

Cytologic Findings of Alveolar Soft Part Sarcoma Presenting with Multiple Pulmonary Masses

– A Case Report with Review of Literature –

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Alveolar soft part sarcoma (ASPS) is a rare malignant soft tissue tumor of uncertain origin, and it has a strong propensity for metastasis to the lungs, bones and brain. We report upon an unusual case of ASPS, presenting as multiple lung nodules with no other detectable primary site, in a 44-year-old man. A fine needle aspiration of the nodules yielded scattered, discohesive cells, each containing an eccentrically displaced nucleus and prominent nucleolus, on a granular background. Tumor cells with numerous bare nuclei, and occasional sheets of epithelioid cells were also found. Under the cytological diagnosis of an unclassified epithelioid malignant tumor, resection of the lung nodules was performed. The histologic findings were consistent with ASPS, showing positive TFE3-nuclear immunoreactivity. There is limited literature concerning cytological findings associated with pulmonary ASPS: especially in cases where the primary site is unknown. Here, we present a cytological review of pulmonary ASPS, investigating the significance of TFE3 staining in the diagnosis of ASPS.

Key Words: Sarcoma, alveolar soft part; Lung; Biopsy, fine needle; Neoplasm metastasis; TFE3 stain

Alveolar soft part sarcoma (ASPS) is a rare, epithelial-like, uniformly malignant tumor, with no benign counterpart, that principally affects young adults in their twenties and thirties. ASPS usually presents as a soft, painless, slow growing soft tissue mass, but it frequently metastasizes to the lungs, brain and bones.¹ ASPS belongs to the high-grade, epithelioid/polygonal sarcoma category: exhibiting characteristic histological and ultrastructural features. However, immunohistochemical findings have been described as nonspecific. Based on morphological resemblance, expression of muscle actin, desmin, MyoD1, myogenin and alpha-striated actin-1, and cytoplasmic granules, a variant of paraganglioma, skeletal muscle origin tumor or a juxtaglomerular tumor, respectively, have been proposed.²⁻⁵ ASPSs are consistently negative for epithelial markers (cytokeratins and epithelial membrane antigen), neuroendocrine markers (chromogranin A and synaptophysin) and melanocytic markers (S-100 protein, human melanoma black-45 [HMB-45] and melan-

A).⁶ Recently, specific translocation as fusion between transcription factor 3 (*TFE3*) gene, belonging to the microphthalmia-TFE subfamily of the basic helix-loop-helix leucine zipper, transcription factors and a novel gene alveolar soft part sarcoma locus (*ASPL*) in ASPS has been reported,⁷ and subsequently the TFE3 antibody has been used to diagnose ASPS.⁸ The characteristic histological findings help pathologists correctly diagnose ASPS, but a cytological diagnosis by fine needle aspiration (FNA) may be difficult as a primary modality because of the rare incidence of ASPS and the limited few cytology literature.⁹⁻¹⁵ In this case, cytological evaluation was even more problematic, because there was no primary tumor connected with presentation of the multiple lung nodules.

In this report, we focused on the cytological features of pulmonary ASPS, along with its cytologic differential points and the diagnostic value of TFE3 immunostain.

CASE REPORT

A previously healthy, 44-year-old man presented with an abnormal chest X-ray, taken due to a traffic accident. A subsequent, 3-dimensional chest computed tomography (CT) revealed multiple scattered nodules in the right middle and lower lobes (Fig. 1A), ranging from 0.5 to 3.7 cm on the longest axis. Magnetic resonance images of the brain showed three enhancing masses at the corticomедullary junctions in the right frontal and parietal lobes, and the genu of the corpus callosum, respectively (Fig. 1B). Thorough examination as well as a whole body positron emission tomography scan did not reveal any masses. Under the clinical impression of the presence of a primary neuroendocrine tumor of the lung or lung carcinoma with intrapulmonary metastasis, CT-guided FNA cytology was taken from the right middle and lower lobes. The smears were air-dried, fixed with alcohol, and stained with a modified Papanicolaou method and hematoxylin-eosin. A subsequent right middle and lower lobes lobectomy was performed. Tissue diagnosis in the brain could not be established because of the patient's non-consent. Cranial radiation and systemic chemotherapy with cyclophosphamide, adriamycin, vincristine and dacarbazine were started. During the three months of follow up, he remained alive with no additional masses or clinical progression.

