Diagnostic Evaluation of Metastatic Rhabdomyosarcoma in Effusion Specimens

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Sarcomas, including rhabdomyosarcoma (RMS), are rarely encountered in effusion specimens; therefore, difficulties in the accurate diagnosis of metastatic sarcomas in effusions can occasionally arise. Immunohistochemistry for myogenin has emerged as a useful adjunct in the diagnosis of RMS, especially in small biopsy specimens. To date, there are no published series describing the utility of immunocytochemistry for myogenin in the diagnosis of RMS in effusion specimens. A total of 15 patients, for whom metastatic sarcomas were diagnosed in effusion specimens between 1998 and 2012, were identified for analysis: alveolar RMS (n = 5); embryonal RMS (n = 1); pleomorphic RMS (n = 1); angiosarcoma (n = 1); Ewing's sarcoma (n = 2); osteosarcoma (n = 1); endometrial stromal sarcoma (n = 1); unclassified spindle cell sarcoma (n = 1); unclassified/undifferentiated pleomorphic sarcoma (n = 1); and leiomyosarcoma (n = 1). Immunocytochemistry for myogenin was performed for each of these cases as well as for 102 effusions that were positive for metastatic carcinoma. Immunocytochemistry for myogenin diffusely and strongly highlighted the nuclei of the tumor cells in six (86%) of seven cases of metastatic RMS; specifically, the five alveolar RMS and one embryonal RMS cases. The one case of pleomorphic RMS, the eight remaining metastatic sarcoma cases, and all 102 cases of metastatic carcinoma were completely negative for myogenin expression. In conclusion, immunocytochemistry for myogenin is a sensitive and specific ancillary adjunct in the diagnostic evaluation of metastatic RMS in effusion specimens. Diagn. Cytopathol. 2013;41:955–959.

Key Words: rhabdomyosarcoma; sarcoma; effusion; cytology; myogenin

Rhabdomyosarcoma (RMS) is an aggressive sarcoma with skeletal-muscle differentiation that primarily affects children and young adults and involves the head/neck, extremities, and soft tissues. Rarely, RMS can exfoliate into body fluids resulting in malignant effusions.[1–4] Histologic subtypes of RMS include embryonal, alveolar, and pleomorphic RMS. Diagnosis of RMS is based on a combination of histologic findings in surgical biopsies or resections along with ancillary studies including immunohistochemistry and cytogenetics. With respect to the former, the clinical utilization of muscle markers such as desmin and myogenin has emerged as valuable adjuncts in the diagnostic confirmation of RMS. As desmin expression is not specific for RMS, myogenin has emerged as a sensitive and specific marker of skeletal muscle differentiation.[5–7]

Myogenin is one of several basic helix-loop-helix myogenic transcription factors, including MyoD, Myf5, and MRF4 that play critical roles in skeletal muscle differentiation and development during embryogenesis.[8] Previous studies of myogenin expression in RMS have cited a sensitivity of 71–100% and high specificity with regards to spindle cell neoplasms.[5,7] The extent of nuclear immunoreactivity in RMS varies depending on the subtype of RMS being examined. For instance, Morgenstern et al. demonstrated that the percentage of myogenin-positive cells is greater in alveolar RMS when compared to embryonal RMS.[7]

Effusions that are positive for metastatic sarcoma are uncommon and account for up to 5% of malignant effusions.[9] The utility of myogenin immunocytochemistry is therefore of particular interest for the diagnosis of RMS in effusion specimens as the cytomorphologic distinction between RMS and its mimics including reactive mesothelium, metastatic carcinoma, and other sarcomas can be challenging.[2,4] In addition, the diagnostic utility of desmin is limited as benign mesothelial cells in the background will also exhibit immunoreactivity for desmin.[10] Although immunohistochemistry for myogenin has been thoroughly studied in biopsies and surgical resection

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specimens, reports in the literature regarding the efficacy of myogenin immunocytochemistry in the diagnosis of RMS in cytology specimens, especially effusions, are sparse and primarily limited to case reports.[2–4,11] Therefore, in this study, we sought to examine a series of metastatic RMS in effusions, especially focusing on the diagnostic utility of immunocytochemistry for myogenin expression.

