micro-organisms and immunostains for IgG4 (performed on both liver and lung biopsies) were negative. The patient was treated with steroids, and there was rapid improvement of her symptoms and radiological infiltrates. Eleven months after biopsy, she is alive with no residual pulmonary disease.

We interpreted this case as a pulmonary localisation of PBC, histologically presenting as cellular NSIP with granulomas. NSIP is a pattern of interstitial lung disease characterised by the uniform expansion of the interstitium by lymphocytes and plasma cells (cellular variant) or fibrosis (fibrosing variant).[4] It can be idiopathic but frequently is secondary to many possible causes, particularly collagen vascular diseases, hypersensitivity pneumonitis and drug reactions. Although we are not aware of other reported cases of NSIP secondary to PBC, we suspect the frequency of NSIP as a pattern of pulmonary involvement in PBC is probably underestimated. In fact, some cases reported as lymphocytic interstitial pneumonia[5,6] or PBC-like lung involvement[7] in patients with PBC are similar to our case; although admittedly the differential diagnosis has a component of subjectivity, in our opinion the relatively mild interstitial infiltrate and the HRCT features of our case favour a designation of NSIP over lymphocytic interstitial pneumonia.

The histological differential diagnosis of cellular NSIP with granulomas is quite broad,[8] but in the clinical context of our case includes particularly an infection and a drug reaction; their exclusion requires a careful correlation of the histology with the clinico-laboratory data. In our patient an infectious agent could not be identified despite a thorough search, and we are not aware of any reported adverse pulmonary reaction to ursodiol, the only drug the patient was taking. Although the possibility that some cases of PBC are part of the spectrum of IgG4-related syndrome deserves further study, it seems quite unlikely in our opinion; in PBC, IgM rather than IgG are typically elevated (both serologically and immunohistochemically[9]), and in the few tested cases including our case, the immunostain for IgG4 has been negative.[10]

In summary, PBC should be included among the many autoimmune diseases which can cause an interstitial lung disease with NSIP pattern. In our opinion, in the correct clinico-radiological context a good-quality transbronchial biopsy can be informative enough to support a diagnosis of cellular NSIP, sparing the patient an unnecessary surgical biopsy.

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1. Kaplan MM, Gershwin ME. Primary biliary cirrhosis. *N Engl J Med* 2005; 353: 1261–73.
2. Shen M, Zhang F, Zhang X. Primary biliary cirrhosis complicated with interstitial lung disease: a prospective study in 178 patients. *J Clin Gastroenterol* 2009; 43: 676–9.
3. Butnor KJ, Khoor A. Collagen vascular diseases and disorders of connective tissue. In: Tomashefski JF, Cagle PT, Farver CF, Fraire AE, editors. *Dail and Hammar's Pulmonary Pathology*. 3rd ed. New York: Springer, 2008; 722–59.
4. Travis WD, Hunninghake G, King TE, *et al.* Idiopathic nonspecific interstitial pneumonia. Report of an American Thoracic Society project. *Am J Respir Crit Care Med* 2008; 177: 1338–47.
5. Weissman E, Becker NH. Interstitial lung disease in primary biliary cirrhosis. *Am J Med Sci* 1983; 285: 21–7.
6. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 14–1998. A 49-year-old woman with primary biliary cirrhosis, pulmonary opacities, and a pleural effusion. *N Engl J Med* 1998; 338: 1293–301.
7. Strobel ES, Bonnet RB, Werner P, *et al.* Bronchiolitis obliterans organizing pneumonia and primary biliary cirrhosis-like lung involvement in a patient with primary biliary cirrhosis. *Clin Rheumatol* 1998; 17: 246–9.
8. Cheung OY, Muhm JR, Helmers RA, *et al.* Surgical pathology of granulomatous interstitial pneumonia. *Ann Diagn Pathol* 2003; 7: 127–38.
9. Daniels JA, Torbenson M, Anders RA, *et al.* Immunostaining of plasma cells in primary biliary cirrhosis. *Am J Clin Pathol* 2009; 131: 243–9.
10. Chung H, Watanabe T, Kudo M, *et al.* Identification and characterization of IgG4-related autoimmune hepatitis. *Liver Int* 2010; 30: 222–31.

