Case Report

Epstein-Barr virus-positive diffuse large B-cell lymphoma, not otherwise specified: A diagnostic challenge

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ABSTRACT

Epstein-Barr virus-positive (EBV+) diffuse large B-cell lymphoma (DLBCL), not otherwise specified (NOS) is an entity included in the 2016 World Health Organization classification of lymphoid neoplasms. EBV+DLBCL, NOS, is an aggressive B-cell lymphoma associated with chronic EBV infection and poor prognosis with standard chemotherapeutic approaches. We present the case of a 71-year-old male hospitalized for generalized lymphadenopathy, unexplained weight loss, and intermittent fever with pancytopenia and a very high EBV viral load. An inguinal lymph node was biopsied and reported at two different centers as Classic Hodgkin lymphoma and EBV associated lymphoproliferative, respectively. The patient was subsequently rebiopsied and a cervical lymph node was subjected to detail histopathologic and immunohistochemical evaluation by clonality studies resulting in a final diagnosis of EBV positive DLBCL, NOS. This case highlights the potential diagnostic pitfalls due to the morphologic heterogeneity of this entity and immunohistochemical overlap with Hodgkin lymphoma, T-cell rich large B-cell Lymphoma, and diffuse large B-cell lymphoma (DLBCL, NOS).

Key words: Aggressive B-cell lymphoma, Epstein-Barr virus-positive diffuse large B-cell lymphoma, Immunohistochemically, Prognosis

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of malignant lymphoma. DLBCL harboring Epstein-Barr virus (EBV) positive monoclonal B-cell proliferation in patients older than 50 years without any known immunodeficiency or prior lymphoma is termed EBV-positive DLBCL of the elderly [1,2]. EBV-positive DLBCL, not otherwise specified (NOS), is an EBV-positive clonal B-cell lymphoid proliferation. Patients with EBV-positive DLBCL of the elderly were initially described by Oyama *et al.* in 2003, in a report of 22 immunocompetent elderly patients [3]. This disease was formerly designated as EBV-positive DLBCL of the elderly, initially described in 2003 and included as a provisional entity in the 2008 World Health Organization (WHO) classification, but the restriction to elderly patients has been removed; although the disease usually occurs in individuals aged >50 years, it can present over a wide age range [2].

In patients with DLBCL, the incidence of EBV among patients of Asian or Latin American origin ranges from 9% to 15% [4-6]. However, the incidence is <5% in the Western population [7,8]. EBV-positive DLBCL of the elderly is mainly identified in patients older than 50 years. However, younger patients without evidence of immunodeficiency have also been reported [4,5]. Patients with EBV DLBCL shared many unfavorable prognostic characteristics, regardless of age. EBV positive patients, both in the elderly and young groups, showed significantly worse overall survival and progression-free survival than negative cases.

The rationale for reporting this case is the potential diagnostic pitfall due to considerable morphologic and immunophenotypic overlap of this entity with both Classic Hodgkin lymphoma (which is also positive for EBV encoded DNA) and with other entities such as T-cell histiocytic rich large B-cell lymphoma and DLBCL, NOS. The need for detailed clinicopathologic correlation and careful morphologic and immunohistochemistry (IHC) evaluation cannot be overemphasized.

CASE REPORT

A 71-year-old male presented to the Hemato-oncology Department of our hospital with a history of generalized lymphadenopathy, fever, unexplained weight loss, and pancytopenia for 6 months. On examination, he was slightly undernourished and cachexic looking but was well oriented and conscious. His vitals were normal and his body mass index was 18.

He underwent complete hematological examination and was found to have hemoglobin – 10 g%, total leucocyte count – 3×10^9/L, and platelet count – 100,000/mcL. His prothrombin

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time and partial thromboplastin time were 16.4 s and 32.9 s, respectively. On serology, a toxoplasma antibodies panel was performed with both Immunoglobulin (Ig) G and IgM level being below 3 IU/ml. Later on, the patient was evaluated for the generalized lymphadenopathy and was diagnosed based on that.

