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Benign TdT-positive cells in pediatric and adult lymph nodes: a potential diagnostic pitfall

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ABSTRACT

Benign TdT-positive cells have been documented in a variety of non-hematopoietic tissues. Scant data are however available on their presence in non-neoplastic lymph nodes. This study is aimed to: (i) characterize the presence/distribution of benign TdT-positive cells in pediatric and adult reactive lymph nodes; (ii) define the phenotype and nature of such elements. This retrospective study considered 141 reactive lymph nodes from pediatric and adult patients without history of neoplastic disease. TdT-positive cells were characterized by immunohistochemical and morphometric analyses and their presence was correlated with the clinical-pathological features. The nature of TdT-positive cells was investigated by: (i) double immunostaining for early lymphoid cell markers; and (ii) assessment of TdT expression in fetal lymph nodes. Sparse TdT-positive cells were documented in all pediatric cases and in most (76%) adult lymph nodes. TdT-positive cell density was higher in children than adults (15.9/mm^2 *versus* 8.6/mm^2; $p<0.05$). TdT positivity did not correlate with any clinical and histological parameter and double immunostaining disclosed a phenotype compatible with early lymphoid precursors (positivity for CD34, CD10 and variable expression of CD7). A very high TdT-positive cell density (802.4/mm^2) was reported in all fetal lymph nodes.

In conclusion, TdT-positive cells are a common finding in pediatric and adult lymph nodes. The interstitial distribution and low number of such cells allows for the differential diagnosis with precursor lymphoid neoplasms. The high density in fetal lymph nodes and the phenotype of such cells suggest their belonging to an immature lymphoid subset gradually decreasing with age.

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1. INTRODUCTION

B and T lymphopoiesis consists of an organized series of biological events, taking place in dedicated anatomic compartments. In adults, B cell maturation mainly occurs in the bone marrow, while T cell development is largely confined to the thymus (1-3). Seminal studies on animal models have documented the presence of T cell progenitors also in non-hematopoietic tissues, such as the gut and peritoneal lymph nodes (4, 5). Similarly, small subsets of murine B cells (i.e. B-1 B cells) likely originate from extra-marrow lymphoid precursors residing in the fetal liver (6).

In keeping with these animal data, benign Terminal deoxynucleotidyl Transferase (TdT)-positive lymphoid precursors have recently been documented in a variety of human non-hematopoietic tissues, including the nasopharyngeal tonsil (i.e. adenoids), the appendix, the spleen and sites of chronic inflammation (e.g. renal parenchyma in chronic interstitial nephritis; synovial membranes in rheumatoid arthritis) (7-12). TdT-positive cells have also been documented in lymph nodes involved by Castleman disease and angioimmunoblastic T cell lymphoma (13). Scant data are as yet available on the presence, density and pathophysiological meaning of these cells in reactive lymph nodes. The only available studies indeed focus on small series of patients with history of neoplastic disease (7, 14). In all such cases, the lymph nodes featured scattered interstitial TdT-positive cells, whose frequency and distribution did not apparently correlate with any clinical-pathological parameter (14).

The results of these studies do not provide data on nodal TdT-positive cells in non-oncologic patients. The present study thus assessed the presence/distribution of nodal TdT-positive cells in a large cohort of pediatric and adult patients with no history of malignancy. It also defined the phenotype and fetal counterpart of such TdT-positive cells, to get hints on their possible biological origin.

2. MATERIALS & METHODS

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2.1 Case selection

Overall, 141 reactive lymph nodes from pediatric (n= 66) and adult patients (n= 75) were considered. Tissue samples were retrieved from the archives of the Surgical Pathology and Cytopathology Unit of Padova University Hospital (Padova – Italy; n= 62) and the Haematopathology Unit of Sant'Orsola University Hospital (Bologna – Italy; n= 79). For each case, the following clinical and laboratory parameters were considered: (i) patient sex and age at diagnosis; (ii) clinical indication for lymphadenectomy; (iii) complete blood cell count at the time of biopsy. The assessment of TdT expression in fetal lymph nodes was performed on autopsy specimens retrieved from the archives of the Surgical Pathology and Cytopathology Unit of Padova University Hospital (n= 12). All cases were re-evaluated by three pathologists (ES, MP and SB), who agreed on the interpretation of immunohistochemical results. The ethic regulations on research on human tissues were followed by the participating Centers, consistent with the declaration of Helsinki.

