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Review Article

Fine Needle Aspiration Cytopathology of Thymoma and Thymic Carcinoma: A Brief Review

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Abstract

Thymic epithelial neoplasms, comprised of thymoma, thymic carcinoma, and others, account for less than 1% of human tumors. Fine needle aspiration (FNA) is performed to diagnose thymic lesions due to its minimal invasiveness. Since classification of thymic epithelial neoplasms usually requires thorough histologic examination of the entire lesion, it is necessary to understand the information FNA can provide as well as its limitations. The advantage of FNA lies in its comparable diagnostic accuracy and minimal invasiveness, but it is almost impossible to subtype thymomas with this technique owing to its sampling issues. This review summarizes the cytologic features of thymoma and thymic carcinoma as well as the potential diagnostic pitfalls associated with FNA.

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Introduction

The thymus is an anterior/superior mediastinal organ, composed predominantly of T lymphocytes, scattered Hassall's corpuscles, and epithelial cells. Thymic lesions include non-neoplastic (thymic cysts, thymic hyperplasia) and neoplastic lesions (thymoma, thymic carcinoma, neuroendocrine tumors, germ cell tumors, and stromal tumors).[1] Fine needle aspiration (FNA) has proven to be a safe and reliable procedure to diagnose thymoma or thymic carcinoma. However, it can be challenging to diagnose and/or classify these lesions due to the overlapping morphologic and immunohistochemical features among the subtypes of thymoma, thymic carcinoma, and other neoplasms.

Thymoma

Thymoma is the most common thymic neoplasm, but its incidence is only 0.13 cases/100,000 population/year.[2–5] Thymoma patients can be asymptomatic or present with local symptoms such as chest pain and superior vena cava syndrome, or paraneoplastic syndromes such as myasthenia gravis, hypogammaglobulinemia, and/or red blood cell aplasia.[6–9]

Thymoma consists of neoplastic epithelial cells and benign T lymphocytes.[2–4] Based on the histologic morphology of neoplastic epithelial cells and the ratio of neoplastic epithelial cells to reactive lymphocytes, thymoma is categorized into type A, type AB, type B (B1, B2, and B3), and rare subtypes (metaplastic thymoma and micronodular thymoma with lymphoid stroma) by the World Health Organization classification system (Table 1).[1,10]

Morphology

Histologically, type A thymoma is composed of predominantly spindled epithelial cells and rare lymphocytes. Similarly, individual cells or clusters of spindle cells with bland nuclei, minimal nucleoli, and smooth chromatin are the predominant cells by FNA cytology of type A thymoma, and small lymphocytes are scant (Fig. 1).

Type AB thymoma exhibits morphologic features of neoplastic epithelial cells of both type A (spindle) and type B (round/polygonal). A sharp transition between these two morphologies is often seen by histology. FNA of type AB thymoma shows mixed spindle and round or polygonal neoplastic epithelial cells with a variable number of small lymphocytes (Fig. 2).

Type B thymoma is divided into three subtypes: B1, B2, and B3 based on the ratio of neoplastic epithelial cells to small reactive lymphocytes. Although the ratio can be roughly evaluated by cytology, it is unclear if it can be used to accurately classify these subtypes.[11–15]

Histologically, type B1 thymoma shows abundant reactive small lymphocytes with medullary islands and few scattered round/polygonal epithelial cells. By FNA cytology, type B1 thymoma demonstrates cellular materials with abundant lymphocytes and very scant round/polygonal neoplastic epithelial cells. Sometimes, immunostaining of a cell block section becomes necessary to distinguish the epithelial cells because they are difficult to visualize on smear slides (Fig. 3).

Type B2 thymoma is also rich in lymphocytes but demonstrates more round/polygonal epithelial cells than type B1. FNA of type B2 shows both round/polygonal neoplastic epithelial cells and reactive lymphocytes (Fig. 4).

Histologically, type B3 is composed of abundant round/polygonal epithelial cells but scant lymphocytes. Similarly, by FNA cytology, type B3 is cellular with abundant round/

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Abbreviations: FNA, fine needle aspiration; NUT, nuclear protein in the testis.
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Table 1. Cytologic features of different subtypes of thymoma

WHO type	Cytologic features	Clinical outcome
A	Predominantly spindle neoplastic epithelial cells with scant lymphocytes	Benign
AB	Mixed spindle and round/polygonal neoplastic epithelial cells	Benign
B1	Predominantly lymphocytes with scattered round or polygonal neoplastic epithelial cells	Low malignant potential
B2	Mixed lymphocytes and round or polygonal neoplastic epithelial cells	Moderate malignant potential
B3	Predominantly round or polygonal neoplastic epithelial cells with minimal lymphocytes	Moderate malignant potential

WHO, World Health Organization.

polygonal neoplastic epithelial cells and scant lymphocytes. In addition, mild atypia can be seen (Fig. 5).

