

## CASE REPORT

### A case of t(14; 18)-negative follicular lymphoma with atypical immunophenotype: usefulness of immunoarchitecture of Ki67, CD79a and follicular dendritic cell meshwork in making the diagnosis

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#### Abstract

Follicular lymphoma is characterised by the t(14;18)(q32;q21) chromosomal translocation causing BCL2 protein overexpression. A proportion of follicular lymphomas do not carry the t(14;18) translocation and lacked BCL2 protein expression. We describe a case of a BCL2 protein- and t(14;18)-negative follicular lymphoma that caused diagnostic difficulty. The usefulness of several immunomarkers including Ki67, CD79a and CD21 in aiding the diagnosis is discussed. The patient is a 51-year-old male who presented with gradually enlarging lymphadenopathy. Histopathological examination of the lymph node showed complete architectural effacement by neoplastic follicles containing expanded CD21-positive follicular dendritic cell meshwork. The neoplastic cells expressed pan-B cell markers (CD20, CD79a) and germinal centre marker (BCL6) but not BCL2 and CD10. Of interest are the staining patterns of Ki67 and CD79a. We observed that the Ki67-positive proliferating cells were evenly distributed within the neoplastic follicles without zonation. In addition, CD79a was homogeneously strong within the neoplastic follicles. These staining patterns were distinctly different from that observed in reactive lymphoid follicles. Fluorescent *in-situ* hybridisation (FISH) analysis however showed absence of BCL2 gene rearrangement. Despite the atypical immunophenotype and lack of BCL2 gene rearrangement, the diagnosis of follicular lymphoma was made based on careful observation of the morphology as well as immunoarchitecture of the Ki67, CD79a and CD21 markers.

**Keywords:** Follicular lymphoma, t(14;18)-negative, BCL2 negative, CD10 negative

#### INTRODUCTION

Follicular lymphoma characteristically carries the t(14;18)(q32;q21) translocation resulting in BCL2 protein overexpression.<sup>1</sup> Interestingly, a proportion of follicular lymphoma lacked the t(14;18) translocation and the BCL2 protein expression is absent.<sup>2</sup> Here we describe a case of t(14;18)-negative follicular lymphoma that lacked BCL2 protein expression, which caused diagnostic difficulty.

#### CASE REPORT

The patient is a 51-year-old man, who presented with gradually enlarging bilateral inguinal and cervical lymphadenopathy of six months duration. This was later associated with fever and night sweats. His past medical history was otherwise

insignificant. A biopsy of the cervical lymph node was performed. This case was referred to our institution for consultation.

#### Pathological findings

Histopathologically, the lymph node showed complete effacement of nodal architecture by neoplastic follicles populated by centroblasts and centrocytes. The follicles were large, closely packed and vaguely formed (Fig. 1A). Germinal centre zonation (light and dark zones) and tingible body macrophages, which are typically seen in reactive germinal centres, were absent here. The centroblast count was more than 15 per high power field. Diffuse transformation occupying approximately 20% of areas was noted (Fig. 1A).

The neoplastic cells strongly expressed CD20, CD79a and BCL6 (Dako, Denmark), the latter confirming its germinal centre cell origin. The pattern of CD79a is of interest because its labelling in the neoplastic follicles was distinct from that generally observed in reactive lymph nodes. In reactive follicles, CD79a is weaker in the germinal centre and stronger in mantle zone (Fig. 1B). In contrast, in neoplastic follicles, CD79a is homogeneously strong in both germinal centre and mantle zone (Fig. 1B). This differential staining pattern is useful in distinguishing neoplastic from reactive follicles. Interestingly, both the neoplastic follicles and transformed area were BCL2-negative (tested with two BCL2 antibodies, clone BCL2/124, Dako, and clone E17, Epitomics, USA that recognised different epitopes) (Fig. 1C). They were also negative for CD10 (Dako). Follicular dendritic cell (FDC) marker, CD21 (Dako) highlighted the intact expanded FDC meshwork within the neoplastic follicles (Fig. 1C), and was absent in the diffuse areas. In addition, CD43 and post germinal centre marker, MUM-1 were negative.

