Journal of the American Society of Cytopathology (2019) 8, 293–298

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jascyto.org/

SHORT COMMUNICATION

Biphasic chest wall synovial sarcoma with epithelial pleural effusion: a diagnostic challenge

Hoda Zeinab M. Amer, MD, FCAP, Rongqin Ren, MD, PhD, Rulong Shen, MD, FCAP*

Cytopathology Department, The Ohio State University Wexner Medical Center, Columbus, Ohio

Received 30 January 2019; received in revised form 8 March 2019; accepted 11 March 2019

KEYWORDS
Synovial Sarcoma;
TLE1;
SS18/SSX2 fusion transcript;
Pleural effusion

Abstract Synovial sarcoma (SS) is a rare malignancy that most commonly involves the extremities and large joints. We describe a 67-year-old woman who presented with shortness of breath and flu-like symptoms, and a chest wall mass. On resection of the mass biphasic morphology of SS was noted, as well as confirmatory immunostains including TLE1 and bcl2. An SS18/SSX2 fusion transcript was detected by reverse transcriptase–DNA amplification. A year later, following chemotherapy, the patient developed a right-sided pleural effusion. Cytological examination of the fluid showed an epithelial population forming clusters and groups. TLE1 was positive, as well as fluorescent in situ hybridization analysis for the SS18/SSX2 fusion transcript. SS can be a challenging diagnosis in fluid-filled cavities, when the epithelial component predominates and its original biphasic quality is not seen. We discuss the diagnostic challenges of monophasic and biphasic SS, and updates to ancillary testing.

Clinical presentation

A 67-year-old white woman presented to the emergency department of an outside institution with shortness of breath and a viral respiratory-like illness. Her history was significant for being a secondary passive smoker for 55 years, and she also had a history of arrhythmia, asthma, gastroesophageal reflux disease, and hypothyroidism. She had no history of malignancy, though her family history included a deceased brother who had Hodgkin lymphoma and a sister who had cervical carcinoma.

A chest X-ray scan performed at the emergency department showed a mass involving the right upper lung with questionable chest wall/rib involvement, and the computed tomography (CT) scan defined the mass to be pleural-based with smooth borders and measuring 3.1 × 2.1 cm. No other

Sources of Support/Funding: None.
*Corresponding author: Rulong Shen, MD, FCAP, Cytopathology Department, The Ohio State University Wexner Medical Center, 410 W 10th Ave, Rhodes Hall S305C, Columbus, OH 43210; Tel.: (614) 293-8687; Fax: (614) 293-8747.
E-mail address: Rulong.Shen@osumc.edu (R. Shen).

2213-2945/$36
https://doi.org/10.1016/j.jasc.2019.03.002

abnormalities or lymphadenopathy were noted. A CT-guided biopsy was performed. Multiple core biopsies were reviewed at an outside institution, and described an "atypical epithelioid proliferation" that was positive for AE1/AE3, CAM5.2, and synaptophysin. Chromogranin was negative and the Ki-67 proliferative index was 1% to 2%. The final diagnosis was "consistent with carcinoid tumor, with a differential of typical and atypical carcinoid." This biopsy was not available for our review.

Two months later, a follow-up CT scan and positron emission tomography (PET) scan showed a PET-avid new T3 paravertebral mass/lymph node measuring 1.8 cm, as well as focal activity within a nodular thickening of the right pleura and a new supraclavicular node. An additional mass-like area of soft tissue thickening was noted posterior to the trachea, with intense PET activity. It was recommended that she undergo resection of the lesion, and she had a right thoracotomy with resection of the chest wall mass including ribs 2 and 3 and wedge resection of the right upper lobe, via right video-assisted thoracoscopy and intercostal nerve field block.

