Gliomatosis cerebri: cytologic and autopsy findings in a case involving the entire neuraxis

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Abstract. We describe the case of a 7-year-old girl who was clinically diagnosed as having a pontine glioma based on magnetic resonance imaging studies. Neoplastic cells were identified upon cytologic examination of cerebrospinal fluid. Autopsy studies revealed an anaplastic astrocytoma (WHO grade III) diffusely infiltrating the cerebral hemispheres, brain stem, cerebellum, leptomeninges, and spinal cord to the level of the conus medullaris. The Ki-67 labeling index focally approached 30%. Although many of the neoplastic cells displayed elongated twisted nuclei reminiscent of microglia, these cells stained intensely for glial fibrillary acidic protein, supporting an astrocytic origin. Unusual features of this case of gliomatosis cerebri include involvement of the entire central neuraxis, correlation with pre-mortem lumbar puncture cytology, and a markedly elevated Ki-67 labeling index.

Introduction

Gliomatosis cerebri is a diffuse infiltrating glioma that may involve the supratentorial, infratentorial, and intraspinal compartments. Historically, diagnosis of this rare tumor was based upon autopsy examination of the central nervous system [Burger and Scheithauer 1994]. Presently, pre-mortem diagnoses can be made using improved neuroimaging techniques and microscopic evaluation of brain biopsies [Ross et al. 1991]. The literature on gliomatosis cerebri is generally restricted to individual case reports and short reports or reviews. One comprehensive review of the literature declared 160 cases reported since 1897 [Jennings et al. 1995]. We had the rare opportunity to correlate pre-mortem clinical, radiographic, and cytologic data with post-mortem gross, microscopic, and immunohistochemical examination of the brain and spinal cord.

Case report

A 7-year-old girl presented with a one-month history of headaches, neck pain, and emesis. She was agitated and confused, and papilledema was noted by fundoscopic examination. Magnetic resonance imaging (MRI) of the brain showed abnormal high signal on the T2-weighted image within the pons and left middle cerebellar peduncle (Figure 1a) with no areas of enhancement. A diagnosis of pontine glioma was made, although the absence of cranial nerve symptoms, ataxia or other focal neurological deficits was unusual. She received 7265 cGy of hyperfractionated radiotherapy. Early in her course of radiation, she had progressive neurological worsening, was diagnosed with hydrocephalus, and underwent placement of a ventriculoperitoneal shunt. Following completion of radiation she improved clinically, however, three months following the initial presentation, a repeat MRI of the brain showed multiple areas of signal abnormality within the cortex and white matter of the cerebral hemispheres (Figure 1b). MRI of the spine showed leptomeningeal enhancement extending to the level of the conus medullaris following administration of gadolinium (Figure 1c). Cerebrospinal fluid from a lumbar puncture contained neoplastic cells. She died five months following her initial presentation, and a com-
Figure 1a (left). Magnetic resonance imaging (MRI) of the brain. T2-weighted image. Abnormal high signal is present in the pons and left middle cerebellar peduncle (arrows). The lesion was non-enhancing on T1-weighted images; b (right): T2-weighted MRI of the brain three months following presentation. Multiple areas of signal abnormality appeared within the cortex and white matter of the cerebral hemispheres (arrows). Subependymal enhancement and slight leptomeningeal enhancement was observed on T1-weighted images; c (below): T1-weighted post gadolinium MRI of the spine showed leptomeningeal enhancement extending to the level of the conus medullaris (arrow).

A complete post-mortem examination was performed.

Materials and methods

Tissue samples obtained at autopsy were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin for routine histological examination. Immunohistochemical analysis was performed on formalin-fixed paraffin-embedded sections, using a rabbit polyclonal antibody for glial fibrillary acidic protein (GFAP) (Dako, Santa Barbara, CA; dilution 1 : 4000), and monoclonal mouse antibodies for Ki-67 proliferation-related antigen (MIB-1, Zymed, San Francisco, CA; dilution 1 : 100), and microglial/macrophage markers (KP-1, Dako; dilution 1 : 50), and (HAM-56, Dako; dilution 1 : 30).

