In conclusion, immunohistochemical detection of spirochetes in paraffin-embedded samples is of great value for proper diagnosis of these types of cases, initially suspected of being a dendritic/reticular cell tumour/sarcoma.

Although *T. pallidum* has already been shown to trigger nodal inflammatory pseudotumour,[5] some cases may show frank sarcomatous changes, as demonstrated here. Pathologists may take this into account in order to avoid overdiagnosing these lesions.

Santiago Montes-Moreno[1]
Olga Acinas García[2]
Gervasio Santiago-Ruiz[2]
Javier Alves Ferreira[3]
Juan Fernando García[4]
Miguel Angel Piris Pinilla[1]

[1]*Lymphoma Group, CNIO, Madrid,*
[2]*Pathology and Internal Medicine Departments, Hospital de Sierrallana, Torrelavega,*
[3]*Pathology Department, Hospital Universitario La Paz and,*
[4]*Pathology Department, MD Anderson Oncological Centre, Madrid, Spain*

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Intrafollicular neoplasia/*in situ* follicular lymphoma: review of a series of 13 cases

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Sir: *In situ* localization of follicular lympmphoma (FL) was described in 2002 as being one of the early events associated with FL development, and renamed in the 2008 version of the World Health Organization lymphoma classification as 'intrafollicular neoplasia/*in situ* follicular lymphoma'.[1,2] It is characterized by the presence of germinal centres (GCs) that strongly express Bcl-2 protein and GC markers (CD10, Bcl-6), while most of the remaining lymph node shows a pattern of follicular hyperplasia, in absence of interfollicular infiltration. The clinical significance of this diagnosis has not yet been fully determined. Here we describe 13 cases of lymph node and spleen biopsies

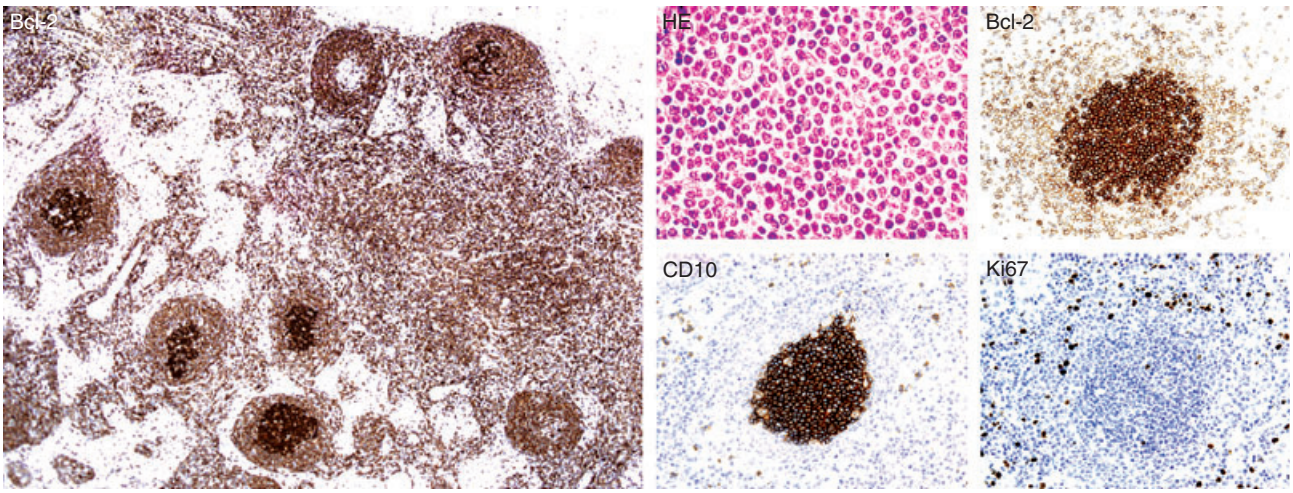

Figure 1. Intrafollicular neoplasia. Note the monomorphic cell composition of the germinal centres (GCs). GCs involved in intrafollicular neoplasia/*in situ* follicular lymphoma are composed mainly of centrocytes with no evident atypia. A feature of these centres is the relative absence of macrophages with tingible bodies and the absence of polarization into light and dark zones. The paucity of large centroblasts reflects their low proliferation rate in *in situ* FLs. Intrafollicular neoplasia/*in situ* follicular lymphoma is characterized by strong co-expression of Bcl-2 and CD10 in the involved GCs.

