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Original Article

The ambiguous boundary between EBV-related hemophagocytic lymphohistiocytosis and systemic EBV-driven T cell lymphoproliferative disorder

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Abstract: Epstein Barr virus (EBV)-related hemophagocytic lymphohistiocytosis (EBV-HLH) is a form of acquired, infection-related HLH which typically represents a fulminant presentation of an acute EBV infection of CD8+ T cells with 30-50% mortality rate. Systemic EBV-positive lymphoproliferative disease of childhood (SE-LPD) is a rare T cell lymphoproliferative disorder predominantly arising in the setting of acute EBV infection, often presenting with HLH. Since both entities have been associated with clonal T cell populations, the discrimination between these diseases is often ambiguous. We report a unique case of a 21 years old female who presented with clinical and laboratory findings of florid HLH in the setting of markedly elevated EBV titers (>1 million) and an aberrant T cell population shown to be clonal by flow cytometry, karyotype, and molecular studies. This case raises the differential of EBV-HLH versus SE-LPD. Review of the literature identified 74 cases of reported EBV-HLH and 21 cases of SE-LPD with associated HLH in 25 studies. Of those cases with available outcome data, 62 of 92 cases (67%) were fatal. Of 60 cases in which molecular clonality was demonstrated, 37 (62%) were fatal, while all 14 cases (100%) demonstrating karyotypic abnormalities were fatal. Given the karyotypic findings in this sentinel case, a diagnosis of SE-LPD was rendered. The overlapping clinical and pathologic findings suggest that EBV-HLH and SE-LPD are a biologic continuum, rather than discrete entities. The most clinically useful marker of mortality was an abnormal karyotype rather than other standards of clonality assessment.

Keywords: Systemic EBV-positive lymphoproliferative disease of childhood, EBV-related HLH, clonal EBV-related HLH, atypical T cell population, EBV-related T cell lymphoma

Introduction

Epstein Barr Virus (EBV) is a ubiquitous Herpes virus with a variety of clinical presentations and associations in both the acute and chronic settings. EBV plays a key pathologic role in several neoplasms, including Burkitt lymphoma, classical Hodgkin lymphoma, post-transplantation lymphoproliferative disorders, NK/T cell lymphoma (nasal type), angioimmunoblastic T cell lymphoma, and nasopharyngeal carcinoma. These are distinct neoplasms where diagnosis is based on clear morphologic and immunophenotypic criteria. However the viral syndromes associated with EBV have a range of clinicopathologic presentations including acute self-limited infectious mononucleosis (IM), rare fulminant infections in patients with X-linked lymphoproliferative disorder (XLP), chronic active EBV infection (CAEB), EBV-related hemophagocytic lymphohistiocytosis (EBV-HLH), and systemic EBV-positive lymphoproliferative disease of childhood (SE-LPD). One main discriminator amongst these entities is the viral integration into CD21+ B cells in IM and XLP versus T cells in the remaining disease categories (**Table 1**). A second main discriminator is the severity of disease. Based upon these criteria, the viral syndromes can be distinguished, with the exception of EBV-HLH and SE-LPD.

IM is an acute EBV infection typically presenting with a triad of fever, pharyngitis and lymphadenopathy (LAD), with self-resolution within 1-2 months. In this setting, EBV typically replicates and survives in CD21+ B cells [1] with systemic

Table 1. Categorization of the spectrum of EBV-related viral syndromes

	Infected cell type	
	B	T
mild disease	IM	CAEB
severe disease	XLP	EBV-HLH, SE-LPD

IM, infectious mononucleosis; XLP, X-linked lymphoproliferative disease; CAEB, chronic active EBV infection; EBV-HLH, EBV-related hemophagocytic lymphohistiocytic syndrome; SE-LPD, systemic EBV-positive lymphoproliferative disease of childhood.

symptoms attributed to the degree of monoclonal or polyclonal [2] CD8+ cytotoxic T cell response to the viremia [3, 4]. There are rare fulminant infections, typically in immunodeficient patients, most notably due to XLP. These patients bear a defect in SAP (signaling lymphocyte activation molecule associated protein, also known as SH2D1A or DSHP) which leads to dysregulated T cell response and results in an uncontrolled proliferation of EBV-infected B cells that clinically resembles EBV-HLH [5, 6].

Rarely, infection with EBV presents as chronic active EBV infection (CAEB). This entity represents persistent and/or recurrent IM for at least six months with an unusual serologic pattern and an accentuated elevation in EBV DNA load. The clinical presentation in this setting shows variable severity with hepatosplenomegaly (HSM), persistent LAD, hypersensitivity to mosquito bites, and hydroa vacciniforme. Laboratory findings include non-specific abnormalities, such as liver dysfunction, thrombocytopenia, and anemia. The pathological changes are variable, including a proliferation and infection of polyclonal predominantly non-CD8+ (CD4+/CD8- and CD4+/CD8+) T cells and CD16+ NK lymphocyte subpopulations, in contrast to infection of B cells seen in IM [7]. These cases are only rarely associated with acute/fulminant death, in contradistinction to EBV-HLH, and are not associated with a clonal T cell proliferation [6]. Cases previously classified as CAEB with a clonal proliferation are now re-classified as systemic EBV-positive lymphoproliferative disease of childhood [8]. In CAEB, mortality generally results from subsequent development of HLH and/or a T/NK lymphoproliferative neoplasm [9].

