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Cytologic Diagnosis of Intraventricular Hemorrhage in a Neonate

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A neonate with autoimmune thrombocytopenia presented with megacephaly and hydrocephalus. The cause remained obscure until hemosiderin-laden macrophages were found in the ventricular cerebrospinal fluid (CSF), indicating prenatal intracranial hemorrhage.

The literature on the diagnosis of intraventricular hemorrhage by the cytologic examination of CSF is reviewed and discussed. The cytologic evaluation of CSF may be helpful in cases of central nervous system dysfunction when the cause is obscure.

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The cytologic analysis of cerebrospinal fluid (CSF) allows the diagnosis of certain neoplasms and infections of the central nervous system. As cytologic techniques are applied more widely, diagnoses can also be made of central nervous system lesions of obscure origin.[1,3] We wish to record the cytologic diagnosis of cerebral intraventricular hemorrhage in a newborn infant who also had hydrocephalus and megacephaly.

Case Report

A 12-hour-old female infant was transferred to the Massachusetts General Hospital (MGH) because of an enlarging head and thrombocytopenia. She was born at 38 weeks' gestation of a healthy mother (gravida 3, paragravida 3). There were no complications except for maternal exposure to varicella in the second trimester of the pregnancy.

The baby was delivered by a vertex presentation under local block anesthesia. She weighed 3.35 kg, with Apgar scores of ten at one and five minutes. No abnormalities were noted except for an enlarged head (39 cm circumference), which was over the 97th percentile. Later in the same day the child was noted to have 7,000 platelets per milliliter and petechiae over the back, front and limbs and was transferred to the MGH.

On admission the infant was megacephalic and had a markedly increased cranial circumference, widened cranial sutures, a full frontal fontanelle and numerous petechiae over the trunk, limbs and palate. Retinal hemorrhages and hepatosplenomegaly were noted. The neurologic examination suggested hydrocephalus. The platelet count was 3,000 per milliliter. The electroencephalogram (EEG) was mildly abnormal, with posterior sharp waves. A computerized axial tomographic (CAT) scan of the head demonstrated cystic lesions in the cerebrum and hydrocephalus and questionable intracerebral calcification (Figure 1). An infectious condition such as toxoplasmosis was considered. Treatment consisted of platelet transfusion, steroids and exchange transfusion. Because of the hydrocephalus, a right cerebral ventricular-peritoneal shunt was performed. Since chronic intraventricular hemorrhage *in utero* was also considered to be a possibility, the ventricular CSF was examined cytologically with a Millipore filter preparation and smears of the deposit obtained by centrifugation. These preparations were stained with Papanicolaou stain and by Prussian blue stain for iron. They demonstrated numerous hemosiderin-laden

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Figure 1
CAT scan, without contrast at the level of the lateral ventricles, showing large cystic lesions, hydrocephalus and an area of increased attenuation values on the right, suggesting calcification.

macrophages (Figures 2 and 3). All the cultures and serologic examinations for the presence of microorganisms were negative.

After the shunt was inserted, the circumference of the head decreased, approaching normal. A follow-up EEG was normal. A repeat CAT scan showed smaller cerebral ventricles. At follow-up one month later the child was thriving, with a stable platelet count of 150,000 per milliliter.

Discussion

The cytologic examination of cerebral ventricular fluid may be helpful in patients who are suspected of having primary or metastatic neoplasms in the brain, who are undergoing craniotomy or who have obscure diagnostic problems. An examination for evidence of previous hemorrhage is rare.[5] This technique seems useful in analyzing cases of obscure hydrocephalus, of central nervous system lesions associated with platelet or coagulation defects and of suspected intracranial hemorrhage. Cytologic analysis might also assist in determining the time of bleed.