Thymoma also includes several rare subtypes: micronodular thymoma with lymphoid hyperplasia and metaplastic thymoma. Micronodular thymoma with lymphoid hyperplasia is composed of discrete nodules of neoplastic epithelial cells separated by lymphoid stroma. The neoplastic epithelial cells can be spindly or round/polygonal in shape. Metaplastic thymoma is composed of islands of neoplastic epithelial cells separated by fibroepithelial spindle cell stroma.[1]

Immunohistochemistry

The neoplastic epithelial cells of thymoma are positive for cytokeratins (AE1/3, CK5/6, etc.) and p63 (p40). The reactive T lymphocytes are positive for CD3. Moreover, CD1a and TdT are positive in thymocytes but may be absent in type A or AB thymoma. Some type A and type AB thymomas may show focal CD20 staining in neoplastic epithelial cells. The neoplastic epithelial cells are negative for CD117 and CD5, except for rare type B3 thymomas that exhibit focal CD117 staining.[16,17] Polyclonal PAX8 has been shown to be positive in thymic epithelial neoplasms, including thymic carcinoma (77%), type A thymomas (100%), and type B thymomas (93%).[18]

Differential diagnosis

The differential diagnosis for type B1 thymoma contains lymphomas such as acute T-lymphoblastic lymphoma (leukemia) (T-ALL/lymphoblastic lymphoma), since both have abundant immature T lymphoid cells that exhibit a similar immunophenotype (TdT^+, $CD3^+$, and $CD99^+$). T-ALL/lymphoblastic lymphoma occurs predominantly in children, while thymoma more often occurs in adults. The presence of neoplastic epithelial cells provides evidence to diagnose thymomas; however, it is often difficult to identify epithelial cells

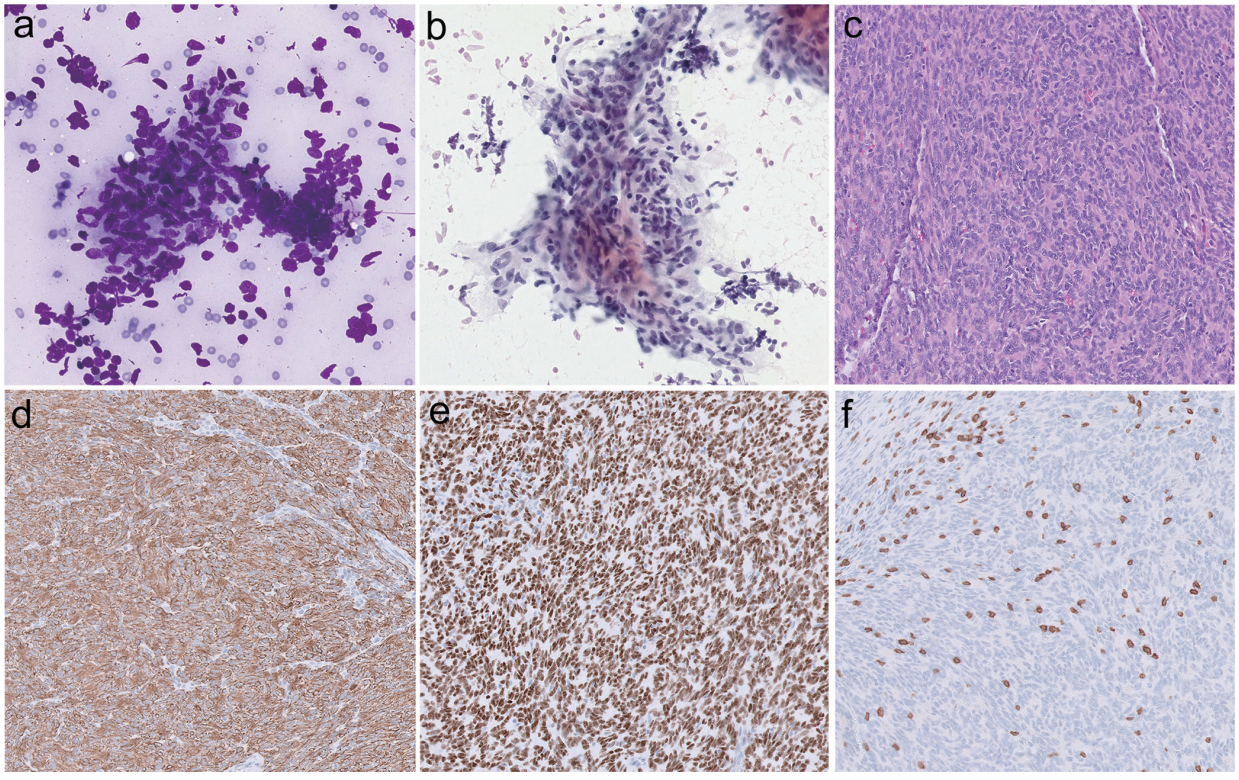

Fig. 1. Type A thymoma. (a) The smear shows a cluster of cells and single spindle-shaped epithelial cells with occasional small lymphocytes, Diff-Quik stain, 400×. (b) The smear shows a cluster of monotonous spindle-shaped epithelial cells, Pap stain, 400×. (c) Hematoxylin and eosin staining shows predominantly spindle epithelial cells, 200×. (d) AE1/3 is diffusely positive in epithelial cells, 200×. (e) p40 is diffusely positive in epithelial cells, 200×. (f) CD3 is positive in scattered lymphocytes, 200×.