Surgical pathology of resection specimen

The resection specimen, per report from outside institution, consisted of chest wall ribs 2 and 3 with soft tissue, measuring in totality 6.3 × 3.3 × 3.2 cm. An intervening somewhat nodular mass surrounded by gray-purple membranous tissue was present between 2 portions of the ribs. The mass measured 4.1 × 2.6 cm, and was bisected longitudinally to reveal an off-white lesion with vague nodularity. The mass was 1.5 cm from the inked anterior margin and 0.8 cm from the inked superior margin. Sections were obtained of the soft tissue margins. An incision is made within the tumor toward the bone; grossly no frank

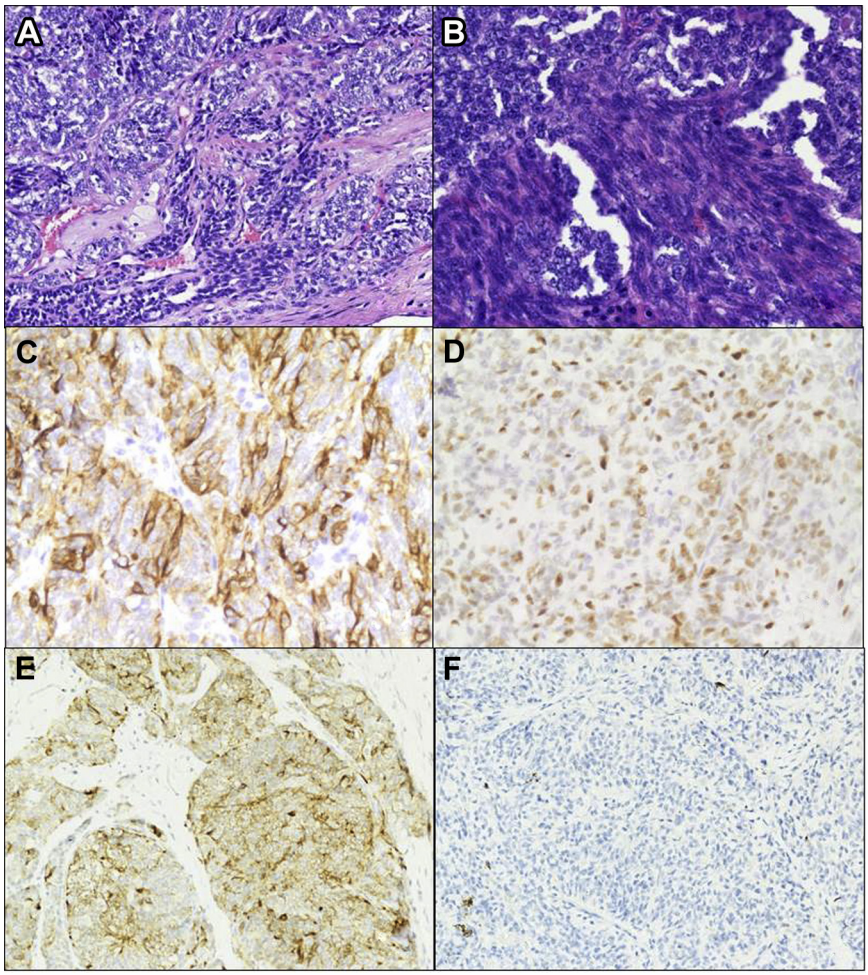

Figure 1 A, Resection specimen showed clusters and groups of epithelioid cells alternating with spindle cells (hematoxylin and eosin stain, 20×). B, Resection specimen showed clusters and groups of epithelioid cells alternating with spindle cells (hematoxylin and eosin stain, 40×). C, Immunohistochemistry performed on the resection specimen showing positive AE1/AE3 (AE1/AE3, 20×). D, Immunohistochemistry performed on the resection specimen showing positive TLE1 (TLE1, 20×). E, Immunohistochemistry performed on the resection specimen showing positive synaptophysin (synaptophysin, 20×). F, Immunohistochemistry performed on the resection specimen showing positive chromogranin. (Chromogranin, 20×).

involvement of the bone was noted, before and after decalcification. It was diagnosed as "atypical carcinoid tumor," with notable mitosis and a Ki-67 of 10%. The case was received by our institution for review, and for continuity of care of the patient.

