

Geographic variation in the prevalence of Epstein–Barr virus-positive diffuse large B-cell lymphoma of the elderly: a comparative analysis of a Mexican and a German population

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Epstein–Barr virus (EBV)-positive diffuse large B-cell lymphoma of the elderly was included as a provisional entity in the 2008 WHO lymphoma classification. Most reports of this disease come from Asia and little is known about it in other regions of the world, including Latin America. Therefore, in this study, 305 diffuse large B-cell lymphomas in patients above 50 years were analyzed, 136 from Mexico and 169 from Germany. EBV was detected by Epstein–Barr early RNA (EBER) *in situ hybridization*. Only cases with EBER+ in the majority of tumor cells were regarded as EBV+ diffuse large B-cell lymphoma. The prevalence of EBV+ diffuse large B-cell lymphoma in Mexican patients was found to be 7% (9 of 136), whereas only 2% (4 of 169) of the German cases were positive. The median age at diagnosis was 66 years in the Mexican cohort, as opposed to 77 years in the German group. The site of presentation was in both groups predominantly nodal in nine cases (70%) and extranodal in four cases (30%). Of the 13 EBV+ cases, 10 (77%) were classified as polymorphic and 3 (23%) as monomorphic type. The polymorphic cases showed a non-germinal center B-cell immunophenotype (CD10– MUM1+). Twelve cases (92%) were LMP1 positive and two (15%) expressed EBNA2. An interesting finding was the high frequency of EBV type B with the LMP1 30bp deletion found in the Mexican cases (50%). Eight of the 11 evaluable cases were B-cell monoclonal by polymerase chain reaction. In summary, we found a similar prevalence of EBV+ diffuse large B-cell lymphoma of the elderly in a Mexican population compared with what has been reported in Asian countries, and in contrast to the low frequency in Western populations (1–3%). However, compared with the Asian series, the Mexican patients were younger at diagnosis, presented predominantly with nodal disease and rarely expressed EBNA2 protein.

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Epstein–Barr virus (EBV) is a ubiquitous herpesvirus that infects >90% of the human population establishing persistent latent infection in the host.¹ Although EBV infection is benign in most individuals, it has been linked to the etiology of a rather broad spectrum of B-cell lymphoproliferations both

in the post-transplant and iatrogenic setting,² as well as to malignant lymphomas in immunocompetent hosts such as Burkitt lymphoma, lymphomatoid granulomatosis and a proportion of classical Hodgkin lymphoma.^{3,4}

In 2003, Oyama *et al*⁵ reported 22 cases of EBV-associated diffuse large B-cell lymphoma that occurred in elderly individuals (>60 years) without apparent immune deficiency, and suggested a relationship with immunological deterioration as a result of the aging process. Further reports from Japan⁶ and Korea⁷ showed that EBV-associated diffuse large B-cell lymphoma accounts for 8–10% of diffuse large B-cell lymphoma in elderly Asian patients without a documented predisposing immunodeficiency. As a result of these reports, EBV-positive diffuse large B-cell lymphoma of the elderly was included in the revised 2008 WHO classification of tumors of hematopoietic and lymphoid tissues⁸ as a 'provisional' category. It is defined as an EBV + clonal B-cell proliferation that occurs in patients older than 50 years and without any known immunodeficiency, immunosuppression, transplantation, autoimmune disease or prior lymphoma. Cases of other well-defined lymphoma entities that may be EBV positive are excluded from this provisional category. Morphologically, EBV + diffuse large B-cell lymphoma of the elderly often shows features of post-transplant lymphoproliferative disorders, although it develops in clinically immunocompetent patients. Helpful pathologic features that suggest EBV association include large transformed cells, polymorphic infiltrate, plasmacytic and plasmablastic morphology, Hodgkin and Reed–Sternberg-like cells, angioinvasion, necrosis and non-germinal center B-cell immunophenotype.

Interestingly, EBV-associated lymphomas like NK/T-cell lymphoma,⁹ T-cell lymphoproliferative disorders of childhood¹⁰ and EBV + classical Hodgkin lymphoma^{11,12} are more commonly observed in Asia and in Latin American countries suggesting an ethnic predisposition for the development of EBV-associated lymphoproliferative disorders. This also seems to be true for EBV + diffuse large B-cell lymphoma of the elderly, which has been reported to account for 8–10% of diffuse large B-cell lymphoma in Asian populations,^{5–7} whereas small series from the United States¹³ and central Europe¹⁴ showed a much lower incidence (1 and 3%, respectively). Analysis of the prevalence of EBV + diffuse large B-cell lymphoma of the elderly in Latin America is still missing. This prompted us to analyze a series of diffuse large B-cell lymphoma in patients above 50 years from four different hospitals in Mexico City.

