Original Article

Image Guided Fine Needle Aspiration Cytology of Thymic Lesions: A Four-Year Study

Malathi Mukund Pai[1], Smrita Singh[1], Akkamahadevi S Patil[1], Ashwini Nargund[1] and Anuradha Kapali[2]

Departments of Pathology[1] and Radiodiagnosis[2], Kidwai Memorial Institute of Oncology, Bangalore (Karnataka), India

Abstract

Background. Image-guided fine needle aspiration cytology (FNAC) has been used to evaluate mediastinal lesions.

Methods. Case records of 28 patients with thymic lesions who had undergone computed tomography (CT) - or ultrasonography-guided FNAC of thymic lesions over a period of four years (January, 2012 – December, 2015), retrieved from the cytology register, were retrospectively studied. Malignant lymphomas were excluded. Four smears were prepared for each case; two were stained with Papanicoulaou (Pap) stain and two were stained with May-Grunwald Giemsa (MGG) stain. A cell block was prepared in cases where adequate material was available using formalin fixation and agar method. Immunohistochemistry on cell block was done wherever required and feasible. Final diagnosis was made on the basis of the cytological and histopathological findings (in those cases that underwent biopsy/surgery), and considering the clinical and radiological findings.

Results. Thymoma accounted for the majority of the cases (n=19; 67.8%), thymic carcinoma (n=6; 21.4%), thymic hyperplasia (n=1; 0.04%), thymolipoma (n=1; 0.04%) and thymic neoplasia (n=1; 0.04%).

Conclusions. Image-guided FNAC with ancillary tests can be a powerful diagnostic tool in the diagnosis of thymic neoplasms. Although a diagnosis by FNAC has often proved challenging in this area, image guided FNAC along with ancillary techniques can provide an accurate diagnosis, especially in thymic neoplasms. **[Indian J Chest Dis Allied Sci 2019;61;19-23]**

Key words: Thymoma, Thymic carcinoma, Mediastinal lesions, FNAC, Cell block, Immunohistochemistry, Cytopathology.

Introduction

Image-guided fine needle aspiration cytology (FNAC) is an established diagnostic modality for evaluating mediastinal lesions. Computed tomography (CT) scan is the primary imaging technique for evaluating suspected thymic abnormalities seen on chest radiographs as well as for detecting occult thymic masses.[1-3] Multiple techniques have been used for the pathologic diagnosis of thymic tumours, including CT-guided core biopsy, mediastinoscopy, mediastinotomy, thoracoscopy and diagnostic techniques, such as endobronchial ultrasound (EBUS) guidance. These techniques, although effective, are relatively invasive with complication rates ranging from 1% to 3%.[4] Available literature suggests a fairly wide range of reliability for FNAC of mediastinal lesions. Published data shows accuracies ranging from 77% to 100%, sensitivities ranging from 71% to 87%, and positive predictive values ranging from 69% to 100%.[4-7] FNAC is vastly under-used as a diagnostic technique for mediastinal masses in general and for thymic epithelial neoplasms specifically.[4,6] We present here a report of image-guided FNAC of thymic lesions with a review of the literature.

Material and Methods

Case records of 28 patients with thymic lesions who had undergone CT- or ultrasonography-guided FNAC of thymic lesions over a period of four years (January, 2012–December, 2015) retrieved from the cytology register were retrospectively studied. Malignant lymphomas were excluded from the study. Majority of the cases of FNAC (n=27) were done under ultrasonography guidance. FNAC was done by pathologists with guidance by a radiologist in all the cases. A clinical resident was present at all occasions to handle any complication that might arise during the procedure. A minimum of four smears were prepared for each case — two were alcohol fixed and stained with Papanicoulaou (Pap) stain and two were air-dried and stained with May-Grunwald Giemsa (MGG) stain. A cell block was prepared in cases where adequate material was available using formalin fixation and agar method. Cytomorphology was studied and immunohistochemistry (IHC) on cell block was done wherever required and feasible. Details of the antibodies used are given in the table below. Final diagnosis was arrived at based on the cytological findings, histopathological findings (in those cases that underwent biopsy/surgery) considering the clinical and radiological findings.