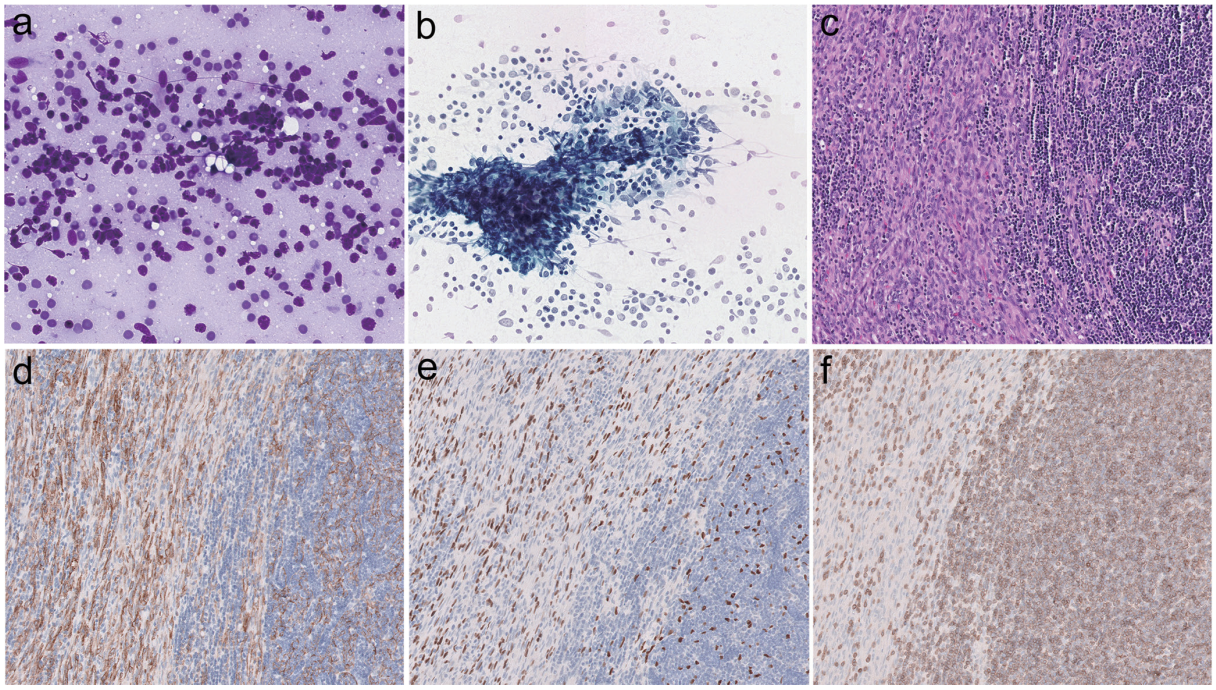

Fig. 2. Type AB thymoma. (a) The smear shows mixed spindle/ovoid and round/polygonal epithelial cells mixed with small lymphocytes, Diff-Quik stain, 400×. (b) The smear shows a cluster of cells and single spindle/ovoid and round/polygonal epithelial cells in a background of small lymphocytes, Pap stain, 400×. (c) Hematoxylin and eosin staining shows spindle-shaped epithelial cells and round epithelial cells mixed with small lymphocytes, 200×. (d) AE1/3 is diffusely positive in epithelial cells, 200×. (e) p40 is diffusely positive in epithelial cells, 200×. (f) CD3 is positive in scattered lymphocytes, 200×.

on aspirate smears. Immunohistochemistry for cytokeratins or others stains in a cell block may help to identify the presence of rare epithelial cells. T-ALL cells are larger with an irregular nuclear contour in comparison with small immature T lymphocytes in type B1 thymoma.