2.3 Immunohistochemical analysis

For immunohistochemical analysis, 3 to 4 μm-thick tissue sections were stained with the following primary antibodies: anti-TdT (clone SEN 28, pre-diluted, Leica Biosystem, Newcastle – UK; and clone EP266, dilution 1:40, Dako Cytomation, Glostrup, Denmark), anti-CD3 (clone LN10, dilution 1:100, Leica Biosystem; and clone SP7, dilution 1:50, Diagnostic BioSystems, CA, USA), anti-CD79a (clone 11E3, pre-diluted, Leica Biosystem; and clone JCB, dilution 1:50, DakoCytomation), anti-CD1a (clone 010, dilution 1:50, Dako, Glostrup – Denmark; and clone EP3622, dilution 1:400, Abcam, Cambridge, UK), anti-CD2 (clone AB75, dilution 1:40, Dako; and clone AB75, dilution 1:50, Leica Biosystem), anti-CD7 (clone LP15, dilution 1:50, Biocare Medical, Concord – USA; and clone 580, dilution 1:50, Leica Biosystem), anti-CD10 (clone 56C6 dilution 1:50 Leica Biosystem), anti-CD34 (clone QBEnd/10, dilution 1:100, Thermo Scientific, Fremont – USA; and clone QBEnd/10 dilution 1:100 Leica Biosystem). Antigen retrieval was

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performed with heat/EDTA in an automated immunostainer (Surgical Pathology and Cytopathology Unit of Padova University Hospital) and with PT-link (PT100/PT101, DakoCytomation) and the EnVision Flex Target Retrieval Solution High pH (K8004, DakoCytomation) (Hematopathology Unit of Sant'Orsola-Malpighi Hospital), as previously described (15).

Immunohistochemical analysis was performed with both single (TdT) and double immunostains (TdT/CD3, TdT/CD79a, TdT/CD7, TdT/CD1a, TdT/CD34 e TdT/CD10). For single immunostains the Bond^{TM} Polymer Refine Detection kit (Leica Biosystem, Newcastle, UK) or Dako Real Detection System Alkaline Phosphatase/RED Rabbit /mouse (K5005, DakoCytomation) were used. Double immunostains were performed using either the Bond^{TM} Polymer Refine Detection and Bond^{TM} Polymer Refine Red Detection kits (Leica Biosystem; Surgical Pathology and Cytopathology Unit of Padova University Hospital) or Dako Real Detection System Alkaline Phosphatase/RED, Rabbit/Mouse and Dako Real EnVision Detection System, Peroxidase/DAB, Rabbit/Mouse (K5007, DakoCytomation; Hematopathology Unit of Sant'Orsola-Malpighi Hospital). Histological pictures were acquired by the DFC420 digital camera and software (Leica Biosystems, Milan – Italy).

2.4 Morphological and morphometric evaluation

Histological evaluation included the following morphological and immunohistochemical parameters: (i) presence/absence of follicular hyperplasia, paracortical hyperplasia and/or sinus histiocytosis; (ii) presence/absence of epithelioid granulomas; (iii) presence and distribution of necrotic areas; (iv) presence and distribution of TdT-positive lymphoid cells.

TdT-positive cell density was assessed as the number of TdT-positive elements per square unit area. TdT-positive cells were manually counted by two pathologists (SB and MP), while the lymph node whole section area was assessed by digital imaging techniques (DMD108 digital microscope and sowftware; Leica Microsystems, Milan - Italy).

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2.6 Statistical analysis

Statistical analysis was performed using non-parametric tests to compare qualitative (Fisher's exact test) and quantitative (Student's t-test) variables. Differences between groups were considered statistically significant for *p*-values below 0.05.

3. RESULTS

3.1 Clinical-pathological features of the study population

The study population consisted of 66 pediatric and 75 adult patients with no history of neoplastic disease. The pediatric population included 49 males and 17 females (male to female [M/F] ratio= 2.9), with a mean age at diagnosis of 8.5 years (range: 0.1-17.0 years). The lymph nodes were obtained from cervical (n= 22), axillary (n= 3), abdominal (n= 11) and inguinal (n=7) stations. The site of biopsy sampling was not reported in 23 cases. Most patients presented with isolated lymph node enlargement and the biopsy was obtained to rule out malignancy (n= 59). In 6 cases, the lymph node was removed for inflammatory and/or infectious disorders. One lymph node was obtained during partial splenectomy for hereditary spherocytosis.

