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Case study

Lymphoblastic lymphoma with a triple-hit profile: a rare but distinct and relevant entity ☆

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Summary Follicular lymphoma with progression to a high-grade lymphoma bears a poor prognosis. We describe a case of a 60-year-old man who presented in 2012 with an epidural mass, diagnosed as a diffuse large B-cell lymphoma (DLBCL) with concurrent low-grade follicular lymphoma. Three years later, the patient presented with a cervical mass, diagnosed as a lymphoblastic lymphoma (LBL). Both the DLBCL and LBL contained a "triple hit" with *BCL2*, *BCL6,* and *cMYC* translocations demonstrated by fluorescence in situ hybridization analysis and a complex karyotype by single-nucleotide polymorphism array analysis. Furthermore, the 2 lymphomas were shown to be clonally related by clonality analysis and single-nucleotide polymorphism array analysis. This case report presents a highly unusual case of an LBL with a triple hit, originating from a DLBCL, which has rarely been described in the literature and deserves further exploration.

1. Introduction

FL accounts for 20% of all lymphomas and shows the highest incidence in Western Europe and the United States. It occurs mostly in middle-aged and elderly patients, who present with widespread nodal disease and involvement of the bone marrow in more than half of the cases. Despite the high stage, low-grade FL has a favorable prognosis with an indolent behavior and a good response to therapy [1,2].

FL is characterized by the t(14;18)(q32;q21) translocation involving *BCL2* and mostly *IGH*, but alternative translocations have also been reported. The t(14;18) is present in up to 90% of low-grade FLs (grades 1-2) and is much less

Abbreviations (B-)LBL, (B-cell) lymphoblastic lymphoma; DHL, double-hit lymphoma; DLBCL, diffuse large B-cell lymphoma; FFPE, formalin fixed and paraffin embedded; FISH, fluorescent in situ hybridization; FL, follicular lymphoma; IgH, immunoglobulin heavy chain; IgL, immunoglobulin light chain; SNP, single-nucleotide polymorphism; THL, triple-hit lymphoma.

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frequent in high-grade FL (3B) [1]. In contrast, the latter often harbors other genetic alterations, such as *cMYC* translocations, *BCL6* mutations, *TP53* mutations, and *CDKN2A* inactivation [3-6].

Transformation of FL to a high-grade lymphoma occurs in approximately 30% of patients, usually to DLBCL [3]. Transformation to other subtypes has also been reported, albeit at much lower frequency, including B-cell lymphoma unclassifiable with features intermediate between DLBCL and Burkitt lymphoma (BCLU), plasmablastic lymphoma, DHL, and B-LBL.

Transformation of FL to B-LBL is rare, with most cases showing a *cMYC* rearrangement in addition to a *BCL2* translocation [7-9]. In contrast, only 8% of FLs that transform to DLBCLs show *cMYC* aberrations [8]. DHLs are characterized by translocation of *cMYC* in combination with translocation of *BCL2* or less frequently *BCL6*. Likewise, THLs are characterized by translocations of *cMYC*, *BCL2*, and *BCL6*.

DHLs frequently present with extensive (stage IV) extranodal disease and involvement of the bone marrow, peripheral blood, and central nervous system, with most cases being therapy resistant [10].

So far, only a few case reports have described double-hit B-LBL in the literature, and only a single case of TdT-positive THL has been published [11].

We here present a case of FL with transformation to high-grade THL with blastoid morphology and TdT expression, and describe the clinical, pathological, and cytogenetic characteristics of this highly unusual case.

2. Materials and methods

2.1. Case presentation

A 60-year-old man with an unremarkable clinical history presented in June 2012 with cervical, inguinal, supraclavicular, and preauricular lymphadenopathy; progressive neurologic complaints; and an epidural mass at C5-Th1 on imaging. Biopsy material was classified as DLBCL, with a triple-hit translocation of *BCL2*, *BCL6*, and *cMYC*, with a minor component of low-grade FL. The bone marrow biopsy showed localization of a low-grade FL, without juxtaposition of a high-grade lymphoma. The patient was treated with 8 courses of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone and central nervous system prophylaxis consisting of 5 doses of intrathecal methotrexate and Diadreson F. Repeat bone marrow biopsy and positron-emission tomography–computed tomography showed complete remission in November 2012, after which the patient received involved field radiotherapy and rituximab maintenance treatment for 2 years.

In July 2015, the patient presented with skin lesions and a cervical mass on the left side, which was diagnosed as lymphoblastic transformation with triple-hit translocation. The same lymphoma was detected in the bone marrow in August 2015. Treatment was started with cytarabine and mitoxantrone in September, followed by vincristine, methotrexate, prednisone, and mercaptopurine in October 2015. This was complicated by a line sepsis with a coagulase-negative staphylococcus, acute kidney failure, and a respiratory syncytial virus infection, leading to respiratory failure. Hereupon, the patient died in December 2015.

2.2. Immunohistochemistry and flow cytometry

Immunohistochemical stainings were performed on FFPE, as well as frozen slides, according to diagnostic procedure protocols used at the pathology department of the University Medical Center in Utrecht, the Netherlands. Stainings were evaluated by 2 independent hematopathologists.

The following stainings were used: CD20 (Roche, Basel, Switzerland; G02369, clone L26, ready to use), CD79a (Dako, United States; M0750, clone JBC117, 1:100), PAX5 (Dako; M7357, clone DAK-PAX5, 1:80), CD21 (Novocastra, United Kingdom; NCL-L-21, clone 2G9, 1:40), CD10 (Novocastra; NCL-L-CD10, clone 56C6, 1:160), BCL6 (Novocastra; NCL-L-BCL6, clone LN22, 1:100), BCL2 (Cellmarque, United States; 226R-14, clone E17, 1:20), Mib (Dako; M7240, clone Mib1, 1:100), TdT (Novocastra; NCL-TDT-339, clone SEN28, 1:40), and EBER (Dako; M7004, clone PE2, 1:400).

Flow cytometry was performed on a bone marrow aspirate at the Laboratory for Translational Immunology of the same institute, according to standard procedures in diagnostic workflow.

2.3. Clonality

DNA was isolated from FFPE slides, and B-cell clonality analysis was performed according to the IdentiClone assay (Invivoscribe, United States). Clonality analysis was performed on the 2012 epidural mass, as well as on the 2015 cervical mass.

2.4. Fluorescence in situ hybridization

FISH was performed on FFPE material from both the 2012 epidural mass and the 2015 cervical mass (slide FISH) according to diagnostic procedures, as well as on cultured cells in metaphase, harvested from blood in September 2015 (metaphase FISH).

For slide FISH, break-apart probes for *BCL6*, *BCL2*, and *cMYC* were used, as well as a fusion probe for *cMYC-IgH* (Cytocell, Cambridge, UK). A cutoff of 10% was used for establishing the presence of a translocation.

For metaphase FISH, the following probes were used: LSI *BCL6* DCBA, LSI *MYC* DCBA, LSIG *IGH* DCBA, LSI *IGH/BCL2* DCDF (Abbott-Vysis, Abbott Park, IL), and *IGL* Break-apart LPH033 (Cytocell, Cambridge, UK).

2.5. SNP array analysis

DNA was isolated from peripheral blood leukocytes collected in September 2015 after informed consent. In addition, tumor