Histopathology and immunophenotyping of surgical resection specimen

Review of the received slides of the resection specimen showed a spindle and epithelioid cell neoplasm with brisk mitotic activity (Figs. 1A and B). Cystic changes and lymphatic permeation were noted. Immunostains performed at the outside institution were positive for CK7, Cam5.2, weakly positive for synaptophysin, and negative for CK20 and TTF-1. Interpretation of Ki-67 showed variability, ranging from 5% to 30%-40% per our institution manual assessment. Additional immunostains were performed, showing focally positive AE1/3 (Fig. 1C), focally/weakly positive TLE1 (Fig. 1D), CD56, bcl-2, and rare positive cells for CK5/6 and p63. CD99 was equivocal. Synaptophysin was repeated, and was focally/partially positive (Fig. 1E). Chromogranin was negative (Fig. 1F), as was CD34, SMA, desmin, S100, PAX8, CK20, and TTF-1.

Molecular testing on surgical resection specimen

Total RNA was extracted from the submitted specimen and transcribed to cDNA with reverse transcriptase. The cDNA was subsequently analyzed using primers to the *SSXl* and *SSX2* (Xp11.2) genes along with a primer to the *SS18* gene (18q11.2), and primers to the *EWSR1* gene (22q12) and a primer to the *FLI1* gene (11q24). Products

