J Neuropathol Exp Neurol
Vol. 79, No. 8, August 2020, pp. 915–920
doi: 10.1093/jnen/nlaa061

BRIEF REPORT

Epstein Virus Barr-Positive Diffuse Large B-Cell Lymphoma Associated with Hemophagocytic Lymphohistiocytosis

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Abstract

Hemophagocytic lymphohistiocytosis (HLH) is a rare and often fatal disease if not diagnosed and treated promptly. HLH can be due to genetic factors or infections, malignancies and collagen-associated vascular diseases. Malignancy-associated HLH is not only more common in the setting of T/NK-cell lymphomas, but may also rarely be seen in the setting of B-cell lymphoma. Here, we describe a unique case of a patient who initially was diagnosed with HLH secondary to Epstein Barr virus (EBV) infection and subsequently developed EBV-positive diffuse large B-cell lymphoma affecting the brain. This case highlights the spectrum of findings associated with EBV infections and the challenges in diagnosing underlying diseases associated with HLH.

Key Words: Epstein Barr virus (EBV), Hemophagocytic lymphohistiocytosis (HLH).

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a rare disease in which macrophages/lymphocytes are inappropriately activated resulting in phagocytosis of all bone marrow-derived cells (1). This inappropriate activation leads to overproduction of cytokines promoting edema, hyperferritinemia, hypertriglyceridemia, hypofibrinogenemia, anemia, and fever (2). If left untreated, it is usually fatal with a mean survival time of <2 months (3). The disease is broadly classified into primary (genetic) and secondary forms (infections, autoimmune diseases, malignancies, or immunosuppression).

In adults, hematologic malignancies are the most common disease implicated in secondary HLH (4). Malignancy-associated HLH has been reported in the setting of T/NK-cell lymphomas (3, 5) and is common in both eastern and western populations (6). However, B-cell lymphomas related to HLH are rare. HLH and B-cell lymphomas are predominantly described in Asian populations and may be related to environmental or genetic factors (6, 7). The majority of B-cell lymphoma associated with HLH are diffuse large B-cell lymphoma (DLBCL), which occurs more commonly in older people (6–8); however, CNS B-cell lymphoma associated with HLH has only rarely been reported.

RESULTS

A 35-year-old previously healthy man presented in September 2017 with a history of 2 weeks of low-grade fever, cold sweats, shortness of breath on exertion, fatigue, aches, and pain. A chest X-ray revealed bibasilar patchy infiltrates and the patient was treated with ceftriaxone 1 mg IM and azithromycin 500 mg/daily for 5 days. During the following weeks, the patient persisted symptomatic and received a course of amoxicillin clavulanate 875–125 mg/BID for 10 days and prednisone 50 mg/day for 5 days, followed by levofloxacin 750 mg/daily for 7 days.

After 4 weeks of failed outpatient treatment and worsening symptoms, he was admitted in the hospital. A chest CT showed bilateral pulmonary consolidation. An extensive work-up for infection, rheumatologic and allergic were negative (Table 1). He received broad-spectrum IV antibiotics with piperacillin and tazobactam 4.5 g IV and levofloxacin 750 mg IV. The patient underwent a bronchoscopy with transbronchial biopsy/bronchoalveolar lavage which was negative for malignancy and culture studies only grew Candida. The patient was switched to itraconazole 200 mg/BID for 15 days and discharged with an improvement in his condition.

In October 2017, the patient was readmitted to the hospital due to elevated lactic acidosis >7, fever and sinus tachycardia, with no focal symptoms. A chest CT showed improved, but persistent bilateral lung infiltrates. Laboratory exams revealed EBV viremia (Table 1) and additional tests were nondiagnostic: (i) Thoracentesis: Mixed inflammatory infiltrate, no evidence of lymphoma; (ii) CT guided lung biopsy: Lymphoplasmacytic infiltrate, nondiagnostic; (iii) Liver biopsy: Revealed steatohepatitis with mild nonbridging pericellular fibrosis; (iv) Bone marrow: Suboptimal biopsy with focally normal cellular, bone marrow with trilineage, concur-

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This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
The authors have no duality or conflicts of interest to declare.

