A distinct subtype of Epstein-Barr virus-positive T/NK-cell lymphoproliferative disorder: adult patients with chronic active Epstein-Barr virus infection-like features

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ABSTRACT

The characteristics of adult patients with chronic active Epstein-Barr virus infection are poorly recognized, hindering early diagnosis and an improved prognosis. We studied 54 patients with adult-onset chronic active Epstein-Barr virus infection diagnosed between 2005 and 2015. Adult onset was defined as an estimated age of onset of 15 years or older. To characterize the clinical features of these adults, we compared them to those of 75 pediatric cases (estimated age of onset <15 years). We compared the prognosis of adult-onset chronic active Epstein-Barr virus infection with that of patients with nasal-type (n=37) and non-nasal-type (n=45) extranodal NK/T-cell lymphoma. The median estimated age of onset of these lymphomas was 39 years (range, 16–86 years). Compared to patients with pediatric-onset disease, those in whom the chronic active Epstein-Barr virus infection developed in adulthood had a significantly decreased incidence of fever (P=0.005), but greater frequency of skin lesions (P<0.001). Moreover, hypersensitivity to mosquito bites and the occurrence of hydroa vacciniforme were less frequent in patients with adult-onset disease (P<0.001 and P=0.0238, respectively). Thrombocytopenia, high Epstein-Barr virus nuclear antigen antibody titer, and the presence of hemophagocytic syndrome were associated with a poor prognosis (P=0.0087, P=0.0236, and P=0.0149, respectively). Allogeneic hematopoietic stem cell transplantation may improve survival (P=0.0289). Compared to pediatric-onset chronic active Epstein-Barr virus infection and extranodal NK/T-cell lymphoma,

adult-onset chronic active Epstein-Barr virus infection had a poorer prognosis (P<0.001 and P=0.0484, respectively). Chronic active Epstein-Barr virus infection can develop in a wide age range, with clinical differences between adult-onset and pediatric-onset disease. Adult-onset chronic active Epstein-Barr virus infection is a disease with a poor prognosis. Further research will be needed.

Introduction

Epstein-Barr virus (EBV) generally infects almost all people by early adulthood. Although EBV infection in childhood is asymptomatic in most people, some develop infectious mononucleosis (the so-called "kissing disease"). Almost all of them recover spontaneously after EBV-specific immunity is established.[1]

In addition, EBV has been reported to be involved in transformation from reactive to neoplastic or abnormal proliferation in some immunocompetent hosts after it infects B cells, T cells, and natural killer (NK) cells. This results in a wide range of conditions known as EBV-associated lymphoproliferative diseases (EBV-LPD),[1,2] including chronic active Epstein-Barr virus infection (CAEBV), which produces infectious mononucleosis-like symptoms such as chronic persistent or recurrent fever, lymphadenopathy, skin rash, liver dysfunction, and hepatosplenomegaly, and has been reported to have a high mortality rate.[3-5] In CAEBV, EBV-infected T cells and NK cells play important roles. Hypersensitivity to mosquito bites, the presence of hydroa vacciniforme (HV), high EBV-related antibody titers, and a high EBV-deoxyribonucleic acid (DNA) copy number in peripheral blood have been reported to be characteristic of CAEBV; however, there is often no discernable immunological abnormality, or history thereof until the patient has been diagnosed, at which point symptoms have rapidly progressed.[6-8] Although the mechanism of onset is still unknown, CAEBV is thought to progress to an EBV-associated T/NK-cell lymphoproliferative disorder by clonal expansion of T cells or NK cells infected with EBV.[9] In addition, CAEBV can have potentially fatal complications such as hemophagocytic syndrome, interstitial pneumonia, malignant lymphoma, myocarditis, and central nervous system infiltration.[4,7,10,11] This entity is currently defined as systemic EBV-positive T-LPD of childhood because almost all diagnoses are made in pediatric patients.[12,13] However, similar clinical conditions have also been reported in adults (EBV-T/NK-LPD with CAEBV-like features in adult; adult-onset CAEBV).[14,15]

Most pediatric patients with EBV-T/NK-LPD show symptoms of CAEBV, although lymphomas are rarely observed.[16] Conversely, adult-onset EBV-T/NK-LPD shows very diverse clinical manifestations, such as extranodal NK/T-cell lymphoma (ENKTL) and aggressive NK-cell leukemia (ANKL), which both follow a relatively rapid clinical course. CAEBV, however, has a chronic clinical course. In cases in which there is clonal expansion due to CAEBV, it may be difficult to distinguish it pathologically from ENKTL without detailed clinical information. A thorough medical interview and careful examination by physicians are, therefore, very important for the diagnosis of adult-onset CAEBV. However, because of its rarity, the clinical features of adult-onset CAEBV have been poorly detailed, and there is no consensus as to whether there are clinical differences between adult-onset and pediatric-onset CAEBV.