EBV-related hemophagocytic lymphohistiocytosis (EBV-HLH) is a form of acquired, infection related HLH which typically presents as a fulminant acute infection by EBV of CD8+ T cells, rather than the B cells seen in IM and XLP [10, 11]. The clinical and laboratory presentation meets HLH-2004 criteria, fulfilling five of 8 criteria which include signs and symptoms of splenomegaly, bi- or pancytopenia, fever, hypertriglyceridemia, hypofibrinogenemia, hyperferritinemia, hemophagocytosis, low/absent natural killer cell activity, or high soluble interleukin-2 receptor levels [7, 12-16]. This entity carries a fatality rate ranging from 30-50% [15, 17], with no apparent statistically significant benefit from stem cell transplant in one meta-analysis evaluating the outcome of 342 patients with EBV-HLH [15]. This may be seen preceding, concurrent with, or subsequent to systemic EBV-positive lymphoproliferative disease of childhood [18]. This entity is most common in East Asia [16, 18]; however, this is also the most common etiology of acquired, infection-related HLH in the West [19]. Aberrant, and even clonal, T cell populations have been reported in association with EBV-HLH, although the presence of these populations is not considered neoplastic [20-23].

Systemic EBV-positive lymphoproliferative disease of childhood (SE-LPD) is a rare T cell lymphoproliferative disorder predominantly arising in the setting of acute EBV infection, although it has also been seen in association with CAEB [24, 25]. It has also been reported in the literature under the names of fulminant EBV-positive T-cell LPD of childhood, sporadic fatal infectious mononucleosis, fulminant hemophagocytic syndrome, fatal EBV-associated hemophagocytic syndrome, and severe chronic active EBV (CAEB) infection [26-28]. The majority of cases have been reported in Asia, with only 15 cases reported in Western countries [28]. The clinical and pathologic picture mimic EBV-HLH with infected CD8+ T cells and common HLH presentation, but it is distinguished by its definitive clonal nature, with morphologies varying from deceptively benign to frankly malignant [18, 23, 26, 28]. This entity portends an abysmal prognosis with nearly 100% mortality [18, 26, 28, 29].

There is considerable overlap between these diagnostic categories, leading to significant clinical confusion. Indeed, fulminant IM in the setting of XLP, EBV-HLH, and SE-LPD may all present with HLH, and clonal aberrant T cells

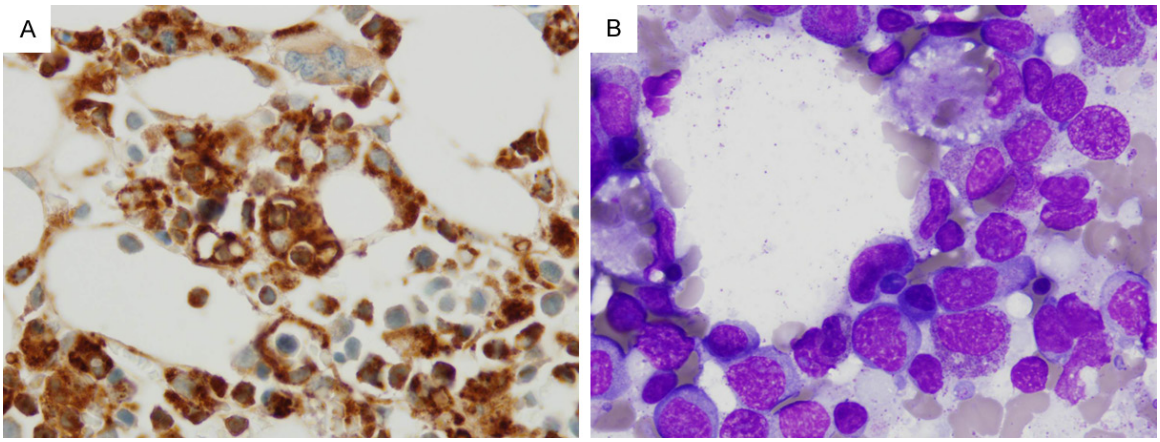

Figure 1. Images of the diagnostic marrow. A. CD68+ immunohistochemical stain highlighting intramedullary histiocytes with intact intracytoplasmic nucleated cells (1000×). B. Wright stain aspirate smear with erythrophagocytosis and frequent intermediate sized lymphocytes with irregular nuclear contours (1000×).

populations have been found in EBV-HLH and SE-LPD. Furthermore, CAEB may transform to either EBV-HLH or SE-LPD. The discrimination between the latter two entities may therefore be particularly challenging but may significantly impact treatment decisions.

