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Diagnostic Challenges in Fine-Needle Aspiration Cytology of Mediastinal Tumors and Lesions

Huihong Xu, MD; Fang Fan, MD, PhD; Yun Gong, MD; Xin Jing, MD; Xiaoqi Lin, MD, PhD; He Wang, MD, PhD; Fan Lin, MD, PhD; Zaibo Li, MD, PhD

- **Context.**—Mediastinal tumors/lesions are frequently encountered in daily cytopathology practice. These lesions are accessible through endoscopic/endobronchial ultrasound-guided or computed tomography-guided fine-needle aspiration cytology and represent a wide range of primary and metastatic tumors. This often poses diagnostic challenges because of the complexity of the mediastinal anatomic structures. Tumors metastatic to mediastinal lymph nodes represent the most common mediastinal lesions and must be differentiated from primary lesions.

- **Objective.**—To provide an updated review on the fine-needle aspiration cytology of mediastinal tumors/lesions, with an emphasis on diagnostic challenges. This review encompasses thymic epithelial neoplasms, mediastinal lymphoproliferative disorders, germ cell tumors, neuroendocrine tumors, soft tissue tumors, and metastatic tumors. Differential diagnoses; useful ancillary studies, including targeted immunohistochemical panels; and diagnostic pitfalls are discussed.

- **Data Sources.**—Data were gathered from a PubMed search of peer-reviewed literature on mediastinal tumors. Data were also collected from the authors’ own practices.

- **Conclusions.**—Fine-needle aspiration cytology plays a vital role in evaluation of mediastinal lesions. Being familiar with the clinical and cytomorphologic features of these lesions, appropriately triaging the diagnostic material for ancillary testing, and correlating with radiologic findings are important in arriving at correct diagnoses and guiding management.

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The mediastinum is the site for a variety of lesions, both benign and malignant. The most frequent primary tumor types of anterior mediastinal masses are thymic tumors and lymphoma, with approximate proportions of 35% and 25% among mediastinal lesions, respectively. Less common tumors of anterior mediastinal masses are thyroid and endocrine tumors (15%), teratomas (10%), malignant germ cell tumors (GCTs; 10%), and benign thymic lesions (5%).

Fine-needle aspiration cytology (FNAC) was first introduced by Martin and Ellis and has been used as an easy and safe procedure for diagnosing primary and metastatic mediastinal diseases. Fine-needle aspiration cytology of mediastinal lesions (5%).

Data were also collected from the authors’ own practices.

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mediastinal lesions (location, size, solid/cystic mass, borders, etc) and laboratory studies, because the incidence and histopathologic features of tumor are closely related to a particular mediastinal anatomic compartment. For example, a large lung carcinoma or pleural-based mesothelioma that extends into the mediastinum may mistakenly be considered as a primary mediastinal tumor/thymic tumor. Although imaging is a quick and noninvasive approach, it is limited by the relatively low sensitivity and specificity. The third step is to analyze the cytomorphology both during and after FNAC procedures (eg, ROSE, EBUS/endoscopic ultrasound, and computed tomography guided). EBUS-TBNA is mostly used in diagnosing mediastinal lymphadenopathy and masses of unknown etiology. The last step is the ancillary testing on the available cytology materials (eg, smears and cell blocks). The cellularity of cell blocks and other cytologic preparations is always a concern for ancillary testing, especially in judiciously selecting the least number of immunostains to support a specific cytology diagnosis, and having sufficient cellular material for further molecular and cytogenetic studies. Therefore, adequate sampling should always be emphasized in order to obtain reliable results from ancillary testing on cell blocks or other cytologic materials. With the new advancements in biotechnology, the use of cytology in the diagnosis of mediastinal lesions is taking the lead role in guiding the postprocedure diagnosis, patient management, and follow-up care.

**THYMIC EPITHELIAL NEOPLASMS**

**Thymoma**

Thymoma is the most common primary tumor of the anterior superior mediastinum and is a neoplasm of adults with an average age of 50 years. Patients with thymoma commonly present clinical paraneoplastic syndromes, such as pure red blood cell aplasia and hypogammaglobulinemia, and up to 40% of patients with thymoma can present with myasthenia gravis. Thymomas are slow-growing tumors, and the most important prognostic factors include tumor size, completeness of resection, and clinical staging (modified Masaoka staging). Histologically, thymoma is a biphasic tumor with neoplastic epithelial cells and benign, reactive immature T lymphocytes. Based on the morphologic features of epithelial tumor cells, the relative proportion of the nontumoral lymphocytic component and resemblance to normal thymic architecture, the World Health Organization (WHO) classification divides thymoma into the following categories. Type A contains spindled or ovoid neoplastic cells, and type B contains predominantly round or polygonal neoplastic cells. Type B is further subdivided into B1, B2, and B3 based on the proportion of lymphocytes (B1 has the highest proportion of lymphocytes and B3 has the lowest) and degree of epithelial atypia. Type AB contains a mixture of types A and B components. Type A and type AB mostly show benign behavior, type B1 has low malignant potential, and types B2 and B3 have moderate malignant potential.
Cytomorphologically, identification of a biphasic lymphoepithelial complex with an admixture of cytologically bland, polygonal/oval/spindled epithelial cells and reactive lymphocytes is generally diagnostic for thymoma. Estimation of the proportion of reactive lymphocytes to neoplastic cells may provide information to further classify subtypes of thymoma. Type A thymoma consists of bland, ovoid/spindle-shaped cells with finely dispersed, homogeneous chromatin and indistinct nucleoli to form whirling fragments, which may also contain arborizing capillaries, scant lymphocytes, and, rarely, Hassall corpuscles (Figure 2, A through C). Type B thymoma usually contains bland, polygonal cells with vesicular nuclei, finely dispersed, open chromatin, and conspicuous nucleoli (Figure 2, E and F). However, neoplastic epithelial cells in type B3 thymoma may show mild atypia.