Results

Their age ranged from 20 months to 75 years (median 47 years); there were 17 (60.7%) males. The most common

[Received: March 14, 2018; accepted after revision: May 18, 2018]

Correspondence and reprint requests: Dr Mukund Malathi, Associate Professor, Cytology Division, Department of Pathology, Kidwai Memorial Institute of Oncology, Dr M.H. Marigowda Road, Bangalore–560 029 (Karnataka), India; E-mail: vgnmaladr@gmail.com

Table. Details of the antibodies used

Antibody	Dilution	Manufacturer	Antibody clone
CK	1:50	BioGenex	C11
Vimentin	1:200	BioGenex	V-9
CD5	Ready to use	Biocare	4C7
TTF1	Ready to use	BioGenex	BGX-397A
EMA	1:100	BioGenex	E29
CK7	1:100	BioGenex	OVTL
CK19	1:100	Biocare	Ks19.1
CK20	1:200	Biocare	KS20.8
CD117	Ready to use	BioGenex	EP10
CK5/6	Ready to use	BioGenex	D5/16B4
p63	1:40	BioGenex	4A4
CEA	1:225	BioGenex	B019411MP
CD3	Ready to use	BioGenex	EP41
TdT	Ready to use	BioGenex	TdT88

Definition of abbreviations: CK=Cytokeratin; CD = Cluster differentiation; TTF=Thyroid transcription factor; EMA=Epithelial membrane antigen; CEA=Carcinoembryonic antigen; Tdt=Terminal deoxynucleotidyl transferase

presenting symptoms were chest pain, cough and dyspnoea. Four patients presented with features of *Myasthenia gravis*. The size of the lesions ranged from 5.1cm to 16.7cm. On cytological examination, thymoma accounted for the majority of the cases (n=19; 67.8%), followed by thymic carcinoma (n=6; 21.4%). The other lesions diagnosed on cytology were thymic hyperplasia in a 20-month-old male child, to rule out lymphoma prior to cochlear implant; thymolipoma in a young adult detected incidentally during routine health check-up; and thymic neoplasia (n=1 each).

Cell blocks were available in four cases with thymoma (two cases with thymoma B2, two cases with thymoma – not sub-typed) and in two cases with thymic carcinoma. IHC was done in the cases with cell blocks.

The case of thymic hyperplasia showed a polymorphous population of lymphoid cells (Figure 1). The single case of thymolipoma that we encountered comprised of a polymorphous population of bland looking epithelial cells and lymphocytes admixed with adipocytes (Figure 2). Histopathological correlation was done in eight cases with thymoma and one case with thymic carcinoma that underwent surgery/biopsy.

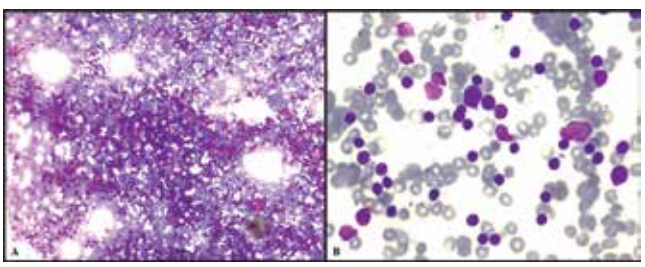

Figure 1. Thymic hyperplasia showing a polymorphous population of lymphoid cells in (A) (May-Grunwald Geimsa stain)x10 & (B)(May-Grunwald Geimsa stain)x40).