The most important differential diagnosis for type B3 thy-

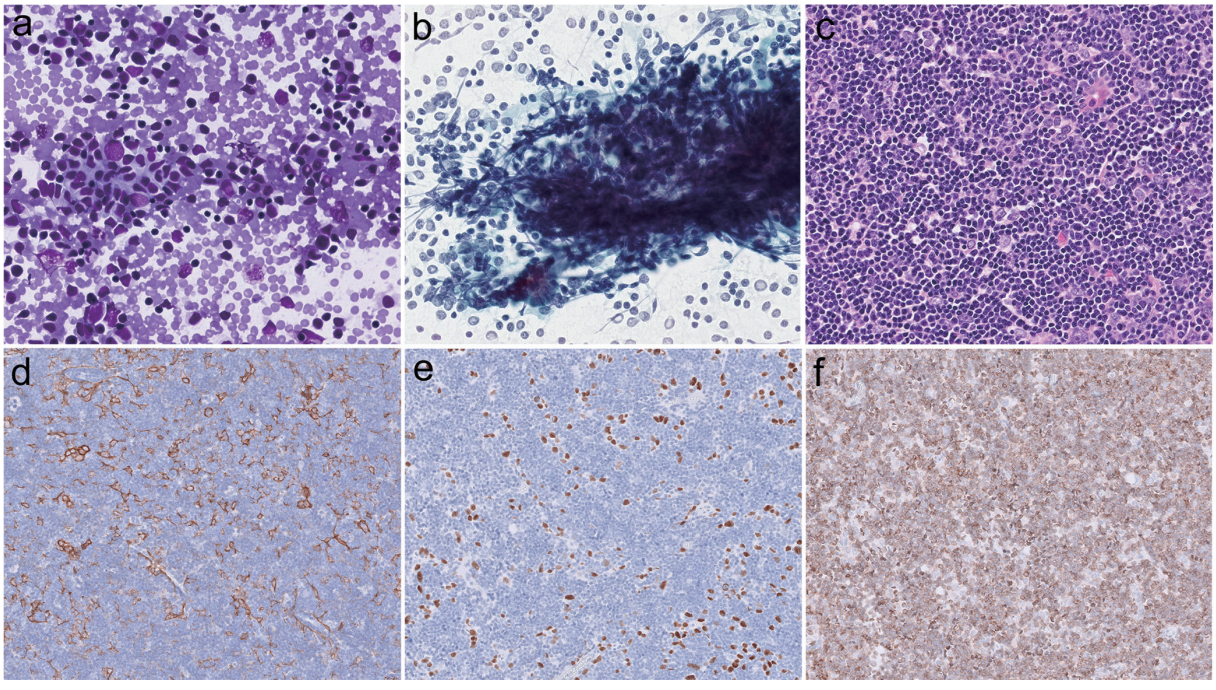

Fig. 3. Type B1 thymoma. (a) The smear shows abundant small lymphocytes with a few scattered round neoplastic epithelial cells, Diff-Quik stain, 400×. (b) The smear shows a cluster of round neoplastic epithelial cells with abundant small lymphocytes, Pap stain, 400×. (c) Hematoxylin and eosin staining shows predominantly small lymphocytes and a few scattered round epithelial cells, 200×. (d) AE1/3 is diffusely positive in epithelial cells, 200×. (e) p40 is diffusely positive in epithelial cells,

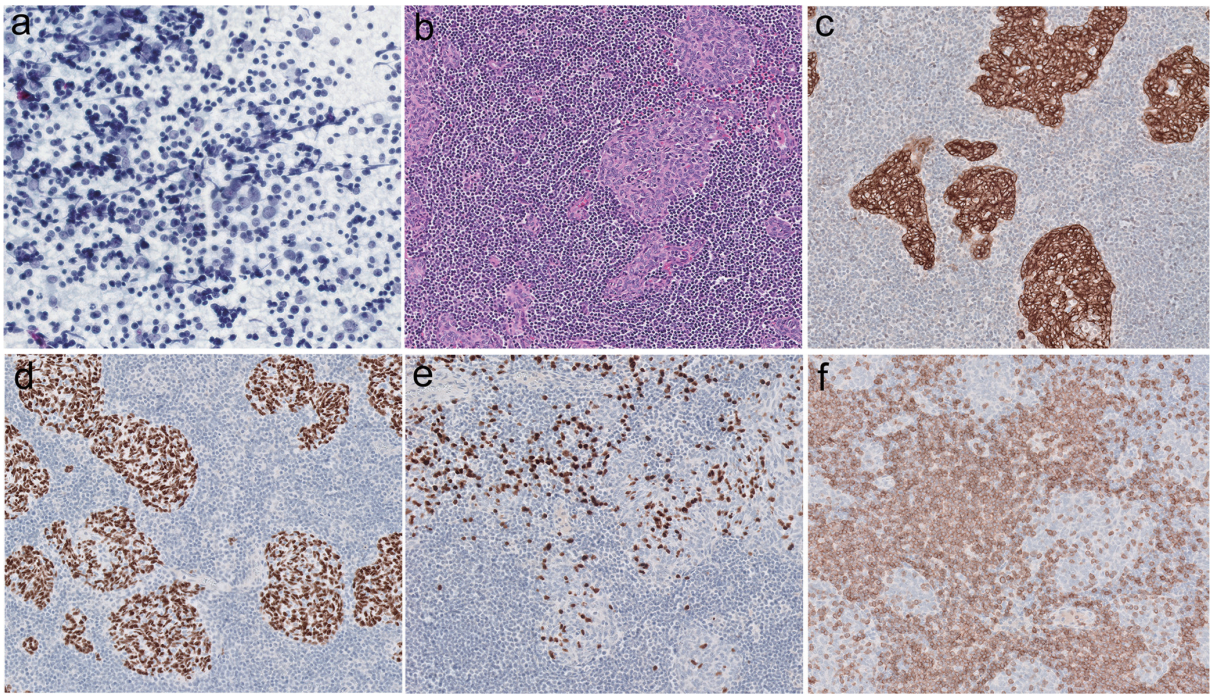

Fig. 4. Type B2 thymoma. (a) The smear shows abundant small lymphocytes with round neoplastic epithelial cells, Pap stain, 400×. (b) Hematoxylin and eosin staining shows islands of round epithelial cells with surrounding small lymphocytes, 200×. (c) AE1/3 is diffusely positive in epithelial cells, 200×. (d) p40 is diffusely positive in epithelial cells, 200×. (e) TdT is positive in thymocytes, 200×. (f) CD3 is positive in scattered lymphocytes, 200×.

