Synchronous discordant Epstein-Barr virus (EBV)–positive nodal T/NK-cell lymphoma and EBV-positive diffuse large B cell lymphoma in a patient with a history of EBV-positive Burkitt lymphoma

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Abstract

In rare cases, patients present with multiple simultaneous lymphomas at one or more anatomic sites. These may be described as *composite* (occurring at one anatomic site) or *discordant* (occurring at different anatomic sites). Although the Epstein-Barr virus (EBV) is often implicated in the development of composite lymphoma, its role in the pathogenesis of discordant lymphoma is less clear. We report a case of discordant Epstein-Barr virus–associated lymphoma consisting of nodal T/NK-cell lymphoma and diffuse large B cell lymphoma in a patient with a history of EBV-positive Burkitt lymphoma post treatment. Simultaneous biopsies of the left and right femoral lymph nodes showed a synchronous nodal T/NK-cell lymphoma (left) and diffuse large B cell lymphoma (right). The nodal T/NK-cell lymphoma was morphologically and immunohistochemical distinct from the diffuse large B cell. Both the T/NK-cell component and the B cell component showed bright nuclear positivity with in situ hybridization for EBER. Molecular studies for C-myc were negative. The role played by the Epstein-Barr virus (EBV) in the pathogenesis of discordant T cell and B cell lymphoma is uncertain but may be clinically significant, particularly in the setting of prior EBV-positive lymphoma. Additional testing for immunodeficiency should be considered in these patients.

Keywords Epstein-Barr virus · T cell lymphoma · Diffuse large B cell lymphoma · Burkitt lymphoma

Introduction

The development of multiple distinct lymphomas within a single patient is an uncommon phenomenon with significant variability in both the patient presentation and in the diagnostic features of the lymphomas themselves. These cases are classified according to temporality (two lymphomas occurring simultaneously are considered *synchronous*, while those occurring sequentially are *metachronous*), anatomic location (two lymphomas existing at a single anatomic site comprise a *composite* lymphoma, while those existing at different anatomic sites comprise a *discordant* lymphoma), and the identifying features of the component lymphomas (including

morphology, immunohistochemical staining pattern, and molecular features) [1, 2].

The most common co-occurring lymphomas are composites of two Non-Hodgkin B cell components (usually including diffuse large B cell lymphoma), or of a B cell and a Hodgkin component (most often of the nodular sclerosing subtype) [3]. Composite B cell and T cell lymphomas, in which distinct B cell and T cell components exist simultaneously at a single anatomic site, are rare but have been reported; most frequently, these consist of a Hodgkin lymphoma component and a non-Hodgkin B cell or T cell lymphoma component [4]. Frequently, composite B cell and T cell lymphomas have been associated with infection by the Epstein-Barr virus (EBV) [5–8].

Synchronous discordant lymphomas are even less common than their composite counterparts, and those consisting of both T cell and B cell components are exceptionally rare. Only one case of discordant T cell and B cell lymphoma has been reported in the literature thus far [9]. No definitive relationship between EBV infection and the development of discordant lymphoma has been established. We describe a new case of

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discordant EBV-positive lymphomas, including an EBV-positive primary nodal T/NK-cell lymphoma and an EBV-positive diffuse large B cell lymphoma, in a patient with a history of EBV-positive Burkitt lymphoma.

Clinical history

A 63-year-old Caucasian woman presented to an outside institution with throat pain and dysphagia. CT imaging showed bilateral tonsillar hypertrophy and cervical lymphadenopathy, and the patient underwent a biopsy of the right tonsil. Immunohistochemical stains were performed on the tissue at the outside institution; these revealed a neoplastic population of small uniform centroblasts that were immunohistochemically positive for CD45, CD10, BCL6, EBV, CD20, and c-MYC; and negative for TdT, MUM1, BCL2, CD5, CD3, Cytokeratin, and Cyclin D1. Ki-67 was positive in greater than 99% of cells. The neoplastic cells were strongly positive for Epstein-Barr virus–encoded RNA by in situ hybridization (EBER ISH). The morphology and immunohistochemical staining pattern were most consistent with a high-grade EBV-positive B cell lymphoma, likely Burkitt lymphoma, but confirmatory cytogenetic testing was not done.

The patient was then transferred to our hospital for treatment. Imaging studies revealed massive splenomegaly and multiple cervical, intrathoracic, intraabdominal, intrapelvic, inguinal, intramuscular, and subcutaneous PET-avid lesions consistent with disseminated neoplastic disease. The patient received 6 cycles of R-EPOCH chemotherapy and intrathecal methotrexate, and follow-up PET imaging studies showed a complete response to the treatment.

