Primary Mediastinal Large B-Cell Lymphoma With Translocations Involving BCL6 and MYC (Double-Hit Lymphoma)

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ABSTRACT

Objectives: Primary mediastinal large B-cell lymphomas (PMLBCLs) are aggressive lymphomas with characteristic clinical, morphologic, and immunophenotypic features. “Double-hit” (DH) lymphomas are B-cell neoplasms characterized by a translocation involving MYC and either BCL2 or BCL6. In the indexed literature, there are no reported cases of PMLBCL associated with DH or triple-hit events.

Methods: Herein, we present a case of a 15-year-old girl with PMLBCL who had typical clinical, morphologic, and immunophenotypic features.

Results: Fluorescent in situ hybridization studies showed rearrangements involving MYC and BCL6. We also excluded the possibility of a reciprocal t(3;8) (3q27;8q24) BCL6/MYC translocation.

Conclusions: This case expands the current spectrum of lymphomas subtypes in which DH can be found and supports the rationale for cytogenetic testing for DH abnormalities in all cases of aggressive large B-cell lymphomas regardless of subtype.

Primary mediastinal (thymic) large B-cell lymphomas (PMLBCLs) are aggressive lymphomas, characteristically seen in young females and usually presenting as a rapidly growing anterior mediastinal mass causing thoracic obstruction, with limited systemic spread and typically without bone marrow involvement. PMLBCLs are considered a separate entity different from diffuse large B-cell lymphoma (DLBCL), are thought to arise from thymic B cells, and share molecular features with classical Hodgkin lymphoma. “Double-hit” lymphomas (DHLs) are B-cell neoplasms characterized by a translocation involving MYC with a second translocation involving another oncogene. The most common combination of “hits” in DHL includes translocations involving MYC and BCL2. Less commonly, translocations may include BCL6.

Up to now, cases of PMLBCL with features of DHL have not been described. Herein we present a case of PMLBCL with separate translocations involving MYC and BCL6. This case expands the spectrum of B-cell lymphomas that can be considered DHL.

Clinical Presentation

The patient is a 15-year-old girl with no medical history who, before admission, had a 2-week-long nonspecific illness with respiratory tract symptoms that were consistent with pneumonia. Despite treatment, her symptoms persisted, and...
she continued to deteriorate. The patient was transferred to our pediatric intensive care unit in critical condition with cardiovascular collapse requiring extracorporeal membrane oxygenation. A chest radiograph showed a large mediastinal mass with right (88 mm) and left (85 mm) components and no lung involvement. Computerized tomography imaging studies failed to demonstrate involvement of other sites. Urgent targeted radiotherapy or chemotherapy was considered, and ultrasound-guided needle biopsy specimens were obtained that yielded the final diagnosis.

Pathologic Findings

Histologic sections of the mediastinal mass biopsy specimens showed involvement by lymphoma of a diffuse pattern with associated fibrosis. Most lymphoma cells were compartmentalized by fine bands of fibrosis, and other areas had thicker bands of fibrosis. The tumor cells were intermediate to large in size and displayed varied morphology, including centroblastic, immunoblastic, and rare Hodgkin-like morphology. Many cells had abundant clear cytoplasm. High cell turnover was evident by the abundant apoptotic and mitotic figures.

Immunohistochemical studies showed that the lymphoma cells were positive for CD45, CD20, CD79a, PAX5, MAL, BCL6, CD10 (weak, focal), CD23, and CD30 (weak, subset: 20%) and negative for synaptophysin, chromogranin, T-cell markers, CD34, and TdT. Keratin immunostaining highlighted the residual thymus (not shown). The Ki-67 proliferation index was approximately 50%, and MYC was positive (variable) in around 40% of tumor cell nuclei. Flow cytometry performed on a cell suspension collected from the tissue biopsy specimen.
The immunophenotype of this lymphoma by immunohistochemical assays: CD20+ (A), CD30+ (weak, ~20%) (B), CD23+ (C), BCL2+ (D), CD10+ (weak and focal) (E), and MAL+ (F).
showed that the lymphoma cells had an unusually increased side-scatter signal (compared with most B-cell lymphomas) and that these were surface λ light chain restricted (dim expression; λ median MFI = 1.31 and κ median MFI = −0.34) and were positive for CD20, CD19, and CD10 (dim).