Materials and Methods

The study was approved by the Institutional Review Board at the University of Michigan. The electronic pathology database was searched to identify cases of metastatic sarcoma diagnosed between 1998 and 2012 (Table I). Fifteen patients for whom metastatic sarcomas were diagnosed in effusion specimens were identified for analysis: alveolar RMS ($n = 5$); embryonal RMS ($n = 1$); pleomorphic RMS ($n = 1$); angiosarcoma ($n = 1$); Ewing's sarcoma ($n = 2$); pleomorphic extraskeletal osteosarcoma ($n = 1$); endometrial stromal sarcoma ($n = 1$); unclassified spindle cell sarcoma ($n = 1$); unclassified/undifferentiated pleomorphic sarcoma ($n = 1$); and leiomyosarcoma ($n = 1$). For the five patients with alveolar RMS, the right arm, peritoneum, retroperitoneum, prostate, and left hand represented the primary sites. The locations of the primary tumors for the embryonal and pleomorphic RMS patients were the mediastinum and left spermatic cord, respectively. The diagnoses of the primary RMS tumors were rendered based on histomorphologic features and confirmatory immunohistochemistry for desmin and myogenin. Desmin immunoreactivity was observed for all seven RMS cases. Myogenin immunohistochemistry was positive in the tumor cells for the five alveolar RMS and one embryonal RMS primaries. Confirmatory molecular studies were performed on the primary RMS tumors in two alveolar RMS cases; a *FOXO1* gene rearrangement was detected via fluorescence in situ hybridization in one case and via reverse transcriptase-polymerase chain reaction in another case. Molecular studies were not performed for the other five RMS primary tumors. Next, the primary sites for the angiosarcoma, osteosarcoma, unclassified spindle cell sarcoma, and unclassified/undifferentiated pleomorphic sarcoma patients were the pericardium, retroperitoneum, right foot, and abdomen, respectively. The uterus represented the primary sites for the endometrial stromal sarcoma and leiomyosarcoma cases. For the two patients with Ewing's sarcoma, the left fibula and left pelvis represented the primary sites.

In addition, 102 effusion cases that were positive for metastatic carcinoma were also collected for analysis (Table I). Primary sites for the metastatic carcinomas were as follows: Müllerian ($n = 35$); lung ($n = 28$); breast ($n = 18$); gastroesophageal ($n = 9$); pancreaticobiliary ($n = 8$); and, colorectal ($n = 4$). Cell blocks were retrieved and the hematoxylin and eosin (H&E) stained sections were examined to confirm the presence of tumor cells in the cell blocks.

Table I. Cases Examined for Myogenin Expression by Immunocytochemistry

Malignancy (*n*)	*# Myogenin-positive (%)*
Rhabdomyosarcoma ($n = 7$)	6 (86%)
Alveolar rhabdomyosarcoma ($n = 5$)	5 (100%)
Embryonal rhabdomyosarcoma ($n = 1$)	1 (100%)
Pleomorphic rhabdomyosarcoma ($n = 1$)	0 (0%)
Sarcoma, non-rhabdomyosarcoma ($n = 8$)	0 (0%)
Angiosarcoma ($n = 1$)	0 (0%)
Ewing's sarcoma ($n = 2$)	0 (0%)
Osteosarcoma ($n = 1$)	0 (0%)
Endometrial stromal sarcoma ($n = 1$)	0 (0%)
Unclassified spindle cell sarcoma ($n = 1$)	0 (0%)
Unclassified/undifferentiated pleomorphic sarcoma ($n = 1$)	0 (0%)
Leiomyosarcoma ($n = 1$)	0 (0%)
Carcinoma ($n = 102$)	0 (0%)
Mullerian ($n = 35$)	0 (0%)
Lung ($n = 28$)	0 (0%)
Breast ($n = 18$)	0 (0%)
Gastroesophageal ($n = 9$)	0 (0%)
Pancreaticobiliary ($n = 8$)	0 (0%)
Colorectal ($n = 4$)	0 (0%)

Immunocytochemistry for myogenin was performed for each of the above cases. Immunostaining was performed on the DAKO Autostainer (DAKO, Carpinteria, CA) using DAKO LSAB+ and diaminobenzadine as the chromogen. De-paraffinized sections of formalin-fixed tissue at 5-μm thickness were labeled with a mouse monoclonal antibody directed against myogenin (clone F5D, 1:50 dilution; DAKO, Carpinteria, CA). Appropriate negative (immunostaining with the primary antibody omitted) and positive controls (tissue sections of known RMS tumors) were stained in parallel. Tumor cells and background mesothelial cells and inflammatory cells were scored for nuclear myogenin immunoreactivity in each case.

