

### Terminal deoxynucleotidyl transferase-positive cells in spleen, appendix and branchial cleft cysts in pediatric patients

We evaluated spleens (n=26), appendices (n=10) and branchial cleft cysts (n=6) for TdT-positive cells in pediatric patients. In spleen, appendix and branchial cleft cysts the range of TdT-positivity was 0-13, 0-96 and 0-6 TdT<sup>+</sup> cells/hpf, respectively. In spleens, scattered TdT<sup>+</sup> cells were seen most frequently in periarteriolar lymphoid sheath regions.

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Terminal deoxynucleotidyl transferase (TdT) plays an important role in the development of lymphoid precursors and in the generation of T-cell receptor diversity. It was thought that the location of TdT<sup>+</sup> lymphoid precursor cells was restricted mainly to the thymus (cortical thymocytes) and bone marrow (precursor B- and T-cells) but recent research by Strauchen and Miller<sup>1</sup> and Onciu *et al.*<sup>2</sup> has shown that TdT<sup>+</sup> cells are present in both tonsils and in reactive lymph nodes.

We evaluated spleen, appendix and branchial cleft cysts for the distribution and number of TdT-positive cells. Each of these sites has prominent lymphoid proliferations. Because previous studies suggested increased numbers of these TdT<sup>+</sup> cells in pediatric patients, we concentrated on specimens from children. Cases evaluated included spleens (n=26), appendices (either acute appendicitis or incidental removal) (n=10) and branchial cleft cysts (n=6), all from patients <18 years old. Spleen specimens (n=13) from adults with benign conditions were selected for comparison. All tissues were fixed in formalin and routine hematoxylin and eosin stains were reviewed. Immunohistochemical staining for TdT was performed using the standard avidin-biotin peroxidase technique with hematoxylin counterstaining. The location, pattern of distribution and frequency of TdT<sup>+</sup> cells were recorded. In areas of highest density, TdT<sup>+</sup> cells were counted and expressed as cells per high-power field (hpf). Dual label immunohistochemical staining (TdT/CD79a, TdT/CD10 and TdT/CD3) was performed on five cases which had increased TdT<sup>+</sup> cells (3 appendices, 2 spleens).

The average age of the patients was 7.8 years (7 days – 17 years). The spleens had an average weight of 372 g (range 20-1358 g). The characteristics of the patients and the results of TdT staining are shown in Tables 1 and 2. The highest numbers of TdT<sup>+</sup> precursors in spleens were present in a 7 day-old and a 12-day old. Two patterns of TdT positivity were seen in spleens. The most frequent was the presence of scattered TdT<sup>+</sup> cells in the periarteriolar lymphoid sheath region of the white pulp. Less commonly, widely scattered positive cells were seen in red pulp. The group of adult spleens had a range of 0-4 TdT<sup>+</sup> cells/hpf, with an average of 0.5 cells/hpf overall. Only three of the 13 adult spleen specimens had any positive cells. There was wide range of positivity in appendix specimens (0-96/hpf). There was no correlation of TdT<sup>+</sup> cells between appendices removed because of appendicitis

**Table 1.** Summary of cases: clinical features, diagnosis and number of TdT-positive cells

Case N.	Age (in years unless stated otherwise)	Site	Weight* (g)	Diagnosis	TdT <sup>+</sup> cells/HPF <sup>o</sup>
1	11	Spleen	626	Sickle cell	0
2	6	Spleen	221	Hemoglobinopathy	1
3	17	Spleen	660	Spherocytosis	1
4	14	Spleen	1358	Spherocytosis	0
5	13	Spleen	n/a	Trauma	2
6	7	Spleen	150	Spherocytosis	2
7	10	Spleen	n/a	Incidental	0
8	1	Spleen	114	Sickle cell	6
9	4	Spleen	n/a	ITP	2
10	3	Spleen	174	Sickle cell	2
11	1	Spleen	72	Sickle cell	5
12	1	Spleen	101	Sickle cell	3
13	6	Spleen	n/a	Possible hemoglobinopathy	3
14	10	Spleen	595	Sickle cell	0
15	7 days	Spleen	n/a	Incidental	9
16	13	Spleen	475	Sickle cell	2
17	1	Spleen	n/a	Incidental	1
18	8	Spleen	191	Spherocytosis	13
19	5	Spleen	385	Unknown etiology	2
20	12 days	Spleen	20	Unknown etiology	8
21	14	Spleen	1200	Sickle cell	0
22	3	Spleen	210	Unknown etiology	0
23	5	Spleen	110	Unknown etiology	2
24	13	Spleen	n/a	Spherocytosis	6
25	7	Spleen	108	Follicular hyperplasia	6
26	16	Spleen	312	Incidental	4
27	2	Neck mass	–	Branchial cleft cyst	0
28	9	Neck mass	–	Branchial cleft cyst	6
29	1	Neck mass	–	Branchial cleft cyst	0
30	8	Neck mass	–	Branchial cleft cyst	0
31	2	Neck mass	–	Branchial cleft cyst	0
32	3	Neck mass	–	Branchial cleft cyst	0
33	8	Appendix	–	Acute appendicitis	4
34	3	Appendix	–	Acute appendicitis	6
35	9	Appendix	–	Incidental	0
36	9	Appendix	–	Incidental	47
37	14	Appendix	–	Acute appendicitis	0
38	13	Appendix	–	Incidental	96
39	3	Appendix	–	Incidental	38
40	2	Appendix	–	Incidental	2
41	7	Appendix	–	Acute appendicitis	26
42	3	Appendix	–	Acute appendicitis	0

