Original contribution

Lymphoid follicle colonization by Bcl-2^{bright+}CD10⁺ B-cells (“follicular lymphoma in situ”) at nodal and extranodal sites can be a manifestation of follicular homing of lymphoma[☆]

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Summary Follicular lymphoma (FL) in situ (FLIS) was first described and proposed as a distinct entity associated with an indolent clinical course in 2002. To gain further insight into the biology of this enigmatic lymphoproliferation, we analyzed morphologic, phenotypic, cytogenetic and molecular features of tissue specimens manifesting a pattern of follicular colonization by Bcl-2^{bright+}CD10⁺ B-cells and associated lymphomas from 13 adults and evaluated their clinical outcomes. We observed this immunoarchitectural pattern in lymph nodes (n = 8), at extranodal sites (n = 4), or at both locations (n = 1) at diagnosis. All except 3 cases showed concomitant bright CD10 expression. Six (46%) patients had synchronous and 2 (15%) developed metachronous B-cell lymphomas, with 5 representing high-grade lymphomas. The Bcl-2^{bright+}CD10⁺ B-cells colonizing reactive follicles and synchronous lymphomas were clonally related in 4/5 (80%) cases analyzed and 5/6 (83%) displayed *BCL2* translocations. Two cases exhibited complex karyotypes in both components; a genetic “triple hit” was detected in one instance and 2 copies of t(14;18) were observed in a lymph node biopsy lacking evidence of lymphoma from an individual with stage 4 disease, suspected on imaging, who subsequently displayed a mantle zone/perifollicular infiltrate of Bcl-2^{bright+}CD10⁺ B-cells in the adenoids. Our findings suggest that bright Bcl-2, and often bright CD10 expression, by B-cells colonizing reactive follicles might represent a phenomenon related to follicular homing of lymphoma, rather than being an attribute of preneoplastic FL precursors. Furthermore, due to the relatively high frequency of overt lymphomas observed, complete staging workup is recommended for patients exhibiting a Bcl-2^{bright+}CD10⁺ B-cell follicular colonization pattern on biopsy.

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1. Introduction

Follicular lymphoma (FL) is genetically characterized by the translocation t(14;18)(q32;q21) in the majority of cases [1], with low-grade FLs displaying a higher frequency of this abnormality [2]. The t(14;18)(q32;q21) is thought to arise in bone marrow B-cell precursors due to aberrant *VDJ* recombination leading to deregulated *BCL2* expression [3]. However, cells harboring only this translocation have limited neoplastic potential.

Folliculotropism, with preferential colonization of germinal centers (GCs) is a unique characteristic of FL, rarely seen in other types of lymphomas. However, other patterns of lymphoid tissue infiltration by FL, eg, interfollicular and diffuse have been described, which can be associated with distinct immunophenotypic features of the neoplastic cells, disease grade and underlying cytogenetic aberrations [1,4]. Moreover, lymph node involvement by FL may be subtle at times, only involving a small portion of the lymph node or a few follicles. In 2002, a novel morphologic and phenotypic pattern of FL was identified, characterized by architecturally intact lymph nodes displaying follicular colonization by Bcl-2^{bright+}CD10⁺ B-cells, referred to as “in-situ localization of follicular lymphoma” or “follicular lymphoma in-situ” (FLIS) [5]. In 40% to 60% of these cases, no evidence of concomitant overt lymphoma was discerned, either at presentation and/or on follow-up [5]. Hence, FLIS was suggested to represent an early stage in FL pathogenesis, associated with indolent clinical behavior [5]. Since the original publication, a few case reports and case series describing this entity and its associated clinical manifestations have been published [6–12].

The aim of our study was to gain further insight into the biologic and clinical significance of the immunoarchitectural pattern referred to as FLIS that we define as the “Bcl-2^{bright+}CD10⁺ B-cell follicular colonization pattern” diagnosed at our institution over the past 10 years. We determined the presence and type of concurrent or subsequent lymphomas occurring in individuals exhibiting this pattern and assessed the cytogenetic, molecular and clinical characteristics of such cases.

2. Materials and methods

2.1. Case selection

We retrospectively searched our departmental database of FL cases from January 2002 to June 2012 to identify biopsies or surgical resection specimens of lymph nodes or extranodal tissue that were coded to document the presence of reactive follicles colonized by Bcl-2^{bright+}CD10⁺ B-cells, as described by Cong et al [5]. Data regarding patient demographics, type of associated or subsequent lymphoma, results of imaging studies, therapeutic regimens and clinical

outcomes were obtained from our laboratory and clinical information systems. This study was approved by our institutional review board.

2.2. Morphology and immunohistochemistry

Morphologic features were evaluated by reviewing hematoxylin and eosin (H&E)–stained sections of formalin-fixed, paraffin-embedded (FFPE) tissue. Immunohistochemical staining was performed using a panel of antibodies. Staining for CD20 (clone MJ1), CD3 (clone LN10), Bcl-6 (clone LN22), Bcl-2 (clone BCL-2/100/D) (all Novocastra, Buffalo Grove, Illinois), MUM1 (clone MUM1p) (Dako, Carpinteria, CA), LMO2 (clone SP51), OCT2 (clone MRQ-2), and BOB1 (clone SP92) (all Cell Marque, Rocklin, CA) was performed after antigen retrieval at pH 9.0 for 20 min, using the Leica Bond III autostainer (Leica, Wetzlar, Germany). Staining for CD10 (clone SP67), CD21 (clone EP3093), and Ki-67 (clone 30–9) (all Ventana Medical Systems, Tucson, AZ) was performed after antigen retrieval at pH 8.3 for 30 minutes, using the Ventana Ultra platform (Ventana Medical Systems, Tucson, AZ). The Bond Polymer Refine Detection (Leica) or the Ultra View Universal DAB Detection Kit (Ventana) was used for visualization, with diaminobenzidine as the chromogen.