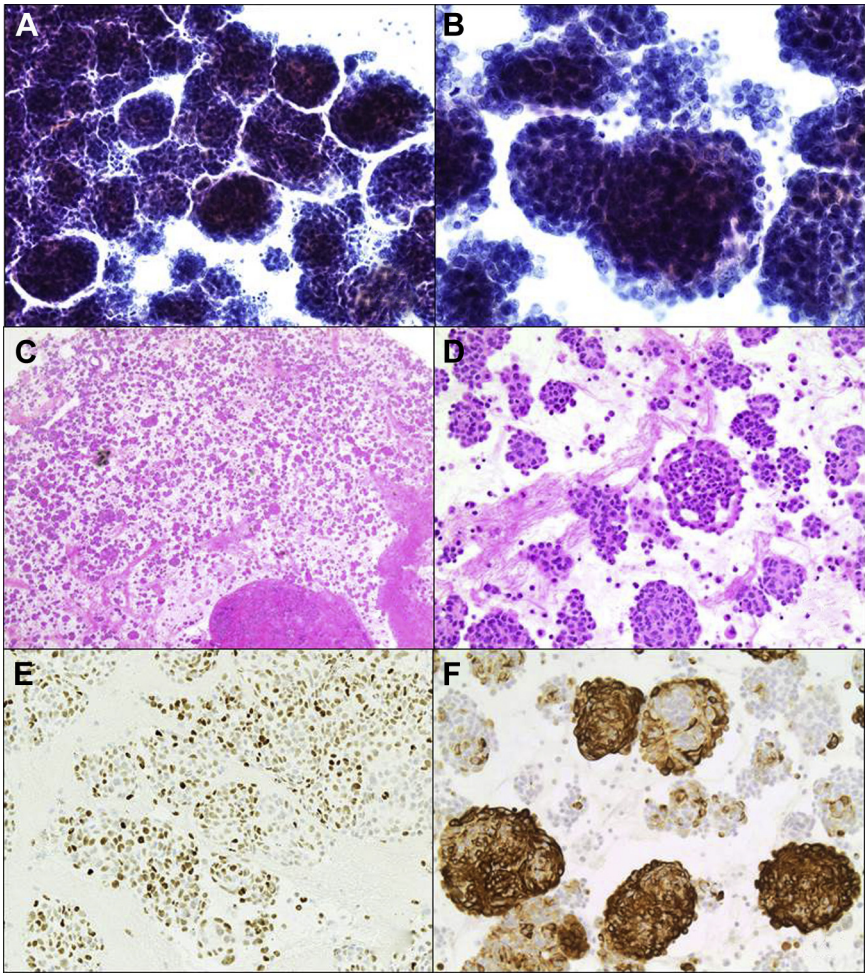

Figure 2 A, Pleural effusion showed clusters and groups of cohesive epithelioid cells showing fine to dense cytoplasm, marked pleomorphism and anisonucleosis (Papanicolaou stain, 20×). B, Pleural effusion showed clusters and groups of cohesive epithelioid cells showing fine to dense cytoplasm, marked pleomorphism, and anisonucleosis (Papanicalaou stain, 40×). C and D, The cell block showed mostly groups and clumps of cells forming glandular-like structures with variable morphology; predominantly rounded, cleaved, epithelioid cells (hematoxylin & eosin stain, C = 10×, D = 20×). E, Immunohistochemistry on cell block of pleural effusion, showing positive TLE1 (TLE 1, 20×). F, Immunohistochemistry on cell block of pleural effusion, showing patchy positive cytokeratin 7 (CK 7, 40×).

were analyzed on an agarose gel. The integrity of the mRNA was assessed to 283 base pairs by an independent amplification using primers to the ubiquitously expressed hypoxanthine phosphoribosyl transferase (*HPRT*) gene. An *SS18/SSX2* fusion transcript was detected by reverse transcriptase–DNA amplification and *EWSR1/FLI1* fusion transcript was not detected. The case was signed out as synovial sarcoma.

Post-treatment clinical presentation

The patient received chemotherapy in the form of doxorubicin and olaratumab, in a total of 15 cycles, as well as pazopanib. Unfortunately, restaging scans revealed progression of the disease. PET scan showed an interval increase in the size of the right supraclavicular lymph node, which now measured 2.6 × 1.4 cm (previously 2 × 1.2 cm). The small paravertebral mass was also present. A right hemithorax and a new small right effusion were noted. She was admitted for thoracocentesis.

Cytopathology of pleural effusion

A total of 900 mL of cloudy yellow fluid from thoracocentesis was received, from which 2 cystospins were prepared and stained with Papanicolaou stain (Pap stain), and 2 cytospins were prepared and stained with Diff-Quik stain (modified Wright-Giemsa stain). A cell block was also prepared.

Cytology and immunophenotyping of pleural effusion

Review of the smears showed clusters and balls of cohesive epithelioid cells showing fine to dense cytoplasm, marked pleomorphism, and anisonucleosis. Scattered discohesive cells were also seen, varying in size. Smaller cells showed more round uniform nuclei. Papanicolaou stain showed a fine chromatin pattern, with small but conspicuous nucleoli. Mitosis was frequent (Figs. 2A and B). Occasional cleaved or indented nuclei were noted. Rosette formation was noticeable on Papanicolaou stain more than Diff-Quik. A background of lymphocytes and rare neutrophils was present.

The cell block showed mostly groups and clumps of cells forming glandular-like structure with variable morphology; predominantly rounded and cleaved epithelioid cells. The majority showed a fine chromatin pattern with small conspicuous nucleoli, and occasional cells with larger dark nucleoli were more apparent (Figs. 2C and D). Occasional Homer-Wright–like rosettes and an occasional ribbon-like architecture with palisading nuclei were seen. In light of the patient's history, immunohistochemistry was ordered, showing positive vimentin, TLE1, CK7 (partial) (Figs. 2E and F), p16 (diffuse strong), and p53 (wild type). It was negative for TTF-1, PAX8, CK20, calretinin.

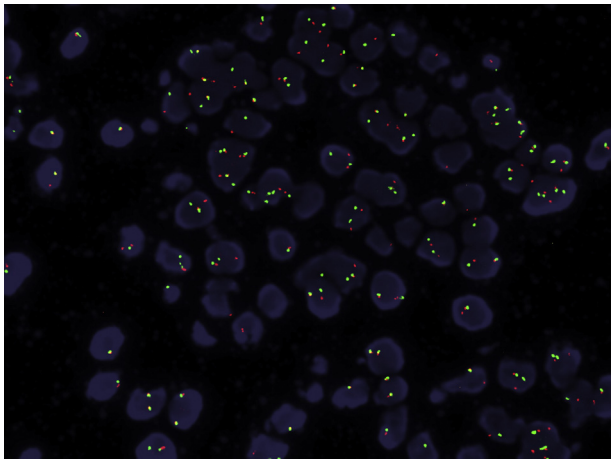

Figure 3 FISH results; using Vysis LSI SS18 (Tel) Spectrum Orange Probe and Vysis LSI SS18 (Cen) Spectrum Green Probe, showing rearrangement involving the SS18 gene at chromosome 18q11.2, in the majority of tumor cells.