The adult population consisted of 38 males and 37 females (M/F ratio= 1.0), with a mean age at diagnosis of 50.0 years (range: 18.2-87.0 years). The adult series included 9 cervical, 8 axillary, 1 mediastinal, 5 abdominal and 10 inguinal lymph nodes. In 42 cases, the site of lymph node biopsy was not specified. In 70 cases, the lymph nodes were removed for isolated nodal enlargement. The remaining lymph nodes were collected during abdominal surgery (cholecystectomy and appendectomy) for non-neoplastic inflammatory disorders.

Histological evaluation of the lymph nodes disclosed a variety of reactive patterns. The majority of both pediatric and adult samples disclosed follicular and paracortical hyperplasia with or without sinus histiocytosis and occasional small epithelioid granulomas. Pure follicular or paracortical hyperplasia and prominent sinus histiocytosis were less common (Table 1; Figure 1A-C). Two pediatric cases were characterized by large necrotizing granulomas, suggestive of

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mycobacterial infection (the diagnosis was subsequently confirmed at molecular and microbiological testing). No cases disclosed features suggestive of malignancy and/or Castleman disease.

3.2 Prevalence, distribution and morphometric evaluation of the TdT-positive cells

TdT-positive cells were documented in all (66/66 [100%]) pediatric lymph nodes and in the vast majority (57/75 [76%]) of adult cases (Table I). The difference in the prevalence between children and adults was statistically significant (Fisher's exact test; $p<0.05$).

In all cases TdT-positive cells were sparse, accounting for a definite minority of the overall lymphoid populations. They were organized singly or in small loose aggregates (<10 cells) and were detectable only by immunohistochemical staining. TdT-positive cells were found close to high endothelial venules in inter-follicular regions (141/141 [100%]), with rare cases also featuring an intra-follicular, mantle zone and/or intra-capsular location (1/141 [0.7%], each). Cytologically, the TdT-positive cells were small with regular nuclear contours, uniform chromatin and scant cytoplasm (Table I; Figure 1 D-E).

In all cases, TdT-positive cells represented <0.5% of all lymphoid elements. Morphometric analysis, however, disclosed a sharp variability in TdT-positive cell density (i.e. number of TdT-positive cells per square unit area). Indeed, the pediatric and adult cohort disclosed a mean TdT-positive cell density of 15.9 ±5.9 cells/mm^2 (range: 0.1-178.0 cells/mm^2) and 8.60 ±3.12 cells/mm^2 (range: 0.1-47.1 cells/mm^2), respectively. The differences in TdT-positive cell density between the two groups were statistically significant (Student's t-test; $p< 0.05$) (Table I).

3.3 Clinical-pathological correlations

The presence and density of TdT-positive cells were correlated with the following clinic-pathological parameters: (i) sex and age at diagnosis; (ii) site of lymph node biopsy; (iii) blood cell

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count values at the time of biopsy; (iv) lymph node histological patterns (i.e. follicular hyperplasia; paracortical hyperplasia; sinus histiocytosis); (v) presence of necrotizing or epithelioid granulomas. None of the above variables did correlate with the presence and density of TdT-positive cells.

4.4 Phenotypic and biological characterization of the TdT-positive cells

The nodal TdT-positive cells likely represent a sub-population of lymphoid precursors residing in para-cortical areas. To get hints on the possible nature of these cells, two parallel approaches were adopted: (i) a thorough phenotypic characterization of TdT-positive cells by double immunostaining (i.e. TdT/CD34, TdT/CD10, TdT/CD7, TdT/CD3, TdT/CD1a and TdT/CD79a); (ii) the assessment of fetal lymph nodes, to test the hypothesis that post-natal TdT-positive cells are in fact residues of an embryonal lymphoid component.

The phenotypic analysis disclosed co-expression of TdT, CD34 and CD10, with occasional positivity for CD7 and consistent negativity for CD3, CD1a and CD79a (Figure 2A-F). This phenotype is in keeping with a common lymphoid precursor (CLPs), *i.e.* an immature hematopoietic cell with skewing toward lymphoid differentiation (16, 17).