The purpose of this study was to characterize the clinicopathological features of EBV-T/NK-LPD with CAEBV-like features in adults (adult-onset CAEBV) by comparing the features to those of patients with pediatric-onset CAEBV and ENKTL. In addition, we compared the prognosis of adult patients with CAEBV to that of patients with ENKTL.

Methods

Patients

We enrolled 54 patients who were diagnosed with adult-onset CAEBV at the Department of Pathology, Kurume University between January 2005 and December 2015. Patients with adult-onset CAEBV were defined as those whose estimated age at onset was 15 years or older, and who met the criteria for the diagnosis of systemic EBV-T-LPD of childhood according to the 2008 and 2016 World Health Organization (WHO) classifications of lymphoid neoplasms.[12] Consequently, patients with pediatric-onset CAEBV were defined as those with an estimated age at onset of less than 15 years. All patients with CAEBV satisfied the following diagnostic criteria, based on the previous report by Kimura *et al.*[17] (with the exception of EBV-DNA viral load, which was measured in plasma in this study as previously reported[18]): (i) sustained or recurrent infectious mononucleosis-like symptoms lasting more than three months: fever (≥38.3°C or ≥101°F), liver dysfunction (elevated liver enzymes), lymphadenopathy, hepatosplenomegaly, cytopenia, interstitial pneumonia, hydroa vacciniforme, and hypersensitivity to mosquito bites; (ii) increased quantities of EBV in affected tissues [i.e. detection of EBV-DNA in tissues or peripheral blood by Southern blot hybridization, or EB-encoded small RNA 1 (EBER)-positive cells detected in affected tissues by microscopy (≥10 cells/high power field)], or in peripheral blood [i.e. EBV-DNA detected in plasma (≥2×10^2 copies/mL in plasma)]; and (iii) no evidence of any previous immunological abnormalities or any other infections that could otherwise explain the condition. We confirmed negativity for human immunodeficiency virus antibody and human T-cell lymphoma virus 1 antibody in all patients. Hemophagocytic syndrome was diagnosed according to the HLH 2004 guidelines;[19] all patients with hemophagocytic syndrome met the study criteria.

This study was carried out in accordance with the recommendations of the Declaration of Helsinki and was approved by the ethics review committee of Kurume University (approval number: 291).

The methodological details regarding determination of the EBV-DNA viral load in peripheral blood,[18] Southern blot hybridization,[8,20] T-cell receptor gamma gene rearrangement,[21,22] *in situ* hybridization for EBER,[21] histology and immunophenotyping,[9] determination of EBV-infected cell type,[6,22] and statistical analysis[23] were based on previous reports, as described in the *Online Supplementary Appendix*.

Epstein-Barr virus-DNA viral load in peripheral blood

A peripheral blood sample was obtained from each patient at diagnosis, in order to investigate the viral load by real-time quantitative polymerase chain reaction. A positive result was defined as an EBV-DNA viral load of ≥200 copies/mL, as previously reported.[18]

Comparison of clinical features with pediatric-onset chronic active Epstein-Barr virus infection and extra-nodal NK/T-cell lymphoma

To compare the clinical symptoms and prognosis of adult-, and pediatric-onset CAEBV, data from 75 patients younger than 15 years who developed CAEBV were extracted from the previously

Table 1. Characteristics of adult-onset chronic active Epstein-Barr virus infection patients.