2.3. Cytogenetic analysis

G-band karyotype analysis was performed on metaphase preparations after unstimulated overnight cultures, and karyotypes were described according to the International System for Human Cytogenetic Nomenclature [13].

Fluorescence in situ hybridization (FISH) analysis was performed on metaphase spreads and/or FFPE sections using *IGH/BCL2* dual color fusion and/or *BCL2* break-apart probes (Abbott Molecular, Des Plaines, IL), per standard protocols. FISH analysis for *BCL6*, and *MYC* (Abbott Molecular) was performed as applicable. Fluorescence signals were analyzed using the Cytovision Imaging system attached to a Nikon Eclipse 600 microscope (Applied Imaging, Santa Clara, CA), and 200–500 cells were scored for each hybridization. To evaluate tissue localization of FISH signals, FFPE sections from 9 cases containing only reactive follicles manifesting the Bcl-2^{bright+}CD10⁺ B-cell colonization pattern were selected, and coordinates of abnormal follicles targeted for FISH signal detection were marked on the underside of the slides after reviewing corresponding serial H&E stained sections.

2.4. Immunoglobulin heavy chain gene rearrangement analysis

Polymerase chain reaction (PCR) to ascertain clonal immunoglobulin heavy chain gene (*IGH*) rearrangements was performed on DNA extracted from fresh or FFPE tissue

using the BIOMED-2 primers, as described [14] followed by high resolution capillary electrophoresis using a 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA).

3. Results

3.1. Case characteristics

Of the 302 patients diagnosed with FL at our institution during the study interval (208 lymph nodes, 94 extranodal sites), we encountered 13 patients that exhibited a distinct pattern of follicular colonization by Bcl-2^{bright+}CD10⁺ B-cells. This pattern was detected at presentation in lymph nodes (n = 8), at extranodal sites (n = 4) or involving both (n = 1) (Table 1); representing 9/208 (4.3%) of nodal and 5/94 (5.3%) of extranodal cases. Extranodal sites at initial presentation included thyroid (n = 1), duodenum (n = 1), jejunum (n = 1), ileum (n = 1), and subcutaneous tissue (n = 1); one patient developed a recurrent lesion in the adenoids.

Follicular colonization by Bcl-2^{bright+}CD10⁺ B-cells in lymph nodes was observed as an isolated finding at presentation in 5 patients (cases 1–5, Table 1); synchronous lymphomas were diagnosed in 6 patients (cases 6–11, Table 1), comprising low-grade FL (n = 2), diffuse large B-cell lymphoma (DLBCL) (n = 2) and “B-cell lymphoma, unclassifiable (BCL-U), with features intermediate between DLBCL and Burkitt lymphoma” (n = 2). Metachronous lymphomas were documented in 2 patients (cases 12 and 13, Table 1), including low-grade FL (n = 2) and DLBCL (n = 1).

3.2. Morphology and phenotype

Lymphoid follicles exhibiting the Bcl-2^{bright+}CD10⁺ B-cell colonization pattern had GCs that lacked polarization, displayed a crowded appearance, due to a predominance of centrocytes, and were surrounded by intact mantle zones. Rare, residual reactive GC components were nevertheless noted, including occasional centroblasts (12/13, 92%), tingible-body macrophages (11/13, 85%), and mitotic figures (5/13, 38%). The paracortical architecture was unaltered in all nodal cases (9/9, 100%).

Ten of 13 (77%) primary cases and the recurrent lesion showed concomitant bright CD10 expression (Table 1), while the intensity of Bcl-6 (n = 13) and LMO2, OCT2 and BOB1 (n = 8) was not brighter than adjacent reactive follicles. The percentage of secondary follicles displaying colonization by Bcl-2^{bright+}CD10⁺ B-cells (determined by Bcl-6, CD10 and Bcl-2 staining) ranged from 18% to 100% (median 79%); 9/13 (69%) cases demonstrated >50% follicular involvement (Table 1). The density of follicular colonization was variable within and between cases; representative examples of near complete replacement and mild infiltration of GCs are shown in Fig 1A–C and D–F, respectively. Of note, the recurrence involving the adenoids

(case 3b) displayed a focal mantle zone/perifollicular pattern of infiltration (Fig 1G, H) despite exhibiting a GC-limited pattern in the prior lymph node biopsy (case 3a). No interfollicular Bcl-2^{bright+}CD10⁺ cellular infiltrate was noted in any case. Staining for Ki-67 revealed a lower proliferation index in colonized GCs (range 5%–25%) compared to uninvolved reactive GCs (40%–50%).