Immunohistochemical characterization of the fetal lymph nodes disclosed TdT-positive cells in all cases, with an interstitial growth pattern akin to that observed in post-natal life. Of note, TdT-positive cell density was several times higher in fetal than in pediatric and adult lymph nodes (mean TdT-positive cell density in fetal lymph nodes: 802.4 ±630.7 cells/mm^2 [range: 21.4 – 4125.0 cell/mm^2]). The differences between fetal and post-natal TdT-positive cell density were statistically significant (p <0.05; Figure 3A-C).

5. DISCUSSION

The positivity for TdT in lymphocytes outside the hematopoietic organs is generally considered a strong hint for the diagnosis of precursor lymphoid neoplasms (i.e. B and T cell

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lymphoblastic leukemia/lymphoma [B-LBL and T-LBL]). This view has recently been challenged by the detection of benign TdT-positive infiltrates in a variety of peripheral tissues. These proliferations span from sparse lymphoid aggregates (7-12) to mass-forming lesions in nodal and extra-nodal sites (18-23). The latter, also known as "indolent T-lymphoblastic proliferations" (iT-LBPs), disclose a precursor T cell phenotype, closely mimicking T-LBL. The indolent clinical course and polyclonal *TCR* rearrangement support a diagnosis of iT-LBP in such cases (18).

The results of our study expand the awareness on benign TdT-positive infiltrates by documenting the presence of sparse TdT-positive cells in all pediatric and most adult reactive lymph nodes (Table I). This acquisition may contribute to avoid misdiagnosing nodal residing TdT-positive cells as minimal infiltration of LBL, especially in small/sub-optimal lymph node core biopsies. The differential diagnosis between LBL and benign nodal TdT-positive cells on lymph node whole section is indeed straightforward and relies on both clinical and pathological parameters. LBL usually presents with large nodal/extra-nodal lesions or peripheral blood involvement. In case of nodal presentation, the neoplastic cells efface the lymph node architecture with little (if any) sparing of the non-neoplastic tissue (24). These findings have never been documented in our series, as TdT-positive cells were scant and only detected by immunohistochemical staining. Unfortunately, the low number of TdT-positive elements did not allow for reliable *TCR*/*IGH* molecular testing. The patients' clinical history, however, excluded a lymphoid malignancy in all cases.

The differential diagnosis with iT-LBP relies on similar clues, as iT-LBP disclose sheets of TdT-positive cells with partial effacement of the involved tissues (18). Notwithstanding, the TdT-positive cells of our series and iT-LBP disclose a similar (yet not overlapping) phenotype. ~~possibly representing the two ends of a biological continuum. In particular, the sparse TdT-positive cells normally residing in the paracortical zone might undergo polyclonal expansion in response to (still unknown) micro-environmental signals, thus producing the macroscopically~~

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~~detectable lesions typical of iT-LBP.~~ Further histological, flow cytometry and molecular studies are needed to ~~test this hypothesis and to~~ highlight any possible relationship between these TdT-positive lymphoid populations.

From a biological perspective, the TdT-positive cells of our series disclosed a CLP-like phenotype with consistent positivity for CD34 and CD10, variable expression of CD7 and negativity for CD3, CD1a and CD79a (Figure 2A-F). The lack of lymphoid lineage markers does not allow to draw any conclusion on the possible B and/or T cell commitment of such cells. Thorough molecular analyses (e.g. single cell PCR; gene expression profile of micro-dissected cells) may however add up to the understanding of their biology. Further studies will address this issue.

The immunohistochemical results of our series are only partially in keeping with prior studies, which documented variable expression of CD3, CD1a (8, 14) or CD79a (10, 25) in most nodal and extra-nodal TdT-positive cells. The reasons for such discrepancy are unknown, but possible explanations may come from: (i) the heterogeneity of the TdT-positive infiltrates in different anatomical and/or clinical settings; (ii) the occurrence of different maturation stages of such TdT-positive cells; and (iii) possible under-/over-estimation of the double immunostainings, due to the small number and high nuclear-to-cytoplasmic ratio of the TdT-positive elements.

