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Immune Deficiency/Dysregulation Associated Lymphoid Proliferations, EBV+ In Persons Living With HIV

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ABSTRACT

Background: The 5th edition of the World Health Organization Classification of Hematolymphoid Tumors recently defined immune deficiency/dysregulation (IDD)-associated-lymphoid-proliferations in HIV settings, where information is scarce, often gone under or misdiagnosed. **Objectives:** To describe the clinical picture, histopathology, and outcomes of IDD-associated-lymphoid-proliferations Epstein–Barr virus+ (EBV) in people living with HIV without organ transplantation, antiretroviral therapy (ART) treated. **Methods:** HIV+ patients diagnosed with IDD-associated-lymphoid-proliferations seen at an academic medical center in Mexico from 2016 to 2019 were included. Immunohistochemical studies, *in situ* hybridization, and polymerase chain reaction analysis for EBV and LMP1 gene deletions were performed and correlated with clinical data. **Results:** We included 27 patients, all men who have sex with men, median age 36 years (interquartile range [IQR] 22-54). The median baseline CD4+ T cells were 113/mL (IQR 89-243), the CD4+/CD8+ ratio was 0.15 (IQR: 0.09-0.22), and the HIV viral load was 184,280 copies/mL (IQR: 76,000-515,707). Twenty patients (74.07%) had IDD-associated-lymphoid-proliferations hyperplasia plasma cell type EBV+, 3 (11.1%) had hyperplasia mononucleosis-like type (IM-type), 1 patient (3.70%) had florid follicular hyperplasia, 3 (11.1%) IDD-associated-lymphoid-proliferations polymorphic type, and there were 22 cases (81.4%) of synchronic Kaposi Sarcoma. Two patients were diagnosed with Hodgkin lymphoma following a second positron emission tomography-computed tomography scan-guided biopsy. The median follow-up was 228 weeks (IQR 50-269); 6 patients died (22.2%) of causes unrelated to IDD-associated-lymphoid-proliferations related. **Conclusion:** IDD-associated-lymphoid-proliferations EBV+ occured in severely immunosuppressed HIV+ patients, a high percentage of whom had concomitant Kaposi sarcoma. The prognosis was good in patients treated only with ART. (REV INVEST CLIN. 2024;76(3):145-58)

Keywords: Post-transplant lymphoproliferative disorders in persons living with HIV. HIV chronic immunosuppression. Epstein–Barr virus. Human Herpesvirus-8. Kaposi sarcoma herpes virus. Immune deficiency/dysregulation-associated lymphoid proliferations.

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INTRODUCTION

HIV infection is the leading cause of immunosuppression worldwide, even in the era of combined antiretroviral therapy (ART). ART has allowed millions of patients to recover immunity to different extents; nonetheless, still, nowadays, patients arrive to seek medical care with severe degrees of immunosuppression due to obstacles in accessing medical care or because of ART abandonment or failure to ART[1,2].

Lymphoproliferative malignant processes were described in persons living with HIV (PLWH) in the early years of the acquired immunodeficiency syndrome (AIDS) epidemic and were the second malignancies included as AIDS-defining events. The non-Hodgkin lymphomas are aggressive B-cell lymphomas, such as Burkitt's lymphoma, diffuse large B-cell lymphoma, primary central nervous system lymphoma, and plasmablastic lymphoma, all of included an AIDS-defining event[3].

In the recently updated World Health Organization (WHO)-HAEM5, published in June 2022[4], the term "lymphoid proliferations and lymphomas associated with immunodeficiency and dysregulation" (IDD) was incorporated into the terminology. In this new reporting system, three points have been integrated: (1) histological diagnosis (hyperplasia with specific subtypes, polymorphic lymphoproliferative disorder, mucocutaneous ulcer, and lymphoma classified as the immunocompetent patient), (2) the presence of an oncogenic virus (Epstein–Barr virus [EBV] and/or KSHV/Human Herpesvirus 8 [HHV8]), and (3) the clinical immunodeficiency setting (inborn error of immunity, HIV infection, posttransplant, autoimmune disease, iatrogenic/therapy-related, immune senescence). Previous to this new classification system, there was no specific category to classify HIV-positive patients with these types of lymphoproliferative disorders; thus, we formerly named them post-transplant lymphoproliferative disorders (PTLD) type, but PLWH have clinical and histopathological characteristics different from transplanted patients; in addition, they receive ART, making their evolution also distinct[4-10].

This study aimed to describe clinical and histopathological features, and patient outcomes of IDD-associated-lymphoid-proliferations EBV+ infection in HIV-positive patients not transplanted, ART treated, seen at the AIDS Cancer Clinic of the National Cancer Institute (Instituto Nacional de Cancerología, INCan), and describe the prevalence of KS (HHV-8 coinfected).

METHODS

The INCan is a 135-bed tertiary-care oncology hospital in Mexico City, with an HIV/AIDS Cancer Clinic founded in 1990. HIV-positive patients with a diagnosis or suspected malignant disease (AIDS and non-AIDS associated cancers) are referred. Up to 2019, 1450 patients have been seen; KS is still the most frequent malignancy. We performed a retrospective study approved by the Institutional Review Board (REV/0014/20). Confidentiality was preserved for all cases.

All cases with a histopathological diagnosis of IDD-associated lymphoid proliferations seen from January 1, 2016, to December 31, 2019, were included, and follow-up was done up to June 30, 2023. Clinical and demographic data were recovered from the electronic clinical record. Eleven patients participated in a randomized clinical trial (RCT)[11]. These patients, as part of the protocol study, had EBV, HHV-8, and cytomegalovirus (CMV) viral load (VL) measured, as well as interleukins 6 (IL6), interleukins 10 (IL-10), tumor necrosis factor (TNF), and gamma interferon at baseline before ART and thereafter at every protocol scheduled visit up to week 48; these data are included in the descriptive clinical study.