A left inguinal lymph node excision biopsy was performed elsewhere and was reported after immunohistochemical evaluation as Classical Hodgkin lymphoma. A subsequent review at another center was reported as atypical EBV associated lymphoproliferation, most likely infectious mononucleosis type. The patient also underwent a positron emission tomography-computed tomography (PET-CT) scan which showed near resolution of the FDG uptake in nodal regions in the absence of definitive therapy. At the same time, the patient continued to be febrile (on and off) and the EBV copies in the blood (15,390 copies/ml) were significantly elevated.

The slides and blocks of the left inguinal lymph node reviewed at our center were reported as atypical lymphoid proliferation on morphologic evaluation. As the residual material in the block was inadequate for IHC to be performed, a repeat biopsy was done from both the left inguinal and left cervical lymph nodes, as the latter revealed increased uptake on PET-CT.

On morphologic evaluation, hematoxylin and eosin-stained sections from the inguinal lymph node showed foci of necrosis with surrounding epithelioid histiocytes and giant cells along with focal areas of atypical large nucleolated cells including Hodgkin-like and Reed-Sternberg (HRS) like cells in a reactive background. Focal areas of cystic change with hemorrhage and neutrophils were also seen with surrounding foreign body giant cell reaction-possibly a reaction to the previous biopsy (Fig. 1a and b).

Sections from the cervical lymph node showed partial effacement of the nodal architecture by clusters and sheets of large atypical nucleolated cells, including HRS like cells with brisk mitosis in a background of aggregates of histiocytes and epithelioid cells with foci of necrosis (Fig. 1c and d). The large cells were positive for CD20 (Fig. 2a), MUM1 (Fig. 2b), CD30 (Fig. 2c), and EBV-encoded RNA (EBER) (Fig. 2d); moreover, they were negative for CD3 (Fig. 3a), CD4, CD8, CD15, c-myc (Fig. 3b), BCL6 (Fig. 3c), CD-10 (Fig. 4a), and ALK-1 and exhibited a high Ki-67 (Fig. 4b) labeling index (Table 1).

A B cell receptor (IgH and Igk) clonality assay performed on the paraffin-embedded block of the cervical lymph node biopsy was positive for clonal Ig heavy chain gene rearrangement. After a thorough histopathological and immunohistochemical examination coupled with clonality studies, a diagnosis of EBV positive DLBCL, NOS was made.

DISCUSSION

According to 2016 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues, EBV+DLBCL, NOS is diagnosed in apparently immunocompetent patients, usually over 50 years of age [4]. This lymphoma was a provisional entity in 2008 WHO, entitled EBV+DLBCL of the elderly, but the "elderly" designation was substituted with "NOS" with the recognition that this entity occurs in younger patients [9]. The NOS designation highlights that the lymphoma must be excluded from more specific entities with neoplastic EBV-positive large B cells, such as lymphomatoid granulomatosis, DLBCL associated with chronic inflammation, and the newly designated entity EBV-positive mucocutaneous ulcer [4].

EBV-positive DLBCL of the elderly is an aggressive postgerminal center B-cell neoplasm characterized by prominent NF-κB activation. Microscopically, the lymph node architecture is effaced and consists of a uniform population of large cells with extensive necrosis, mitoses, and apoptoses. If minimal to no reactive

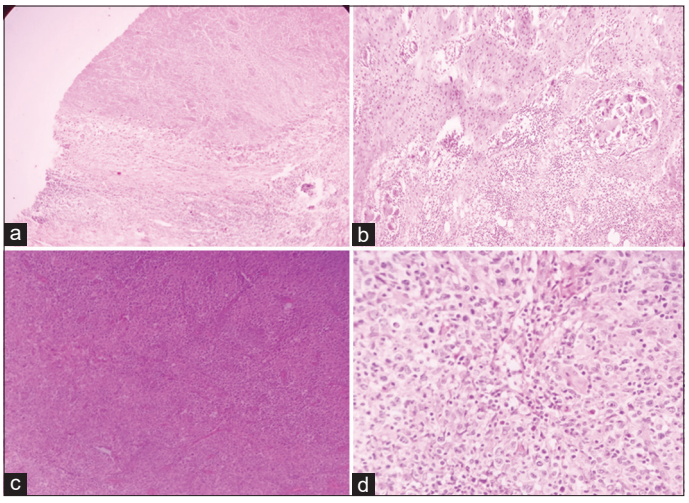

Figure 1: (a) Foci of necrosis with epithelioid histiocytes and giant cells in in inguinal lymph node biopsy (hematoxylin and eosin [H&E], ×10); (b)Atypical large nucleolated cells with foreign body giant cell reaction in inguinal lymph node biopsy (H&E, ×40); (c) Partial effacement of nodal architecture by clusters and sheets of large atypical nucleolated cells in cervical lymph node (H&E, ×10); (d)Atypical nucleolated cells with aggregates of histiocytes and epithelioid cells in cervical lymph node (H&E, ×40)