TABLE 1. Laboratory Data At Time of Admission and in October

Laboratory Data	Admission	October
Hb	14 g/dL	8.7 g/dL
Hematocrit	39.4%	26.6%
Absolute lymphocytes:	0.6 (10e9L) and the normal reference range for absolute lymphocytes is 0.8–5.3 (10e9/L)	
Absolute neutrophil count:	7.6 (10e9/L)→this is within normal limits. Reference range is 1.6–8.3 for absolute neutrophil count.	
Platelet count	225 (10e9/L). Normal reference range for platelets is 150–450 10e9/L	
White blood cell count:	9.0 (10e9/L). Normal reference range for WBCs is 4–11.	
RDW	16.2%	17.3%
ALT	77 U/L	49 U/L
AST	119 U/L	118 U/L
Sodium	127 mmol/L	134 mmol/L
Calcium	7.6 mg/dL	7.4 mg/dL
Albumin	2.3 g/dL	1.5 g/dL
Protein total	5.1 g/dL	4.5 g/dL
Mononucleosis screen	Negative	
Lactic acid	3.3 mmol/L	6.2 mmol/L
Lactate dehydrogenase		597 U/L
Sputum culture aerobic bacterial	Moderate growth for Candida albicans	
INR	1.18	1.16
PTT	33	43
HIV	Negative	
Respiratory virus panel by PCR	Negative	
Antineutrophil cytoplasmic Aby IgG	Negative	
Triglycerides	295 mg/dL	379 mg/dL
Fibrinogen	108 mg/dL	245 mg/dL
Bronchial culture aerobic bacterial	Negative	
Ferritin	2060 ng/mL	3440 ng/mL
Aspergillus galactomannan Agn bronchial	Negative	
Aspergillus antibody blood	None detected	
Mycoplasma pneumoniae by PCR bronchial	Not detected	
Legionella species PCR	Not detected	
Vasculitis panel—blood (myeloperoxidase antibody IgG and proteinase 3 antibody IgG)	Negative	
GBM antibody IgG—blood	Negative	
C3	38 mg/dL	
C4	8 mg/dL	
Blastomyces Agn quant EIA blood	Negative	
Hepatitis C antibody	Nonreactive	
Hepatitis B surface antigen	Nonreactive	
AFB stain non blood bronchoalveolar	Negative	
DNA double stranded antibodies	Negative	
Anti-nuclear Aby IgG by IFA with reflex	Negative	
Fungal antibodies (blastomycosis, coccidiomycosis, histoplasmosis, aspergillus)	Negative	
Neutrophil cytoplasmic IgG antibody	Negative	Negative
Viral culture respiratory bronchial	Negative	
Sputum	Candida albicans and Candida dubliniensis	
Fungus culture bronchial lavage	Candida albicans and Candida dubliniensis	
Mycoplasma pneumonia abys IgG and IgM		Negative
CMV antibody IgG		Positive
CMV DNA quantification		Not detected
EBV capsid antibody IgG		Positive

(continued)

TABLE 1. Continued

Laboratory Data	Admission	October
EBV DNA PCR quantitative whole blood		1593
Cryptococcus antigen		Negative
Beta 2 microglobulin		14.6 mg/dL
GBM antibody IgG		Negative
Jo 1 antibody IgG		Negative
SSA Ro ENA antibody IgG		Negative
SSB La ENA antibody IgG		Negative
HTLV I and 2 antibody with reflex		Negative
Direct antiglobulin test		Negative
M tuberculosis by quantiferon! follow QTB collection process		Negative
Anti-nuclear Aby IgG by IFA with reflex		Negative
Legionella pneumophila aby types 1–6 IgG		Negative
Coxiella B antibody IgG phase 1 and 2		Negative

rent flow cytometry was performed (IF17-4039) and showed no immunophenotypic evidence of malignancy but with very few B lymphocytes present; and (5) PET scan: Increased bilateral lower lobe consolidations with new scattered areas of parenchymal hypodensity, associated with FDG uptake surrounding these hypodensities, mild hepatosplenomegaly. Ultimately, a presumptive diagnosis of HLH was made and the patient's condition improved after receiving IV dexamethasone 40 mg/4 doses.

