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Case Report

Double-hit and triple-hit lymphomas arising from follicular lymphoma following acquisition of MYC: report of two cases and literature review

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Abstract: Double-hit or triple-hit B-cell lymphomas (DHL and THL) are rare subtype lymphomas usually associated with poor prognosis. It is defined by two or three recurrent chromosome translocations; *MYC*/8q24 loci, usually in combination with the t (14; 18) (q32; q21) bcl-2 gene or/and BCL6/3q27 chromosomal translocation. DHL was often observed both in de-novo diffuse large B cell lymphomas (DLBCL). It is otherwise unclassifiable, showing features intermediate that of large B-cell lymphoma and Burkitt lymphoma. Here, we present two follicular lymphoma patients; one transformed to THL, another transformed to DHL. Both cases revealed aggressive clinical courses with poor prognosis and associated with acquisition of c-Myc gene (MYC) and central nervous system (CNS) involvement. We reviewed the related literature, correlated the immunophenotype and clinical manifestations such as response to therapy and prognosis. Although the incidence of DHT and THL is low, cytogenetic and FISH analyses should be included when B-cell lymphoma patients experience relapse or refractory course of disease. We concluded that c-Myc may contribute to aggressive transformation, and more mechanism-based therapy should be explored.

Keywords: Double hit, follicular lymphoma, *MYC*, *BCL2*, *BCL6*, cases report

Introduction

High-grade non-Hodgkin lymphomas have been associated with multiple cytogenetic abnormalities including *MYC* with *BCL6* or *BCL2* rearrangement. For example, it has been reported DLBCL contains chromosomal translocations involving the *BCL2* gene, which is the hallmark of follicular lymphoma (FL) in 10–40% of lymphoma cases [1]. It also contains the *MYC* proto-oncogene, the hallmark of Burkitt lymphoma (BL) in 5–15% of cases [2]. In 2011, Aukema et al. showed that chromosomal translocations were biological and diagnostic hallmarks of many B-cell lymphoma [3]. Recently, a new subset of lymphoma, one with concurrent *BCL2* and *MYC* translocations, has received great attention. It has been proposed that this unclassifiable entity could be called ''double-hit'' or ''triple-hit'' lymphoma depending on the number of aberrations present, such as *MYC* translocation in the setting of a complex karyotype with the addition of rearrangements in *BCL2* and, less commonly, *BCL6* [4]. DHL has the double disadvantage of *MYC* (proliferation) and *BCL2* (anti-apoptosis) [5]. For this reason, it has been shown to be highly aggressive with poor prognosis and a minimal response to therapy [6, 7]. Here, we report two cases of patients with double- and triple-hit lymphoma arising from low grade follicular lymphomas and aggressive clinical courses with CNS involvement.

Case report

Case 1

This is a 44-year-old female presented with pharyngeal discomfort for more than one month without any B symptoms such as generalized weakness, fever, weight loss, or night sweats in