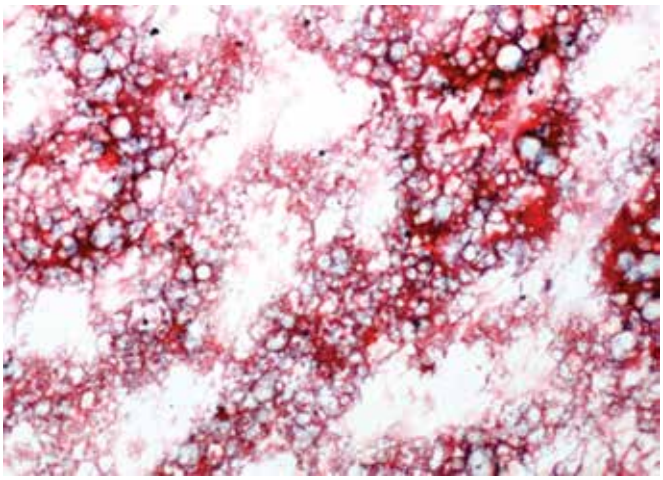

Figure 2. Photomicrograph showing predominantly adipocytic component with scattered lymphocytes (Papanicolaou × 10).

Concordance was noted between seven cases with thymoma diagnosed on FNAC with histopathological diagnosis while one case with thymoma diagnosed on cytology, turned out to be a case of malignant lymphoma on histopathology. Of these, two cases had type A thymoma, one had type B2 thymoma, one case had type B3 thymoma and three cases had type AB thymoma.

The cases diagnosed as type A thymoma were composed of clusters of predominantly spindled to oval epithelial cells with bland oval, occasionally round nuclei, evenly dispersed chromatin and inconspicuous nucleoli. Mitotic figures were rare. The lymphocytic component was very scant with occasional scattered lymphocytes (Figure 3). The cases of type B2 thymoma showed dyscohesive clusters of cortical epithelial cells with immature lymphocytes in the background. The epithelial cells were mitotically inactive with bland looking round to ovoid nuclei, delicate nuclear membrane, pale chromatin, and ill-defined cell borders. Cell block showed large epithelial cells with a prominent lymphocytic component. The epithelial cells were large and polygonal in shape with vesicular nuclei and prominent nucleoli.

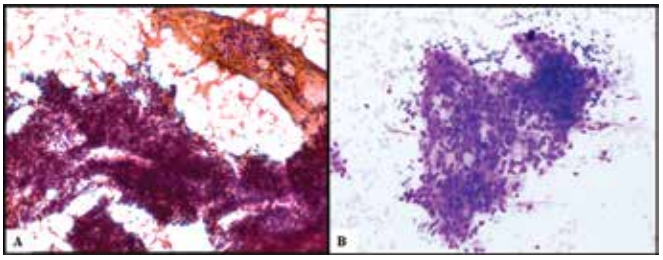

Figure 3. Thymoma type A; clusters of spindle cells with scant lymphocytes (A) (May-Grunwald Giemsa stain × 10) and (B) (May-Grunwald Giemsa × 40).

The epithelial cells were smaller compared to those in type B1, B2 or B3 thymoma. The cases of type AB thymoma were composed of clusters of epithelial cells and lymphocytes in almost equal frequency. Nuclear atypia

and mitoses were rare. The epithelial cells had round, oval or spindle pale nuclei with dispersed chromatin and indistinct nucleoli. Cell block showed nodules of type A and type B component as well as areas of admixed type A and type B cells (Figure 4). IHC was done on the cell block. The spindle cells were positive for cytokeratin (CK) and Vimentin with focal epithelial membrane antigen (EMA) positivity. The epithelial cells were negative for CD5 and thyroid transcription factor-1 (TTF1).