Nine months after completing the chemotherapy regimen, the patient presented again with recurrent lymphadenopathy. She underwent additional imaging studies at our hospital, which showed newly PET-avid femoral and inguinal lymph nodes with additional PET-avid lesions in the skin and subcutaneous tissue. Needle core biopsies of the enlarged left and right femoral lymph nodes were performed.

Following diagnosis, the patient was started on RICE chemotherapy and referred to an outside institution for stem cell transplant.

Materials and methods

Approval was obtained from the institutional IRB at the University of Missouri Hospitals and Clinics, Columbia, Missouri. Paraffin-embedded H&E slides were prepared by standard methods after formalin and B+ fixation. Immunostaining was performed following the standard protocol on a Dako Immunostainer (Dako, Carpinteria, CA) (Table 1). Flow cytometric immunophenotyping was

performed on a FACS Canto II flow cytometer (Becton-Dickinson, Franklin Lakes, NJ) using standard protocols. Molecular studies (including a T cell lymphoma FISH panel, B cell lymphoma FISH panel, and PCR for T cell receptor gene rearrangement) were performed at Mayo Medical Laboratories (Rochester, MN).

Slides from the patient's prior tonsillar biopsy, including immunohistochemical stains performed at the outside hospital, were reviewed at our institution.

Results

Histologic and immunohistochemical studies

Review of H&E and PAS-stained slides revealed two morphologically distinct cell populations. Tissue from the left femoral lymph node showed marked paracortical hyperplasia with expansion of interfollicular areas by a uniform population of intermediate-sized cells (Fig. 1). In contrast, tissue from the right femoral lymph node showed total effacement of the normal architecture by sheets of intermediate-to-large-sized centroblasts with pale cytoplasm, vesicular chromatin, and prominent nucleoli (Fig. 2).

Table 1 Antibody clones used for IHC

Specificity	Antibody (clone)	Source
CD34	QBEnd10	Dako
CD10	56C6	Dako
TdT	EP266	Dako
CD20	L26	Dako
CD19	LE CD19	Dako
PAX-5	DAK-PAX5	Dako
MUM1	MUM1p	Dako
CD79	JCB-117	Dako
bcl-2	124	Dako
bcl-6	PG-B6P	Dako
Cyclin D1	EP121	Dako
CD5	4C7	Dako
CD7	CBC37	Dako
CD3	IS503	Dako
CD8	C8/144B	Dako
CD56	123C	Dako
CD57	TB01	Dako
TIA	2G9A10F5	Biogenex
Granzyme B	EPR8260	Abcam
c-myc	Y69	Dako
p53	DO-7	Dako
Ki-67	Mib-1	Dako

Fig. 1 Left femoral lymph node: a) Periodic acid-Schiff (PAS) staining ($\times 400$) showing the expansion of interfollicular areas by a population of intermediate-sized cells. Neoplastic cells display b) loss of CD5, c) strong expression of CD3, and d) bright positive signaling for EBER ISH

The immunohistochemical staining pattern was likewise indicative of two distinct cell populations (Table 2). The neoplastic cells from the left femoral lymph node showed strong immunohistochemical positivity for CD3, CD8, p53, TIA, and Granzyme B and were negative for CD5 and CD7. Very

rare CD56-positive and CD57-positive lymphocytes were identified. Ki-67 was positive in approximately 60% of neoplastic cells. In contrast, the neoplastic cells from the right femoral lymph node showed immunohistochemical positivity for CD20, PAX-5, MUM1, CD79a, CD19, CD30 (focal), and

Fig. 2 Right femoral lymph node: a) HE staining ($\times 100$) and b) PAS staining ($\times 400$) showing effacement of the normal architecture by sheets of intermediate-to-large-sized centroblasts with pale cytoplasm, vesicular chromatin, and prominent nucleoli. c) Neoplastic cells demonstrating strong immunohistochemical positivity for CD20 and d) bright positive signaling for EBER ISH

Table 2 Results of immunohistochemical staining and in situ hybridization (ISH) for Epstein-Barr virus–encoded small RNAs (EBER)

Antibodies and ISH	Right tonsil (January 2017)	Left femoral lymph node (April 2018)	Right femoral lymph node (April 2018)
Cytokeratin	–		
CD45	+		
CD34		–	–
CD10	+		–
TdT	–	–	–
CD20	+	–	+
CD19			+
PAX-5		–	+
MUM1	–	–	+
CD79			+
bcl-2	–		–
bcl-6	+	–	–
Cyclin D1	–		–
CD5	–	–	–
CD7		–	
CD3	–	+	–
CD8		+	
CD56		+ (rare)	
CD57		+ (rare)	
c-myc	+	+ (10%)	+ (10%)
p53		+	+ (weak)
Ki-67	+ (99%)	+ (60%)	+ (90%)
EBER ISH	+	+	+

p53 (weak) and did not stain for BCL6, BCL2, CD10, CD34, TdT, Cyclin D1, or CD3, or CD5. Ki-67 was positive in approximately 90% of neoplastic cells. In both lymph nodes, C-myc was positive in approximately 10% of neoplastic cells.

