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Childhood florid follicular hyperplasia with immunoglobulin light-chain restriction in the gastrointestinal tract.

Running title: Light-chain restriction in follicular hyperplasia

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AML designed research, performed research, analyzed the data and wrote the paper. SMM designed research, performed research and analyzed the data, RR performed research, JLAM performed research, FM performed research, SGV performed research, AB performed research, TMG performed research, analyzed data and contributed essential reagents, MAP designed research, performed research, analyzed the data and wrote the paper.

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Abstract

Aims: Immunoglobulin light-chain expression is used routinely as an indirect marker of clonality for recognizing B cell lymphoproliferative disorders.

Methods and results: Here we describe four floral follicular hyperplasia cases in the gastrointestinal tract (appendix and rectum) of children (4 to 6 years). Immunohistochemical studies revealed lambda light-chain restriction that was associated with polyclonal IgH pattern. Clinical features and follow-up of the patients did not reveal any other systemic symptoms, laboratory abnormalities or organ alterations.

Conclusions: Recognition of this phenomenon is useful in the diagnosis of nodular lymphoid hyperplasia of the gastrointestinal tract, for avoiding overdiagnosis of lymphoid malignancies, and raises concerns that the identification of light-chain restriction is not necessarily a marker of monoclonality.

Key words

light-chain restriction, reactive, follicular hyperplasia, lambda-chain restriction, children, gastrointestinal tract, clonality, monotypia.

Introduction

Diagnosis of lymphoproliferative disorders is based on the integration of clinical data from patients with the morphological and molecular features of their lesions, an approach that is routinely adopted to differentiate malignant and benign lesions, and then for accurately recognizing the lesion. It is often difficult to distinguish between a reactive process and a malignant lymphoproliferative disorder, and many immunohistochemical and molecular tests

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have been developed to address this. Particular relevance is ascribed to the recognition of monoclonality as a marker suggesting malignancy^{1,2}. In this context, light-chain restriction has been considered to be a surrogate of monoclonality and is routinely used in the diagnosis of lymphoid malignancy. Thus, the demonstration of monotypic (kappa or lambda) B cells is a routine test in the diagnosis of lymphoid and plasma cell disorders, and, in the vast majority (90%) of mature B cell malignancies, single immunoglobulin (Ig) light-chain expression indicates the clonal origin of the malignancy³.

In spite of this, monotypic or monoclonal B cells have been described in reactive hyperplastic tonsils and rare lymphadenitis, where marginal zone B cells and isolated follicles may display light-chain restriction^{4,5}.

Here, we describe four childhood cases (two appendixes and two rectal polyps) in which prominent populations of monotypic B cells are present in the context of a florid follicular lymphoid hyperplasia, with features mimicking the morphology and phenotype of pediatric lymphoma.

The differences between this lesion and follicular pediatric lymphoma are discussed.

Material and Methods

Case selection

Four cases with lymphoid hyperplasia, studied between 2009 and 2013, were retrieved from the consultation archives of the Hospital Universitario Marques de Valdecilla, Santander, Spain. They were studied by morphological, immunohistochemical and molecular methods. Approval and/or informed consent were not required for the study.

Hematoxylin-eosin (HE) and immunohistochemistry slides were examined under an Olympus Bx41 microscope (Olympus Europe GmbH). Images were photographed using an Olympus DP70 camera device (Olympus Europe GmbH) and DP controller (Olympus Europe GmbH).

Immunohistochemistry

Paraffin sections (3- μ m thick) were immunostained using the Envision method (Dako, Glostrup, Denmark) and diaminobenzidine (DAB) chromogen following a heat-induced antigen retrieval step and avidin-biotin peroxidase detection on an automated immunostainer (Dako, Glostrup, Denmark). A panel of antibodies (all ready-to-use) against human immunoglobulin K (polyclonal rabbit) and L (polyclonal rabbit) light chains, CD20 (L26), CD3 (polyclonal rabbit), CD10 (56C6), CD5 (4C7), BCL2 (124), BCL6 (PG-B6p), CD23 (SP23), Ki67 (MIB-1), Ig A (polyclonal rabbit) and Cyclin D1 (EP12) was used.

