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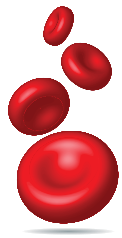
CME Information: EBV-positive diffuse large B-cell lymphoma of the elderly: 2016 update on diagnosis, risk-stratification and management



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Upon completion of this educational activity, participants will be better able to:

- Understand the current knowledge and limitations regarding the diagnostic criteria, incidence, clinical features and pathological findings of patients with EBV+ diffuse large B-cell lymphoma (DLBCL) of the elderly
- Discuss the current management and potential future therapeutic options of patients with EBV+ DLBCL of the elderly

Activity Disclosures

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EBV-positive diffuse large B-cell lymphoma of the elderly: 2016 update on diagnosis, risk-stratification, and management

Jorge J. Castillo,[1]* Brady E. Beltran,[2] Roberto N. Miranda,[3] Ken H. Young,[3] Julio C. Chavez,[4] and Eduardo M. Sotomayor[5]

Epstein–Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL) of the elderly is a provisional entity included in the 2008 WHO classification of lymphoid neoplasms. It is a disease typically seen in the elderly and thought to be associated with chronic EBV infection and severe immunosuppression with a component of immunosenescence. Recent research, however, has suggested that EBV-positive DLBCL can be seen in younger, immunocompetent patients.

The diagnosis of EBV-positive DLBCL of the elderly is made through a careful pathological evaluation. The differential diagnosis includes infectious mononucleosis (specifically in younger patients), lymphomatoid granulomatosis, Hodgkin lymphoma, and gray zone lymphoma, among others. Detection of EBV-encoded RNA (EBER) is considered standard for diagnosis; however, a clear cutoff for positivity has not been defined.

The International Prognostic Index (IPI), and the Oyama score can be used for risk-stratification. The Oyama score includes age >70 years and presence of B symptoms. The expression of CD30 is emerging as a potential adverse, and targetable, prognostic factor.

Patients with EBV-positive DLBCL should be staged and managed following similar guidelines than patients with EBV-negative DLBCL. It has been suggested, however, that EBV-positive patients have a worse prognosis than EBV-negative counterparts in the era of chemoimmunotherapy. There is an opportunity to study and develop targeted therapy in the management of patients with EBV-positive DLBCL.

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Introduction: Disease Overview

Epstein–Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL) of the elderly is a provisional category included in the 2008 World Health Organization classification of lymphoproliferative disorders (WHO) [1]. The seminal report by Oyama and colleagues reported on 22 patients with large cell lymphoma that expressed the EBV-encoded RNA (EBER) in the nuclei of the malignant cells [2,3]. These patients tended to be elderly, and had a poor response and short survival with standard combination chemotherapy. In recent years, data on EBV-positive DLBCL of the elderly continue accumulating, and not only have helped advance our understanding of this condition but has also generated areas of uncertainty.

Epstein–Barr virus (EBV) infection is common worldwide with a prevalence ranging between 80% and 95%, depending on the geographical area. In the case of patients with DLBCL, the prevalence of EBV infection is unknown, as no large population-based studies have been performed to date. However, small studies and case series have rendered disparate results with prevalence rates of less than 5% in Western countries (United States and Europe) to 10–15% in Asia and South America [4–7]. The reasons for this difference are unclear but it is likely that virological (e.g., EBV strain) and genetic factors (e.g., HLA types) play a role.

EBV was the first oncogenic virus ever identified, and EBV infection has been associated with a number of malignancies, such as nasopharyngeal carcinoma and Burkitt lymphoma, among others. EBV infection is associated with immunosuppression and chronic antigenic activation, which are key components of the neoplastic process. Patients with EBV-positive DLBCL of the elderly usually present with an EBV latency pattern type III, in which all EBV-associated proteins (i.e., latent membrane proteins and nuclear antigens) are expressed [8]. EBV latency pattern III is associated with a marked immunodeficiency state. For example, other lymphomas associated with EBV latency pattern III are post-transplant and HIV-associated lymphomas.

Immunosenescence is a process associated with physiological aging characterized by a series of changes in the function of the immune system. T-cell response dysregulation, thymic atrophy, reduced output of new T-cells, development of anergic memory cells, loss of immunosurveillance, and deficiencies in cytokine production as well as limitations in the T-cell receptor repertoire are processes that have been associated with immunosenescence. Such processes might accelerate in the context of chronic infections such as EBV infection. It is likely; however, that other not previously identified factors might also play a role in the pathogenesis of EBV-positive DLBCL of the elderly.

