

Exfoliation of Alveolar Rhabdomyosarcoma Cells in the Ascites of a 50-Year-Old Woman: Diagnostic Challenges and Literature Review

Norio Motoda¹, Yuji Nakamura¹, Mutsumi Kuroki², Koichi Yoneyama²,
Saiko Isshiki³, Ryuji Ohashi¹ and Zenya Naito⁴

¹Department of Diagnostic Pathology, Nippon Medical School Musashi Kosugi Hospital, Kanagawa, Japan

²Department of Obstetrics and Gynecology, Nippon Medical School Musashi Kosugi Hospital, Kanagawa, Japan

³Department of Radiology, Nippon Medical School Musashi Kosugi Hospital, Kanagawa, Japan

⁴Department of Integrated Diagnostic Pathology, Nippon Medical School, Tokyo, Japan

Alveolar rhabdomyosarcoma (ARMS) is a nonepithelial tumor with skeletal muscle differentiation and typically affects adolescents and young adults. The cytological features of ARMS in body fluid have not been well characterized, which complicates diagnosis. Here, we describe the cytological features of ARMS in the ascites of a 50-year-old woman with an intra-abdominal mass and abundant ascites. Aspiration cytology of ascitic fluid revealed numerous small discohesive round cells with mild nuclear atypia and prominent nucleoli. Rhabdomyoblastic cells, characteristic of rhabdomyosarcoma, were identified rarely. Cannibalism and ‘window’ formation, as seen in reactive mesothelial cells, complicated the diagnosis of ARMS. Histological examination established the diagnosis of ARMS, which was confirmed by immunohistochemical expression of myogenic markers. When diagnosing ARMS from effusion samples, the diagnostic problems associated with the morphological similarity of ARMS cells to reactive mesothelial cells should be considered. (*J Nippon Med Sch* 2019; 86: 236–241)

Key words: alveolar rhabdomyosarcoma, ascites, cytology, mesothelial cells, myogenin

Introduction

Rhabdomyosarcoma (RMS) is a malignant tumor with skeletal muscle differentiation and commonly occurs during childhood and adolescence¹. Histologically, RMS can be classified into three subtypes: alveolar, embryonal, and pleomorphic. Alveolar rhabdomyosarcoma (ARMS) was originally described by Riopelle and Theriault in 1956² and later established by Enzinger and Shiraki³ as a tumor affecting the extremities or the perirectal/perianal regions of patients aged 10 to 20 years. Histopathologically, ARMS is characterized by poorly differentiated round cells with rhabdomyoblastic differentiation and an irregular alveolar pattern⁴. However, the cytological features of ARMS have not been well characterized, which poses a challenge for diagnostic cytology.

Sarcomas can occasionally exfoliate into body fluids, causing malignant effusions that account for less than 5%

of malignant effusions. ARMS cells rarely appear in body fluids, and thus experience with ARMS cytology in effusions is extremely limited. To our knowledge, only 15 cases of ARMS in body fluids have been reported^{5–10}. Herein we describe an adult case of ARMS exfoliated into ascites, which presented diagnostic challenges because of ambiguous cytological findings that mimicked those of reactive mesothelial cells. We then compare the cytological and clinicopathological findings of our case with those of previously reported ARMS cases.

Case Presentation

A 50-year-old woman presented with abdominal fullness persisting for 3 months. She had a history of uterine leiomyoma that was locally resected 5 years previously. Initially, recurrence of leiomyoma was suspected, and treatment was started with a gonadotropin-releasing hormone

Correspondence to Norio Motoda, MD, PhD, Department of Diagnostic Pathology, Nippon Medical School Musashi Kosugi Hospital, 1–396 Kosugi-cho, Nakahara-ku, Kawasaki, Kanagawa 211–8533, Japan

E-mail: n-motoda@nms.ac.jp

https://doi.org/10.1272/jnms.JNMS.2018_86-404

Journal Website (<https://www.nms.ac.jp/sh/jnms/>)