Results

A total of seven cases of metastatic RMS diagnosed in effusion specimens were examined in this study: five alveolar RMS; one embryonal RMS, and one pleomorphic RMS (Fig. 1). The tumor cells in all five alveolar RMS cases exhibited a predominantly discohesive pattern of small, round blue cells with moderate to high nuclear-to-cytoplasmic ratios (Fig. 1A). In cells with moderate amounts of cytoplasm, the nuclei were eccentrically placed. Occasional small, tight clusters were appreciated. The chromatin within the nuclei was granular in texture.

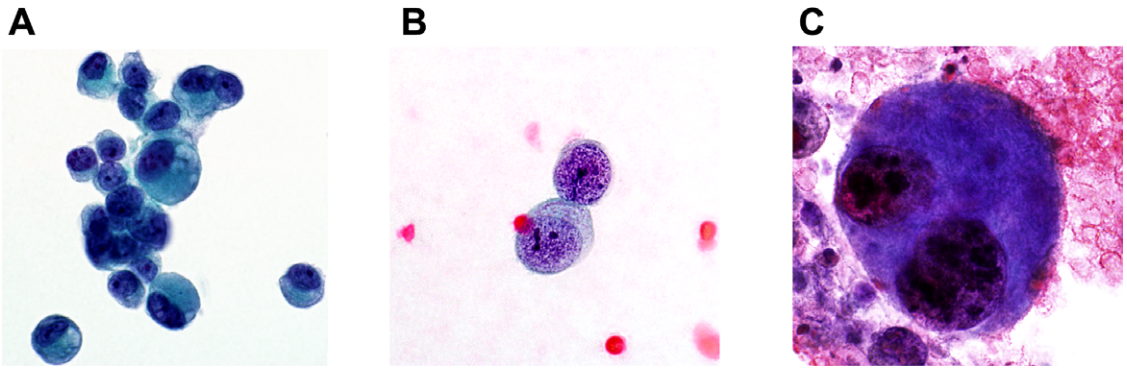

Fig. 1. Cytomorphologic features of metastatic alveolar, embryonal, and pleomorphic rhabdomyosarcoma (RMS) in effusion specimens. Representative photomicrographs obtained from Papanicolaou-stained slides prepared from cases of (**A**) alveolar, (**B**) embryonal, and (**C**) pleomorphic RMS are shown. All photomicrographs were obtained at 1000× magnification. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Small but conspicuous nucleoli were evident in four of five cases. Multinucleated cells were present but few in number in all five cases. The one case of embryonal RMS was hypocellular but significant for the presence of single neoplastic cells with scant to moderate amounts of cytoplasm (Fig. 1B). Nuclei were eccentrically placed in cells exhibiting moderate amounts of cytoplasm. The chromatin texture was granular and small, conspicuous nucleoli were present. Multinucleated cells and tumor cell clusters were not evident in this case. The tumor cells for the one case of pleomorphic RMS were markedly enlarged with moderate to abundant cytoplasm, contained eccentrically placed nuclei, and were present in a discohesive pattern. Multinucleation and prominent macronucleoli were frequently encountered (Fig. 1C).

Immunocytochemistry for myogenin diffusely and strongly highlighted the nuclei of the tumor cells in six (86%) of seven cases of metastatic RMS; specifically, the five alveolar RMS cases and the one case of embryonal RMS (Figs. 2A–D). The one case of pleomorphic RMS was completely negative for myogenin expression (Figs. 2E–F). To determine the specificity of myogenin immunocytochemistry for the detection of metastatic RMS in effusion specimens, eight cases of non-RMS metastatic sarcoma effusion specimens were also examined for myogenin expression. These metastases were from primary sarcomas originally diagnosed as: angiosarcoma ($n = 1$); Ewing's sarcoma ($n = 2$); osteosarcoma ($n = 1$); endometrial stromal sarcoma ($n = 1$); unclassified spindle cell sarcoma ($n = 1$); unclassified/undifferentiated pleomorphic sarcoma ($n = 1$); and leiomyosarcoma ($n = 1$). None of these cases exhibited immunoreactivity for myogenin in the tumor cells. To further investigate the specificity of myogenin immunocytochemistry, 102 cases of metastatic carcinoma were analyzed for myogenin expression as negative controls. The tumor cells in all 102 cases were myogenin-negative (Table I). Immunoreactivity for myogenin in background mesothelial cells and inflammatory cells was not observed in any instances.