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Clinically, patients tend to be diagnosed at an older age; hence the term elderly, and have a worse survival than would be expected in patients with EBV-negative DLBCL. Several clinical aspects might be associated with such worse prognosis. Typically, in addition to common nodal involvement that usually present with high International Prognostic Index (IPI) scores, and patients tend to have higher rates of extranodal involvement with the gastrointestinal tract, skin and bone marrow being the most commonly affected sites. Also, there is higher proportion of patients with elevated LDH levels, and more advanced clinical stage as well as worse performance status than patients with EBV-negative DLBCL.

Expectedly, the definition of EBV-positive DLBCL of the elderly continues evolving. Recent evidence suggests that EBV-positive DLBCL can be seen in young, immunocompetent individuals [9–12]. These studies have shown similar virological and pathological findings between younger and older patients with EBV-positive DLBCL. Interestingly, the outcome of younger patients with EBV-positive DLBCL appears similar to younger patients with EBV-negative DLBCL while the outcome in older patients seems worse. Finally, it is important to note that there is no clear cutoff for a positive expression of EBER as previously published studies have used rates of positivity ranging from 10% to 50% [13].

Diagnosis

The World Health Organization (WHO) recognizes two main morphologic subtypes, polymorphous and large-cell lymphoma (monomorphic). The polymorphous subtype demonstrates large B cells that include centroblasts, immunoblasts, and plasmablasts admixed with a variable number of reactive cells. The reactive cells include small lymphocytes, plasma cells, and histiocytes. The monomorphic subtype is less common, and is characterized by monotonous sheets of large transformed B-cells. Hodgkin and Reed–Sternberg-like cells can be admixed in both the polymorphic and monomorphic types [14]. Geographic necrosis is common. Recently, Montes-Moreno et al. subdivided the polymorphic subtype into three further subgroups: one called large cell type composed of numerous large cells, another shows that the large cells are Hodgkin or Reed–Sternberg (HRS)-like cells, and another subtype shows only few or no HRS-like cells (Fig. 1). However, no prognostic significance has been associated with the morphologic subgroups [14]. The lymphoma cells express B-cell markers such as CD19, CD20, CD22, and CD79. CD30 is expressed in about 40% of cases. Most cases have an activated B-cell phenotype, expressing MUM1/IRF4, and are negative for CD10 and BCL6 [14]. Expression of NF-κB component p50 and phosphorylated signal transducer and activator of transcription (pSTAT3) are more commonly seen compared with EBV-negative DLBCL. LMP-1 is expressed in 2/3 of cases while EBNA-2 in 1/3 of cases, hence cases show type II or III latency patterns [15]. Gene expression profiling shows that EBV-positive DLBCL is molecularly distinct from EBV-negative DLBCL. The gene set enrichment assay (GSEA) demonstrated an enhanced Toll-like receptor signaling pathway (which has many similarities to the NF-κB pathway) and the JAK-STAT pathway [16]. Clonal rearrangement of the immunoglobulin gene is seen in approximately 60% of cases, and a subset of cases shows rearrangements of the T-cell receptor [14].

Differential diagnosis

Plasmablastic lymphoma (PBL): PBL is an aggressive lymphoma with immunoblastic morphology and plasmacytic immunophenotype [17,18]. The lymphoma cells typically are of large size with round-to-oval centrally or eccentrically located nucleus, dispersed chromatin, prominent single nucleolus, and amphophilic cytoplasm with perinuclear hof. Apoptotic cells with accompanying tingible-body macrophages can be seen, imparting a starry-sky pattern at low magnification. Mitotic figures are frequently seen, consistent with a high Ki-67 proliferation index (90–100%). The lymphoma cells mostly express plasmacytic markers such as CD38 (100%), MUM1 (100%), and CD138 (84%). CD79a is uncommonly expressed (14%); and CD20 is virtually not expressed (3%) in contrast with EBV+ DLBCL of the elderly. CD45 expression is seen in 1/3 of the cases. Frequent (80%) expression of epithelial membrane antigen (EMA) is observed. HHV-8 LANA is negative, which is very helpful for differentiating PBL from morphologically similar large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease [19]. Most PBLs are positive for EBER (78%) with a predominance of type I latency pattern in contrast with type II and type III in EBV+ DLBCL of the elderly. By FISH, *MYC* rearrangement is detected in about 50% of cases; the most common partner is *IgH* [20]. Of note, *MYC* rearrangement was more commonly seen in EBER-positive PBL compared with EBER-negative PBL. Clonal rearrangement of the immunoglobulin gene is seen in most cases.