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Biclonal follicular lymphoma: histological, clinical and molecular characteristics

Sir,
The t(14;18) translocation is the cytogenetic hallmark of follicular lymphomas (FLs).[1] At a molecular level, it determines the juxtaposition of *B-cell lymphoma 2* (*BCL2*) gene in 18q21.3, with one of the immunoglobulin heavy-chain (*IGH@*) gene joining regions (J_H) on chromosome 14q32.33, resulting in overexpression of the anti-apoptotic Bcl2 protein. Most chromosome 18 breakpoints cluster at two sites: a 150 bp sequence located in the 3′ untranslated region of *BCL2*, named the major breakpoint region (MBR), and a more distal minor cluster region (mcr), a 500 bp region placed 20 kb downstream of *BCL2*. In addition, long distance polymerase chain reaction (PCR) has revealed breakpoints scattered between MBR and mcr.[2]

In FL patients, the coexistence of double *BCL2* rearrangement is an infrequent event, the characteristics and prognostic importance of which are not well established. Different mechanisms can explain the presence of double *BCL2* rearrangements: (1) the appearance of a second translocation in a cell already involved in the original clone bearing a primary t(14;18) translocation; (2) the generation of a new malignant clone *de novo* in an uninvolved cell; and (3) the second translocation appears in a formerly benign cell with t(14;18) originating a malignant clone. Herein we report a case of FL showing two *BCL2*

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rearrangements at MBR and mcr breakpoint clusters, and its associated clinical features, along with a review of the literature.

All studies were carried out on an axillary lymph node. Histological classification and grading of our series were performed according to the criteria stated in the World Health Organization (WHO) classification.[3] The Follicular Lymphoma Prognostic Index (FLIPI)[4] was determined using age, Ann Arbor stage, haemoglobin, number of nodal areas involved, and serum lactate dehydrogenase (LDH). The study was approved by the local Ethical Committee. The patient provided informed written consent.

Genomic DNA was isolated by conventional phenol-chloroform extraction, according to standard protocols. DNA purity, quality and concentration were estimated by ultraviolet spectrophotometry and agarose gel electrophoresis. Samples were subjected to a highly sensitive detection of MBR/J_H and mcr/J_H rearrangements by nested-PCR (N-PCR) using primers and conditions previously described.[2]

Column purified PCR products (GFX PCR DNA and Gel Band purification kit; Amersham-Biosciences, USA) were bi-directionally DNA sequenced using forward and reverse primers: J_H-in, MBR-in, mcr-in[5] and mcr-2in (5′-GTTACTCTTGCAGGGTTCT-3′) and analysed on an automated DNA sequence analyser (ABI3130xl Genetic Analyzer; Applied Biosystems, USA).

In silico DNA sequence analysis (nucleotide alignments, editions and annotations) were performed using EditSeq, MapDraw (LaserGene; DNA Star, USA), BLAST (Basic Local Alignment Search Tool) online (http://www.ncbi.nlm.nih.gov/blast/, accessed July 2009) and the Smith-Waterman algorithm online (http://jalingner.sf.net, accessed July 2009). Primer mcr-2in was designed using PrimerSelect software (LaserGene; DNA Star). Updated genomic DNA sequence versions encompassing the *BCL2* and breakpoint clusters on chromosome 18 and *IGH@* germline regions on chromosome 14 were obtained from GenBank accession NC_000018.8 and X97051.1, respectively.