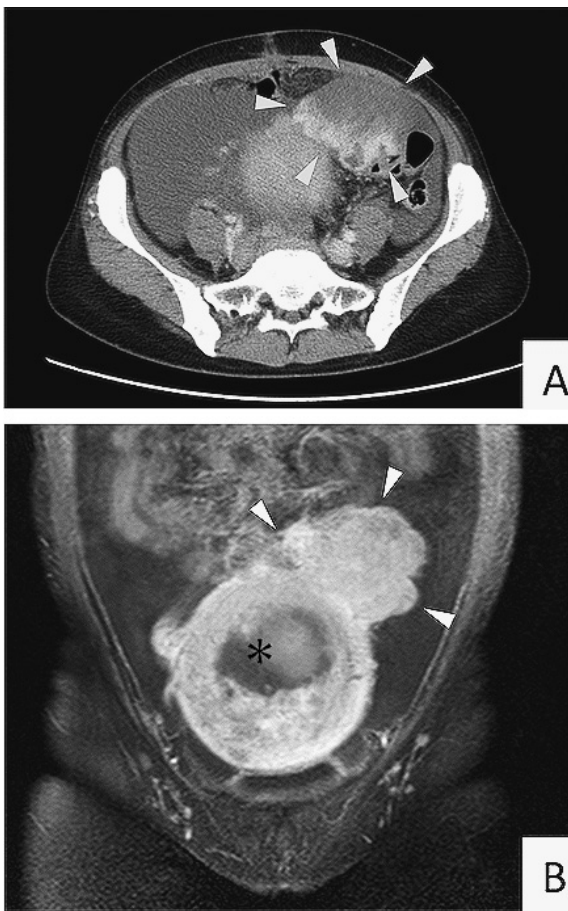


Fig. 1 A. Enhanced CT image showing a peripherally enhanced mass at the left anterolateral wall of the uterus (arrowheads), with abundant ascites. B. Contrast-enhanced fat-suppressed T1-weighted MR image showing an irregularly shaped mass attached to the uterine fundus (arrowheads). An intrauterine mass believed to be a leiomyoma was also observed (asterisk).

analog. This had no clinical benefit, and abdominal fullness worsened. Computed tomography and magnetic resonance imaging revealed multiple intra-abdominal masses with abundant ascitic fluid, which suggested an aggressive malignant gynecological disease such as carcinosarcoma (Fig. 1A, B).

Cytological and histological examination of the endometrium was undiagnostic. Abdominal paracentesis was used to collect ascitic fluid for cytological evaluation. The sample was visualized by Papanicolaou and periodic acid-Schiff (PAS) with BD CytoRich™ preparations. The sample consisted of small to medium-sized loosely cohesive cell clusters, accompanied by single discohesive cells, in a bleeding background (Fig. 2A). Mitotic figures were easily identified, and a few binuclear and multinuclear cells were noted (Fig. 2B). Most of these atypical cells had round-to-ovoid, eccentric nuclei with fine chro-

matin and small, prominent nucleoli. The cytoplasm was thick and the nuclear-cytoplasmic ratio was slightly increased. We also identified pairs of tumor cells with 'window' formations and cannibalism, which mimicked reactive mesothelial cells (Fig. 2C and D). Some cells contained cytoplasmic glycogen, which was detected by PAS staining (Fig. 2E). Rhabdomyoblastic cells, characteristic of ARMS, were observed rarely (Fig. 2F). Although malignant disease was suspected, we could not reach a definitive diagnosis, as we were unable to confidently distinguish tumor cells from reactive mesothelial cells. Thus, a laparotomy was performed for further analysis, 9 days after admission.

Laparotomic observation revealed a greyish mass, approximately 10 cm in diameter, with a hemorrhagic and necrotic appearance, and about 4,000 mL of bloody ascites (Fig. 3A). The mass was located at the left lateral aspect of the uterus and extended into the small intestine and sigmoid colon. The surgeon decided that radical surgery was impossible and performed a partial resection, so that, at the very least, a histological diagnosis could be performed.

The sample obtained consisted of fragmented whitish tissue with hemorrhagic necrosis. Histological examination showed proliferation of small atypical cells with eosinophilic cytoplasm and round hyperchromatic eccentric nuclei and discohesiveness, arranged in an irregular alveolar pattern (Fig. 3B-D). A small number of rhabdomyoblastic cells and multinucleated cells were also identified. Immunohistochemical analysis showed that the tumor cells were strongly positive for desmin (Fig. 3E), myoglobin, myogenin (Fig. 3F), vimentin, and CD99 and focally positive for epithelial membrane antigen, smooth muscle actin, and calretinin and negative for leukocyte common antigen, chromogranin, synaptophysin, HMB-45, and S-100. Epithelial components suggestive of adenosarcoma or carcinosarcoma were absent, as indicated by the absence of CK7- and CK20-positive cells. Mitosis was frequently identified, and the Ki-67 labeling index was approximately 80%. The diagnosis of ARMS was based on these morphological and immunohistochemical findings.

