Quiz Case

Fine needle aspiration of hematolymphoid lesions of the thyroid: Onsite adequacy and ancillary testing

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CASE REPORT

A 7-year-old girl presented with an enlargement of the thyroid gland. Thyroid hormones and serologic testing were normal. A thyroid ultrasound showed a right 0.8 cm thyroid nodule with microcalcifications and well-defined borders. Fine needle aspiration (FNA) revealed polymorphic lymphocytes without Hurthle cells [Figure 1a-d]. Flow cytometry showed a population of CD2+, CD5+, CD8+, CD7+, CD4+, and CD1a+ cells. Immunohistochemistry on cell block section showed immunoreactivity for TdT without immunoreactivity for CD34.

Figure 1: Polymorphic population of lymphocytes and histiocytes, with squamoid cells (yellow arrows in a and b). A: PAP stain ×10; (b) PAP stain ×100; (c) Higher magnification of histiocytes and polymorphic lymphocytes (red arrowhead-lymphoblast, green arrowhead-small lymphocytes) (Diff Quick stain ×100); (d) Polymorphic population of small lymphocytes (red arrowhead-lymphoblast, green arrowhead-lymphocytes) (PAP stain ×100).

QUESTION 1

What is your interpretation?
A. Colloid nodule
B. Primary thyroid extranodal NK/T-cell lymphoma
C. Extranodal mucosa-associated lymphoid tissue (MALT) lymphoma of the thyroid
D. Squamous cell carcinoma chronic inflammation
E. Ectopic intrathyroidal thymic tissue

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Answer to question 1: Option E (ectopic intrathyroidal thymic tissue).

The aspirates were moderately cellular and showed predominantly polymorphic lymphocytes with occasional histiocytes. The lymphocytes were predominantly small to medium in size with mature clumped chromatin and a smaller subset featuring large nuclei with open chromatin (lymphoblasts). Furthermore, evaluation of the aspirate smear showed occasional aggregates of squamoid cells consistent with Hassall corpuscles [Figure 1].

Immunophenotyping by flow cytometry analysis [Table 1] revealed the presence of CD45/SS populations consistent with mature lymphocytes (46%), monocytes (1%), granulocytes (3%), and a CD45dim region (29%). The gated CD45dim area was comprised largely of immature/maturing T-cell subsets that were CD3 negative but expressed CD2, CD5, CD7 and variably expressed CD4+, CD8+, and CD4+/CD8+ subsets, along with partial CD1a expression. The gated CD45brt lymphocytes were predominantly mature CD3+ mixed T cells that displayed normal CD4/CD8 content (4:8 ratio = 1.9) without aberrant expression of associated markers. The gated minority granulocytes and monocytes appeared immunophenotypically normal. The flow cytometry interpretation illustrated a cellular makeup that was consistent with normal thymus tissue and likely represented ectopic intrathyroidal thymic tissue.\[1\] Flow cytometry was negative for any obvious abnormal or significant clonal populations [Table 1]. Cellblock sections showed polymorphic lymphocytes that were immunoreactive for TdT without immunoreactivity for CD34 [Figure 2].

![Figure 2](image_url): The lymphocytes were immunoreactive for TdT and non-immunoreactive for CD34 (a and b, ×40, immunohistochemistry on cell-block sections).

| Target cell population | MoAb/CD# | CD45dim Lymphs | CD45brt Lymphs | Gate type: CD45 versus SS-log |
|------------------------|----------|----------------|----------------|-----------------------------|
| % of total cell population |          | 29             | 46             | Sample preparation: density-gradient; mononuclear isolation with RBC lysis |
| CD45       | 64       | 100            | Commonly found on: Pan-Leukocytes |
| T cell markers |
| CD1a       | 34       | 17             | Cortical thymocytes |
| CD5+       | 91       | 97             | Pan T cells |
| CD7        | 95       | 90             | Pan T cells |
| CD2        | 94       | 99             | Pan T cells |
| CD3        | 4        | 98             | Mature T cells |
| CD4        | 64       | 65             | Helper/Inducer T cells |
| CD8        | 30       | 38             | Suppressor/cytotoxic T cells |
| CD4+/CD8+  | 27       | 6              | T cell precursor subset |
| CD56       | 1        | 1              | NK cells; T cell subset |
| CD57       | 0        | 2              | NK cell subset; T cell subset |
| B cell markers |
| HLA-DR     | 10       | 10             | B, NK, Myeloid, Activated T cells |
| CD10       | 3        | 0              | Early B lineage, Early T cells |
| CD20       | 1        | 0              | Late B lineage cells |
| CD22       | 2        | 0              | Pan-B cells |
| CD19       | 2        | 1              | Pan-B cells |
| Additional markers |
| CD38       | 95       | 76             | Broad lineage; Activation marker |
| CD34       | 25       | 0              | Early precursors, Stem cells |

*Markers for Myeloid (CD15, CD16, CD11b, CD14, CD13, CD33, CD34, CD64); Monocytic, Platelet/Megakaryocytic (CD41, 61+/CD14-, CD61, CD36); and Erythroid Markers (CD36, Gly A) negative. brt: Bright, Lymphs: Lymphocytes, MoAb/CD#: Monoclonal antibody/CD number.