Patients' characteristics	All patients (n = 54)	Infected-cell type T-cell (n = 22)	NK-cell (n = 32)	$P^{\S}$
Sex				
Male, n (%)/female, n (%)	31 (57.4)/23 (42.6)	12 (54.5)/10 (45.5)	19 (59.4)/13 (40.6)	0.784
Median age, years (range)	39 (16-86)	37 (19-86)	41 (16-78)	0.672
≥ 50 years, n (%)	22 (40.7)	10 (0.455)	12 (0.545)	0.585
Symptoms and involvement sites				
Fever, n (%)	35 (64.8)	15 (68.2)	20 (62.5)	0.775
Bone marrow, n (%)	31 (57.4)	13 (59.1)	18 (56.3)	0.783
Splenomegaly, n (%)	28 (51.9)	13 (59.1)	15 (46.9)	0.418
Hepatomegaly, n (%)	22 (40.7)	12 (54.5)	10 (31.3)	0.101
Lymphadenopathy, n (%)	21 (38.9)	13 (59.1)	8 (25.0)	0.0202*
Skin rash, n (%)	21 (38.9)	4 (18.2)	17 (53.1)	0.0119*
Abdominal disturbance, n (%)	11 (20.4)	5 (22.7)	6 (18.8)	0.743
Laryngopharynx, n (%)	11 (20.4)	4 (18.2)	7 (21.9)	1
Lung, n (%)	8 (14.8)	2 (9.1)	6 (18.8)	0.449
Gastrointestinal tract, n (%)	5 (9.3)	2 (9.1)	3 (9.4)	1
Oral lesion, n (%)	2 (3.7)	0 (0)	2 (6.3)	0.508
Central nervous system, n (%)	1 (1.9)	1 (4.5)	0 (0)	0.407
Myocarditis, n (%)	1 (1.9)	0 (0)	1 (3.1)	1
Past medical history				
Hypersensitivity to mosquito bites, n (%)	4 (7.4)	1 (4.5)	3 (9.4)	0.638
Hydroa vacciniforme, n (%)	2 (3.7)	0 (0)	2 (6.3)	0.508
ECOG PS high (2-4), n (%)	17 (31.5)	10 (45.5)	7 (21.9)	0.0814
Laboratory test at initial diagnosis				
Anemia (Hb <10.5 g/dL), n (%)	15 (27.8)	7 (31.8)	8 (25.0)	0.758
Thrombocytopenia (< 100×10^9/L), n (%)	26 (48.1)	13 (59.1)	13 (40.6)	0.268
LDH elevation, n (%)	40 (74.1)	16 (72.7)	24 (75.0)	1
Transaminase, elevation, n (%)	22 (40.7)	12 (54.5)	10 (31.3)	0.101
Hemophagocytic syndrome, n (%)	25 (46.3)	12 (54.5)	13 (40.6)	0.407
EBV-related antibody to				
VCA-IgG, median titer (range)	320 (10-5120)	160 (10-2560)	160 (40-5120)	na
VCA-IgM, median titer (range)	<10 (<10-60)	<10 (<10-40)	<10 (<10-60)	
EBNA, median titer (range)	40 (<10-320)	10 (<10-80)	20 (<10-320)	
Unknown, n (%)	13 (24.1)	4 (18.2)	9 (28.1)	
EBV-DNA, in plasma (copies/mL)				
Median (range)	1×10^4(nd-1×10^6)	1×10^4(2×102-1.1×10^6)	1×10^4(nd-3.7×10^5)	na
Unknown, n (%)	17 (31.5)	8 (36.4)	9 (28.1)	
EBV monoclonality by Southern blot, n (%)	25/30 (81.3)	16/17 (94.1)	9/13 (69.2)	na
EBER+cell counts /HPF, median (range)	58 (2-487)	32 (2-435)	61(3-487)	0.180
Histological classification[9]				
A1, n (%)	16 (29.6)	10 (45.5)	6 (18.7)	0.127
A2, n (%)	20 (37.0)	6 (27.2)	14 (43.8)	
A3, n (%)	18 (33.3)	6 (27.2)	12 (37.5)	
Immunophenotype				
CD4	19 (35.2)	12 (63.2)	7 (36.8)	na
CD8	20 (37.0)	11 (55.0)	9 (45.0)	
CD56	34 (61.8)	8 (23.5)	26 (76.5)	
TCRab (n = 7)	7/7 (100)	7/7 (100)	na	
Allogeneic HSCT	9 (16.7)	3 (13.6)	6 (18.8)	0.723

CAEBV: chronic active EBV infection; EBV: Eptein-Barr virus; EBNA: Epstein–Barr virus nuclear antigen 1; EBV-DNA: EBV-deoxyribonucleic acid; ECOG PS: Eastern Cooperative Oncology Group Performance Status; EBER: Epstein-Barr virus-encoded RNA; nd: not detected (EBV-DNA < 2×10^2 copies/mL); HPF: high power field; HSCT: hematopoietic stem cell transplantation; Hb: hemoglobin; LDH: lactate dehydrogenase; TCR: T-cell receptor; VCA: viral capsid antigen. *Statistically significant; na: not avalilable; $P^{\S}$: T-cell type *versus* NK-cell type.