When blood enters the CSF, a leptomeningeal cell reaction takes place[3] with the appearance of granulocytes. After about four hours the first signs of phagocytosis appear. Monocytes, the first to undergo cytoplasmic activation, initiate phagocytosis but are not effective in eliminating the numerous red blood cells (RBCs). Active proliferation of leptomeningeal tissues occurs at about 12 to 18 hours. Many macrophages appear, and RBCs in contact with them are engulfed within a few hours. Enzymatic destruction of the hemoglobin occurs first, with RBCs appearing as empty cytoplasmic vacuoles in the macrophages. Destruction of the more resistant stroma of RBCs takes longer. At about four days the first hemosiderin granules are seen in the cytoplasm of phagocytes,[4,6] often referred to as "hemosiderophages." Hemosiderin usually appears in the cytoplasm as large, coarse, dark brown to gray-black granules or finely distributed minute granules.[6] Hematoidin is deposited in the cytoplasm somewhat later, usually as brownish yellow granules.

The life span of phagocytes is relatively long, and they can still be found in the subarachnoid space more than six months after the initial hemorrhage.[2] A long life span can be simulated when aging macrophages and their contents are in turn phagocytized by younger cells. Macrophages can provide evidence not only of a single hemorrhage but also of repeated hemorrhages. In the case of repeated hemorrhage, recently ingested RBCs, bleached RBCs, hemosiderin and hematoidin are simultaneously present in the cytoplasm.

The Prussian blue stain is specific for iron in the ferric form. It provides conclusive evidence of the presence of hemosiderin and distinguishes it from other types of brown granules in the cytoplasm (Figure 3).

In the newborn infant reported above, suspected intraventricular cerebral hemorrhage was confirmed by cytologic examination of ventricular CSF. In addition, since the macrophages contained granules rather than RBCs, the hemorrhage could be dated as having occurred *in utero*. The Prussian blue stain demonstrated conclusively that the granules were derived from the breakdown of hemoglobin rather than from some other source since they contained iron. Cytologic examination and specific staining may establish such a diagnosis in other obscure cases.

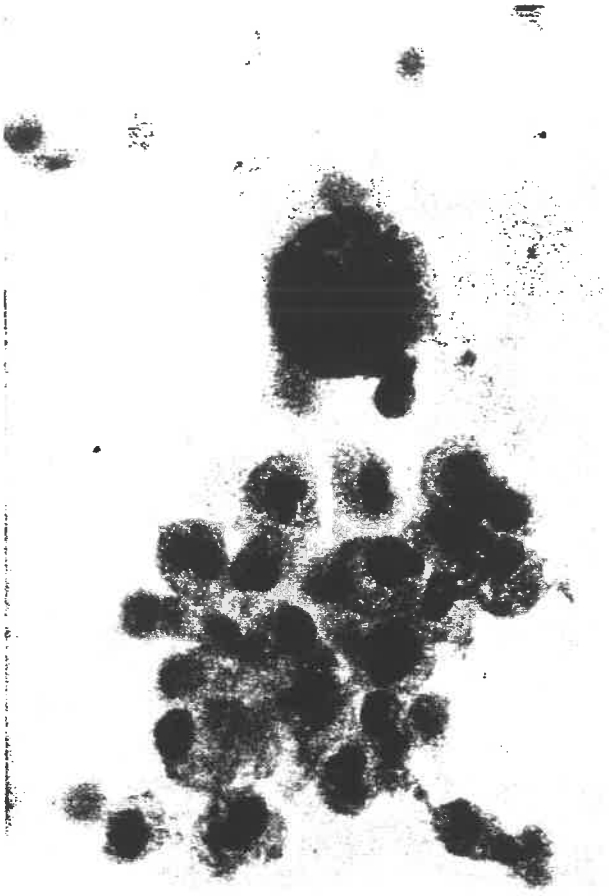

Figure 2
Ventricular CSF showing macrophages with brownish granules of hemosiderin (Papanicolaou stain, × 570).

Figure 3
Ventricular CSF, with Prussian blue reaction confirming the presence of iron (stained blue) in the granules (× 570).

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