DLBCL associated with chronic inflammation: This DLBCL is mostly associated with EBV infection, and arises in patients with a long-standing chronic inflammatory process such as pyothorax (pyothorax associated lymphoma, PAL), chronic osteomyelitis, metallic implant, or chronic skin ulcers [21]. Morphology is typical for DLBCL. Most lymphoma cells are positive for CD20 and CD79 but can be negative in cases with plasmacytic differentiation. MUM-1 and CD138 are positive in such cases. EBER and EBNA-2 are positive in most cases, illustrating a type III latency program [22,23]. Clonal rearrangement of the immunoglobulin gene is seen in most cases. Comparative genomic hybridization on PAL tumor samples demonstrated gain of chromosome 8q24, and *MYC* amplification was found by southern blot technique [24]. Sequencing of the *TP53* gene (exons 5–8) using paraffin-embedded tissue found mutations in 2/3 of cases, with most being single-nucleotide substitution [25]. By gene expression profiling, PAL was shown to be molecularly different from nodal DLBCL, with increased expression of activated B-cell-like signature [26]. DLBCL associated with chronic inflammation is an aggressive lymphoma, with 5-year survival rate of 22% [22].

Primary effusion lymphoma (PEL): PEL is a rare B-cell neoplasm mostly affecting immunosuppressed patients who present with a lymphomatous effusion in pleural, pericardial, or peritoneal cavities, usually without a detectable tumor mass [27]. In cytospin samples, the lymphoma cells show a morphologic range from immunoblastic to markedly irregular or anaplastic features and some cells resemble Reed–Sternberg cells. A prominent Golgi zone adjacent to the nucleus is often present in the lymphoma cells. In tissue sections, the lymphoma cells have round or oval shapes, moderate to large amounts of cytoplasm, and round to variably indented or multilobated nuclei with one or more prominent nucleoli. Multinucleated giant tumor cells and lymphoma cells with wreath-like nuclei, resembling "hallmark cells" can be seen. Mitotic figures are numerous. The lymphoma cells usually express CD45 without expression of pan-B markers (CD19, CD20, CD22, and CD79) or T/NK cell markers. Surface and cytoplasmic immunoglobulins are generally absent. CD30, EMA, CD38, CD138, and HLA-DR are variably positive. Latency-associated nuclear antigen-1 (LANA-1) of HHV8 is typically positive. EBER is positive in about 70% of cases, but LMP-1 is negative. Extracavitary PEL shares a similar immunophenotype but with more common expression of B-cell markers. Recurrent cytogenetic abnormalities have not been reported. Comparative genomic hybridization of eight PEL cases showed gain of chromosomes 12 and X in three and two cases, respectively, and amplification within the 1q region in two cases [28]. *BCL-2, BCL-6,* and *MYC* genes were not rearranged, and mutations in *MYC, HRAS, KRAS, NRAS*, and *TP53* genes were not found [29]. Clonal rearrangement of the immunoglobulin gene is

