

Characteristic Flow Cytometric Profile of Ectopic Intra-thyroidal Thymic Tissue in Children

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Key words: Ectopic thymic tissue, thyroid nodule, immunophenotyping

Running title: Ectopic thymic tissue in thyroid nodule

Word count: 1144

Figures/Tables: 1 figure + 1 table

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as doi: [10.1002/cyto.b.21954](https://doi.org/10.1002/cyto.b.21954)

Dear Editor:

Ectopic intrathyroidal thymic tissue (EITT) occurs due to aberrant cervical migration during embryogenesis. It is a benign and self-limiting condition that is not uncommon in pediatric age group and may present as a thyroid nodule. The immature lymphocytes present in thymoma and T-Lymphoblastic Lymphoma (T-LBL) may have morphological resemblance to and share some immunophenotypic characteristics with normal thymocytes. Therefore, diagnosis of EITT based on flow cytometry could be challenging.

Normal thymic tissue has a specific T cell maturation pattern; the characteristic CD4+CD8+ double positive (DP) either CD3- or more mature CD3+ thymocytes predominate. On a CD3/CD4/CD8 dot plot, CD3+ thymocytes produce characteristic “classic wing pattern” with CD3+ DP thymocytes forming the body and maturing helper and cytotoxic T-cells, the wings (1, 2). This typical pattern is useful in differentiating normal thymic tissue from benign thymoma or T-LBL. Here, we describe two cases of EITT in children, one with tuberous sclerosis and the other with Graves’ disease, which displayed a unique flow cytometric profile with the presence

of tightly clustered immature T cells, raising the concern about a possible T-LBL along with skewed distribution of more mature thymic subsets.

We reviewed clinical features, ultrasonography, fine needle aspiration (FNA) biopsy specimens for histopathology, 10-color flow cytometry and T cell receptor gene (*TCR*) rearrangement, which was available in one patient. First patient is an 8-year-old euthyroid male with tuberous sclerosis and on melatonin and diphenhydramine (Case #1). Second patient is a 4-year-old male with Graves' disease (Case #2) who is being treated with methimazole and atenolol.

FNA biopsies were carried out after finding a hyperechoic thyroid nodule in Case#1 and soft tissue mass with solid heterogeneous, speckled pattern in Case#2 on ultrasonography. The flow cytometry of FNA biopsies displayed CD45bright lymphocytes consisting of 77% CD3+ mature T cells with 52% CD4+, 21% CD8+ cells and 1% DP with a CD4/CD8 ratio of 2.5. CD3 expression on mature T cell was relatively homogeneous (Figure 1A). Interestingly, it also displayed a tightly clustered population of CD45dim immature T lymphocytes (22%) that were predominantly CD3-, CD5dim+, CD2dim+, CD4partial+, CD8-, CD1a+, and CD34partial+. Fifty-eight percent of gated CD45dim population was CD4+, 19% was CD4+CD8+ (DP) and 42% was CD4-CD8-CD34+ (Figure 1B). Intracytoplasmic CD3 (iCD3) and TdT was not assessed due to insufficient specimen.

Case #2 showed a population of CD45bright lymphocytes consisting of 58% CD3+ mature T cells with 33% CD4+, 18% CD8+ and 1% DP with a CD4/CD8 ratio of 1.8. CD3 expression on mature T cell was relatively homogeneous (Figure 1C). Case #2 also showed a tightly clustered population of CD45dim immature T lymphocytes (32%) that were predominantly iCD3+, surface CD3-, CD5dim+, CD2dim+, CD4partial+, CD8-, CD1a+, CD34partial+ and TdT+. Fifty four percent of gated CD45dim population was CD4+, 5% DP and 46% CD4-CD8-CD34+ (Figure 1D).

Both specimens displayed an absence of CD10 expression on CD34+ immature T cells, decreased presence of DP T cells within CD45dim population and absence of DP maturing T cells. There was no aberrant B cell or myeloid marker expression seen in either case and *TCR* rearrangement study in Case #2 did not show clonality.