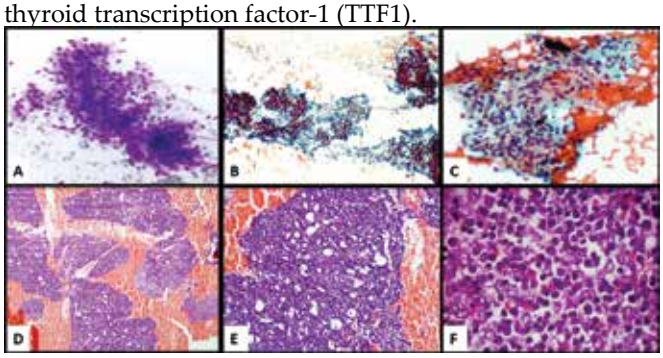

Figure 4. Photomicrographs of a case of thymolipoma showing (A) almost equal proportions of epithelial cells and lymphocytes in clusters (May-Grunwald Giemsa stain × 10); (B&C) (Papanicolaou×4 & ×10 respectively); (D) the cells are forming nodules in cell block (Haematoxylin and Eosin×4); (E) (Haematoxylin and Eosin×10); and (F) histopathology showing admixture of epithelial cells and lymphoctyes with minimal nuclear atypia (Haematoxylin and Eosin×40).

The case of type B3 thymoma comprised of sheets of epithelial cells with mild to moderate atypia and scant lymphocytes. The cells had round to oval irregular nuclei which were smaller than those in type B2 thymoma. The cells had pale to clear cytoplasm with some showing focal keratinisation. The few lymphocytes that were present were mostly immature T cells. Mitotic figures were less than 2 per high power field (hpf) or focally 4-10/10 hpf, which were assessed on histological sections. IHC was done on the histological sections. The epithelial cells were positive for CK7, CK19, EMA (focal) and negative for CD117, CD5 and CK20. The lymphoid cells were positive for terminal deoxynucleotidyl transferase (TdT), CD5 and negative for CD3 confirming them to be immature T cells.

The case with thymic carcinoma that underwent surgery, correlated with the histopathological diagnosis of squamous cell carcinoma with the tumour cells showing large nuclei with prominent nucleoli and keratinisation. The tumour cells were positive for CD5, CD117, CK5/6 and p63 on the cell block and negative for CK7, CK20 and TTF1 (Figure 5).

One patient had primary thymic adenocarcinoma where glandular differentiation was seen (Figure 6). IHC was done on the cell block. The tumour cells were positive for CD5, CD117, CEA and CK7 and were negative for TTF1, CK20 (Figure 7). On follow-up for a period of a median of 13.5 months (6 – 18 months), eight patients (thymoma 6, 1 patient each with 1, thymic hyperplasia) are doing well; eight patients died (thymic carcinoma 4, thymoma 3, malignant lymphoma 1). Twelve patients were lost to follow-up.

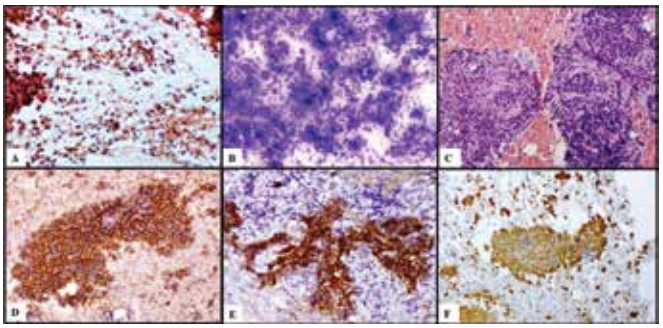

Figure 5. Photomicrograph showing (A) dyscohesive tumour cells with prominent nucleoli (Papanicolaou × 10); (B) (May-Grunwald Giemsa × 10); (C) cell block showing tumour cells forming nests (Haematoxylin and Eosin, × 4); (D) tumour cells showing positivity for CD5; (E) for CD117; and (F) for CK5/6.