Table 1. Details of antibodies used and antigen retrieval methods

Antibody	Clone	Source	Antigen retrieval/visualization method	Dilution
Bcl-2	Clone 124	Dako, Glostrup, Denmark	Citrate 10 mM pH 6.5/Novolink (Novocastra)	1:50
Bcl-6	GI191E/A8	CNIO, Madrid, Spain	Citrate 10 mM pH 6.5 and Proteinase K/ Novolink (Novocastra)	0.25
CD10	56C6	Novocastra, Newcastle, UK	Citrate 10 mM pH 6.5/Novolink (Novocastra)	1:10
CD20	L26	Dako	Citrate 10 mM pH 6.1/FLEX (Dako)	1:50
Ki67	MIB 1	Dako	Citrate 10 mM pH 6.1/FLEX (Dako)	1:50

Table 2. Summary of immunohistochemical quantification of Bcl-2 and molecular results

Case	Bcl-2 IHQ quantification	PCR Bcl-2 (MBR and mcr)	FISH Bcl-2 (BA)
1	4c	POS	POS
2	2a	POS	NEG
3	1a	NEG	POS
4	2a	POS	POS
5	2b	POS	POS
6	4b	POS	NEG
7	2a	POS	NEG
8	3a	NEG	NEG
9	4c	NEG	AMPLIF
10	3a	NEG	POS
11	4c	NEG	POS
12	3b	POS	NEG
13	3c	POS	POS

Immunohistochemical quantification: cases were classified into one of four groups, depending on the number of strongly immunoreactive Bcl-2+ follicles: group 1, <5; group 2, 5–10; group 3, 11–20; group 4, >20. According to the relative abundance of these aberrant follicles relative to the other secondary hyperplastic germinal centres, cases were classified as 'a' (<20%), 'b' (20–50%) or 'c' (>50%).
POS, positive; NEG, negative; AMPLIF, amplified without translocation.

with the diagnosis of 'intrafollicular neoplasia/*in situ* FL' seen in consultation between January 2002 and January 2008. Diagnostic criteria for *in situ* FL were those previously described[1] (Figure 1). Details of immunohistochemistry methods used are shown in Table 1. Fluorescence *in situ* hybridization (FISH) and polymerase chain reaction for Bcl-2 translocation were also performed in all cases (Table 2).

The series was comprised of seven women and six men, whose median age at diagnosis was 55 years (range 40–85 years) (Table 3). Of the 13 cases, 10 showed no evidence of FL at diagnosis after staging procedures and the discovery of their intrafollicular neoplasia was considered to be incidental in the context of diverse clinical situations (i.e. inguinal hernia, unrelated gastric tumour, follow-up after initial diagnosis of breast or laryngeal carcinoma). Three of these 13 cases were discovered to present full-blown FL in another lymph node or bone marrow location after staging procedures (nos 6, 7 and 8). Interestingly, one case featured the association of intrafollicular lymphoma and classic Hodgkin's lymphoma (cHL) in the first diagnostic sample (no. 8). Additionally, one case in this series developed a conventional FL (stage IV) that arose 15 months after the initial diagnosis of intrafollicular neoplasia/*in situ* FL (no. 9). In summary, only four of the 13 cases featuring intrafollicular neoplasia/*in situ* FL developed full-blown FL after a median follow-up of 12 months (range 8–60 months).

Five cases showed association with lymphoproliferative disorders other than FL. Case 10 developed stage III diffuse large B-cell lymphoma (DLBCL) 3 months after the diagnosis of *in situ* FL, without any evidence of FL in the diagnostic sample. Additionally, four cases showed a simultaneous association of intrafollicular neoplasia with cHL (two cases) or other low-grade non-Hodgkin B-cell lymphomas [in this case splenic marginal zone lymphoma (SMZL)] (two cases). The association of intrafollicular neoplasia with SMZL was considered to be an incidental finding after splenectomy for splenomegaly and pathological lymphocytosis in two cases, where discordant light chain restriction