The aims of this study were (1) to determine the EBV-association rate in a Mexican cohort of diffuse large B-cell lymphoma of the elderly, (2) to compare it to an analogous cohort from Germany, (3) to determine the EBV strain type and (4) to define the pathological characteristics of the disease.

Patients and methods

Case Selection

Cases with the diagnosis of diffuse large B-cell lymphoma, in patients age 50 years and above, from which tissue blocks were available for EBV *in situ* hybridization, immunohistochemical and molecular analysis were included in the study. From four different Pathology Departments from Mexico City, Mexico, 136 cases were collected. For comparison, 169 cases of diffuse large B-cell lymphoma in patients 50 years or older were selected from the files of the Institute of Pathology, Tübingen, Germany from 2000 to 2009. All cases were reviewed by two pathologists (LQ-M and PA). The medical records were reviewed with special attention to previous medical history.

Immunohistochemical and *In Situ* Hybridization Analyses

All cases were subjected to *in situ* hybridization using oligonucleotides complementary to Epstein–Barr early RNA (EBER) transcripts in paraffin embedded tissue in an automated stainer (Ventana Medical Systems, Tucson, Arizona). EBER positivity in the majority of tumor cells was required to diagnose a case as EBV + diffuse large B-cell lymphoma. A tissue microarray was constructed with the EBV + cases for further immunohistochemical characterization. The paraffin blocks were sampled using 1.0 mm diameter tissue cores and the cores were arrayed in triplicates. Immunohistochemical analysis was performed on an automated immunostainer (Ventana Medical Systems) according to the company's protocols. The following panel of antibodies was used: BCL-2 (Zytomed Systems, Berlin, Germany), BCL-6 (Zytomed Systems), CD5 (Novocastra, Newcastle, UK), CD10 (Novocastra), CyclinD1 (clone SP6; Epitomics, Burlingame, CA, USA), MUM1 (DAKO Deutschland GmbH, Hamburg, Germany), MIB1 (DAKO), CD20 (clone L26; DAKO), CD79a (DAKO), LMP1 (clone CS.1–4; DAKO), EBNA2 (Novocastra).

Polymerase Chain Reaction Analyses for the Detection of EBV Strain Type and *IGH* Rearrangements

DNA used for polymerase chain reaction (PCR) was extracted from 10 μ m paraffin sections after dewaxing and proteinase K digestion applying standard phenol/chloroform purification procedures. PCR analysis for EBV A and B strain typing was performed, as previously reported.¹⁵ Primers flanking a region of the *EBNA2* gene differing between type A and type B EBV were used. Each reaction was performed in duplicate with 200 or 500 ng of extracted DNA in a 50- μ l volume. The expected fragments for EBV types A and B are 168 and 184 bp, respectively. PCR analysis for the 30-bp *LMP1* gene

deletion was performed by using primers flanking the characteristic 30bp deletion, as previously described,¹⁶ rendering a 161-bp product for wild-type *LMP1* and 131 bp for the deletion variant.

The PCR for *IGH* gene rearrangements was performed as previously described with BIOMED primers for the framework regions 2 and 3 (FR2 and FR3) using 0.5 U Phusion Hot Start DNA Polymerase (Finnzymes, Woburn, MA, USA) and 200 ng of genomic DNA.¹⁷ The JH primer was modified with D4 fluorescent dyes (Sigma-Aldrich, St Louis, MO, USA). The products were separated by capillary electrophoresis on the GenomeLab GeXP Genetic Analysis System and analyzed by the GenomeLab GeXP software 10.2 (Beckman Coulter, Brea, CA, USA).

Results

Patient Characteristics

EBER positivity was detected by *in situ hybridization* in the majority of tumor cells in 11 of 136 Mexican cases analyzed. After carefully reviewing the medical records, two patients were excluded from the study because both had a clinical history of rheumatoid arthritis. The remaining nine patients (7%) had no history of immunodeficiency, immunosuppressive therapy, prior lymphomas or autoimmune diseases. The clinical data are summarized in Table 1. The group consisted of five males and four females with a median age of 66 years (range 51–75 years). Six patients presented predominantly with lymph node involvement. Three patients presented with extranodal involvement, one in the skin of the face, one with a tumor mass in the tongue and one with spleen involvement. Five patients presented in stage IV disease, one patient in stage III and two patients in stage II. Four patients were lost to follow-up after diagnosis. One patient (case 6) was lost to follow-up with disease in very bad clinical condition, after six cycles of chemotherapy