In December 2017, he again developed recurrent fevers, shortness of breath and received dexamethasone 40 mg daily for 4 days, with a plan to taper over the following weeks. In January 2018, the patient continued having worsening symptoms with rising levels of ferritin suggesting an impending relapse; he received dexamethasone 40 mg daily/2 weeks. A CT chest imaging study showed worsening of his pulmonary disease with cavitary lesions and a sputum culture grew MSSA. The steroid treatment was stopped due to concern of fungal pneumonia and he received a 14-day course of ceftriaxone. The patient returned in January 2018, for an outpatient schedule transbronchial and cryoprobe biopsy of LLL. Results revealed lmphohistiocytic infiltrate that was T cell and histocyte predominant admixed with occasional B cells, and plasma cells that appear polytypic, not diagnostic of lymphoma. His BAL culture did not grow any organism except for Candida. A repeated EVB virus PCR was 675 and the decision was to continue monitoring the patient.

In February 2018, the patient's treatment was switched to mycophenolate 500 mg/BID, due to prolonged steroid complications including cushingoid appearance and delayed wound healing. CT control of the chest revealed improved bilateral lung consolidation with remaining cavitary lesions in LLL, likely remaining cavitary lesion after focal necrosis associated with HLH lung involvement (Fig. 1).

In May 2018, he presented to the emergency department with a 1-week history of left-sided facial droop, weakness, and slurred speech, at which time MRI of the brain revealed right-sided periventricular lesions involving the thalamus, basal ganglia, caudate, and putamen with perilesional edema (Fig. 2). He was started on high-dose dexamethasone and underwent brain biopsy, which confirmed EBV-positive DLBCL (Fig. 2). PET scan showed subtle enhancement for metastatic disease in gastrohepatic lymph nodes and a left lingular pulmonary nodule, these lesions were monitored. His treatment was as followed, illustrated in Table 2.

In July 2018, a brain MRI showed tumor progression involving the right basal ganglia and the adjacent corona radiata, with more well-defined peripheral enhancement and consolidation of previously separated areas of enhancing tumor. The patient was started on whole brain radiotherapy plus focal radiation. He then underwent the first phase of planned radiotherapy to a cumulative dose of 3060 cGy delivered in 17 fractions to the whole brain with 10 MV photons via a 3D conformal radiotherapy technique. He then underwent mid treatment MRI to define his boost volume. The study showed a 3.1 × 2.2 × 2.7 cm rim-enhancing irregular mass involving the right thalamus, right putamen, caudate, and the corona radiata. There was associated vasogenic edema measuring 5.9 × 4.4 cm. A 9 Gy boost was delivered in 5 fractions to the residual disease with 6 MV photons via a volumetric arc therapy technique in an effort to minimize dose delivered to the uninvolved head and neck structures. In summary, the patient first reported in September of 2017 with bibasilar patchy infiltrates, followed by a diagnosis of HLH in October of the same year. In the following months, the patient experienced worsening symptoms and was ultimately diagnosed with EBV-positive DLBCL as shown in Figure 3.