In situ hybridization

Neoplastic cells from both the left and right femoral lymph nodes were strongly positive for EBER by ISH.

Flow cytometry

Flow cytometric analysis again showed different immunophenotypes expressed by the neoplastic cells in the left and right lymph nodes. Analysis performed on tissue taken from the left femoral lymph node (viability 64.2%) identified no monotypic B cell populations, phenotypically abnormal T cell populations, or blast cell populations. T cells comprised 24.8% of the specimen, with an approximately equal distribution of CD4 and CD8 positive cells showing no aberrant antigen expression. The analysis performed on tissue taken from the right femoral lymph node (viability 69.3%) identified a monotypic population of B cells expressing CD19 and CD20 but not expressing CD5, CD10, or surface/cytoplasmic

immunoglobulin light chains. B cells comprised 4.3% of the specimen. No phenotypically abnormal T cell populations or blast cell populations were identified.

Molecular analysis

A T cell lymphoma FISH panel performed on tissue from the left femoral lymph node showed no ALK or TCL1A rearrangements and no abnormalities of chromosomes 7 and 8. PCR for T cell receptor gene rearrangement, performed on the same tissue, detected the presence of clonal T cell receptor gene rearrangement in a polyclonal background. A B cell lymphoma FISH panel performed on tissue from the right femoral lymph node showed no MYC rearrangement and no gene fusion of MYC and IGH. IgH PCR was not performed.

Discussion

The morphologic features, immunohistochemical staining pattern, flow cytometry results, and molecular results in this case were most consistent with two synchronous and discordant lymphomas: an EBV-positive primary nodal T/ NK-cell lymphoma in the left femoral lymph node,

and an EBV-positive diffuse large B cell lymphoma, activated B cell phenotype, in the right femoral lymph node. Given the patient's history, the primary clinical concern at the time of biopsy was for recurrence of Burkitt lymphoma; however, the features exhibited in the femoral lymph node biopsy specimens were not consistent with the patient's previous biopsy-proven lymphoma.

A B cell lymphoma FISH panel performed on the right femoral lymph node showed no MYC rearrangements or MYC:IGH fusion products, rendering unlikely a diagnosis of Burkitt lymphoma; additionally, the morphology and immunohistochemical staining pattern in both lymph nodes (including CD10 negativity) were markedly different from the patient's prior biopsy.

The diagnosis of an EBV-positive primary nodal T/NK-cell lymphoma in the left femoral lymph node was likewise confirmed by the presence of clonal T cell receptor gene rearrangement by PCR. The immunophenotypic and morphologic features of this component (including strong positivity for CD8, negativity for both CD56 and CD57, and absence of tumor necrosis and angiocentricity) suggested that it may best be characterized as an EBV-positive primary nodal T/NK-cell lymphoma rather than as a variant of EBV-positive extranodal T/NK-cell lymphoma [10].

All three lymphomas—the Burkitt's lymphoma, the EBV-positive primary nodal T/NK-cell lymphoma, and the diffuse large B cell lymphoma—showed strong positivity for EBV. Given these findings, the patient was diagnosed with an EBV-associated synchronous discordant lymphoma with both B cell and T cell components in a setting of prior EBV-positive Burkitt lymphoma.

The pathogenesis of discordant lymphoma is poorly understood, in part because of its rarity. Zhang et al. estimated that fewer than 20 reports of discordant lymphoma have been published since the phenomenon was first classified in 1974, though they suggested that it is likely under-diagnosed [2, 11]. The diagnosis of discordant lymphoma requires evidence of at least two unique lymphomas in at least two anatomically distinct sites. These sites may be two different lymph nodes, as in our case and others, or may include non-lymphoid tissues such as the nasopharynx, skin, salivary glands, stomach, abdominal cavity, mediastinum, and bone marrow [2, 12–18]. Because not all lymphoma patients will undergo biopsies at multiple anatomic locations, there is significant potential for under-diagnosis, especially in patients who are asymptomatic or without diffuse lymphadenopathy at presentation. Elucidating the pathogenesis of discordant lymphoma is rendered even more challenging by the wide variability in the diagnostic features of the component lymphomas themselves. Some researchers have utilized molecular analysis to identify a possible clonal component in discordant small and large B cell lymphomas with

both lymph node and bone marrow involvement, but the results of these small studies are difficult to apply to all discordant lymphomas—particularly those that arise in different tissues and from different cell lines from those studied [18]. For example, prior studies which have shown a clonal association between a Hodgkin lymphoma component and a diffuse large B cell component may have little relevance to our case, in which the neoplastic B cell and T cell components (by definition) arise independently of one another.