Discussion

The literature on the cytologic diagnosis of RMS in effusion specimens is sparse and limited to case reports owing to the rarity of metastatic RMS presenting in body fluids.[2–4] These case reports all focused on cases of metastatic alveolar RMS. This in addition to the difficulty in distinguishing RMS from background mesothelial cells and inflammatory cells as well as other metastatic neoplasms contribute to difficulties in accurately diagnosing metastatic RMS in effusions. The accurate diagnosis is essential for timely, appropriate management in patients afflicted with these aggressive tumors. In tissue specimens, myogenin immunohistochemistry has been shown to be a sensitive and specific marker for RMS.[5–7,12] However, the current knowledge regarding the utility of this marker in effusion cytology is limited.

Although the cytologic features of alveolar RMS have not been extensively described in effusion specimens, two cytomorphologically distinct types of RMS cells have been described in fine-needle aspiration and small biopsy specimens: (1) small, round blue cells with scant cytoplasm and hyperchromatic nuclei and (2) differentiated rhabdomyoblasts with eosinophilic cytoplasm, some exhibiting prominent cytoplasmic cross-striations.[13,14] The former cell type is more commonly encountered in effusion specimens[2–4]; our cytomorphologic observations corroborate this notion. Nelson et al.[2] reported a case of metastatic alveolar RMS in an ascites specimen that was composed of the former cell type that was initially misinterpreted as reactive mesothelial cells. Further workup revealed the presence of neoplastic cells that exhibited immunoreactivity for myogenin ultimately leading to a diagnosis of metastatic RMS. Two additional case reports describe pleural effusion specimens with cytologically