Immunoglobulin gene rearrangement

Polymerase chain reaction (PCR) analysis of the rearranged Ig H genes DNA samples extracted from formalin-fixed, paraffin-embedded tissues was carried out. Sections of 5 μ m thickness were cut from each paraffin block, deparaffinized and digested in 80 μ l solution containing 50 mmol TRIS-HCl, pH 8 and 2.5 μ l of proteinase K (Qiagen, Crawley, UK) at 55 C overnight. PCR for IgH clonality detection (FR1-JH, FR2-JH and FR3-JH) was performed using commercial BIOMED-2 multiplex PCR master mixes and control primers sets (In Vivo-Scribe Technologies, San Diego, CA, USA) following conventional procedures ³. Results were analyzed with ABI Genescan (ABI 3100 Sequencer) and interpreted according to recently published guidelines ⁶.

FISH analysis

Interphase FISH analysis for the detection of translocations affecting BCL2 and BCL6 (LSI dual-color break-apart probes, Abbott Molecular, Illinois, USA) were performed on FFPE sections from whole-tissue sections.

EBV hybridization

EBV by chromogenic *in situ* hybridization (EBER) was performed following conventional procedures (Dako, Glostrup, Denmark).

ISH kappa /lambda

Kappa and Lambda were also performed by *in situ* hybridization (ISH) conventional procedures (Ventana, Tucson, Arizona).

Results

Clinical features

The clinical features of these patients at diagnosis and the follow-up are summarized in Table 1. All four patients were children, between 4 and 6 years of age, and none of them had a previous history of known immunodeficiency or any other related condition.

Cecal appendixes were extirpated in the context of a clinical suspicion of acute appendicitis, while rectal polyps were discovered in the course of a clinical exploration for identifying the causes of unexplained gastrointestinal bleeding.

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Patient N1 was a 4-year-old boy with an elevated value of antinuclear antibody (ANA) (1/160) and no other personal or familial data of interest. After an episode of 24 h of abdominal pain with leukocytosis, he was diagnosed with acute appendicitis and an appendectomy was performed.

Patient N2 was a 4-year-old boy, also without personal or familial history of interest and positive for a culture for *Haemophilus influenzae* (6.8 mg/ml), who presented with abdominal pain of 30 h evolution. He was diagnosed with acute appendicitis, and an appendectomy was subsequently performed.

Patient N3 was a 6-year-old girl who was studied because of previous episodes of upper gastrointestinal bleeding and rectorrhagia. A polypoid lesion in the rectum was discovered and subsequently excised.

Patient N4 was a 5-year-old boy who was studied after an episode of digestive hemorrhage. A rectal polypoid lesion was excised.

Immunosuppression was not found in any of the cases and IgA levels were within the normal range in all cases.

None of the patients showed any clinical evidence of autoimmune disorder.

Histopathological findings

The histopathological findings are summarized in Figure 1, 2, 3 and 4.

In the first two cases, the wall of the appendix was markedly thickened, with expansion of the mucosal and submucosal layers because of a conspicuous hyperplasia of lymphoid tissue. The first patient also had an acute inflammatory infiltrate that reached the periappendicular adipose tissue. At low magnification, both lesions were characterized by large, expanded,

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well-delimited follicles with floral shape. At high magnification, germinal centers exhibited typical polarization, with centroblasts segregated to one pole of the follicle. There were also visible macrophages with tingible bodies that conferred a starry-sky pattern. Of particular note were an attenuated mantle zone and a hyperplastic interfollicular B cell component. Numerous aggregates of lymphoid cells were also present in the appendiceal lumen.

With respect to the rectal lesions, the microscopy findings were similar to those previously described as nodular lymphoid hyperplasia of the intestinal tract in infancy and childhood, with a striking follicular hyperplasia-forming tumor that expanded the submucosa without infiltrating the mucosa. Lesions were composed of expanded secondary follicles with floral germinal centers, without atypical cells, granulomas or an acute inflammatory component. Lymphoepithelial lesions were not observed.

An immunohistochemical study was performed in all cases and showed no abnormalities of the architecture or phenotype, other than lambda light-chain restriction by the germinal center and interfollicular B-cells, this phenomenon being observable in most of lymphoid follicles. There were appropriate distributions of CD20+, Pax5+ B cells in follicles with mostly CD3+ T cells in the interfollicular regions. In all cases, BCL2 was expressed by mantle zone B cells and the T cells, but not in the germinal centers. BCL6 and CD10 expression was restricted to the germinal centers, with no interfollicular population of BCL6+ or CD10+ lymphocytes, highlighting the irregular and floral shape of the follicles. CD23 showed follicular dendritic meshworks of irregular shape, formed by the invagination of the mantle zone cells. Only rare IgA-positive plasma cells were found. Ki67 showed proliferating cells within the germinal centers and stained few nuclei outside. Cyclin D1 and MUM1 immunostaining revealed no evidence of abnormal cell populations.