The neoplastic epithelial cells of thymoma are positive for pan-cytokeratin (AE1/3 and CAM5.2) and p63 (Figure 2, D and G). PAX8 is consistently expressed in thymic epithelial neoplasms, including both thymoma and thymic carcinoma. The reactive T lymphocytes in thymoma are positive for TdT, CD3, CD99, and CD1a. CD20 can be positive in up to 50% of type A and type AB thymomas.

There are several pitfalls in diagnosing thymoma by FNAC. The first one is to distinguish epithelial-rich type B3 thymoma from thymic carcinoma. Type B3 thymomas are believed to have intermediate biologic potential between other types of thymomas and thymic carcinomas. Type B3 thymomas show relatively benign-appearing nuclei or mild nuclear atypia without overt cytomorphic atypia, relatively high smear cellularity in the proper clinical context, and lack of necrosis. In contrast, thymic carcinoma is monophasic, and its epithelial cells contain large, irregular nuclei with coarse chromatin, with or without prominent nucleoli. Ancillary studies are helpful because thymoma epithelial cells are negative for CD5, CD117, Glut1, and MUC1, which are positive in thymic carcinoma cells.

Differentiating lymphocyte-rich type B1 thymomas from lymphoma is another challenging scenario in cytology diagnosis. In such cases, demonstration of immature T-lymphocyte component (TdT+, CD3+, CD99+, CD1a+) on flow cytometry and a neoplastic epithelial component (smears, hematoxylin-eosin stain [H&E], and immunohistochemistry with cytokeratins) will help make a diagnosis of thymoma. Most acute lymphoblastic lymphoma/leukemia (ALL) cells are T-cell–derived with an immunophenotype of T lymphocytes (TdT+, CD3+, CD99+), and are larger than mature lymphocytes with enlarged nuclei containing evenly dispersed chromatin, but ALL usually occurs in children. Although type B1 thymomas can also show a large population of immature T lymphocytes with similar immunophenotype, they usually occur in adults, are slow growing, and show thymic neoplastic epithelial cells. A diagnosis of T-cell ALL (T-ALL) in a mediastinal mass should be rendered with extreme caution because both thymoma and T-ALL can exhibit an immature T-cell phenotype, such as CD1a, TdT, CD34, CD10, CD45dim, CD99, cCD3, partial sCD3, and double CD4/CD8 expression. It is important to note that expression of CD3, CD45, CD4, and CD8 in thymocytes shows a smearing pattern ranging from high density to negative, reflecting all stages of T-cell maturation, whereas T-ALL cells express these antigens in a tight clustering pattern. In addition, loss of T-cell antigens, and expression of CD10 and CD34, are in favor of T-ALL.

Thymic Carcinoma

Thymic carcinoma is very rare and only accounts for less than 1% of thymic tumors. It mostly occurs in middle-aged patients (30–60 years) with a slight male predilection (male to female ratio of 1.5). Patients with thymic carcinoma usually present with nonspecific symptoms, such as weight loss, anorexia, dyspnea, and chest pain, or without any symptoms. Thymic carcinoma is highly aggressive, with a median survival of 18 months in high-grade carcinomas. Thymic carcinoma contains heterogeneous groups with variable histologic features, including keratinizing/nonkeratinizing squamous cell (most common subtype), basaloïd squamous cell, anaplastic large cell, mucoepidermoid, adenosquamous, clear cell, small cell, lymphoepithelioma-like, and papillary and nonpapillary adenocarcinoma.

For low-grade, well-encapsulated thymic carcinoma, surgical resection may be curative. However, for most thymic carcinomas, radiation and chemotherapy are commonly used as adjuvant therapy after surgery.

The histopathology of each of these thymic carcinoma variants mimics its counterpart in other anatomic sites. Cytomorphologically, smears are variably cellular and demonstrate poorly differentiated carcinoma with loosely cohesive, large, polygonal malignant cells with irregular nuclear contours (Figure 2, H). Necrosis and mitoses are common. Thymic carcinoma cells are large epithelial cells with overtly malignant nuclei with coarse chromatin, with or without prominent nucleoli. Thymic carcinomas may show mature lymphocytes in the background, which are mostly mature B lymphocytes, although the lymphocytes in lymphoepithelioma-like carcinoma are mostly mature T lymphocytes.

Thymic carcinoma cells are positive for cytokeratin, PAX8, p63, CD5, and CD117. In addition, thymic carcinoma cells may also be positive for Glut1 and MUC1.

The differential diagnosis for thymic carcinoma includes type B3 thymoma, metastatic malignancies, and NUT (nuclear protein of the testis) carcinoma. Differentiation between thymic carcinomas and type B3 thymoma is described as in thymoma. To differentiate from metastatic malignancies, demonstration of positive PAX8, CD5, and CD117 staining in tumor cells would be consistent with the diagnosis of a thymic carcinoma. NUT carcinoma represents a small but appreciable percentage of poorly differentiated or undifferentiated mediastinal malignancies, including examples of both epithelioid and round cell types. NUT nuclear staining and molecular study for NUT gene translocation will confirm the diagnosis of NUT carcinoma.

MEDIASTINAL LYMPHOPROLIFERATIVE DISORDERS

Lymphomas are relatively common malignancies involving the mediastinum, but most are of secondary involvement. Less common are primary mediastinal lymphomas, including Hodgkin lymphoma, primary mediastinal large B-cell lymphoma, lymphoblastic lymphoma, and others. Hodgkin lymphoma is the most common primary mediastinal lymphoma, accounting for up to 70%. When lymphoproliferative disorder is suspected during ROSE, additional passes with fresh tissue materials should be reserved for potential flow cytometry.