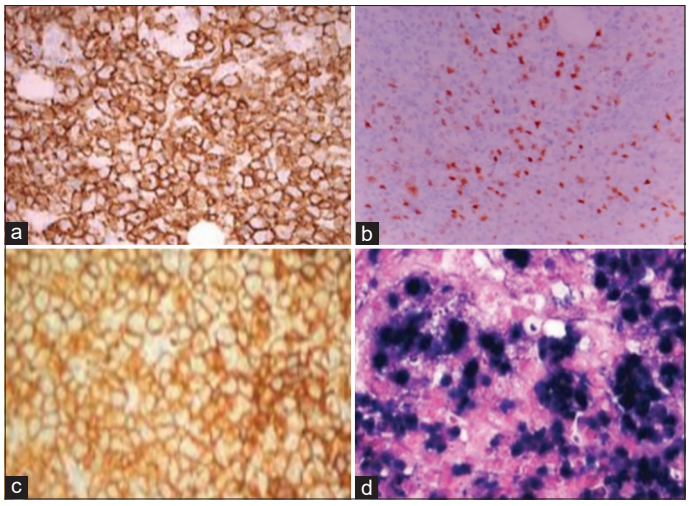

Figure 2: (a) Immunoreactive score 4+in large nucleolated cells (immunohistochemistry [IHC] stain, CD20; ×40); (b) Immunoreactive score 2+in large nucleolated cells (IHC stain, MUM-1; ×40); (c) Immunoreactive score 3+in large nucleolated cells (IHC stain, CD30; ×40); (d) Immunoreactive score 4+in large nucleolated cells (IHC stain, EBER; ×40)

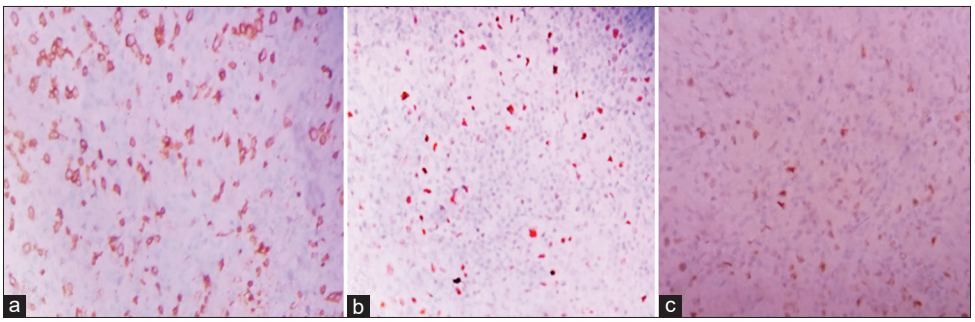

Figure 3: (a) Non-immunoreactive in large nucleolated cells (immuno reactive in t-cells) (immunohistochemistry [IHC] stain, CD3; ×40); (b) Immunoreactive score 1+in large nucleolated cells (negative) (IHC stain, c-myc; ×40); (c) Immunoreactive score 1+in large nucleolated cells (IHC stain, bcl6; ×40)

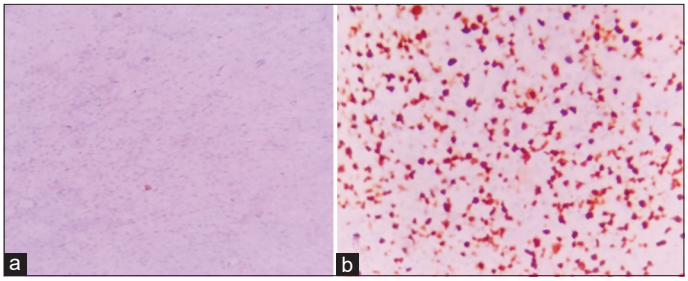

Figure 4: (a) Non-immunoreactive score 0 in large nucleolated cells (immunohistochemistry [IHC] stain, CD10; ×40); (b) Immunoreactive in 80% of neoplastic cells (IHC stain, ki-67; ×40)