Herein we report an illustrative case which highlights the diagnostic overlap between EBV-HLH and SE-LPD, in addition to a review of the literature of 95 reported cases of these entities. We demonstrate that karyotypic abnormalities are the most robust way to predict adverse clinical outcome, but suggest that there is a pathologic spectrum of these EBV-related disorders rather than two distinct diagnostic categories.

Materials and methods

Morphologic studies

Peripheral blood smears and bone marrow aspirate smears were air dried and stained with Wright-Giemsa. Bone marrow biopsy specimens were fixed in B-Plus, decalcified, and embedded in paraffin. Clot sections were fixed in B-Plus and embedded in paraffin. Sections (5 μm) of both bone marrow biopsy and clot were obtained and stained with H & E.

Immunohistochemical analysis

Immunohistochemical analysis was performed on 5 μm sections from the bone marrow biopsy. The Leica Bond Polymer Refine Detection (DAB) Kit (Leica, Buffalo Grove, IL), mouse or rabbit, was used for antibody staining. The following antibodies were used: CD3 and Granzyme B (Ventana, Tucson, AZ); CD30, CD68, and E-cadherin (Leica, Buffalo Grove, IL), and TIA-1 (Beckman Coulter, Brea, CA).

In situ hibridization

In situ hybridization for EBER was performed on 5 μm sections from the bone marrow biopsy using a RTU EBER probe (Leica, Buffalo Grove, IL). The Leica Bond Polymer Refine Detection (DAB) Kit, mouse or rabbit, was used for staining with a Mouse Anti-FITC secondary antibody (Leica, Buffalo Grove, IL).

Flow cytometry

Cells from bone marrow aspirate were analyzed by flow cytometry. Immunophenotyping was performed using 8-color flow cytometric analysis (FACSCanto II flow cytometer, Becton Dickinson, San Jose, CA) according to standard protocol. The following directly conjugated monoclonal antibodies were used: CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD16, CD56, CD57, CD19, CD33, CD38, CD45, TCR-Alpha-Beta, and TCR-Gamma-Delta (Becton Dickinson, San Jose, CA); Kappa and Lambda (Biolegend, San Diego, CA); and IO Test Beta Mark TCR Repertoire Kit (Beckman Coulter, Brea, CA). Flow cytometric data were analyzed by using Winlist software (Verity Software House, Topsham ME).

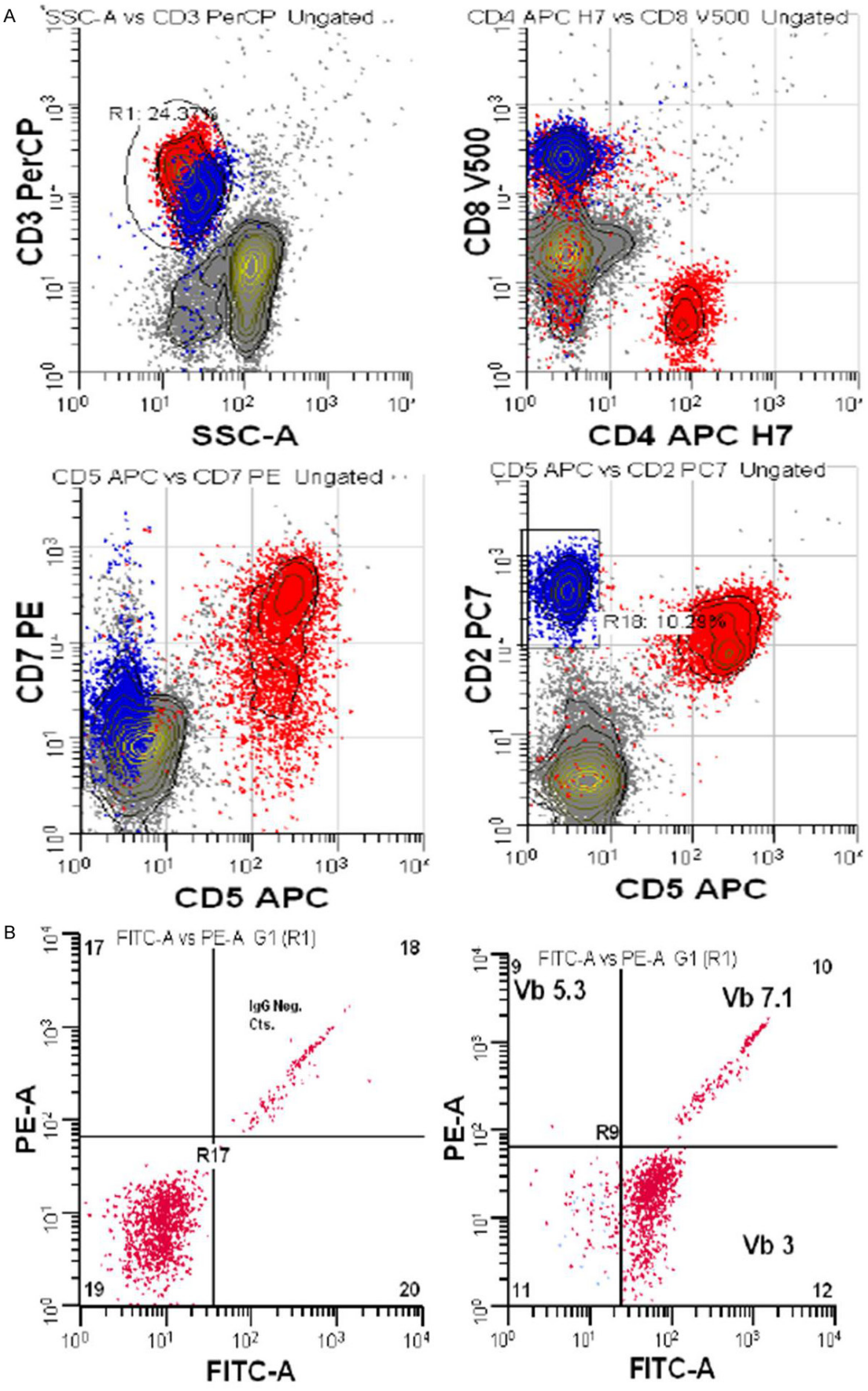
A
SSC-A vs CD3 PerCP Ungated
R1: 24.37%
CD3 PerCP
SSC-A
CD4 APC H7 vs CD8 V500 Ungated
CD8 V500
CD4 APC H7
CD5 APC vs CD7 PE Ungated
CD7 PE
CD5 APC
CD5 APC vs CD2 PC7 Ungated
R18: 10.29%
CD2 PC7
CD5 APC
B
FITC-A vs PE-A G1 (R1)
17
18
IgG Neg. Cts.
R17
19
20
PE-A
FITC-A
FITC-A vs PE-A G1 (R1)
9
10
Vb 5.3
Vb 7.1
R9
Vb 3
11
12
PE-A
FITC-A