Pathologic findings

Cytologic analysis of pre-mortem CSF obtained via lumbar puncture revealed neo-
Figure 2a. Malignant cells identified in CSF obtained by lumbar puncture (original magnification in each panel, x 1700); a: cluster of three anaplastic cells showing irregular nuclei with regions of abnormal chromatin clearing and vacuolated cytoplasm (Papanicolaou-stained filter preparation); b: pair of anaplastic cells with irregular regions of chromatin condensation and clearing. Note the tongue-like nuclear protrusion (arrow). (Papanicolaou-stained filter preparation); c: pair of neoplastic cells with eccentric skirts of fibrillary cytoplasm (Papanicolaou-stained filter preparations).

plastic cells with varied morphology. The cells were clustered in small discohesive groups. Some displayed polygonal, vesicular nuclei with chromatin clumping and vacuolated cytoplasm (Figure 2a). Others had round convoluted nuclei with tongue-like protrusions of chromatin (Figure 2b). Many of the cells had an eccentric skirt of fibrillar cytoplasm (Figure 2c). Rare ovoid or curled cells with elongated C-shaped nuclei were also observed.

The fresh brain weighed 1440 grams. The leptomeninges were cloudy and congested. The vessels at the base of the brain were
normal. Bilateral uncal and cerebellar tonsillar herniation were evident. The left olfactory bulb was expanded to 1 cm in diameter. Coronal sections revealed that the corpus callosum and the body and crus of the fornix (Figure 3a) were expanded by a diffusely infiltrating tumor. The cortex was grossly unremarkable, and the gray-white matter junction was well preserved. The pons was enlarged eccentrically to the left, and both middle cerebellar peduncles were expanded by tumor (Figure 3b). The spinal nerve roots exiting the lumbosacral spinal cord were encased by tumor.

Microscopically, tumor diffusely infiltrated all lobes of the cerebral hemispheres, cerebellum, fornix, corpus callosum, olfactory bulb, brainstem, leptomeninges, and spinal cord to the level of the conus medullaris (Figure 4a). Tumor was present in all histologic sections, with the fornix and olfactory bulb displaying the greatest degree of anaplasia and cellularity. Infiltrating tumor cells were seen in normocellular regions of the cortex and along white matter tracks. The glial neoplasm was composed of cells displaying fibrillary or vacuolated cytoplasm. The nuclei ranged from round and rod-shaped to irregular, lobulated hyperchromatic forms, similar to those observed by CSF cytology (Figure 4b). Twisted rod-shaped cells predominated in some regions of the white matter and leptomeninges. Mitotic figures were numerous, yet there was no evidence of necrosis, vascular endothelial proliferation or secondary structures.

Immunohistochemical staining for the Ki-67 proliferation antigen using the MIB-1 antibody revealed a labeling index of 30% in the septal region and fornix (Figure 5a). Immunolabeling for the glial fibrillary acidic protein (GFAP) was particularly intense within elongated cells with rod-shaped nuclei that infiltrated the leptomeninges, although a number of cells with rounded or bizarre nuclei stained as well (Figure 5b). Immunostains for the microglial markers KP-1 and Ham-56 were negative in the neoplastic cells, thus excluding diffuse macrophage infiltrate secondary to therapeutic effects and the even more rare tumor, microglioma [Hulette 1996].

General autopsy findings included bilateral acute bronchopneumonia, pulmonary edema, and pulmonary congestion.
Figure 4b. In this histologic section, note the round, oval, C-shaped, and bizarre multilobulated nuclear forms similar to those observed on cytologic filter preparations. In addition, thin, elongated, and twisted nuclei were more common in histologic sections than in the CSF cytology specimen. A mitotic figure is evident (arrow) (HE, original magnification 520 x).

Figure 5a. Immunohistochemical staining for the Ki-67 proliferation antigen revealed a labeling index of 30% in the paraventricular region and the fornix. In the center of the figure, tumor cells appear to rupture through the ependymal lining to form a mass within ventricular space (upper right portion of image). The nuclear counterstain reveals an intact ependymal layer, which abruptly ends (arrow), and then re-appears in the lower third of the image (MIB-1 immunostain, original magnification 170 x).

Discussion

In the early years of the 20th century, there were reports of cases characterized pathologi-
in the CSF was a 9-year-old girl with diffuse cerebrospinal gliomatosis extending to the lower thoracic level (T11) [Onal et al. 1996]. Cytologic study was reported as negative in another case of gliomatosis cerebri extending to the lower thoracic spinal cord (T9) [Kawano et al. 1978]. The identification of malignant cells within the CSF of this case is particularly striking given the fact that positive cytologies are obtained in only about 20% of symptomatic metastatic meningeal gliomatosis associated with astrocytomas in general [Packer et al. 1983].

