Unusual immunophenotypic variant of large B-cell lymphoma associated with HHV-8 and EBV in an HIV positive patient

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Abstract Human herpesvirus type 8, also known as Kaposi’s sarcoma-associated herpesvirus (HHV-8/KSHV) has been associated with several lymphoproliferative disorders including Kaposi’s sarcoma, primary effusion lymphoma (PEL), cases of multicentric Castleman’s disease (MCD) including plasmablastic lymphoma associated with MCD, and germinotropic lymphoproliferative disorder. These lymphoproliferative disorders, with the exception of the latter, usually arise in HIV-positive or profoundly immunosuppressed patients. Herein, we describe an unusual large B-cell lymphoma in a 43 year-old male infected with HIV who presented with multiple lymphadenopathies. The tumor cells were positive for EBV, HHV-8/KSHV, CD20 (small subset), PAX5, and IgM and negative for CD138, and IgG. This lymphoma is difficult to classify following the 2008 WHO criteria and expands the current spectrum of viral-associated lymphomas.

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1. Introduction

HHV-8/KSHV positive lymphomas include primary effusion lymphoma (PEL), plasmablastic lymphoma associated with multicentric Castleman’s disease (MCD), and germinotropic lymphoproliferative disorder. PEL is a distinct clinicopathologic entity that accounts for approximately 4% of all AIDS-related lymphomas [1]. It usually
involves one specific body cavity (pleural, peritoneal or pericardial) in the form of an effusion without the presence of a tumor mass [2]. This type of lymphoma occurs most commonly in HIV and HHV-8/KSHV positive middle-aged males, is frequently associated with EBV, and has a dismal survival [2,3]. Less commonly, HIV positive patients may present with HHV-8/KSHV positive lymphomas that involve solid organs, lack a concurrent effusion, and exhibit morphologic, genotypic, and immunophenotypic features of classic PEL [4]. This distinct presentation, also known as the extracavitary/solid form, may involve lymph nodes, gastrointestinal tract, skin, lungs, and the central nervous system as the only site of involvement; however, it may also precede or follow the development of a malignant effusion of typical PEL [4]. Solid PEL can be difficult to diagnose. The tumor cells have variable cytomorphology ranging from plasmablastic or immunoblastic to frankly anaplastic and usually lack expression of pan-B-cell markers as well as surface and cytoplasmic immunoglobulins; nevertheless B-cell lineage can be demonstrated by the presence of monoclonal IgH gene rearrangements [4,5]. Detection of HHV-8/KSHV in tumor cells is required to establish the diagnosis [6].

Herein, we report the clinicopathologic features of a very unusual case of HIV-associated large B cell lymphoma, which is difficult to classify following the 2008 WHO criteria and discuss the divergence in the clinicopathologic features of this unusual case between the most important differential diagnoses (solid PEL, plasmablastic lymphoma associated with MCD, and germinotropic lymphoproliferative disorder).

2. Case report

The patient is a 43-year-old homosexual male with a remote history of syphilis who initially presented to an outside hospital in August of 2013 complaining of a four-month history of fevers, chills, and night sweats. He was admitted and found to be HIV positive for which he was started on highly active antiretroviral therapy. At presentation his absolute CD4 count was 99 cells/mL and HIV viral load was 243 copies (log 2.39). A CT scan revealed diffuse lymphadenopathy (axillary, mesenteric, root, and retroperitoneum) and mild hepatosplenomegaly. An excisional biopsy of two right axillary lymph nodes was performed. A bone marrow biopsy near the time of diagnosis was negative for involvement by lymphoma. Chemotherapy with R-EPOCH (rituximab, etopoxide, vincristine, doxorubicin cyclophosphamide, prednisone) protocol was initiated three months after his initial presentation, for a total of six cycles. To assess response to therapy, a CT scan of the neck, chest, abdomen, and pelvis was performed after the fourth cycle and showed an increase in the number and size of chest and abdominal lymph nodes, indicating progressive disease. The patient underwent salvage chemotherapy but unfortunately passed away seven months after initial presentation.

Two blocks of formalin fixed paraffin embedded (FFPE) tissue containing two excisional lymph node biopsies, measuring 2 and 1.2 cm in greatest dimension were sent in consultation to the Division of Hematopathology at the University of Miami/Sylvester Comprehensive Cancer Center. Multiple 4-μm thick tissue sections were prepared and stained with hematoxylin and eosin, and specific immunohistochemical stains. Molecular studies were performed on FFPE to determine IgVH, IgH, and T-cell receptor (TCR) gamma genes rearrangements by gene sequencing and fragment analyses.

Histologic sections revealed lymph nodes involved by lymphoma. The neoplasm had vaguely nodular pattern in one of the lymph nodes (Fig. 1A and B) and a diffuse pattern in the other lymph node (Fig. 1C). The lymph node sinuses were open and free of tumor cells. The tumor cells were large, mostly immunoblastic with occasional plasmablastic appearance. Mitotic figures and apoptotic bodies were common (Fig. 1D–F). A “starry sky” pattern was focally noted. Kaposi sarcoma or morphologic changes reminiscent of Castleman’s disease were not identified.