Classic Hodgkin Lymphoma

Histologically, classic Hodgkin lymphoma is classified into 4 variants: nodular sclerosis, lymphocyte rich, mixed...
Figure 2. Thymic epithelial neoplasms. A, Type A thymoma with spindle-shaped epithelial cells. B, Type A thymoma with spindle-shaped epithelial cells, cell block. C, Follow-up surgical resection shows typical type A thymoma with spindle tumor cells. D, AE1/3 is positive in tumor cells. E, Type B1 thymoma with round neoplastic cells and abundant small lymphocytes. F, Type B1 thymoma with round neoplastic cells and abundant small lymphocytes. G, Type B1 thymoma with positive AE1/3 staining in neoplastic epithelial cells. H, Thymic carcinoma with loosely cohesive, large, polygonal malignant cells with irregular nuclear contours (Diff-Quik, original magnifications x100 [A] and x400 [F]; hematoxylin-eosin, original magnifications x100 [B] and x200 [C]; Papanicolaou stain, original magnifications x100 [E] and x200 [H]; original magnifications x200 [D] and x100 [G]).
cellularity, and lymphocyte depleted. Distinction between the 4 variants is generally not possible by FNAC. The nodular sclerosis variant accounts for the vast majority of mediastinal classic Hodgkin lymphomas and comprises approximately 35% of all mediastinal lymphomas.\(^3\) Classic Hodgkin lymphomas most commonly present in young females aged 15 to 34 years.\(^3\)

Cytologically, classic Hodgkin lymphomas are best characterized by the presence of Reed-Sternberg/Hodgkin cells in a background of polymorphous lymphoid population comprising small lymphocytes, plasma cells, eosinophils, and histiocytes. Reed-Sternberg cells are large mononucleated or binucleated cells with prominent inclusion-like nucleoli (macronucleoli), and moderate delicate cytoplasm (Figure 3, A and B). Granulomatous inflammation may rarely be present.

Misdiagnosis of primary thymic Hodgkin lymphoma as thymoma has been reported in the literature, especially in cases when Hodgkin lymphoma developed in the background of residual thymic parenchymal cells.\(^2\) Reed-Sternberg cells are believed to be of germinal center B-cell origin, yet they have lost most of their B-cell-specific antigen expression. Reed-Sternberg cells are positive for CD15, CD30, PAX-5 (weak), and MUM1, negative for CD45, and show decreased or negative expression of B-cell markers, including CD20, CD79a, OCT2, and BOB1.

**Primary Mediastinal Large B-Cell Lymphoma**

Primary mediastinal large B-cell lymphoma (PMBCL) is derived from thymic B cells and is the second most common non-Hodgkin lymphoma arising in the anterior mediastinum (after T-ALLs).\(^3\) PMBCL mainly affects women between ages 20 and 50 years.\(^3\)

Cytologically, PMBCL is identical to diffuse large B-cell lymphomas. Fine-needle aspiration cytology of PMBCL reveals a population of large pleomorphic cells with scant cytoplasm, irregular nuclear membranes, and prominent nucleoli. Lymphoglandular bodies are often seen in the background.

PMBCL cells are positive for panlymphoid marker CD45 and pan–B-cell markers, such as CD19, CD20, PAX5, and CD79a, but expression of CD5, CD10, CD30, BCL6, MUM1, and BCL2 is variable.

Fine-needle aspiration cytology samples of PMBCL can be paucicellular with only fragments of collagen and very few diagnostic cells because of sclerosis, which is common in PMBCL.

**Acute Lymphoblastic Lymphoma/Leukemia**

Acute lymphoblastic lymphoma/leukemia comprises 80% to 90% of all anterior mediastinal non-Hodgkin lymphomas and 40% of childhood lymphomas.\(^2,3\) Most ALLs are T-cell derived (80%). Cytomorphologically, lymphoblast cells are usually 1.5- to 2-fold larger than mature lymphocytes, and they show a high nuclear to cytoplasmic ratio, evenly distributed fine chromatin, and inconspicuous nucleoli. Immunophenotypically, ALL cells express immature thymic T-cell markers, including TdT, CD3, and CD99.

**MEDIASTINAL GERM CELL TUMORS**

Germ cell tumors (GCTs) comprise 15% of adult mediastinal tumors as the second most common tumor of the mediastinum after thymoma. Most GCTs arise in the anterior mediastinum, whereas a small portion involves the posterior mediastinum.\(^2,37,38\) Germ cell tumors are predominantly seen in male individuals (97%) with an average age of 31 years.\(^39,40\) Mediastinal GCTs include teratoma, seminoma, and nonseminomatous GCTs (embryonal carcinoma, yolk sac tumor [YST], and choriocarcinoma), with teratoma as the most common entity, followed by seminoma.\(^39,40\)

**Teratomas**

Mediastinal teratomas are usually located in the anterior mediastinum. Biochemical markers are typically not elevated in benign teratomas. Mediastinal teratomas contain mature and/or immature somatic elements derived from ectodermal, mesenchymal, or endodermal cells. Endodermal elements include gastrointestinal, respiratory, and endocrine gland tissue. Mesodermal elements are represented by bone, cartilage, and muscle. Ectodermal tissue can

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**Figure 3.** Classic Hodgkin lymphoma. A, Large mononucleated and binucleated Reed-Sternberg cells with prominent nucleoli are present in a background of small lymphocytes. B, binucleated Reed-Sternberg cells with prominent nucleoli are present in a background of a polymorphous lymphoid population of small lymphocytes, plasma cells, and histiocytes (Diff-Quik, original magnification ×200 [A]; Papanicolaou stain, original magnification ×200 [B]).
The components.38,41 Components, because additional chemotherapy is usually nondiagnostic or negative interpretation, because these elements may be seen from the needle track. However, FNAC interpretation can be very difficult when sampling occurs in cystic areas without yielding enough diagnostic material. For example, the presence of only keratin debris, bone, or calcified materials may lead to a nondiagnostic or negative interpretation, because these elements may be seen from the needle track.