The Ki67 staining pattern was closely studied. In neoplastic follicles, the proliferative rate was approximately 50%. Of note, it was observed that the Ki67-positive proliferating cells were evenly distributed throughout each follicle (Fig. 2A). The typical zonation observed in reactive germinal centre was absent (Fig. 2A). Furthermore, the interfollicular proliferation fraction was lower (approximately 30%) than that in neoplastic follicles, but higher than that expected in a reactive lymph node. Within the transformed area, proliferation rate was 60%. The T cell marker CD3 was also useful. Although absent in the neoplastic cells, it highlighted the follicular pattern with reactive T cells rimming the unstained follicles (Fig. 2B). CD5 and cyclin D1 (Dako) were negative ruling out other low grade B-cell lymphomas especially chronic lymphocytic leukaemia/small lymphocytic lymphoma, mantle cell lymphoma and marginal zone lymphoma.

Fluorescent *in-situ* hybridisation (FISH) analysis using *BCL2* DNA break-apart probes (Dako) showed normal signals i.e. there was no evidence of *BCL2* gene rearrangement (Fig. 2B). Final diagnosis of diffuse large B-cell lymphoma (20%) with follicular lymphoma, grade 3A (80%) was made. The patient unfortunately was lost to follow-up.

## DISCUSSION

This report describes a case of follicular lymphoma with unusual immunophenotype (BCL2-negative, CD10-negative) and cytogenetic profile (normal *BCL2* gene status) in a 51-year-old man. The diagnosis was aided by immunohistological pattern of FDC meshwork, CD79a and distribution of the (Ki67-positive) proliferating cells.

Absence of the *BCL2* gene rearrangement, which is more frequently observed in high grade follicular lymphomas,<sup>3</sup> caused absence of BCL2 protein expression. Of interest, BCL2 protein may also be “absent” in a minority of t(14;18) translocation-positive lymphomas due to somatic mutations, inhibiting epitope recognition by the standard BCL2/124 antibody.<sup>4,5</sup> In the present case we had ruled out the possibility of pseudonegative BCL2 expression by testing with both BCL2/124 and E17 antibodies for the protein and by FISH analysis.

Being a germinal centre-derived tumour, most follicular lymphomas express CD10 and BCL6, within the neoplastic follicles as well as in interfollicular regions.<sup>6</sup> CD10 expression however is variable in different grades of follicular lymphoma<sup>7,8</sup> particularly in high grade tumours, posing considerable diagnostic difficulty.<sup>8</sup>

Careful study of the architecture and cell morphology in the present case including attenuation of mantle zones and absence of tingible body macrophages provided important clues to diagnosis.<sup>3</sup> Reactive lymph nodes on the other hand display variable-sized secondary follicles with tingible body macrophages and a well-defined mantle zone. CD21 was useful because it highlighted the FDC meshwork, which are large and expanded in the follicular area and absent in the transformed zone.<sup>3</sup>

Follicular lymphoma proliferative index increases with higher histological grade and is a predictor of poor survival.<sup>9</sup> Most grade 1–2 follicular lymphomas show low proliferation fraction while grade 3 cases exhibit high proliferation rate. However, to date, there is no standard cut-off value for Ki67 proliferative index (varies from 20% to 45%) to distinguish indolent from aggressive lymphoma.<sup>3,9</sup> Hence, it should not be used as a determinant of tumour grading.

Of importance in this report is the unique pattern of the proliferating cell distribution as highlighted by Ki67. The zonation which is