Table 3. Summary of clinical features

Case	Age/sex	Localization of intrafollicular neoplasia	Other diseases	Time to diagnosis (months) and location of associated neoplasm(s)	Stage	Follow-up (months)	Current status
1	46/M	Mesenteric LN	Gastric GIST			25	NEL
2	55/F	Mesenteric LN	ND			12	NEL
3	85/F	R Axillary LN	Breast Ca.			14	NEL
4	68/M	R Axillary LN	Glottis Ca.			9	Exitus*
5	78/M	Inguinal LN	Sigma Ca.			10	NEL
6	54/F	Axillary/RTP	FL	(0) L inguinal LN	IIIA	8	NR
7	40/F	Cervical LN	FL	(0) Subm. mass	III	27	CR
8	40/F	Axillary LN	FL and HL	(2) Retroperitoneal LN	IV	60	CR
9	65/M	Mesenteric LN	FL	(15) Axillary LN	IV	32	SD
10	60/F	R. Inguinal LN	Brucellosis, DLBCL	(3) Abdominal LN	III	3	–
11	49/F	Inguinal LN	HL	(0) Inguinal LN	IIIB	12	CR
12	42/M	Spleen	SMZL	(0) Spleen (relapsed 55 m)	IV	58	Relapse
13	74/M	Spleen	SMZL	(0) Spleen	IV	9	PR

*Exitus unrelated to lymphoma.
F, female; M, male; LN, lymph node; FL, follicular lymphoma; DLBCL, diffuse large B-cell Lymphoma; HL, Hodgkin lymphoma; SMZL, splenic marginal zone lymphoma; CR, complete response; NEL, no evidence of lymphoma; SD, stable disease; ND, no data.

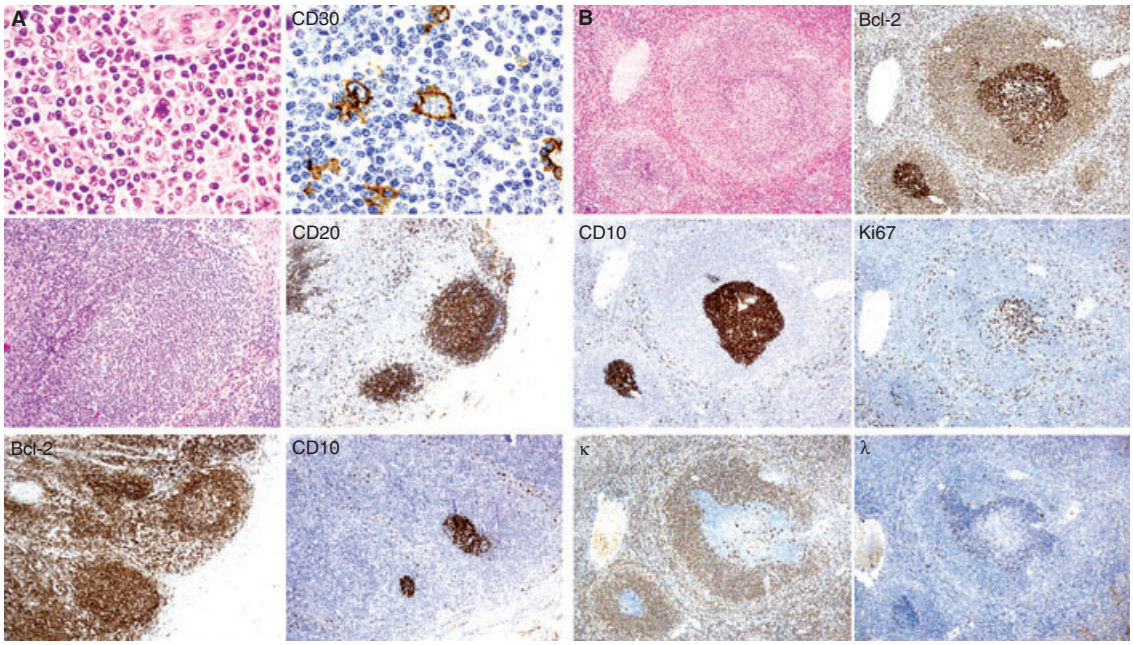

Figure 2. A, Intrafollicular neoplasia associated with interfollicular classic Hodgkin's lymphoma. Interfollicular Reed–Sternberg cells (CD30+) with a lymphocyte-rich background are admixed with isolated germinal centres (GCs) with the features of *in situ* follicular lymphoma. **B,** Intrafollicular neoplasia associated with splenic marginal zone lymphoma. Together with a biphasic neoplasm in the white pulp that expands into the marginal zone (and exhibits κ chain restriction), the GCs strongly express Bcl-2 and CD10. GC cells express monotypic λ.

between the SMZL neoplastic population and intrafollicular neoplasia cells in both cases – and lack of t(14;18) by FISH in case 13 – favoured the interpretation of composite neoplasia (Figure 2).