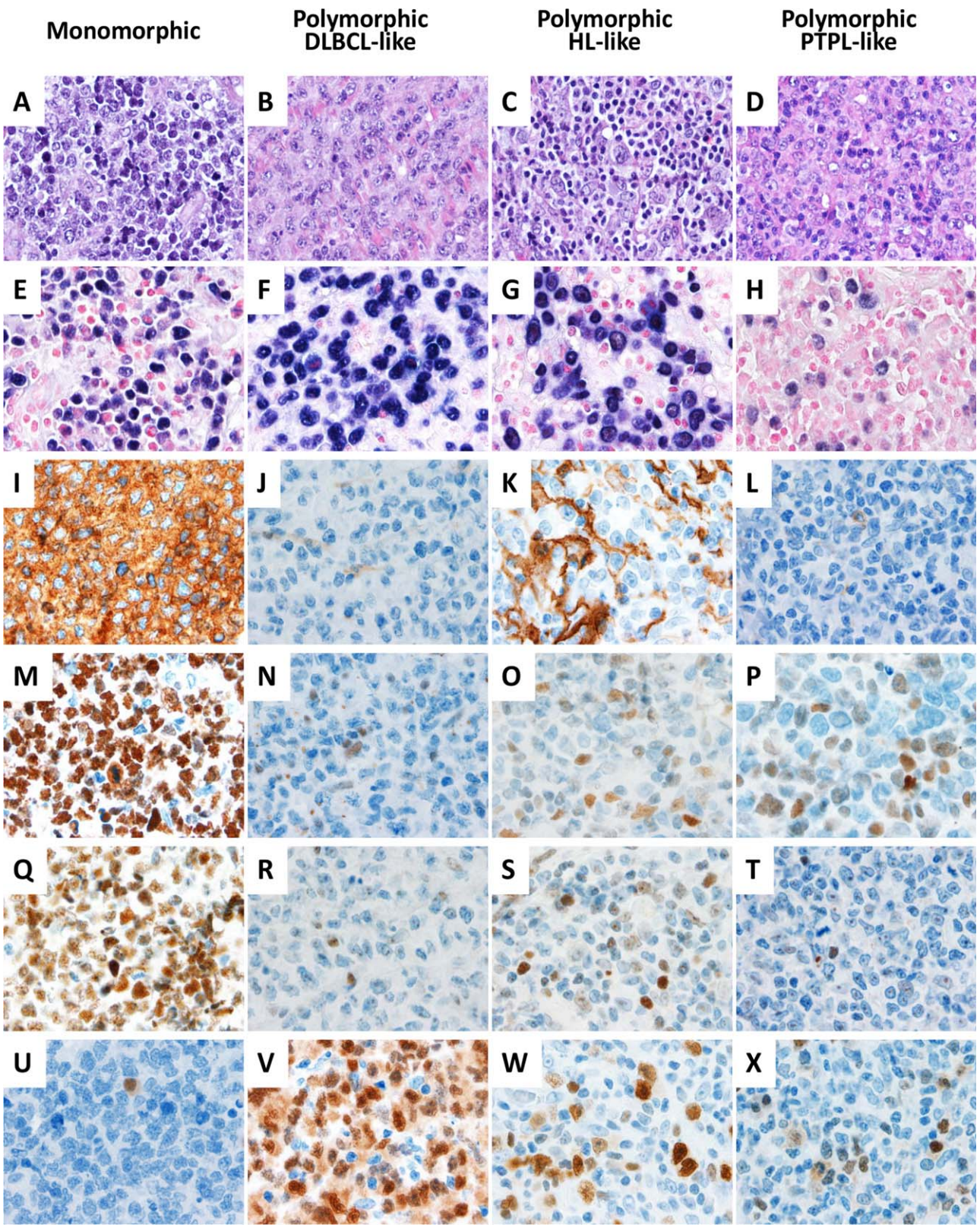

Figure 1. EBV-positive diffuse large B-cell lymphoma of the elderly, subtypes according to Montes-Moreno and Chi Y. Ok [12,14,16]. A–D. The lymph node is completely effaced by large lymphoma cells, with a monomorphic (A) or a polymorphic (B) population. Montes-Moreno suggested two additional variants for the polymorphic cases: Hodgkin lymphoma-like (C) and lymphoproliferative disorder -like (D). Hematoxylin and eosin, x400. E–H. *In situ* hybridization for EBER shows positivity in large lymphoma cells, x400. I–T: Panel shows that the neoplastic cells are positive for CD10 (I), BCL6 (M) and FOXP1 (Q) in monomorphic subtype, largely negative for CD10 (J), BCL6 (N) and FOXP1 (R) in polymorphic subtypes by immunohistochemistry, x400. U–X: Panel show expression of different subtypes with MUM1: Negative in monomorphic subtype, and variably positive in the polymorphic subtype, x400. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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TABLE I. Differential Diagnosis of EBV-Positive DLBCL of the Elderly