published papers by Kimura *et al.*[17] The clinical features of all 75 patients are shown in *Online Supplementary Table S2.*

Furthermore, 82 patients, who were diagnosed with ENKTL at our institution according to the WHO classification were used for the prognostic comparison.[12] These patients' clinical features are shown in *Online Supplementary Table S3*. ENKTL cases were divided into "nasal-type" (n=35) and non-nasal-type (n=47), according to the anatomical sites of the lesions

Results

Clinical characteristics of patients with adult-onset chronic active Epstein-Barr virus infection

The clinical characteristics of all patients with CAEBV are summarized in Table 1. Detailed characteristics including estimated age at onset, EBV-related antibody titers, EBV-DNA copy number in plasma, T-cell receptor gamma rearrangement status, EBV Southern blot analysis, EBER-positive cell counts, histological classification, treatment, and outcome of the 54 patients with CAEBV are presented in *Online Supplementary Tables S5-S8*.

The median period from estimated onset to diagnosis was approximately 12-24 months (range, 3- >120 months). Figure 1 shows the periods from the estimated onset to the diagnosis of CAEBV and discontinuation of observation, which was more than one year in most cases. The median age at diagnosis was 39 years (range, 16–86 years). The number of cases by age at onset is shown in *Online Supplementary Figure S2.* The onset of CAEBV was observed at all ages. However, as the age distribution of adult-onset CAEBV appeared to be bimodal, we also investigated the clinicopathological factors of CAEBV patients diagnosed at ≥50 years of age. In EBV-infected cells, lymphadenopathy was significantly more frequent in patients with the T-cell type (P=0.0202), whereas those