Cytogenetics by FISH was performed in all samples where intrafollicular neoplasia was diagnosed. In one of these cases (no. 9) the same cytogenetic aberration was found by FISH (Bcl-2 amplification) in both intrafollicular neoplasia and FL lesions, consistent with a clonal relationship. FISH studies were not performed in the other cases with associated FL or DLBCL due to unavailability of tissue samples.

All cases were characterized by the strong co-expression of Bcl-2 and CD10 in the involved GCs (Figures 1 and 2). The proportion and disposition of the Bcl-2+ GC cells varied from case to case. We counted the Bcl-2+ follicles in order to investigate correlations with diverse clinical presentations (Table 2).

These results confirm the existence of a subset of patients carrying the features of this rare condition known as intrafollicular neoplasia/*in situ* FL.[1] In this series, only four out of 13 cases featuring intrafollicular neoplasia/*in situ* FL developed full-blown FL after a median follow-up of 12 months. However, the rate of association with other non-FL lymphoid neoplasias was surprisingly high, with five out of 11 cases being associated with either cHL, SMZL or DLBCL. Interestingly, the amount of infiltration of the lymph node by this intrafollicular neoplasia was associated with the probability of it actually being associated with FL in the follow-up and with the presence of other lymphoma subtypes, as previously proposed.[1]

An interesting observation is the association in five cases of *in situ* FL with classical Hodgkin (two cases), non-Hodgkin low-grade B cell malignancies (SMZL, two cases) and DLBCL (one case). Furthermore, one case associated with Hodgkin lymphoma was actually a composite lymphoma in which overt FL was demonstrated after bone marrow biopsy. Together with two previous cases that described the coexistence of *in situ* FL with other low-grade B-cell malignancies (chronic lymphocytic leukaemia and lymphoplasmacytic lymphoma),[1] these associations with other Hodgkin and non-Hodgkin lymphomas in the absence of FL may suggest that intrafollicular neoplasia may be a sign of an increased tendency to develop lymphoid malignancies due to underlying molecular abnormalities.

The immunophenotypical data of this series of patients lend additional support to the interpretation that this condition may represent something else than merely a precursor for common FL. Thus, cases described here show strong simultaneous expression of Bcl-2 and CD10 in these neoplastic GCs, with an intensity of Bcl-2 immunoreactivity greater than that usually observed in FL cases bearing t14;18 and reminiscent of that observed in duodenal FL cases, a tumour distinguished by a very low tendency to spread to other organs. These results support the consideration of this lesion as intrafollicular neoplasia, a separate disease entity that may precede or be associated with FL or other lymphoproliferative conditions, although in some cases it may simply be diagnosed as an isolated finding, after extensive staging and follow-up. A subset of these intrafollicular neoplasia cases behave like genuine *in situ* FLs, a preneoplastic condition associated with FL.

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Santiago Montes-Moreno
Yolanda Castro[1]
Socorro M Rodríguez-Pinilla
Juan F García[2]
Manuela Mollejo[3]
Maria E Castillo
Agueda Bas-Vernal[4]
Carlos Barrionuevo-Cornejo[5]
Lidia Sanchez-Verde
Javier Menarguez[1]
Juan C Cigudosa
Miguel A Piris

Lymphoma Group, Spanish National Cancer Research Centre (CNIO), [1]*Pathology Department, Hospital General Universitario Gregorio Marañón, and* [2]*Pathology Department, MD Anderson Clinical Cancer Centre, Madrid,* [3]*Pathology Department, Hospital General Virgen de la Salud, Toledo, and* [4]*Hospital Universitario Virgen de la Arrixaca, Murcia, Spain, and* [5]*Pathology Department, Instituto de Enfermedades Neoplásicas, Lima, Peru*

S.M-M. and Y.C. contributed equally to this work.