Immunohistochemical and *in situ* hybridization (ISH) analyses

In all cases, *in situ,* hybridization was performed using oligonucleotides complementary to Epstein–Barr early RNA (EBER) transcripts in paraffin-embedded tissue in an automated stainer (Ventana Medical Systems, Tucson, Arizona). The paraffin blocks were sampled using 2.0 mm diameter tissue cores. Immunohistochemical analysis was performed on an automated immunostainer (Ventana Medical Systems) according to the company's protocols and was carried out in all samples with a panel of monoclonal and polyclonal antibodies. The following panel of antibodies was used: CD20 (Clone L26, Dilution 1:400, DAKO), CD3 (Dilution 1:400, DAKO), CD138 (Clone

M115, Dilution 1:75, DAKO), kappa y lambda (Dilution 1:20000, DAKO), Lambda, CD30 (Clone Ber-H2, Dilution 1:50, DAKO), HHV8 (Clone 13B10, Dilution 1:20, BioSB), LMP1 (clone CS. 1-4; DAKO), EBNA2 (Novocastra), Ki67 (Clone SP6, Dilution 1:50, Biocare Medical).

To determine the number of plasma cells and histiocytes, 10 fields were evaluated with the highest magnification and were categorized as follows: mild: 0-10% cells, moderate: 11-40% cells, severe: > 40% cells. EBER ISH was determined by semiquantitative histological score: EBV-1: rare positive cells; EBV-2: occasional scattered positive cells, including in multifocal clusters where the positive cells account for up determination of the type of % of cells/HPF; EBV-3: few scattered positive cells, multifocal clusters, where the positive cells account for up to 20%/HPF; EBV-4: frequent positive cells, diffusely distributed or in multifocal clusters where the positive cells account for > 20-50%/HPF and EBV-5, and as most when the positive cells account for > 50% of the cells/HPF EBER was performed with polymerase chain reaction (PCR); DNA was extracted from 10 mm paraffin sections after dewaxing and proteinase K digestion applying standard phenol/chloroform purification procedures. PCR analysis was performed for EBV A, B, and AB strain typing. Primers flanking a region of the EBNA2 gene differing between types A, B, and AB were used[8]. Each reaction was performed in duplicate with 200 or 500 Zg of extracted DNA in a 50-mL 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 using primers flanking the characteristic 30 bp deletion, as previously described, rendering a 161-bp product for wild-type LMP1 and 131 bp for the deletion variant. All patients with Kaposi sarcoma diagnosis had a positive HHV-8 on tissue biopsy.

Statistical methods

Categorical variables were described using frequencies and proportions; numerical variables were reported as either means and standard deviation (± SD) or medians and interquartile range (IQR), depending on the data distribution. Survival was estimated with the non-parametric Kaplan–Meier statistical function. Statistical analysis was performed using STATA 11.1

RESULTS

Twenty-seven patients were diagnosed with IDD-associated-lymphoid proliferation during the study period. The 27 cases represent 7.56% of 357 patients admitted to the clinic during 2016-2019; all were men who have sex with men (MSM); the median age was 36 years (IQR 22-54). The most frequent B symptom was weight loss at 88.8% (24/27), followed by fever at 51.85% (14/27) and night sweats at 48.15%. Hepatosplenomegaly was present in 12 (44.4%). All patients had lymph node enlargement, 88.8% in ≥ 2 sites. Positron emission tomography-computed tomography (PET-CT) scan with FDG study was performed in 12 patients, all of whom had concomitant KS; the study was positive in all; 9 (81.8%) of them had supra and infra diaphragmatic nodal involvement. The median SUVmax was 8.89 (IQR 3.9-16.4).

Twenty-two (81.4%) patients had concomitant KS at the time of IDD-associated-lymphoid-proliferations diagnosis, 20 (74.07%) with disseminated KS, and 16 (59.2%) of them received chemotherapy for KS. Sixty-six percent of the patients had an opportunistic infection or another coinfection documented at the time of the IDD-associated-lymphoid-proliferations diagnosis, the most frequent being histoplasmosis and syphilis (22.2%), 8 (29.63%) patients had two or more coinfections (Table 1).

All patients had anemia with median hemoglobin of 8.89 g/L (IQR 3.94-16), 9 (33.3%) had thrombocytopenia, and 4 (14.8 %) had pancytopenia. Fifteen (55.5%) patients had elevated levels of lactic dehydrogenase (LDH) with a median of 381 IU/mL (IQR 51-892 IU/mL) (Table 1).

The baseline median HIV VL was 184280 copies/mL (IQR: 76000-515707), the CD4 + 113 cells/mL (IQR 89-243), and CD4/CD8 ratio 0.15 (IQR: 0.09-0.22) (Table 1). Eight (29%) patients had < 100 CD4 + cells/mL, 13 (48.14%) patients between > 100 and < 200 CD4+ cells/mL, and only 7 (25.9%) had > 200 CD4+ cells/mL. The median time from HIV diagnosis to DDI diagnosis was 57 days (IQR 31-334). Three patients had abandoned ART years before the DDI diagnosis; for these 3 cases, the date of ARVs reinitiation was considered the closest date when the patient restarted ARVs. The median time from DDI diagnosis to ARV initiation was 58 days (IQR 28-87.5).