Figure 2. Flow cytometry histograms. A. Four panels showing a discrete population (10.29% of total events, indicated in blue) with greater side scatter (and forward scatter, not shown) compared to background CD3+ T lymphocytes (red) with the following atypical immunophenotype: CD5-, CD7 dim, CD8+, CD4-, and CD2 bright. B. Histograms showing isotype controls (left) and clonality of the neoplastic population by Vbeta3 (right).

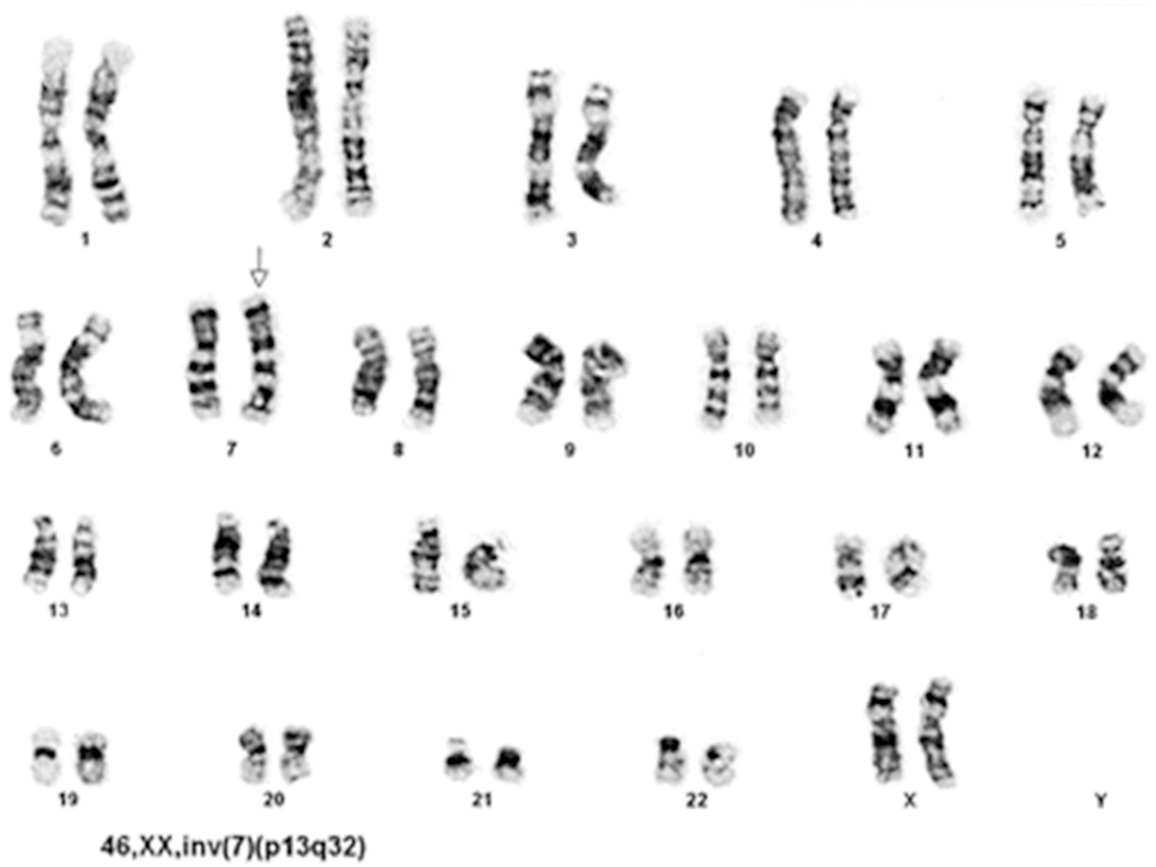

Figure 3. Karyogram showing inv (7) (p13q32) in 10 of 20 metaphases examined.

Cytogenetic analysis

Two cultures of the patient bone marrow were set up in marrow max media (Gibco, Grand Island, NY) in two separate incubators for overnight at 37°C. The cultures were harvested following day after colcemid (10 μg/mL) for 60 minutes and hypotonic solution (0.56% KCl) treatments. The cells were then fixed in methanol/acetic acid (3:1 ratio) and metaphase preparations were banded using Pancreatin-Giemsa. A total of 20 cells were analyzed, 10 cells from each of the two cultures. Nomenclature of all karyotypes was described according to the *International System for Human Cytogenetic Nomenclature* (ISCN, 2013).

Molecular studies

For analysis of *TRG* VJ rearrangements, PCR amplification of multiple sites in the T cell receptor region was performed using previously reported primers and modified reaction conditions [30]. Amplicons were radioactively labeled, separated using a 6% denaturing polyacrylamide gel, and visualized using autoradiography.

Literature review

A literature search of related published studies was conducted. An analysis was performed to determine overall correlation between clonality studies, immunophenotypic aberrations, and karyotypic abnormalities with patient outcomes. Studies were selected using the following search terminology "EBV-HLH", "EBV-HLH clonal", and "SE-LPD".

