Hydroa vacciniforme-like lymphoma: a chronic EBV+ lymphoproliferative disorder with risk to develop a systemic lymphoma

Short title: Hydroa vacciniforme-like lymphoma

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Key Points:

1. Hydroa vacciniforme-like lymphoma is a chronic EBV+ lymphoproliferative disorder of childhood with risk to develop systemic lymphoma.
2. The disease shows favorable response to conservative therapy despite the presence of a T- or NK-cell monoclonal proliferation.

Abstract:

Hydroa vacciniforme like-lymphoma (HVLL) is an EBV-positive T-cell lymphoproliferative disorder of childhood that occurs mainly in Central-/South America and Asia. We present the clinicopathological features of 20 Mexican children with HVLL with a median age of 8 years at diagnosis (range: 1 – 15). All patients presented with skin lesions involving sun-exposed areas but not exclusively. Fever, lymphadenopathy and hepatosplenomegaly were often observed. Most patients were treated with immunomodulators and/or immunosuppressive agents resulting in temporary remissions. For thirteen patients follow-up was available with a median of 3 years (range 1 month -13 years). Three patients with long follow-up (9-13 years) are alive with disease. Four patients died, two after developing systemic lymphoma. Histologically, the skin showed a predominantly angiocentric and periadnexal EBER+ lymphoid infiltrate with variable atypia and subcutaneous involvement. Fifteen patients showed a T-cell phenotype (12 αβ, two γδ, one silent phenotype) and monoclonal TCRγ rearrangements, whereas six exhibited a NK-cell phenotype. Four patients had hypersensitivity to mosquito bites. One patient showed both phenotypes. HV like-lymphoma is an EBV-associated lymphoproliferative disorder of αβ-, γδ or NK-cell phenotype with a broad clinical spectrum, usually prolonged clinical course, and risk for progression to systemic disease.

Key words: Hydroa vacciniforme, Hydroa vacciniforme-like lymphoma, EBV+ lymphoproliferative disorder, TCRγ monoclonality, hypersensitivity to mosquito bites
INTRODUCTION

Hydroa vacciniforme (HV) is characterized, in its classic form, by light-induced vesicles that evolve to crusts and leave varicelliform scars after healing. Systemic symptoms are not observed and the disease usually remits spontaneously in adolescence or young adulthood. In the past years a peculiar group of vesicopapular eruptions that mimics HV was recognized in children mainly from Mexico, Peru and Asia. These lesions present with marked facial edema, vesicles, crusts, and large ulcers with severe scarring and disfigurement in sun-exposed and non-exposed skin areas. The patients usually have systemic symptoms including fever, weight loss and asthenia. Hepatosplenomegaly and lymphadenopathy are frequently observed in the acute phase, and association with hypersensitivity to mosquito bites (HMB) was noted. In the initial description, because panniculitis and/or vasculitis were the predominant histological features, it was suggested to call this condition edematous scarring vasculitic panniculitis (ESVP) to separate it from the classical form of HV. Later studies demonstrated that these lesions called “severe” HV or ESVP were associated with Epstein-Barr virus (EBV) infection and often showed monoclonal rearrangements of the T-cell receptor (TCR) genes, and therefore, the term HV-like lymphoma was suggested.