During the laparotomy, we again collected ascitic fluid, which contained atypical cells with a morphology similar to that observed histologically. These findings indicated that the atypical cells observed preoperatively in ascites were ARMS cells. After the laparotomy, her systemic condition and abdominal fullness worsened (Fig. 4), and she developed a leukemoid reaction and renal failure. She

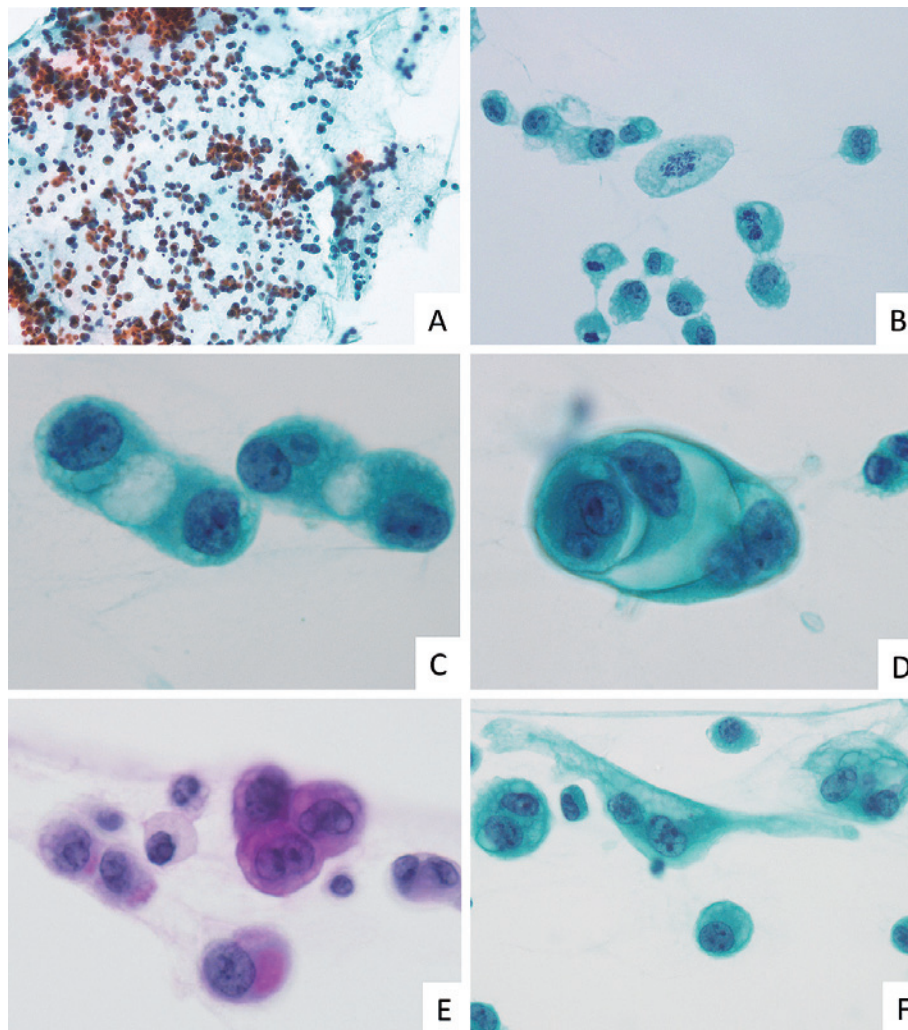


Fig. 2 A. Smears of the ascitic fluid (Papanicolaou stain) showed small to medium-sized loosely cohesive cell clusters and single discohesive cells in a bleeding background. B. Tumor cells had round to ovoid eccentric nuclei with fine chromatin and small prominent nucleoli. Mitotic figures were frequently observed. C. 'Window' formation and D. cannibalism of tumor cells, as seen in mesothelial cells. E. PAS staining revealed cytoplasmic glycogen in some cells. A few spindle-shaped cells resembling rhabdomyoblasts were observed. F. Cytoplasmic cross-striations were not apparent.

died on the 19th postoperative day.

Discussion

RMS is a nonepithelial malignancy with skeletal muscle differentiation and is classified as embryonal, alveolar, or pleomorphic¹¹. About 70% of RMS cases are embryonal RMS (ERMS)-the most common subtype-and 20% to 30% of cases are ARMS⁴. ERMS typically occurs in younger children, while ARMS commonly affects children aged 10 to 15 years. The common sites for ARMS are the extremities and central areas of the body, such as the head/neck and urogenital organs. In children, ARMS has a worse prognosis than ERMS; however, in adults, all RMSs are associated with a poor prognosis¹, particularly when tu-

mors are large (diameter >5 cm) and surgically unresectable¹². The current case involved a 50-year-old woman with a rapidly growing large mass (10 cm) that eventually resulted in death, which highlights the aggressiveness of ARMS in adults.

