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Original contribution

Double-hit follicular lymphoma with *MYC* and *BCL2* translocations: a study of 7 cases with a review of literature☆

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Summary Follicular lymphoma with *MYC* and *BCL2* translocations, so-called double-hit follicular lymphoma (DH-FL), is rare. Here, we report the clinicopathological features of 7 cases of DH-FL. All neoplasms had a follicular pattern (1 partially diffuse). Five cases were predominantly low grade, 4 of which had focal (≤20%) grade 3A areas, and 2 cases were of grade 3. All cases were positive for pan–B-cell antigens, CD10, and BCL6; 6 cases were positive for BCL2. Ki-67 was less than or equal to 50% in 6 cases and 90% in 1 grade 3 case. Three patients presented with stage IV disease and 3 had a Follicular Lymphoma International Prognostic Index score of greater than 2. Six patients received immunochemotherapy, and 1 is still under induction therapy with rituximab, ibrutinib, and lenalidomide. Four achieved complete remission and two had a partial response with persistent or refractory disease. The median follow-up time was 25 months (range, 8.5-53.7 months). Two patients treated with standard regimen for follicular lymphoma had relapsed or refractory disease, and 1 died from complications of allogeneic stem cell transplant administered for relapse. In contrast, all 4 patients treated with more intensive regimen for double-hit lymphoma achieved complete remission. In summary, despite predominantly low-grade histology, cases of DH-FL in this study were aggressive and responded better to more intensive than standard treatment regimens, suggesting DH-FL is part of the spectrum of double-hit high-grade lymphoma.

1. Introduction

Follicular lymphoma (FL) is the most common low-grade lymphoma in the United States and in Western Europe [1]. The prognosis of affected patients is highly variable, and long-term 5-year survival rate range from 53% to 90% depending on risk factors [2,3]. Approximately 25% to 35% of patients with FL eventually progress to diffuse large B-cell lymphoma (DLBCL) [4]. Transformation is associated with acquisition of additional genetic abnormalities, and *MYC* translocation is one well-known additional genetic hit. The combination of *MYC* translocation with another oncogene translocation, most often *BCL2* (*MYC/BCL2*), is best known to occur in aggressive B-cell lymphomas, the so called

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double-hit lymphoma (DHL) [5,6]. In the literature, there are rare reports of low-grade B-cell lymphoma, including FL, with double-hit genetics, particularly *MYC* combined with *BCL2*. The term double-hit follicular lymphoma (DH-FL) has been used for these rare tumors [7-14]. The overall frequency of *MYC/BCL2* rearrangements is reported to be about 2% in FL, but the actual frequency may be higher as studies for *MYC* and *BCL2* gene rearrangements are not routinely performed in all cases [2,7]. As the standard regimen for FL (both low-grade and high-grade) and DHL are totally different, a better understanding of DH-FL has practical implications [15-17]. In this study, we report a series of 7 patients with DH-FL accessioned at our institution over a 10-year period. This experience suggests that cases of DH-FL, regardless of morphologic grade, tend to have an aggressive clinical course.

2. Materials and methods

2.1. Case selection

The database of our institution was searched for cases of FL associated with *MYC*/8q24 rearrangement and *IGH@BCL2*/t(14;18)(q32;q21) from January 2006 to February 2016. The presence of translocations was confirmed by conventional cytogenetic and/or fluorescence in situ hybridization (FISH) analysis. Three cases had been reported in a previous study from our institution as a part of a larger study on DHL [18,19]. Each case was diagnosed and classified using the 2008 World Health Organization (WHO) criteria [20,21]. The corresponding medical records were reviewed to obtain clinical data, including age, sex, sites of involvement, tumor stage at diagnosis, serum lactate dehydrogenase (LDH) levels, treatment regimens, response to therapy, and follow-up data. This study was approved by the internal review board of our institution.

2.2. Immunophenotyping

Immunohistochemical studies were performed using formalin-fixed, paraffin-embedded tissue sections either at the time of diagnosis or retrospectively for this study. After deparaffinization and dehydration of the sections in graded alcohols and xylene, endogenous peroxidase was blocked with hydrogen peroxide. Heat-induced epitope retrieval was performed using citrate buffer, pH 6.0. The panel of monoclonal antibodies used in this study included: CD3 (rabbit polyclonal), CD5 (clone 4C7), CD10 (clone 56C6), CD20 (clone L26), BCL2 (clone 124), BCL6 (clone PG-B6p), and Ki-67 (clone MIB-1) (Dako, Carpinteria, CA) and MYC (clone Y69; Ventana, Tucson, AZ).

2.3. Fluorescence in situ hybridization analysis

FISH was performed using formalin-fixed, paraffin-embedded tissue sections according to the manufacturer's protocols. FISH probes used in this study were all obtained from the same commercial source (Abbott Molecular, Downers Grove, IL) and included the following: LSI *C-MYC* dual-color, break-apart rearrangement probe; LSI *IGH@BCL2* dual-color, dual-fusion translocation or LSI *BCL2* dual-color, break-apart rearrangement probes; and LSI BCL6 dual-color break-apart rearrangement probe. A total of 200 interphase nuclei were analyzed for each probe. Most cases of low-grade FL were analyzed for *MYC* rearrangement by FISH at the outside institutions. Cases that were analyzed in our institution usually had a grade 3 component or a high Ki-67 rate.

3. Results

3.1. Clinical characteristics

The clinical features are summarized in Table 1. The study group included 7 patients, 2 men and 5 women, with a median age of 47 years (range, 34-64 years). All patients had lymph node involvement, and 4 patients also had extranodal disease involving the bone marrow, tonsil, spleen, and peripheral blood. Evidence of bone marrow lymphoma was documented in 3 (42.9%) of 7 patients. Three presented with stage IV disease, 3 stage III, and 1 stage II. Three patients had a Follicular Lymphoma International Prognostic Index score greater than 2. Serum LDH level was elevated in 3 of 6 patients with available data.

3.2. Morphological and immunophenotypic features

The morphological and immunophenotypic features are summarized in Table 2. Using the 2008 WHO classification criteria, all cases were classified as FL [21]. Morphologically, 6 neoplasms had an entirely or nearly exclusive follicular pattern (>75%), and only 1 case presented with a follicular and partially diffuse pattern (Fig. 1A). The follicles were composed of a mixture of centrocytes and centroblasts without blastoid features (Fig. 1B-C). Five cases were classified as predominantly low-grade FL (grade 1-2), though 4 of them also showed varying degrees of focal (≤20%) grade 3A (cases 3, 4, 6, and 7). In case 7, although a diffuse area was noted, the grade 3A components were confined to the follicles and therefore not considered DLBCL. The other 2 cases displayed mainly grade 3A (case 5) or 3A and 3B morphology (case 1). In case 5, the grade 3A DH-FL was diagnosed 33 months after an initial diagnosis of grade 2 FL that was untreated and observed and therefore considered disease progression.

Immunophenotypically, all tumors were of B-cell lineage, positive for one or more pan–B-cell antigens and negative for pan–T-cell antigens. BCL2 was positive in 6 of 7 neoplasms, and CD10 and BCL6 were positive in all 7 cases. No tumors analyzed showed more than 30% MYC-positive cells. Only 1 case showed a very high proliferation (Ki-67)