Table 1. Patient's characteristics and laboratory parameters

Variable	(n = 27)	%
Age (median)	36	IQR 22-54
Male (MSM)	27	100
Weight loss	24	88.8
Fever	14	51.8
Night sweats	13	48.1
Hepatosplenomegaly	12	44.4
Lymph node enlargement	27	100
≥ 2 sites lymph node involvement	24	88.8
Kaposi sarcoma	22	81.4
Disseminated KS	20	74.07
Chemotherapy for KS	16	59.2
Number of chemotherapy cycles received for	2 (IQR 1-9)	
Coinfections	18	66.6
Histoplasmosis	6	22.2
Syphilis	6	22.2
Helicobacter pylori	4	14.8
Cytomegalovirus end-organ disease	2	7.41
Disseminated *Mycobacterium* avium complex	2	7.41
Anal condylomas	2	7.41
Herpes zoster infection	2	7.41
PET-CT study	12	44.4
Positive PET-CT	12	44.4
Supra and infra diaphragmatic nodal involvement	9	81.8
*SUVmax + (median)	8.89	IQR 3.96-16.4
Hemoglobin (median)	8.89 g/L	IQR 3.9-16.4
Leukocytes (median)	4590 cells/mL	IQR 3800-6300
Platelets (median)	199.000 cells /mL	IQR 11000-505000
Neutrophils (median)	2600 cells/mL	IQR-2000-3200
Lymphocytes (median)	1400 cells/mL	IQR 300-2900
CD4+ cells/mL (median)	113	IQR 89-243
CD4/CD8 (median)	0.15	IQR 0.09-0.22
HIV VL copies /mL. (median)	184280	IQR 76000-515707
Lactic dehydrogenase** (median)	381 IU/mL	IQR 51-892 IU/mL

*The SUVmax is the relationship: uptake/volume of the voxel with maximum uptake and has been agreed upon as the way to express uptake quantification. The most used algorithm is the maximum Standardized Uptake Value (SUVmax) and in PET readings this measure expresses the amount of deposit, the greater the deposit, the greater the number.
**References values for LDH expressed with IU/mL (reference normal values 120-246). MSM: men who have sex with men; IQR: interquartile range; PET-CT: positron emission tomography-computed tomography.

Table 2. Epstein Barr, HIV, HHV-8, and cytomegalovirus viral loads, cytokines (IL6, IL10; TNF and IFN), CD4+ and CD8+ cells were measured at baseline in 11 patients participating in a randomized clinical trial with an IDD-associated lymphoid-proliferations diagnosis, compared to 29 patients from the same cohort without IDD-associated lymphoid-proliferations

Variable	IDD associated-lymphoid-proliferations (n = 11)	Non-IDD associated-lymphoid-proliferations (n = 29)	p =
EBV copies/mL	296	117	0.37
IQR	232-2539	53-1512	
HHV8 copies/mL	300	4004	0.68
IQR	250-4284	561-13494	
HIV copies/mL	198847	206565	0.47
IQR	43903-687750	40607-605433	
CMV copies/mL	80	247	0.55
IQR	40-898	40-923	
CD4+ cells/mL	90	60	0.42
IQR	38-122	36-84	
CD8+ cells/mL	653	851	0.58
IQR	337-1156	418-1276	
IL 6 pg./mL	18.81	20	0.65
IQR	9.02-22.02	11.74-51.24	
IL10 pg./mL	22.99	17.35	0.40
IQR	14.08-42.3	10.46-32.13	
TNF pg./mL	4.69	18.25	0.05
IQR	2.47-27.74	10.85-31.12	
IFN pg./mL	10.74	9.47	0.45
IQR	6.94-50.42	6.18-23.96	

IQR: interquartile range. Epstein Barr virus (EBV), Human Herpes virus 8 (HHV-8), HIV and cytomegalovirus (CMV) viremia, and cytokines. Normal reference values: IL6 3.4 pg./mL, IL10 9.1 pg./mL, TNF 8.1 (for IFNγ we use the media measure in samples of 30 healthy subjects 1.8 pg/mL SD0.8 pg/mL), HIV VL with Abbott Real-time, HHV-8, CMV, and Epstein-Barr virus (EBV) VL ELITe MGB KIT by ELITe InGenius Software, CD4+, and CD8+ cells count (flow cytometry, Facs Canto II, Becton Dickinson). Plasma levels of interleukin 6, 10 (IL-6, IL-10), tumor necrosis factor (TNF), and interferon-gamma (IFN-ϫ) were measured using a sandwich-type immunoassay, ELISA (Biolegend).

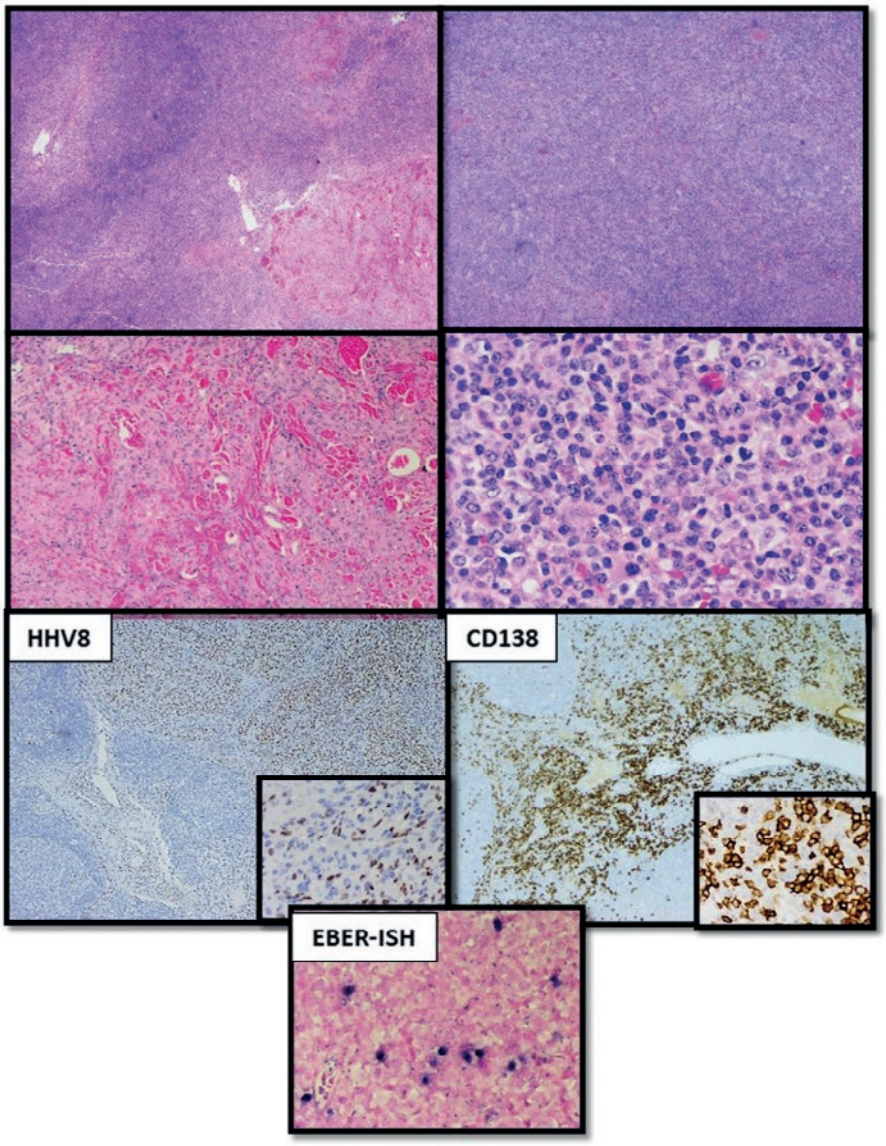
For the group of 11 patients that participated in RCT, information about EBV, CMV, and HHV-8 VL and levels of IL-6, IL10, IFNγ, and TNF are summarized in table 2. There were no differences in cytokines levels among RCT participants with IDD-associated-lymphoid-proliferations compared to the participants without it, as it is shown in Table 2.