DISCUSSION

To the best of our knowledge, this is the first case of an EBV-positive DLBCL in the CNS associated with HLH. In our literature search, we found only 2 previously reported cases of primary CNS lymphoma related to HLH (3, 9), neither of which were reported to be EBV-positive. Both previous cases were diagnosed as primary HLH, in contrast to our case in which the patient had a presumptive diagnosis of secondary HLH due to EBV infection. Although, in all 3 cases, the presentation was insidious with nonspecific symptoms and the diagnosis of lymphoma followed several weeks/months after the

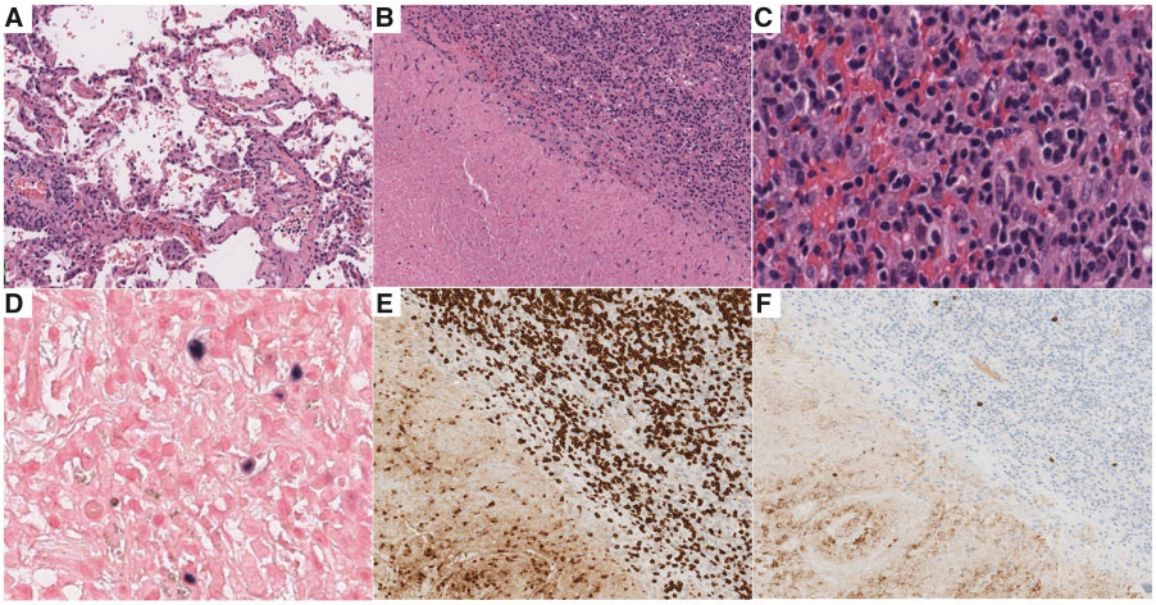

FIGURE 1. Lung biopsy. Pneumonia with diffuse necrosis and EBV-infected cells. **(A)** H&E lung reveals focal acute fibrinous organizing pneumonia. **(B)** H&E staining (10×) reveals infiltrative process (top right) and diffuse necrosis (bottom left), 10×. **(C)** H&E staining (40×) reveals immune cells which have **(D)** EBER-ISH positivity, **(E)** diffuse positivity for CD3, and **(F)** sparse positivity for CD20.