The histologic subtypes of synchronous (n = 6) and metachronous (n = 3) lymphomas are listed in Table 1. In brief, all FL were low grade, all DLBCL had centroblastic morphology, and the two BCL-U exhibited blastoid morphology. All lymphomas displayed a GC phenotype (Bcl-6⁺, CD10⁺, MUM1[−]), and the BCL-U had markedly elevated Ki-67 proliferation indices (70% and 90%). The Bcl-2^{bright+}CD10⁺ B-cell follicular colonization pattern was noted in lymph nodes involved by lymphoma, in adjacent or regional uninvolved lymph nodes or at mucosal sites at a distance from a primary intestinal (or non-intestinal) lymphoma; some cases exhibited this pattern at multiple sites (Table 1). Representative examples of the different types of lymphomas and sites of lymphoid follicles manifesting the Bcl-2^{bright+}CD10⁺ B-cell colonization pattern are illustrated in Figs. 2–4.

3.3. Cytogenetic analysis

The results of cytogenetic analysis are presented in Table 2.

3.3.1. Follicles exhibiting colonization by Bcl-2^{bright+}CD10⁺ B-cells

Karyotype analysis performed in 4 primary cases exhibiting the Bcl-2^{bright+}CD10⁺ B-cell follicular colonization pattern (cases 3a, 5, 10, and 12) displayed a normal karyotype (case 5), complex karyotype (cases 3a and 10, Fig. 5) or a non-clonal karyotype (case 12). Both cases with a complex karyotype demonstrated t(14;18)(q32;q21); one exhibited 2 copies of the translocation (case 3a) and the other an additional translocation t(3;8)(q27;q24) representing a potential genetic “triple-hit” (case 10). Metaphase FISH detected *IGH/BCL2* rearrangements in 3 of 4 cases (cases 3a, 10, and 12). The adenoid sample (case 3b) revealed a normal karyotype, and no *IGH/BCL2* rearrangement was detected by FISH (due to lack of lesional tissue).

Interphase FISH analysis, performed on FFPE sections in 9 cases (cases 1, 2, 4, 6, 7, 8, 9, 10, and 11), detected *BCL2* rearrangements in 5 (56%) samples (cases 6, 8, 9, 10, and 11), 2 (22%) lacked rearrangements (cases 4 and 7), and 2 (22%) had inconclusive results due to hybridization failure (cases 1 and 2). FISH for *BCL6* and *MYC* confirmed *BCL6* and *MYC* rearrangements and verified their localization to the colonized follicles in the lymph node lacking BCL-U (case 10).

In summary, FISH analysis (metaphase and/or interphase) confirmed the presence of *BCL2* rearrangements within GCs of colonized follicles in 7 of 10 (70%) informative cases (cases 3a, 6, 8, 9, 10, 11, 12).

Table 1 Clinical and pathologic characteristics of cases

Case	Clinical features			Bcl-2 ^{bright+} CD10 ⁺ follicles			Lymphoma					ECOG Score	Ann Arbor Stage	BM	Therapy	Outcome
	Age	Sex	Symptoms/biopsy indication	Site	n/total (%)	CD10	Sync	Meta	Site	Type	Time to NHL					
1	56	F	Cholelithiasis	Cystic Duct LN	72/73 (99%)	Bright	No	No	N/A	N/A	NA	0	UNK	No	None	Alive, NED (61 mo)
2	78	M	Skin Lesion	Skin	3/17 (18%)	Bright	No	UNK	N/A	N/A	NA	UNK	UNK	ND	UNK	LTF
3a	60	F	Lymphadenopathy, parotid mass	Cervical LN	75/95 (79%)	Bright	No*	No	N/A	N/A	NA	0	4	ND	Rituximab	Alive, AWD (96 mo)
3b			Breathing difficulty	Adenoids	2/15 (13%)	Bright	No	No**	N/A	N/A	NA	NA	4	ND	None	
4	49	F	Thyroid nodule	Thyroid	6/20 (20%)	Bright	No	UNK	N/A	N/A	NA	UNK	1	ND	UNK	LTF
5	54	M	Incidental, radiographic imaging	Mediastinal LN	9/22 (41%)	Bright	No	No	N/A	N/A	NA	1	1A	ND	None	Alive, NED (50 mo)
6	65	M	Axillary lymphadenopathy	Axillary LN	9/13 (69%)	Bright	Yes	No	Axillary LN	Low-grade FL	NA	0	1A	ND	None	Alive, NED (18 mo)
7	43	F	Pelvic pain	Ileum	14/14 (100%)	Normal	Yes	No	Ileum	Low-grade FL	NA	0	2A	ND	Rituximab	Alive, NED (13 mo)
8	59	F	Abdominal pain	Mesenteric LN & Jejunum	65/65 (100%)	Bright	Yes	No	Mesenteric mass, jejunal involvement	DLBCL	NA	1	1A	ND	R-CHOP (3 Cycles)	LTF
9	58	F	Hematuria, radiographic imaging	Duodenum	1/4 (25%)	Bright	Yes	No	Duodenum	DLBCL	NA	2	1A	ND	R-CHOP (4 Cycles) Local Radiation	Partial Response, DOD (7 mo)
10	50	M	Abdominal pain	Cervical LN	38/54 (75%)	Normal	Yes	No	Cervical LN	BCL-U	NA	0	4A	Yes	R-CEOP (1 Cycle) CODOX-M/IVAC (2 Cycles)	Relapse, DOD (8 mo)
11	76	F	Pancreatic mass	Peripancreatic LN	20/21 (95%)	Normal	Yes	No	Pancreas, duodenum, stomach	BCL-U	NA	1	1E	ND	R-CHOP (1 Cycle)	AWD (9 mo)
12	69	F	Incidental radiographic imaging	Para-aortic LN	39/45 (87%)	Bright	No	Yes	Paraaortic LN	Low-grade FL	5.3 years	1	3A	ND	Rituximab + Bendamustine for 0.5 years	Alive, NED (71 mo)
13	58	F	Unknown	Postauricular LN	12/14 (86%)	Bright	No	Yes	A. Inguinal LN B. Arm subcutis	A. Low-grade FL B. DLBCL	A. 9.6 years B. 10.1 years	0	1E	ND	Local Radiation	DOD (122 mo)