Few studies of cytological diagnosis of ARMS in effusion specimens have been published: only 15 cases have been reported in the English literature (Table 1). Nelson et al reported that RMS is characterized cytomorphologically by the presence of two distinct cell types-small, round blue cells with scant cytoplasm and hyperchromatic nuclei, resembling lymphocytes, and rhabdomyoblasts, including strap cells, tadpole cells, or ribbon-shaped cells with abundant eosinophilic cytoplasm⁸. In

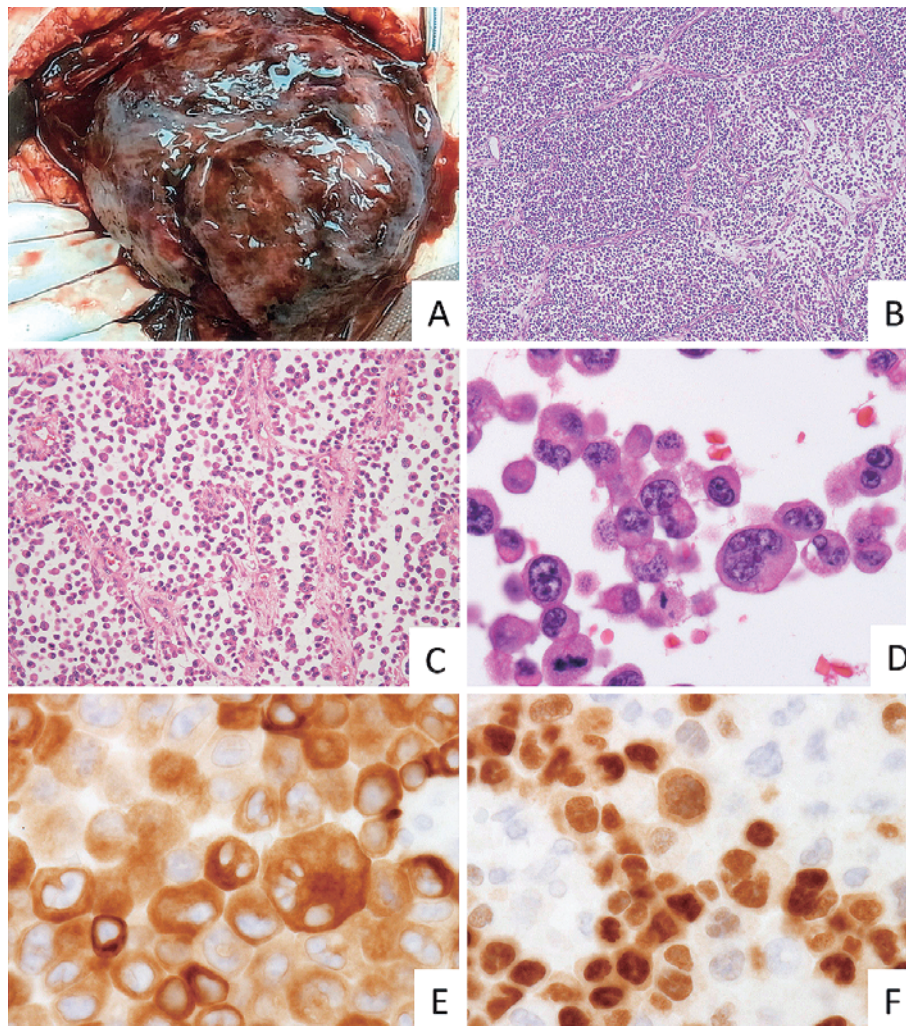


Fig. 3 A. Intraoperative observation revealed a bulky fragile mass with marked hemorrhage and necrosis. B. The resected specimen showed small to medium-sized round cells with high nuclear/cytoplasmic (N/C) ratios, which formed an alveolar structure separated by fibrovascular septa. C. Most tumor cells were discohesive, but a single layer of tumor cells adhered to fibrous septa. D. At high magnification, the tumor cells had eccentric eosinophilic cytoplasm and round hyperchromatic nuclei, resembling atypical cells observed in cytological specimens. In addition, a few multinucleated cells were observed. Tumor cells exhibited myogenic markers, such as E. desmin and F. myogenin.

effusion specimens from patients with ARMS, the former cell type is observed frequently, but the latter may be scarce or even absent. In our case, single small to medium-sized, loosely cohesive, discohesive cells with hyperchromatic round nuclei were predominant, although a few binucleated and multinucleated cells were observed. The scarcity of typical rhabdomyoblastic cells further complicated the diagnosis of ARMS.