The above findings, together with the age, sex, anatomic location, and clinical presentation, were considered most consistent with the diagnosis of DLBCL, best classified as PMLBCL. A concurrent bone marrow examination showed no involvement by lymphoma.

Fluorescence in situ hybridization (FISH) studies performed on formalin-fixed, paraffin-embedded tissue sections from the mediastinal biopsy specimen showed the presence of BCL6 (3q27) and MYC (8q24) gene rearrangements in around 50% of the cells Image 3. Reflex testing for MYC/IGH fusion was negative, indicating that the MYC gene rearrangement involved a partner different from IGH. No IGH/BCL2 rearrangement was identified. For FISH analyses, 100 cells were counted per probe; a cutoff of 10% was used to determine a positive IGH/BCL2 fusion, and a cutoff of 15% was used for BCL6 and MYC break-apart probes.

To exclude the possibility of a reciprocal t(3;8) (3q27;8q24) BCL6/MYC gene rearrangement, we performed additional FISH studies with single-color probes for MYC and BCL6. The test showed lack of BCL6/MYC fusion, and the presence of a cytogenetic DHL was confirmed (Image 3D).

**Treatment and Clinical Course**

The patient was started on chemotherapy immediately after diagnosis and was enrolled in a clinical trial (COG-ANHL1131) for patients with high-risk disease that examines the addition of rituximab to the standard etoposide, vincristine, doxorubicin, and cyclophosphamide therapy. Soon after commencement of treatment, she had a quick recovery with no further requirements for intensive care support.

**Discussion**

PMLBCL is known to be an epidemiologically, biologically, clinically, histologically, and immunophenotypically distinct from DLBCL.1 PMLBCL arises in the anterior mediastinum, from thymic B cells, and occurs most frequently in young female patients. PMLBCL presents aggressively with development of large anterior mediastinal masses that invade local structures, often leading to superior vena cava syndrome.1 Although local bulky masses and involvement of supraclavicular and cervical lymph nodes may be seen, there is typically an absence of other lymph nodes and bone marrow involvement. Extrathoracic manifestations can occur often involving the kidneys, adrenals, or liver.1

Our patient is a 15-year-old girl with a large anterior mediastinal mass associated with thoracic obstruction and no involvement with other structures or bone marrow. Biopsy specimens of the mediastinal mass confirmed the presence of a DLBCL most consistent with PMLBCL. Features of classic Hodgkin lymphoma were not present. A mature B-cell origin and surface λ light chain restriction was confirmed by immunophenotyping. In addition, the lymphoma cells had a characteristic protein expression profile that included positivity for CD30 (weak), BCL6, CD23, and MAL. The diagnosis of PMLBCL was supported by the localization of the mass in the anterior mediastinum with...
the presence of residual thymus, as well as morphologic and immunophenotypic findings with partial expression of CD30 and expression of MAL. MAL expression is seen in up to 70% of PMLBCLs, but it is rare in DLBCL, thus being one of the most specific immunomarkers for PMLBCL.\(^4\)

