Case Report
Utility of immunohistochemistry with an antibody against MYC at the initial diagnosis of follicular lymphoma, grade 3A, for predicting a more aggressive clinical course: a case report and review of the literature

Kunimoto Ichikawa1,2, Nanae Aritaka3, Kanako Ogura4, Masaru Hosone5, Yasunori Ota6, Eriko Sato3, Norio Komatsu1, Takao Hirano3

1Department of Internal Medicine, Division of Hematology, Juntendo University School of Medicine, Tokyo, Japan; 2Department of Hematology, Juntendo University Urayasu Hospital, Chiba, Japan; 3Department of Hematology, Juntendo University Nerima Hospital, Tokyo, Japan; 4Department of Pathology, Juntendo University Nerima Hospital, Tokyo, Japan; 5Department of Diagnostic Pathology, Juntendo University Nerima Hospital, Tokyo, Japan; 6Department of Pathology, Institute of Medical Science, The University of Tokyo, Tokyo, Japan

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Abstract: Follicular lymphoma (FL) is the most common indolent lymphoma, and associated with the chromosomal translocation t(14;18)(q32;q21). While, FL harboring both BCL2 and MYC translocation at diagnosis is very rare. The evaluation of MYC expression in typical FL at presentation using southern blot, G-banded karyotyping or fluorescence in situ hybridization (FISH) analyses has been described so far. However, there are no reports about the use of immunohistochemistry (IHC) to evaluate MYC protein expression in FL at presentation. Here, we present a FL patient who transformed to a B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt's lymphoma, accompanied by concurrent BCL2, BCL6, and MYC translocations; i.e., triple-hit lymphoma. Paraffin-embedded tissue section-FISH analysis demonstrated that the FL was negative for MYC, but MYC protein expression was subsequently detected in the lymph node specimen obtained at the initial diagnosis using IHC. This case revealed aggressive clinical course and central nervous system involvement. In the literature concerning MYC positive FL five out of 8 patients were dead within 24 months. The detection of MYC protein expression in FL using IHC might be useful to predict more aggressive clinical course.

Keywords: BCL2, MYC, BCL6, transformation, follicular lymphoma, triple-hit lymphoma

Introduction
Follicular lymphoma (FL) is a common subtype of indolent B-cell lymphoma and characterized by the translocation t(14;18)(q32;q21) and BCL2 gene rearrangements in cytogenetic studies [1]. MYC translocations are found in 80-90% of Burkitt’s lymphoma cases, 5-15% of diffuse large B-cell lymphoma (DLBCL) cases [2, 3] and 2-8% of FL cases [4,5-6]. However, many of MYC positive FL were FL, grade 3B. While, typical low-grade FL harboring both BCL2 and MYC translocations at diagnosis is very rare [4, 5, 7-9]. In the past reports MYC translocation in FL at diagnosis was detected using southern blot, G-banded karyotyping or fluorescence in situ hybridization (FISH) analyses.

Herein, we describe a case of FL, grade 3A, in which MYC split signal was negative in paraffin-embedded tissue section (PS)-FISH analysis but MYC protein expression was detected in immunohistochemistry (IHC) in the lymph node (LN) specimen at diagnosis, and rapidly progressed to triple hit lymphoma (THL), resulting in an early death.

Case report
An 80-year-old woman presented with fever and systemic lymphadenopathies. Her labora-
Myc protein expression in follicular lymphoma