Cytologic findings

The fine needle aspirates from the lung disclosed paucicellular and bloody smears (Fig. 2A). A few sheets of oval round epithelioid cells in a trabecular arrangement were noted (Fig. 2B), but most of the aspirates were composed of singly scattered, discohesive, large round to plasmacytoid tumor cells in the hemorrhagic background. These tumor cells had relatively abundant eosinophilic cytoplasm and eccentrically located nuclei. Scattered single tumor cells had a round to polyhedral shape and bare nuclei, and occasionally, prominent nucleoli (Fig. 2C). Cytoplasmic fragility resulted in detached cytoplasmic fragments and a flocculent and finely granular background. Occasionally, tumor cells with nuclear pleomorphism, prominent nucleoli and binucleated cells were also found (Fig. 2D). No mitosis or necrosis was found. Cytoplasmic vacuoles were not identified. Cytological diagnosis was an epithelial tumor with oncocytic features.

Histologic findings

The resected pulmonary masses measured up to 3.7 cm and were well demarcated from the pulmonary parenchyma. The cut surface was smooth and pale gray-tan with no necrosis or hemorrhage. Microscopically, each tumor was composed of large

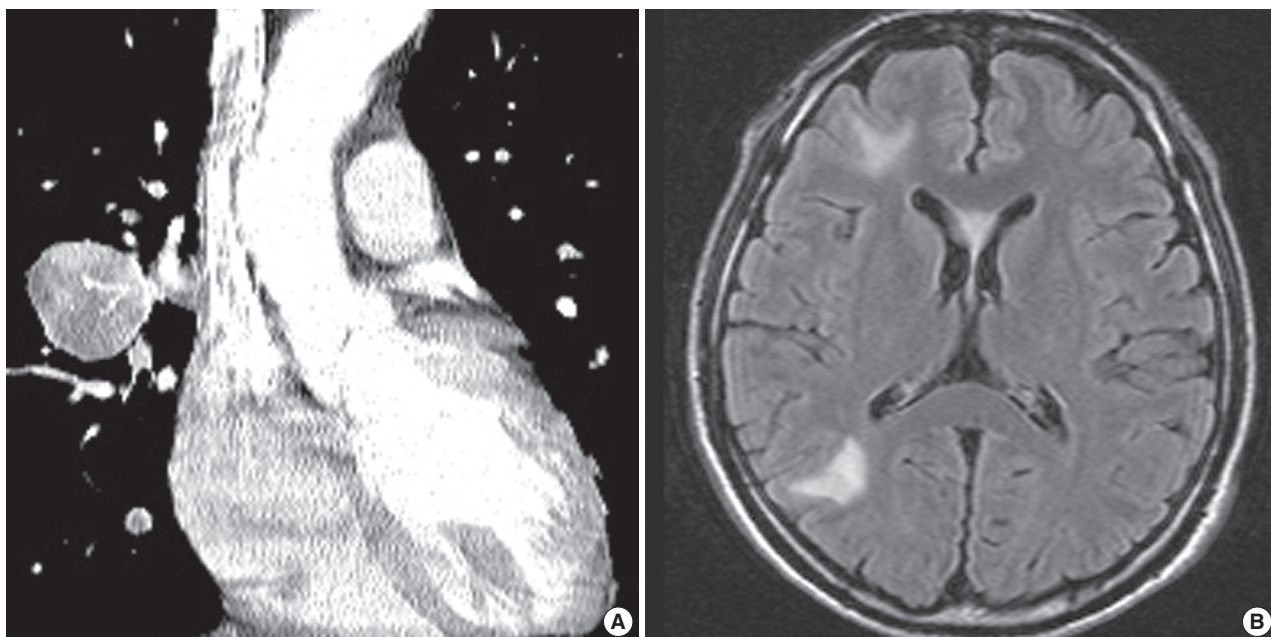


Fig. 1. (A) Chest computed tomography shows multiple round shaped nodules with dilated, heterogeneously enhanced tortuous vessels within the mass. (B) The T2-weighted magnetic resonance image shows three masses located at the corticomедullary junctions in the right frontal and parietal lobes, and the genu of the corpus callosum, respectively.