HV-like lymphoma (HVLL) and systemic EBV-positive T-cell lymphoproliferative disease (LPD) of childhood were incorporated for the first time in the 2008 World Health Organization (WHO) classification of tumours of haematopoietic and lymphoid tissues in the subgroup of EBV-positive T-cell LPD of childhood. HVLL is defined as an EBV-positive cutaneous T-cell lymphoma occurring in children and less often in young adults, and may be associated with hypersensitivity to insect bites. Clinically, patients present with facial edema and recurrent vesiculopapular rash followed by ulceration and crusting. Although the skin lesions are not exclusively limited to sun-exposed areas and do not only appear after sun-exposure, like HV skin lesions, there is a seasonal increased occurrence during the summer. Most cases reveal a CD8+ T-cell phenotype, however, a small proportion of cases have been reported to have a natural killer (NK)-cell phenotype. In contrast, only rare cases of CD4+ T-cell phenotype have been described. The lymphoid cells regardless of the cell-type derivation are positive for cytotoxic markers like granzyme B and TIA-1. CD30 expression was found in a part of the reported cases in the literature.
Since the incorporation of HVLL into the WHO lymphoma classification several controversial issues have been raised that remain to be clarified. It is not known whether HVLL represents a true lymphoma from its first clinical manifestation or a preneoplastic disorder with risk to develop into a systemic lymphoma. It is also uncertain whether HVLL is a de novo disease or develops in patients with long-standing HV, as has been suggested. Classic HV in western countries is considered a benign photodermatosis with spontaneous improvement or remission during adolescence. These cases are rarely biopsied and in the original series clonality and EBV status were not investigated. Subsequent studies in Asian populations showed that “classic” HV was also an EBV-associated disorder, and therefore, it was proposed to include it as part of the clinical spectrum of chronic active EBV infection. Nevertheless, it is unclear whether what has been called “classic” HV in Asian populations corresponds to the same disease described in Western population and/or Mexico, where the disease is self-limited and progression to HVLL has not been observed. This discrepancy has contributed to the uncertainty in the differential diagnosis between classic HV and HVLL. It has been proposed that the most useful criterion to separate these two entities is monoclonality of the TCR genes. Nevertheless, the differential diagnosis seems to be arbitrary posing serious diagnostic and therapeutic problems for pathologists, dermatologists and hematologists.

A related cutaneous disorder, mainly described from Japan is severe mosquito bite allergy. Severe mosquito bite allergy is defined as an EBV+ NK-cell lymphoproliferation characterized by high fever after bites, ulcers, skin necrosis and deep scarring with potential to progress into overt lymphoma or leukemia in the long-standing clinical course. Severe mosquito bite allergy is not recognized as a distinct entity in the 2008 WHO classification, and its relationship to HVLL is not well defined. Accumulating evidence indicates that these two cutaneous disorders might represent different manifestations within the spectrum of disorders encompassed under the umbrella of chronic active EBV infection (CAEBV) of T/NK-cell type. The aim of this study was to analyze the clinical, histological and molecular features of 20 Mexican children mostly diagnosed, treated and followed at the Instituto Nacional de Pediatria in Mexico City, Mexico with the diagnosis of ESVP or HVLL, to define more precisely the diagnostic criteria and prognosis of the disease in order to facilitate the development of more effective treatments. Our study reveals that EBV+
HV-like LPD, regardless of the presence or absence of systemic symptoms, and the severity of the skin lesions, is a monoclonal disorder of T-cells and/or NK-cells with a broad clinical spectrum, a usually protracted clinical course, and long-term risk to progress to a systemic lymphoma.

**Material and Methods:**

*Case selection*

Twenty-eight biopsies from 20 Mexican children with the diagnosis of ESVP, from which tissue blocks were available for EBV *in situ hybridization* (ISH), immunohistochemical and molecular analyses were included in the study. Cases were collected from the files of the Department of Pathology, Instituto Nacional de Pediatria, Mexico City (17 cases) in the time period from 1976 to 2009, and from the Department of Pathology, Instituto Nacional de Ciencias Medicas y de la Nutricion Salvador Zubiran (3 cases). Some of the cases have been previously reported but the biopsies were completely and independently analyzed in the current series. Clinical information and follow-up were obtained from patient records or treating physicians. The study was conducted in accordance with the Declaration of Helsinki. The study was approved by the ethics committee from the National Institute of Pediatria in Mexico. The cases belong to a local disease registry.

*Immunohistochemistry and EBV in-situ hybridisation (ISH)*

The morphological and immunohistochemical features were analyzed on formalin-fixed and paraffin-embedded tissue sections. Immunohistochemical stains were performed using an automated immunostainer (Ventana Medical Systems, Tucson, AZ, USA) according to the company’s protocol. The following panel of antibodies was used: CD3 (clone SP7; DCS, Hamburg, Germany; dilution 1:100), CD4 (clone SP35; Zytomed, Berlin, Germany), CD8 (clone C8/144B; Dako, Glostrup, Denmark), CD30 (clone Ber-H2; Dako), CD56 (clone 123C3; Dako), LMP-1 (clone CS 1-4; Dako), T-cell intracellular antigen 1 (TIA-1) (clone 4i389; Zytomed), Beta F1 (Thermo Scientific, Fremont, CA, USA), TCRgamma (Thermo Scientific) and Epstein-Barr virus nuclear antigen 2 (EBNA-2) antibody (clone PE2; Novocastra, Berlin, Germany).