**Table 1:** Flow cytometry report on needle rinse of FNA of the lesion.
The aspirates did not show thyroid follicular cells and any colloid in the background (option A). Primary thyroid lymphomas are extremely rare and account for <1% of cases of extranodal lymphomas. Most primary thyroid lymphomas are non-Hodgkin lymphomas of B-cell origin, and primary thyroid extranodal NK/T-cell lymphomas are exceedingly rare. Cytologic examination would reveal a diffusely infiltrating small to medium-sized lymphoid cells, prominent mitotic figures, and areas of necrosis with apoptotic debris, not observed in the current case (option B). Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type is characterized by morphologically heterogenous B-cells and may be encountered in 0.5% of patients with Hashimoto thyroiditis. It is postulated to occur as a response to autoimmune processes, such as in patients with Hashimoto thyroiditis and patients with *Helicobacter pylori* infection. FNA would show thyroid follicular atrophy with Hurthle cell changes and follicular cells with lymphocytic infiltration as lymphoepithelial structures, which were not detected in the current case (option C). Primary squamous cell carcinoma, typically common in elderly patients, is a rare entity which carries poor prognosis (may be a component of anaplastic thyroid carcinoma). FNA would reveal follicular cells infiltrated or replaced by morphologically malignant cells. The malignant cells may be cohesive and arranged in sheets, nests, cords, islands, or a trabeculae pattern. The cells would exhibit large, pleomorphic, hyperchromatic to vesicular nuclei which may show eosinophilic nucleoli and abundant eosinophilic cytoplasm. Individual keratinization, mitotic figures (including the atypical forms), and tumor necrosis may also be noted. The cell-block sections may show intercellular bridges. This morphology was not evident in the current case (option D).

**QUESTION 2**

The thymus is the primary organ of T lymphocyte development and partly develops from the endoderm of the third pharyngeal pouch. Which of the following organs have the same embryologic origin as the thymus?

A. Thyroid gland
B. Superior parathyroid glands
C. Inferior parathyroid glands
D. Ultimobranchial bodies

Answer to question 2: Option C (inferior parathyroid glands).

The inferior parathyroid gland originates from the endoderm of the third pharyngeal pouch just like the thymus. The thyroid originates from the endodermal cells of the primitive pharynx between the first and second pharyngeal pouches (option A), the superior parathyroid glands originate from the fourth pharyngeal pouch (option B), and the ultimobranchial bodies which give rise to the calcitonin producing parafollicular cells of the thyroid originate from the fourth pharyngeal pouch (option D).

**QUESTION 3**

According to the World Health Organization classification, the obligatory criteria for diagnosing type B1 thymoma includes:

A. Occurrence of bland spindle-shaped epithelial cells (at least focally) and paucity or absence of immature (TdT+) T cells throughout the tumor
B. Increased numbers of single or clustered polygonal or dendritic epithelial cells intermingled with abundant immature T cells
C. Thymus-like architecture and cytology with abundance of immature T cells, areas of medullary differentiation (medullary islands), and paucity of polygonal or dendritic epithelia cells without clustering (i.e.,<3 contiguous epithelial cells)
D. Occurrence of bland, spindle-shaped epithelial cells (at least focally), and abundance of immature (TdT+) T cells focally or throughout tumor
E. Sheets of polygonal slightly to moderately atypical epithelial cells; absent or rare intercellular bridges; paucity or absence of intermingled TdT+ T cells

Answer to question 3: Option C (thymus-like architecture and cytology with abundance of immature T cells, areas of medullary differentiation (medullary islands), and paucity of polygonal or dendritic epithelia cells without clustering (i.e.,<3 contiguous epithelial cells)).

Occurrence of bland spindle-shaped epithelial cells (at least focally) and paucity or absence of immature (TdT+) T cells throughout the tumor is classified as Type A thymoma (option A).