component (small lymphocytes, plasma cells, or histiocytes) is seen, the disease is subclassified as monomorphic. The disease is classified as polymorphic if a reactive component is present [2,3]. This morphologic subclassification has not been shown to have prognostic implications [3]. The immunohistochemical profile is generally positive for B-cell markers CD20, CD19, CD79a, and PAX-5. CD10 and BCL6 are usually negative, while MUM1 is commonly positive. Cases with immunoblastic or plasmablastic features may lack CD20 expression [5]. *In situ* hybridization for Epstein-Barr encoding region (EBER) is positive and is considered the most important test for diagnosis, with the highest diagnostic sensitivity [2].

EBV-positive DLBCL of the elderly is caused by the senescence of the immune system as a part of the normal aging process, based largely on shared features with immunodeficiency-associated lymphoproliferative disorders. These shared features include EBV infection, similar EBV latency pattern, morphologic similarities, and presence of monoclonal T-cell populations [3,5].

The median survival rate is of 2 years in the Asian population. EBV-positive DLBCL of the elderly commonly involves extranodal sites. Site of primary extranodal involvement includes the skin, soft tissue, bones, nasal cavity, pharynx/hypopharynx, tonsils, tongue, lung, pleura, stomach, liver, spleen, peritoneum, cecum, and bone marrow [5,9]. Subsequently, several studies have shown that lymph node involvement is very common and seen in up to 70% of patients [5,9]. The standard treatment for DLBCL is the combination of rituximab, etoposide, prednisolone, vincristine, cyclophosphamide, and doxorubicin (R-EPOCH) [10].

Table 1: Immunohistochemistry interpretation on the excision biopsy sample

Antibody -	[Clone] -	Interpretation
CD3 -	[PS1] -	Non Immunoreactive in large nucleolated cells (Immuno reactive in T-cells)
CD4 -	[EP204] -	Non Immunoreactive in large nucleolated cells (Immuno reactive in T-cells &histiocytes)
CD8 -	[EP334] -	Non Immunoreactive in large nucleolated cells (Immuno reactive in T-cells)
CD15 -	[BRA4F-1] -	Non Immunoreactive score 0 in large nucleolated cells
CD20 -	[L-26] -	Immunoreactive score 4+in large nucleolated cells
CD30 -	[BerH2]-	Immunoreactive score 3+in large nucleolated cells
CD56 -	[123C3] -	Non Immunoreactive score 0 in large nucleolated cells
CD68 -	[KP-1] -	Non Immunoreactive score 0 in large nucleolated cells [Immuno reactive in interspersed histiocytes]
ALK-1 -	[CD246] -	Non Immunoreactive score 0 in large nucleolated cells
EBER -	[ISH] -	Immunoreactive score 4+in large nucleolated cells
CD10 -	[56C6]-	Non Immunoreactive score 0 in large nucleolated cells
Bcl6 -	[EP278]-	Immunoreactive score 1+in large nucleolated cells
Cmyc -	[EP 121]-	Immunoreactive score 1+in large nucleolated cells (Negative)
Ki67 -	[30-9]-	Immunoreactive in 80% of neoplastic cells
Mum-1-	[EP190]-	Immunoreactive score 2+in large nucleolated cells

CONCLUSION

EBV+DLBCL has a poor response to treatment and poor survival outcomes and so a rapid diagnosis is essential. Detection relies on clinical suspicion and looking for EBV in every case of DLBCL. The prognosis of EBV+DLBCL is worse than that

of EBV-negative tumors, with a median survival of 2 years. Prognosis is worse in patients 70 years or older and in those with B symptoms. At present, there is no uniformly accepted treatment for EBV+DLBCL beyond the current standard therapy for DLBCL. This case highlights the importance of a detailed morphologic evaluation and comprehensive IHC, including *in-situ* hybridization for EBER along with awareness of potential morphologic and IHC overlap with Hodgkin lymphoma on one hand as well as DLBCL, NOS, and T-cell/histiocyte rich large B-cell lymphoma on the other.

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