Histopathology of FNA biopsies showed polymorphic lymphocytes admixed with cohesive epithelia groups and lack of thyroid tissue in Case #1, and polymorphic lymphocytes admixed with cohesive epithelial cells with no evidence of papillary thyroid carcinoma in Case #2. Immunohistochemical stains were not feasible in both cases because biopsy specimens were hemodiluted and showed scant number of cells (Figure 1E-H). Both cases were concluded as EITT.

Thymocyte maturation process is greatly regulated by thymic epithelial cells and follows a specific maturation pattern (1). During normal development, early maturing thymocytes are CD3-CD7+CD2+CD5dim+CD4-CD8-CD1a-CD34+. Maturing thymocytes gradually start expressing CD1a, CD4 followed by CD8; CD10 expression is upregulated with simultaneous decrease in CD34 expression. Then, gain of expression of CD3 follows and finally loss of CD10, CD1a and either expression of CD8 or CD4 occurs leading to development of mature helper or cytotoxic T cells. Therefore, normal thymus typically displays CD3-CD34+CD4-CD8- (DN), CD3-CD34-CD4dim+CD8-, CD3-CD34- DP, and CD3+ DP, CD3+CD4+CD8- helper, CD3+CD4-CD8+ cytotoxic T cells subsets with CD3+ thymocytes producing characteristic “classic wing pattern” by flow cytometry. Understanding the characteristic pattern of thymocyte subsets is very important for accurate diagnosis of ectopic thymic tissue.

Primary thyroid T cell lymphomas are extremely rare and can involve thyroid gland without distant metastases. Thymomas are epithelial tumors that contain a large number of immature T cells and are frequently associated with various autoimmune disorder, including Graves' disease. Ectopic thymomas are rare tumors that originate in areas including the thyroid, posterior or middle mediastinum. The flowcytometric features of normal thymus, T-LBL, thymoma and two cases of EITT are described in Table1.

Two cases of thyroid nodule that we describe showed tight clustering of CD45dim T cells on CD45/side scatter graph and lack the characteristic smearing pattern of CD3. CD45dimCD3- cells were predominantly either single CD4+ or DN. Moreover, there was an absence of CD3-CD34+CD10+ subset and CD3+ DP subset seen in a normal thymus. Although atypical presentation seen in these two cases share some features of T-LBL, first immature cells comprised a fraction of the lymphoid cells in the mixture lacking homogeneity in cellular composition. Secondly, there were no aberrant B cell or myeloid antigen expression, and no clonal TCR rearrangement at least in one case.

The percentage of DP thymocytes are always greater than 50% in normal pediatric thymus, 40% to 95% in types A/B and B thymomas and <20% in type A thymoma (2). However, type A thymomas are predominantly composed of thymic epithelial cells with only scant immature T lymphocytes. In thymoma, the distribution of maturing thymocytes may be skewed by the absence of normal epithelial support (1). The two cases that we evaluated showed mature/maturing lymphocytes with significantly lower population of CD3- DP cells, absent CD3+ DP subset in more mature cell population, as well as lack of CD34+CD10+ and CD34-CD10+ subsets, differing from an ectopic thymoma profile.

Flow cytometric findings of these two cases presented are unique and appear almost a copy of one and other (Figure 1A-1D). The difference in the thymocyte maturation pattern including lack of CD34+CD10+ and CD34-CD10+, significantly decreased CD3- DP cells and

absent CD3+ DP subset could be partially due to influence of the host tissue along with absent and/or hypo-/dysfunctional thymic epithelium. In fact, the ectopic thymus tissue flow cytometric profile in our cases is quite different than the reported case in adenoid tissue (1) and also differ from the previously reported EITT cases (2). Interestingly, both immature CD3-negative and more mature CD3-positive populations were predominated by CD4-positive thymocytes. Peripheral blood contamination as evidenced by granulocyte population on CD45/SS plots may be a factor in increased CD4-positive T cells in mature gate; however, CD4 predominance in immature CD3-negative thymocytes is unusual. Therefore, potential effect of ectopic microenvironment on the final composition of the EITT tissue warrants further investigation.