Another unprecedented finding of our study is the progressive decrease of nodal TdT-positive cells with aging. The mean TdT-positive cell density was indeed much higher in fetal than post-natal lymph nodes and in pediatric than adult cases (Figure 3A-C). Despite a thorough biological interpretation of such findings is beyond the scopes of the present study, these results pinpoint the TdT-positive cells as an early lymphoid population possibly homing from primary hematopoietic organs (i.e. bone marrow and thymus) to lymph nodes during fetal life and progressively decreasing after birth. As such, the TdT-positive cells in the lymph nodes, Waldeyer ring, appendix and other non-lymphoid tissues may represent an immature lymphoid

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reservoir, whose decrease may be mediated by either re-circulation or maturation to distinctive T or (less likely) B cell subsets (8).

In conclusion, small subsets of TdT-positive cells are a normal finding in the lymph nodes of both pediatric and adult patients and their occurrence does not apparently correlate with any clinical-pathological parameter. The low number and sparse paracortical distribution of such cells allow for the differential diagnosis with precursor lymphoid neoplasms and/or other mass-forming TdT-positive cell proliferations. The origin of these cells is still largely unknown, but immunohistochemical studies on both fetal and post-natal lymph nodes suggest their belonging to an immature lymphoid population, progressively decreasing from the fetal to the pediatric and adult age.

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FIGURE LEGENDS

Figure 1. Representative histological and immunohistochemical features of the studied lymph nodes. A-C. The majority of lymph nodes disclosed follicular hyperplasia (**A**) with either paracortical hyperplasia (**B**) or sinus histiocytosis (**C**). **D-E.** TdT-positive cells were documented by immunohistochemical staining as sparse/isolated non-descript lymphoid elements. Most of these cells were located within paracortical areas (**D**; *arrowheads*); occasional cases also featured intra-capsular location (**E**). (Histological images have been obtained from adult lymph nodes; H&E and horseradish peroxidase stains; original magnification, 5x and 20x).

Figure 2. Immunophenotype of the TdT-positive cells. Double immunostaining disclosed co-expression of TdT, CD34 (**A**) and CD10 (**B**). TdT-positive cells were also variably positive for CD7

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(**C**) and consistently negative for CD3 (**D**), CD79a (**E**) and CD1a (**F**). (Alkaline phosphatase *plus* LRP [i.e. *red*] stain: TdT; horseradish peroxidase *plus* DAP [i.e. *brown*] stains: CD34, CD10, CD7. CD3, CD79a, CD1a; original magnification, 40x).

Figure 3. Variation in TdT-positive cell density among fetal, pediatric and adult lymph nodes. Fetal lymph nodes (**A**) were characterized by a high density of TdT-positive cells, with frequent aggregates and clustering of cells. These features were not typical of post-natal life, despite the number of TdT-positive cells was higher in pediatric (**B**) than adult (**C**) lymph nodes. (Horseradish peroxidase stain; original magnification, 10x).

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Table I. Clinic-pathological features of the study population

	Pediatric cases (n=66)	**Adult cases (n=75)**
Mean age (years)	8.5 (*range*: 0.1-17.0)	50.0 (*range*: 18.2-87.0)
M/F ratio	2.9:1	1:1
Lymphadenectomy site *Cervical* *Axillary* *Mediastinal* *Abdominal* *Inguinal* *Unknown*	 22/66 (33.3%) 3/66 (4.5%) - 11/66 (16.7%) 7/66 (10.6%) 23/66 (34.8%)	 9/75 (12.0%) 8/75 (10.6%) 1/75 (1.3%) 5/75 (6.7%) 10/75 (13.3%) 42/75 (56.0%)
Lymph node histology *Follicular Hyperplasia* *Paracortical Hyperplasia* *Sinus Histiocytosis* *Mixed pattern*	 10/66 (15.2%) 2/66 (3.0%) 4/66 (6.0%) 50/66 (75.8%)	 18/75 (24.0%) 6/75 (8.0%) 10/75 (13.3%) 41/75 (54.7%)
Prevalence of TdT-positive cases*	66/66 (100%)	57/75 (76%)
Density of TdT-positive cells (cells/mm^2)*	15.9 ±5.9	8.6 ±3.12
TdT-positive cell distribution: *Interfollicular (IF)* *IF & intrafollicular* *IF e mantle zone* *IF e capsular*	 63/66 (95.5%) 1/66 (1.5%) 1/66 (1.5%) 1/66 (1.5%)	 57/57 (100%) - - -

* Parameters with statistically significant differences between the pediatric and adult groups

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Highlights

- Scattered TdT-positive cells are very common in pediatric and adult lymph nodes;
- The prevalence and density of TdT-positive cells are higher in children than adults;
- TdT-positive cells disclose the phenotype of common lymphoid precursors;
- The awareness of these cells prevent their misdiagnosis as precursor lymphoid neoplasms.