Increased numbers of single or clustered polygonal or dendritic epithelial cells intermingled with abundant immature T cells are classified as type B2 thymoma (option B). Occurrence of bland, spindle-shaped epithelial cells (at least focally); abundance of immature (TdT+) T cells focally or throughout tumor is classified as type AB thymoma (option D), and sheets of polygonal slightly to moderately atypical epithelial cells; absent or rare intercellular bridges; paucity or absence of intermingled TdT+ T cells is classified as type B3 thymoma (option E).

**QUESTION 4**

TdT immunostain is useful in delineating which of the following combinations?
Thymic carcinomas, thymomas, and epithelial cells of normal thymus
B. Immature T cells of normal thymus, >90% of thymomas, and neoplastic T cells of T lymphoblastic lymphoma
C. Epithelial cells of normal thymus, thymomas, thymic carcinomas, neuroendocrine tumors, many germ cell tumors, and dendritic cell tumors
D. Normal and neoplastic B cells and epithelial cells of Type A and AB thymoma

Answer to Question 4: Option B (immature T cells of normal thymus, >90% of thymomas, and neoplastic T cells of T lymphoblastic lymphoma).

Thymic carcinomas, thymomas, and epithelial cells of normal thymus may be identified with cytokeratins (option A). Epithelial cells of normal thymus, thymomas, thymic carcinomas, neuroendocrine tumors, many germ cell tumors, and dendritic cell tumors may be identified with cytokeratins (option C). and normal and neoplastic B cells and epithelial cells of type A and AB thymoma may be identified with CD20 (option D).

BRIEF REVIEW OF THE TOPIC

Thyroid nodules are uncommon in the pediatric population as compared to adults and account for 0.2–2% of cases. However, 20–73% of nodules found in children are malignant and exhibit higher risks of regional and distant metastases than in the adult population. Thyroid cancer is the eighth most common cancer in adolescents and the second most common cancer in girls.

Advances in ultrasonographic examinations have resulted in increased numbers of incidental thyroid gland lesions than previously reported. Ultrasound findings that suggest malignancy include microcalcifications, irregular borders, and hypoechogeticity. Features which indicate malignancy may be determined using the thyroid imaging reporting and data system (TI-RADS). TI-RADS refers to any of several risk stratification systems for thyroid lesions usually based on ultrasound characteristics. Points are assigned to the lesion based on its composition, echogenicity, shape, margin, and echogenic foci. A score of 0 is considered benign (TR1), 2 is considered not suspicious (TR2), 3 is considered mildly suspicious (TR3), 4 to 6 is considered moderately suspicious (TR4), and 7 points or more is considered highly suspicious (TR5). Identification of some of these features on ultrasound prompts the provider to pursue testing to rule out malignancy. Rarely, ectopic non-thyroidal tissue may be detected on ultrasound as a thyroid nodule with concerning features. Thus, in accordance with current guidelines, any thyroid lesion found in a child, except for pure cysts, requires thorough evaluation including FNA.

The differential diagnosis for thyroid lesion in children typically includes nodular goiter, lymphocytic thyroiditis, colloid cysts, follicular adenomas, degenerating nodules, and malignant thyroid nodules. However, other considerations for an intrathyroidal nodule, which occur less frequently, include ectopic thymic tissue, lymphomas, and even metastatic malignancies from other anatomic locations. Making the correct diagnosis through minimally invasive procedures, especially in instances of benign lesions, would prevent the performance of more invasive and complicated procedures, with their attendant consequences.

Thymus is a primary lymphoid organ that plays an important role in the differentiation of T-cells. During embryogenesis, the thymus is formed from the ectoderm of the third branchial cleft and the endoderm of the third pharyngeal pouch. The definitive thymus is formed by fusion of the right and left thymic primordial tissues before it descends to the upper anterior mediastinum. Aberrant persistence of thymic tissue along migration may lead to ectopic thymus location, including intrathyroidal locus. The prevalence of ectopic thymic tissue in children is very low and has been reported to range between 0.99% and 1.8%. Ectopic thymic tissue may manifest as a neck mass or an incidentally detected lesion.

The two main differential diagnoses of ectopic thymic tissue (immature/maturing T-cells) include T-lymphoblastic leukemia (T-ALL) and thymoma. Since both the normal thymic tissue as well as the background lymphocytes in thymoma would have identical immunophenotypic profile, the distinction cannot be made solely based on immunophenotypic grounds, rather it is made based on morphological examination showing infiltrating "dispersed" epithelial cells, which is consistent with thymoma. Benign thymic maturing T-cells demonstrate a characteristic maturation expression pattern on flow cytometric immunophenotyping, which allows for reliable distinction from T-ALL.