Table 1 Clinical features of EBV+ DLBCL of the elderly from Mexico

Case	Sex	Age	Stage	Localization	Treatment	Outcome
1	F	58	IIAe	Face, LN	—	LFU
2	M	71	IVB	LN	—	LFU
3	F	67	IIIBe	Spleen, LN	CHOP ^a	DwD (1 month)
4	M	66	IVB	LN	MACOP B	DwD (57 months)
5	M	62	IVA	LN	CHOP+RT	DwD (22 months)
6	M	51	IIA	LN	CHOP	LFU ^b (18 months)
7	F	71	IVB	LN	—	LFU
8	F	60	IIBe	Tongue, LN	—	LFU
9	M	75	IVB	LN	—	DwD ^c (1 month)

AwD: alive with disease; DwD: died with disease; LFU: lost to follow-up; LN: lymph node; RT: radiotherapy.

^aOnly one cycle was given.

^bThe patient was lost to follow-up with disease after six cycles of CHOP.

^cThe patient died before treatment was started.

with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP); he never achieved complete remission and refused further treatment. Two patients (cases 3 and 4) died within 1 month of the diagnosis. Patient 3 died with disease while receiving the first cycle of chemotherapy. The remaining two patients died with disease 22 and 57 months after diagnosis. The median age of all 136 patients analyzed was 69 years (range 50–92 years).

For comparison, 169 cases of diffuse large B-cell lymphoma in patients above 50 years from Germany were analyzed. EBER positivity was detected in six of 169 cases. Two cases were excluded from the study because of a previous history of marginal zone lymphoma in one patient and a known HIV infection in the second patient. Only four cases fulfilled the criteria of EBV+ diffuse large B-cell lymphoma of the elderly, representing 2% of the German cases, as opposed to the 7% found in the Mexican cases. The clinical data of these four patients are summarized in Table 2. The patients consisted of two males and two females with a median age of 77 years (range 63–88 years). Three patients presented with stage II and one with stage III. Predominant lymph node involvement was found in three patients, whereas one patient presented with extranodal involvement of the intestine. The latter (case 11) died of sepsis secondary to intestinal perforation before treatment could be started. Two additional patients died with disease (cases 10 and 12). Patient 10 received two cycles of rituximab and bendamustine. This patient had a clinical history of a prostate carcinoma, treated with surgery, hormonal ablation and radiotherapy, 5 years before the lymphoma was diagnosed. Patient 12 received four cycles of CHOP, achieving partial remission; however, 1 month later recurred with massive lymphadenopathy and a tumor mass in the breast. The patient received bendamustine with poor response. Only one patient (case 13) is alive, and in complete remission following R-CHOP therapy 35 months after the primary diagnosis. The average age of all 169 cases analyzed was 72 years (range 51–92 years).

Histological and Immunophenotypic Features

The histological and immunophenotypic features are summarized in Table 3. Of the nine EBV-positive

Table 2 Clinical features of EBV+ DLBCL from Germany

Case	Sex	Age	Stage	Localization	Treatment	Outcome
10	M	77	IIIA	LN	R-Benda	DwD (3 months)
11	F	88	IIBe	Intestine, LN	—	DwD ^a
12	F	77	IIA	LN	CHOP Benda	DwD (11 months)
13	M	63	IIA	LN	R-CHOP	CR (35 months)

Benda: bendamustine; CR: complete remission; DwD: died with disease; LN: lymph node; R: rituximab; RT: radiotherapy.

^aThe patient died before treatment was started.

Table 3 Pathological features of EBV-positive B-cell lymphomas of the elderly

Case	Pattern	Necrosis	CD20	BCL2	BCL6	CD10	MUM1	LMP1	EBNA2	EBV type	LMP1 del	IGH clonality
<i>Mexican cases</i>												
1	Poly	—	+	—	—	—	+	Rare	—	A	—	ND
2	Mono	—	+	—	+	+	—	+	—	A	—	Monoclonal
3	Poly	—	+	+/-	—	—	+	+	—	NA	+	Polyclonal
4	Poly	+++	+	+	—	—	+	+	—	B	+	Monoclonal
5	Poly	—	+	—	—	—	—	+	Rare	A	—	ND
6	Poly	—	+	—	—	—	+	+	—	A	—	Monoclonal
7	Poly	—	+	—	—	—	+	+	—	B	+	Monoclonal
8	Poly	+++	+	—	+	—	+	+	—	B	+	Polyclonal
9	Poly	+++	+	—	+	—	+	+	—	B	+	Monoclonal
<i>German cases</i>												
10	Poly	—	+	+	—	—	+	+	+	B	—	Monoclonal
11	Mono	—	+/- ^a	+/-	+	—	+	+	—	A	—	Monoclonal
12	Mono	—	+	+	—	+	—	+	—	A	+	Monoclonal
13	Poly	—	+	—	—	—	+	—	—	A	—	Polyclonal

Mono: monomorphic type; NA: not assigned; ND: not done; Poly: polymorphic type.