	DLBCL Elderly	PBL	DLBCL CI	PEL	DLBCL, NOS	DLBCL Leg Type	HHV8 MCD
Median age, years (range)	71 (50–91)	50 (7–65)	64 (46–82)	Young or middle age	7th decade	7th decade	Young adults
Gender: Male to female ratio	1.4	7	12.3	Male > female	Male > female	0.3	Male > female
Clinical features	B-symptoms	B- symptoms; oral mucosa mass	Chronic inflammation, pyothorax	Immunosupressed, coexistent Kaposi sarcoma	Lymphadenopathy	Skin tumors that disseminate	Multicentric Castleman disease, immunodeficiency
Geographic distribution	Asia > Europe and America	Worldwide	Asia	Worldwide	Worldwide	Worldwide	Worldwide
Disease distribution	Nodal and common extranodal	Oral, gastrointestinal tract	Pleural	Pleural and pericardial cavities	Nodal > extranodal	Skin lower extremeties	Nodal, spleen
Stage of disease at diagnosis	III or IV	III or IV	I or II	III or IV	III or IV	I or II	III or IV
Association with HIV infection	No	~70%; no in elderly	No	Yes	No	No	Yes
Pathogenesis	Immune senescence, NF kappa B	EBV, HIV, IL10	EBV, IL-10, IL6	HHV8 oncogenesis; EBV more limited	NF kappa B dysregulation	NF kappa B dysregulation	HHV8 oncogenesis; IL-6 signaling
Microscopic features	Polymorphic > monomorphic	Diffuse growth; plasmablastic	Plasmablastic or immunoblastic	Plasmablastic or immunoblastic	Diffuse large cells	Diffuse infiltrate	Plasmablastic morphology
Positive markers	CD20, CD30+/−, CD79, PAX5,	CD138, MUM1/IRF4, IgG, EMA	CD20, CD4	CD45, CD30-/+, CD138+/-, MUM-1	CD20, CD10-/+, PAX5	CD20, BCL2, PAX5, MUM1,	CD20−/+, CD138+/−, IgM-lambda
Negative markers	CD10	CD10, CD20, PAX5	CD10, CD138, ALK	CD10, CD19, CD20, CD138, Ig,	CD138, Ig	CD10	CD79a, CD138
Proliferation rate (Ki67)	High	>90%	>90%	>90%	40 −90%	High	>90%
Cytoplasmic immunoglobulin	Negative	50-70%	Uncommon	Uncommon	Uncommon	Negative	IgA lambda
Association with EBV infection	EBER 100%; LMP1 70%	EBER 80%, LMP1 40%	Common	70% (EBER)	3%-10%	Uncommon	No
Latency pattern EBV infection	II > III	I, II	III	I	I	NA	NA
HHV8	Negative	Negative	Negative	Positive	Negative	Negative	Positive
Comparative genomic hybridization	c-REL amplification; trisomy 3 and gain of *BCL6*		Gain 8q24, MYC amplification	Gain chr. 12 and X		18q21 amplification; deletion 9q21	
Molecular genetics	Infrequent *MYC, BCL2*, and *BCL6* GR	*MYC* GR, hypermutated immunoglobulin	*TP53* mutations	No *BCL2, BCL6 or MYC* GR; hypermutated immunoglobulins,	NK-kB c-REL amplification	*MYC* and *BCL6* GR	Unmutated immunoglobulin
Gene expression profiling	Toll-like receptor and JAK-STAT		ABC-like signature		GCB > ABC type	ABC type	

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TABLE II. Selected Case Series on the Use of Chemo(immuno)Therapy in Patients with EBV-Positive DLBCL

Study	EBER cutoff	Regimen	*N*	OR/CR rate	OS
Oyama, 2007	>50%	CHOP	56	80%/66%	5-year: 25%
Park, 2007	>20%	CHOP	25	72%/NR	5-year: 48%
Beltran, 2011	>20%	R-CHOP	8	NR/66%	3-year: 40%
		CHOP	12	NR/33%	3-year: 40%
Ahn, 2014	>50%	R-CHOP	18	72%/61%	3-year: 57%
Ok, 2014	>10%	R-CHOP	28	89%/NR	5-year: 54%
Sato, 2014	>30%	R-CHOP	8	50%/25%	3-year: 38%
		CHOP	3	33%/33%	3-year: 0%
Lu, 2015	>20%	R-CHOP	35	66%/NR	3-year: ~30%
Song, 2015	NR	R-CHOP	8	63%/50%	3-year: 70%
		CHOP	8	50%/38%	3-year: 25%

EBER: EBV-encoded RNA; OR: overall response; CR: complete response; OS: overall survival; CHOP: cyclophosphamide, doxorubicin, vincristine and prednisone; R-CHOP: rituximab and CHOP; NR: not reported.

seen in most cases and can be used for determining lineage. Gene expression profiling showed that PEL is in the differentiation stage of plasmablasts because the gene expression profile showed features of immunoblasts, between EBV-transformed lymphoblastoid cell lines or AIDS immunoblastic lymphoma, and plasma cells from multiple myeloma cell lines [30].

Other entities that present at nodal or extranodal sites and show similar histopathology, immunophenotype, such as DLBCL, NOS and primary cutaneous DLBCL, leg type, and HHV8-associated Castleman disease are shown in Table I.