Abbreviations: M, male; F, female; LN, lymph node; BM, bone marrow involvement; NA, not applicable; Sync, synchronous; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CEOP, rituximab plus cyclophosphamide, etoposide, vincristine, and prednisone; CODOX-M/IVAC, cyclophosphamide, vincristine, doxorubicin, and methotrexatek/ifosfamide, etoposide and cytarabine; Meta, metachronous; normal, similar staining intensity as reactive germinal centers; UNK, unknown; ND, not done; LTF, lost to follow-up; NED, no evidence of disease; AWD, alive with disease; DOD, dead of disease; *, FL suspected on imaging; **, mantle zone colonization pattern observed.

Fig. 1 Reactive lymphoid follicles can show variable degrees and patterns of colonization by Bcl-2^{bright+}CD10⁺ B-cells. A-C, Lymphoid follicle (case 12) demonstrating complete colonization of GC by centrocytic Bcl-2^{bright+}CD10^{bright+} B-cells. (A: H&E, 100×; B: CD10, 100×; C: Bcl-2, 100×). D-F, Follicle (case 12) exhibiting a milder GC infiltrate of centrocytic Bcl-2^{bright+}CD10^{bright+} B-cells (D: H&E, 100×; E: CD10, 100×; F: Bcl-2, 100×). G-I, Adenoid biopsy (case 3b) showing a lymphoid follicle with focal mantle-zone and perifollicular infiltrate of centrocytic Bcl-2^{bright+}CD10^{bright+} B-cells. (G: H&E, 40×; H: CD10, 40×; I: Bcl-2, 40×).

3.3.2. Synchronous and metachronous lymphomas

Karyotype analysis performed in 3 of 6 synchronous lymphomas (cases 7, 8, and 10) detected t(14;18)(q32;q21) in two cases (cases 7 and 10); one (case 8) was a failure. Case 10 also demonstrated t(3;8)(q27;q24). Metaphase FISH confirmed *BCL2* rearrangements in cases 7 and 10, while metaphase FISH for *MYC* and *BCL6*, performed in the latter, confirmed the genetic “triple-hit.”

Interphase FISH performed in 4 cases (cases 6, 8, 9, and 11) detected *BCL2* rearrangements in all (100%). Additionally, interphase FISH performed on a peripheral blood sample from one patient (case 11) demonstrated *BCL2* rearrangement at a very low frequency (8/1608 cells), despite the inability of flow cytometry to detect circulating neoplastic cells (data not shown).

Karyotype analysis of the 2 metachronous lymphomas (cases 12 and 13) revealed t(14;18)(q32;q21) in the FL and DLBCL occurring in one individual (case 13), but failed in one (case 12). Metaphase FISH confirmed the presence of *BCL2* rearrangements in all informative cases.

3.4. Immunoglobulin heavy chain gene rearrangement analysis

The results of PCR analysis for *IGH* gene rearrangement performed using whole tissue FFPE sections from 11 cases are listed in Table 2. Clonal products were detected in 5 of 9 (55%) informative samples only displaying Bcl-2^{bright+}CD10⁺ B-cell follicular colonization (cases 3a, 7, 8, 9, and 11). A clonal relationship between the primary and recurrence, exhibiting only Bcl-2^{bright+}CD10⁺ B-cell follicular colonization (case 3), could not be established due to exhaustion of lesional tissue in the recurrence sample.

Four of 5 (80%) synchronous lymphomas analyzed yielded clonal products (cases 7, 8, 9, 11) of a similar size as the Bcl-2^{bright+}CD10⁺ B-cell colonized follicles.

PCR performed on the peripheral blood of one patient (case 11) detected a similar clonal product as the sample manifesting follicular colonization by Bcl-2^{bright+}CD10⁺ B-cells and the synchronous lymphoma.

Fig. 2 A-D, Mesenteric lymph node biopsy (case 8). A, Low magnification showing scattered, small reactive lymphoid follicles and preserved nodal architecture, B, GC displays predominance of centrocytic cells. C, GCs showing colonization by CD10^{bright+} B-cells. D, GC infiltrate exhibits bright Bcl-2 expression (A: H&E, 20 $\times$; B: H&E, 400 $\times$; C: CD10, 20 $\times$; D: Bcl-2, 20 $\times$). E-H, Intramucosal lymphoid follicles demonstrating GC colonization by Bcl-2^{bright+}CD10^{bright+} B-cells. E, The mantle zone is preserved while the GCs are colonized by centrocytic cells. F, GC exhibiting dense infiltrate of centrocytic cells. G, GC-colonizing B-cells show bright CD10 expression. H, The GC infiltrates also show bright Bcl-2 expression (E: H&E, 20 $\times$; F: H&E, 400 $\times$; G: CD10, 20 $\times$; H: Bcl-2, 20 $\times$). I-L, Mesenteric DLBCL showing infiltration of small bowel wall. I, Large neoplastic lymphocytes infiltrating small bowel submucosa with focal extension into mucosa. J, The neoplastic cells display centroblastic morphology. K, Lymphoma cells lack CD10 expression. L, Lymphoma cells express Bcl-2 (I: H&E, 20 $\times$; J: H&E, 400 $\times$; K: CD10, 400 $\times$; L: Bcl-2, 400 $\times$).