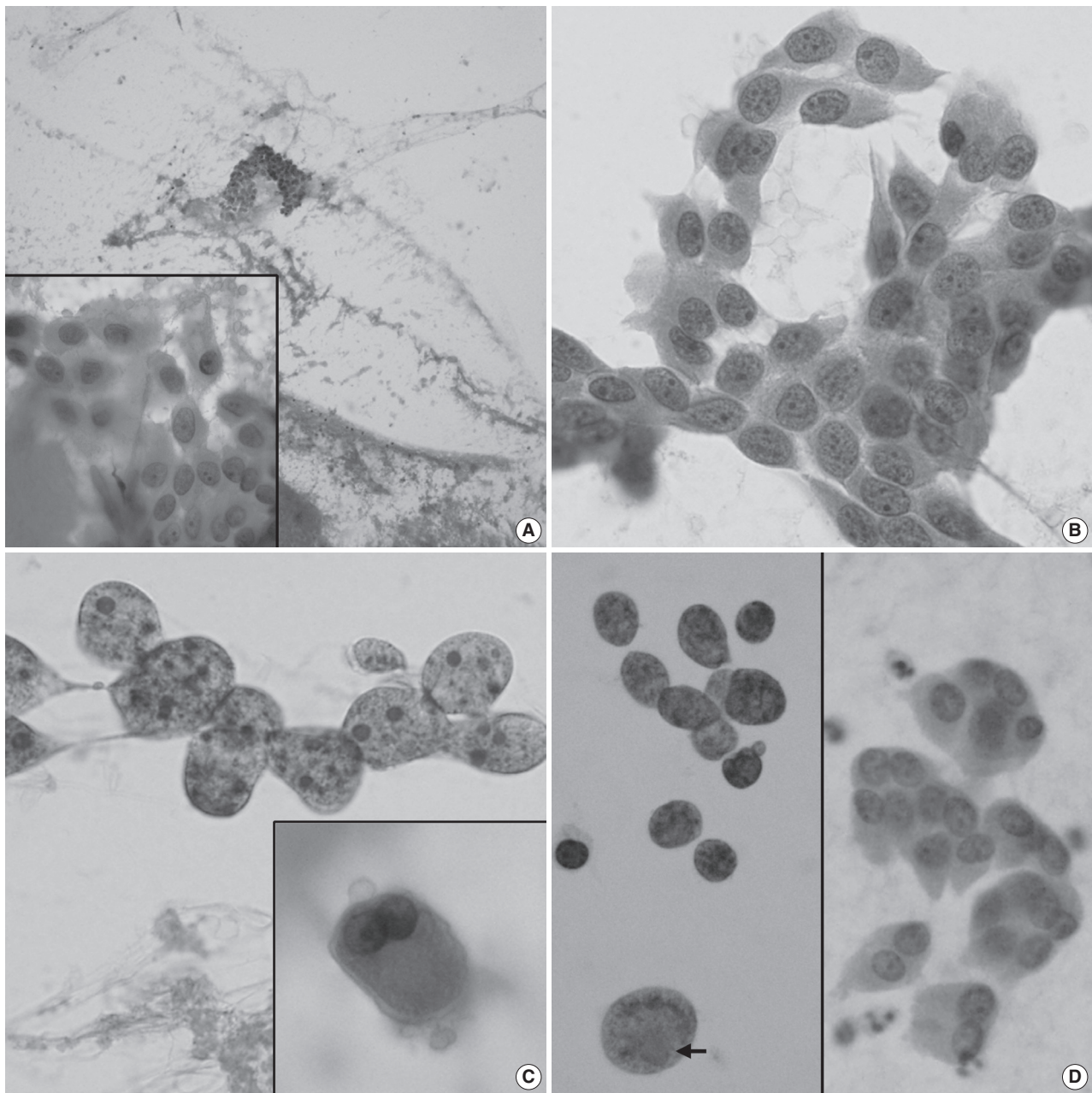


Fig. 2. (A) A sheet of aggregated tumor cells is seen on a bloody background (Papanicolaou stain). Inset indicates tumor cells as having abundant eosinophilic cytoplasm with a relatively well defined cell border (Papanicolaou stain). (B) High magnification reveals vaguely trabecular clusters with abundant, finely granular cytoplasm and round, heterochromatic nuclei (Papanicolaou stain). (C) Scattered, large bared nuclei are shown to have prominent nucleoli, and, occasionally, binucleated tumor cells, with abundant granular cytoplasm (inset). Note the fragmented granular materials in the background (Papanicolaou stain). (D) Naked nuclei are shown to occasionally have prominent nucleoli (left, arrow, Papanicolaou stain), and some tumor cells have oncocytic cytoplasm with eccentrically located nuclei, which have a plasmacytoid appearance (right, Papanicolaou stain).