Risk Stratification

Several groups have shown that patients with EBV-positive DLBCL have worse prognosis when compared with patients with EBV-negative DLBCL, making EBV *per se* an adverse prognostic factor. A Japanese study compared the outcomes between 96 patients with EBV-positive and 107 with EBV-negative DLBCL [3]. Approximately 60% of the EBV-positive patients achieved CR after chemotherapy, in contrast with 90% in EBV-negative patients. EBV-positive DLBCL patients had worse survival than EBV-negative DLBCL patients with estimated 5-year OS rates of 25% versus 65%, approximately. Similar results were found in a Korean study that evaluated 34 EBV-positive patients out of 380 patients with DLBCL [7]. EBV positivity was associated with median OS of 36 months versus not reached in EBV-negative DLBLC patients. A smaller Peruvian study also showed EBV positivity associated with worse prognosis in de novo DLBCL. Out of 74 patients with DLBCL, 11 patients were EBV-positive. The median OS in EBV-positive patients was 7 months compared with 47 months in EBV-negative patients [31]. Of note, only a minority of patients, however, received rituximab as part of their therapy in these studies.

More recently, there have been a number of reports on the use of chemoimmunotherapy in patients with EBV-positive DLBCL with disparate results. In a multicenter consortium study on DLBCL patients treated uniformly with R-CHOP, the response and survival rates of 28 patients with EBV-positive DLBCL was compared with 695 EBV-negative patients, and showed no statistical differences [16]. Interestingly, there were no differences in clinical presentation between the two groups. Pathologically, there was a higher rate of CD30 expression in EBV-positive patients. Similar results were found in a Korean study that evaluated 18 patients with EBV-positive DLBCL, who had similar OS rates to 204 EBV-negative patients with 3-year OS rates of 57% and 60%, respectively [32]. Conversely, a recent Japanese study showed a median OS of 9 months in 8 patients with EBV-positive DLBCL treated with R-CHOP while the median OS for EBV-negative patients was not reached [33]. An Spanish study on 47 patients with EBV-positive DLBCL of the elderly mostly treated with R-CHOP-like regimens showed 2-year OS rate of 40%, which appeared lower than patients with EBV-negative DLBCL [14]. A recent Chinese study also found worse outcomes in patients with EBV-positive DLBCL with median OS of 18 months versus median OS that was not reached in EBV-negative patients, although it was not specified the proportion of patients who received chemotherapy and chemoimmunotherapy [10].

In summary, patients with EBV-positive DLBCL have a worse prognosis than EBV-negative patients when treated with chemotherapy. The outcomes appear less different, however, when treated with chemoimmunotherapy. It is important to note that none of these studies was prospective, and the inclusion criteria have inherent differences that could have biased the results. Additionally, geographic differences could be explained by different strains of EBV. For example, a high frequency of EBV type B with the LMP1 30bp deletion was found in Mexican cases [6].

Other prognostic factors

The IPI score is one of the most commonly used risk stratification tools in DLBCL. In an early report by Oyama and colleagues [3], the IPI score appeared to be of limited significance on prognosticating survival in patients with EBV-positive DLBCL. The research group designed a prognostic index that consisted on two factors, age older than 70 years and presence B symptoms. Patients with zero, one or two factors showed median OS times of 56, 25, and 9 months, respectively. In a smaller study, Beltran and colleagues identified higher IPI and higher Oyama scores to be associated with worse outcome in patients with EBV-positive DLBCL [4]. In this study, a notable adverse prognostic factor was lymphopenia defined as an absolute lymphocyte count of $<1.0 \times 10^9$/L. Of potentially clinical implication is the finding that expression of CD30 is not only increased but also associated with a worse OS in patients with EBV-positive DLBCL [16]. In this study, EBER+/CD30+ DLBCL patients had worse outcome than EBER+/CD30- or EBER-/CD30+ DLBCL patients. A clinical trial aimed at evaluating the anti-CD30 antibody-drug conjugate brentuximab vedotin in EBV-positive DLBCL was initiated in August 2012 but withdrawn due to lack of accrual in July 2013 (NCT01671813). More recently, a number of studies have evaluated the presence of EBV-positive DLBCL in younger, apparently immunocompetent individuals. However, it is unclear from the data available if younger age is associated with a better or worse survival [34].

Management of EBV-Positive DLBCL of the Elderly

The addition of the anti-CD20 chimeric monoclonal antibody, rituximab, to anthracycline-based chemotherapy has clearly improved

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survival outcomes in patients with DLBCL in different clinical settings (e.g., early and advanced stage, and older and younger patients) [35–37]. The response to combination chemotherapy appears lower in EBV-positive DLBCL than in EBV-negative DLBCL patients. Overall response (OR) rates to cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) range from 30% to 80%, and complete response (CR) rates from 30% to 50%. More recent data suggest higher rates of response to chemoimmunotherapy, specifically rituximab and CHOP (R-CHOP) with OR rates of 50–90% and CR rates of 30–70%. However, no prospective comparative studies have been performed to date. Response rates to chemotherapy and chemoimmunotherapy in selected studies of patients with EBV-positive DLBCL are shown in Table II. Thus, there is not standard approach for EBV-positive DLBCL of the elderly and treatment options usually are in concordance with current strategies for de novo DLBCL.