Molecular testing on pleural effusion

For confirmation, the case was sent for interphase fluorescent in situ hybridization (FISH) on the formalin-fixed paraffin embedded tissue, using a combination of Vysis LSI *SS18* (Tel) Spectrum Orange Probe and Vysis LSI *SS18* (Cen) Spectrum Green Probe (Vysis, Abbott Molecular, Des Plaines, IL). A pattern consistent with a rearrangement involving the *SS18* gene (formerly *SYT*) at chromosome 18q11.2 was seen (Fig. 3).

Discussion

Synovial sarcomas (SSs) are rare malignant tumors, accounting for 5% to 14% of soft tissue sarcomas.[1] The entity was originally described in 1865 by Simon et al, with the descriptor name of "hypertrophied sarcomatous synovial fringe.[2] Jones and Whitman in 1914, as well as Sabrazes in 1934, coined the term "synovial sarcoma," noting its histologic features that were reminiscent of embryologic synovium, and the propensity of SS to involve large joints.[3-5] Although the exact cell of origin of SS remains unclear, it likely arises from pluripotent mesenchymal cells that acquire the ability to differentiate along epithelial lines through mutation of *SS18-SSX2* and repression of E-cadherin.[4,6,7]

From a clinical standpoint, SS occurs in a variable range of ages, with a median age of 35 years, and a slight male predominance. Occurring most commonly near large joints, 70% of SSs are seen in extremities.[6] Metastatic disease is common in SS, occurring in almost one-half of patients (47%), with a median time to occurrence of 4.5 years from the initial diagnosis. Metastases occur mainly to the lungs

(79%). Lymph nodes, chest wall, and abdomen, and less common areas such as kidney, pancreas, and brain, have been described. Local recurrence is also a common occurrence in nearly half of patients (47%), approximately 4 years after the initial diagnosis, and sometimes even as late as 10 years.[8] SS can have a variable prognosis. Adverse prognostic factors include male sex, truncal location, larger-sized lesions, and older age. Monophasic tumors have a worse prognosis than biphasic tumors, and other histologic features such as increased mitosis, necrosis, neurovascular invasion, and inadequate margins noted on the resection, are poor prognostic indicators.[9]

SS only rarely involves the chest wall, with fewer than 10 cases reported in the literature,[1] dating as far back as 1950.[10] Common presenting symptoms of chest wall SS are chest pain, dyspnea, and cough, depending on the thoracic organ or structure involved.[1] On imaging, SS of the chest wall is generally similar to SS seen elsewhere, a heterogenous enhancing mass with well-defined margins and internal calcifications, and fluid−fluid levels that are referred to as "bowl-of-fruit," better demonstrated on magnetic resonance imaging.[11] Cortical bone destruction is common, as well as invasion of muscle.[11,12]

SS of the chest wall poses unique challenges, largely because of its wide differential, which includes lung and pleural-based tumors. SS with a prominent spindle cell component must be distinguished from other tumors such as solitary fibrous tumor, sarcomatoid mesothelioma, or other entities such as fibrosarcoma, leiomyosarcoma, or neuroendocrine carcinoma spindle cell variant. Those with a prominent epithelial component must be distinguished from epithelioid mesothelioma, adenocarcinoma, metastatic carcinomas, or even melanoma.[12-14] SS rarely involves the body cavities, and its cytomorphology has not been thoroughly described. It is presumed however, that it would be cytomorphologically similar to corresponding soft tissue fine-needle aspiration cytology.[13] On cytologic evaluation, the monophasic fibrousness of SS type shows elongated oval to spindle cells with unipolar or bipolar cytoplasmic processes, arranged in clusters, with occasional single naked nuclei. Nuclei are variable, showing bland chromatin pattern with inconspicuous nucleoli, or hyperchromatic pleomorphic types. Common architectural patterns include branching tumor fragments a long vessels as well as acinar forms. The background can show scant mucin, mast cells, and/or calcification. Occasionally, whorling and pericytic patterns can be seen. The biphasic subtype and purely epithelial SS, which is rare, will show an epithelial component consisting of larger cells with vesicular nuclei, pale cytoplasm, and well-defined borders. The epithelial cells form balls, nests, glandular structures, and tubular formations. Glandular structures can occasionally have mucin-like material.[13-15] It has been noted that biphasic SS tumors on metastases lose their epithelioid morphology, retaining their spindle cell component.[15,16] We report a different occurrence, where the biphasic morphology of the SS chest wall lesion was altered in the pleural effusion, showing a loss of the spindle cell morphology, while retaining the epithelia component. This has not been previously described.

