Cytological Diagnosis of Metastatic Glioblastoma in the Pleural Effusion of a Lung Transplant Patient

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The extracranial metastasis of glioblastoma is a rare event. We report the case of a patient who developed metastatic glioblastoma in pleural effusion 15 months after lung transplant, with emphasis on differential diagnosis based on cytological material. In our case, tumor cells had pleomorphic nuclei, prominent nucleoli, and fine vesicular chromatin. Some were arranged in a poorly formed pseudo-glandular architecture, mimicking a poorly differentiated adenocarcinoma. The cytological diagnosis of metastatic glioblastoma is difficult and depends critically on clinical history and suspicion, particularly in the transplant setting. Review of the literature indicates that transmission/metastasis of intracranial malignancy occurs rarely following organ transplantation, with some debate on the suitability for transplant of organs from affected donors. Although the situation is uncommon, this report of the cytological findings of extracranial glioblastoma may extend our current knowledge and provide additional differential diagnostic information for this entity. Diagn. Cytopathol. 2014;42:619–623.

Key Words: cytology; pleural effusion; metastatic intracranial neoplasm; glioblastoma; lung transplant

Glioblastoma is the archetypal high-grade intracranial malignancy (ICM).[1,2] Similar to other central nervous system (CNS) malignant neoplasms, extracranial metastasis of glioblastoma is rare. It usually occurs when the tumor invades the dural veins,[1,2] though it may also be more likely due to dural disruption for shunt placement, biopsy, or debulking.[3–5] The extracranial spread of glioblastoma can be divided into regional and/or distal metastasis,[6] with the bone and marrow being common sites of distal spread. Metastases to spleen, skin, heart, cervical lymph nodes, and lung have also been reported.[5–8] The diagnosis in most cases was based on the clinical history, the fine-needle aspiration (FNA) cytology, and the immunohistochemical profile of the tumor cells.[9,10]

Nonetheless, only a handful of cases have been reported in the cytopathological literature describing the cytological findings of metastatic glioblastoma.[8–12] Most commonly seen were sheets of heterogeneous malignant cells with tumor necrosis and prominent transversing blood vessels.[8–12] These features are often seen in other malignant tumors, rendering cytological diagnosis and differentiation of this seldom-seen metastasis from other commonly encountered tumors challenging. Although rare, the literature does contain reports of transmission/metastasis of ICM following organ transplantation.[13–18]

Herein, we report an unusual case of metastatic glioblastoma to the pleural effusion after lung transplant. In addition to the classic cytological features described above, our case revealed tumor cells with pleomorphic nuclei, prominent nucleoli, fine vesicular chromatin, scant cytoplasm, and areas with poorly formed pseudoglandular architecture, mimicking a poorly differentiated adenocarcinoma. These cytologic findings may extend our current knowledge, providing additional diagnostic information for metastatic glioblastoma.

Case Report

Clinical History

A 57-year-old man with severe chronic obstructive pulmonary disease received a lung transplant from a donor

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subsequently found at autopsy to have glioblastoma. At the time of autopsy no extracranial metastasis was found in the donor. The recipient declined to be listed for retransplantation. He initially did well post-transplant, with considerably improved quality of life. Fifteen months after the transplant, the patient suffered progressive dyspnea and underwent imaging of the chest. This revealed bilateral lung masses, enlarged mediastinal lymph nodes and pleural effusion. Positron emission tomography (PET) scan showed avidity in the mediastinal nodes and the pulmonary masses. Multiple lesions in the liver were also identified. Cranial imaging at this time showed no intracranial pathology. The patient underwent lung biopsy. Initial transbronchial FNA showed "atypical epithelial cells." The pleural fluid cytology was diagnosed as "a poorly differentiated carcinoma." Tumor cells were negative for multiple epithelial markers. The following month FNA of a paratracheal lymph node revealed similar tumor cells, found to express glial fibrillary acidic protein (GFAP). The patient expired shortly thereafter.