There were no differences in cytokines levels or measures in patients with DSK and IDD-associated lymphoid proliferations compared to those in the same cohort without. All measures are shown in Table 2.

Histological and immunophenotypic features

The most frequently biopsied sites were the lymph nodes, in 23 patients (85.91%), followed by 3 gastrointestinal mucosa (11.1%), and one nasal mucosa (3.7%). Twenty cases (74.07%) had IDD-associated-lymphoid proliferations plasma cell hyperplasia type associated with EBV infection; 3 cases (11.1%) had IDD-associated-lymphoid-proliferations mononucleosis-like type (IM-type); 3 (11.1%) corresponded to IDD-associated-lymphoid-proliferations polymorphic type, and only 1 patient (3.70%,) had florid follicular

Figure 1. Case 4, lymph node biopsy. **A-D:** H/E. Plasma cell hyperplasia type non-destructive immune deficiency/dysregulation-associated-lymphoid proliferation and concomitant KS, **E:** human herpesvirus 8: positive in KS (+++). **F:** CD138: positive in plasma cells (moderate), **G:** Epstein–Barr early RNA-*in situ* hybridization: positive (Epstein-Barr virus-1 score).



hyperplasia (Fig. 1). Twenty-two cases (81.4%) had concomitant KS diagnosis and IDD-associated-lymphoid proliferations in the same tissue (Table 3). There were no differences in the histopathologic characteristics between KS and non-KS IDD-associated-lymphoid-proliferations biopsies.

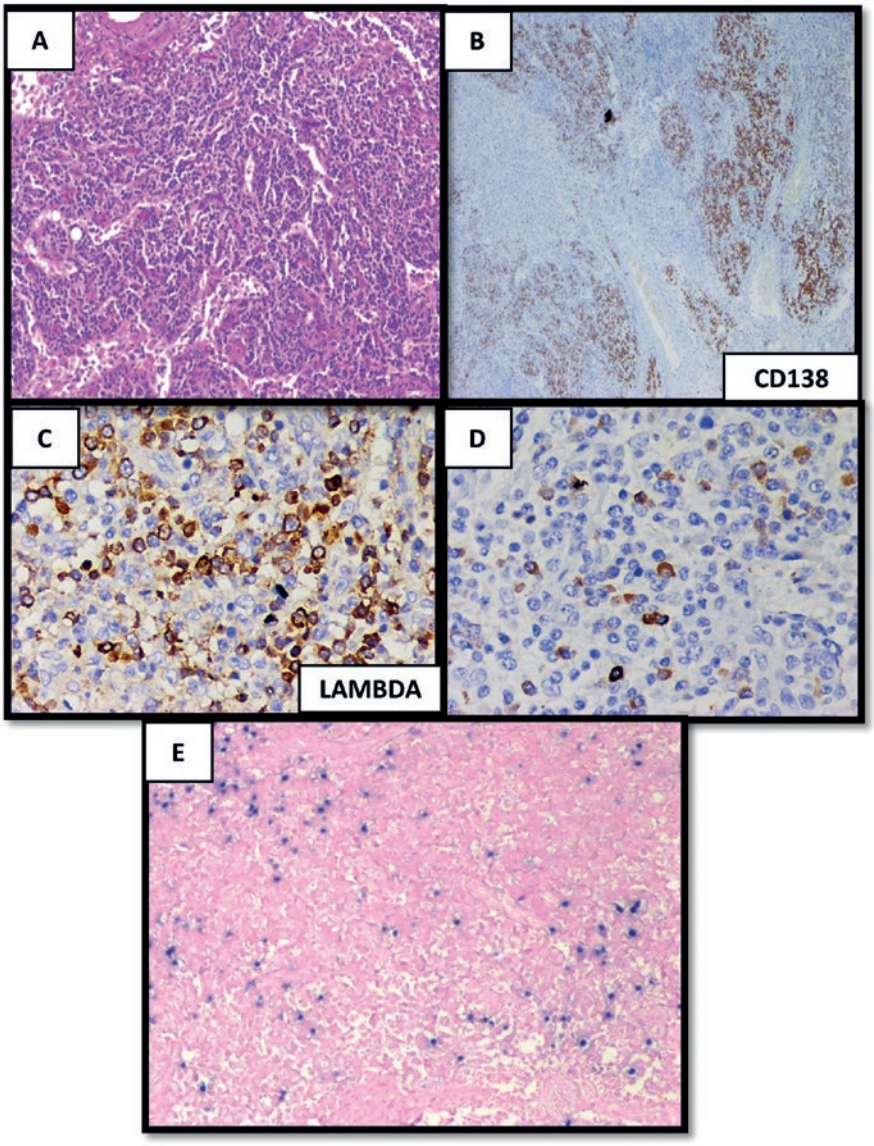
In 16 (59.25%) patients, a different diagnosis was initially given; 10 (37.03%) were only reported as KS, 3 (11.1%) as reactive lymphoid hyperplasia, one as plasmablastic lymphoma, one as acute proctitis, one as classic Hodgkin lymphoma mixed cellularity variant EBV-associated, and one as Castleman disease rich in plasma cells. The clinicians requested a second review from the hematopathologist to discard other malignancies besides KS and, in another case, because there was a discrepancy between the clinical presentation and the initial histopathologic report (a patient was initially diagnosed with plasmablastic lymphoma, but the clinical features and the good general condition of the patient were not compatible with this diagnosis).