Other rare types of SS include the undifferentiated small round blue cell type, which must be distinguished other small round cell tumors. Up to 20% of SSs can show undifferentiated areas.[15]

Ancillary testing has become invaluable for the accurate diagnosis of SS. Immunohistochemistry is a convenient method that can be easily performed on paraffin blocks. TLE1 is an immunostain that shows expression in 95% of SS, although the specificity ranges from 63.7% to 72%.[17] Decreased expression of TLE is noted particularly in subtypes such as pleuropulmonary SS. In the undifferentiated round cell SS, Calponin has been found to be of use.[17] Other immunostains helpful for ruling out other spindle cell tumors include EMA, BCL2, MIC2, CD34, and CK.[17] In the epithelial component, the immunostains of CK7, CK19, and CK8/18 are frequently expressed. Other useful immunostains depend on the differential diagnosis and site of the tumor. For example, in this case, a TTF-1, Napsin A, Calretinin, and WT-1 were negative, thus ruling out adenocarcinoma or mesothelioma of the lung and pleural cavity. The biopsy and resection specimen of this particular case of SS both showed a weak staining of synaptophysin. This unconventional staining pattern in combination with the unique chest wall location misled the pathologist in the direction of a neuroendocrine tumor, of atypical carcinoid. Recently, a rare subset of SS tumors with "granular cell" morphology was described to be synaptophysin-positive.[18] This "granular cell" morphology was not overtly evident in the sections available for our review. Further studies are necessary to explore possible other rare subtypes of SS that may be synaptophysin-positive.

Molecular testing through gene fusion analysis, or by evidence of break-apart test FISH, has become standard in the diagnosis of SS. Ninety-five percent of SSs are characterized by expression of the *SS18-SSX* gene fusion oncogene, formed by the chromosomal translocation t(X;18) (p11.2;q11.2). The correlation between the histologic subtype and its corresponding fusion gene has been described, the *SS18−SSX1* fusion is found predominantly in biphasic tumors, while *SS18−SSX1* and *SS18−SSX2* fusions are found equally in monophasic tumors.[6,7] Other forms of molecular testing have been recently introduced, including gene expression profiling of SS, in particular that of complexity index in sarcomas (CINSARC). CINSARC is a prognostic panel of 67 genes for sarcomas that has been shown to be of use in defining the genomic character of metastatic versus primary SS tumors.[17] Whole-blood microRNA signature has also provided a useful tool to assess metastatic risk in SS, noting that the expression levels of seven microRNAs decreased following tumor resection, and increased in metastases.[19] Several biomarkers have been also been described in SS, and can potentially be used to evaluate for recurrence or metastatic disease, and help to determine prognosis. Examples include insulin-like growth factor-binding protein-7 (IGFBP7) and matrix metalloproteinases (MMPs), both of which are found to be increased

in metastatic SS, and Secernin-1 (SCRN1), a 50-kDa cytosolic protein that was found to be negative in metastatic disease.[17,19]

Conclusions

SS of the chest wall is a rare occurrence, and involvement of fluid-filled cavities can pose a diagnostic challenge on fine-needle aspirations, particularly when the predominant component of the pleural effusion is epithelial rather than biphasic. Ancillary testing with immunohistochemistry and molecular methods are essential tools for accurate diagnosis.