Another problem with effusion samples is that tumor cells are difficult to distinguish from reactive mesothelial cells. In fact, similar difficulties were reported in other studies of ARMS^{7,8}. In general, identification of malignant cells in effusion usually relies on detection of “non-

mesothelial cells” in the background of abundant mesothelial cells. However, some types of malignant cells can present in a discohesive manner or create pairs that form “windows,” thus mimicking reactive mesothelial cells. In addition, reactive mesothelial cells can resemble tumor cells by exhibiting atypical nuclei or multinucleation, which increases the difficulty of identifying malignant cells. To ensure correct diagnosis of malignant tumors such as RMS in effusion specimens, these confounding factors need to be identified and shared among diagnostic cytopathologists.

To differentiate ARMS cells from reactive mesothelial cells, immunohistochemical detection of myogenic regu-

latory factors such as myogenin and MyoD1 is helpful, as these are specifically expressed by ARMS but are absent in reactive mesothelial cells¹³. Myogenin is particularly useful because its expression in ARMS is stronger than in ERMS, which allows the two subtypes to be distinguished^{14,15}. A previous study reported that myogenin was expressed in 86% of ARMS cases⁹. In the present

case, myogenin was strongly positive in most tumor cells in histological sections, confirming the diagnosis of ARMS. Unfortunately, we could not perform immunohistochemical analysis on a cell block, as the amount of ascitic fluid obtained was insufficient.

Histologically, sarcomatous overgrowth of adenosarcoma or carcinosarcoma could be the differential diagnosis in the present case. To assess this possibility, we carefully examined all 21 specimens, which had similar populations of highly cellular, monomorphous primitive cells with round nuclei and features of arrested myogenesis without epithelial elements, thus strictly fulfilling the ARMS diagnostic criteria¹¹. Furthermore, the absence of tumor elements in preoperative endometrial curettage samples did not support a diagnosis of adenosarcoma or carcinosarcoma, which typically involves the endometrium or cervical mucosa^{16,17}. These findings suggest that the primary site of ARMS could be other organs, such as the retroperitoneum, instead of the uterus. Ideally, we should have thoroughly investigated the uterine corpus, to identify the tumor location and histological type, but were unable to do so because hysterectomy was not performed, which is a limitation of this study.

In this study, we described a case of ARMS in an adult patient, which was difficult to diagnose from analysis of ascitic fluid. A reason for this difficulty was the similar cytological features of ARMS and reactive mesothelial cells, which are often observed in fluid samples from



Fig. 4 An unenhanced coronal CT image at 8 postoperative days revealed multiple new metastases (arrows) and abundant ascites (arrowheads).

Table 1 ARMS effusion cytology: Review of present and past cases

Case	Age (yr)/ Sex	Site	Cytological findings	Mitosis	Multi-nuclear cells	Rhabdomyoblastic cells	Initial cytological diagnosis	Ref.
1	19/ male	Pleural effusion	Single round cells with round nuclei and scanty vacuolated cytoplasm.	N/A	N/A	N/A	N/A	5
2	4/ male	Pleural effusion	Isolated and clustered cells with scanty cytoplasm and large, round, slightly polymorphous nuclei.	Present	N/A	N/A	Atypical inflammatory cells	6
3	29/ female	Pleural effusion	Loosely cohesive clusters and single discohesive, small to medium-sized cells with round to oval, hyperchromatic nuclei.	N/A	Present	N/A	Reactive mesothelial cells	7
4	17/ female	Ascites	Small-to-very large single cells with single or multiple hyperchromatic nuclei and high nucleocytoplasmic ratios.	Absent	Present	Present	Reactive mesothelial cells	8
5-9	N/A	N/A	Predominantly discohesive pattern of small, round blue cells with moderate to high nuclear-to cytoplasmic ratios.	N/A	Present (5/5)	N/A	N/A	9
10-15	15-35 (average 23)	Ascites (all cases)	Single scattered neoplastic cells and cellular clusters.	N/A	N/A	N/A	N/A	10
16	50/ female	Ascites	Medium-sized loosely cohesive clusters and single discohesive cells with round to ovoid and eccentric nuclei.	Present	Present	A few	Atypical cells	*

* Present case

ARMS patients. The lack of typical rhabdomyoblastic cells in the ascites was another confounding factor. Although effusion cytology is useful for preoperative diagnosis, awareness of these diagnostic problems and limitations is necessary when tumors such as ARMS are clinically suspected.

Conflict of Interest: The authors declare no conflict of interest.

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(Received, October 25, 2018)

(Accepted, January 22, 2019)

(J-STAGE Advance Publication, April 26, 2019)