Table 3. Histopathological features

Variable	(n = 27)	%
Morphological characteristics		
Biopsy site		
Lymph node	23	85.9
GI tract	3	11.1
Nasal zone	1	3.70
Type of IDD-associated-lymphoid proliferations		
Non-destructive	24	88.8
PH	20	74.07
MI	3	11.1
HFL	1	3.70
Polymorphic	3	11.1
Distribution pattern		
Diffuse	24	88.8
Partial	3	11.1
R-S like cells		
None	22	81.4
Few	3	11.1
Moderate	2	7.41
Histiocytes		
Few	22	81.44
Moderate	4	14.81
Abundant	1	3.70
Plasmatic cells		
Few	6	22.2
Moderate	9	33.33
Abundant	12	46.44
Type of IDD-associated-lymphoid- proliferations		
Non-destructive type PH		
CD138 +	20	74.07
Partial light chain restriction	20	74.07
(Kappa+/Lambda+++)	20	74.07
KI67 >30%	3	11.11
Non-destructive IM		
CD30+	3	11.11
LMP-1+	3	11.11
Polymorphic		
Ki67 >30%	3	11.11
In situ hybridization		
EBER-ISH	27	100

EBER-ISH: Epstein–Barr early RNA-in situ hybridization.

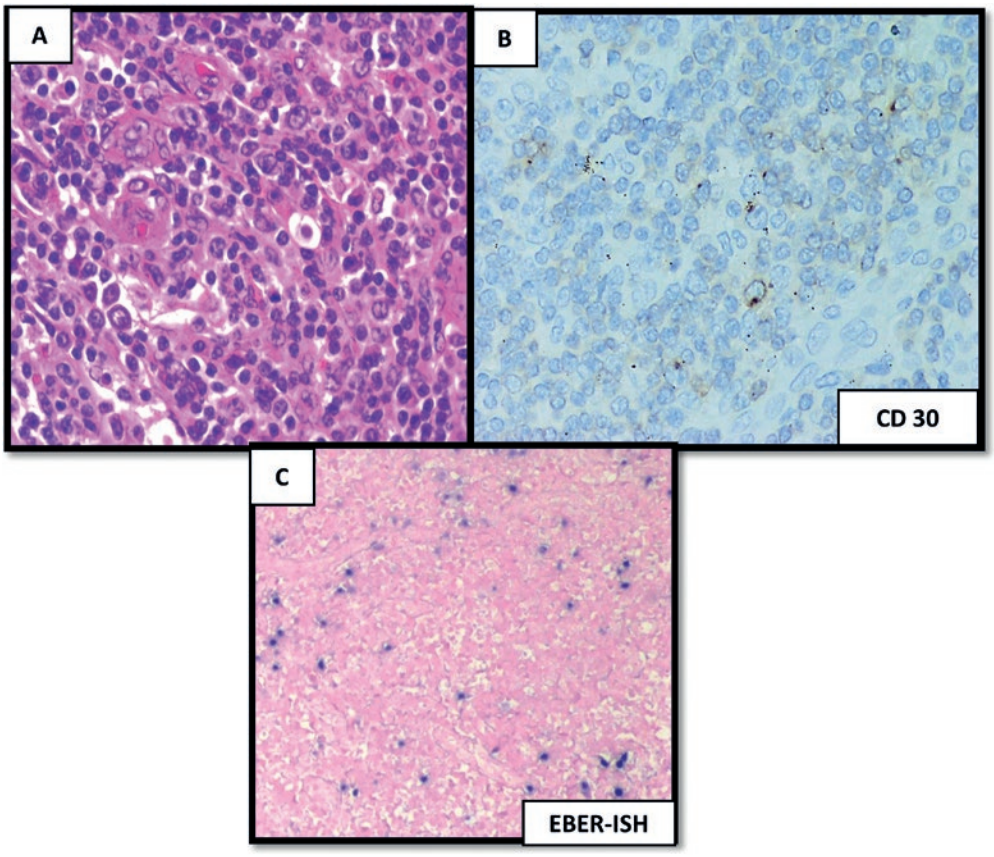
Figure 2. Case 5, lymph node biopsy. **A:** H/E. Plasma cell hyperplasia type non-destructive immune deficiency/dysregulation-associated-lymphoid-proliferations. **B:** CD138: positive in plasma cells (severe). **C:** LAMBDA positive in plasma cells (+++). **D:** KAPPA positive in plasma cells (+). **E:** Epstein–Barr early RNA-in situ hybridization: positive (Epstein–Barr virus-2 score).