Future Directions

Novel therapies that will address viral replication, targeting specific pathways in EBV-DLBCL and improving/modulating the immune response against EBV have been investigated and seem promising in the treatment of this condition.

Antiviral therapy in combination with EBV lytic phase induction

Typical anti-herpes therapies are not effective in eradicating EBV from chronically and transformed B-cells. In order for ganciclovir and acyclovir to show antiviral activity, they require proteins of lytic phase to be active, however EBV maintains a latent-phase in B-cell infected cells [38]. Thus, induction of EBV into a lytic phase could lead to a effective exposure to antiviral therapy and has been studied with variable success[39]. Inducers of lytic phase include methylase transferase inhibitors, histone deacetylase inhibitors (HDACs), and proteasome inhibitors among others [13,39]. Arginine butyrate, which has HDACs properties, and gancyclovir were administered to 15 patients with refractory EBV-positive B-cell lymphomas. There were 10 responses with four complete responses [40]. Other HDACs, such as panobinostat and belinostat (FDA approved for refractory T-cell lymphomas and refractory multiple myeloma, respectively) have shown a synergistic effect by sensitizing EBV-positive lymphoma cell lines to ganciclovir, however clinical efficacy is yet to be proven [41].

Targeting specific pathways prominently activated in EBV-associated DLBCL

EBV-positive DLBCL of the elderly has an activated B-cell (ABC) DLBCL profile and is characterized by increased activation of the NF-kB pathway. Bortezomib inhibits the 26S proteasome, important in the degradation of the NF-kB inhibitor IkB [42]. In EBV transformed B-cells and mouse models, bortezomib has induced apoptosis [43]. In a phase I/II study of combination of bortezomib with standard R-CHOP the progression-free survival (PFS) and overall survival (OS) was not significantly different between GCB and ABC DLBCL [44]. Also, a recent randomized phase II study compared R-CHOP and VR-CAP (vincristine substituted by bortezomib) in 164 patients with ABC DLBCL [45]. There were no differences in response rates, PFS and OS between the two groups. Lenalidomide can also target the ABC DLBCL by down regulating interferon regulatory factor (IRF4) and the NF-kB pathway [46]. Early clinical data showed preferential activity in refractory/relapsed ABC vs. GCB DLBCL, with an overall response rate (ORR) of 53% and 9%, respectively [47]. Given these results, two studies adding lenalidomide to R-CHOP in newly diagnosed DLBCL have recently been completed [48,49]. Ibrutinib is an inhibitor of the Bruton Tyrosine Kinase (BTK), an important component of the B-cell receptor (BCR) pathway. In a study of 80 patients with refractory/relapsed DLBCL, ibrutinib was given at 560 mg daily until disease progression or unacceptable toxicity. The ORR rate in ABC DLBCL and GCB DLBCL were 37% and 5%, respectively [50]. The PI3K kinase pathway seems to be critical for upstream signaling of NF-kB in ABC DLBCL cell lines, thus inhibition of the PI3K pathway appears to be another approach for EBV-positive DLBCL with ABC signature [51,52].

Although treating EBV-positive DLBCL of the elderly by targeting the COO of ABC-DLBCL seems an attractive therapeutic approach, none of these studies were specifically designed for EBV-related lymphomas. In addition, determining the COO by IHC does not seem to be the ideal approach, and gene expression profiling studies might be needed to identify patients with ABC DLBCL [53,54].