moma (also known as atypical thymoma) includes primary thymic carcinoma and metastatic carcinoma because the neoplastic epithelioid cells from type B3 thymoma may resemble thymic carcinoma cells or metastatic carcinoma cells. Type B3 thymoma cells usually show mild-to-moderate pleomorphic nuclei. On the contrary, thymic carcinoma or meta-

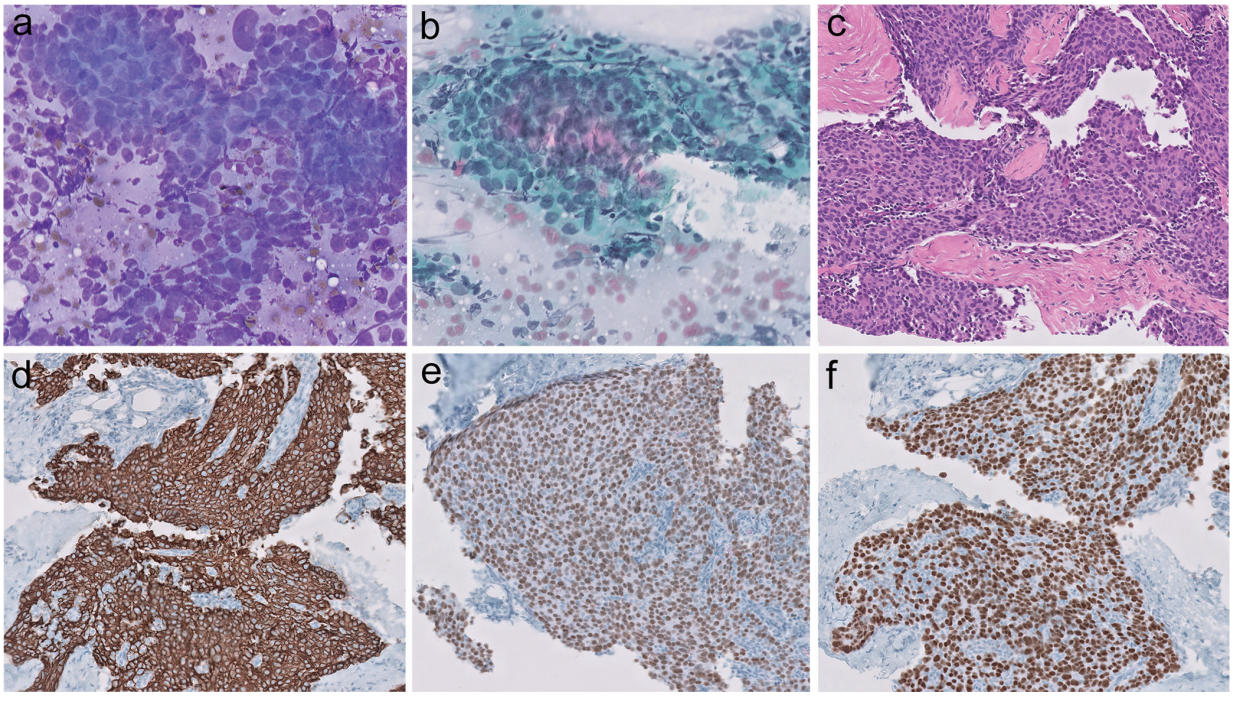

Fig. 5. Type B3 thymoma. (a) The cellular smear shows predominantly round neoplastic epithelial cells, Diff-Quik stain, 400×. (b) The smear shows a cluster of large round neoplastic epithelial cells, Pap stain, 400×. (c) Hematoxylin and eosin staining shows islands of round epithelial cells separated by stroma, 200×. (d) AE1/3 is diffusely positive in epithelial cells, 200×. (e) p40 is diffusely positive in epithelial cells, 200×. (f) p63 is positive in epithelial cells, 200×.