A 40-year-old woman was referred to our institution in October 2005. Physical examination showed cervical and axillary adenopathies. Histological analysis of an axillary lymph node biopsy was consistent with FL, grade 1, according to the WHO classification. Bone marrow (BM) infiltration was also observed. Peripheral blood showed haemoglobin 13 g/dL, platelets 215×10^9/L, and white blood count 6.3×10^9/L. Serum level of LDH was 361 IU/L (normal $\leq$460 IU/L). For immunohistochemistry analysis, the following monoclonal antibodies were used: CD10, CD23, CD5 (Novocastra Laboratories, UK) and CD20 (Dako, Denmark). No Bcl-2 protein expression could be detected in tumour cells with the standard Bcl-2 antibody (Clone 124; Dako), which was raised against an epitope on residues 41–54 of Bcl-2 protein. Interestingly, when an alternative Bcl-2 (N-19) Sc-492 antibody (Santa Cruz Biotechnology, USA) was used, which epitope maps at the N-terminus of the human Bcl-2 protein, Bcl-2 protein expression was detected in all tumour follicles (Fig. 1a,b). Molecular study of the same lymph node biopsy showed the presence of both MBR and mcr rearrangements. The patient was diagnosed as FL, clinical stage IVA, FLIPI 1, and started with FND (fludarabine-mitoxantrone-dexamethasone) regimen (6 cycles). A complete clinical remission was achieved and after 44 months of follow up she had a favourable clinical course. The molecular analysis on BM cells after chemotherapy showed the persistence of mcr/J_H rearrangement, with reduced signal intensity, and complete loss of the MBR/J_H breakpoint, suggesting the presence of two different clones with a differential response to treatment.

The specificity of the double rearrangement was confirmed by repeated independent PCR amplifications analysed by agarose gel electrophoresis, and DNA sequencing (Fig. 2a,b, respectively). To rule out contamination with amplimers obtained from other positive samples, several MBR/J_H and mcr/J_H rearrangements from the same period of time as our case were DNA sequenced and no evidence of matching between breakpoints or N-segment sequences were observed. In this case, MBR and mcr breakpoints occurred on nucleotides 58,944,441 and 58,915,444 (NC_000018.8), and J_{H2} and J_{H6b} on nucleotides 87,785 and 89,773 (X97051.1), respectively (Fig. 2B).

In addition, both *BCL2*/*IGH@* junctions (i.e., MBR/J_H and mcr/J_H rearrangements) were BLASTed against all human genomic databases using non-stringent parameters in order to explore the presence of template nucleotide

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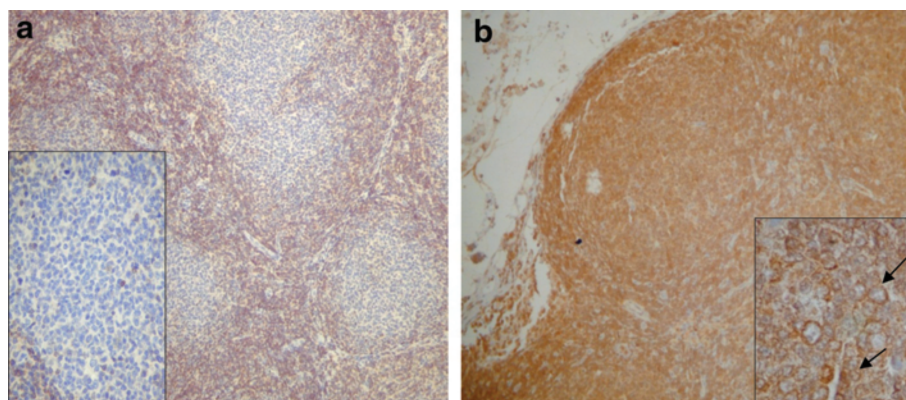

Fig. 1 Immunohistochemical staining of follicular lymphoma biopsy for the Bcl-2 protein. (a) No Bcl-2 protein was detected with the standard antibody (clone 124; Dako) Inset: higher power. (b) Bcl-2 protein expression in all tumour follicles with the alternative antibody (Clone N-19 Sc-492; Santa Cruz). Inset: BCL2 positive centroblasts (long arrow) and centrocytes (short arrow).