Laboratory data were as follows: hemoglobin (Hb), 10.5 g/dl (normal range: 11.1-15.2 g/dl); lactate dehydrogenase (LDH), 527 IU/l (normal range: 119-221 IU/l); ferritin, 207 ng/ml (normal range: 3.6-114.0 ng/ml); and soluble interleukin-2 receptor (sIL2R), 5020 IU/l (normal range: 145-519 U/ml). A contrast-enhanced computed tomography (CT) scan detected systemic lymphadenopathies, the largest of which (diameter: 2.5 cm) was located in the inguinal region. A pathological investigation of the left inguinal lymph node (LN) biopsy specimen demonstrated a mixture of large centroblast-like cells and medium-sized cells with irregular nuclei (Figure 1A). The neoplastic cells were positive for CD10, CD20, CD79a, PAX5, BCL2, BCL6, and IRF4/MUM1, but negative for CD3, CD5, CD30, and Epstein-Barr virus-encoded RNA-1 (EBER-1) (CD20; Figure 1B), and displayed an MIB1 proliferation index of 30% (Figure 1C). In part of the specimen, a few irregular neoplastic follicles, which contained follicular dendritic cell (FDC)-like cells (CD21/CD23/CD35), were observed. FISH analysis indicated that 4% of the cells were positive for a fusion signal involving the immunoglobulin heavy chain (IGH) and BCL2 (IgH/BCL2). PS-FISH analysis demonstrated that the cells were negative for the MYC split signal, but 32% of them were positive for BCL6. The karyotype of the specimen could not be obtained due to a lack of mitotic cells. The patient was diagnosed with FL, grade 3A. Fluorodeoxyglucose (FDG)-positron emission tomography (PET) detected significantly intense FDG uptake (maximum standardized uptake value (SUVmax): 6.0) in multiple lesions. A bone marrow (BM) aspirate examination conducted 3 weeks after the LN biopsy showed that 30% of the cells were large abnormal lymphocytes with irregular nuclei, prominent nucleoli, and cytoplasmic vacuoles (Figure 2A). During IHC, the BM specimen demonstrated diffusely distributed proliferating atypical large cells, which were positive for...
CD20, BCL2, and BCL6, but negative for EBER-1 (Figure 2B and 2C), and displayed an MIB1 proliferation index of 50% (Figure 2D). Chromosomal analysis involving G-banded karyotyping and spectral karyotyping (SKY) detected complex chromosome abnormalities (Figure 3A and 3B), including the addition of an MYC translocation. By the time she was admitted for treatment (40 days after the LN biopsy), the patient's laboratory findings had deteriorated markedly (Hb, 6.9 g/dl; LDH, 5782 IU/l; and ferritin, 6063 ng/ml). Based on these findings and the observed histological and cytogenetic alterations, it was considered that the FL had transformed to a B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt's lymphoma, accompanied by concurrent BCL2, BCL6, and MYC translocations in the BM; i.e., THL.

The patient was initially treated with the rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone regimen. Her clinical findings improved, but somnolence appeared on the first day of the third course of treatment. Contrast-enhanced magnetic resonance imaging (MRI) of the brain detected several lesions in the bilateral frontal lobes and dilatation of the third ventricle. The cerebrospinal fluid contained large lymphocytes with irregular nuclei, prominent nucleoli, and cytoplasmic vacuoles. Flow cytometry showed that these cells expressed lambda type immunoglobulin light chains. Although the patient was treated with dexamethasone plus 40 Gy of whole brain irradiation, she died at 5 months after being diagnosed because of disease progression.

IHC subsequently demonstrated that the LN tissue obtained at presentation exhibited 40-50% positivity for the MYC protein (Figure 1D).

Discussion

In our case, although PS-FISH analysis found that the FL was negative for the MYC split sig-
nal, IHC detected MYC protein expression in the LN specimen, and the lesion rapidly progressed to THL, resulting in an early death.

Gene expression studies of paired samples demonstrate that FL transformation is associated with changes in expression of MYC and its

### Table 1. The characteristics of MYC-positive follicular lymphoma or low-grade lymphoma with t(14;18) (q32;q21) cases reported before