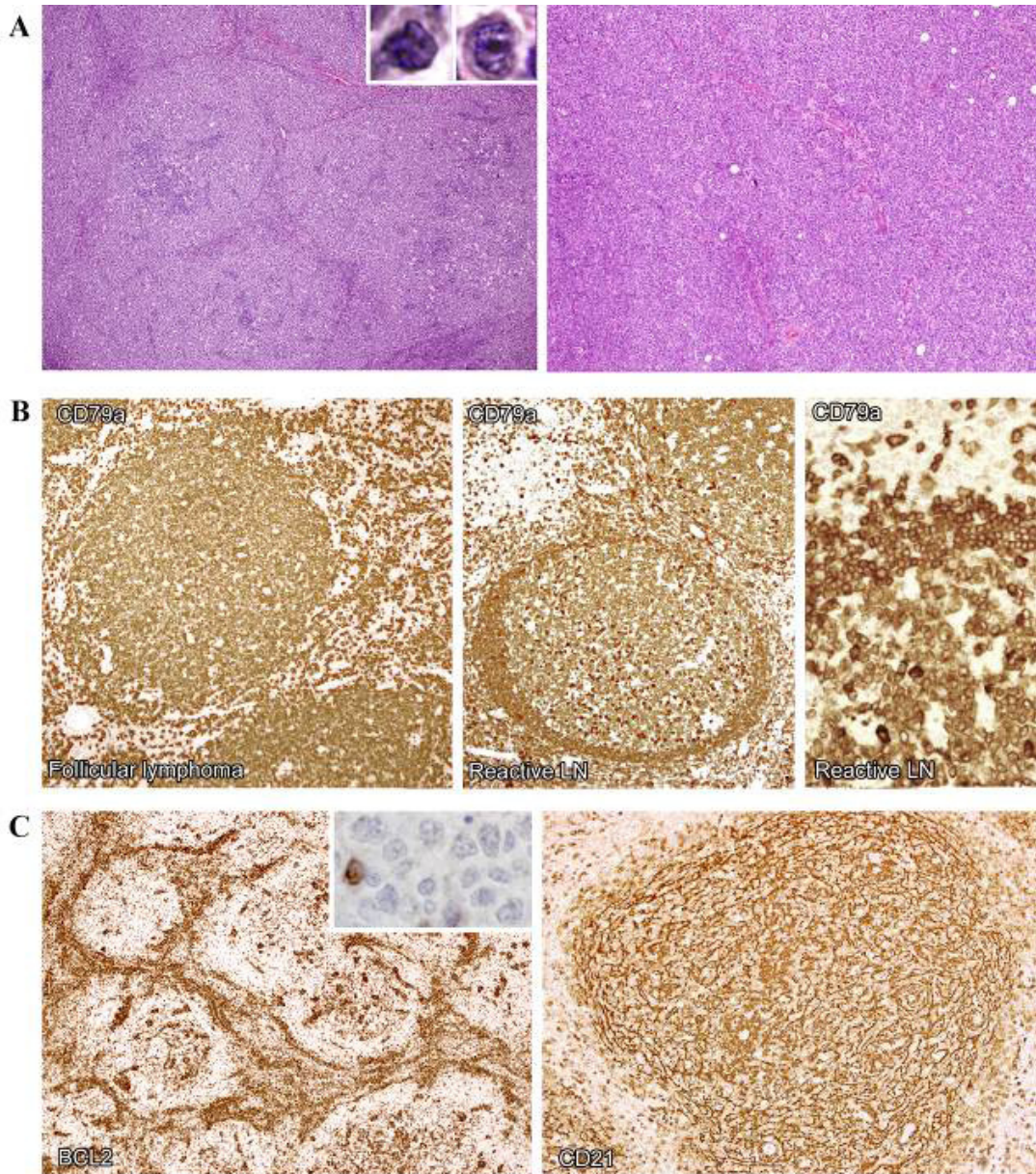


FIG. 1: Follicular lymphoma. (A) (Left) There is complete effacement of normal nodal architecture, replaced by vaguely formed neoplastic follicles, H&E x40. The neoplastic cells consist of centroblasts and centrocytes (inset, H&E x600). (Right) Diffuse area consistent with large cell transformation, H&E x40. (B) (Left) Labelling for CD79a shows strong expression in both neoplastic follicles and surrounding cells, CD79a x100. (Middle and right) In contrast, the staining pattern of CD79a in a reactive lymph node shows strong labelling of the mantle zone cells and weaker staining of the germinal centre cells, CD79a x100 (middle), x400 (right). (C) (Left) The neoplastic follicles are negative for BCL2, BCL2 x100. Inset shows BCL2-negative tumour cells at higher magnification, BCL2 x400. (Right) CD21 highlights the intact and expanded follicular dendritic cell meshwork of the neoplastic follicles, CD21 x100.