Case report

We report a case of a 21 years old Caucasian female with 3 weeks history of weakness,

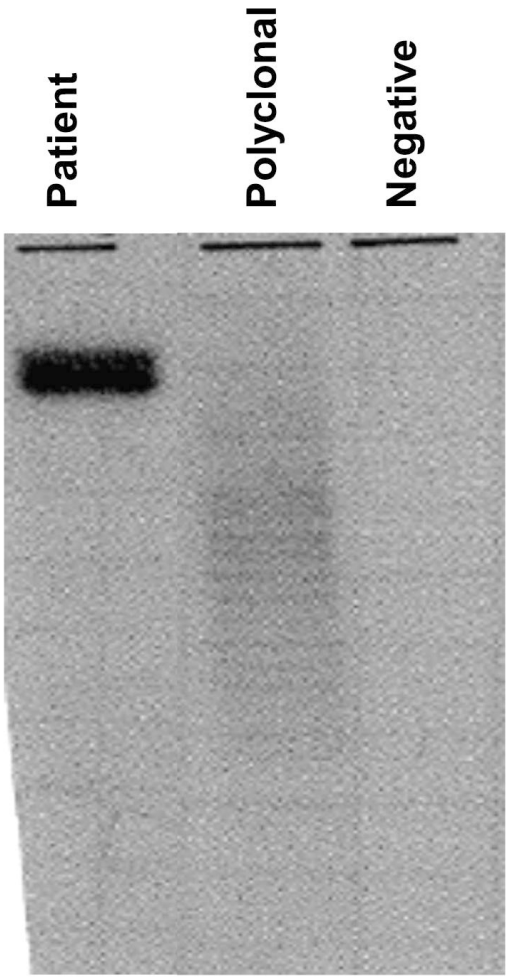

Figure 4. T-cell receptor gamma studies showing a distinct clonal band on V10 studies.

fatigue, left neck lymphadenopathy (LAD) and hepatosplenomegaly with persistent cyclic fever. Initial laboratory work-up revealed severe pancytopenia (WBC: 1.3 K/μl, Hgb: 10.2 g/dL and PLT: 29 K/μl), with mild liver enzyme elevation (LDH: 992 U/L, Total Bilirubin: 0.8 mg/dL, Alkaline Phosphatase: 187 U/L, and AST: 131 U/L) and a positive monospot test. At that time, EBV PCR quantitation demonstrated 250,000 nucleic acid copies. The pancytopenia prompted diagnostic consideration of hemophagocytic syndrome, and a bone marrow biopsy was performed at an outside institution (not shown) which was significant for multiple necrotizing granulomata. Special stains did not highlight any infectious etiologies. EBER ISH (with a suboptimal control) did not highlight any EBV infection. The patient was then transferred to Vanderbilt University Medical Center (VUMC). Additional work up at our institution, demonstrated markedly elevated EBV real time PCR quantitation studies: 1,038,918 copies/mL despite no detection of IgM or IgG antibodies against EBV viral capsid or antinuclear antibodies. A subsequent bone marrow biopsy, almost a week from the first biopsy, showed readily identifiable hemophagocytosis (**Figure 1A** and **1B**). At this time, the patient was more severely pancytopenic (WBC: 0.3 K/ul, Hgb: 7.6 g/dL and PLT: 17 K/ul) and febrile with an elevated ferritin level (14,294 ng/mL), low fibrinogen (<60 mg/dL), and a markedly elevated soluble CD25 level (43,879 u/mL). In this clinical setting, these findings were consistent with hemophagocytic lymphohistiocytosis (HLH).