All cases were subjected to *ISH* using oligonucleotides complementary to Epstein-Barr early RNA (EBER) transcripts in paraffin embedded tissue in and automated
stainer (Ventana Medical Systems). Double staining for EBER ISH and immunohistochemistry were performed following the above-described methods. ISH was performed first without counterstain, followed by immunohistochemistry in an automated immunostainer.\textsuperscript{25}

\textit{Polymerase Chain Reaction (PCR) Analyses of TCRγ and δ gene rearrangements}

T-cell clonality was analyzed by PCR amplification of the T-cell receptor (TCR) gamma chain genes with 2 distinct protocols according to McCarthy\textsuperscript{26} and Trainor\textsuperscript{27} with some modifications. DNA used for PCR was extracted from 10µm paraffin sections after dewaxing and proteinase K digestion applying standard phenol/chloroform purification procedures. Two different PCRs of TCRγ rearrangements were performed in duplicates (30 and 100 ng DNA) using Phusion Hot Start DNA Polymerase with Phusion GC Buffer containing MgCl\textsubscript{2} and 30ng and 100ng of DNA respectively. Selected samples were additionally analyzed in duplicate for TCRδ rearrangements using the commercially available BIOMED-2 assay\textsuperscript{28} (InVivoScribe Technologies Inc., San Diego, CA, USA) according to the manufacturer’s instructions. 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).

\textbf{RESULTS}

\textit{Clinical findings:}

The clinical features of the 20 patients are summarized in Table 1. The group consisted of 14 males and six females (ratio 2.3:1) with a median age at the time of diagnosis of eight years (range 1 – 15 years). The mean duration of disease at the time of consultation was 2.4 years (1 – 5 years). All patients presented with a dermatosis characterized by vesicles, blisters, erythema, ulcerations, crusts, and vacciniforme scars involving mainly the face and ear lobes (Figure 1A-B). Facial and hand edema was a prominent feature. Patients often presented with severe, deep skin lesions with disfiguring scars in both covered and sun-exposed areas (Figure 1C-D). Light avoidance did not prevent the development of skin lesions. None of the patients had light hypersensitivity. Three patients were clinically diagnosed either as cutaneous lymphoma of childhood, bullous urticaria or psoriasiform dermatitis. Ten
patients (50%) presented with disseminated dermatosis and systemic symptoms such as fever, wasting, lymphadenopathy and hepatosplenomegaly. These symptoms were often observed in patients with severe cutaneous lesions. Ten patients (50%) presented only with skin lesions without systemic symptoms. In four patients HMB, was documented. For 13 patients follow-up is available with a median of 3 years (range 1 month -13 years). However, five of these patients were lost during the follow-up (1 – 24 months), mostly after treatment with good response (Cases 6, 10, 12, 13 and 14). Three patients (Cases 1, 4 and 15) with long follow-up (9-13 years) are alive with waxing and waning disease. Four patients died, two after developing systemic lymphoma (2 – 4 years), including a sinonasal NK/T-cell lymphoma, and a peripheral T-cell lymphoma, not otherwise specified (PTCL, nos). One patient died secondary to hepatic failure (Case 9) without active skin lesions. No autopsy was performed. One patient died from complications of chemotherapy (Case 20). Seven patients were lost to follow-up after the skin biopsy was taken and a diagnosis was rendered.