polygonal-shaped cells distributed in nests in an organoid arrangement, which were separated by delicate fibrovascular septa (Fig. 3A). These tumor cells were separated by thin-walled sinusoidal vascular spaces in a focal pseudoalveolar pattern. The tumor cells were round with eccentrically located vesicular nu-

clei, and the cytoplasm was abundant and eosinophilic with a finely granular appearance. Special stain for reticulin delineated the tumor as alveolar or forming solid nests. Periodic acid Schiff stain (PAS) and diastase-resistant PAS stains revealed tumor cells with abundant cytoplasmic needle-like, pink-colored, crystal-

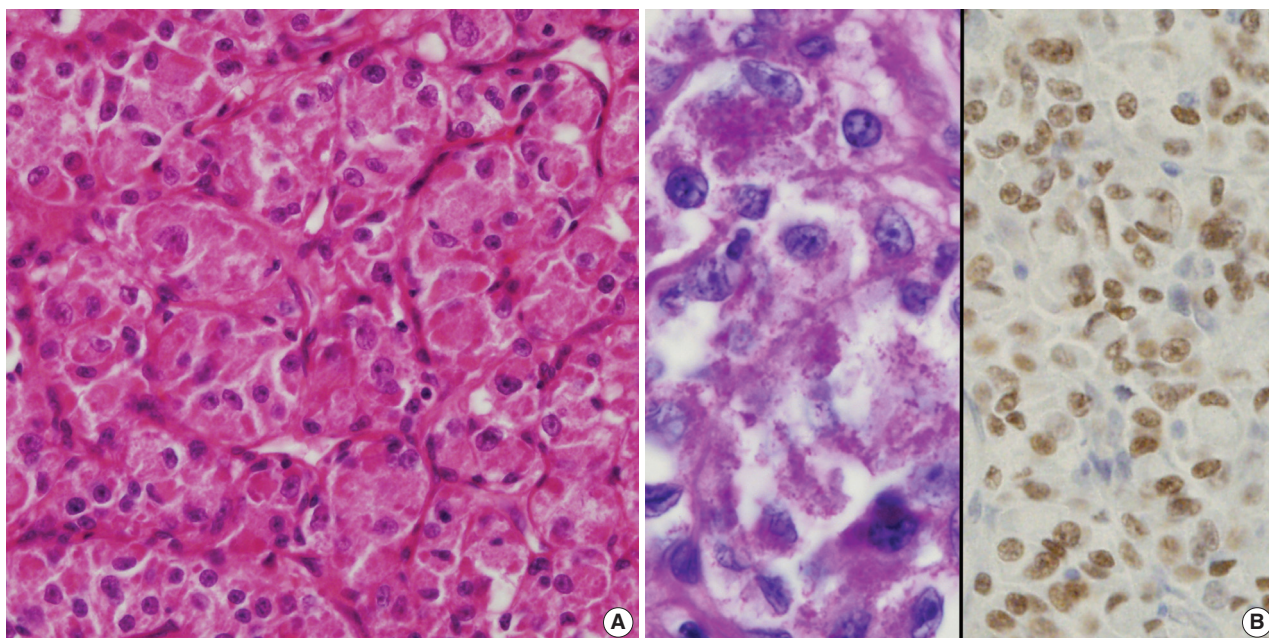


Fig. 3. (A) The pulmonary masses are composed of solid nests of large polygonal cells separated by sinusoidal vascular channels. (B) Diastase-resistant periodic acid-Schiff (PAS) stain highlights the intracytoplasmic, needle-like, crystalline structures (left, diastase-resistant PAS stain). The tumor cells show strong, positive nuclear reactivity for transcription factor E3 (TFE3) (right, TFE3 immunostain).