Fig. 3 A-D, Cervical lymph node showing effaced architecture due to an infiltrate of BCL-U (case 10). E-H, Another lymph node from the same region showing reactive follicles, with a few exhibiting GC colonization by Bcl-2^{bright+}CD10⁺ B-cells. A, Diffuse lymph node infiltrate of neoplastic lymphocytes. B, Lymphocytes are medium to large in size and have blastoid morphology. C, The infiltrating lymphocytes express CD10. D, The neoplastic lymphocytes also express Bcl-2. E, A neighboring lymph node shows scattered small reactive lymphoid follicles with preserved mantle zones and intact lymph node architecture. F, Some GCs show dense infiltrates of centrocytic cells. G, Colonized GCs show similar intensity of CD10 expression as the BCL-U. H, GC-infiltrating cells show bright Bcl-2 expression (A, E: H&E, 20×; B, F: H&E, 400×; C: CD10, 40×; D: Bcl-2, 40×; G: CD10, 40×; H: Bcl-2, 40×).

3.5. Clinical characteristics and outcomes

The clinical characteristics, including biopsy indication, therapy and outcomes, are summarized in Table 1. The 13 study patients comprised 4 males and 9 females (age range 43–78 years, median 58 years). Clinical follow-up ranged from 7–122 months (median 18 months); 3 patients were lost to follow-up shortly after biopsy.

Two of 3 patients (cases 1 and 5) lacking biopsy-proven synchronous or metachronous lymphomas, with follow-up information, are alive without therapy and have no evidence of disease to date. Of interest, one patient (case 3), whose lymph node biopsy exhibited follicular colonization by Bcl-2^{bright+}CD10⁺ B-cells and 2 copies of t(14;18) on karyotype analysis, had multiple, deep-seated enlarged lymph nodes on imaging (not biopsied) suggesting stage 4 disease (presumably FL). Radiologic remission was achieved with single agent rituximab; however, adenoidectomy performed 96 months later for difficulty in breathing revealed follicular hyperplasia and a focal mantle-zone/perifollicular infiltrate of Bcl-2^{bright+}CD10^{bright+} B-cells. All overt synchronous lymphomas and one metachronous lymphoma (case 12) were observed in the same region as the tissue or organ displaying Bcl-2^{bright+}CD10⁺ B-cell colonized follicles. Seven of 8 (87%) lymphomas were associated with >50% follicles demonstrating colonization by Bcl-2^{bright+}CD10⁺ B-cells.

All three patients with FL (cases 6, 7, 12, Table 1) are currently alive, while 3 of 4 patients with follow-up information who had or developed aggressive lymphomas died shortly after diagnosis despite immunochemotherapy. One patient with BCL-U (case 11, Table 1) is alive with disease after immunochemotherapy.

4. Discussion

The entity of FLIS was first described by Cong et al to represent the finding of intense Bcl-2 expression by CD10⁺ B-cells infiltrating reactive follicles in lymph nodes with otherwise preserved architecture [5]. The GCs of these follicles had a crowded appearance due to a predominance of centrocytes, often lacking other reactive GC constituents [5]. Montes-Moreno et al, in their series of FLIS, commented on the intense CD10 co-expression by the Bcl-2^{bright+} B-cells [11]. Similar observations were also reported by Jegalian et al in a recent large series, which revised (and added to) the original series of Cong et al [10].

Since its original description, different terminology has been used to describe this peculiar follicular lymphoproliferation, ranging from “in-situ localization of follicular lymphoma” [5,8] to “intrafollicular neoplasia/in situ follicular lymphoma” [11], to “follicular lymphoma in-situ” [6,9,10]. The term “in situ” has been used in many tumor systems, mostly epithelial, to denote a tissue localized, and often the earliest morphologically recognizable, stage in

neoplastic evolution, associated with an indolent course. Since (1) the genesis of t(14;18) is thought to occur in the bone marrow, (2) often more than one lymphoid follicle shows colonization by Bcl-2^{bright+}CD10⁺ B-cells and (3) the biology of this lymphoproliferation is not well understood at present, we have used a descriptive, non-committal term for this immunoarchitectural pattern, ie, follicular colonization by Bcl-2^{bright+}CD10⁺ B-cells.