**Histological and immunophenotypical features:**
Twenty-eight biopsies from 20 patients were analyzed. All biopsies showed similar histological findings, characterized by a lymphoid infiltrate predominantly in the dermis that extended sometimes deep into the subcutaneous tissue (Figure 2A). The infiltrate was mainly located around adnexae and blood vessels, often with angiodestructive features. The intensity of the infiltrate and atypia of the lymphocytes varied from case to case. There were biopsies with relative few reactive-appearing lymphocytes (Figure 2B) and biopsies with dense infiltrate (Figure 2C), and obvious cytological atypia characterized by large cells with irregular nuclei, prominent nucleoli and abundant clear cytoplasm. In 11 of the 20 biopsies an intraepidermal spongiotic vesicle was observed without epidermotropism (Figure 2D).

The immunohistochemical findings are summarized in Table 2. In all 28 biopsies the infiltrate was predominantly CD3+ with very few scattered CD20+ cells. The cytotoxic marker TIA1 was positive in all biopsies analyzed. In 11 of 20 patients (55%), the initial biopsy showed a proliferation of cytotoxic TCR αβ T-cells with expression of CD8+, Beta F1+, TIA1 and negativity for CD4 and CD56 (Figure 2E-H). Two cases (10%) (Cases 6 and 13) were double negative for CD4/CD8 and Beta F1 but positive for TCRγ indicative of a γδ T-cell derivation (Figure 3A-G). One of these cases was
focally positive for CD56. One case (Case 10) was TCR silent. In five cases the CD3+TIA1+ cells were double negative for CD4/CD8, BetaF1 negative and homogeneously strong CD56+ indicative of a NK-cell phenotype (Figure 4 A-E). The lymphoid infiltrate in the cases with a NK-cell phenotype tended to involve the subcutaneous tissue with rimming of the neoplastic cells surrounding individual fat cells mimicking subcutaneous panniculitis-like T-cell lymphoma (SPTCL) (Figure 4B). Three of the NK-cell cases revealed numerous eosinophils scattered within the lymphoid infiltrate, all had history of HMB (Figure 4F). In case 15 three biopsies were available. At diagnosis the lymphoid infiltrate was composed of cytologically bland cells (Figure 4 I); however, the atypia of the infiltrating cells increased in the follow-up biopsies one and seven years after the original diagnosis (Figure 4J-K). In Case 20 the infiltrating lymphocytes showed expression of CD8, Beta F1 and CD56 (Supplemental Figure 1). The lymphoid infiltrate was intermingled with abundant eosinophils. Eleven cases (55%) showed a variable number of CD30+ cells (Figure 4G). Few scattered CD4+ reactive lymphocytes were observed in all cases analyzed. LMP1 was positive only in three cases (Figure 4H), whereas EBNA-2 remained negative in all cases analyzed. CD30 expression was more frequently found in biopsies with a NK-cell phenotype (6 of 7; 86%) than in biopsies with a T-cell phenotype (6 of 13; 46%).

In situ hybridization and molecular analysis:
The results of EBER ISH and TCRγ and δ clonality analyses are summarized in Table 2. ISH for EBV using EBER1 probe showed numerous EBER+ cells in all cases; however, in most cases the amount of EBER+ cells represented only a subpopulation of the CD3+ infiltrating cells (Figure 2J). The amount of EBER+ cells was similar in cases with T- and NK-cell phenotype. EBER+ cells concentrated mainly around the blood vessels and adnexae in the dermis (Figures 2-3), in the subcutaneous tissue (Figure 4C), and in the basal epithelial layer in cases with intraepidermal vesicles. In Case 20, due to the expression of CD8 and CD56 in the lymphoid infiltrate, double stainings with EBER and CD8 or CD56 were performed. EBER+ cells were predominantly CD56+, whereas few cells were CD8+ suggesting a NK-cell derivation of the infiltrating cells (Supplemental Figure 1). However, molecular analysis revealed a monoclonal rearrangement of the TCRγ genes favouring a T-cell derivation. Due to the latter finding the case was classified as most probably of T-cell phenotype,
although the presence of two different EBV infected populations cannot be excluded. Case 8 had two biopsies, the first biopsy showed a predominantly CD8+/EBER+ dermal infiltrate; however, the second biopsy taken three years later showed a more panniculitic infiltrate with predominance of CD56+/EBER+ cells with very few CD8+ cells (Supplemental Figure 2). This case demonstrates that both EBV+ T- and NK-cell populations can occur in the same patient.