^aThis case was mainly CD79a positive.

diffuse large B-cell lymphomas identified in Mexican patients, eight revealed a polymorphous infiltrate characterized by a mixture of small lymphocytes, histiocytes, plasma cells and lymphoid cells with plasmacytic differentiation, immunoblasts and large atypical lymphoid cells and Reed–Sternberg-like cells (Figure 1a and b). In three cases (33%) (cases 4, 8 and 9), geographic areas of necrosis with apoptotic tumor cells, surrounded by EBER+ cells, were observed (Figure 1c and d). All polymorphic cases expressed CD20 and CD79a (Figure 1e), and showed a non-germinal center B-cell phenotype characterized by CD10 negativity and in general MUM1 positivity (Figure 1f). BCL-2 was generally negative. The large Reed–Sternberg-like cells were also CD20 positive, usually CD30 positive (Figure 1g) and lacked CD15. Only one case (case 2) showed a monomorphic morphology with sheets of centroblasts (Figure 2a). Almost 100% of tumor cells were EBER+ (Figure 2b). Phenotypically, the tumor cells revealed a germinal center B-cell phenotype (CD20+, CD10+ and BCL-6+) (Figure 2c–e). LMP1 was positive in all cases (Figure 2f), whereas EBNA2 was positive in rare cells in only one case (11%) (case 5).

Of the four EBV+ German cases, two had a polymorphic and two a monomorphic cytology. All cases were CD20 and CD79a positive. Case 10, a polymorphic type showed a non-germinal center B-cell phenotype (BCL-2+, CD10–, MUM1+) and was positive for LMP1 and EBNA2 (Figure 1h). Case 13, a polymorphic type was unusual in that LMP1 and EBNA2 were both negative and that the patient achieved complete remission.

Morphologically and phenotypically the Mexican and German cases were very similar. Of the 13 cases combined, 10 (77%) were of the polymorphic type and 3 (23%) were monomorphic. Twelve (92%) were LMP1 positive and two (15%) were EBNA2 positive.

Interestingly, seven additional samples of the 136 Mexican DLBCL (5%) showed EBER positivity in <20% of cells (Figure 3). The patients were five females and two males with a median age of 70.5 years (range 56–87 years). These cases were not regarded as EBV+ diffuse large B-cell lymphoma of the elderly. In contrast to the Mexican cases, only one German case showed partial EBER positivity. (5% vs 0.5%, respectively).

EBV Genotype and Clonality Analyses

Of the nine Mexican cases analyzed (lanes 1–9), four of the eight evaluable cases were EBV strain type A, and four cases type B EBV (Figure 4). The 30-bp del-*LMP1* variant was identified in five of the nine cases including the four cases with type B EBV. All four type A EBV carried the wild-type *LMP1* gene. Of the four German cases, three were EBV type A (lanes 11–13) and one was EBV type B (lane 10). One EBV type A case carried the *LMP1* 30 bp deletion (lane 12). The results of clonality analysis are summarized in Table 3. Of the 11 evaluable cases, 8 showed a B-cell monoclonal population and three were polyclonal.

Discussion

In this study, we analyzed a series of diffuse large B-cell lymphoma in patients aged 50 years or greater for the presence of EBV infection by *in situ hybridization* in a Mexican population, and compared it with a German population. The prevalence of EBV+ diffuse large B-cell lymphoma of the elderly in the Mexican patients was significantly higher than in the German cohort (7% vs 2%), and the lymphomas occurred approximately one decade earlier (66 and 77 years, respectively). The preva-