line accumulations (Fig. 3B). The tumor cells exhibited strong positive nuclear staining for TFE3/TFEB (1 : 600, P16-polyclonal sc-5958, Santa Cruz Biotechnology, Santa Cruz, CA, USA) (Fig. 3B) and vimentin (prediluted, V9, Dako, Glostrup, Denmark): but, were negative for neuron specific enolase (prediluted, BBS/NC/VI-H14, Dako), CD99 (prediluted, 12E7, Dako), smooth muscle actin (1 : 100, 1A4, Dako), desmin (1 : 100, D33, Dako), pancytokeratin (prediluted, AE1/AE3, Dako), cytokeratin (CK)7 (1 : 100, OV-TL 12/30, Dako), CK20 (1 : 100, K²⁰8, Dako), epithelial membrane antigen (prediluted, E29, Dako), CD34 (prediluted, QBEnd10, Dako), HMB-45 (prediluted, HMB-45, Dako), synaptophysin (1 : 40, SY38, Dako), chromogranin (1 : 2,000, DAKA3, Dako), S-100 protein (prediluted, polyclonal, Dako), CD30 (prediluted, Ber-H2, Dako) and leukocyte common antigen (prediluted, 2B11+PD7/26, Dako).

ASPSs were diagnosed based on the presence of characteristic morphological findings, PAS positive crystalline structures and positive TFE3 immunostaining. Although the remote possibility of primary pulmonary ASPS with intrapulmonary metastasis could not be excluded, it was thought to be metastatic disease due to the multiple lung nodules and rare occurrence of primary pulmonary ASPS. The brain lesions were also considered to be metastatic ASPS.

DISCUSSION

ASPS is a rare neoplasm of the soft tissue that mainly occurs in teenagers and young adults, and its pulmonary and cerebral presentation, as in the present case, is even rarer.¹⁶ Pulmonary ASPS is quite rare, and it typically shows dilated, tortuous and tubular structures within the mass, representing engorged vessels on CT.¹⁷ Most previous cases have been metastatic disease, and primary pulmonary ASPS without evidence of soft tissue tumor elsewhere is very exceptional.^{1,6,16} The present case had been diagnosed as metastatic pulmonary ASPS due to well demarcated multiple nodules despite no palpable and visible primary lesion elsewhere. The unusual clinicoradiologic features, the variable and unfamiliar cytologic features with abundant bared nuclei and cytoplasmic fragility of the epithelioid cells and the lack of clinical awareness of ASPS due to the rarity of the lesion have created a diagnostic dilemma in this case.

In past cytological reports, ASPS commonly exhibits cytoplasmic fragility and naked nuclei.^{10,12} Cellularity of FNA of ASPS varies according to the included amount of blood because ASPS is a highly vascular malignant tumor.^{17,18} Cytoplasmic features of ASPS are variable; mostly granular cytoplasm and, rarely vacuolated cytoplasm.^{9,10,12,14,15,18} Pseudoalveolar patterns that can be frequently found in excised tissue specimens of ASPS were not observed in FNA of the present case, except for

vaguely trabecular epithelioid clusters. These findings are same as those of previously reported cytologic studies of ASPS.^{12,13} Rod-shaped extracellular and cytoplasmic crystalline structures are a relatively uncommon finding in aspirated or imprint smears of ASPS, although they are important when identified.¹² The epithelioid morphology of ASPS represents a serious potential pitfall in the cytological diagnosis. There have been reports of the cytology of ASPSs being similar to malignant melanoma, paraganglioma, neuroendocrine tumor, synovial sarcoma, epithelioid sarcoma, oncocyctic neoplasm, granular cell tumor and plasmacytoma.⁹⁻¹⁵ Epithelioid sarcoma has more nuclear pleomorphism, and the tumor cells are variably shaped, occurring singly or in clusters with irregular nuclei, nuclear folds, macronucleoli and a high nucleus-cytoplasm ratio.¹⁹ These tumor cells are intermixed with a few inflammatory cells and necrotic tissue fragments. A perinuclear pale zone, well-defined cell borders and granuloma-like structure may be integral for diagnosing epithelioid sarcoma. Paraganglioma is composed of Zellballen patterns separated by vessels and surrounded by S-100 protein-positive sustentacular cells: these findings are rarely identified on the cytology slides, and paraganglioma tends to have more striking anisonucleosis with no prominent nucleoli as seen in ASPS. Granular cell tumor can mimic ASPS, but ASPS has a more epithelioid appearance, while granular cell tumor has more coarsely granular cytoplasm and more elongated and rectangular shaped tumor cells in the cytologic materials.¹⁸ Deeply eosinophilic and granular cytoplasm of ASPS tumor cells should be distinguished from those of oncocyctic neoplasm and plasmacytoma, in cytological studies. Plasmacytoma shows atypical