Conflict of interest: Nothing to report

References

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Table1

Table1: Flow cytometric features of normal thymus, T-lymphoblastic lymphoma, thymoma and EITT (current cases)				
Flow cytometry parameter	Normal Thymus	T-Lymphoblastic Lymphoma	Thymoma	Ectopic Intra-Thyroidal Thymus (current cases)
CD45/Side Scatter	Smearing pattern of CD45dim to bright thymocytes	Tight cluster of CD45dim lymphoblasts	Smearing pattern of CD45dim to bright thymocytes	Tight cluster of CD45dim subpopulation
sCD3 expression	Smearing pattern of immature to mature Thymocytes	Most commonly sCD3-negative, may show partial CD3dim expression	Smearing pattern of immature to mature Thymocytes	Distinct CD3-negative and CD3-positive populations
CD4+CD8+ dual positive	greater than 50%	homogeneous dual positive or dual negative lymphoblast	40% to 95% in types A/B and B thymomas; <20% in type A thymoma	Distinct CD3-negative thymocytes with < 20% of DP expression and absent CD3-positive DP thymocytes
CD10 expression	positive in early and late thymocytes precursors	Either positive or negative in lymphoblast	Positive in early and late thymocytes precursors	Negative
CD3-CD4+ single positive T cells	Low level (~ 10-15%)	May be present in some T-LBL	variable	Predominant
CD2/CD5/CD7 T cell antigen expression	positive	Loss of one or more T cell antigen expression	positive	positive or decreased
Aberrant B cell and/or Myeloid antigen expression	Absent	May be present	Absent	Absent

Figure Legend:

Figure1. Flow cytometry dot plots of thyroid nodule fine needle aspiration biopsy of two cases of ectopic intra-thyroidal thymus tissue. (A) Case #1 mature lymphocyte gate, (B) Case #1 CD45dim gate, (C) Case #2 mature lymphocyte gate, (D) Case #2 CD45dim gate. The dot plots show T cell marker profile on CD45dim immature lymphocytes and CD45bright more mature lymphocytes. Mature T cells show normal expression of CD3, CD5, more prominent CD4 in comparison with CD8, and absence of CD4+CD8+ double positive (DP) subset. CD45dim Immature T cells showing greater CD3-CD4+, CD3-CD4-CD8-, significantly low CD3- DP subsets, diminished CD5 expression, absent CD10 expression on CD34+ thymocytes and CD1a+ expression.

Fine needle aspiration (FNA) histological findings. Predominantly small lymphocytes with no atypical features or large cells in Case#1 (E) and Case#2 (G) (Hematoxylin/Eosin stain, Objective 40x).

Polymorphous lymphocytes (arrows) without atypical features admixed with cohesive epithelia groups (black triangles). There is no evidence of colloid or microscopic features of papillary thyroid carcinoma in Case#1 (F) and Case #2 (H) (Hematoxylin/Eosin stain, Objective 40x).