In addition, it is very important to specify any non-teratomatous components, such as other germ cell components (YST, embryonal carcinoma, choriocarcinoma, and seminoma), carcinoma components, and sarcomatous components, because additional chemotherapy is usually required because of more aggressive behavior of these components.38,41

### Seminomas

Seminoma is the second most common mediastinal GCT. The β-hCG serum levels are elevated in approximately one-third of patients with seminoma. Metastasis occurs in most patients at the time of diagnosis, with the most common involvement in adjacent lymph nodes.38,42 It is important to correctly distinguish seminomas from nonseminomatous GCTs, because seminomas usually respond well to radiation- and/or platinum-based chemotherapy.

Histologically, mediastinal seminoma mimics the seminoma/dysergoninoma in other anatomic sites, manifested by proliferation of round/polygonal cells with indistinct cell borders, clear/eosinophilic cytoplasm, round/oval nuclei, and prominent nucleoli. The most common cytologic findings of YST on smears include glandular formations of large cells with pleomorphic nuclei and distinct nucleoli. The cytoplasm may show microvacuoles, and pink hyaline globules may be present. Schiller-Duval bodies are papillary vascular structures with a surrounding cyst and hyaline globules; however, most cases lack these bodies.43,44

Immunophenotypically, YST cells are usually positive for α-fetoprotein, Glypican 3, and SALL4, and variably positive for cytokeratins. The YST cells are negative for OCT4, CD30, CD117 and PLAP (Table 1).

### Choriocarcinoma

Choriocarcinoma may show multinucleated syncytiotrophoblasts and cytotrophoblasts. Large areas of hemorrhage and necrosis with a rim of trophoblasts may be present.44

Immunophenotypically, choriocarcinoma cells are positive for β-hCG and GCT markers (SALL4 and LIN28) and variable for epithelial membrane antigen and Glypican 3, but negative for CD30 (Table 1).

### Embryonal Carcinoma

Embryonal carcinoma consists of sheets, acinar structures, and papillary fragments of large cells with basophilic and vacuolated cytoplasm, vesicular nuclei, and prominent large nucleoli (Figure 4, G and H). Necrosis is usually present.43,44

Embryonal carcinoma cells are positive for cytokeratin, CD30, OCT4, and SALL4 (Table 1).

### Mediastinal Neuroendocrine Tumor

Neuroendocrine tumors of the thymus (tNETs) account for only about 5% of mediastinal tumors and are potentially aggressive neoplasms that are capable of local recurrence and distant metastasis.45 According to the 2015 WHO classification of tumors of the lung, pleura, thymus, and heart,46 tNETs are subclassified into typical carcinoid (low grade), atypical carcinoid (intermediate grade), large cell neuroendocrine (high grade), and small cell (high grade) carcinomas based on neuroendocrine cytomorphology, architecture patterns, expression of neuroendocrine markers, presence of necrosis, and mitotic activity. The features of cytomorphology remain the key to diagnosing small cell carcinoma. A tNET may simultaneously contain mixed components of low-grade NET to high-grade neuroendocrine carcinoma, or may coexist with a thymoma or thymic carcinoma in rare cases.47 tNETs are positive for neuroendocrine markers, including synaptophysin, chromogranin, CD56, and INSM1.48-52 Clinical information is essential to confirm the tNET’s primary origin. Most common local symptoms are chest pain, cough, and respiratory distress. Paraneoplastic syndromes (eg, Cushing syndrome and hypercalcemia) are mainly seen in low-grade or intermediate tNETs (typical carcinoid and atypical carcinoid). History of lung NET or multiple neuroendocrine neoplasia syndrome 1 (MEN-1) should be noted. About 25% of typical carcinoid and atypical carcinoid cases are associated with MEN-1 syndrome.53 Patients with both pulmonary and thymic NETs are at an increased risk to develop other malignancies, such as carcinomas of the breast or prostate. Although typical carcinoid is the predominant NET in lung, tNET mostly is atypical carcinoid with a male predominance. The genetic profile of thymic carcinoids is different from that of their pulmonary counterparts.44,54 Thus far there is no reliable staging system of tNETs.
Figure 4. Mediastinal germ cell tumors. A, Seminoma. Two populations of cells in a “tigroid” background. One population contains clusters of or individual tumor cells, which are larger in size with abundant, glycogen-rich basophilic cytoplasm and nucleoli. The other population contains small mature lymphocytes. B, Seminoma with 2 populations of cells. C, Seminoma with 2 populations of cells. Cell block. D, Seminoma cells are positive for PLAP immunostain. E, Follow-up surgical resection shows typical seminoma morphology. F, OCT3/4 is positive in tumor cells. G, Embryonal carcinoma. Sheets and papillary fragments of large cells with basophilic and vacuolated cytoplasm are present. H, Embryonal carcinoma. Cluster of large cells shows vesicular nuclei and prominent large nucleoli. Cell block (Diff-Quik, original magnification ×400 [A]; Papanicolaou stain, original magnifications ×400 [B] and ×200 [G]; hematoxylin-eosin, original magnifications ×200 [C, E, and H]; original magnification ×200 [D and F]).
Table 1. Immunostains in Mediastinal Germ Cell Tumors