The morphological characteristics of cases with plasma cell hyperplasia type were predominantly the partial distribution pattern, with plasma cell roads and no evidence of necrosis (Fig. 1). All cases showed an increased number of plasma cells distributed forming roads. Twenty (74.07%) showed partial restriction of light chains kappa +, lambda +++ (Fig. 2). Regarding the hyperplasia mononucleosis-like type non-destructive IDD-associated-lymphoid proliferation, it had an increase in activated immunoblasts and Redd Sternberg-type cells that were positive for EBER-ISH (Fig. 3). In the case of IDD-associated-lymphoid-proliferations of the polymorphic type, the distribution pattern was diffused with loss of architecture and B lymphocytes in different degrees of maturation with different shapes and sizes mixed with activated immunoblasts (Fig. 4 and Table 3).

Reactive immunoblasts with the expression of CD30 were observed in all cases, regardless of the type of lymphoproliferative disorder; however, in the IM-type disorder, the presence of reactive immunoblasts was more prominent (Fig. 3).

Figure 3. Case 5, lymph node biopsy: **A:** H/E. Hyperplasia mononucleosis-like type non-destructive immune deficiency/dysregulation-associated-lymphoid-proliferations. **B:** CD30: positive (++). **C:** Epstein–Barr early RNA-*in situ* hybridization: positive (Epstein–Barr virus-3 score).



All cases were positive for EBER by ISH, and the score was determined by semiquantitative histological scoring: 13 (48.14%) were EBV-1, 11 (40.74%) were EBV-3, and 3 (11.11%) were rated EBV-4. The polymorphic disorders, those that showed the highest EBER expression, were scored as EBV-5 (Fig. 4).

The results of EBV strain and *LMP-1* gene deletion are summarized in table 4. Most IDD-associated-lymphoid-proliferations hyperplasia type forms were associated with EBV type B (11/20), of which 4 carried the 30 bp LMP1 deletion. In contrast, type A EBV was associated with the polymorphic form (2/2). However, in 10 patients, EBV typing and LMP-1 deletion could not be detected due to scarcity of tissue.

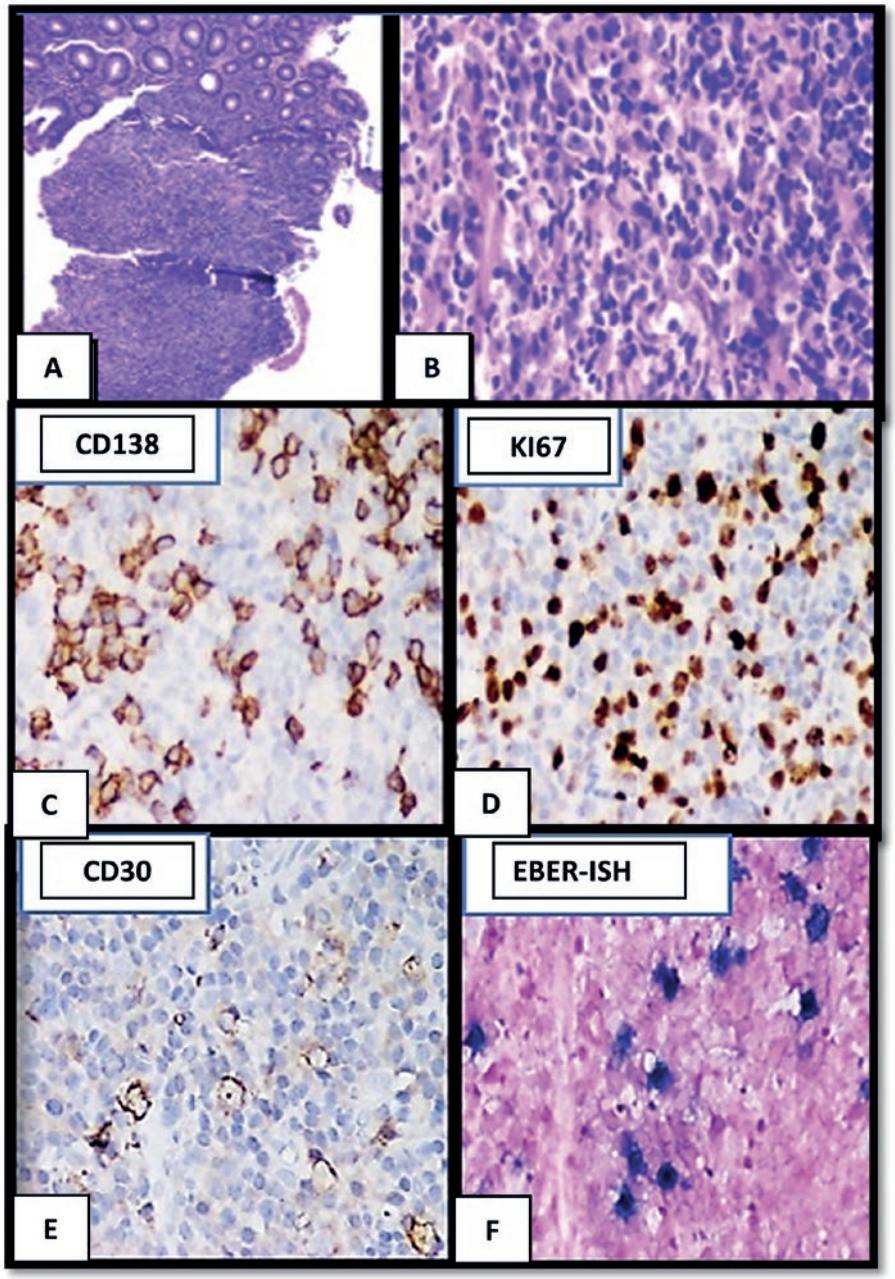
In all the cases in which LMP1 detection was performed by immunohistochemistry, this was negative in 26/27 (96.2%). Therefore, most cases (> 96%) had a type 1 latency.

Outcomes

Twenty-two patients (81.4%) started ART within a month of IDD-associated-lymphoid-proliferations diagnosis; 5 were on ART with a median time of 7 weeks (IQR 2-8 weeks). The median time from HIV diagnosis to IDD-associated-lymphoid-proliferations was 114 weeks (IQR 3.7-790.3 weeks). The overall survival at 4 years was 70%.