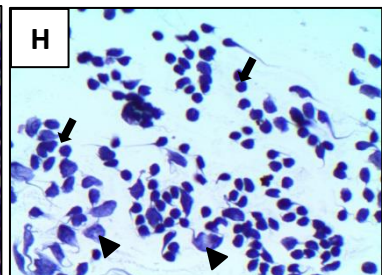
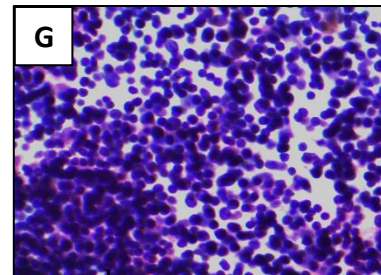
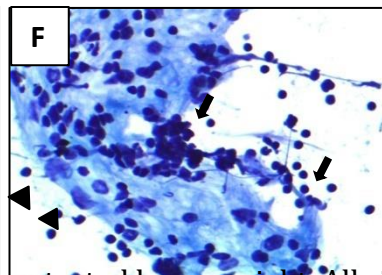
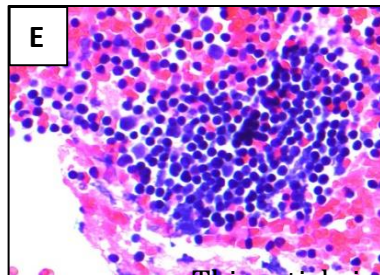
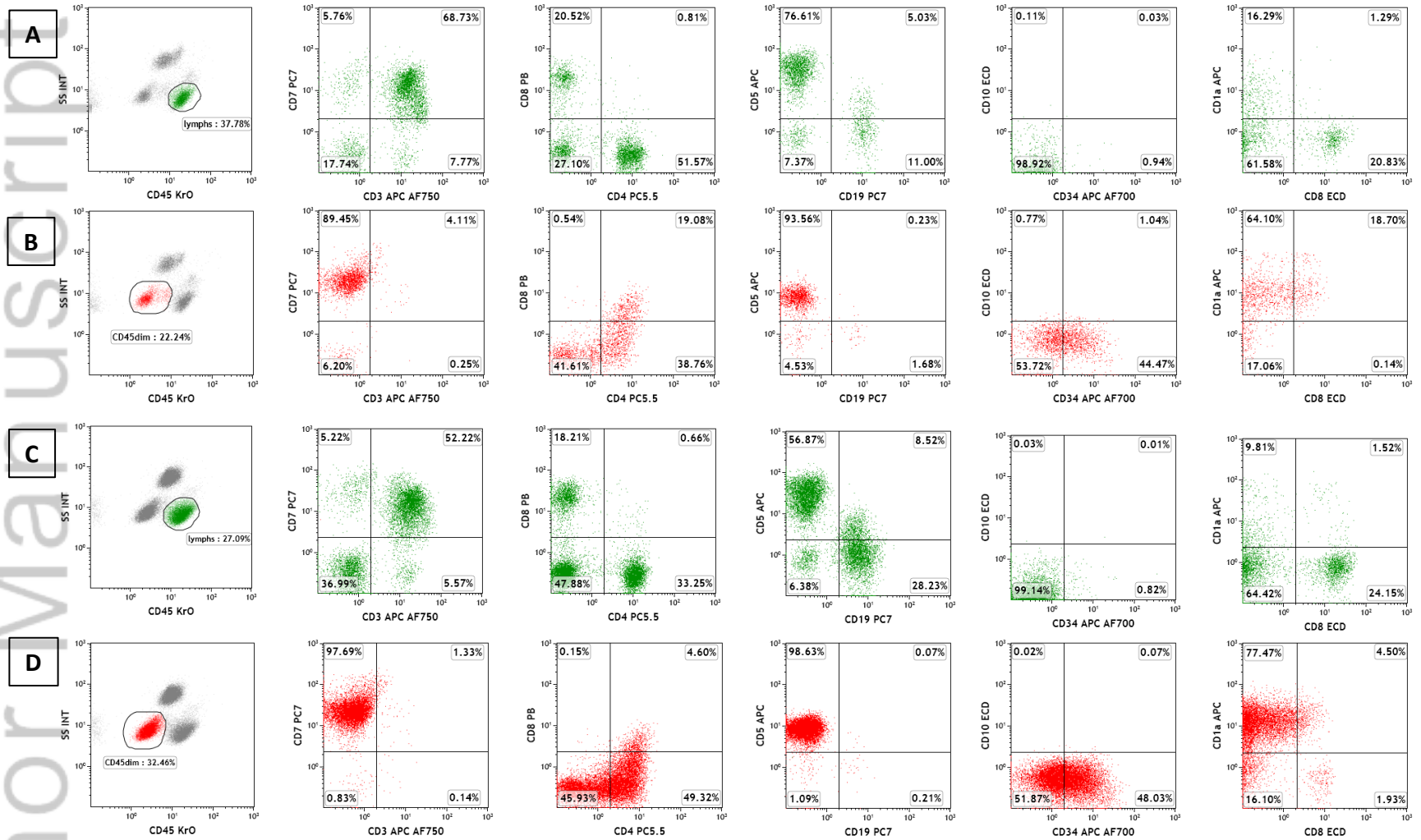


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CD10 expression	positive in early and late thymocytes precursors	Either positive or negative in lymphoblast	Positive in early and late thymocytes precursors	Negative
CD3-CD4+ single positive T cells	Low level (~ 10-15%)	May be present in some T-LBL	variable	Predominant
CD2/CD5/CD7 T cell antigen expression	positive	Loss of one or more T cell antigen expression	positive	positive or decreased
Aberrant B cell and/or Myeloid antigen expression	Absent	May be present	Absent	Absent