Our series comprised 14 cases, including 13 primary cases and one recurrence, involving lymph nodes and extranodal sites, and all except three displayed concomitant bright CD10 expression. The density of follicular colonization was variable within and between cases, but the majority of our cases demonstrated a higher frequency of follicles manifesting the Bcl-2^{bright+}CD10⁺ B-cell immunoarchitectural pattern compared to prior reports [5,11]. Most publications have described this pattern only in lymph nodes or in the spleen. Jegalian et al recently reported FLIS at extranodal sites, one case each involving the thyroid and jejunum [10]. We corroborate and extend this observation. In our series, the small bowel was the most common primary extranodal site (60%) manifesting this pattern, and isolated cases involving the thyroid and subcutaneous tissue were noted. We also document a mantle-zone/perifollicular pattern of infiltration of the adenoids for the first time.

The frequency of synchronous or metachronous lymphomas in patients exhibiting follicular colonization by Bcl-2^{bright+}CD10⁺ B-cells has been quite variable in prior reports. In the series of Cong et al, consisting of 25 cases, synchronous FL was diagnosed in 20% involving another lymph node; 12% subsequently developed FL, and 8% had other synchronous low-grade non-GC derived B-cell lymphomas (chronic lymphocytic leukemia/small lymphocytic lymphoma [CLL/SLL] and lymphoplasmacytic lymphoma) [5]. Montes-Moreno et al, in their study of 13 cases, reported synchronous FL in 23% and other types of synchronous lymphomas, including classical Hodgkin lymphoma and splenic marginal zone lymphoma in 31%, while 15% developed subsequent lymphomas (FL and DLBCL) [11]. In the recent large series of Jegalian et al, comprising 34 cases, synchronous FL was detected in 15% (2 Bcl-2⁺ and 3 Bcl-2[−] FL), 15% were composite lymphomas with other low-grade non-GC derived B-cell lymphomas or interfollicular Hodgkin lymphoma; subsequent FL developed in 5%, and only 3% developed a DLBCL [10]. Our findings are similar to those of Montes-Moreno et al, with overt synchronous lymphomas observed in 46% of our cases, which were clonally related to samples exhibiting follicular colonization by Bcl-2^{bright+}CD10⁺ B-cells in 80% of analyzed cases. An additional lymphoma was suspected on imaging that wasn't sampled, while 15% developed subsequent lymphomas. However, in contrast to previous studies, the majority of synchronous lymphomas (67%) in our series represented “high-grade” lymphomas (DLBCL and BCL-U).

Cong et al confirmed the presence of t(14;18) localized to the Bcl-2^{bright+} GCs in 1 of 5 (20%) FLIS cases using laser capture microdissection and in 43% using whole-tissue sections by PCR analysis [5], while Montes-Moreno et al detected *BCL2* rearrangements in 85% of cases using both PCR and FISH analysis [11]. Consistent with prior studies, a high percentage of our informative samples manifesting follicular colonization (70%) harbored the t(14;18) by karyotype and/or FISH analysis, and this translocation was detected in both components, colonized follicles and synchronous lymphomas, in 83% of cases. We found a targeted FISH approach, albeit cumbersome, to yield the highest detection rate.

Two different pathophysiologic hypotheses for the FLIS pattern were proposed by Cong et al: 1) a phenomenon related to homing of lymphoma cells to reactive GCs or 2) a pre-neoplastic entity requiring additional hits for transformation [5]. Henopp et al, on detecting a 2.3% prevalence of FLIS in reactive lymph nodes, which is much higher than the prevalence of FL (1/10 000), suggested that FLIS could represent an early stage of lymphomagenesis, with the majority of patients exhibiting this pattern not progressing to overt lymphoma [9]. Cheung et al shared a similar view, based on their study of a patient with FLIS lacking evidence of disease progression by imaging on prolonged follow-up, but the presence of circulating cells harboring an identical t(14;18) led them to argue against FLIS being a limited process [8].

Jegalian et al speculated that FLIS could be the tissue counterpart of circulating t(14;18) carrying cells, which can be observed in healthy individuals [10]. The t(14;18) can be detected by PCR-based methods in reactive lymphoid tissue (lymph nodes and tonsils) in up to 54% [15] and the peripheral blood in up to 55% of normal individuals [16]. The frequency of detecting this translocation in circulating lymphocytes increases with age [16] and in certain disease states [17–19]. Currently, there is no definitive evidence linking FLIS to the presence of circulating cells harboring t(14;18) in healthy individuals. Follicular colonization by Bcl-2^{bright+}Bcl-6⁺ B-cells was not seen in the spleen of an individual who had circulating cells with t(14;18) [20]. Moreover, transgenic mice with deregulated *BCL2* expression, although not the best models to clarify early histopathologic alterations of human FL, demonstrate florid follicular hyperplasia in lymphoid organs early in disease course rather than a pattern resembling FLIS [21,22]. Validation of FLIS as an early pathogenetic stage in FL evolution will be challenging, requiring not only sequencing

of *IGH* genes, but also concurrent genomic analysis of circulating cells carrying t(14;18), as well as GCs colonized by Bcl-2^{bright+}CD10⁺ B-cells in the same individual. Complicating such analysis is also the recent revelation that FL progression patterns are complex and not linear as previously thought [23].

Bonzheim et al provided evidence in support of FLIS being a precursor to overt FL [6]. They detected the same *IGH* gene rearrangement and *BCL2* translocations in a lymph node with FLIS and a synchronous Bcl-2[−] FL in another lymph node; however, unlike the FLIS, the Bcl-2[−] FL had acquired multiple numerical chromosomal aberrations and a *BCL2* mutation [6]. Carbone et al described a similar case, where Bcl-2[−] FL and FLIS, observed as distinct components in the same lymph node, had similar *IGH* gene rearrangement products by PCR, but they suggested that bright Bcl-2 acquisition by FLIS likely reflected a homing-related phenomenon of FL cells to reactive GCs [7,24].