Additionally, examination of the bone marrow showed a population of intermediate sized morphologically atypical lymphocytes (**Figure 1B**) which corresponded to a population of atypical intermediate sized T lymphocytes identified on flow cytometry with the following immunophenotype: CD2 bright, CD3 (+), CD4 (-), CD5 (-), CD7 dim, CD8 (+), CD16 (-), CD56 (-), TdT (-), gamma-delta (-), and alpha-beta (+) (**Figure 2**, selected markers shown). Additionally, flow cytometry analyzing 16 separate TCR beta clones showed clonality for Vb3 (**Figure 2**). Metaphase cytogenetic studies revealed that ten of the twenty metaphases analyzed demonstrated an abnormal karyotype with a pericentric inversion of chromosome 7 (**Figure 3**). The remaining 10 cells were normal. The karyotype was described as 46, XX, inv (7) (p13q32) [10]/46, XX [10]. Finally, molecular studies showed a clonal population of cells utilizing the variable 10 region of the T cell receptor gamma chain locus (**Figure 4**).

An etoposide and dexamethasone based regimen per HLH-94 protocol resulted in symptomatic improvement and resolution of laboratory abnormalities. One month following initial treatment, a bone marrow study was negative for T cell lymphoma by morphology, flow cytometry, molecular, and cytogenetic studies. However, the patient suffered rising ferritin levels and EBV titers two months after diagnosis, and a bone marrow biopsy at that time demonstrated HLH and the presence of a small T cell clone. Following this second bone marrow, the patient received a single cycle of CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy, with weekly rituxan, steroids

Table 2. Summary of the literature on EBV-HLH and SE-LPD with HLH

Number patients	Diagnosis	MC	IP	AK	Outcome	Treatment	Reference
7	EBV-HLH	N/A	5	N/A	4/5 with IP DOD, 4/7 DOD overall	N/A	McCall et al. [22]
1	EBV-HLH	1	1	N/A	Alive, 3 years later	IVIG, INF alpha2b, Ganciclovir & Prednisolone	Lin et al. [21]
1+	EBV-HLH	1	1	N/A	N/A	N/A	Fukada et al. [20]
5	SF-IM	4	N/A	N/A	4/4 with MC DOD	N/A	Wick et al. [27]
1	EBV-HLH	1	1	None	Alive	Prednisolone, VP, CsA	Wada et al. [23]
1	EBV-HLH	1	N/A	N/A	Dead; No disease at autopsy#	VP Prednisolone, IT MTX	Owen et al. [39]
1	EBV-HLH	1	N/A	N/A	1/1 with MC DOD	Antibiotics	Dolezal et al. [35]
1	EBV-HLH	1	N/A	1	1/1 with MC and AK DOD	HLH94	Ito et al. [38]
3	EBV-HLH	N/A	N/A	3	3/3 with AK DOD	VP based chemo	Chen et al. [41]
4	EBV-HLH	N/A	N/A	1	1 with AK DOD	N/A	Kaneko et al. [44]
1	EBV-HLH	0	N/A	1	1/1 with AK DOD	VP and Dexamethasone, CsA	Ishii et al. [43]
27	EBV-HLH	25	N/A	6	6/6 with AK DOD; 9/25 with MC DOD (11 total DOD)	Immunochemotherapy	Imashuku et al. [37]
16	EBV-HLH	5	N/A	N/A	12/16 DOD overall; 3/5 with MC DOD	9 VP and Steroid +/- IVIG, 3 with Steroid/IVIG/CsA, 2 Conservative, 2 Lymphoma therapy	Ahn et al. [34]
1	EBV-HLH	1	N/A	N/A	1/1 with MC DOD	CsA, Steroid, IVIG	Bird et al. [40]
7**	EBV-HLH and SE-LPD	2	N/A	N/A	2/2 MC DOD 4 other DOD	Variable: Steroids to CHOP to HLH94, to 106B & VHR, to IMVP-16 & PD	Hong et al. [8]
1	SE-LPD	1	N/A	N/A	1/1 with MC DOD	VP, CsA and prednisolone, then CHOP	Yoshii et al. [25]
2	SE-LPD	2	N/A	1	1/1 with MC and AK DOD; 1/1 with MC DOD	CHOP +/- Bleomycin & Precarbazine	Su et al. [53]
1	SE-LPD	1	N/A	N/A	1/1 with MC DOD	Steroids, acyclovir	Gaillard et al. [36]
1	SE-LPD	1	N/A	1	1/1 with MC and AK DOD	Antibiotics	Chan et al. [54]
1	SE-LPD	1	N/A	N/A	1/1 with MC DOD	Supportive	Craig et al. [51]
1+	EBV-HLH	1	N/A	N/A	Relapse at 15 mo, then LTFU	VP	Noma et al. [52]
3	EBV-HLH	3	N/A	2 normal; 1 N/A	3/3 with MC DOD	N/A	Kawaguchi et al. [10]
6	SE-LPD	5	N/A	N/A	5/5 with MC DOD; 1 LTFU+	Variable: 1 supportive therapy; 1 plasmapheresis; 1 VCP & prednisolone; 1 VP & antibiotics; 1 valcyclovir	Quintanilla-Martinez et al. [26]
1	SE-LPD	1	N/A	N/A	1/1 with MC DOD	VP; then CsA	Tabanelli et al. [29]
1	SE-LPD	1	N/A	N/A	1/1 with MC DOD	Antibiotics, steroids, colectomy	Abdul-ghafar J et al. [55]