FISH studies identified the presence of separate, non-reciprocal MYC and BCL6 rearrangements. In this case, the partner gene involved in the MYC translocation was not IGH. The presence of surface light chain expression detected by flow cytometry indicates that at least one IGH allele is functional and argues against the possibility that both IGH alleles are involved in rearrangements with BCL6 and MYC. IGH rearrangement with MYC was excluded using MYC/IGH fusion probes. In DHL, MYC translocations with non-IGH occur in around 40% of cases and often involve PAX5 and BCL6.\(^6\) In the setting of DLBCL, the identification of simultaneous MYC, IGH/BCL2, or, less frequently, BCL6 rearrangements is considered a genetic double hit (DH) or “triple hit” depending on the number of translocations. Cytogenetic DHs have been specifically recognized in the context of DLBCL due to the associated aggressiveness and poor response to standard chemotherapy.\(^7\) DHLs
represent 1% to 12% of all DLBCLs, and the types of DHLs thus far recognized include a spectrum of morphologies and lymphoma subtypes such as large B-cell lymphomas with features intermediate between DLBCL, follicular lymphoma, and lymphoblastic lymphomas.

DHLs with MYC and BCL6 rearrangements are less well described than those with MYC and BCL2 rearrangements. Pillai et al and Turakhia et al each described six cases. Patients in these series had de novo disease, most frequently extranodal, and were usually women; the most common histologic types were DLBCL, B-cell lymphoma unspecified, and other aggressive large B-cell lymphomas. It is important to recognize that when a lymphoma in which MYC and BCL6 rearrangements (assessed with break-apart probes) are simultaneously such lymphoma as a DHL. The Mitelman database reports including follicular lymphoma, chronic lymphocytic leukemia, of DLBCL and seven cases of other mature B-cell neoplasms, 12 cases of B-cell lymphomas with t(3;8), including five cases other aggressive large B-cell lymphomas. It is important to recognize that when a lymphoma in which MYC and BCL6 rearrangements (assessed with break-apart probes) are simultaneously identified, such as the case presented here, the possibility of a reciprocal t(3;8) must be excluded before labeling such lymphoma as a DHL. The Mitelman database reports 12 cases of B-cell lymphomas with t(3;8), including five cases of DLBCL and seven cases of other mature B-cell neoplasms, including follicular lymphoma, chronic lymphocytic leukemia, and other aggressive large B-cell lymphomas.

DHL events in cases of PMLBCL are currently considered a rare finding, and thus the clinical implications of DH events in the context of PMLBCL are unknown. To further support the low frequency of this event, a search for all DH and triple-hit large B-cell lymphomas diagnosed at our institution yielded 39 cases for the past 2 years, and notably, none of these cases was classified as PMLBCL. MYC abnormalities have been previously reported to occur in PMLBCL. Scarpa et al reported six cases of PMLBCLs in which MYC abnormalities were identified in three cases, two with mutations or small rearrangements and one with a major MYC rearrangement. A more recent study including 32 patients with PMLBCL showed that a frequent level of MYC protein expression was seen in up to 94% (n = 30), of whom one-third (n = 10) had high MYC expression. However, MYC rearrangements by FISH were negative in the 10 cases assessed. Rearrangements in BCL6 but not in BCL2, although not characteristic, can occasionally occur in PMLBCLs. This may in principle favor the MYC and BCL6 gene rearrangement combination in the setting of PMLBCL.

Comparative genomic hybridization of PMLBCL shows gains of chromosome 9p, including amplification of the REL oncogene and JAK2. Other molecular genetic abnormalities that have been identified in PMLBCL include TP53 mutations, BCL2 and MAL gene overexpression, and somatic mutations of IGVH, BCL6, PIM1, PAX5, and MYC. To date, none of these abnormalities are required for diagnosis.

Herein, we present a case of PMLBCL in which the DHs involving MYC and BCL6 rearrangement were identified. This case expands the current spectrum of lymphoma subtypes in which DHs can be found. We believe this observation should raise awareness that the clinical aggressiveness associated with DHLs can occur outside of the more common DLBCL not otherwise specified (NOS). These findings support that cytogenetic testing for DH abnormalities should be performed in all cases of diffuse large B-cell lymphomas regardless of subtype. Such identification may shed light on the incidence as well as therapeutic and prognostic implications of these cytogenetic abnormalities in the context of aggressive large B-cell lymphomas other than DLBCL NOS.

References


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