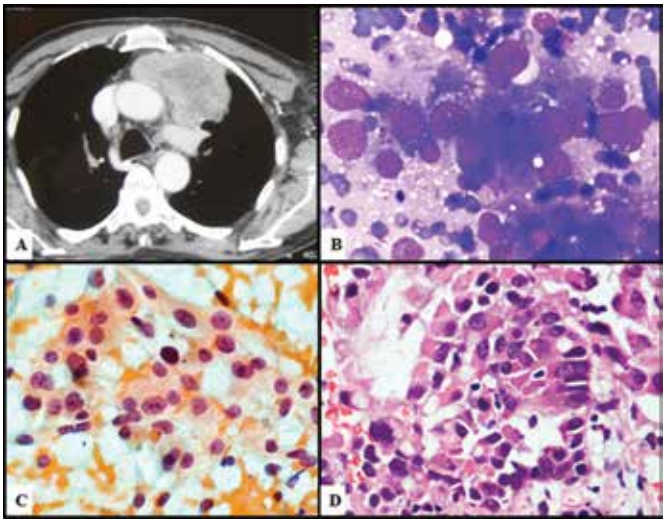

Figure 6. (A) Computed tomography showing a large heterogenous mass in the anterior mediastinum. Photomicrograph showing (B) tumour cells arranged in a vague glandular pattern with large nuclei and prominent nucleoli (May-Grunwald Giemsa × 40); (C) (Papanicolaou × 40); and (D) cell block with the tumour cells in glandular and solid pattern (Haematoxylin and Eosin × 40).

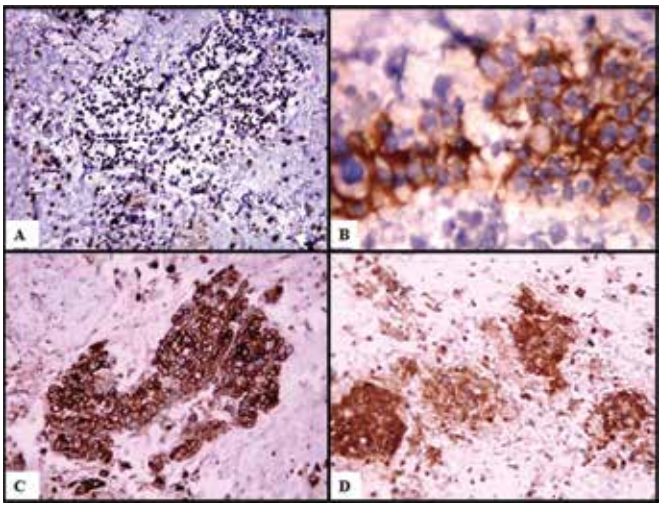

Figure 7. Photomicrograph of tumour cells showing positivity (A) for CD5; (B) for CD117; (C) for CK7; and (D) for CEA.

Discussion

Thymic tumours represent 0.2%–1.5% of all malignancies with an incidence of 0.15 per 100,000 population.[8] Thymomas are the most common primary neoplasms of the anterior mediastinum in adults with an incidence of 1.5 cases per million, and the overall incidence of thymic neoplasms is 0.13 per 100,000 person years.[9,10] These are usually seen in adults in the 5th and 6th decade. Their occurrence in children is rare. Thymoma is a distinctive biphasic tumour containing neoplastic epithelial cells and benign reactive, immature T-cell lymphocytes.[5,11] These are slow growing tumours with a potential for local extension and late recurrence. Metastases are most frequent in the pleura, pericardium, and diaphragm.[12] Among thymic carcinomas, squamous cell carcinoma is the most common. Other non-neoplastic lesions include thymic hyperplasia and thymic cysts.

Available literature suggests a fairly wide range of reliability for FNAC of mediastinal lesions. Published data show accuracies ranging from 77% to 100%, sensitivities ranging from 71% to 87%, and positive predictive values ranging from 69% to 100%.[4-7] Diagnostic pitfalls previously noted in the evaluation of thymic neoplasms by image guided FNAC can be overcome by proper sampling and the appropriate use of ancillary studies.