ACCEPTED MANUSCRIPT

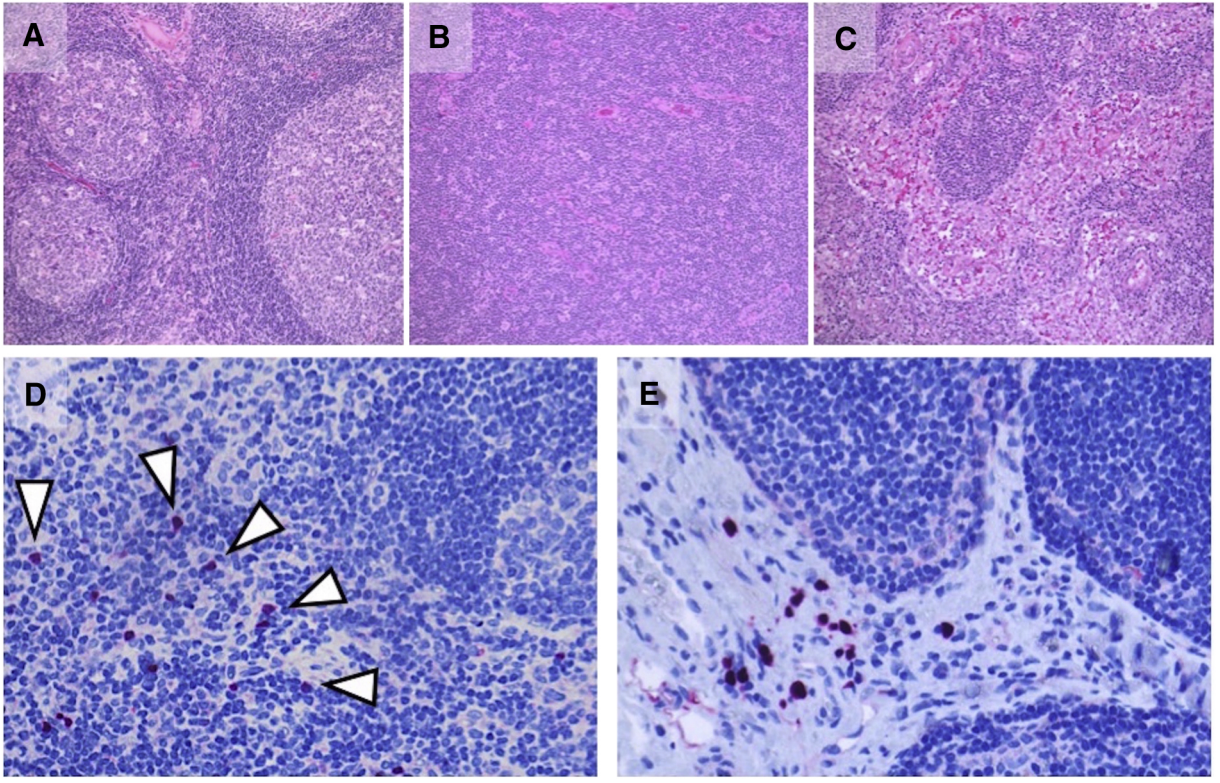

Figure 1