The median time of follow-up was 228 weeks (IQR 50-269). Six patients died due to the following causes: disseminated *Mycobacterium* avium complex (one), H1N1influenza pneumonia (one), community-acquired pneumonia and sepsis (one), a patient initially diagnosed as plasmablastic lymphoma died of sepsis secondary to severe neutropenia after chemotherapy administration, and two due severe immune reconstitution inflammatory syndrome associated Kaposi sarcoma (S-IRIS-KS) after starting ART. Of the 18 patients

Figure 4. Case 17, rectal biopsy: **A and B:** H/E. polymorphic type destructive immune deficiency/dysregulation-associated-lymphoid-proliferations. **C:** CD138: Positive in plasma cells (mild) **D:** KI67. 40%. **E:** CD30 (++) positive. **F:** Epstein–Barr early RNA-*in situ* hybridization: positive (Epstein–Barr virus-5 score).



with KS who survived, 76.9% achieved complete remission and 15.3% had partial remission, with no KS relapse.

Ten patients (37.03%) had no lymphadenopathy found after a year on ART, and 9 (33.3%) had a decrease in lymphadenopathy with lymph node sizes < 1 cm. Two patients were diagnosed 1 month after IDD-associated-lymphoid-proliferations with mixed-cellularity classic Hodgkin lymphoma EBV+ and one patient with castleman disease; the diagnosis was established after a second lymph node biopsy was obtained guided by high metabolic SUVmax on PET-CT.

Table 4. PCR analysis of EBV and LPM-1 gene deletion

EBV strain	Total n = 20 (20/27)	IDD-associated-lymphoid-proliferations - IM (1/20)	IDD-associated-lymphoid-proliferations - PH (17/20)	DLPT-polymorphic type (2/20)
EBV subtype A	6/20	–	4/20	2/20
LMP-1 wild type	4/20	–	4/20	–
LMP-1 30-bp deletion	2/20	–	–	2/20
EBV subtype B	12/20	1/20	11/20	–
LMP-1 wild type	7/20	–	7/20	–
LMP-1 30-bp deletion	4/20	–	4/20	–
EBV subtype AB	2/20	–	2/20	–
LMP-1 wild type	.	–	–	–
LMP-1 30-bp deletion	2/20		2/20	

PCR: polymerase chain reaction; IDD: immune deficiency/dysregulation; EBV: Epstein–Barr virus; *Bp:* base pair. In 10 patients, EBV and LMP-1 30bp deletion typing tests could not be performed since the material was scarce and was consumed.

DISCUSSION

We describe 27 PLWH with morphological characteristics that resemble PTLD but in non-transplanted settings in the former WHO-HAEM classification, currently called IDD-associated-lymphoid-proliferations, in the fifth version of the WHO hematolymphoid tumor classification. This is the most extensive series of patients described, with features that had not been reported, since 81% of them had synchronic KS. Thus, these patients were coinfected with two oncogenic viruses, and 50% had at least one concomitant opportunistic infectious process, reflecting severe immunosuppression and a milieu of inflammatory cytokines.

Before this new HAEM5 classification was created, there was little information, and few studies had been published on what was previously called lymphoproliferative disorders associated with EBV infection, similar to that described in PTLD. One of the largest series is that of Nador et al., who reported 10 HIV-infected patients with PTLD of polymorphic type, two of them associated with KS[4,12]. The CD4+ T-cell count was only reported in 3 patients with a range of 54-188 cells/mL, similar to our series median 113 cells/mL (IQR 89-243 cells/mL), reflecting severe immunosuppression. From the histopathological point of view, findings were similar; the diffuse pattern prevailed with a trend to plasma differentiation and light chain restriction in 50% of cases and EBV determination in 60%. These findings are similar to the cohort reported here, but follow-up was unknown for most of the cases[12].

Other case reports have been published in the pediatric population: Kingma et al. in 1999 reported a case of low-grade monoclonal lymphoproliferative disorder in the brain associated with EBV in an HIV+ patient[13]. Tao and Valderrama analyzed two HIV+ children with polymorphic B-cell lymphoproliferative disorders associated with EBV with lung involvement; in one, the course was indolent, and in the other, it was aggressive, and both patients were on ART[2,6,8,9,14].

Tao and Wasik published 12 cases of polymorphic lymphoproliferative disorders associated with EBV in non-transplanted patients; four of them were HIV+. In three cases with polymorphic type PTLD, the histopathological characteristics showed a diffuse distribution pattern, with areas of necrosis and an increased number of plasma cells, in accordance with what has been described. Five patients died from disease-related causes. In the present series, we found a predominance of the non-destructive variety, diffuse pattern, and abundant infiltrate of plasma cells, which is similar to Tao's report; none of our patients died from disease-related causes.

Nador et al. proposed that lymphoproliferative disorders constitute a continuous morphological spectrum

that can develop into true lymphoma in which IL-6 is increased, and coinfections could play a role in the pathogenesis of this disease[12]. The role of IL-6 in the pathogenesis of PTLD has been described in the context of transplanted patients. IL-6 seems to be produced by monocytes, endothelial cells, and/or fibroblast, cell types known to produce large quantities of IL-6 upon induction with a variety of stimuli, including IL-10, IL-8, TNF and IFN, it can also act as a growth factor for EBV immortalized cells and, at high concentrations, it inhibits cytotoxic functions and further depresses immunity that may contribute to tumor development[12,14,15]. In the subgroup of patients of the RCT in whom cytokines were measured, we found that IL6, IFN, and IL-10 were all increased. High levels of IL-6 had been found in solid organ transplant patients with PTLD; the median level of IL-6 was 17 times higher than the reference value in the patients of this cohort in whom it was measured; IL6 was 5.5 times the reference value. The high production of IL-6 has been imputed as a pathogenic mechanism for PTLD development[15].