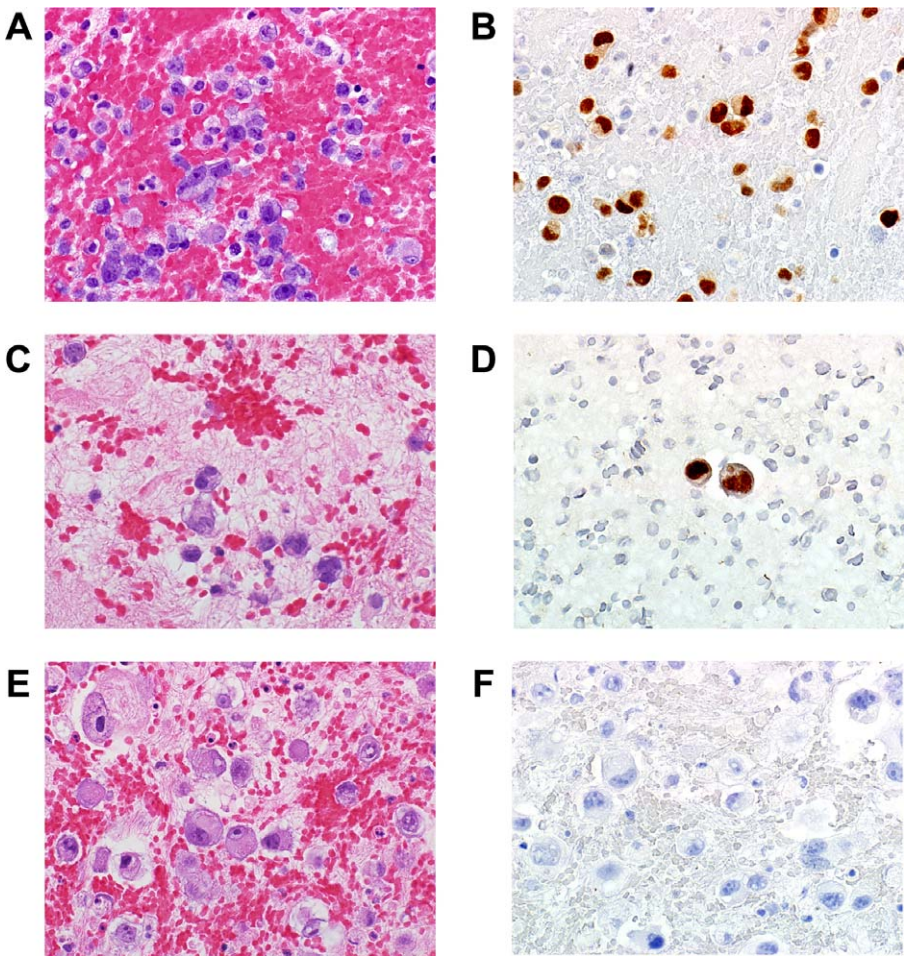

Fig. 2. Immunocytochemistry for myogenin in effusion specimens positive for metastatic rhabdomyosarcoma (RMS). (**A**, **C**, and **E**) Representative photomicrographs obtained from H&E stained cell block sections derived from cases of alveolar, embryonal, and pleomorphic RMS, respectively. (**B**, **D**, and **F**) Corresponding immunohistochemical stains for myogenin expression for the cases of alveolar, embryonal, and pleomorphic RMS, respectively. The tumor cells in the cases of alveolar and embryonal RMS are positive for myogenin expression whereas the one case of pleomorphic RMS is negative for myogenin expression. All photomicrographs were obtained at 600× magnification. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

suspicious cells that exhibited nuclear immunoreactivity for myogenin thereby confirming a diagnosis of metastatic RMS.[3,4]

The results of our study, which examine a series of RMS cases, corroborate the findings in the aforementioned case reports. All five of our alveolar RMS cases exhibited diffuse, strong, nuclear immunoreactivity for myogenin in the tumor cells. In addition, despite previous studies reporting patchy immunoreactivity for myogenin in embryonal RMS,[6,7] we observed diffuse immunoreactivity for this marker in our one case of metastatic embryonal RMS. Next, myogenin immunoreactivity was not detected in the tumor cells in our one case of metastatic pleomorphic RMS. In this patient, the spermatic cord represented the primary site; this site has been recognized as a site within which this tumor can arise.[15] Prior immunohistochemical workup revealed that the tumor cells in this resection specimen were negative for cytokeratins but positive for desmin. The cytomorphology of the tumor cells in this effusion specimen was identical to that in the primary resection specimen. We retrospectively performed immunohistochemistry for myogenin on this specimen. Similar to the findings in the effusion specimen, we observed that none of the tumor cells exhibited immunoreactivity for this marker. Of note, Furlong and colleagues observed that 44% of pleomorphic RMS are negative for myogenin expression on immunohistochemistry.[15]

Overall, we observed a sensitivity of 86% for myogenin immunocytochemistry in the detection of RMS tumor cells in effusion specimens. Next, we sought to examine the specificity of this marker in exfoliative fluid cytology. We approached this issue by performing immunocytochemistry on eight malignant effusion cases of metastatic sarcoma other than RMS. None of the tumor cells in these eight

cases exhibited myogenin immunoreactivity. As reactive mesothelial cells and metastatic carcinomas can occasionally mimic RMS, we also tested 102 cases of carcinomatous effusions. Again, immunocytochemistry for myogenin was completely negative in all of these cases. Of note, immunostaining for desmin, in addition to myogenin, is also used to confirm myogenic differentiation in RMS. In effusion specimens, desmin immunocytochemistry is of limited value; desmin immunostains are difficult to interpret in this setting as background mesothelial cells exhibit immunoreactivity for desmin.[10,16] In our study, we did not observe any immunoreactivity for myogenin in the background mesothelial cell population.

The limited number of effusion cases with metastatic sarcoma, including RMS, represents a limitation to this study. This is expected as metastatic sarcoma is rarely encountered in body fluids, compared to reactive and carcinomatous effusions. Nonetheless, our study demonstrates that careful cytomorphologic evaluation with adjunct immunocytochemistry for myogenin can accurately diagnose metastatic RMS in effusion specimens. Myogenin represents a sensitive and specific marker for the workup of RMS in effusion cytology.

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