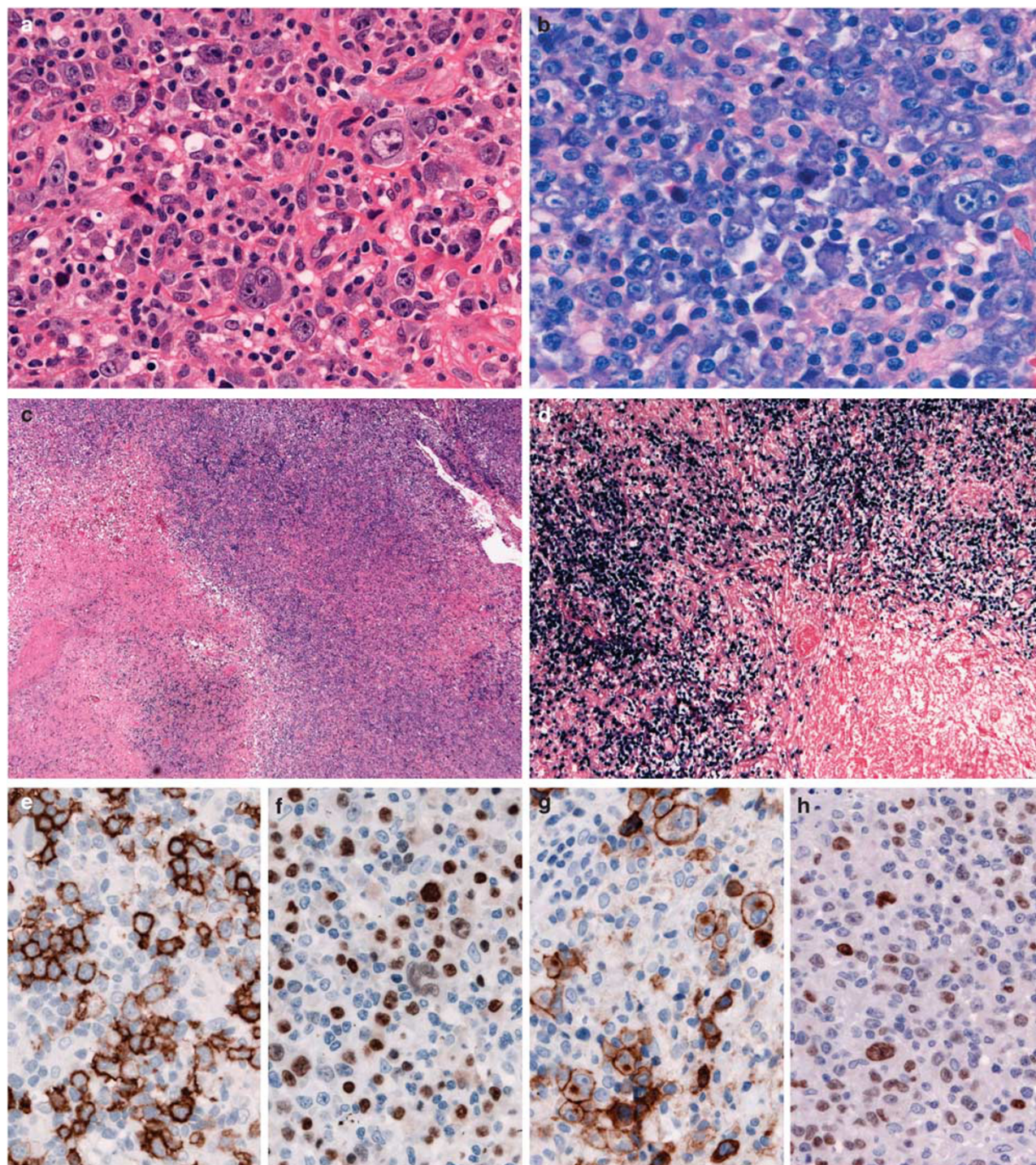


Figure 1 Polymorphic type of EBV-positive B-cell lymphoma of the elderly. (a) A polymorphous proliferation characterized by small lymphocytes, plasmacytic and plasmablastic cells, and scattered large transformed cells mimicking Hodgkin and Reed–Sternberg cells is observed (hematoxylin–eosin; original magnification $\times 400$). (b) Giemsa stain highlights the plasmacytic differentiation of the tumor cells. (Giemsa stain; original magnification $\times 400$). (c) Low magnification of a lymph node diffusely infiltrate with large geographic areas of necrosis (hematoxylin–eosin; original magnification $\times 50$). (d) EBER *in situ* hybridization shows diffuse EBV-positive tumor cells surrounding the necrosis (original magnification $\times 100$). (e) CD20 stain highlights the large, transformed cells (original magnification $\times 400$). (f) Note that most of the tumor cells are positive for MUM1 (original magnification $\times 400$). (g) CD30 stain is positive in most of the large transformed Hodgkin and Reed–Sternberg-like cells (original magnification $\times 400$). (h) Case 10. EBNA 2 stain is positive in many small and large tumor cells (original magnification $\times 400$).

lence of EBV+ diffuse large B-cell lymphoma of the elderly in Mexican patients is comparable with the data published in the Asian literature

(7% vs 8–10%).^{5–7} However, the median age at presentation in Mexican patients was relatively low (66 years) when compared with the Japanese series