<table>
<thead>
<tr>
<th>Markers</th>
<th>Seminoma</th>
<th>EBC</th>
<th>YST</th>
<th>ChorioCA</th>
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<tr>
<td>AE1/AE3</td>
<td>or +</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>CD117 (c-kit)</td>
<td>+</td>
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<td>OCT4</td>
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<td>LIN28</td>
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<td>SALL4</td>
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<td>Nanog</td>
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<td>α-Fetoprotein</td>
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<td>Glypican 3</td>
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<td>-</td>
<td>+</td>
<td>or -</td>
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<td>PLZF</td>
<td>-</td>
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<tr>
<td>β-hCG</td>
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<tr>
<td>5-hmC</td>
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Abbreviations: ChorioCA, choriocarcinoma; EBC, embryonal carcinoma; YST, yolk sac tumor. +, positive; −, negative; +/-, positive/negative.

Radiologic imaging is commonly used in evaluation of tNETs. On computed tomography scan, the appearance of tNETs is nonspecific and typically demonstrates a large, well-circumscribed enhancing soft tissue density mass of heterogeneous attenuation in the anterior mediastinum. Necrosis, hemorrhage, and calcification may also present. Tumor extension into the lungs, liver, pericardium, local blood vessels, and lymph nodes is seen in some cases. On magnetic resonance imaging, these tumors are enhancing masses that usually demonstrate heterogeneous signal intensity and various degrees of cystic changes and enhancement. tNETs can also be slightly T1 hyperintense or hypointense. On fluoro-deoxyglucose-positron emission tomography (FDG-PET), tNETs are FDG-avid and may appear similar to their nonneuroendocrine counterparts.

However, 68Ga-DOTATATE-PET may detect additional tNET lesions that are not visible on FDG-PET. tNETs share lots of common characteristics with their counterparts, pulmonary NETs. However, tNETs may also present with their own unique features. Clinical information, cytomorphic and histopathologic features, and cytogenetic alterations are summarized in Table 2. Representative images of atypical carcinoid and small cell carcinoma are illustrated in Figure 5, A through H, and Figure 6, A through F, respectively.

MEDIASTINAL SOFT TISSUE TUMORS

Mesenchymal neoplasms of the mediastinum are very rare, and they only account for about 2% to 6% of mediastinal tumors. Except for site- or organ-specific tumors (eg, gastrointestinal stromal tumor), essentially all entities encountered in peripheral soft tissues can also occur in mediastinum. Primary mediastinal soft tissue sarcomas should be differentiated from secondary etiologies, such as status after radiation therapy, or as “somatic-type” malignancy in a mediastinal GCT, or as part of sarcomatous component of a thymic sarcomatoid carcinoma or pseudosarcomatous stroma in a thymoma. Here, we would like to focus on site-specific cytologic features in some entities of primary mediastinal soft tissue sarcoma.

Lipomatosus tumors are common in the mediastinum and can be found in any compartment. Thymolipoma is usually seen in young adults with median age of 29 years. It is a circumscribed mediastinal mass lesion, one which is often found in the anterior mediastinum. Histologically it is composed of both mature adipose tissue and variable portion of thymic tissue, lymphoid follicles, and Hassall corpuscles. On FNA smears and cell block sections, clusters of mature adipocytes, thymic epithelial cells, immature TdT+ lymphocytes (thymocytes), and Hassall corpuscles can be seen. It is common that a definitive diagnosis of thymolipoma may be precluded because of limited tissue sampling of FNAC or small biopsy. This entity should be included in the differential diagnosis as site-specific tumor. If thymic epithelial components and atypical changes are absent, lipoma should be included in the differential as well.

Liposarcoma, a malignant adipocytic tumor with various subtypes, is the most common primary malignant mesenchymal tumor of the mediastinum. It occurs in all mediastinal compartments, mostly in anterior and posterior mediastinum; it can be originated from thymus (thymoliposarcoma) too. Dyspnea, pain, and cough are the most common symptoms, and are helpful to rule out benign mimics (eg, lipoma). Imaging studies often show large tumor mass with mature lipomatous component. Histologically it often presents as a well-circumscribed, multinodular tumor mass with mixed lipomatous and myxoid areas. Well-differentiated and dedifferentiated liposarcoma are the most common subtypes in the mediastinum. Extensive sampling is a key in FNAC. Smears and/or cell block sections show scattered or aggregates of variably sized, univacuolated adipocytes mixed with expelled lipid. Smaller multivacuolated cells with atypical scalloped nuclei (lipoblasts) can be seen, which is not required for establishing the diagnosis. Atypical enlarged hyperchromatic nuclei can be found in stromal cells and large adipocytes (Figure 7). The molecular signatures of mediastinal liposarcomas are similar to those occurring in other sites. Immunostaining of MDM2, CDK4, and p16 shows they are often overexpressed in well-differentiated and dedifferentiated liposarcoma. The gold standard test is MDM2 amplification by fluorescence in situ hybridization analysis. The (12;16) FUS-DDIT-3 fusion is present in myxoid liposarcoma. The mortality of mediastinal liposarcomas ranges from 30% to 50%.

Very few fibroblastic/myofibroblastic tumors (eg, desmoid tumors, solitary fibrous tumor, and inflammatory myofibroblastic tumor) are reported to occur in mediastinum. Desmoid tumor is an aggressive type of fibromatosis, locally invasive with myofibroblastic proliferation but never metastasis. It usually occurs in young patients (median age, 38 years), with a slight male predominance. Fine-needle aspiration cytology smears show variable cellularity, bland spindle-shaped and polygonal cells, oval to elongated nuclei, rare mitotic activity, and dense collagenous stroma. Because of the location and common local recurrence, unresectable case is often fatal.