Molecular analysis demonstrated monoclonal rearrangement of the TCRγ genes in 13 of the 14 cases analyzed with a T-cell phenotype. In the two cases with TCRγ expression (Case 6 and 13), a monoclonal TCRδ gene rearrangement was also demonstrated further supporting the γδ T-cell derivation of these two cases. In two cases with several biopsies (Cases 4 and 8) clonality analyses showed the same monoclonal TCRγ gene rearrangement in the two biopsies analyzed taken several years apart (4 and 3 years apart, respectively), despite the subtle infiltrate found in subsequent biopsies (Figure 5 and Supplemental Figure 2). The five cases with a NK-cell phenotype (Cases 15-19) showed a polyclonal rearrangement of the TCRγ and δ genes.

**Treatment**

Due to the long study period, patients had received a variety of different treatments; however, 13 patients were primarily treated with immunomodulating or immunosuppressive therapy such as thalidomide, steroids and/or chloroquine. The skin lesions usually improved with these treatments, and even though the patients usually recurred with new skin lesions, the amount of the infiltrate and the amount of EBV+ cells, in general, decreased in follow-up biopsies (Figure 5). The three patients with the longest follow-up (9, 12 and 13 years) have been treated with a combination of thalidomide and steroids repeatedly during acute flare-ups of their disease. Two patients (Case 4 and 15), additionally had received at some point chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP) with only a transient effect. Due to side effects, both patients stopped the chemotherapy and continued their treatment with thalidomide and steroids with relatively good response. In Case 8 due to an initial diagnosis of vasculitis, the patient was treated with cyclophosphamide and prednisone with good results after 3 years of follow-up. Two patients (Cases 11 and 16) developed systemic lymphoma under treatment with thalidomide, both died of disease. Patient 20 was treated with CHOP because of the
diagnosis of HVLL. He had a two year history of skin lesions before receiving chemotherapy. The patient died while receiving chemotherapy of infectious complications.

**Differences between patients with a T-cell and NK-cell phenotype**

We compared the clinical features between cases with a T-cell vs NK-cell phenotype. The male to female ratio was similar in both groups (T-cell: 2.5:1 vs NK-cell: 2:1). The median age at consultation tended to be younger in patients with a NK-cell phenotype (8 vs 10 years). The skin lesions in both groups were very similar; however, patients with a T-cell phenotype had more often systemic symptoms at presentation (57% vs 33%). HMB was documented in three patients with a NK-cell phenotype and in one patient with a T-cell phenotype and expression of CD56 (Case 20). Progression to NK/T-cell lymphoma manifested in the nasal region occurred in one of three patients with NK-cell phenotype for whom follow-up was available, whereas only one of six patients with a T-cell phenotype developed a PTCL, nos.