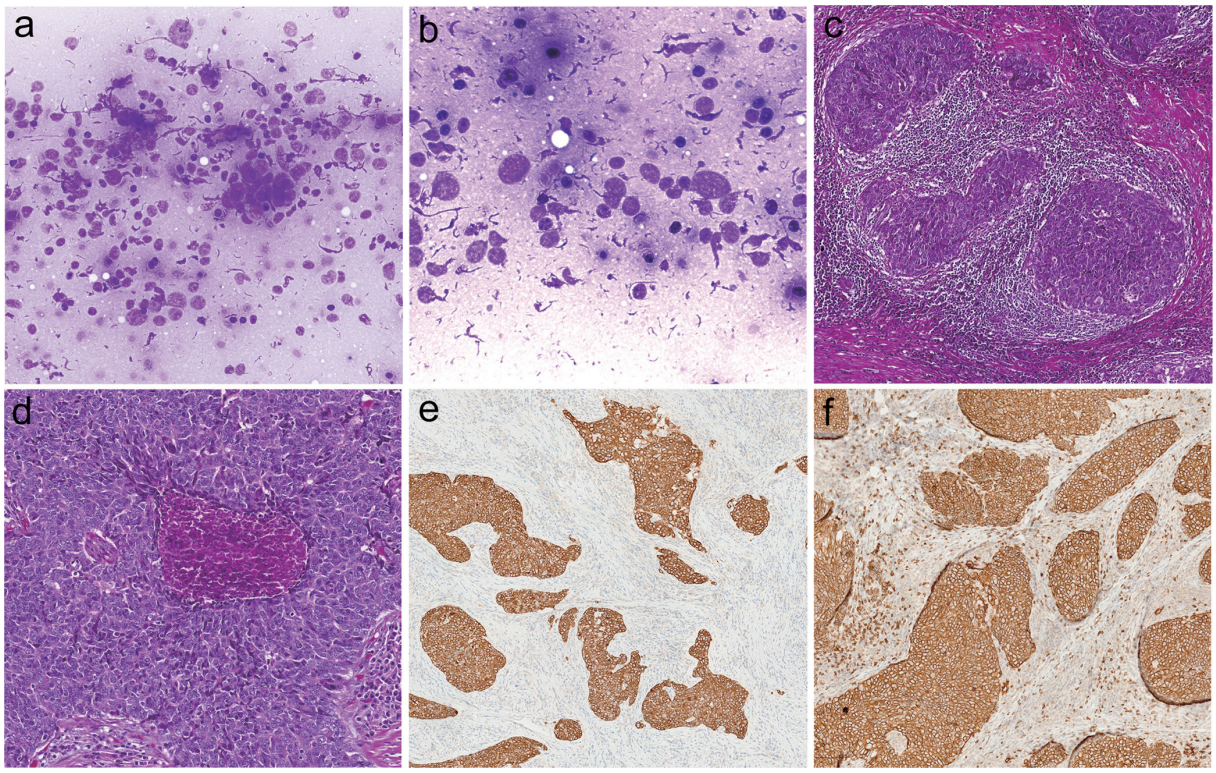

Fig. 6. Thymic carcinoma (lymphoepithelioma-like). (a) The smear shows abundant loosely cohesive, large, polygonal malignant epithelioid cells with nuclear pleomorphism. Scattered small lymphocytes are present, Diff-Quik stain, 400×. (b) High magnification shows significant nuclear pleomorphism, 600×. (c) The surgical resection shows irregular cords and islands of malignant polygonal epithelial cells separated by connective tissue stroma containing dense lymphoplasmacytic infiltrates, hematoxylin and eosin stain, 100×. (d) Comedonecrosis is present, 200×. (e) AE1/3 is positive in epithelial cells, 100×. (f) CD5 is also positive, 100×.

static carcinoma cells have irregular nuclei with prominent pleomorphism, distinct nucleoli, and coarse chromatin. Necrosis and mitosis are less common in type B3 thymoma. Immunophenotypically, type B3 thymoma epithelial cells are negative for CD5 and CD117, which are usually positive in thymic carcinoma. Metastatic carcinomas are usually positive for their specific site markers. For example, breast carcinoma is positive for GATA3, lung adenocarcinoma is positive for TTF1, gynecologic carcinoma is positive for PAX8, etc.

Type A thymoma may need to be differentiated from other spindle cell lesions such as a solitary fibrous tumor, schwannoma, and spindle neuroendocrine tumor, etc. Immunohistochemical studies can provide necessary information to differentiate type A thymoma from these entities.

Thymic carcinoma

Thymic carcinomas are rare and constitute approximately 20% of thymic epithelial neoplasms.[19,20] Patients usually present with mass-related symptoms, weight loss, and anorexia, or they are asymptomatic. Paraneoplastic syndromes are uncommon in thymic carcinoma patients. Those with thymic carcinomas usually have poor clinical outcomes.[19,20]

Thymic carcinomas include multiple histological types, with squamous cell carcinoma accounting for 80% of cases. Nonsquamous thymic carcinomas include basaloid carcinoma; lymphoepithelioma-like carcinoma; mucoepidermoid carcinoma; adenocarcinoma, not otherwise specified; low-grade papillary adenocarcinoma; enteric-type adenocarcinoma; adenosquamous carcinoma; clear cell carcinoma; nuclear protein in the testis (NUT) carcinoma; sarcomatoid carcinoma; undifferentiated carcinoma; thymic carcinoma with adenoid cystic carcinoma-like features; and thymic carcinoma, not otherwise specified.[21–23] These carcinomas morphologically resemble their counterparts in other anatomic sites.