Cytological and Histological Findings

Cytological slide preparation and immunohistochemistry. Material was obtained by thoracentesis of the pleural effusion. Cytospin slides were prepared, then fixed in 95% ethanol, and stained by the Papanicolaou method. Cell blocks were also prepared, fixed in formalin, and processed in the histology laboratory according to standard protocols. Sections of the cell block were stained with hematoxylin and eosin (H&E). Lymph node excision specimens were fixed in formalin and processed in the histology laboratory. Immunohistochemistry (IHC) studies were performed on the cell block material using the Dako autostainer. The sections were cut at 4 micron thickness and deparaffinized prior to incubation with primary antibodies. Heat antigen retrieval at 70°C for 40 min was also used to enhance signal detection. Primary antibodies were diluted according to standard protocols and manufacturer suggestions. These antibodies included: GFAP (1:500 dilution, DAKO, Carpinteria, CA), calretinin (prediluted, Cell Marque, Hot Springs, AK), Ber-EP4 (1:30 dilution, DAKO, Carpinteria, CA), CK5/6 (prediluted, Ventana, Tucson, AZ), CK7 (1:500 dilution, DAKO, Carpinteria, CA), CK20 (prediluted, DAKO, Carpinteria, CA), and thyroid transcript factor 1 (TTF1, prediluted, Ventana, Tucson, AZ). All antibodies were monoclonal, except calretinin which was polyclonal.

Cytological findings. Cytological examination of the effusion specimens revealed malignant cells in a background of numerous reactive mesothelial cells, mixed inflammatory cells, and blood (Fig. 1). They were arranged predominantly as small loosely cohesive clusters. The tumor cells had an epithelial appearance, with high nuclear-cytoplasmic (N/C) ratio and large pleomorphic nuclei. The nuclei were often eccentrically located with irregular nuclear membranes, and had prominent nucleoli. The chromatin was finely granular to vesicular in character. The cytoplasm was scant and vacuolated (Fig. 2). Glial cytoplasmic processes, a critical diagnostic feature in many intracranial malignancies, were not seen in tumor cells in the effusion. In some areas, tumor cells were also arranged in acinar configurations, mimicking a poorly differentiated adenocarcinoma, but without true glandular formation (Fig. 3). IHC showed that tumor cells were diffusely positive for GFAP (Fig. 4), but negative for calretinin, Ber-EP4, CK5/6, CK7, CK20, and TTF1 (data not shown).

Histological findings. In the cell block section, tumor cells had large hyperchromatic nuclei, irregular nuclear membranes, fine chromatin, and prominent nucleoli (Fig. 5). However, the N/C ratio was reduced in the cell block section relative to the cytospin preparation, and consistent with proportions encountered in intracranial glioblastoma. Tumor necrosis and desmoplastic stromal reaction were present. The biopsy specimen of lung mass and a lymph node showed a similar morphology; tumor cells were strongly and diffusely positive for GFAP and CD56, and were negative for cytokeratin AE1/AE3, thyroid transcription factor-1, melanoma marker HMB-45, cytokeratins 7 and 20, chromogranin, CD45, CAM5.2, and prostate-specific antigen (data not shown).

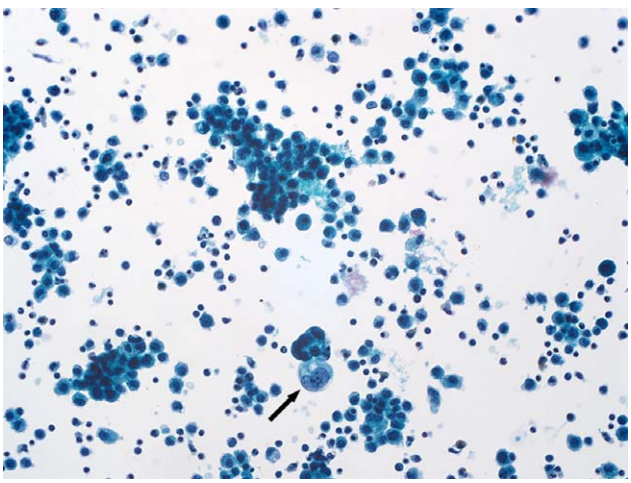

Fig. 1. Metastatic GBM in the pleural effusion seen at low magnification. The specimen shows an increased cellularity. The tumor cells (arrow) are seen as scattered individual cells or arranged in loosely cohesive clusters. Reactive mesothelial cells are numerous in the background. (cytospin, Papanicolaou stain, 100×). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