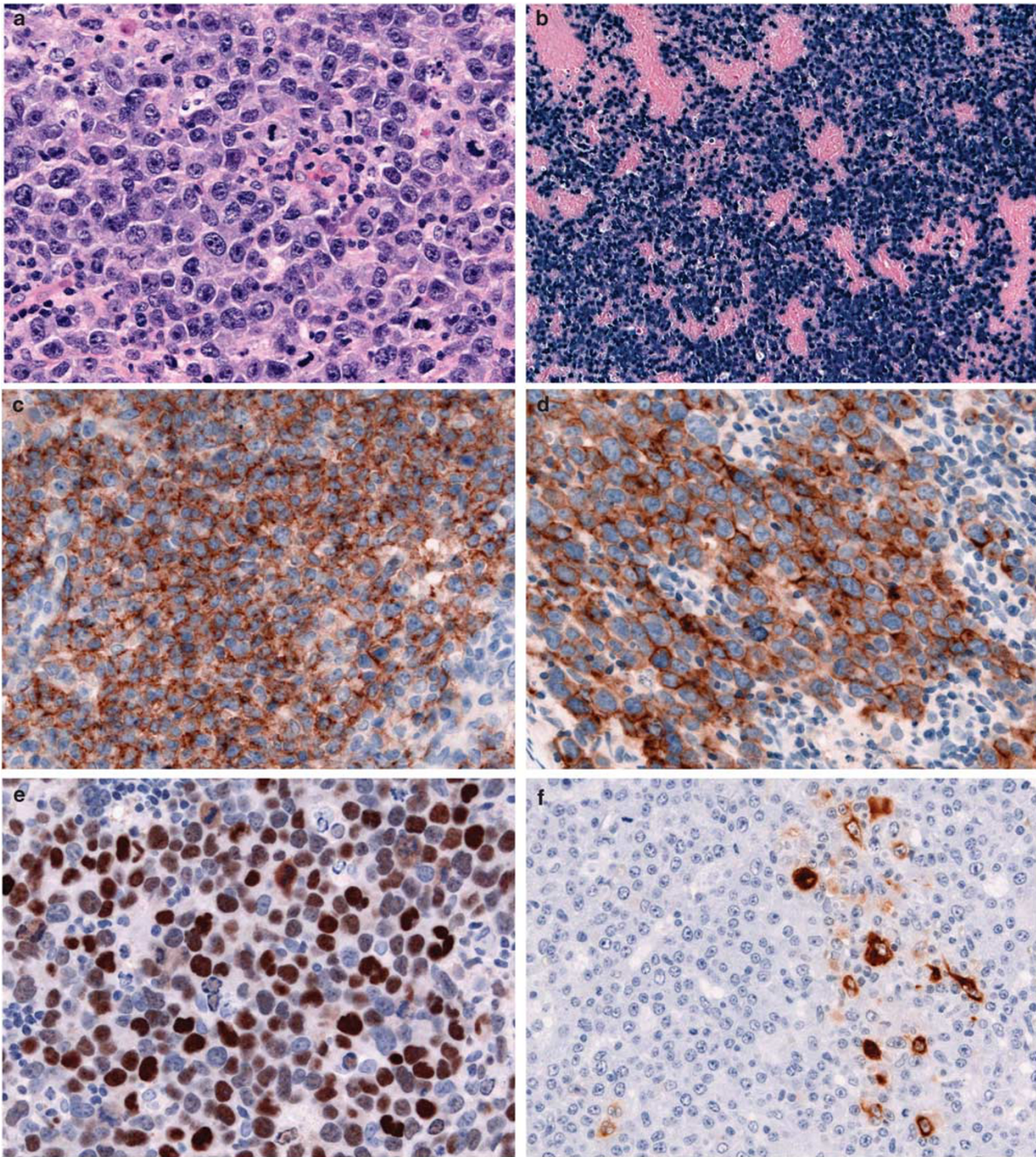


Figure 2 Monomorphic type of EBV-positive B-cell lymphoma of the elderly (case 2). (a) The lymph node is diffusely replaced by a monomorphic infiltrate of large centroblastic cells with abundant mitosis (hematoxylin–eosin; original magnification $\times 400$). (b) EBER *in situ* hybridization shows diffuse EBV-positive tumor cells. Note that almost 100% of the tumor cells are EBER positive (original magnification $\times 100$). (c) CD20 stain is positive in the large tumor cells (original magnification $\times 400$). (d) The tumor cells are strongly positive for CD10 (original magnification $\times 400$). (e) BCL-6 is also positive in the tumor cells (original magnification $\times 400$). (f) LMP1 stain shows specific cytoplasmic positivity in a minority of the tumor cells (original magnification $\times 400$).