**Discussion:**

HVLL is a rare LPD originally described in Mexican children under the name of ESVP. In the first series, it was described as a benign but clinically severe systemic disease with malignant potential that was not related to classic HV. Because subsequent studies demonstrated that these lesions often showed monoclonal rearrangements of the TCR genes, the term HVLL was proposed. The 2008 WHO classification recognized the increasing awareness of EBV associated monoclonal disorders of T-cell or NK-cell origin in children and young adults, and incorporated for the first time the group of EBV positive T-cell LPD of childhood into the lymphoma classification. In this study, we investigated the clinico-pathological features of 20 Mexican children diagnosed as HVLL. The severity of the skin lesions and the clinical presentation varied among the patients and showed a broad spectrum. In contrast to classic HV, the lesions were larger and deeper and produced in some cases extensive tissue loss and disfigurement and were not associated with light hypersensitivity. Systemic symptoms like fever, lymphadenopathy and/or hepatosplenomegaly were common especially in patients with severe cutaneous lesions and a T-cell phenotype. Central to the disease was the relatively long clinical course before patients sought medical attention (range 1 – 5 years), underlining the
chronic nature of the disorder. We now demonstrate that the infiltrating EBV+
lymphocytes can consist of T-cells either of αβ or γδ derivation or NK-cells.
Occasionally, coexistence of more than one phenotype of EBV+ infected cells can be
observed. Regardless of the severity of the skin lesions and the presence or absence
of systemic symptoms, cases with a T-cell phenotype constantly showed monoclonal
rearrangement of the TCRγ genes. Furthermore, in two cases with long follow-up and
several biopsies, the same T-cell clone was demonstrated several years apart
indicating that monoclonality and clonal persistence is not predictive of an aggressive
disease or of a progressive clinical course. Accordingly, in a recent report from
Japan, Kimura et al24 reported four cases of “classic” HV defined as patients with a
characteristic dermatosis without systemic symptoms or cellular atypia; nevertheless,
all four cases were reclassified as HVLL based on the monoclonality of the TCRγ
genesis. Our study, and those of others15, 29 suggest that EBV+ HV-like lesions are
often monoclonal in nature and have overlapping histological features regardless of
the presence or absence of systemic symptoms. Furthermore, no difference in the
amount of infiltrating EBER+ cells has been found between these disorders.15
Interestingly, recently it has been reported that HV and/or HVLL are associated with
increased numbers of EBV infected γδ T-cells in peripheral blood.24, 30-31 Kimura et
al,24 demonstrated that the EBV-infected cells in peripheral blood in almost half of
HVLL patients (6 of 13 cases) were γδ T-cells. Nevertheless, in none of these studies
the phenotype of the skin infiltrating cells was described. In our study, the vast
majority of cases with a T-cell phenotype (12 of 15 cases) showed a αβ phenotype,
whereas only a minority (2 of 15) were of γδ derivation. It is not clear whether the
difference in the prevalence of EBV+ γδ T-cells between the Japanese population
and ours is related to racial differences or only reflect discrepancies between the
EBV-infected cells in the peripheral blood and the cells infiltrating the skin.
Another interesting aspect of this study is that 30% of all HVLL cases revealed a NK-
cell phenotype, similar to the Peruvian series32 and the 38% recently reported by the
Japanese group24 indicating that a third of all HVLL are of NK-cell phenotype, which
is more than previously appreciated. Patients with NK-cell phenotype tended to have
more “panniculitic” lesions with increased amounts of infiltrating eosinophils, often
associated with HMB. Morphologically, these lesions can mimic SPTCL, primary
cutaneous γδ T-cell lymphoma or cutaneous involvement by an extranodal NK/T-cell
lymphoma, nasal type. Without clinical information, the differential diagnoses with the
latter might be impossible to resolve because the morphology and phenotype of the EBV+ infiltrating cells are indistinguishable. Our study confirms previous results indicating that HMB is usually associated with a proliferation of EBV infected NK-cells. Interestingly, patients with NK-cell phenotype in this study rarely presented with systemic symptoms despite of the sometimes alarming histology. Accordingly, previous studies have shown that patients with NK-cell phenotype show a relatively indolent clinical course when compared with patients with a T-cell phenotype. Nevertheless, patients with a NK-cell phenotype seem to have a higher risk to develop a systemic lymphoma such as aggressive NK-cell leukemia or extranodal NK-cell lymphoma, nasal type on the long clinical course. The severity of the clinical presentation has been proposed to prognosticate which cases will eventually progress to a systemic disease. In the series of Iwatsuki et al 5 of 11 patients diagnosed as severe HV developed NK/T-cell lymphoma 2 – 14 years after the onset of the disease. Of note, all cases were associated with NK-cell lymphocytosis, HMB and/or hemophagocytosis. Two of these cases were classified as “subcutaneous lymphomas” without further specifications, which raises the possibility that these lesions represented further manifestations of the disease and not a progression to a systemic lymphoma. In this study, only two patients, one with a T-cell phenotype and one with a NK-phenotype developed a systemic lymphoma relatively soon after the initial diagnosis (2 and 4 years, respectively). However, long clinical follow-up was available only in three patients, two with a T-cell phenotype and one with a NK-cell phenotype.