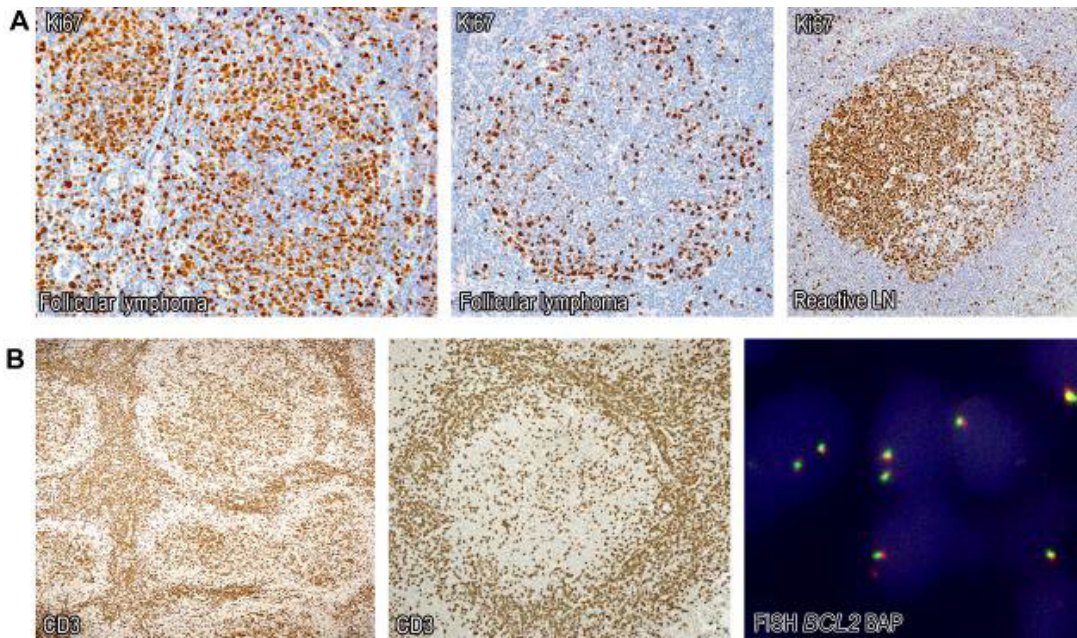


FIG. 2: Follicular lymphoma. (A) (Left and middle) Ki67-positive proliferating cells are evenly distributed and show a peripheral distribution within the neoplastic follicles, Ki67 x40. The zonation pattern that is seen in a reactive lymph node (right) is lost in the neoplastic follicles, Ki67 x100. (B) (Left and middle) CD3 highlights clearly the follicular pattern of the neoplastic follicles since the reactive T cells rim the unstained follicles, CD3 x40 (left), x100 (middle). (Right) Fluorescent *in situ* hybridisation (FISH) analysis using *BCL2* break-apart probe (BAP) shows normal red/green signals in the nuclei of neoplastic cells confirming the absence of *BCL2* gene rearrangement, FISH x1000. LN = lymph node; FISH = fluorescent *in situ* hybridisation; BAP = break-apart probe

usually seen in reactive germinal centres as a result of different cell proliferation rate is lost in neoplastic follicles. In follicular lymphoma, the proliferating cells are diffusely and evenly distributed without obvious zonation pattern.<sup>10</sup> In addition, the frequent rimming of Ki67-positive proliferating cells around neoplastic follicles (not present in this case but observed in other cases) (Fig. 2A) is a helpful pattern to note. Finally it was noted that CD79a labelling was uniformly strong in the neoplastic follicles, without the contrast observed between germinal centre and mantle zone in reactive follicles.

#### Conclusion

The diagnosis of follicular lymphoma that lacked the characteristic immunophenotype and *BCL2* gene rearrangement, although challenging, can be achieved with careful observation of lymph node architecture and cellular morphology, patterns of follicular dendritic cell meshwork and CD79a expression as well as absence of “germinal centre pattern” of the proliferating cells.

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