(71 years).^{5,6} An interesting finding was that an additional 5% percent of the diffuse large B-cell lymphoma in Mexican population showed EBV positivity in $<20\%$ of the large cells. Although these cases did not fulfill the criteria of EBV+

diffuse large B-cell lymphoma of the elderly, it suggests that in rare cases, secondary EBV infection of an established B-cell clone can occur.¹⁸ A similar scenario has been reported in a T-cell lymphoma.¹⁹ Alternatively, these cells could represent clonally

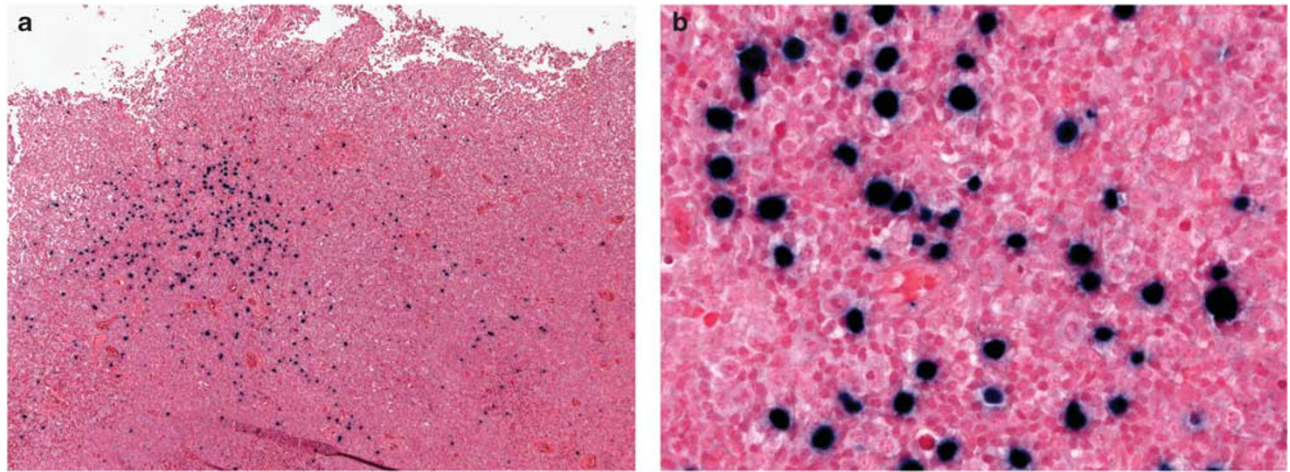


Figure 3 Partial EBV positivity in diffuse large B-cell lymphomas. (a) EBER *in situ hybridization* shows focal EBV-positive tumor cells (original magnification $\times 50$). (b) At higher magnification, the large positive tumor cells and small lymphocytes are depicted (original magnification $\times 400$).

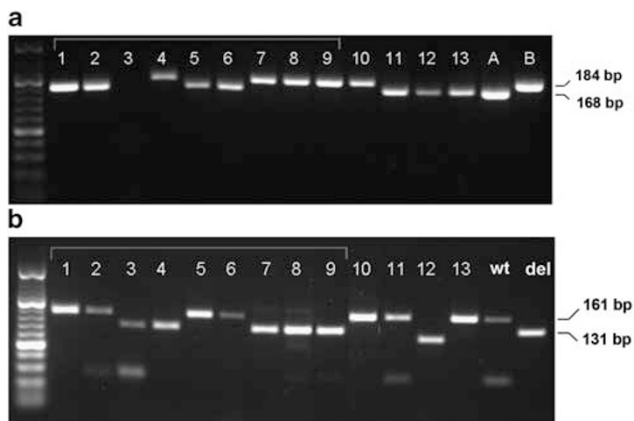


Figure 4 (a) EBV subtyping by PCR analysis from *EBNA2* gene sequences. The primers generate a specific 168 bp fragment for specimens containing type A EBV, and a specific 184 bp fragment for samples infected by type B EBV. Lanes 1–9 represent the Mexican cases, lanes 10–13 the German cases. (a, b) are the positive controls. (b) PCR analysis of the 30-bp *LMP1* gene deletion. The 161-bp fragment represents the full-length (wild-type) *LMP1* gene configuration, whereas the 131-bp fragment represents the 30-bp *LMP1* deletion. Lanes 1–9 represent the Mexican cases, lanes 10–13 the German cases. wt, wild-type control and del, *LMP1* 30 bp deletion control.

unrelated EBV transformed B cells and might reflect the higher incidence of and/or susceptibility for EBV infection in Mexican population. The prevalence of 2% found in the German cohort corroborates previous data published from central Europe.¹⁴ Furthermore, in a small series from the Midwestern USA, the overall rate of EBV positivity in otherwise typical diffuse large B-cell lymphoma in older adults was approximately 1%.¹³ The published studies until now indicate a lower prevalence of EBV+ diffuse large B-cell lymphoma of the elderly in Western populations, as compared with Asians and the Mexican population analyzed in this study. These results are not that unexpected, since other EBV-associated disorders have been

shown to be more prevalent in Asian and Latin American populations.