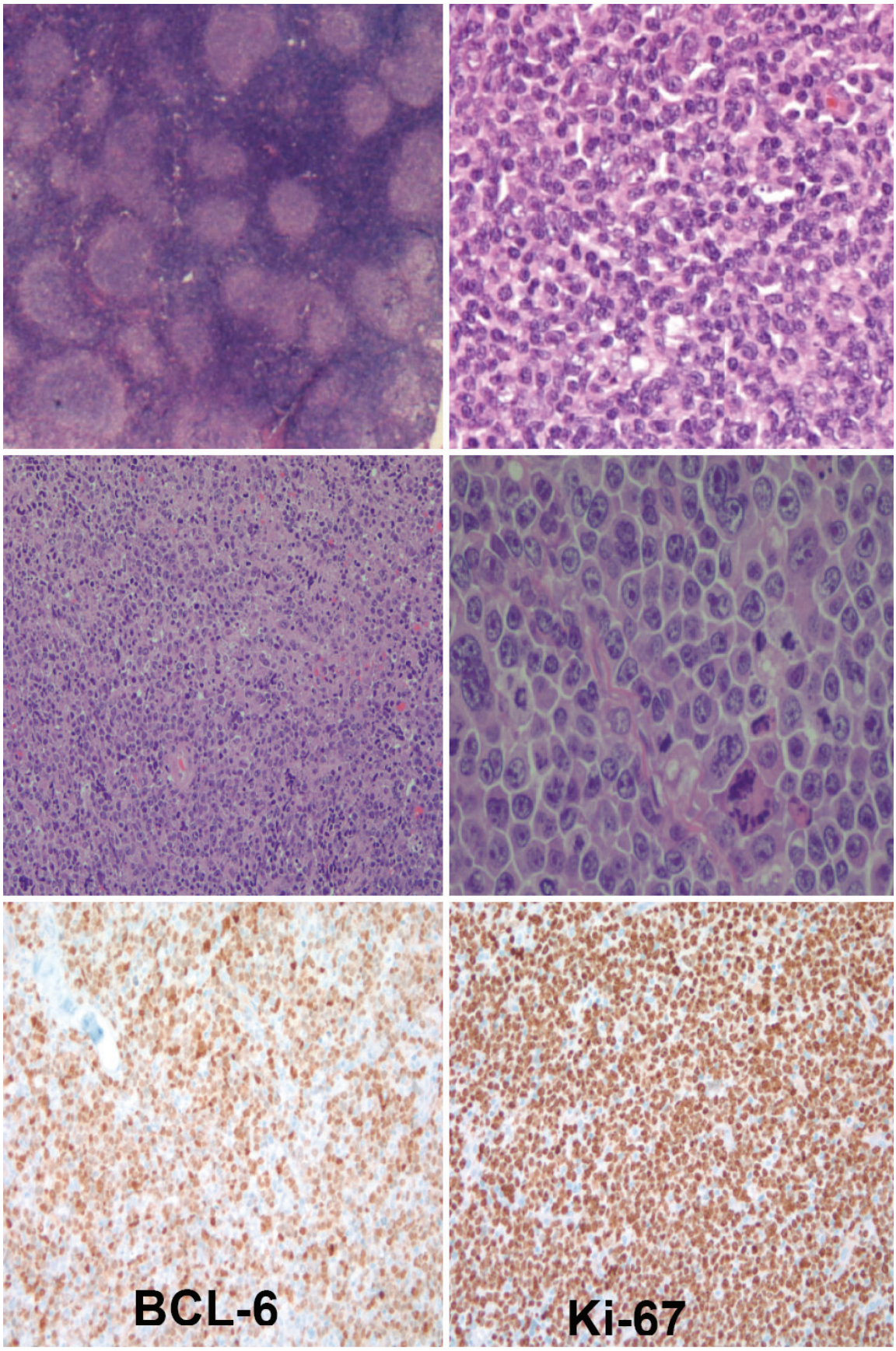
BCL-6
Ki-67

Figure 1. Case 1. Upper panel; tonsil mass excisional biopsy at the onset of disease showing follicular lymphoma, grade II. Left, low power (x40), and right, high power (x400) H&E staining showing follicular infiltrates and cleaved small and some large lymphocytes. Middle panel; scalp tumor resection at recurrence showing high grade large cell transformation. Left, low power (x40) shows diffuse large lymphocytic infiltrate with necrosis, and right, high power (x400) showing sheets of large atypical lymphocytes with prominent nucleoli and increased mitosis. Lower panel; Immunohistochemical analysis of the recurrent lymphoma showing BCL-6 (left) and Ki-67 (right) stain.

June 2011. Physical exam revealed a right tonsil mass of a soybean size. She was given antibiotics at a local hospital. No improvement was observed. She was then admitted to our hospital for further examination. Right-side tonsillectomy was performed and the pathology of tonsil mass showed morphology of a low grade follicular lymphoma. Immunohistochemical stains performed on paraffin blocks revealed CD20(+), BCL-2(+), CD10(+) and CD3(-) and Ki67 20-30%. A pathological diagnosis of follicular lymphoma, grade II was rendered (**Figure 1**). The laboratory examinations of peripheral blood, including the level of lactate dehydrogenase (LDH) and liver and kidney function were all within the normal range. B-ultrasonography showed multiple solid masses in the bilateral cervical, axillary, and inguinal regions and a subcutaneous mass on the left back. Bone marrow smear and biopsy were both within normal limit. The patient was staged IIIA and started with 6 cycles of R-CHOP chemotherapy in July 2011. After chemotherapy completion, the patient's PET-CT revealed no significant radioactive activity; she had achieved complete remission (CR) and underwent autologous hematopoietic stem cell transplantation (Auto-HSCT) in December 2011 and was discharged.