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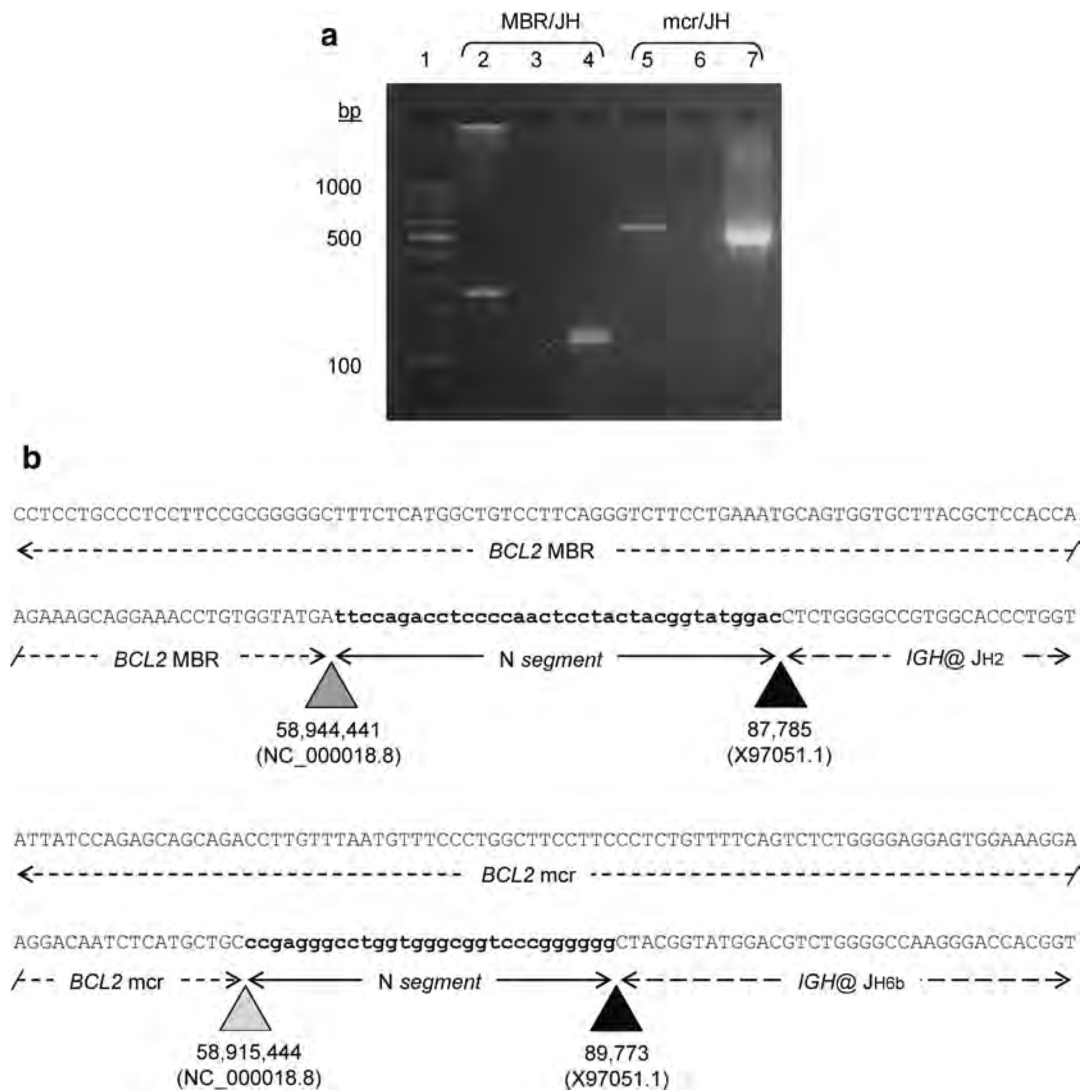