Appropriate internal and external controls for kappa and lambda staining were used. Patient N3 was also studied with ISH for kappa and lambda, and the results nicely confirmed lambda light-chain restriction (Figure 4)

There was no clinical or histological evidence of past or present EBV infection, and *in situ* hybridization studies for EBV gave negative results in all cases.

The IgH polymerase chain reaction (PCR) was performed. This revealed polyclonal rearrangements in three cases (Figure 5). The second case yielded no useful results because of poor DNA quality. FISH analysis to identify translocations in BCL2 and BCL6 loci gave negative results in all cases.

Patients were followed up for periods between 3 and 54 months, with no evidence of lymphoproliferative disorder being observed in any of them.

Discussion

We describe four similar lesions, arising in the cecal appendix or in the rectum, affecting similarly aged children, characterized by a submucosal expansion of hyperplastic lymphoid tissue with prominent follicles of a floral shape, in which immunohistochemical study has demonstrated lambda light-chain restriction by the reactive germinal center and interfollicular B cells, with PCR identifying a polyclonal heavy chain immunoglobulin gene. The floral morphology of these follicles and the starry-sky pattern are similar to those described in pediatric follicular lymphoma. Nevertheless, these four cases featured polarized germinal centers with macrophages and a proper mixture of centrocytes and centroblasts without the presence of atypical monotonous blastoid cells. We also recognized an attenuated mantle cuff in our cases, and a hyperplastic interfollicular B-cell component that was more apparent after immunostaining.

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The clinical presentation and follow-up, together with the previously described morphological features, are indicative of the benign nature of these lesions, which are more closely related to follicular hyperplasia than to a true malignant lymphoma. This is also supported by the polyclonal PCR results and by the failed of FISH to detect any genetic abnormalities. The light-chain restriction by the germinal center cells is an unexpected finding and has no clear explanation.

Lymphoid hyperplasia in the gastrointestinal tract is relatively common in the first decade of life ^{7,8}. Lymphoid tissue is usually present in the appendix and reaches its maximum stage of development in childhood and adolescence. Hyperplasia of the colonic lymphoid tissue is usually greater in the colon and rectum and is much more prominent in children than in adults. When they enlarge and become polypoid, these lesions are considered benign lymphoid polyps or rectal tonsils, by analogy with the lymphoid tissue of the tonsil. Although it has been reported related to immunodeficiency states, inflammatory bowel disease and Hirschsprung's disease, in most cases it is considered merely to represent a normal response of colonic lymphoid tissue to antigenic stimulation ^{9,10}. To our knowledge, the presence of light-chain restriction in the lymphoid follicles of these lesions has not been described before ¹¹.

Monotypic cells in the context of follicular hyperplasia in healthy patients have been previously reported ^{12,13,14}. All these cases were described in lymph node, and most were found amongst the adult population. A large majority of these cases were incidental findings, with the follow-up not disclosing new lesions or increased risk of lymphoma development, with a few exceptions. Although most of the studies have used immunohistochemistry, some have been done using flow cytometry, giving similar results ¹⁵, thereby confirming that this is not a technical artifact.

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The presence of light-chain restriction in extranodal lymphoid hyperplastic tissue was first reported in 2004 by Attygalle et al ⁴, who described the presence of atypical marginal zone hyperplasia with light-chain restriction in tonsils and appendices. In all cases, molecular studies revealed polyclonality, affecting children aged between 3 and 11 years, who had not received any therapy and were alive and well after a median follow-up of 35.3 months. Interestingly, both cases described by Attygalle and these cases exhibit lambda-chain restriction.

These lesions bear a striking similarity to pediatric follicular lymphoma, and could provide some basis for the interpretation that some cases of pediatric follicular lymphoma correspond to atypical lymphoid hyperplasia with light-chain restriction. Nevertheless, we cannot rule out the possibility that these lesions are manifestations of early lymphoma cases, and that follow-up of a larger series could reveal an increased risk of lymphoma development.