The two hypotheses of Cong et al are not necessarily mutually exclusive [5]. Although, a pre-neoplastic or a distinct precursor stage of Bcl-2^{bright+}CD10⁺ B-cells colonizing reactive follicles can neither be proven nor refuted for many of our cases, unique features of some cases suggest that follicular colonization by Bcl-2^{bright+}CD10⁺ B-cells may represent a peculiar lymphoid tissue homing-related phenomenon of certain lymphomas. Complex cytogenetic aberrations were detected in two cases. In one instance, a clonally related aggressive BCL-U involving a neighboring lymph node harbored an identical abnormality, a rare type of genetic “triple-hit” comprising t(14;18)(q32;q21) and t(3;8)(q27;q24), which is associated with adverse clinical outcomes [25]. In the other case, imaging studies suggested the presence of systemic, unsampled lymphoma. Variability in staining intensity of Bcl-2 and CD10 between colonized follicles and clonally related lymphomas was also evident in some cases. Hence, besides FL, aggressive lymphomas of GC origin or phenotype may also traffic to reactive GCs, on occasion, where microenvironment-specific signals could modulate the cytologic appearance, proliferative activity and expression of Bcl-2 and other antigens. Modulation of a variety of antigens upon GC entry and exit by normal or neoplastic B-cells is a well described phenomenon [26].

The clinical significance of cases manifesting the Bcl-2^{bright+}CD10⁺ B-cell follicular colonization pattern remains poorly understood at present. Many patients exhibiting this pattern do not develop overt lymphoma for long periods, while others have synchronous lymphoma, including

Fig. 4 BCL-U involving the head of the pancreas (case 11) (A–D) and a peripancreatic lymph node exhibiting GC colonization by Bcl-2^{bright+}CD10⁺ B-cells (E–H). A, A dense infiltrate of BCL-U in the pancreas. B, Neoplastic lymphocytes are intermediate in size and exhibit blastoid morphology. C, Lymphoma cells express CD10. D, Lymphoma cells express Bcl-2. E, Peripancreatic lymph node displaying scattered, small reactive lymphoid follicles with preserved mantle zones and intact lymph node architecture. F, GC-colonizing cells have centrocytic morphology. G, GC-infiltrating cells express CD10, which is similar in intensity to adjacent reactive follicles. H, The GC B-cells show bright Bcl-2 expression (A, E: H&E, 20×; B, F: H&E, 400×; C: CD10, 400×; D: Bcl-2, 400×; G: CD10, 200×; H: Bcl-2, 200×).

Table 2 Cytogenetic and molecular findings

Case	Bcl-2 ^{bright+} CD10 ⁺ follicles			Lymphoma						
	Karyotype	FISH metaphase	FISH FFPE	<i>IGH</i> PCR	Sync	Meta	Karyotype	FISH metaphase	FISH FFPE	<i>IGH</i> PCR
1	ND	NA	<i>BCL2</i> F	ND	No	NA	NA	NA	NA	NA
2	ND	NA	<i>BCL2</i> F	F	No	NA	NA	NA	NA	NA
3a	47-50, XX,hsr(3)(q13),+i(8)(q10), +11,+18,t(14;18)(q32;q21)x 2,+der(18)t(14;18)(q32;q21) [cp6]/46,XX [14]	<i>IGH/BCL2</i> R, <i>BCL6</i> NR	ND	C	NA		NA	NA	NA	NA
3b					NA	Bcl-2 ^{bright+} CD10 ⁺ B-cells ^b	F	F	F	PC
4	ND	NA	<i>BCL2</i> NR	F	No	NA	NA	NA	NA	NA
5	46,XY [20]	<i>IGH/BCL2</i> NR	ND	PC	No	No	NA	NA	NA	NA
6	ND	NA	<i>IGH/BCL2</i> R, <i>BCL6</i> NR	ND	Low-grade FL	NA	ND	NA	<i>IGH/BCL2</i> R, <i>BCL6</i> NR	ND
7	ND	NA	<i>BCL2</i> NR	C ^a	Low-grade FL	No	46,XX,t(11;14)(q23.1;q32), t(14;18)(q32;q21)[10]/46, XX[8]	<i>IGH/BCL2</i> R, <i>BCL6</i> NR	ND	C ^a
8	ND	NA	<i>BCL2</i> R	C ^a	DLBCL	NA	F	ND	<i>BCL2</i> R	C ^a
9	ND	NA	<i>BCL2</i> R	C	DLBCL	NA	ND	NA	<i>BCL2</i> R	C
10	46,XY, t(3;8)(q27;q24), t(14;18)(q32;q21) [2]/46,XY[6]	<i>IGH/BCL2</i> R, <i>BCL6</i> R, <i>MYC</i> R	<i>BCL2</i> R, <i>BCL6</i> R, <i>MYC</i> R	PC	BCL-U	No	46,XY,t(3;8)(q27;q24), t(14;18)(q32;q21) [24]/46,XY [3]	<i>IGH/BCL2</i> R, <i>BCL6</i> R, <i>MYC</i> R	ND	PC
11	ND	NA	<i>IGH/BCL2</i> R, <i>BCL6</i> NR	C ^a	BCL-U	No	ND	NA	<i>IGH/BCL2</i> R, <i>BCL6</i> NR, <i>MYC</i> NR	C ^a
12	46,XX [9]/46,XX, t(18;20)(p11.3;q11.2)[1]	<i>IGH/BCL2</i> R	ND	PC	No	Low-grade FL	F	<i>IGH/BCL2</i> F	NA	ND
13	ND	ND	ND	PC	No	A: Low-grade FL B: DLBCL	A: t(14;18)(q32;q21),+7 B: 53~56,XX,add(2)(q37),+4, +6,+7,+7,+add(8)(q24),?del(9) (p13),+12,+13, der(17)t(?X;17) (?q13;p13),t(14;18)(q32;q21), +21,+1~4mar [20]	A: <i>IGH/BCL2</i> R, <i>BCL6</i> NR B: <i>IGH/BCL2</i> R, <i>MYC</i> R	NA	A: C B: C