Fig. 2 (a) EtBr-stained 2% agarose gel electrophoresis showing double MBR/J_H and mcr/J_H rearrangements by nested-PCR. Lane 1, ladder of 100 bp (Biodynamics, Argentina; Cat# B040-50). Lanes 2 and 5, positive controls for MBR and mcr, respectively. Lanes 3 and 6, negative controls. Lanes 4 and 7, MBR and mcr rearrangements of our patient (approximately 140 bp for MBR and 500 bp for mcr). (b) DNA sequences of MBR/J_H (upper panel) and mcr/J_H (lower panel) rearrangements from our case. Grey and black triangles indicate the exact breakpoint locations on chromosomes 18 and 14, respectively. N-segments are highlighted in bold. The involved *BCL2* cluster region and the specific J_H unit are indicated.

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junction insertions. No evidence of such homologous templates for these N-junction insertions was observed.

The *BCL2/IGH@* rearrangement is the molecular marker of FL, and it is a very useful tool to monitor early recurrence as well as minimal residual disease. A small number of patients show a double *BCL2* gene rearrangement, an event that has been scarcely reported in the literature. This report describes a biclonal FL patient with both MBR and mcr rearrangements, who was detected among a series of 110 FL patients studied at the molecular level in our laboratory, thus representing 0.9% of total cases. FL is characterised by t(14;18)(q32;q21) that directly results in Bcl-2 overexpression. Our case did not show Bcl-2 protein expression with the standard Bcl-2 antibody. However, Bcl-2 expression was observed when the alternative Bcl-2 (N-19) Sc-492 antibody was used. Schraders *et al.*[6] found that a subset of FL cases with a t(14;18) contained somatic mutations in the translocated *BCL2* gene, resulting in amino acid substitutions in the region of the epitope recognised by the antibody, that determined false negative cases. These findings suggest that our patient may have somatic mutations on both translocated *BCL2* genes; to the best of our knowledge, this is the first case reported with these characteristics.

The presence of double rearrangement was accurately demonstrated by N-PCR and DNA sequencing. Molecular studies also included the analysis of *BCL2/IGH@* junctions to identify template nucleotide insertions, which were not found in our case. Of note, a previous study of t(14;18) in FLs showed templated nucleotide insertions in more than 30% of the breakpoints junctions,[7] suggesting that this translocation is a more complex process than previously thought, involving the interaction and/or subversion of V(D)J recombination with its enzymatic machinery.

The coexistence of PCR products from both MBR and mcr rearrangements has been scarcely reported in the literature,[8–10] representing only 0.5–4.0% of *BCL2/IGH@* positive FL patients (Table 1). These frequencies mostly

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Table 1 Double *BCL2/IGH@* gene rearrangements in follicular lymphoma patients

Reference	Method	Total cases	*BCL2* positive cases (%)	Double rearrangement (no. cases)	Frequency (%)
Price *et al.*[8]	N-PCR	44	24 (54)	MBR-MBR (6)	6/24 (25)
				MBR-mcr (1)	1/24 (4)
Peth *et al.*[9]	N-PCR	62	42 (68)	MBR-mcr (1)	1/42 (2)
Weinberg *et al.*[10]	QRT-PCR	236	185 (78)	MBR-icr (5)	8/185 (3)
				MBR-3′BCL2 (2)	2/185 (1)
				MBR-5′mcr (1)	1/185 (0.5)
Present study	N-PCR	110	85 (77)	MBR-mcr (1)	1/85 (1)

MBR, major breakpoint region; mcr, minor cluster region; N-PCR, nested PCR; QRT-PCR, real time PCR.

coincide with our data (1/85), which was fully checked by DNA sequencing.

There is no reference in the literature about the clinical characteristics of patients with double *BCL2/IGH@* breakpoints. The only exception is the case reported by Nomdedéu *et al.*[11] with both MBR and vcr rearrangements that showed a blastic transformation related to a p53 gene mutation. Our case showed a favourable clinical course, suggesting that the presence of two different clones would not be related to a more aggressive disease. Moreover, the presence of mcr-J_H rearrangement in the last molecular study may represent residual t(14;18) positive cells that can still be found in patients who experience major clinical responses. Finally, *BCL2/IGH@* positive cells can be detected in healthy individuals. Although no link to presence of this translocation and later development of FL could be established,[12] this possibility cannot be discarded in this case.