The relationship of EBV in the pathophysiology of lymphoproliferative neoplasm in HIV+ patients is well known. HIV infection has been associated with decreased immunosurveillance, which allows a decrease in the containment of EBV infection to B lymphocytes. In addition, a decrease in CD8+ lymphocytes versus EBV has been described in HIV+ patients[16,17].

High EBV VL has been described in patients with lymphoproliferative diseases[18]. Fan et al. reported a median EBV VL of 3,210 copies/mL (range 34-1,500,000) in patients with lymphoma18. A study from Johns Hopkins University reported a median EBV VL of 54,960 copies/mL (170-961,520) in patients with PTLD-EBV+[18,19]. The median EBV VL was 296 copies/mL (IQR 232-2,539) of the patients from the RCT, much lower than those described in the studies previously mentioned; in our study, the essay for EBV was done using whole blood.

On the other hand, the various phases of the viral cycle have been related to various lymphoproliferative disorders. In this study, all patients were EBER positive; the majority of patients had a type I latency pattern, unlike those described in post-transplant patients and HIV lymphoma patients, in whom an association with the type III latency pattern has been observed.

More questions arise when trying to explain the development of these lesions since the type I latency period is related to infection of memory B cells, with a low replication rate that is concordant with the VLs described in this series, which could indicate that this disorder is linked to a more chronic *pauci* EBV replicative infection and not to a state of active replication.

Several studies have reported the variable geographical incidence and distribution of EBV virus and its strains, mainly EBV type B strain in the Mexican population, unlike other Latin American countries such as Argentina or Peru, where type A strain is more frequent, or as in European countries such as Germany[20]. According to the study by Frank et al., most IDD-associated-lymphoid-proliferations in immunocompetent patients contain EBV type A, which is consistent with what is described as the efficient immortalization of B cells in vitro by EBV type A virus and the importance of the product of the EBNA-2[21]. Unlike what has been described in HIV+ patients, in the Western hemisphere, there is evidence that these patients may harbor either EBV type A or B, or both types simultaneously[21-23]. In this study, we found that 60% of the patients had EBV type B, and this was correlated with the IDD-associated-lymphoid-proliferations hyperplasia type, unlike the 2 patients who had IDD-associated-lymphoid-proliferations polymorphic type in which the associated strain was type A. According to the study published by Nador et al., in which 10 cases of polymorphic type PTLD were analyzed, 4 patients were classified as EBV+, and 3 were type A, which differs from the results of the present study[12]. Independently from the geographical factor, it must be considered that a particular HIV+ population was studied; all patients were MSM, since Kaposi sarcoma is mainly reported in MSM in Mexico[24]. Ibrahim et al. reported EBV B type strain with 30-bp LMP1 deletion in the MSM population[25].

Second, the role of HHV-8 in the pathophysiology of these disorders is not clear either. In patients with KS, HIV-Tat activity induces the production of INFγ, with proangiogenic and inflammatory properties characterized by infiltrates of T-CD8+ lymphocytes and macrophages. In addition, it promotes the activation of cell surface adhesion molecules, such as cytokines that induce an inflammatory state. In patients with HIV, EBV, and HHV-8 coinfection, the inflammatory state could be perpetuated, and this could lead to the development of IDD-associated-lymphoid-proliferations[26].

Primary effusion lymphoma (PEL) models in HIV-positive B cells that are infected by HHV-8 also showed coinfection by EBV in a lytic state (where all genes are expressed). These PEL cells express LMP-1 by regulating the latency periods of both viruses, which translates into a decrease in protein expression by both viruses, which in turn promotes the immune escape of the cells, in addition to the production of proteins such as KSVH-LANA and LMP1 that inhibit p53 and promote NFKB activation, respectively, promoting tumorigenesis[17].

According to the results from this study, the IDD-associated-lymphoid-proliferations hyperplasia type has a favorable prognosis when treated with ART; there were no deaths attributable to IDD-associated-lymphoid-proliferations. However, we found two cases of HL that were diagnosed early; the rest of the patients did not develop lymphoproliferative malignant disease during long follow-ups (median 170 weeks; IQR 13-937); all received ART. In previous studies, no follow-up or therapy is reported, so it is impossible to compare the course of the disease or the treatment received.

When diagnosing these cases, mistakes are frequent, and there is no clarity regarding their management because there are currently no guidelines or information addressing therapy[1,13]. In three cases in which the biopsy was repeated, it was done because the clinician had a high suspicion of malignant disease; two had high SUV max in PET-CT, and in another case with Castleman disease, the SUV max was much lower. We understand that this disease could evolve in the same patient and have different stages in different parts of the body, so when there is a high grade of suspicion, more than one biopsy should be performed to avoid missing a malignant diagnosis that requires treatment. The PET-CT scan helps guide biopsy site lesions with the highest SUV max.

We recognized the limitations of this study as it is a single referral hospital, and thus, the number of patients here reported is larger than in other series, probably also related to the fact that in Mexico, patients are still diagnosed with advanced HIV disease, something that is no frequent in high-income countries with programs for earlier HIV detection and prompt access to ARV therapy. Furthermore, the fact that all are MSM is related to referral bias, as our institution is a referral center for Kaposi sarcoma. This entity is mostly diagnosed in MSM in Mexico[24].

A strength of this study is the long follow-up and the fact that all patients had received ARVs, which promotes immune reconstitution; this fact has not been previously described.

IDD-associated-lymphoid-proliferations are disorders that can occur in patients with HIV without organ transplantation, which requires a differential diagnosis with lymphoproliferative neoplasms such as lymphoma that requires chemotherapy and have a very different course and prognosis. The cooperation between EBV and HH8-V has not been understood in this entity. In this cohort, the prognosis was good once a neoplasm was discarded. HIV control with ART and treatment of concurrent infections contributed to the control of the disorder, in which an inflammatory state has been proposed in its pathogenesis. The implications these lesions may have for developing future lymphoproliferative neoplasms are still unknown.

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