The unique variable pattern of maturing T-cells mainly includes the following markers: CD3, CD1a, CD34, CD4, and CD8. The earliest maturing thymic T-cells are initially negative for CD3, CD1a, CD4, and CD8. Later, they acquire CD1a, CD4, and CD8, followed by expression of surface CD3. Finally, they will commit to either CD4 or CD8 and will lose CD1a with retention of surface CD3 (i.e., mature T cells). The normal progression of maturing, non-neoplastic thymocytes, is from CD4(−)/CD8(−), to CD4(dim+)/CD8(−), to CD4(+)/CD8(+), finally to a mature helper T-cell CD4(+)/CD8(−) or CD4(−)/
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CD8(+) cytotoxic T cells. Similarly, thymocytes progress from CD34(+)CD1a(-)/CD3(-), to CD34(-)/CD1a(+)/CD3(-), to CD34(-)/CD1a(+)/CD3(+), and eventually to mature T-cell immunophenotype CD1a(-)/CD3(+). In this case, the immunophenotypic analysis is consistent with a population of immature/maturing T-cells (CD3-/CD2+/CD5+/CD7+, with variably express CD4+, CD8+, and CD4+/CD8+ subsets, along with partial CD1a expression) which, in the given patient scenario, is most consistent with ectopic thymic tissue in the thyroid gland [Table 1].

Rapid onsite evaluation (ROSE) is a critical component of FNA procedure by pathologists and cytotechnologists to increase the diagnostic yield of FNA procedures. Multiple studies have reported improved specimen adequacy with this technique further emphasizing its unique role in patient care. In addition to improving diagnostic adequacy, ROSE also facilitates triaging of specimens that require ancillary diagnostic tests. In the current case, ROSE determined that the specimen was devoid of colloid and follicular cells and mainly consisted of singly scattered lymphocytes. A determination to triage a portion of the specimen for flow cytometric analysis was made during ROSE for evaluating lymphoproliferative processes. Flow cytometry was critical in making the right diagnosis and confirmed the non-neoplastic nature of the lesion, consistent with aberrant ectopic intrathyroidal thymic tissue with additional help from cell block.

FNA procedure without ROSE assessment may put the patient at risk for a more invasive procedure, especially if the cellblock contains insufficient diagnostic material. Therefore, the role of ROSE in evaluating and triaging cytopathology specimens cannot be overemphasized since it enhances diagnostic yield. One particular case illustrated here highlights ROSE’s critical role during the FNA procedure for effective patient care by submitting the needle rises for flow cytometry. The cases such as the one illustrated here highlight the critical role of ROSE during FNA procedure for effective patient care.

SUMMARY

Thyroid nodules are uncommon in the pediatric population and when they do occur, are more likely to be malignant and associated with local, regional, or distant metastasis.

Ectopic thymic tissue in the thyroid is a rare occurrence in children and may be mistaken for other B and T lymphocytic lesions on FNA procedures due to morphologically overlapping low-grade lymphoproliferative lesions. ROSE during an FNA procedure is critical because it can initiate appropriate triage of the specimen for ancillary testing and increase diagnostic accuracy. This would minimize the potential of invasive procedures and interventions.

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COMPETING INTERESTS STATEMENT BY ALL AUTHORS

The authors declare that they have no competing interest. The co-author Vinod Shidham, MD, the co-editor-in-chief of this journal does not have any competing interests.

AUTHORSHIP STATEMENT BY ALL AUTHORS

Each author has participated sufficiently in the work and takes public responsibility for appropriate portions of the content of this article. All authors read and approved the final manuscript. Each author acknowledges that this final version was read and approved.

ETHICS STATEMENT BY ALL AUTHORS

As this is case without identifiers, our institution does not require approval from the Institutional Review Board (IRB) (or its equivalent).

LIST OF ABBREVIATIONS (In alphabetic order)

brt – bright
FNA – Fine needle aspiration
Lymphs – Lymphocytes
MALT – Mucosal associated lymphoid tissue
MoAb/CD# – Monoclonal antibody/CD number
NK – Natural killer
ROSE – Rapid onsite evaluation
T-ALL – T- acute lymphoblastic leukemia
TdT+ – TdT immunoreactive
TI-RADS – Thyroid imaging reporting and data system
WHO – World Health Organization.

EDITORIAL/PEER-REVIEW STATEMENT

To ensure the integrity and highest quality of CytoJournal publications, the review process of this manuscript was conducted under a double-blind model (authors are blinded for reviewers and vice versa) through automatic online system.

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