Squamous cell carcinoma of the thymus can be keratinized or nonkeratinized and has a similar morphology as squamous cell carcinoma from other organ systems. It shows large malignant squamoid cells with round or ovoid nuclei and coarse chromatin. Necrosis and mitosis are common.

Lymphoepithelioma-like carcinoma shows irregular cords and islands of tumor cells separated by connective tissue stroma containing dense lymphoid infiltrates (lymphoepithelioma-like pattern) or minimal lymphoid infiltrates (desmoplastic pattern). The neoplastic epithelial cells are poorly differentiated with round or ovoid nuclei, vesicular chromatin, and prominent eosinophilic nucleoli. Pleomorphic and multinucleated tumor cells as well as comedonecrosis can be seen. Cytologically, the smears are cellular with predominant variable-sized round or ovoid epithelial cells and scattered small lymphocytes. The neoplastic epithelial cells have round-to-ovoid nuclei, vesicular chromatin, and prominent eosinophilic nucleoli. Lymphoepithelioma-like carcinoma cells are positive for cytokeratins, CD5, and CD117 (Fig. 6).

NUT midline carcinoma, also called NUT carcinoma, is a rare thymic carcinoma with a poor prognosis.[24] NUT carcinoma is poorly differentiated with small-to-intermediate basaloid cells and abrupt squamous differentiation with keratinization. By FNA cytology, it shows discohesive monotonous cells that have a scant cytoplasm and small-to-intermediate round or ovoid nuclei. Dyskeratotic cells may be present on

smears, but squamous differentiation and keratinization are not seen.[25–27] A diagnosis of NUT carcinoma can be confirmed by nuclear staining of NUT by immunohistochemistry or *NUT*1 gene rearrangement by fluorescence *in-situ* hybridization.

Besides type B3 thymoma, thymic carcinomas must be differentiated from metastatic carcinomas, which are much more common. Morphological analysis is not very helpful due to significant overlapping. A history of malignancy in another site favors metastasis, while positive staining of CD5 and CD117 favors thymic carcinomas.

Conclusion

FNA has increasingly become an important technique to acquire tissue in order to diagnose thymic lesions of the mediastinum; however, a systemic approach should be considered, including the medical history, clinical and radiologic findings, cytomorphologic findings, and ancillary results. In the meantime, it is always important to obtain adequate materials for both diagnostic and therapeutic purposes using ancillary testing.

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Conflict of interest

The authors have no conflicts of interest related to this publication.

Author contributions

Manuscript writing and critical revision (ZL and PW). All authors have made a significant contribution to this study and have approved the final manuscript.