Interestingly, all the four cases exhibited lambda light-chain restriction. Kappa and lambda are usually distributed in a 2:1 ratio. Abnormal kappa:lambda (K:L) ratios have been reported in association with infections, autoimmune disorders and immunodeficiency ^{16,17}. Only one of our patients had a positive test for an infectious microorganism and none showed kappa-chain deficiency. It is possible that the predominance of lambda light chain is associated with an immature immune system in which an anomalous K:L ratio may persists ^{18,19}.

Our findings indicate that Immunoglobulin light-chain restriction, although of recognized value in the diagnosis of malignant lymphoproliferative lesions, may definitely be present in follicular hyperplasia, making it more difficult to distinguish it from pediatric follicular lymphoma ^{20,21,22,23,24}. This raises some concerns about the use of light-chain immunohistochemistry in the

diagnosis of lymphoid neoplasms, and suggests that more sensitive techniques could reveal physiological monotypic subpopulations that should not be considered malignant.

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References

1. Collins RD. Is clonality equivalent to malignancy: specifically, is immunoglobulin gene rearrangement diagnostic of malignant lymphoma? *Hum Pathol.* 1997; 28: 757-759.
2. Kroft, S. H. Monoclonones, monotypes, and neoplasia pitfalls in lymphoma diagnosis. *Am J Clin Pathol.* 2004; 121(4): 457-459.
3. van Dongen JJ, Langerak AW, Brüggermann et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia.* 2003; 17(12): 2257-2317.

- Accepted Article
4. Attygalle AD, Liu H, Shirali S et al. Atypical marginal zone hyperplasia of mucosa-associated lymphoid tissue: a reactive condition of childhood showing immunoglobulin lambda light-chain restriction. *Blood*. 2004; 104(10): 3343-3348.
 5. Nam-Cha SH, San Millán B, Mollejo M et al. Light-chain-restricted germinal centres in reactive lymphadenitis: report of eight cases. *Histopathology*. 2008; 52(4): 436-444.
 6. Langerak AW, Groenen PJ, Brüggemann M et al. EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. *Leukemia*. 2012; 26(10): 2159-71.
 7. Smith T A. Lymphoid hyperplasia of the appendix in children; its relation to recurrent appendicitis. *Ann Surg*. 1924; 79(6): 871-878.
 8. Farris AB, Lauwers GY, Ferry JA et al. The rectal tonsil: a reactive lymphoid proliferation that may mimic lymphoma. *Am J Surg Pathol*. 2008; 32(7): 1075-1079.
 9. Kaplan B, Benson J, Rothstein F et al. Lymphonodular hyperplasia of the colon as a pathologic finding in children with lower gastrointestinal bleeding. *J Pediatr Gastroenterol Nutr*. 1984; 3(5): 704-708.

- Accepted Article
10. Kokkonen J and Karttunen TJ. Lymphonodular hyperplasia on the mucosa of the lower gastrointestinal tract in children: an indication of enhanced immune response? *J Pediatr Gastroenterol Nutr.* 2002; 34(1): 42-46.
 11. Kojima M, Nakamura N, Itoh H et al. Histological variety of localized lymphoid hyperplasia of large intestine: histopathological, immunohistochemical and genotypic findings of 16 cases. *J Clin Exp Hematopathol.* 2009; 49 (1): 15-21.
 12. Palutke M, Schnitzer B, Mirchandani I et al. Increased numbers of lymphocytes with single class surface immunoglobulins in reactive hyperplasia of lymphoid tissue. *Am J Clin Pathol.* 1982; 78(3): 316-323.
 13. Levy N, Nelson J, Meyer P et al. Reactive lymphoid hyperplasia with single class (monoclonal) surface immunoglobulin. *Am J Clin Pathol.* 1983; 80(3): 300-308.
 14. Bain VG and Bain GO. Lymphocyte populations with abnormal kappa:lambda ratios in reactive lymphoid hyperplasia. *J Surg Oncol.* 1985; 29(4): 227-230.
 15. Kussick SJ, Kalnoski M, Brazier RM et al. Prominent clonal B-cell populations identified by flow cytometry in histologically reactive lymphoid proliferations. *Am J Clin Pathol.* 2004; 121(4): 464-472.