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IgG4+ Rosai–Dorfman disease of the lung

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Sir: we note with interest a review article[1] and editorial[2] regarding IgG4-related systemic sclerosing disease. Other conditions have also been found with a significant IgG4+ plasma cell population, including Rosai–Dorfman disease (RD), suggesting a possible overlap between RD and IgG4-related disease.[3]

We describe a case of pulmonary RD with a prominent IgG4+ plasma cell population. The patient was a 61-year-old non-smoking man who, 2 months previously, had undergone a radical prostatectomy for Gleason grade 4+3 = 7 prostatic adenocarcinoma. Follow-up magnetic resonance imaging identified a suspicious spiculated nodule within the upper lobe of the left lung (Figure 1). The radiological differential diagnosis was between a pulmonary metastasis and a synchronous lung cancer.

The patient was referred to the cardiothoracic surgeons, and an open biopsy produced a wedge of lung measuring 64 × 26 × 14 mm that contained a well-circumscribed, solid, white nodule measuring 21 mm in diameter. Microscopically, the nodule was fibrotic (Figure 2A) and composed of sheets of large histiocytoid cells with vesicular nuclei, prominent nucleoli and abundant eosinophilic cytoplasm (Figure 2B). Focal lymphocyte phagocytosis was seen in a minority of these histiocytoid cells (Figure 2C). In addition, the fibrotic nodule was composed of numerous plasma cells and lymphocytes. Immunohistochemistry showed these histiocytoid cells to be S100+ (Figure 2D). IgG4 immunohistochemistry was positive and, using the quantification method described by Shrestha *et al.*,[3] had a significant score of 2 (Figure 2E). Three months following surgery, the patient remains asymptomatic and clinically well.

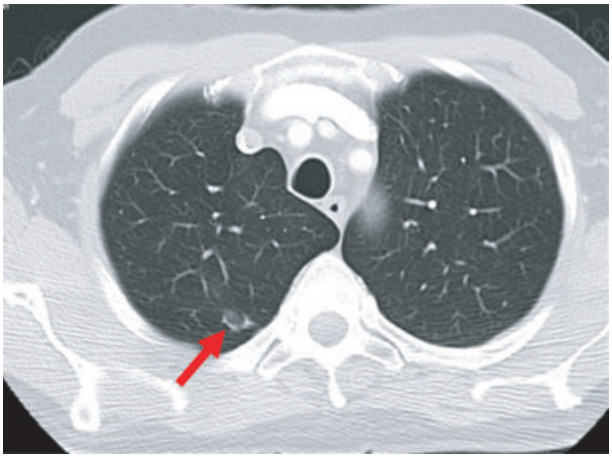

Figure 1. Spiculated mass within the upper lobe of the left lung.

RD is a benign proliferation of histiocytes of unknown aetiology.[4] It is a rare condition typically affecting lymphoid tissue, hence its other more descriptive clinicopathological name of massive lymphadenopathy with sinus histiocytosis.[5] Extranodal disease with or without nodal involvement was reported in 43% of the cases in the RD Registry, but only 2% had any lower respiratory tract involvement.[4]

In contrast to the demographics of our index case, pulmonary RD usually occurs in females within the teenage years. When symptomatic, patients present with shortness of breath and occasionally stridor.[4] The radiological appearance is variable, hence tissue is required for a definitive diagnosis.

Pulmonary RD has a significantly worse prognosis compared with the nodal form. Approximately one-third die of the disease and more than one-third have a progressive condition.[4] There is no ideal treatment and interventions are reserved for those cases with obstructing symptoms. Surgery, radiotherapy and drugs, including prednisolone and α-interferon, have all been used with varying success.[6]

There has been a reported overlap in the histopathological appearance and IgG4 status of RD and IgG4-related disease.[3] This latter condition is characterized by raised serum IgG4 levels with histological evidence of extensive IgG4+ plasma cells and T-lymphocyte infiltration together with fibrosis.[1] The condition is also very responsive to treatment with corticosteroids.[2,7] There are emerging manifestations of IgG4-related disease in the lung from inflammatory pseudotumour,[7,8] non-specific interstitial pneumonitis and RD.[1,3]

Our case has the morphological and immunohistochemical appearance of RD with IgG4+ plasma cells, which presented as a pseudotumour. The significance of this specific IgG subgroup is uncertain. However, this does add to the need for further investigations into these 'overlap' conditions and clarification of any diagnostic and/or therapeutic significance.