References

[1] International Agency for Research on Cancer. Thoracic tumours: WHO classification of tumours. 5th edition, volume 5. Lyon: International Agency for Research on Cancer; 2021.
[2] Gerein AN, Srivastava SP, Burgess J. Thymoma: a ten year review. Am J Surg 1978;136(1):49–53. doi:10.1016/0002-9610(78)90199-x, PMID:677390.
[3] Morgenthaler TI, Brown LR, Colby TV, Harper CM Jr, Coles DT. Thymoma. Mayo Clin Proc 1993;68(11):1110–1123. doi:10.1016/s0025-6196(12)60907-0, PMID:8231276.
[4] Mullen B, Richardson JD. Primary anterior mediastinal tumors in children and adults. Ann Thorac Surg 1986;42(3):338–345. doi:10.1016/s0003-4975(10)62751-8, PMID:3530162.
[5] Engels EA. Epidemiology of thymoma and associated malignancies. J Thorac Oncol 2010;5(10 Suppl 4):S260–S265. doi:10.1097/JTO.0b013e3181f1f62d, PMID:20859116.
[6] Duwe BV, Sterman DH, Musani AI. Tumors of the mediastinum. Chest 2005;128(4):2893–2909. doi:10.1378/chest.128.4.2893, PMID:16236967.
[7] Thomas CR, Wright CD, Loehrer PJ. Thymoma: state of the art. J Clin Oncol 1999;17(7):2280–2289. doi:10.1200/JCO.1999;17(7):2280-2289, PMID:10561285.
[8] Couture MM, Mountain CF. Thymoma. Semin Surg Oncol 1990;6(2):110–114. doi:10.1002/ssu.2980060209, PMID:2315600.
[9] Salyer WR, Eggleston JC. Thymoma: a clinical and pathological study of 65 cases. Cancer 1976;37(1):229–249. doi:10.1002/1097-0142(197601)37:1<229::aid-cncr2820370133>3.0.co;2-2, PMID:1247958.
[10] den Bakker MA, Roden AC, Marx A, Marino M. Histologic classification of thymoma: a practical guide for routine cases. J Thorac Oncol 2014;9(9 Suppl 2):S125–S130. doi:10.1097/JTO.0000000000000297, PMID:25396309.
[11] Gerhard R, Kanashiro EH, Kliemann CM, Juliano AG, Chammas MC. Fine-needle aspiration biopsy of ectopic cervical spindle-cell thymoma: a case report. Diagn Cytopathol 2005;32(6):358–362. doi:10.1002/dc.20258, PMID:15880711.
[12] Ryu HS, Koh JS, Park S, Kim MS, Cho SY, Lee SS. Classification of thymoma by fine needle aspiration biopsy according to WHO classification: a cytological algorithm for stepwise analysis in the classification of thymoma. Acta Cytol 2012;56(5):487–494. doi:10.1159/000339001, PMID:23075888.
[13] Wakely PE Jr. Cytopathology of thymic epithelial neoplasms. Semin Diagn Pathol 2005;22(3):213–222. doi:10.1053/j.semdp.2006.02.005, PMID:16711402.
[14] Wakely PE Jr. Cytopathology-histopathology of the mediastinum: epithelial, lymphoproliferative, and germ cell neoplasms. Ann Diagn Pathol 2002;6(1):30–43. doi:10.1053/adpa.2002.29341, PMID:11842377.
[15] Alexiev BA, Drachenberg CB, Burke AP. Thymomas: a cytological and immunohistochemical study, with emphasis on lymphoid and neuroendocrine markers. Diagn Pathol 2007;2:13. doi:10.1186/1746-1596-2-13, PMID:17498299.
[16] Li Z, Xu H, Fan F. Approach to Mediastinal Fine Needle Aspiration Cytology. Adv Anat Pathol 2022;29(6):337–348. doi:10.1097/PAP.0000000000000355, PMID:35838636.
[17] Xu H, Fan F, Gong Y, Jing X, Lin X, Wang H, *et al*. Diagnostic Challenges in Fine-Needle Aspiration Cytology of Mediastinal Tumors and Lesions. Arch Pathol Lab Med 2022;146(8):960–974. doi:10.5858/arpa.2021-0108-RA, PMID:34402861.
[18] Weissferdt A, Moran CA. Pax8 expression in thymic epithelial neoplasms: an immunohistochemical analysis. Am J Surg Pathol 2011;35(9):1305–1310. doi:10.1097/PAS.0b013e3182260735, PMID:21836478.
[19] de Jong WK, Blaauwgeers JL, Schaapveld M, Timens W, Klinkenberg TJ, Groen HJ. Thymic epithelial tumours: a population-based study of the incidence, diagnostic procedures and therapy. Eur J Cancer 2008;44(1):123–130. doi:10.1016/j.ejca.2007.11.004, PMID:18068351.
[20] Greene MA, Malias MA. Aggressive multimodality treatment of invasive thymic carcinoma. J Thorac Cardiovasc Surg 2003;125(2):434–436. doi:10.1067/mtc.2003.133, PMID:12579125.
[21] Chhieng DC, Rose D, Ludwig ME, Zakowski MF. Cytology of thymomas: emphasis on morphology and correlation with histologic subtypes. Cancer 2000;90(1):24–32. PMID:10692213.
[22] Finley JL, Silverman JF, Strausbauch PH, Dabbs DJ, West RL, Weaver MD, *et al*. Malignant thymic neoplasms: diagnosis by fine-needle aspiration biopsy with histologic, immunocytochemical, and ultrastructural confirmation. Diagn Cytopathol 1986;2(2):118–125. doi:10.1002/dc.2840020205, PMID:3522136.
[23] Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. Introduction to The 2015 World Health Organization Classification of Tumors of the Lung, Pleura, Thymus, and Heart. J Thorac Oncol 2015;10(9):1240–1242. doi:10.1097/JTO.0000000000000663, PMID:26291007.
[24] French CA, Kutok JL, Faquin WC, Toretsky JA, Antonescu CR, Griffin CA, *et al*. Midline carcinoma of children and young adults with NUT rearrangement. J Clin Oncol 2004;22(20):4135–4139. doi:10.1200/JCO.2004.02.107, PMID:15483023.
[25] Bishop JA, French CA, Ali SZ. Cytopathologic features of NUT midline carcinoma: A series of 26 specimens from 13 patients. Cancer Cytopathol 2016;124(12):901–908. doi:10.1002/cncy.21761, PMID:27400194.
[26] Lee T, Choi S, Han J, Choi YL, Lee K. Abrupt Dyskeratotic and Squamoid Cells in Poorly Differentiated Carcinoma: Case Study of Two Thoracic NUT Midline Carcinomas with Cytohistologic Correlation. J Pathol Transl Med 2018;52(5):349–353. doi:10.4132/jptm.2018.07.16, PMID:30056636.
[27] Policarpio-Nicolas ML, de Leon EM, Jagirdar J. Cytologic findings of NUT midline carcinoma in the hilum of the lung. Diagn Cytopathol 2015;43(9):739–742. doi:10.1002/dc.23291, PMID:26138851.