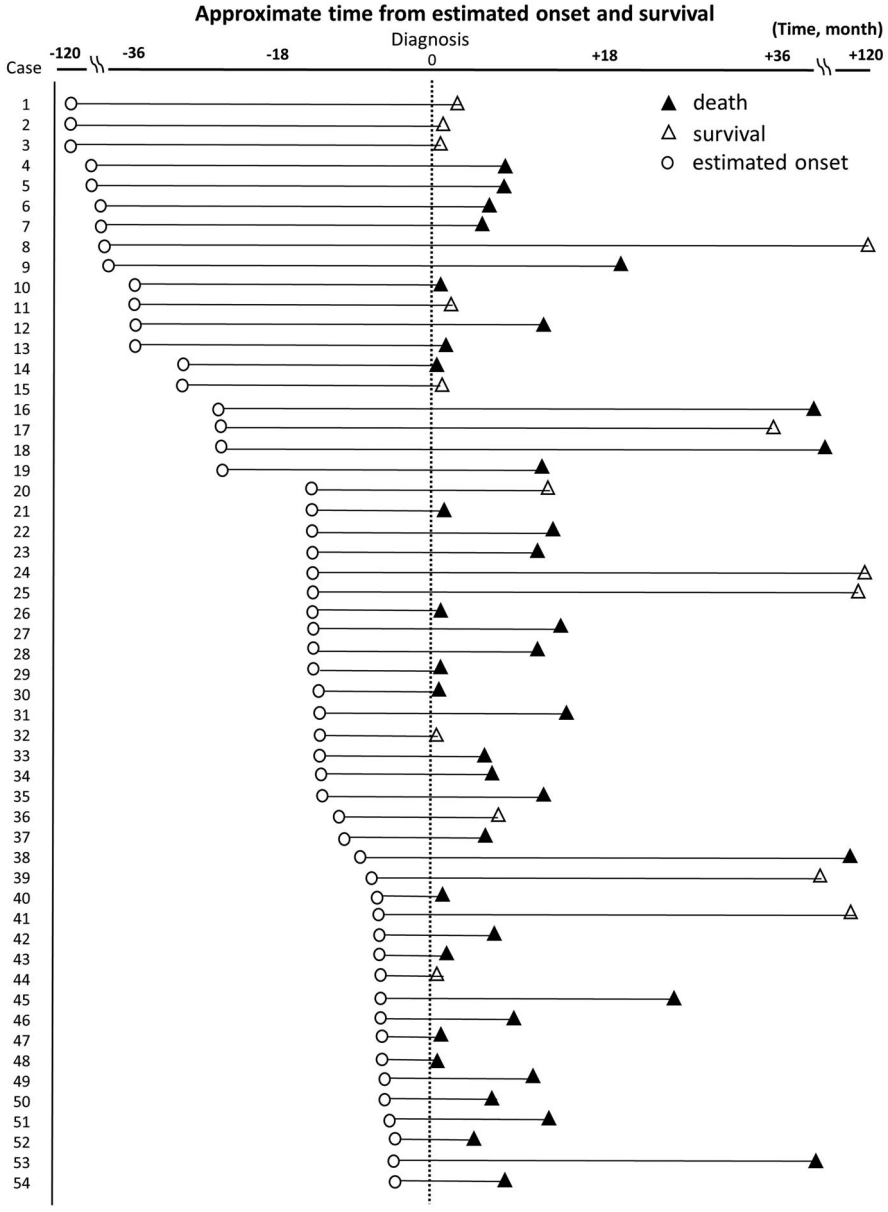


Figure 1. Approximate time from estimated onset and survival. The figure shows the periods from estimated onset to diagnosis of chronic active Epstein-Barr virus infection/discontinuation of observation. In most cases more than one year elapsed from the estimated onset to the diagnosis.

with the NK-cell type had significantly more skin lesions (P=0.0119). A comparison of the clinicopathological features of CAEBV with nodal lesions *versus* CAEBV with extranodal lesions is shown in *Online Supplementary Table S2*. There were no clinicopathological differences between adult-onset CAEBV with nodal lesions and those with extranodal lesions. In addition, the anti-viral capsid antigen (VCA)-IgM antibody tended to be low or less than the level of detection. The median count of EBER-positive cells was 53 per high power field (range, 2–487), and 86.3% (44/51) of the cases showed ten or more positive cells per high power field. Furthermore, 97.3% (36/37) of the cases had ≥10^2 EBV copies/mL, and only 2.7% (1/37) had levels below that of the level of detection (2×10^2 copies/mL).

Table 2. Characteristics of patients aged over 50 years with adult-onset chronic active Epstein-Barr virus infection.

Patients' characteristics	All patients (n = 22)	Infected-cell type T-cell (n = 10)	NK-cell (n = 12)	P^{s}
Sex				
Male, n (%)/female, n (%)	12 (54.5)/10 (45.5)	5 (50.0)/5 (50.0)	7 (58.3)/5 (41.7)	1
Symptoms and involved sites				
Fever, n (%)	9 (40.9)	5 (55.6)	4 (44.4)	0.666
Bone marrow, n (%)	8 (36.4)	4 (50.0)	4 (50.0)	1
Splenomegaly, n (%)	7 (31.8)	4 (57.1)	3 (42.9)	0.652
Hepatomegaly, n (%)	5 (22.7)	4 (80.0)	1 (20.0)	0.135
Lymphadenopathy, n (%)	10 (45.5)	7 (70.0)	3 (30.0)	0.0212
Skin rash, n (%)	11 (50.0)	2 (18.2)	9 (81.8)	0.03
Abdominal disturbance, n (%)	3 (13.6)	1 (33.3)	2 (66.7)	1
Laryngopharynx, n (%)	5 (22.7)	2 (40.0)	3 (60.0)	1
Lung, n (%)	0 (0)	0 (0)	0 (0)	na
Gastrointestinal tract, n (%)	0 (0)	0 (0)	0 (0)	na
Oral lesion, n (%)	0 (0)	0 (0)	0 (0)	na
Central nervous system, n (%)	0 (0)	0 (0)	0 (0)	na
Myocarditis, n (%)	0 (0)	0 (0)	0 (0)	na
Past medical history				
Hypersensitivity to mosquito bites, n (%)	2 (9.1)	0 (0)	2 (100)	0.481
Hydroa vacciniforme, n (%)	1 (3.7)	0 (0)	1 (100)	1
ECOG PS high (2-4), n (%)	6 (27.3)	4 (66.7)	2 (33.3)	0.348
Laboratory test at initial diagnosis				
Anemia (Hb <10.5 g/dL), n (%)	6 (27.3)	3 (50.0)	3 (50.0)	1
Thrombocytopenia (< 100×10^9/L), n (%)	16 (72.7)	5 (31.3)	11 (68.7)	0.0557
LDH elevation, n (%)	13 (74.1)	6 (72.7)	7 (75.0)	1
Transaminase, elevation, n (%)	4 (18.2)	3 (75.0)	1 (25.0)	0.293
Hemophagocytic syndrome, n (%)	5 (22.7)	4 (80.0)	1 (20.0)	0.135
EBV-related antibody to:				
VCA-IgG, median titer (range)	160 (80-5120)	160 (10-1280)	160 (80-5120)	na
VCA-IgM, median titer (range)	<10 (<10-10)	<10 (<10-10)	<10 (<10-10)	
EBNA, median titer (range)	40 (<10-80)	10 (<10-40)	40 (<10-80)	
Unknown, n (%)	9 (40.9)	3 (33.3)	6 (66.7)	
EBV-DNA, in plasma (copy/mL)				
Median (range)	1×10^4(nd-1×10^6)	2×10^5(5×10^4-1×10^6)	1.1×10^3(nd-7.3×10^4)	na
Unknown, n (%)	13 (59.1)	7 (53.8)	6 (46.2)	
EBV monoclonality by Southern blot, n (%)	8/9 (88.9)	3/4 (75.0)	5/5 (100)	na
EBER+cell counts /HPF, median (range)	87 (2-487)	58 (2-435)	168 (15-487)	0.308
Histological classification[9]				
A1, n (%)	5 (22.7)	3 (60.0)	2 (40.0)	0.864
A2, n (%)	7 (31.8)	3 (42.9)	4 (57.1)	
A3, n (%)	10 (45.5)	4 (40.0)	6 (60.0)	
Immunophenotype				
CD4	10 (45.5)	7 (70.0)	3 (30.0)	na
CD8	10 (45.5)	4 (40.0)	6 (60.0)	
CD56	10 (45.5)	5 (50.0)	5 (50.0)	
TCRab (n = 3)	3/3 (100)	3/3 (100)	na	
Allogeneic HSCT	2 (9.1)	1 (50.0)	1 (50.0)	na