Mediastinal solitary fibrous tumor is rare and is commonly seen in anterior mediastinum. Histologic features are similar to its counterpart of pleural origin. STAT6 is the most specific and reliable marker for solitary fibrous tumor. Cytologically, bland spindle cells with a fusiform nucleus are found in a background of a meshwork of irregular fascicles. The mediastinal solitary fibrous tumors more frequently show aggressive behavior compared with the nonmediastinal ones. The factors for predicting malignancy include...
Table 2. Classification and Features of Neuroendocrine Tumor of the Thymus

<table>
<thead>
<tr>
<th>Classification</th>
<th>TC</th>
<th>AC</th>
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<tr>
<td><strong>Sex</strong></td>
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<td>Male &gt; Female</td>
<td>Male &gt; Female</td>
<td>Male = Female</td>
</tr>
<tr>
<td><strong>Age, median; range, y</strong></td>
<td>49; 31–66</td>
<td>48–55; 18–82</td>
<td>51; 16–79</td>
<td>58; 37-63</td>
</tr>
<tr>
<td><strong>Percentage</strong></td>
<td>20</td>
<td>40–50</td>
<td>15–25</td>
<td>10</td>
</tr>
<tr>
<td><strong>Local symptoms</strong></td>
<td>Pain, cough, dyspnea, or respiratory distress</td>
<td>Pain, cough, dyspnea, or respiratory distress</td>
<td>50% with chest pain, dyspnea, upper inflow congestion</td>
<td>Weight loss, sweating, chest pain, cough, and vena cava superior syndrome</td>
</tr>
<tr>
<td><strong>Paraneoplastic phenomena</strong></td>
<td>Yes (Cushing syndrome, hypercalcemia)</td>
<td>Yes (Cushing syndrome, hypercalcemia)</td>
<td>Rare</td>
<td>Yes (Cushing syndrome, SIADH, superior vena cava syndrome)</td>
</tr>
<tr>
<td><strong>MEN-1 association</strong></td>
<td>25%</td>
<td>Less associated</td>
<td>Not associated</td>
<td>Not associated</td>
</tr>
<tr>
<td><strong>Grade</strong></td>
<td>Low grade</td>
<td>Intermediate grade</td>
<td>High grade</td>
<td>High grade</td>
</tr>
<tr>
<td><strong>Cytomorphology</strong></td>
<td>Uniform small, round to oval, scant cytoplasm, salt-and-pepper chromatin, loose arrangement, delicate angulated blood vessels</td>
<td>Slightly more atypia (Figure 5)</td>
<td>Large tumor cells, anaplastic giant cells often seen</td>
<td>Small tumor cells, high N/C ratio, scant cytoplasm, often fusiform, ill-defined cell membranes, fine granular nuclei chromatin, inconspicuous nucleoli, nuclear molding, crush artifacts, numerous apoptotic bodies (Figure 6)</td>
</tr>
<tr>
<td><strong>Other variants</strong></td>
<td>Spindle cell, pigmented, amyloid, oncocytic, mucinous, angiomatoid, and sarcomatous</td>
<td>Combined LCNEC with a second component of either thymoma or nonneuroendocrine thymic carcinoma</td>
<td>Combined SCC with a second component of either thymoma or nonneuroendocrine thymic carcinoma</td>
<td></td>
</tr>
<tr>
<td><strong>Histomorphology</strong></td>
<td>Trabecular, festoons, solid nests, and glandular growth patterns and rosette formation</td>
<td>More diffuse pattern</td>
<td>Common growth patterns are less obvious, less organized, variable</td>
<td>Common growth patterns are less obvious, less organized, variable</td>
</tr>
<tr>
<td><strong>Necrosis</strong></td>
<td>No</td>
<td>Yes, focal</td>
<td>Yes, extensive</td>
<td>Yes, extensive</td>
</tr>
<tr>
<td><strong>Mitoses/2 mm²</strong></td>
<td>&lt;2</td>
<td>≥2 to 10</td>
<td>Often &gt;20</td>
<td>Often &gt;50</td>
</tr>
<tr>
<td><strong>IHC</strong></td>
<td>Strong and diffuse +: keratins, NE markers, Pax8; ---: TTF-1, GATA-3, napsin A</td>
<td>Strong and diffuse +: keratins, NE markers, Pax8; ---: TTF-1, GATA-3, napsin A</td>
<td>Strong and diffuse +: keratins, NE markers, CD117, TTF-1; ---: CD5</td>
<td>Most cases express keratins and NE markers, but not required for diagnosis</td>
</tr>
<tr>
<td><strong>Differential diagnosis</strong></td>
<td>Paragangliomas, thymomas (type A), medullary thyroid carcinoma, pulmonary carcinoid with mediastinal involvement</td>
<td>Paragangliomas, thymomas (type A), medullary thyroid carcinoma, pulmonary carcinoid with mediastinal involvement</td>
<td>Atypical carcinoid, basaloid squamous cell carcinoma, poorly differentiated non–small cell lung carcinoma, small cell carcinoma, synovial sarcoma</td>
<td>T-lymphoblastic lymphoma, small cell sarcoma (PNET/Ewing tumors)</td>
</tr>
<tr>
<td><strong>Genetic alterations</strong></td>
<td>The fewest</td>
<td>Higher</td>
<td>Higher</td>
<td>Most</td>
</tr>
<tr>
<td><strong>Regional and distal metastasis</strong></td>
<td>50%</td>
<td>&gt;50%</td>
<td>75%</td>
<td>Most</td>
</tr>
<tr>
<td><strong>Prognosis (5-y OS)</strong></td>
<td>Slightly better, 50%–100%</td>
<td>Slightly worse, 20%–80%</td>
<td>Worse, 30%–66%</td>
<td>Poor, 0%</td>
</tr>
</tbody>
</table>