Despite a lack of evidence for a clear clonal relationship between component lymphomas, our patient's metachronous development of synchronous discordant lymphoma following diagnosis with (and treatment for) Burkitt lymphoma suggests a possible common etiology, especially given that all three of her lymphomas demonstrated strong positivity with *in situ* hybridization for Epstein-Barr virus-encoded RNA. The association between EBV and the development of malignant lymphomas (particularly Burkitt, Hodgkin, and diffuse large B cell lymphomas) is well known [19]. The virus infects B cells in the oropharynx and remains latent for a period of time until, in some patients, it induces malignant transformation of its host cells at varying stages of germinal center maturation [20]. The stage at which germinal center maturation is arrested largely determines the type of lymphoma that arises; Burkitt lymphoma and some types of diffuse large B cell lymphoma are derived from centroblasts, while Hodgkin lymphoma and other types of diffuse large B cell lymphoma originate in post-centroblastic B cells [19]. The etiologic relationship between EBV and T/NK-cell lymphomas is less clear, but some studies have found evidence to suggest that EBV may be capable of “accidentally” infecting NK/T cells [20]. Others have suggested that infection of B cells by EBV induces subtle molecular changes that may promote the proliferation of T cell clones [21].

By definition, the diagnosis of a discordant lymphoma first requires that the presence of a composite lymphoma be definitively ruled out. In our case, the immunohistochemical profiles of the component lymphomas favored a discordant rather than composite presentation. The right femoral lymph node (identified as an EBV-positive diffuse large B cell lymphoma) was immunohistochemically negative for CD3 and CD5, which rendered less likely the existence of a composite T/NK-cell component within the same lymph node. Confirmatory molecular testing (such as PCR for TCR gene rearrangement or clonal IgH) could not be performed on all three lymph nodes in our case; however, such testing may be useful in future cases to better exclude a strong clonal relationship between the components, which could favor a diagnosis of composite lymphoma.

Like many discordant lymphomas, composite lymphomas occur synchronously—and, because they are relatively more

common (with an incidence between 1 and 4.7%), there is a greater body of published research about their pathogenesis and etiology [22]. There is a strong precedent for the association of EBV infection with the development of composite lymphoma, particularly those with both B cell and T cell components. Xu et al. described a composite lymphoma consisting of an angioimmunoblastic T cell lymphoma and a diffuse large B cell lymphoma in which both components showed strong nuclear positivity with in situ hybridization for EBER [6]. Hirose et al. reported a similar case of a composite lymphoma consisting of a peripheral T cell lymphoma and an anaplastic variant of a diffuse large B cell lymphoma; again, both components demonstrated strong EBER positivity [5].

Various types of immunodeficiencies cause known predisposition to the development of EBV-associated lymphomas. These include combined T cell and B cell immunodeficiencies such as hyper-IgM syndrome, common variable immunodeficiency, Wiskott-Aldrich syndrome, and diseases of immune regulation (such as X-linked lymphoproliferative disease and autoimmune lymphoproliferative syndrome) [23]. Certain drugs have also been implicated in lymphomagenesis via immunosuppression, including methotrexate and some TNF inhibitors [23, 24]. Our patient had never been diagnosed with any immunodeficiency syndrome. She underwent treatment with methotrexate in association with chemotherapy for her original diagnosis of Burkitt lymphoma, but it is unclear if this exposure was of significant duration and intensity to lead to the later development of her discordant lymphoma.

The association between EBV re-activation and lymphomagenesis is of particular interest in discordant lymphomas with both B- and T cell components, in which it is clear that one lymphoma does not arise directly from a clone of the other. B and T cell discordant lymphomas are described only rarely in the existing literature. Our patient's combination of EBV-positive primary nodal T/NK-cell lymphoma and EBV-positive diffuse large B cell lymphoma (both existing within lymph nodes) appears to be unique among published reports of discordant lymphomas, but other cases have described peripheral T cell lymphomas found in combination with Hodgkin lymphoma [2]. In at least one of these cases, the discordant lymphoma displayed strong EBV positivity [13]. In our opinion, discordant lymphomas with both B and T cell components should prompt investigation into underlying immunodeficiency disorders, particularly when (as in this case) the component lymphomas are EBV-positive, and when the patient has a history of a third EBV-positive lymphoma.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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