperplastic arachnoid cells in the cyst wall.

In a study of five cases, Miyagami and Tsubokawa\textsuperscript{13} reported that the structure of the arachnoid cyst wall was similar to that of the normal arachnoid membrane and that the inner surface of the arachnoid cyst wall was formed of one or several layers of arachnoid cells with slender processes, which contained large extracellular spaces but no microvilli. These cysts appeared to be truly intraarachnoid in location and to be formed by splitting or duplication of the arachnoid membrane.\textsuperscript{14,15} In a study of nine cases, Schachenmayr and Friede\textsuperscript{17} found that the dominant phenomenon in the cyst wall was an absence of the normal trabeculation of the subarachnoid space, the trabeculae being replaced by tightly packed collagen fibrils and a few scattered cells in between. They found no evidence of a tight sealing of the extracellular spaces in the cyst wall.

A number of interesting theories have been proposed to explain the expansion of arachnoid cysts. According to the ball-valve hypothesis, an anatomical fissure of the cyst wall functionally acts as a one-way valve, allowing free entrance of CSF but preventing its exit into the subarachnoid space. One-way pulsatile movement of CSF has been demonstrated by cine-mode magnetic resonance imaging studies and confirmed at endoscopic operations by inspecting the inside of the cyst wall.\textsuperscript{16} The theory of an osmotic gradient between cystic contents and CSF lacks support given that the cystic content is similar in composition to CSF.\textsuperscript{17} There has been no evidence of either a tight sealing of the extracellular spaces in the cyst wall or an active transcellular fluid movement in some studies.\textsuperscript{14,15}

Fluid production by the cells lining the cyst wall is another aspect that has been considered. There is morphological and ultrastructural evidence to support the secretory nature of the cyst wall. Ultrastructurally, the cyst lining cells demonstrate the presence of microvilli on the luminal surface and cytoplasmic vesicles that are consistent with fluid secretion.\textsuperscript{8} Moreover, enzyme immunocytochemistry demonstrated Na-K adenosine triphosphatase in the plasma membranes lining the cavity at the luminal side and near the intercellular clefts at the basolateral side, a structural organization consistent with fluid transport toward the lumen.\textsuperscript{6} The argument against continuous secretion lay in the fact that cysts often remain static in size and sometimes disappear, thus demonstrating that secretion is neither universal nor likely the only mechanism involved. Schuhmann and colleagues\textsuperscript{18} have described choroid plexus ectopia inside a growing arachnoid cyst. Our results seem to put the hypersecretion theory into the right perspective. Considering our previous data, we tested AQ1, which seems to be the most realistic member expressed in this kind of disorder. Given the technical limits of immunohistochemistry, it is impossible to preclude the expression of other members of the AQ1 family at the subcellular level. There was no staining for AQ1 in the arachnoid villi (Fig. 6), where CSF absorption occurs, thus offering more evidence for the mechanical theory of CSF pressure gradient absorption and for translation to the ball-valve mechanism of cyst development.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.jpg}
\caption{Photomicrograph revealing a normal arachnoid membrane. H & E, original magnification $\times$ 200.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig5.jpg}
\caption{Photomicrograph showing the results of immunohistochemical staining with AQ1 epitope (clone ab9566, Abcam) for AQ1 in an arachnoid cyst membrane. It is possible to depict AQ1 expression in red blood cells of an arachnoid capillary but not in the cells lining the cyst wall. Original magnification $\times$ 100.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig6.jpg}
\caption{Photomicrograph revealing immunohistochemical staining with AQ1 epitope (clone ab9566, Abcam) for AQ1 in an arachnoid villus. Note the complete absence of AQ1 expression in this anatomical structure. Original magnification $\times$ 200.}
\end{figure}
References


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