Abbreviations: AC, atypical carcinoid; IHC, immunohistochemistry; LCNEC, large cell neuroendocrine carcinoma; MEN-1, multiple endocrine neoplasia type 1; N/C, nuclear to cytoplasmic ratio; NE, neuroendocrine; OS, overall survival rate; PNET, primitive neuroectodermal tumor; SCC, small cell carcinoma; SIADH, syndrome of inappropriate antidiuretic hormone secretion; TC, typical carcinoid.
Figure 5. Mediastinal atypical carcinoid. A, Cellular smear with loosely arranged clusters of and individual round to oval, variably sized cells. B, The neoplastic cells show slight pleomorphism, scant cytoplasm, salt-and-pepper chromatin, and loose arrangement. C, Cell block shows trabecular and papillary clusters of neoplastic cells. D, AE1/3 is positive in neoplastic cells. E, Synaptophysin is diffusely and strongly positive in neoplastic cells. F, TTF1 is positive in neoplastic cells. G, Follow-up surgical resection shows carcinoid tumor cells with cytologic atypia. H, Chromogranin is positive in tumor cells (Diff-Quik, original magnification ×400 [A]; Papanicolaou stain, original magnification ×400 [B]; hematoxylin-eosin, original magnification ×200 [C and G]; original magnification ×200 [D through F, and H]).
Figure 6. Mediastinal small cell carcinoma. A, The smear shows small clusters of and individual tumor cells with relatively small size, high nuclear to cytoplasmic ratio, scant cytoplasm, ill-defined cell membranes, inconspicuous nucleoli, nuclear molding, and crush artifacts. B, Tumor cells show fine granular nuclei chromatin and inconspicuous nucleoli. The background is necrotic with numerous apoptotic bodies. C, Cell block shows small clusters of and individual tumor cells in a necrotic background. D, Synaptophysin positive in tumor cells. E, Follow-up surgical resection shows typical small cell carcinoma morphology. F, Chromogranin is positive in tumor (Diff-Quik, original magnification ×400 [A]; Papanicolaou stain, original magnification ×400 [B]; hematoxylin-eosin, original magnification ×200 [C and E]; original magnification ×200 [D and F]).
high mitotic count (>4 mitoses per 2 mm²), high cellularity, pleomorphism, and necrosis. Because of the spindle cell morphology, mediastinal solitary fibrous tumor should be differentiated from type A thymoma, mesothelioma (desmoplastic or sarcomatoid), sarcomatoid carcinoma, synovial sarcoma, and malignant peripheral nerve sheath tumor (MPNST).

Vascular neoplasms in the mediastinum are more frequently seen among other mesenchymal tumors, and they account for 1% to 4.5% of all mediastinal tumors. They can involve both anterior and posterior mediastinum and tend to be large (3–20 cm). All vascular lesions resemble the correspondent entities in other body parts. Lymphatic or vascular markers (eg, CD31, CD34, ERG, and lectin) are usually expressed. Most benign mediastinal hemangiomas are seen in young adults, localized in the anterior mediastinum. Half of the patients present with retrosternal pain, cough, or stridor. Lymphangioma can present as a cystic mass in young children, which is also positive for D2-40 besides common vascular markers. Vascular endothelial tumors of intermediate malignancy (eg, kaposiform hemangioendothelioma) and high-grade malignancy (eg, epithelioid hemangioendothelioma and angiosarcoma) are reported in the mediastinum. Pure angiosarcoma should be differentiated from those that arise as part of a mediastinal GCT tumor with somatic-type malignancy, which often have poor prognosis.

Peripheral nerve sheath tumors are most common in the mediastinum among the other soft tissue tumors, often arising in the posterior mediastinum. The most common lesions are benign schwannoma and MPNST. Most cases are asymptomatic and are incidentally detected by routine imaging studies. The schwannoma usually presents as circumscribed encapsulated lesions, sometimes associated with cystic degeneration and degenerative (ancient) changes. The cellular variant of schwannoma with high cellularity and without Verocay bodies is very common in the mediastinum. Mediastinal MPNST often shows divergent mesenchymal differentiation, with components of skeletal muscle (rhabdomyosarcoma, Triton tumor), osteosarcoma, and chondrosarcoma. H3K27me3 is an emerging sensitive marker (loss of expression) to confirm MPNST (loss of expression in 80%–90% of high-grade MPNST). In addition, MPNST and schwannoma are positive for SOX10, helping in diagnosis. In contrast, expression of S100 may be focal or lost in MPNST.

Figure 7. Mediastinal liposarcoma. A, The smear shows a small aggregate of variably sized vacuolated spindle cells with atypical nuclei. B, The smear shows a small aggregate of variably sized vacuolated spindle cells with atypical nuclei. C, Cell block shows clusters of spindle cells with atypical nuclei. D, Ki-67 shows increased staining in spindle cells (Diff-Quik, original magnification ×400 [A]; Papanicolaou stain, original magnification ×200 [B]; hematoxylin-eosin, original magnification ×200 [C]; original magnification ×200 [D]).
Mediastinal paraganglionic tumors of the autonomous nervous system are often reported in case reports. The tumors located in the anterior mediastinal compartment are associated with aorticopulmonary, vagal, subclavian, carotid, and coronary vessel paraganglia. The tumors involving the posterior mediastinal compartment derive from the sympathetic chain and secrete catecholamines in up to 50% of cases. Although there are no distinctive clinicopathologic features compared with the paravertebral paraganglioma from other body sites, the prognosis of mediastinal paraganglionic tumors is usually worse because of their close proximity to the major vessels and organs in the mediastinum.