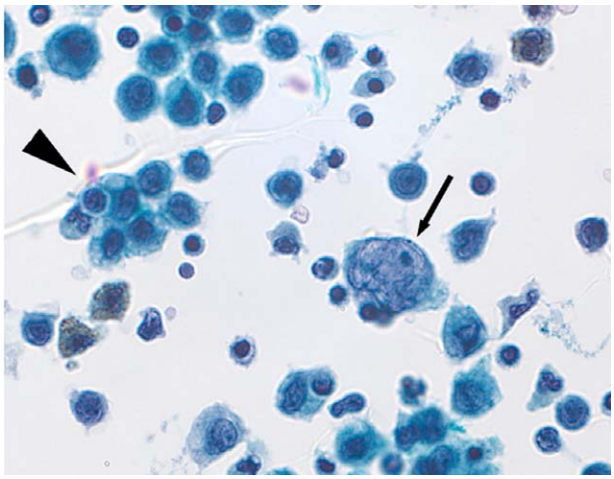

Fig. 2. Metastatic GBM in the pleural effusion seen at high magnification. The tumor cells (arrow) have large hyperchromatic nuclei, irregular nuclear membranes, fine to vesicular chromatin, and one prominent or several smaller nucleoli. The cytoplasm is scant and vesicular. Reactive mesothelial cells (arrowhead) are also prominent. (cytospin, Papanicolaou stain 260×). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Although we were unable to review the morphology of the tumor from the donor, the immunoprofile and the histomorphology confirmed the diagnosis of glioblastoma.

Discussion

Malignant pleural effusion affects over 150,000 patients each year in the United States; it is a manifestation of advanced malignant disease and associated with a poor prognosis.[19] The most common metastases to the pleura are lung adenocarcinomas in both men and women and breast carcinomas in women. Other common tumors that

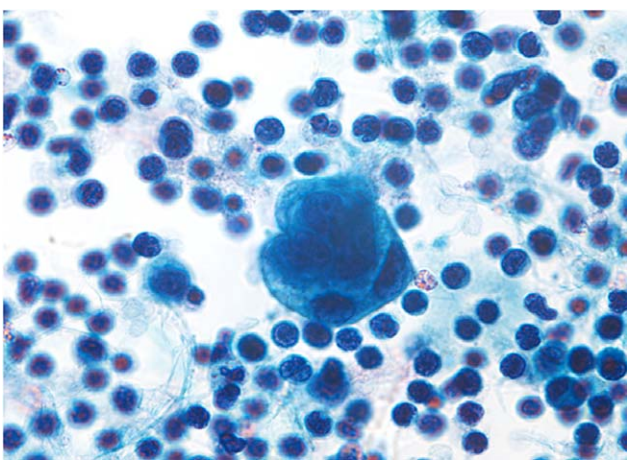

Fig. 3. Metastatic GBM in the pleural effusion seen at high magnification. The tumor cells also form pseudo-acinar arrangements and mimic a poorly differentiated adenocarcinoma, but, there is no true glandular formation (cytospin, Papanicolaou stain 260×). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

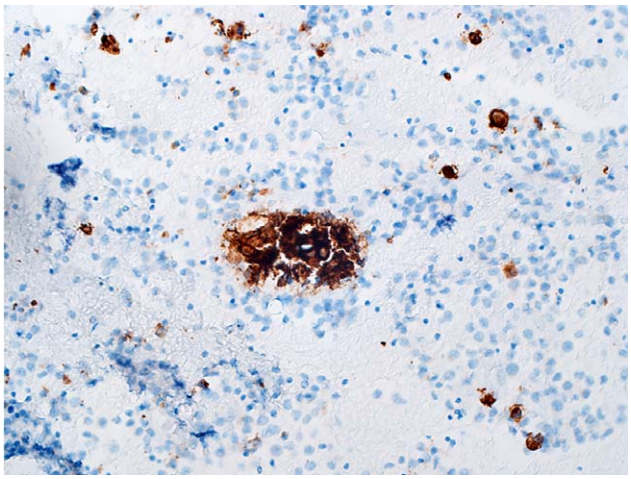

Fig. 4. Immunohistochemical stain of tumor cells. IHC stain shows that the tumor cells are positive for GFAP (160×). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

may present as malignant effusions include lymphoma, mesothelioma, and melanoma.[19] In addition, pleomorphic tumor cells may lead to the consideration of a metastatic sarcoma. In our case, the pleural effusion occurred after lung transplantation. The large tumor cells showed pleomorphic nuclei and prominent nucleoli, scant and vacuolated cytoplasm, and pseudo-acinar arrangement but no true gland formation.

In exfoliatvie cytology of adenocarcinoma of the lung, the tumor cells form three-dimensional tight clusters and acinar structures. Some tumor cells may also have intracytoplasmic mucin. These features indicate glandular differentiation. The nuclei in adenocarcinoma have coarse