In thymomas, if the epithelial component is not identified, it might be mistaken for a benign lymph node, a reactive process or a lymphoma. Spindle cell thymomas pose a challenge to diagnosis, as these can be confused for a carcinoid, low-grade sarcoma or sclerosis in large cell lymphoma.[13] The lymphocytes of a thymoma are T-cells. On IHC, these stain for TdT, CD3, CD1a and CD99, but these lack the blastic chromatin of lymphoblasts. The epithelial cells are reactive for p63 and CK5. These do not react for CD5 or c-KIT, as do thymic carcinomas.[14]

The term "thymic carcinoma" encompasses a heterogeneous group of rare and very aggressive tumours. These have the cytomorphological appearance of a poorly-differentiated large cell carcinoma. Thymic carcinomas are often morphologically and immunohistochemically indistinguishable from a metastatic carcinoma.[15] These can be mis-diagnosed as squamous cell carcinoma of lung or a germ cell tumour.[12] In such cases, accurate radiological diagnosis with proper sampling of the lesions and IHC stains are helpful in arriving at the correct diagnosis. Thymic carcinoma distinguishes itself among carcinomas by its expression of CD5 and c-KIT (CD117). Carcinoembryonic antigen (CEA) is seen to be positive in cases with glandular differentiation. It is negative for TTF- 1.[7] The favourable prognostic factors are non-invasiveness, completeness of resection, younger age, and *Myasthenia gravis*.[7] We encountered six cases of thymic carcinoma, out of which, IHC was feasible in two cases with cell blocks. These two cases were subsequently diagnosed as thymic squamous cell carcinoma and thymic adenocarcinoma.

Thymic hyperplasia is of two types — true hyperplasia and lymphoid hyperplasia. Thymic lymphoid hyperplasia refers to an increased number of germinal centres predominantly in the interstitium and at the cortico-medullary junction. It is most frequently seen in association with *M. gravis*. This may, however, be idiopathic or seen in other autoimmune diseases, like systemic lupus erythematous, scleroderma and rheumatoid arthritis.[16] We had one patient (20-month-old male child) with thymic hyperplasia, who was referred to us to rule out lymphoma prior to cochlear implant surgery.

Cases of mediastinal lymphoblastic lymphoma can be mistaken for thymoma or thymic hyperplasia, lymphoid type, where the epithelial component is very small and inconspicuous. We encountered a case of lymphoblastic lymphoma which was initially thought to be a thymoma. This entity still poses a challenge for cytological diagnosis even with ancillary techniques.

One drawback of FNAC is that invasion cannot be determined. Zakowski *et al*,[13] studied 22 cases of thymic lesions and reported that the diagnosis on cytology correlated well with the histopathology, especially when correlated with clinical and radiological findings. In our study as well, the cytological diagnosis correlated well in those cases where histopathology was available (87.5%).

Till date, the management of thymic neoplasms is based on a multi-modal therapeutic strategy which includes surgery, chemotherapy and radiotherapy. The prognostic evaluation is mainly based on the Masaoka staging[17] and the World Health Organization [WHO] classification. Poor prognostic factors include presence of associated endocrinopathies, incomplete resection of tumuor, high grade tumours. The prognosis in patients with primary thymic neuroendocrine tumours (NETs) remains poor due to aggressive nature and high incidence of recurrence following surgery.[17]

Data indicate that vascular endothelial growth factor molecules, insulin-like growth factor 1 receptor (IGF1R), cyclin-dependent kinases (CDK) and mammalian target of rapamycin (mTOR) may be potentially useful as targets for biological therapy.[14] Other molecular targets include epidermal growth factor receptor (EGFR) expression in 80% thymomas and 50% thymic carcinomas, c-Kit in 73% thymic cancers and 5% thymomas. Gefitinib, cetuximab, dasatinib are being evaluated and case reports and small series have shown some therapeutic benefit.[18] In our study, we were not able to do molecular studies due to financial constraints and lack of facilities, which may be the only drawback.

Conclusions

Image-guided FNAC as a powerful initial diagnostic test which can be used in cases of mediastinal lesions to rule out a thymic neoplasm, especially in cases where there is a clinical suspicion. This holds good especially when ancillary tests are done on cell blocks along with

cytomorphology. One should note that it is important, that clinical and radiological correlation is a must before giving a cytological diagnosis.

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