16. Bernier GM, Gunderman JR, Ruymann FB. Kappa-chain deficiency. *Blood*. 1972; 40(6): 795-805.

17. Barandun S, Morell A, Skvaril F et al. Deficiency of kappa- or lambda-type immunoglobulins. *Blood*. 1976; 47(1): 79-89.

18. Renckens ALJM, Jansen MJH, Van Munster PJJ et al. Nephelometry of the kappa/lambda light-chain ratio in serum of normal and diseased children. *Clin Chem*. 1986; 32(12): 2147-2149.

19. Herkner KR, Salzer H, Böck A et al. Pediatric and perinatal reference intervals for immunoglobulin light chains kappa and lambda. *Clin Chem*. 1992; 38(4): 548-550.

20. Lorscheich RB, Shay-Seymore D, Moore J, et al. Clinicopathologic analysis of follicular lymphoma occurring in children. *Blood*. 2002; 99(6): 1959-1964.

21. Swerdlow SH. Pediatric follicular lymphomas, marginal zone lymphomas, and marginal zone hyperplasia. *Am J Clin Pathol*. 2004; 122 Suppl: S98-109.

22. Campo E, Swerdlow SH, Harris NL et al. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. *Blood*. 2011; 117(19): 5019-5032.

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23. Louissaint A Jr, Ackerman AA, Dias-Santagata D et al. Pediatric-type nodal follicular lymphoma: an indolent clonal proliferation in children and adults with high proliferation index and no BCL2 rearrangement. *Blood*. 2012; 120(12): 2395-2404.

24. Liu Q, Salaverria I, Pittaluga S et al. Follicular lymphomas in children and young adults: a comparison of the pediatric variant with usual follicular lymphoma. *Am J Surg Pathol*. 2013; 37(3): 333-343.

Case no	Age (years)/sex	Prior history	Biopsy site	Histological findings	Light-chain restriction	IgH rearrangement	Follow-up (months)	Current status
1	4/Male	Abdominal pain	Appendix	FH + Acute appendicitis	Lambda	Polyclonal	36	Alive and well
2	4/Male	Abdominal pain	Appendix	FH	Lambda	NE	54	Alive and well
3	6/Female	Rectal bleeding	Rectum	Polypoid lesion with FH	Lambda	Polyclonal	6	Alive and well
4	5/Male	Rectal bleeding	Rectum	Polypoid lesion with FH	Lambda	Polyclonal	3	Alive and well

Table 1

Figure Legends

Figure 1. Histological and immunophenotypic features of florid follicular hyperplasia, patient N 1. Architecture of the appendix with a marked expansion of the submucosa by prominent lymphoid tissue (A). Follicular aggregates are composed of CD20-positive B cells (B). CD3 stains T cells surrounding the follicles (C). Hyperplastic lymphoid follicles with prominent germinal centers showing a floral morphology (D). High magnification of germinal centers (G). Germinal centers are positive for CD10 and BCL6 (E, F) and negative for BCL2 (I). Ki67 staining with high proliferation in germinal centers (H).

Figure 2. Histological and immunophenotypic features of rectal polyp with florid follicular hyperplasia, patient N3. Architecture of the polypoid lesion with a marked expansion of the submucosa by prominent lymphoid follicles (A, D), with a floral shape as highlighted by CD20 (B) and CD3 (C) staining. CD10 and BCL6 staining delineates the shape of the reactive germinal centers (E, F), which are negative for BCL2 (I). Ki67 staining in germinal centers (H).

Figure 3. Hyperplastic follicles showing light-chain restriction for lambda (B, F) compared with kappa (A, E) in both patients N3 and N4. High magnification of kappa (C, G) and lambda (D, H) staining.

Figure 4. Patient N3. Lambda chain restriction confirmed by ISH probes for both Kappa and Lambda (A). High magnification of predominance of lambda probe in a germinal center (C). External control: Detail of lambda and kappa ISH in a tonsil sample (B, D).

Figure 5. PCR analysis of the rearranged Ig H genes, patient N3. Polyclonal rearrangements in IgH F1 (A), IgH F2 (B) and IgH F3 (C).

Table 1: Summary of clinical features and follow up. FH: Follicular hyperplasia. NE: not evaluable.

FIGURE 1.

FIGURE 2.

FIGURE 3.

FIGURE 4.

FIGURE 5.