References

1. Braham E, Aloui S, Aouadi S, Drira I, Kilani T, El Mezni F. Synovial sarcoma of the chest wall: a case report and literature review. *Ann Transl Med*. 2013;1:9.
2. Simon G. Exstirpation einer sehr grossen, mit dicken Stiele angewachsenen Kneigelenkmaus mit gluklichem Erfolge. *Archiv.fur Klin Chir*. 1865;6:573–576.
3. Jones SF, Whitman RC. Primary sarcoma of the lower end of the femur involving the synovial membrane: with a critical review of the literature of synovial sarcoma report of one case with complete pathological and radiographic examination. *Ann Surg*. 1914;60:440–450.
4. Brand RA. 50 years ago in CORR: synovial sarcoma. Kirk J. Anderson, MD and Orliss Wildermuth, MD CORR 1961;29:55-70. *Clin Orthop Relat Res*. 2013;471:1064–1066.
5. Sabrazes J, Loubat E, de Grially R, Magendie J. Synovial sarcomas. *Gaz Hebd Sc Med Bordeaux*. 1934;55:754–762.
6. Nielsen TO, Poulin NM, Ladanyi M. Synovial sarcoma: recent discoveries as a roadmap to new avenues for therapy. *Cancer Discov*. 2015;5:124–134.
7. Saito T. The SYT-SSX fusion protein and histological epithelial differentiation in synovial sarcoma: relationship with extracellular matrix remodeling. *Int J Clin Exp Pathol*. 2013;6:2272–2279.
8. Krieg AH, Hefti F, Speth BM, et al. Synovial sarcomas usually metastasize after >5 years: a multicenter retrospective analysis with minimum follow-up of 10 years for survivors. *Ann Oncol*. 2011;22:458–467.
9. Sharp SJE, Choong PFM. Factors predictive of survival in synovial sarcoma; a review of the literature. *Clin Oncol*. 2016;1:1051.
10. Eisenberg RB, Horn RC. Synovial sarcoma of the chest wall; report of a case. *Ann Surg*. 1950;131:281–286.
11. Fujimoto K, Hashimoto S, Abe T, et al. Synovial sarcoma arising from the chest wall: MR imaging findings. *Radiat Med*. 1997;15:411–414.
12. De Paoli L, Quaia E, Poillucci G, Gennari A, Cova MA. Imaging characteristics of pleural tumours. *Insights Imaging*. 2015;6:729–740.
13. Wakely Jr P. Pulmonary spindle cell lesions: correlation of aspiration cytopathology and histopathology. *Ann Diagn Pathol*. 2001;5:216–228.
14. Leona A, Doyle MD, Jason L, Hornick MD. *Epithelioid and Epithelial-Like Tumors in Practical Soft Tissue Pathology: a Diagnostic Approach*. 2nd ed. Philadelphia, PA: Elsevier; 2019.
15. Bhagat V, Tailor H, Baldwa V, Unjiya R. Cytopathological diagnosis of primary synovial sarcoma of lung–a rare case report. *Int J Med Sci Public Health*. 2013;2:1123–1124.
16. Enzinger FM, Weiss SE. Synovial sarcoma. In: Enzinger FM, Weiss SE, eds. *Soft Tissue Tumors*. 4th ed. St. Louis, MO: C.V. Mosby; 2006:1483–1502.
17. Rekhi B, Basak R, Desai SB, Jambhekar NA. Immunohistochemical validation of TLE1, a novel marker, for synovial sarcomas. *Indian J Med Res*. 2012;136:766–775.
18. Setsu N, Kohashi K, Endo M, et al. Inhibin-α and synaptophysin immunoreactivity in synovial sarcoma with granular cell features. *Hum Pathol*. 2012;43:850–857.
19. de Necochea-Campion R, Zuckerman LM, Mirshahidi HR, Khosrowpour S, Chen CS, Mirshahidi S. Metastatic biomarkers in synovial sarcoma. *Biomark Res*. 2017;5:4.