Although HVLL is characterized by a monoclonal proliferation of T- or NK-cells, the best approach for treatment remains uncertain. Chemotherapy and/or radiotherapy have been used in many patients, but have been shown to be of little or no benefit. The effect is usually transient and does not induce sustained remission in most cases. Furthermore, patients receiving chemotherapy have been reported to have a worse prognosis with short survival. In the series of Barrionuevo et al, five of eight patients that received chemotherapy died mainly of sepsis and liver failure with only slight improvement of the skin lesions. Similar results were reported by Rodriguez-Pinilla et al, where eight of 11 patients that received chemotherapy and/or radiotherapy died, five or these secondary to infectious complications. Accordingly, in this study one patient died under chemotherapy of infectious complications. In contrast, immunomodulating therapies such as prednisolone, cyclosporine A
interferon $\alpha$, chloroquine and thalidomide have been shown to result in temporary remissions or improvement of the symptoms. These results indicate that a conservative approach should be recommended as first-line therapy in these patients.

In conclusion, HVLL is considered an EBV+ cutaneous T-cell lymphoma based solely on the demonstration of a monoclonal T-cell proliferation; however, the long, waxing and waning clinical course and the relatively good response to immunomodulating therapy challenges the concept of a full-blown malignant lymphoma at onset. Criteria like presence of systemic symptoms, T-cell clonality, amount of EBV+ cells and/or density of the infiltrate do not help in predicting which patients will eventually progress into a systemic disease. Our data support the concept that EBV+ HV-like lesions represent different clinical severities of the same disease within the spectrum of EBV-associated cutaneous LPD. In order to avoid unnecessarily aggressive treatment, and the stigma of a lymphoma diagnosis, the term HV-like EBV+ LPD to encompass the different clinical manifestations of the EBV associated HV-like cutaneous lesions both of T-cell and NK-cell origin would be preferable for clinical usage. The challenge remains to identify morphological or clinical markers to predict which patients are at risk to progress into a systemic lymphoma.

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Authorship contributions:
L.Q-M. and F.F. design the study, analyzed data and wrote the manuscript. C.R. provided samples and help writing the manuscript. F.N, G.A and P.G. performed the pathology work. I.B. Performed and analyzed the molecular analysis. MS-O., C.D-M., R. R-M., and C. L-M., provided samples and clinical data.

Disclosure/conflict of interest
The authors declare no conflict of interest
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HVLL: Hydroa vacciniforme-like lymphoma; ESVP: edematous, scarring vasculitic panniculitis; HMB: hypersensitivity to mosquito bites; IFN: interferon; AwD: alive with disease; DoD: died of disease; LFU: Lost to follow-up; DwD: died without disease § died of hepatic failure. No active skin lesions. FTTH: Failure to thrive. HS: Hepatosplenomegaly
Table 2. Immunophenotype and molecular analysis of 20 cases of Hydroa vacciniforme-like lymphoma

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*History of HMB; n.d.: not done; # Only HE stain available. & nasal mucosa biopsy; § positive for TCR gamma by immunohistochemistry. +/- a minority of cells; +/- many cells; +: most cells

αβ?: Most probably αβ lineage
Legends:

Figure 1: Skin lesions in Hydroa vacciniforme-like lymphoma. (A-B) Multiple skin lesions in face and ear lobes characterized by vesicles, erythema and crusts. (C-D) The lesions produce in some cases extensive tissue loss and disfigurement (C) and/or vacciniforme scars (D) in sun exposed areas.