plasma cells with eccentric locations arranged in a dissociative fashion, but ASPS usually has centrally located nuclei only occasionally exhibiting eccentric nuclei. The monomorphic, large, round oncocyctic cells in oncocyctoma have eccentric nuclei and abundant dense granular cytoplasm arranged in confluent sheets. Malignant melanoma is another tumor included in the differential diagnosis, and shows cytological features such as high cellularity with epithelioid cells, spindle cells and clear cells, singly dispersed round to oval cells, eccentric nuclei, prominent nucleoli and nuclear pseudoinclusions, intracytoplasmic melanin pigment, marked pleomorphism, bi- and multinucleated giant cells, and a focal microacinar pattern. Rarely, ASPS findings include binucleated cells, which resemble Reed-Sternberg-like cells. However, melanoma has a better preserved cytoplasm, and pigmentation with no granular background, while the naked nuclei of dispersed tumor cells cytologically characterize ASPS. The different cytological characteristics have been summarized in Table 1.

Immunohistochemical assay plays only a limited role in the histological diagnosis of ASPS. However, recent cytogenetic studies have revealed that ASPS shows a unbalanced translocation, i.e., der (17)t(X;17) (p11.2;q25) fusing the *TFE3* gene at Xp11 to the *ASPL* gene at 17q25, creating an *ASPL-TFE3* fusion protein.⁷ Antibodies directed against the *ASPL-TFE3* fusion protein are expressed in the nuclei of ASPS tumor cells,⁸ and diagnosing ASPS using immunohistochemistry with *TFE3* antibody has shown to be highly accurate. Rare pediatric Xp11.2 translocation renal cell carcinomas, granular cell tumor and adrenocortical carcinoma are also labeled strongly by *TFE3*.²⁰

Table 1. Summary of cytological features compared to those of pulmonary ASPS

	ASPS	Epithelioid sarcoma	Granular cell tumor	Plasmacytoma	Oncocyctoma	Melanoma
Cellularity	Variably cellular	Moderately cellular	Variably cellular	Variably cellular	Variably cellular	Highly cellular
Background	Granular background	Necrotic, inflammatory, bloody	Granular background			Not granular background
Nuclear location	Central location	Eccentric location	Eccentric location	Eccentric location	Central or eccentric location	Eccentric location
Nuclei	Vesicular nuclei with prominent nucleoli, binucleation	Highly pleomorphic, and irregular shaped cells, perinuclear pale region, binucleation	Round, or spindle small cells with stripped nuclei and inconspicuous nucleoli	Nuclear atypia, moderate pleomorphism, and open chromatin, paranuclear hof, multinucleation	Monomorphic cells with fine, granular chromatin, inconspicuous nucleoli	Highly pleomorphic, vesicular, large nucleoli and nuclear inclusions, multinucleation
Cytoplasm	Fragile membrane, eosinophilic, granular cytoplasm	Dense and squamoid with distinct cell boundaries	Coarse granular, faintly basophilic cytoplasm	Basophilic cytoplasm	Abundant clear, granular cytoplasm, red granule on Papanicolaou stain	Melanin pigment
Others	Crystals, high vascularity, pseudoalveolar pattern	Granuloma-like structure, necrosis				

ASPS, alveolar soft part sarcoma.

In summary, we have described the FNA cytological features of pulmonary ASPS, an uncommon epithelioid/polygonal sarcoma, of idiopathic origin, that shares a large portion of features with other sarcomas with epithelioid cells. Awareness of the existence of this rare disease and knowledge of its cytologic features as well as diagnostic utility of TFE3 stain are important in correct diagnosis.

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