SMARCA4-deficient thoracic sarcoma is a relatively new entity with an aggressive clinical course and specific genetic alterations of the BAF chromatin remodeling complex, and it can arise in mediastinum. Morphologically, SMARCA4-deficient thoracic sarcoma usually shows round to epithelioid cells arranged in a solid pattern with variably prominent rhabdoid differentiation. Prominent cytologic atypia and mild to moderate nuclear pleomorphism are present. Immunohistochemistry markers are useful and indispensable because tumor cells typically show dual loss of SMARCA4 and SMARCA2, but diffuse positivity of SOX2.

MEDIASTINAL METASTATIC TUMORS

Essentially, all tumors may metastasize to mediastinum, mainly to mediastinal lymph nodes. Metastases in mediastinum make the known primary tumors an advanced stage. Sometimes, mediastinal metastasis is the first presenting manifestation before an underlying primary tumor is identified. EBUS-FNAC and endoscopic ultrasound–FNAC are useful tools in obtaining materials for diagnosis and molecular testing to guide treatment. It is important to know clinical history and be familiar with tumor morphology of various types for evaluating mediastinal metastatic tumors.

Figure 8. Mediastinal metastatic clear cell sarcoma. Fine-needle aspiration from a large mediastinal mass in a 20-year-old man. A, The smear is cellular, showing dispersed tumor cells with abundant pale cytoplasm. A binucleated cell is shown in the center. B, Tumor cells are uniform with round to oval nuclei, vesicular chromatin, and prominent nucleoli. C, The cell block section shows abundant uniform tumor cells associated with necrotic debris. D, Tumor cells stain positive for SOX-10. Fluorescence in situ hybridization is positive for EWSR1 rearrangement (image not shown). A 3-cm soft tissue mass was identified later in dorsal left foot (Diff-Quik, original magnification ×400 [A]; Papanicolaou stain, original magnification ×400 [B]; hematoxylin-eosin, original magnification ×200 [C]; immunohistochemistry, original magnification ×200 [D]).

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Metastatic carcinoma of the lung, especially small cell carcinoma, is the most common source of metastatic carcinoma in mediastinum. Other primary sites include breast, head and neck, kidney, urinary bladder, and prostate. Clinical history and appropriate immunohistochemical stains can help to identify primary sites in most cases. Metastatic melanoma should always be considered in the differential diagnoses when encountering a malignant neoplasm in mediastinum. Typically, tumor cells are in loose clusters and dispersed forms. Cells have centrally or eccentrically located nuclei and prominent nucleoli. Intranuclear pseudoinclusions and cytoplasmic melanin pigments may be present. Positive immunohistochemical stains for HMB-45, Melan-A, S100, and SOX-10 further support the diagnosis. Sarcomas may metastasize to mediastinum, sometimes as an initial presentation when the primary sarcoma is small or occult. Figure 8 illustrates a metastatic clear cell sarcoma in mediastinum in a 20-year-old man; the primary soft tissue tumor in the left foot is identified after the metastasis is diagnosed with FNAC. Metastatic synovial sarcoma is shown as dispersed cells alternating with cell clusters on cytology smears. Cells have uniform, oval to spindle nuclei with scant tapering cytoplasm and inconspicuous nucleoli. Tumor cells stain positive with epithelial membrane antigen and TLE-1, stain negative for INI1, and demonstrate t(X;18) on cytogenetic study. In addition, 2 new and more specific immunostain markers (SS18 and SS18-SSX) have been recently discovered. Metastatic epithelioid sarcoma contains uniform tumor cells on the smears. Tumor cells have distinct cell borders, eccentrically located nuclei, hyperchromatic chromatin, and inconspicuous nucleoli. Clinical history and positive staining for cytokeratin and CD34 as well as negative staining for IN1 confirm the diagnosis. Rarely, there are metastatic tumors in mediastinum that are undifferentiated and do not show a definitive lineage of differentiation.

CONCLUSION

Various lesions, such as congenital/acquired cysts, benign tumors, and primary and metastatic malignancies, can develop within mediastinum, and diagnosis of mediastinal lesions has been challenging. Image-guided FNAC and needle core biopsy have been used increasingly and successfully to obtain sufficient tissue materials for diagnosing mediastinal lesions and ancillary studies for targeted therapy. Previous studies have demonstrated that image-guided FNAC is a safe, minimally invasive, and cost-effective procedure to provide a precise diagnosis for mediastinal lesions and ancillary studies for targeted therapy when adequate sampling is obtained. This review summarizes the FNAC findings of the major mediastinal lesions, including epithelial tumors, lymphoproliferative disorders, GCTs, neuroendocrine tumors, and metastatic tumors of the mediastinum, and it emphasizes the pitfalls and limitations of cytologic diagnosis. It is noteworthy to mention that accurate diagnosis and subclassification of mediastinal lesions rely not only on microscopic examination of adequately sampled specimens but also on clinical and radiologic correlation, that is, a patient’s age, clinical manifestations, radiologic features, and specific anatomic location. Nevertheless, metastatic mediastinal tumors are much more common than primary mediastinal tumors.

In summary, adequate sampling and close communication with radiologists and clinicians cannot be overemphasized when interpreting FNAC specimens of mediastinal lesions because of the wide spectrum of tissue types and the broad varieties of cytologic morphology.

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