EBV-specific adoptive cellular immunotherapy

The high immunogenicity of EBV provides the basis for the development of immunotherapy to prevent EBV reactivation and overcome immune escape mechanisms by the virus. To survive, EBV develops a long period of latency by evading the immune system recognition and host immune antiviral response. EBV blocks the expression of the highly immunogenic proteins during latency and expresses lytic proteins that impairs antigen processing by infected cells and by producing viral cytokines that impairs the immune system [55]. Efficacy of adoptive T-cell therapy using specific EBV cytotoxic T-cells (CTLs) has been demonstrated in patients with EBV-related post-transplant lymphoproliferative disorder (PTLD) back in 1995 [56,57]. The infusion of EBV specific CTLs was effective in patients as a treatment, in cases of PTLD, and as a prophylaxis, in patients undergoing solid organ transplant and allogeneic stem cell transplantation [58,59]. The initial strategy of administering unselected donor lymphocytes from EBV seropositive stem cells donors was associated with the high risk of developing graft versus host disease (GVHD). To reduce risk of GVHD, EBV-CTLs grown from peripheral blood healthy donors on a best HLA-match basis and banked (Edinburgh CTL bank). The most common method is to establish lymphoblastoid cell lines (LCLs) by in vitro EBV infection and used as stimulators for development of specific EBV-CTLs [60]. Best HLA-matched EBV-CTLs were administered to PTLD post SOT with a response rate of 50–60% [61]. Although effective, the prolonged time to manufacture CTLS (up to 6–12 weeks) may be too long of a time for a patient with an aggressive lymphoma. To generate CTLs in a rapid manner, donor-derived EBNA1- specific T-cells were developed by a faster method using cytokine secreting system with selection of interferon-gamma secreting EBNA1-specific cells. The process to generate these EBNA1- specific EBV-CTLs is approximately 3 days. In a study of 10 patients with EBV-related refractory PTLD after allogeneic HSCT, the administration of EBNA1 specific EBV-CTLs was able to restore T-cell immunity against EBV and produced impressive clinical efficacy with a response rate of 70% [62]. In immunocompetent patients, generation of CTLs using this methodology did not yield the same efficacy when was administrated to EBV-related Hodgkin lymphoma (HL) patients (latency type II) with a response rate of 30%, despite expressing the EBNA1 antigen, due to poor immunogenicity of latency II type EBV-HL cells (such as LMP1 and LMP2) [63]. This same latency type is seen in EBV-positive DLBCL of the elderly.

In an effort to improve immunogenicity and expand the EBV antigen profile potentially targeted by CTLs, Bollard et al developed a methodology with the goal of increasing the frequency of relevant EBV-latency specific antigens (LMP1 and LMP2). Dendritic cells (DCs) transduced by adenovirus vector and EBV transformed LCL as antigen-presenting cells were used to activate and expand LMP 1/2

specific T-cells. In this trial of 50 patients with EBV associated lymphomas (HL, DLBCL NK T-cell lymphomas) received LMP1/2 specific CTLs. The overall efficacy was 96% in the 29 patients as adjuvant treatment (high risk of relapse) and 64% in patients with refractory/relapsed EBV associated lymphomas [64].

More recently, chimeric antigen receptor (CAR) T-cells directed against tumor-associated markers, such as CD19, are undergoing clinical development in leukemia and lymphoma [65,66]. There is preclinical evidence of efficacy of LMP1-directed CAR T-cell in nasopharyngeal carcinoma, a malignancy associated with EBV infection [67]. Thus, immunotherapy seems to offer an interesting and effective alternative for patients with EBV-related lymphomas, in particular EBV-positive DLBCL of the elderly given its success and tolerability.

Conclusion

In summary, EBV-positive DLBCL of the elderly is an uncommon aggressive lymphoma subtype that seems to be associated with a worse prognosis in the era of chemoimmunotherapy. Current studies have shown that EBV impacts the outcome of individuals with different ethnic background. Patients from Asia, Latin America and East Europe seem to have relatively poor survival, whereas North American patients showed no survival difference from EBV-negative DLBCL patients. This preliminary observation suggests that future clinical and biological analyses on EBV-positive DLBCL should be stratified per ethnic background. EBV-positive DLBCL appears to also occur in young, immunocompetent individuals, which might lead to reconsideration of the current provisional diagnostic criteria. The incidence of EBV-positive DLBCL is likely underestimated as EBV testing is not routinely performed and LMP1 has lower sensitivity than EBER detection assays. Furthermore, an accepted cutoff for EBV positivity has not been defined. Particular signaling pathways such as CD30, NF-kB, BCR, and PD-1 appear closely related to mechanistic dysregulation in EBV-positive DLBCL patients. Due to the rarity of EBV-positive DLBCL, the development of multi-institutional prospective studies is warranted. Potential directions could include adding lenalidomide, bortezomib, brentuximab vedotin, ibrutinib, or nivolumab to chemoimmunotherapy. As many abnormal signaling pathway activation are driven by EBV, to develop novel EBV-targeting approaches, such as CTLs or CAR T-cells will be experimentally intriguing in future research efforts.

Disclosures

The authors have no conflict of interest to disclose.

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