<table>
<thead>
<tr>
<th>Case</th>
<th>Diagnosis</th>
<th>Investigation tools and results for MYC</th>
<th>CNS involvement</th>
<th>Outcome</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FL</td>
<td>Southern blot</td>
<td>MYC rearrangement</td>
<td>NA</td>
<td>Dead (20M) [5]</td>
</tr>
<tr>
<td>2</td>
<td>FL, G1</td>
<td>G-banded karyotyping</td>
<td>t(8;22)(q24;q11)</td>
<td>Yes</td>
<td>Dead (24M) [7]</td>
</tr>
<tr>
<td>3</td>
<td>FL, G1</td>
<td>G-banded karyotyping</td>
<td>Not detected</td>
<td>Yes</td>
<td>Dead (6M) [8]</td>
</tr>
<tr>
<td>4</td>
<td>Low-grade</td>
<td>G-banded karyotyping</td>
<td>t(8;14)(q24;q32)</td>
<td>NA</td>
<td>Alive (7M) [9]</td>
</tr>
<tr>
<td>5</td>
<td>FL, G2</td>
<td>G-banded karyotyping</td>
<td>c-MYC rearrangement</td>
<td>No</td>
<td>Alive (62M) [4]</td>
</tr>
<tr>
<td>6</td>
<td>FL, G2</td>
<td>G-banded karyotyping</td>
<td>t(2;8)(p12;q24)</td>
<td>No</td>
<td>Alive (42M) [4]</td>
</tr>
<tr>
<td>7</td>
<td>FL, G2</td>
<td>G-banded karyotyping</td>
<td>Add(8)(q24)</td>
<td>Yes</td>
<td>Dead (49M) [4]</td>
</tr>
<tr>
<td>8</td>
<td>FL, G3</td>
<td>G-banded karyotyping</td>
<td>Lack of mitotic cells</td>
<td>Yes</td>
<td>Dead (5M)</td>
</tr>
<tr>
<td>(Present case)</td>
<td>PS-FISH</td>
<td>Not detected</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IHC</td>
<td>Yes, 40-50%</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Ref., references; FL, follicular lymphoma; NA, not available; M, months; G1, G2, and G3, grade 1, 2, and 3; FISH, fluorescence in situ hybridization; BCL, B-cell lymphoma; PS-FISH, paraffin-embedded tissue section-FISH; IHC, immunohistochemistry.
target genes [10-12]. It has been reported a lot concerning high grade transformation of FL due to acquisition of MYC. However, MYC positive FL cases at the initial diagnosis are quite rare, in which it has been proposed that FL harboring both BCL2 and MYC translocations using southern blot, G-banded karyotyping or FISH analyses at presentation might represent a more aggressive subtype of the disease, even if they exhibit a low-grade morphology [4, 5, 7-9]. However, no reports have described the use of IHC to evaluate MYC protein expression in FL at the initial diagnosis. This case report, therefore, has a precious value.

According to the laboratory findings, B symptom and pathological investigation, it is difficult to perfectly deny just the observation of a high percentage of MYC protein-high tumor cells in the indolent phase of a synchronously present transformed FL in a single patient. However, the FDG uptake values (SUVmax) detected in the PET were as low as 6.0. Generally, in DLBCL much more intense FDG uptake would be shown in the PET. Furthermore, the dramatic deterioration in the laboratory findings was seen after the BM biopsy. Therefore, it was suggested that the explosive disease progression occurred between the LN and the BM biopsies. However, if MYC IHC high at the initial LN biopsy in this patient assumes synchronous present of transformed FL cells in typical FL, the utility of IHC with an antibody against MYC at the initial diagnosis of FL might enable the early identification of FL that has a more aggressive clinical course.

Tapia et al. detected significantly higher MYC protein expression in translocated DLBCL compared with non-translocated DLBCL (61% vs. 28%) [13]. MYC protein expression was demonstrated to be high in the context of an underlying MYC translocation, however up to 50% of MYC IHC-high cases are known to lack a MYC translocation. In the present case, the FL exhibited greater MYC IHC-high at presentation than the median value for non-translocated DLBCL [13].

In over-expression of MYC protein is not rare in non-Hodgkin’s lymphoma and immunostaining does not correlate directly with MYC translocation or prognosis [14]. Prognostication by MYC IHC and Southern analysis had yielded negative results [15]. On the other hand, it was reported that MYC and BCL2 protein expression in IHC predicts survival in patients with DLBCL treated with R-CHOP like regimens [16, 17].

The characteristics of MYC positive FL cases reported before were summarized in Table 1 [4, 5, 7-9]. At least four out of 8 patients who were diagnosed with FL concurrent with MYC at the initial diagnosis experienced transformation to aggressive lymphoma or leukemia. Central nervous system (CNS) involvement occurred in at least four of 8 patients. Five out of 8 patients were dead within 24 months. This summary is very small case series but suggests FL concurrent with MYC translocation might have a more aggressive clinical course in comparison to that of the typical FL without MYC. In FL MYC protein expression in IHC might not be necessarily correlated to the presence of MYC translocation, and which needs to be researched in larger series. MYC protein expression, however, might also contribute to the poor prognosis like the present case. It was suggested that the treatment strategy for MYC IHC high FL might need to be distinguished from that of typical FL without MYC protein expression.

In conclusion, the detection of MYC protein expression in FL using IHC might be useful to predict more aggressive clinical course. However, this needs to be confirmed in a larger trial.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Kunimoto Ichikawa, Department of Hematology, Juntendo University Urayasu Hospital, 2-1-1, Tomioka, Urayasu-shi, Chiba 279-0021, Japan. Tel: +81 47 353 3111; Fax: +81 47 381 5054; E-mail: kichika@juntendo.ac.jp

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