CAEBV: chronic active EBV infection; EBV: Eptein-Barr virus; EBNA: Epstein–Barr virus nuclear antigen 1; EBV-DNA: EBV-deoxyribonucleic acid; ECOG PS: Eastern Cooperative Oncology Group Performance Status; EBER: Epstein-Barr virus-encoded RNA; nd: not detected (EBV-DNA < 2×10^2 copies/mL); HPF: high power field; HSCT: hematopoietic stem cell transplantation; Hb: hemoglobin; LDH: lactate dehydrogenase; TCR: T-cell receptor; VCA: viral capsid antigen. *Statistically significant; na: not avalilable; P^{s}: T-cell type *versus* NK-cell type.

Clinicopathological analysis of patients diagnosed at over 50 years of age with chronic active Epstein-Barr virus infection

The clinical characteristics of CAEBV patients diagnosed at over 50 years of age are presented in Table 2. Eight out of 9 cases (88.9%) showed EBV monocolonality by Southern blot analysis. However, in our study, there was no difference in clinical features between all CAEBV cases and those aged over 50 years. Furthermore, as shown in *Online Supplementary Figure S4*, we performed a prognostic analysis based on the log-rank test by comparing CAEBV in patients aged 50 years or older and those under 50 years. However, there was no difference in overall survival between these two groups (P=0.922).