In February 2012, the patient was hospitalized again with the presentation of enlarging right forehead subcutaneous mass. Emission computed tomography (ECT) showed abnormal accumulation in the right frontal bone. The cranial CT scan revealed occupied lesions in the skull and subcutaneous tissues in the right forehead. The patient underwent scalp tumor resection and repair of skull defect with inactivated autogenous cranial bone flap. The biopsy showed sheets of large atypical lymphocytes with brisk mitosis. Immunohistochemical analysis of the biopsy mass showed CD79a(+), KI-67>95%(+), CD3(+), CD20(+), CD10(+), BCL2(+), and BCL6(+) (**Figure 1**). Fluorescence in situ hybridization (FISH) confirmed both c-MYC/IGH and BCL2/IGH rearrangement and BCL6 (3q27) +. The patient was pathologically diagnosed with B-cell lymphoma, unclassifiable, with features intermediate between large B-cell lymphoma and Burkitt lymphoma. One week after the operation, the patient again found subcutaneous mass on the right size of her forehead. It was about 1×1 cm in size. PET-CT studies revealed multiple abnormal accumulations in the right frontal bone and right temporal bone, tumors in the anterior cranial fossa protruding into the sphenoid sinus, and multiple lymphadenopathies in the portacaval and retroperitoneal regions and around the abdominal aorta. These findings appeared highly suggestive of recurrence of lymphoma involving central nervous system and other systems. It was recommended that the patient undergo an intense chemotherapy regimen. However, she declined further treatment and expired in May 2012.

Case 2

This is a 61 year-old female initially presented with a 1-month history of pain in left chest in January 2011. A CT scan showed enlargement of the lymph node in the retroperitoneal area, area around the aorta, and in the left inguinal region. There was also a 3.4×2.5 cm mass in left rib area. Lymph node biopsy demonstrated follicular lymphoma, grade I, and immunohistochemical analysis revealed CD20(+) and BCL6 (+). Upon completion of the staging workup, the patient was treated with 8 circles of R-CHOP, and achieved CR. In February 2012, the patient experienced weakness in her right leg and palpated masses on her right hip and left back. These were not accompanied by fever, night sweats, or other expected symptoms. A CT scan showed masses in the left chest wall, left psoas muscle, and right iliac fossa, invading the right iliac muscle and lumbar muscles. Pathological examination of the mass in the left chest wall showed sheets of large atypical lymphoid cells with prominent nucleoli. Mitotic activity is high. Immunohistochemistry of the biopsy revealed CD10(+), BCL2(+), BCL6(-), PAX-5(+), MUM-1(-), and Ki-67>95%(+). FISH showed MYC/IgH rearrangement and Bcl-2/IgH rearrangement (**Figure 2**). Finally, the patient was diagnosed