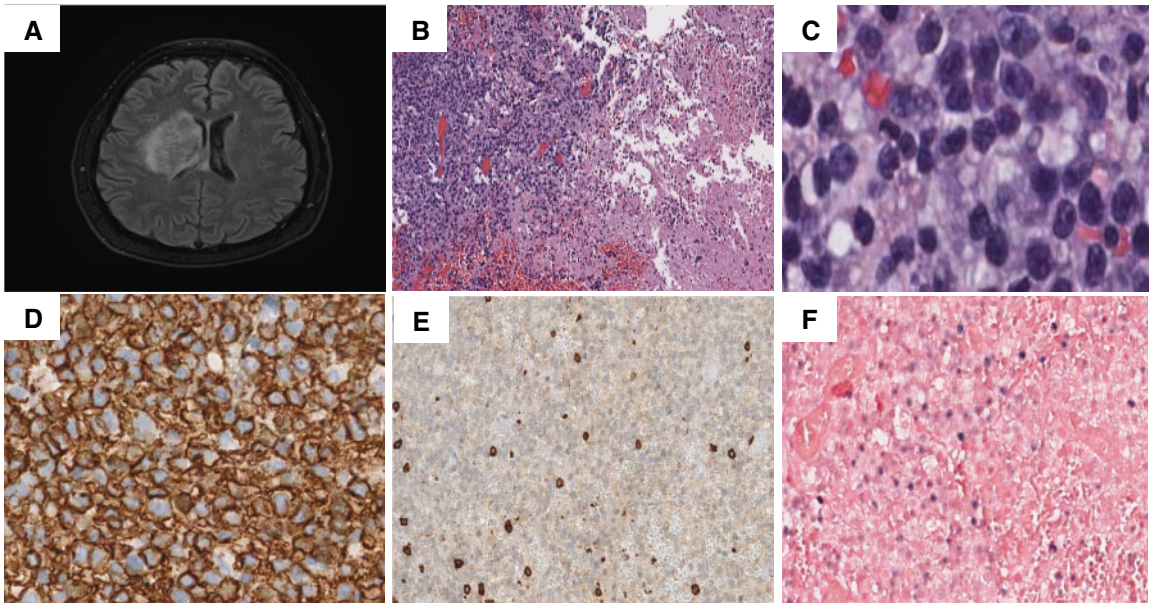

FIGURE 2. Brain biopsy—primary CNS lymphoma. **(A)** MRI of brain shows abnormal heterogeneous mass-like enhancing lesion centered in the right basal ganglia. **(B)** Normal brain (right) adjacent to diffuse large B cell lymphoma (left). **(C)** Large atypical lymphoid cells with slightly vesicular nuclear chromatin, conspicuous nucleoli and irregular nuclear outline. **(D)** The large atypical lymphoid cells show strong staining for CD20. **(E)** CD3 stain highlights scattered reactive T cells intermixed with atypical lymphoid cells. **(F)** EBER-ISH positive in atypical large cells.

HLH presentation/diagnosis. The case reported by Dzoljic et al, was a 25-year-old man diagnosed with DLBCL affecting the brain (10). This patient was a heterozygous carrier of the perforin mutation (the genetic marker for HLH) and his brother died from a confirmed primary HLH (homozygous/compound heterozygous perforin mutations). This case report raises the discussion of patients with partial activated perforin gene and their association with lymphoid malignancies, but not HLH. Unfortunately, the patient has not been tested for perforin mutations.

This case emphasizes the challenges when searching for possible underlying diseases, especially in the setting of hematologic malignancies. HLH can precede by many years the diagnosis of an underlying malignancy, particularly lymphoma; however, it can also occur at the same time of initial presentation, or even after definitive treatment/remission (6, 11). Our patient initially presented as secondary HLH due to EBV viremia, with no evidence suggesting lymphoma in the lung, although further imaging studies were not pursued upon initial diagnosis. Therefore, we cannot completely exclude the possibility of early lymphoma at the time of HLH diagnosis. Immunosuppression controlled the cytokine storm secondary to EBV infection; however, it may be possible that our patient presented in the preclinical stages of lymphoma triggered by EBV affecting the B-lymphocytes at the time of HLH presentation. While EBV-positive DLBCL can occur in immunocompetent patients, it is mostly commonly seen in elderly patients (≥65 years of age). In immunocompromised patients, it is predominantly in the setting of HIV and commonly seen in the CNS. Our patient initially received immunosuppressive therapy for HLH which may have contributed to the development of the lymphoma in the CNS. A previous study discusses that hematological conditions, like autoimmune hemolytic anemia, can precede the appearance of lymphomas, especially after immunosuppression therapy is given for the presenting condition. This suggests that HLH presenting as a primary-like form can represent a similar event (9).