Microglioma is a rare histiocytic neoplasm of the central nervous system which is highly infiltrative and appears to arise from resident tissue histiocytes in the brain [Hulette 1996]. The principle differential diagnosis of microglioma is gliomatosis cerebri. Neoplastic microglia are usually long and slender with twisted nuclei, however, there can be histological overlap with neoplastic glia. Defining the cell of origin may depend on immunohistochemistry. In our case, focal areas of tumor cells displayed nuclear morphology reminiscent of microglial cells with elongated rod-shaped nuclei. Thick elongated processes associated these elongated nuclei, however, were intensely highlighted by immunostains for GFAP, while all neoplastic cells remained negative for KP-1 and HAM-56, supporting an astrocytic, rather than a microglial origin.

The remarkably diffuse nature of gliomatosis cerebri, which in fact defines this group of neoplasms, supports the concept of diffuse neoplastic transformation as an alternative to the more conventional notion of single-cell transformation [Scherer 1940]. Alternatively, extraordinary motility of clonally transformed cells has been suggested as a possible explanation [Leenings et al. 1995]. Molecular events contributing to increased motility may include amplification or mutation of the epidermal growth factor receptor, which is involved in regulating cell proliferation, differentiation, and motility through several intersecting signaling pathways [Xie et al. 1998]. A deletion mutant of this receptor found in 50–60% of high-grade astrocytomas utilizes an altered spectrum of signaling cascades [Chu et al. 1997], which may hypothetically influence adhesion or motility. The exceptional diffuseness observed in this
particular case, however, would favor multifocal synchronous transformation. Future molecular genetic analyses of widely separated regions within these glial neoplasms may help resolve these issues.

The Ki-67 antigen is present within the nuclei of cells in the proliferating phases of the cell cycle. MIB-1 is a monoclonal antibody directed against recombinant parts of the Ki-67 antigen, and has the advantage of detecting proliferating cells in formalin-fixed paraffin sections [Cattoretti et al. 1992]. A general correlation exists between histologically determined tumor grade and the Ki-67 labeling index [Burger et al. 1986]. The Ki-67 labeling index for anaplastic astrocytoma WHO III has ranged in the literature from 1.6% [Burger et al. 1986] to 11.2% [Karamitopoulou et al. 1994], with individual cases of 32% [Hsu et al. 1997]. The utility of Ki-67 immunolabeling has not been well studied in autopsy material. We have observed reliable labeling in three autopsied gliomas, although serial stains performed in this case revealed a decrease in nuclear staining intensity with long fixation times (> 1 month).

We are aware of only two cases of gliomatosis cerebri in which the Ki-67 labeling index was examined. One was a 52-year-old man who was histologically diagnosed with a low-grade glioma with a MIB-1 labeling index of 3.57% [Kannuki et al. 1997]. Thirty months following whole brain irradiation and chemotherapy, he had a presumed complete remission. The second case was a 46-year-old woman diagnosed with a low-grade astrocytoma. Pre-mortem MIB-1 index was 6.3%, and post-mortem MIB-1 index was 7.6%. The pre-mortem biopsy showed a monotonous increase of gemistocyte-like cells; in contrast, the post-mortem study showed higher cellularity, nuclear atypia, and focal necrosis [Nishioaka et al. 1996]. The present case had a Ki-67 labeling index of ~ 30%. While cases of gliomatosis cerebri often exhibit morphologies reminiscent of low-grade gliomas, the extensive regions of high cellularity, pleomorphic nuclear atypia, mitotic activity, and Ki-67 labeling indices in this case were indicative of a WHO grade III astrocytic neoplasm.

In summary, we have presented an example of gliomatosis cerebri with correlation of pre-mortem and post-mortem evaluations. This case was notable for unusually extensive permeation of the entire central neuraxis in addition to extensive leptomeningeal involvement, correlating with pre-mortem spinal MRI scans and lumbar puncture cytologies. The clinical aggressiveness of this tumor may be reflected by the high Ki-67 proliferation index and histologic identity with anaplastic (WHO Grade III) astrocytoma. GFAP immunostains further support an astrocytic histogenesis for gliomatosis cerebri, as suggested by recent classification schemes [Bigner et al. 1998].

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