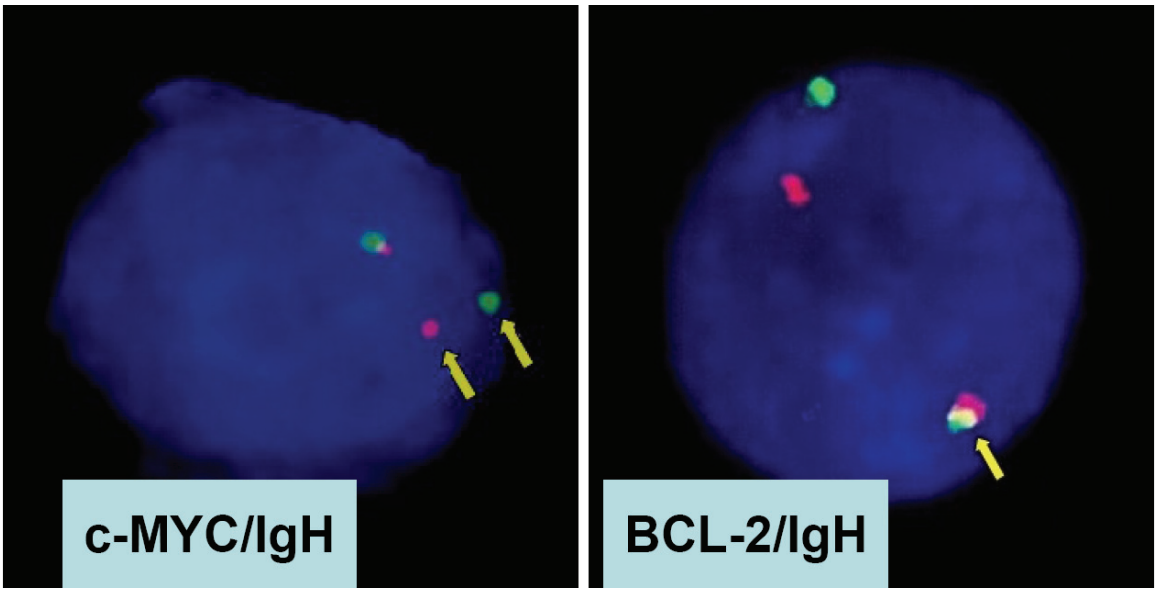

Figure 2. Case 2. Fluorescence in situ hybridization analysis of *BCL2* and *MYC* translocation. Green-labeled DNA probe and red-labeled DNA probe bound to both sides of MYC and BCL2, respectively. Translocation events of these genes split signals with two colors. Left, split signal induced by c-myc/IgH translocation and right, split signal induced by Bcl-2/IgH translocation.

with diffuse large B-cell lymphoma, unclassifiable, with features intermediate between large B-cell lymphoma and Burkitt lymphoma. Then lumbar puncture was performed and cerebrospinal fluid examination revealed atypical lymphocytes with DLBCL phenotype, suggesting the involvement of the central nervous system. ESHAP chemotherapy in combination with regular intrathecal injection of cytarabine was provided. In June 2012, after 4 rounds of R-ESHAP, CT scan showed changes consistent with partial response (PR) with worsening clinical course.

Discussion

Double-hit and triple-hit lymphomas (DHL and THL) are a heterogeneous group characterized by highly aggressive clinical behavior, complex karyotypes, and a spectrum of pathological features overlapping with Burkitt lymphoma (BL), diffuse large B-cell lymphoma (DLBCL), and B-lymphoblastic lymphoma/leukemia (B-LBL). Generally, these cases present with variable morphologies, including acute lymphoblastic leukemia/lymphoma (ALL), DLBCL, BL, B-LBL, and rarely FL [8-11]. Most reported patients have de novo disease, while a minority have a history of grade 1-2 FL and develop DHL secondarily, presumably by acquisition of a *MYC* translocation [6]. *BCL2* translocation may precede *MYC* events in lymphomagenesis of DHL and the acquisition of a *MYC* translocation may be an important oncogenic event in the transformation of FL to high-grade lymphoma [12]. Marin et al. and Tanaka et al. have both demonstrated in vivo that BCL2 deregulation caused by *BCL2* translocation increases cell survival by preventing apoptosis, predisposing the cell to acquire secondary chromosomal aberrations [13, 14]. *MYC* induces DNA stress and activates the *TP53* checkpoint, leading to apoptosis. In the cases reviewed here, MYC was shown to translocate with IGH, which is consistent with most reports of DHL, which show MYC to be frequently translocated with IGH or a non-IG partner [6]. It is also consistent with the majority of BL, in which IGH is the most common MYC translocation partner, and confer the recurrent lymphoma more aggressive and drug resistance features [15].

In published cases, the incidence of concurrent *BCL2/MYC* translocations in patients with DLBCL ranges from 11%, as reported by Pedersen, down to 3%, as reported by Savage [16, 17]. Because technology like FISH has not been used on a large, unselected series, and because the published cytogenetic data may be biased to specific categories of lymphomas,

the incidence of aggressive B-cell lymphomas with *MYC* breakpoints, double hits in particular, is difficult to assess and secondary DHL has been rarely reported. Pedersen et al. have reported that, in transformed lymphoma, DHL was very frequent (21%), and these patients all had FL [16]. Consistent with this, our two patients evaluated here had both been primarily diagnosed as FL and only later with transformed to B-cell lymphoma, unclassifiable, with features intermediate between large B-cell lymphoma and Burkitt lymphoma upon relapse.