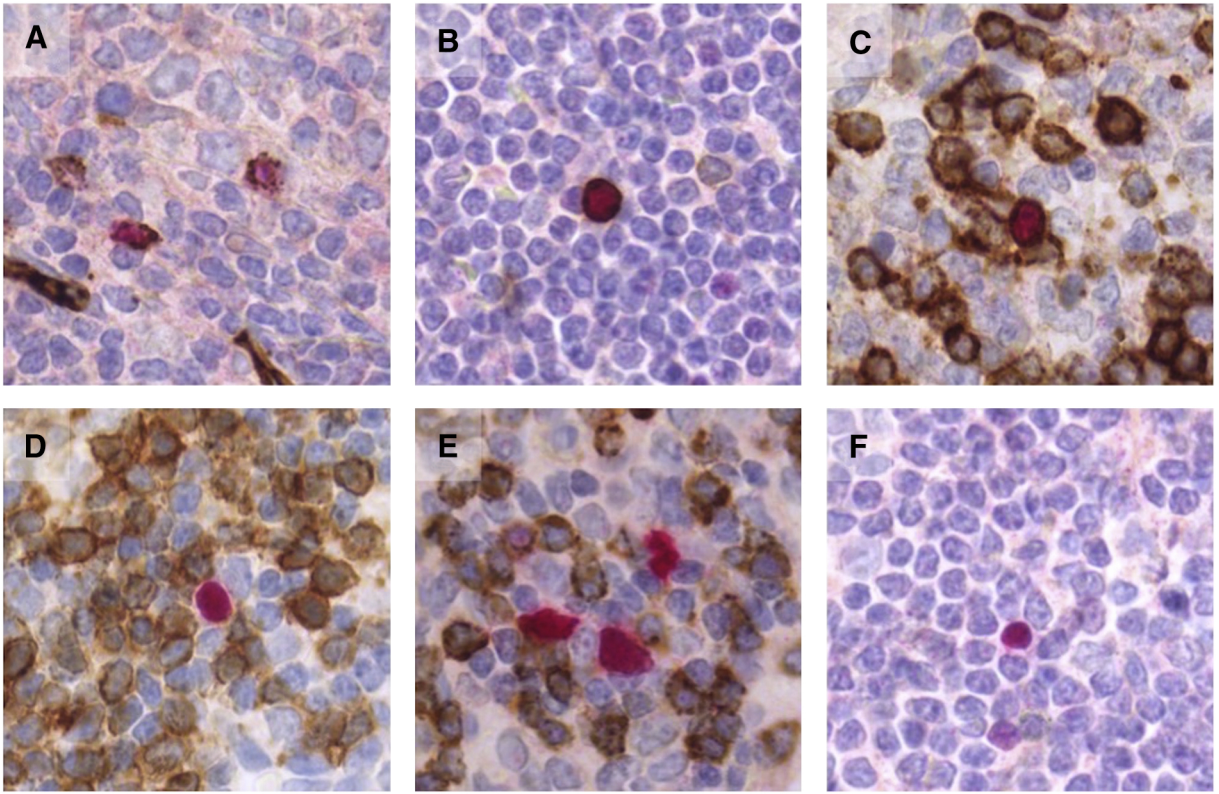

Figure 2

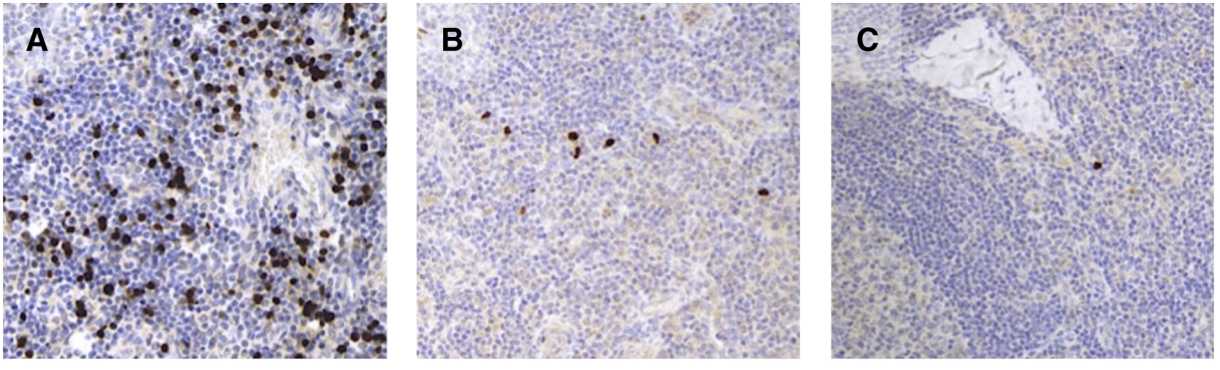

Figure 3