\*Weights noted only for spleen cases. <sup>o</sup>Assessed in the area of the highest density of TdT<sup>+</sup> cells. n/a: not available.

or as incidental specimens. Age did not affect the number of TdT<sup>+</sup> cells seen in appendices. TdT<sup>+</sup> cells were most often located between follicles adjacent to small blood vessels. In branchial cleft cysts, only one specimen, from a 9-year old patient, had 6<sup>+</sup> cells/hpf. The remaining cases (5/6) had no TdT<sup>+</sup> cells.

The results of the dual-label immunohistochemical stains were only marginally successful, despite several attempts. In all spleens evaluated, only rare positive cells were identified. The few cells identified showed co-expression of TdT/CD79a, and TdT/CD10 but no cells were identified with TdT/CD3. In 3/3 appendices, no co-labeling of CD10, CD3 or CD79a was identified.

Immunohistochemical staining for TdT is an excellent marker of immature lymphoid precursors, being both sensitive and specific.<sup>1,2</sup> Studies have characterized TdT<sup>+</sup>

**Table 2.** Summary of results of TdT staining.

Site	TdT <sup>+</sup> cells/hpf
Spleen (n=26)	3.1 (0-13)
Appendix (n=10)	12.6 (0-96)
Branchial cleft cyst (n=6)	1* (0-6)
Tonsils (Strauchen and Miller, n=15) <sup>1</sup>	90.4 (8-223)
Lymph node (Strauchen and Miller, n=6) <sup>1</sup>	5.3 (1-13)
Lymph node, pediatric (Onciu <i>et al</i> , n=26) <sup>2</sup>	31.8 (1-180)

\*The majority of cases were negative (5/6).

cells in the bone marrow, thymus, and more recently in tonsil and reactive lymph nodes.<sup>1-5</sup> Our study shows that these cells are present, albeit in small numbers, in spleen, appendices and branchial cleft cysts.

Meru *et al.* suggested that increased numbers of precursors seen in tonsils are a result of higher antigenic stimulation.<sup>5</sup> We consider that appendix would have high antigenic stimulation and increased TdT<sup>+</sup> cells for similar reasons.

TdT<sup>+</sup> immature B cells are a normal component of fetal spleen.<sup>6</sup> Cattoretti *et al.* suggested that immature B cells seen in spleen are not resident in the spleen but are deposited by circulation. The results of our dual stains indicated that the few successfully stained cells were probably B-cell precursors (e.g. CD10<sup>+</sup>/TdT<sup>+</sup> or CD79a<sup>+</sup>/TdT<sup>+</sup>, with no CD3<sup>+</sup>/TdT<sup>+</sup>). The concentration of these cells in the white pulp of the spleen, with only rare cells in the red pulp, supports the idea that these cells circulate, as suggested by Cattoretti.<sup>6</sup> Their low numbers, with increases in reactive conditions, suggest that they are capable of increasing with immune stimulation.

The presence of TdT<sup>+</sup> cells in spleen, appendix and branchial cleft cysts in low numbers is not surprising in the light of finding these cells in other sites, such as ton-

sil and lymph node. It is likely that there will be small numbers of TdT<sup>+</sup> cells in any lymphoid organ or site of lymphoid proliferation, and these cells will probably be more numerous in pediatric patients than in adult ones.

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