TABLE 2. Treatment Following Confirmed EBV-Positive DLBCL

Day 1	Methrotexate 8 g/m^2
Day 2	Rituximab 375 mg/m^2
Day 7	Temozolomide 150 mg/m^2/day
Day 10	Rituximab 375 mg/m^2
Day 15	Methrotexate 8 g/m^2
Day 17	Rituximab 375 mg/m^2
Day 24	Rituximab 375 mg/m^2

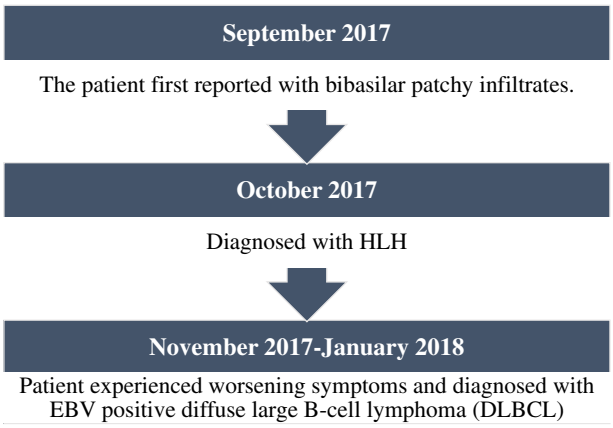

FIGURE 3. Summarized progression of EBV-positive DLBCL diagnosis.

B-cell lymphomas associated with HLH include various types of large lymphoma (LBCL), including intravascular lymphoma and DLBCL not otherwise specified, especially the immunoblastic morphologic variant. HLH secondary to B-cell lymphoma has a less aggressive disease course with improved survival, compared with HLH secondary to T/NK lymphoma. Nonetheless, the overall prognosis of HLH secondary to B-cell lymphoma remains poor with a median survival of 9–11 months (6, 12).

Our patient met diagnostic criterial for HLH but did not have demonstrable hemophagocytosis on bone marrow biopsy. According to the HLH 2004 diagnostic criteria, the diagnosis of HLH requires 5 of the following 8 findings, as presented in Table 3. Our patient fulfilled 5 of 8 diagnostic criteria: Fever, cytopenia, hypertriglyceridemia/low fibrinogen hyperferritinemia, and elevated IL2Ra. Of note, the NK cell level proportion was on the low end of normal at 0.2% NK cells. The patient experienced triglyceride levels of 226 mg/dL (normal triglyceride reference range is <150 mg/dL), while also reporting fibrinogen levels of 120 mg/dL (normal fibrinogen reference range is 200–420 mg/dL).While hemophagocytosis is a diagnostic criteria, it is neither pathognomonic of nor required for the diagnosis of HLH (13) and in many cases, including ours, bone marrow aspiration may fail to demonstrate hemophagocytosis at the first examination because of its patchy involvement (14). Of note to the cytopenia, hemoglobin, absolute lymphocytes, white blood cells, platelet count, and absolute neutrophil count were decreased as well. The treatment of HLH should be aimed at suppressing the overactive immune system and treating the underlying disorder. In patients who present with HLH secondary to malignancy, the goal of treatment is to target the malignancy.

HLH is a rare disorder and potentially lethal if not diagnosed and treated in a timely manner. HLH is being recognized with increasing frequency in adults. Establishing a diagnosis of HLH may be difficult due to nonspecific symptoms on presentation and requires a high degree of clinical

TABLE 3. HLH Criteria, Patient Exhibited 5 of 8 Criteria (Bolded)

Fever ≥ 38.5°C	**Peripheral blood cytopenia**	Hemophagocytosis in the bone marrow, spleen, lymph node, or liver	Low or absent NK cell activity.
Splenomegaly	**Hypertriglyceridemia and/or hypofibrinogenemia**	Elevated ferritin	**Elevated soluble CD25 (soluble IL-2 receptor alpha)**

suspicion. When HLH is suspected, an underlying cause must be identified which can include underlying primary factors, for example, genetic, or secondary processes, for example, EBV infections or underlying malignancies, especially NK/T-cell lymphoma. Our report highlights the spectrum of findings associated with EBV infections, and the clinical overlap between EBV-driven HLH and EBV-driven lymphoma related with HLH.

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