N/A, not available; DOD, dead of disease; MC, molecular clonalilty found; Molecular clonality demonstrated by EBV genome clonality, T cell receptor beta and/or gamma clonality, or immunoglobulin heavy chain gene clonality; AK, abnormal karyotype found defined by ISCN 2013, criteria; IP, immunophenotypic abnormality found as defined by abnormal expression of pan-T cell markers only; *4/23 with MC overlap with AK cases; **SE-LPD defined by clonality; +Excluded from outcome analysis; #treated as remission in analysis. VHR, prednisolone, cyclophosphamide, daunorubicin, vincristine, L-asparaginase, intrathecal methotrexate; L-Asp, L-asparaginase; SF-IM, sporadic fatal infectious mononucleosis, considered SE-LPD; INF, interferon; CsA, cyclosporine; IVIG, intravenous immunoglobulin; HLH94. HLH protocol, IT, intrathecal; VP-Etoposide; VCP, vincristine; MTX, Methotrexate; LTFU, lost to follow-up; IMVP-16/PD, ifosfamide, methotrexate, etoposide, prednisolone; 106B, prednisolone, cyclophosphamide, daunorubicin, vincristine, L-asparaginase, CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone.

and etoposide. The patient achieved remission and proceeded to ablative matched unrelated donor allogeneic peripheral blood stem cell transplantation (PBSCT) 4 months following her original diagnosis. The patient is currently day +300 from PBSCT with complete engraftment and no evidence of the clonal T cell population in four post-transplantation bone marrow studies.

Results and discussion

In this case, the overwhelming evidence of clonality established by immunophenotypic aberrancy (loss of CD5, dim CD7, bright CD2, and restricted Vb3 expression), molecular T cell receptor gamma clonality (single V10 clone), as well as a clonal aberration by cytogenetic studies (inv (7) (p13q32)) supported a final diagnosis of a SE-LPD. However, this case illustrates the difficulty in distinguishing SE-LPD and EBV-HLH. The overlapping clinical and morphologic features of these entities create a challenging diagnostic dilemma. These diagnoses are typically separated by identification of a clonal T/NK cell population; however, using T/NK cell clonality to distinguish EBV-HLH and SE-LPD is problematic [31].

Gorczyca *et al*. has described several helpful single and combination markers suggestive of T cell aberrancy, such as diminished or loss of CD45 expression, complete loss of one or more pan-T antigens; dim expression of more than two pan-T antigens in the setting of altered light scatter properties; and CD4/CD8 dual-positive or dual-negative expression [31]. However, in contrast to B cell counterparts where light chain restriction serves as a reliable surrogate for clonality, there are no specific flow cytometric findings diagnostic of T cell malignancy. Furthermore, using flow cytometric detection of immunophenotypic aberrancy as a marker T clonality is limited since several case series and case reports identify variable loss of CD5 [20-23, 32], CD7 [22, 32], and occasionally CD3 [22] singularly or in combination in both IM and EBV-HLH.

The assessment of T cell clonality is similarly complicated by a significant proportion of false positive molecular clonality results due to the detection of clonal populations of cells in reactive settings. One small study by Cairns demonstrated a false positive clonal T cell receptor gamma molecular study rate of 14% in reactive lymphadenopathy (2 of 14 reactive lymph nodes) [33]. Additionally, case reports claiming evidence of clonality based on molecular analysis of T cell receptor and/or immunoglobulin gene rearrangements in the setting of EBV-HLH further complicates differentiation of these entities [8, 10, 23, 34-40]. Other markers of clonality, including EBV genome terminal repeat analysis [33, 35] and karyotype [10, 11, 37, 38, 41-44] have also been reported in EBV-HLH.