In terms of immunophenotype, DHL and THL have been found to express B-cell markers, generally and often with a germinal center phenotype (BCL-6+, CD10+) even though this is not specific to DHL [18]. The high Ki-67 proliferation index and positive BCL2 staining which is not typical Burkitt lymphoma should prompt FISH and cytogenetic analysis for *MYC* and *BCL2* rearrangements to identify DHL, particularly if the results of tissue biopsy are unexpected or for ant recurrent lymphomas [19].

DHL is probably related to *MYC*-induced growth promotion combined with the anti-apoptotic effect conferred by *BCL2* overexpression. This renders it highly aggressive and associates it with poor prognosis and a lack of response to therapy [6, 7]. In most published articles, DHL and THL seem to present with elevated lactate dehydrogenase (LDH) levels and to have a high incidence of extranodal lesions (bone marrow, central nervous system, and gastrointestinal tract involvement), high international prognostic index (IPI≥3), and significantly shorter median overall survival rate [20]. Likewise, the patient in case 1 received 6 rounds of R-CHOP chemotherapy and achieved CR. This was followed by Auto-HSCT. Unfortunately, she relapsed with CNS involvement within 9 months and died after an additional 3 months. In case 2, after 8 rounds of R-CHOP, the patient achieved CR, but she also relapsed 5 months later. In line with the published literature, these patients are generally refractory to standard chemotherapy regimens and have a poor prognosis with a median overall survival of only 0.2–1.5 years [3, 20]. These lymphomas seem to behave more aggressively and to suffer early relapse, even if a complete response to initial chemotherapy is observed. In a large, population-based, R-CHOP-treated study cohort, *MYC* rearrangement was also associated with inferior outcome, and additional *BCL2*-translocation was found to have no additional impact on survival [2]. In contrast, Pedersen reported no statistically significant differences in clinical presentations were found between patients with DH or non-DH lymphoma but gave no underlying reasons [16]. The reason for these differences is still unclear, but it may be attributable to technical factors related to the tissue samples and the different compositions of patient cohorts.

Until today, the optimal treatment for these lymphomas has remained undefined. In the cases reviewed here, DHL and THL showed a high resistance to intensive chemotherapy, including high-dose chemotherapy followed by SCH [21]. In recent reports, patients have been treated with conventional chemotherapeutic regimens for DLBCL, intensive chemotherapeutic regimens, high-dose chemotherapy with Auto-HSCT, and radiation therapy. However, DHL responds poorly to R-CHOP regimens (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone), CODOX-M/IVAC regimens, and Hyper-CVAD chemotherapy [22]. Tomita et al. reported that the median survival time was only 4 months from diagnosis for patients with DHL [21]. In most previously published studies, the inclusion of Rituximab in standard DLBCL therapy resulted in a shift in predictive and prognostic factors [17]. Parker et al. reported 2 cases of DHL successfully treated with aggressive immunochemotherapy followed by autologous stem cell transplantation and radiation therapy. The first patient received post-transplant mediastinal radiation and developed recurrence in multiple areas outside of the radiation field. The second patient received total body irradiation as part of the conditioning regimen, and has not experienced recurrence as of 18 months after transplant, 24 months after diagnosis of the dual translocation lymphoma. These results suggest a role for total body irradiation in the management of this highly aggressive non-Hodgkin lymphoma [23]. However, due to the small number of cases, the value of radiation therapy for the treatment of DHL still needs to be assessed in large-scale clinical studies before it can gain recognition. In conclusion, more studies into new therapeutic approaches to the treatment of this disease are acutely needed [5]. Due to the high risk of CNS involvement, inclusion of CNS-directed therapy should be considered.

Taking all of the above into consideration, timely diagnosis plays a vital role in patient outcome and more mechanism-based therapy should be explored.

Conflict of interest

All authors have no conflict of interest to report.

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