In the original description of EBV+ diffuse large B-cell lymphoma of the elderly, the frequent extranodal presentation was emphasized as a hallmark feature of this disease (up to 80%).⁵ Nevertheless, in an extended study from the same Japanese group,⁶ the authors described a high but similar frequency of extranodal involvement in elderly patients with both EBV-positive and EBV-negative DLBCL. In our study, only 30% of the cases showed extranodal involvement; however, all of them had lymph node involvement at the time of diagnosis. Accordingly, most recent series have found a predominantly nodal involvement in EBV+ diffuse large B-cell lymphoma of the elderly.^{13,14,20}

Although the mechanism underlying the development of these lymphomas is not understood, it is believed that these lesions arise secondary to deterioration of the immune system due to the aging process. Probably, the best evidence that acquired immunodeficiency might have an etio-pathogenic role is the fact that around 30% of EBV+ diffuse large B-cell lymphoma of the elderly reported from Asia express EBNA2, and, therefore, show a latency III EBV infection, typical of immunodeficiency-associated lymphomas. Of note, 4 of the original 17 EBV+ cases, all of them EBNA2+, had to be excluded from our study because after carefully reviewing the medical records, a cause for immunosuppression or immune dysregulation was identified (autoimmune disease, two cases; previous lymphoma one case, HIV positivity, one case). Moreover, one of the remaining two cases with EBNA2 expression (case 10) had a history of prostate carcinoma treated with surgery, hormonal ablation and radiotherapy 5 years prior to development of the EBV+ diffuse large B-cell lymphoma of the elderly. Although previous history of carcinoma does not preclude the diagnosis of EBV+ diffuse large B-cell lymphoma of the elderly, it is tempting to speculate

that this fact influenced the development of the EBV + diffuse large B-cell lymphoma of the elderly in this patient. Furthermore, besides the EBNA2 positivity, this was the only case with type B EBV without *LMP1* deletion. Type B EBV without *LMP1* deletion can only effectively transform B cells in immunodeficient patients, as has been shown in HIV + patients.^{16,21,22} This evidence strongly suggests that this patient might have had a more profound immunosuppression than the presumed age-related immune dysfunction. In our experience, EBNA2 expression in EBV + diffuse large B-cell lymphoma of the elderly is rather rare. We strongly believe that patients with EBV + diffuse large B-cell lymphoma with EBNA2 expression should be studied carefully for an underlying cause of immunosuppression.

Another point of interest is the high frequency of type B EBV with the *LMP1* 30bp deletion in the Mexican cohort (50%, four of eight cases). These results corroborate our previous findings in classical Hodgkin lymphoma in a Mexican population without obvious immunodeficiency.²³ This finding most probably reflects the geographic variation in EBV strain type rather than differences in the pathogenesis of the disease or the immune status of the host.

Morphologically, the cases analyzed in this study did not differ from previous reports.^{5,6,13,14} Most of the cases corresponded to the polymorphic variant of EBV + diffuse large B-cell lymphoma of the elderly (10 of 13 cases). The tumor cells were in all instances CD20 positive, often CD30 positive, and lack CD15 expression. The strong, homogeneous CD20 positivity combined with the lack of CD15 are useful features in the differential diagnosis with EBV + classical Hodgkin lymphoma.²⁴ As already described,¹³ all polymorphic cases revealed a non-germinal center B-cell phenotype. In contrast, two of the three EBV + diffuse large B-cell lymphoma of the elderly with monomorphic type showed a germinal center B-cell phenotype with expression of CD10 and BCL-6 or BCL-2. As reported in the literature, the majority of our cases were monoclonal, as demonstrated by *IGH* PCR (8 of 11 cases).

In conclusion, we found a similar prevalence of EBV + diffuse large B-cell lymphoma of the elderly in a Mexican population compared with what has been reported from Asian countries, and in contrast to the low frequency in Western populations. However, compared with the Asian series, the Mexican patients were younger at diagnosis, presented predominantly with nodal disease and rarely expressed EBNA2. An interesting finding was the high frequency of EBV type B with *LMP-1* deletion found in the Mexican cases.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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