In conclusion, to our knowledge, this is the first evidence of the coexistence of MBR and mcr *BCL2* rearrangements confirmed by DNA sequencing. This FL patient was negative for the standard Bcl-2 antibody but positive for an alternative Bcl-2 epitope and presented a favourable clinical course, although more reports on the clinical characteristics of similar FL cases with confirmed biclonal *BCL2* rearrangements are necessary to determine its prognostic significance.

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1. Rowley JD. Chromosome studies in the non-Hodgkin's lymphomas: The role of the 14;18 translocation. *J Clin Oncol* 1988; 6: 919–25.
2. Noriega MF, De Brasi C, Narbaitz M, Slavutsky I. Incidence of BCL-2 gene rearrangements in Argentinean non-Hodgkin lymphoma patients: Increased frequency of breakpoints outside of MBR and mcr. *Blood Cells Mol Dis* 2004; 32: 232–9.
3. Harris NL, Swerdlow SH, Nathwani BN, *et al.* Follicular lymphoma. In: Swerdlow SH, Campo E, Harris NL, *et al.*, editors. *WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues*. Lyon: IARC Press, 2008: 220–6.
4. Solal-Céligny P, Roy P, Colombat P, *et al.* Follicular lymphoma international prognostic index. *Blood* 2004; 104: 1258–65.
5. Gribben JG, Freedman AS, Woo SD, *et al.* All advanced stage non-Hodgkin's lymphomas with a polymerase chain reaction amplifiable breakpoint of bcl-2 have residual cells containing the bcl-2 rearrangement at evaluation and after treatment. *Blood* 1991; 78: 3275–80.
6. Schraders M, de Jong D, Kluin P, Groenen P, van Krieken H. Lack of Bcl-2 expression in follicular lymphoma may be caused by mutations in the *BCL2* gene or by absence of the t(14;18) translocation. *J Pathol* 2005; 205: 329–35.
7. Jäger U, Böcskör S, Le T, *et al.* Follicular lymphomas BCL-2/IgH junctions contain templated nucleotide insertions: novel insights into the mechanism of t(14;18) translocation. *Blood* 2000; 95: 3520–9.
8. Price CG, Tuszynski A, Watt SM, Murdoch SJ, Lister TA, Young BD. Detection of additional JH/BCL2 translocations in follicular lymphomas. *Leukemia* 1991; 5: 548–54.
9. Peh SC, Shaminie J, Tai YC, Tan J, Gan SS. The pattern and frequency of t(14;18) translocation and immunophenotype in Asian follicular lymphoma. *Histopathology* 2004; 45: 501–10.
10. Weinberg OK, Weiyun ZA, Mariappan MR, Isaacson PG, Piris MA, Berger F. Minor BCL2 breakpoints in Follicular Lymphoma. Frequency and correlation with grade and disease presentation in 236 cases. *J Mol Diagn* 2007; 9: 530–7.
11. Nomdedéu J, Baiget M, Gaidano G, *et al.* p53 mutation in a case of blastic transformation of follicular lymphoma with double bcl–2 rearrangement (MBR and vcr). *Leuk Lymph* 1998; 29: 595–605.
12. Schmitt C, Balogh B, Grundt A, *et al.* The bcl-2/IgH rearrangement in a population of 204 individuals: Occurrence, age and gender distribution, breakpoints, and detection method validity. *Leukemia Res* 2006; 30: 745–50.

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Balloon cell neurofibroma

Sir,
Neurofibroma with clear cell change ('balloon cell neurofibroma') has only once been reported in the literature.[1] We report the second case of this unusual lesion.

A healthy 29-year-old woman presented with a raised skin lesion on the upper back, which was long standing, but had recently increased in size. She had no history of

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