Figure 2: Hydroa-vacciniforme-like lymphoma morphology and immunophenotype. (A-B): Case 1. Skin biopsy showing an infiltrate mainly in the dermis that extends into the subcutaneous fat. Note that the infiltrate surrounds adnexae (H&E stain, original magnification 25x). (B) Higher magnification shows a small lymphoid infiltrate without atypia surrounding a blood vessel (H&E stain, original magnification 400x) (C): Case 15. Skin biopsy showing a dense infiltrate with atypical medium to large lymphoid cells with rather abundant cytoplasm. (H&E stain, original magnification 400x). Insert: The atypical cells have irregular nuclei with large eosinophilic nucleoli (H&E stain, original magnification 630x). (D): Case 11. Skin biopsy with intra-epidermal bullae and a dense infiltrate in the upper dermis surrounding adnexae and blood vessels. (H&E stain, original magnification 100x). Immunohistochemical analysis in a case with a $\alpha\beta$ T-cell phenotype (Case 9). (E) CD3 stain shows that the cells surrounding the adnexa are strongly CD3 positive. Additionally, the infiltrating cells are TIA1 positive (F), CD8 positive (G), and negative for CD4 (H) (E-H immunoperoxidase, original magnification 200x). (I) CD30 staining reveals many positive cells (immunoperoxidase, original magnification 400x). (J) EBER in situ hybridization is positive in the infiltrating lymphocytes. Note that the number of EBER positive cells is less than those positive for CD3 (original magnification 200x).

Figure 3: Immunohistochemical analysis, EBER in situ hybridization and molecular analysis in a case with $\gamma\delta$ T-cell phenotype (Case 6). (A) Skin biopsy showing a dense lymphoid infiltrate in the dermis surrounding blood vessels and adnexae (H&E: original magnification 100x). (B): EBER in situ hybridization reveals that practically all lymphoid cells are EBER positive (original magnification 100x). (C-G) The cells are CD3 (C) and TIA1 positive (D) but negative for CD8 (E) and Beta F1 (F). Note that the lymphoid cells are TCRgamma positive (G) (C-G:
immunoperoxidase, original magnification 400x) (Insert F-G: immunoperoxidase, original magnification 630x) (H) Higher magnification of the EBER in situ hybridization (original magnification 400x). (I): TCRδ gene rearrangement shows a dominant monoclonal peak of 220 base pairs.

Figure 4. Immunohistochemical analysis and EBER in situ hybridization in a case with NK-cell phenotype (Case 15). (A) H&E stain of a skin biopsy with a dense infiltrate extending from the upper dermis deep into the subcutaneous tissue (original magnification 12.5x). (B-E) Higher magnification shows atypical lymphoid cells with rimming of individual fat cells mimicking subcutaneous panniculitis-like T-cell lymphoma (B). The cells are EBER positive (C), strongly and homogeneously CD56 positive (D) and TIA1 positive (E). (B-E: original magnification 400x). (F) Higher magnification of the dermis reveals angioinvasion with abundant eosinophils (original magnification 400x). (G) CD30 stain shows many positive cells. (H) LMP1 is positive only in few scattered cells (G-H: immunohistochemistry, original magnification 400x). (I-K) Comparative morphology of three skin biopsies in case 15. (I) Skin biopsy at diagnosis shows a lymphoid infiltrate of rather small lymphocytes without atypia. (J) Skin biopsy one year after diagnosis shows a lymphoid infiltrate composed of medium-sized cells with abundant clear cytoplasm. (K) Skin biopsy seven years after the original diagnosis with rather atypical large cells, abundant clear cytoplasm and prominent nucleoli (I-K: H&E stain, original magnification 400x).

Figure 5. Morphology and comparative TCRγ clonality analysis in Case 4. (A-B) H&E stain in two recurrent skin biopsies taken 4 years apart at the age of 20 and 24 years old. Note that the first biopsy (A) shows a denser lymphoid infiltrate when compared to the second biopsy (B) (A-B: original magnification 100x). (C) Higher magnification of the second biopsy reveals a discrete infiltrate surrounding blood vessels with bland morphology. (D) Some of the lymphoid cells are EBER positive (C-D: original magnification 400x). (E-F) Amplification of the CD3 region of the TCR gamma chain gene showed an identical monoclonal peak of 195 base pairs in both biopsies.
Figure 1
Figure 2
Figure 4
Figure 5
Hydroa vacciniforme-like lymphoma: a chronic EBV+ lymphoproliferative disorder with risk to develop a systemic lymphoma

Leticia Quintanilla-Martinez, Cecilia Ridaura, Florian Nagl, Marimar Sáez-de-Ocariz, Carola Durán-McKinster, Ramon Ruiz-Maldonado, Georgia Alderete, Peter Grube, Carmen Lome-Maldonado, Irina Bonzheim and Falko Fend