Immunohistochemical stains showed that the tumor cells were positive for CD45/LCA, CD20 (weak, small subset) (Fig. 2A), PAX-5 (subset), MUM-1 (Fig. 2B), IgM (strong) (Fig. 2C), CD30 (subset) (Fig. 2D); and were negative for CD3, CD4, CD5, CD7, CD34, CD56, CD138 (Fig. 2E), EMA, and IgG (Fig. 2F). They were also positive for HHV-8/KSHV (nuclear granular pattern) (Fig. 3A and insert), which highlighted the nodular pattern of the malignant cells. In situ hybridization analysis for EBV-encoded RNA (EBER) was positive (Fig. 3B and insert). The tumor cells were also moderately to strongly positive for nuclear expression of c-MYC detected by immunohistochemistry, rearrangement and were negative for monoclonal IgH gene rearrangement and were negative for monoclonal TCR gamma gene rearrangement. Despite the strong expression of c-MYC detected by immunohistochemistry, rearrangements of the MYC gene were not detected by fluorescence in situ hybridization. An attempt to determine the mutational status of IgVH gene to support the presence of somatic
mutations and germinal/postgerminal center cell origin of the neoplasm as seen in PEL by sequence analysis was performed. Unfortunately, due to the poor preservation of the genomic DNA, we were unable to retrieve a meaningful genetic sequence.

3. Discussion

The last decade of the twentieth century ushered in major discoveries, such as the identification and characterization of HHV-8/KSHV as an oncogenic virus causing AIDS-associated Kaposi’s sarcoma [7], PEL [8], MCD [9], plasmablastic microlymphoma [10], and germinotropic lymphoproliferative disorder [11]. Although all these previously described entities have specific clinical, demographic, morphologic, and immunophenotypic hallmarks, their accurate and reproducible classification remains a challenge. This is particularly the case for HHV-8/KSHV-associated large B-cell lymphomas presenting as solid masses in extracavitary sites [12].

The differential diagnosis of HHV-8/KSHV-associated lymphoproliferative processes presenting as solid masses in extracavitary sites includes solid PEL, plasmablastic lymphoma associated with MCD, and germinotropic lymphoproliferative disorder. Lymphoma cells in the extracavitary/solid form of PEL are usually EBV, HHV-8/KSHV, and CD138 positive, may express IgG, and commonly lack expression of B cell markers. In general, PELs are considered to be of postgerminal center B-cell derivation based on the fact that most PELs have detectable somatic hypermutation of Ig variable region genes (which could not be confirmed in our case due to

![Fig. 1 Morphologic findings. (A–B) The neoplasm had vaguely nodular pattern in one of the lymph nodes that was better noted with the stains for EBV and HHV-8/KSHV (see below). (C) A diffuse pattern was noticed in the other lymph node. (D–F) The tumor cells were large, predominantly immunoblastic in appearance, with basophilic cytoplasm, prominent nucleoli, and occasional plasmablastic features. Mitotic figures and apoptotic cells were noted.](image)
technical difficulties). Additionally, somatic hypermutation of the noncoding region of the BCL-6 gene is also present [13]. Expression of IgG immunoglobulin and plasma cell markers, such as CD138, as well as previous results of gene expression profiling analysis of PELs from HIV positive patients, further supports a postgerminal center cell or “plasmablastic” phenotype [12,14]. Unusual features for solid PEL in our case include, the presence of multiple lymphadenopathies, negativity for CD138, and expression of cytoplasmic IgM (most cases of PEL lack cytoplasmic Ig) [2,4,5].

The differential diagnosis also includes large B-cell lymphoma arising in HHV-8/KSHV-associated MCD, where the HHV-8/KSHV positive plasmablasts harbor unmutated Ig variable region genes, express high levels of cytoplasmic IgM and lambda light chain (as in our case), and are negative for CD138 and EBER [15]. The positivity for EBER and the lack of features and/or clinical history of MCD make this diagnosis unlikely. Germinotropic lymphoproliferative disorder is a rare HHV-8/KSHV associated process that occurs mostly in immunocompetent adults who usually present with localized nodal enlargement [11]. The plasmablasts are present in large numbers, involve germinal centers (germinotropism), are positive for MUM-1, LANA-1, and EBER, and are negative for CD20, CD79a, BCL-2, CD10 or BCL-6. Molecular analysis of the plasmablasts usually demonstrates a polyclonal or oligoclonal IgH gene rearrangement pattern. Features that argue against this diagnosis in our case include the HIV positive status of the patient, his clinical presentation with multiple lymphadenopathies, tumor cells harboring monoclonal IgH gene rearrangement, lack of germinotropism, and the clinical outcome.

To the best of our knowledge there are only 3 cases documented in the literature of HHV-8/KSHV associated lymphomas with expression of IgM (interestingly all lambda light chain restricted as in our case). One case resembled classical Hodgkin lymphoma, another was a

![Fig. 2 Immunohistochemical findings. (A) A subset of the tumor cells was moderately positive for CD20 and (B) strongly positive for MUM-1 and (C) IgM, and (D) a subset was positive for CD30. The tumor cells were negative for CD138 (E) and IgG (F).](image-url)
case of intravascular large B-cell lymphoma, and the third case showed hybrid features between multicentric plasmablastic microlymphoma and germinotropic lymphoproliferative disorder in the context of MCD [16,17]. Our case increases the number of unusual neoplasms that may be found in association with HHV-8/KSHV infection.

4. Conclusion

The present case of HHV-8/KSHV and EBV positive large B-cell lymphoma in the setting of HIV infection, involving lymph nodes is difficult to classify following the current 2008 WHO schema. Nonetheless, we believe that this case may represent an unusual phenotypic variant of solid PEL expressing IgM.

Disclosure

The authors have stated that they have no conflicts of interest.

References