Abbreviations: Sync, synchronous lymphoma, Meta, metachronous lymphoma, NA, not applicable; ND, not done; F, failure; R, rearranged; NR, not rearranged; C, clonal; PC, polyclonal.

^a, Identical clones shared between Bcl-2^{bright+} CD10⁺ colonized GCs and synchronous lymphoma; ^b, mantle zone/perifollicular infiltrate.

Fig. 5 A, G-band karyotype obtained from a lymph node displaying GC colonization by Bcl-2^{bright+}CD10⁺ B-cells (case 10) that was adjacent to a lymph node involved by BCL-U [black arrows indicate t(14;18)(q32;q21), and white arrows demonstrate t(3;8)(q27;q24)]. B, Metaphase FISH using *IGH/BCL2* dual-color fusion probes [yellow arrow indicates *IGH/BCL2* fusion signal; green arrow shows intact *IGH* locus; red arrow shows normal copy of *BCL2*]. C, Metaphase FISH using *MYC* break-apart probes [yellow arrow indicates intact *MYC*, green arrow shows 3' *MYC* on der(3), and red arrow shows 5' *MYC* on der(8)]. D, Interphase FISH targeting colonized follicles (FFPE sections) with *BCL2* break-apart probes [yellow arrow indicates intact *BCL2*, green arrow shows 3' *BCL2*, and red arrow shows 5' *BCL2* confirming *BCL2* rearrangement]. E, Interphase FISH targeting colonized follicles (FFPE sections) with *MYC* break-apart probes [yellow arrow indicates intact *MYC*, green arrow shows 3' *MYC*, and red arrow shows 5' *MYC* confirming *MYC* rearrangement]. F, G-band karyotype of a lymph node showing GC colonization by Bcl-2^{bright+}CD10^{bright+} B-cells without histologic or phenotypic evidence of lymphoma (case 3a), but imaging studies suggested stage 4 disease [black and white arrows show multiple copies of t(14;18)(q32;q21)]. G, Interphase FISH using *IGH/BCL2* probes showed 2 copies and rare cells displayed multiple copies of rearranged *IGH/BCL2* [yellow arrows – *IGH/BCL2* fusions].

aggressive variants, or they may subsequently develop lymphoma. Jegalian et al proposed histopathologic and phenotypic criteria to distinguish more indolent proliferations from partial lymph node involvement by FL (PFL) [10]. Using a combination of features, including subtle alterations in follicle architecture, extent of GC infiltration, cytology of infiltrating cells, presence of interfollicular infiltrate, and variability in Bcl-2 and CD10 intensity, the authors reclassified 9 of 25 (36%) previously reported FLIS cases [5] as having PFL, of which 8 were found to have concurrent or subsequent FL [10]. A correct diagnosis of FLIS was suggested to portend a low risk of developing lymphoma, however, a significant fraction of untreated

individuals with PFL (47%) also lacked evidence of disease on follow-up [10]. Our cases lacked morphologic criteria of PFL; however, 50% of cases associated with concurrent lymphomas did not express bright CD10. Additionally, we observed a high frequency of follicles manifesting the colonization pattern in samples from virtually all patients with synchronous or metachronous lymphomas. Molecular or cytogenetic analysis might be helpful in predicting concomitant overt or aggressive lymphomas elsewhere when biopsies only exhibit the follicular colonization pattern, as exemplified in this study. Given the small number of cases and relatively short follow-up duration, future studies are warranted to confirm our observations. In the

absence of definitive pathologic and molecular features enabling risk stratification at present, we support the recommendation of Carbone and Santoro, who advocate full staging workup to rule out coexistent lymphoma [27]. Scheduled clinical follow-up evaluation might also be prudent to detect disease progression or transformation, as is the case for individuals with FL.

In summary, we present a series of cases exhibiting a pattern of follicular colonization by Bcl-2^{bright}+CD10⁺B-cells in architecturally intact lymph nodes and at extranodal sites. We describe a higher incidence of synchronous lymphomas, especially high-grade variants than prior reports. Some of the unique characteristics of our cases suggest that this distinct immunoarchitectural pattern might reflect a phenomenon associated with homing of neoplastic B-cells to reactive follicles, at times representing evolved clones, rather than indicate a particular stage in FL development.

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