Proposed mechanisms of the pathogenesis of EBV-driven HLH give insight into the reason for the overlapping characteristics and diagnostic challenges. Both entities result from the infection of CD8+ T cells by EBV with expression of latency II proteins (LMP-1, LMP-2, EBNA-1). Studies suggest that LMP-1 (part of the tumor necrosis factor superfamily) recruits Tumor Necrosis Factor Receptor (TNFR) associated factors (TRAF) which may activate NF-κB and promote proliferation through up-regulation of downstream proteins, TNF-alpha and IFN-gamma, while simultaneously inhibiting TNF-alpha mediated apoptosis through suppression of complex formation between TNF-alpha and TNFR-1, selectively in T cells [5, 45]. Up-regulation of TNF-alpha has been implicated in the pathogenesis of HLH [46-48]. Furthermore, LMP-1 can significantly inhibit the expression of SAP, linking EBV-HLH with the genetic disease, XLP, and providing an explanation for a shared pathogenesis between these entities [5, 45, 49, 50].

This survival advantage for T cells provides possible insight into the mechanism of evolution of T cell lymphoproliferative disorder from EBV-HLH. Additionally, it illustrates the diagnostic dilemma of ascribing a specific title to a process which evidence suggests may represent a biologic continuum. This possible biologic continuum raises two essential questions: 1) how do we reliably separate these entities and 2) does separation of these entities provide prognostic data relevant in the clinical setting?

We reviewed the literature, identifying 74 of EBV-HLH and 20 cases of SE-LPD with associated HLH (**Table 2**). Of these, there were 92 cases with available outcome data. Due to the overlap between the clinical designations of EBV-HLH and SE-LPD, we intentionally examined outcome data of all cases together. Of all

cases with outcome data, 62 cases resulted in disease related fatality (67%, 43 of 74 cases (58%) designated EBV-HLH and 19 of 21 cases (90%) designated SE-LPD). In 60 patients with evidence of molecular clonality, 37 cases showed disease related fatality (62%). This mortality rate is slightly higher than what other studies suggest is the mean mortality rate for all HLH (53%) [16, 17]. Many of these cases with a cytogenetic abnormality received alternative treatments rather than the standard HLH-94 protocol that is typically utilized for a diagnosis of EBV-HLH. Therefore, it is unclear if mortality might have been reduced by using standard HLH treatment protocols. It is also difficult to ascertain which of these cases were treated by stem cell transplantation from the available data since often initial treatments only were noted.

Studies examining EBV genome terminal repeat analysis showed no clinical prognostic significance [33, 35]. Although case reports detailing 11 different patients with symptoms of EBV-HLH/SE-LPD using molecular methods to establish clonality showed an anecdotal trend of universal fatality [10, 11, 26, 35, 36, 51-53], Imashuku *et al.* compared molecular clonality, as assessed by T cell receptor and immunoglobulin heavy chain gene rearrangements, in 15 of their 32 patients (including 5 cases which were negative for EBV) and demonstrated no statistical significant difference in overall survival between molecularly clonal and non-clonal cases of HLH [37]. Furthermore, Ahn *et al.* compared the clinical significance of clonality established by *TRG* or *IGH* gene rearrangement studies in 28 patients with HLH (including 14 patients without EBV) and found no statistical significant prognostic impact [34].

By contrast, several case reports of EBV-HLH and SE-LPD harboring cytogenetic abnormalities demonstrated universal fatality [10, 11, 37, 38, 41-44]. In our analysis of 14 cases with karyotypic abnormalities, all 14 patients died of disease (100%). Imashuku *et al.* compared the risk associated with EBV-clonality, TCR-clonality, and cytogenetic clonality, and only demonstrated adverse risk for those cases with a cytogenetic abnormality [37]. Our results, which incorporate other studies and are limited only to EBV-positive cases, support these conclusions. Interestingly, nearly all cases with karyotypic abnormalities were diagnosed as EBV-HLH (12 of 14 cases) by the original paper authors, raising the question that these cases might more accurately be diagnosed as SE-LPD.

These studies, in combination with our data, seem to suggest that important prognostic information is derived from karyotype information rather than other assessments of clonality. We suggest that it may be more clinically useful to consider these entities as a biologic continuum, and to regard the cases with karyotypic abnormalities as either "high risk" malignant counterparts, rather than using other standards of clonality assessment to make the distinction between EBV-HLH and SE-LPD, or to reserve the distinction of SE-LPD for only those cases with karyotypic abnormalities. Notably, this trend seems specific to SE-LPD arising in association with EBV-HLH, as monoclonality in CAEB by molecular rearrangements studies has been shown to be prognostically significant, perhaps by identifying those cases which have progressed to either EBV-HLH or SE-LPD and carry correspondingly higher mortality rates [8].

This case represents a rare example of SE-LPD in a western country with an atypical non-fatal outcome. Despite adverse variables including increased EBV titers (>1 million) and karyotypic abnormality, this patient is in remission 15 months following diagnosis in the setting of a matched unrelated donor stem cell transplant. Early diagnosis, intervention, and stem cell transplantation may explain the good outcome of this case.

Disclosure of conflict of interest

None.

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