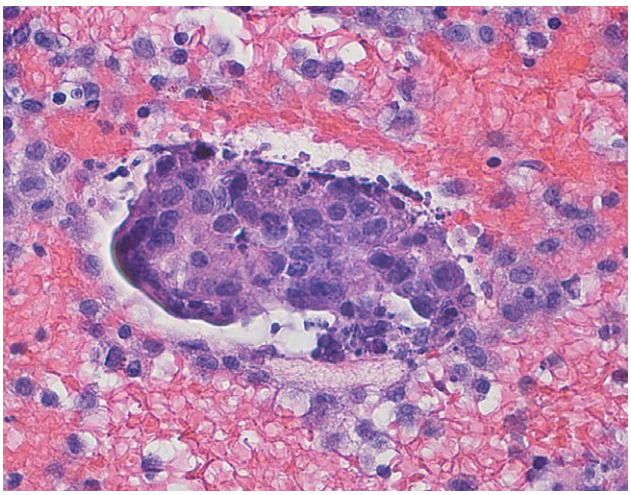

Fig. 5. Cell block section. Tumor cells had large hyperchromatic nuclei, irregular nuclear membranes, fine chromatin, and prominent nucleoli (H&E stain 160×). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

chromatin, irregular nuclear membranes, and prominent nucleoli. The cytoplasm of the tumor cells is moderate and has a feathering or lacy appearance, and the cells do not have distinct cell borders.[20,21] Immunohistochemical stains of tumor cells are positive for cytokeratin, BerEP4, TTF-1, and Napsin-A in metastatic lung adenocarcinoma,[21] but negative for calretinin. Malignant mesothelioma tends to diffusely involve the serous cavities.[22] It may show hypercellularity, with clusters of tumor cells. The tumor clusters of malignant mesothelioma have scalloped edges and are usually much larger than those of adenocarcinoma.[22] Individual tumor cells can be relatively uniform and intermediate in size with scant to moderate amounts of cytoplasm. The chromatin is finely granular in texture. Nucleoli are small and inconspicuous. In IHC studies these tumors are usually positive for cytokeratin, calretinin, WT1, p53, and D2–40.[20,22] Helpful diagnostic features of metastatic sarcomas in effusion cytology are markedly pleomorphic malignant cells, prominent nucleoli, and the presence of cytoplasmic features such as striation (for muscular differentiation) or dense material (for cartilaginous differentiation).

Glial cytoplasmic processes, a critical diagnostic feature in many intracranial malignancies, were not seen in tumor cells of our case. They may not be as prominent due to cell rounding in effusions and/or due to the less differentiated nature of the metastatic tumor. In our case, the GFAP positivity was critical to render the diagnosis. GFAP has a molecular weight of 50 kDa and belongs to the family of intermediate filament proteins. It is expressed by astrocytes and ependymal cells of CNS and is a diagnostic marker of glial differentiation.[23,24] It has also been expressed in several other cell types, including glomerular cells of the kidney, Leydig cells of the testis, osteocytes, and chondrocytes.[24,25] In addition to GFAP-producing brain tumors, a few other tumors have been reported to express GFAP, such as salivary gland tumors,[26] gastrointestinal stromal tumors,[27] soft tissue myoepitheliomas, osteosarcoma, and chondrosarcomas.[24] Therefore, in addition to glial neoplasms, the above-mentioned tumors should be considered when interpreting a positive GFAP immunostain.

An uncommon aspect of our case is the patient's status as a lung transplant recipient. Due to immunosuppression, the risk of development of malignancy is greater following organ transplantation. Although primary intracranial malignancies are generally viewed as having low risk of extracranial metastasis,[1,2] many studies have been conducted to assess the risk of transmission of such tumors from the donor following transplantation.[13–18] Recently, Watson et al. evaluated transplants from 177 donors with primary ICM in the British health system over a 16-year period; they found that none of the 448 recipients developed an intra- or extracranial malignancy.[17] Similarly, a study of 642 donors with ICM revealed that 175 organs were harvested from donors with glioblastoma, and among them no tumor transmission was documented.[28] A US study, by Nalesnik, et al., characterized the transmission risk from organ donors with ICM as follows: low-grade malignancies (WHO grades I and II) confer low risk; and high-grade malignancies (WHO grades III and IV) confer high risk of transmission.[18] However, the authors commented that some grade IV tumors (e.g., glioblastoma) may be better regarded as intermediate risk, whereas others (e.g. medulloblastoma) are truly high risk of transmission of tumor. In contrast, other studies recommend that glioblastoma should be specifically designated as high-risk tumor.[6] Taken together, organ transplantation fulfills a vital need, and demand continually outpaces supply; therefore, when potential organ donors are known to have cancer at the time of death, the likely benefit of receiving functional organ(s) must be weighed against the risk of transmission of malignancy.

In summary, this report presents a rare, informative cytopathologic observation in pleural effusion. The presence of pleomorphic tumor cells with fine granular chromatin and immunoreactivity with GFAP engender the important differential diagnosis of metastatic ICM. Correlation with clinical and radiologic findings, a high index of suspicion, and adjunct ancillary studies are critical. It is important to differentiate this malignancy from others for optimal clinical management.

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