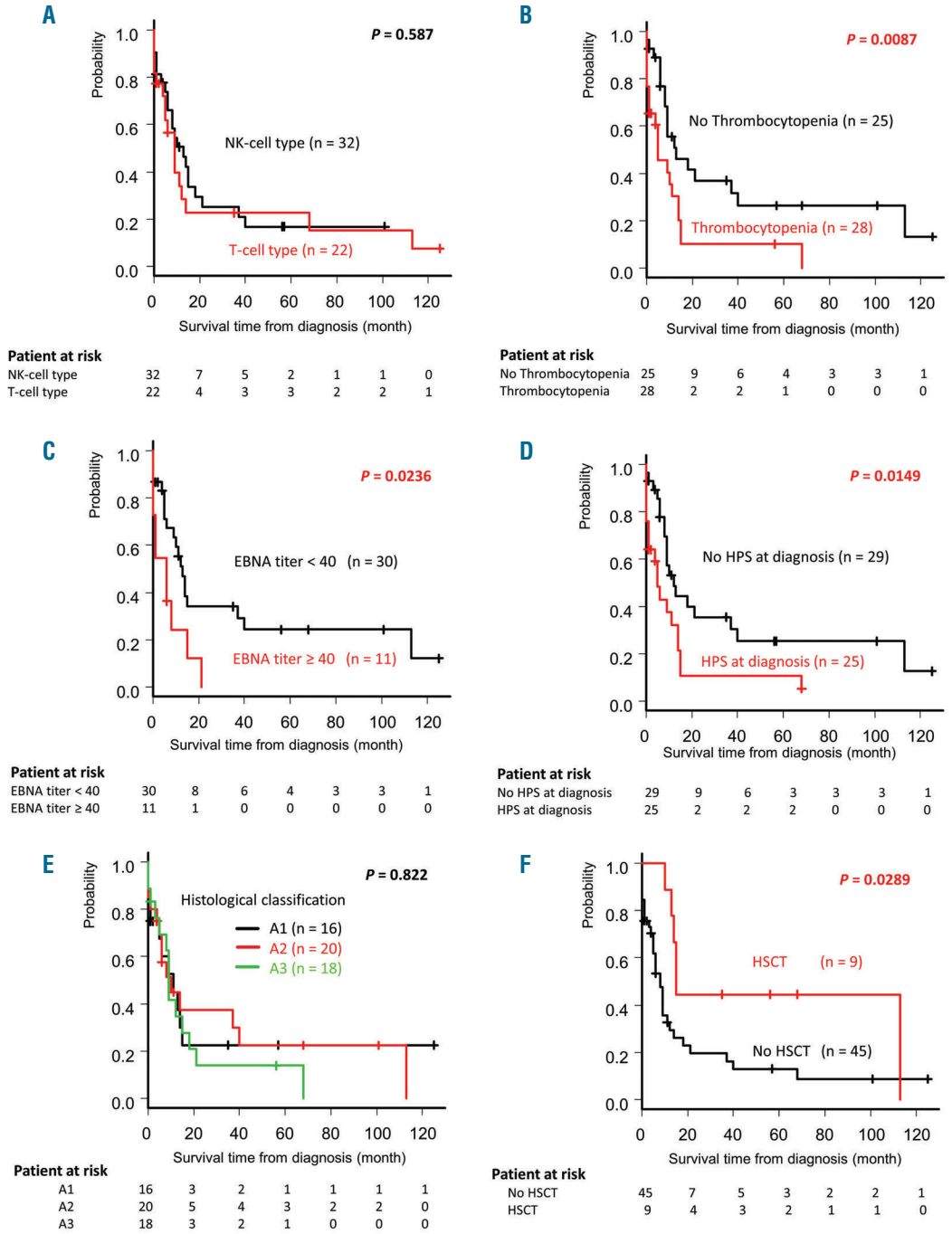


Figure 2. Indicators for predicting prognosis in terms of overall survival in adult-onset chronic active Epstein-Barr virus (EBV) infection patients. Although infected-cell type (A) and histological classification (B) were not prognostic factors for overall survival (P=0.587 and P=0.822, respectively), thrombocytopenia (B), platelet count < 100×10^9/L), EBNA antibody titer ≥ 40 (C), the presence of hemophagocytosis syndrome (HPS) (D) at the initial diagnosis were poor prognostic indicators for overall survival (P=0.0087, P=0.0236, and P=0.0149, respectively). With regards to treatment, allogeneic HSCT improved survival (F) (P=0.0289). CAEBV: chronic active EBV infection; EBNA: Epstein–Barr virus nuclear antigen 1; EBV: Epstein-Barr virus; HPS: hemophagocytic syndrome; HSCT: hematopoietic stem cell transplantation.

Comparative analysis of clinical features of adult-onset and pediatric-onset patients

Comparison of clinical features of adult-, and pediatric-onset patients is shown in Table 3. Patients with adult-onset CAEBV had a significantly lower frequency of fever, and more frequent occurrence of skin lesions (erythema), compared to pediatric-onset patients (P=0.005 and P<0.001, respectively). Hypersensitivity to mosquito bites and hydroa vacciniforme were also statistically less frequent in patients with adult-onset CAEBV (P<0.001 and P=0.0238, respectively). As regards laboratory results at initial diagnosis, while elevated liver enzymes were more frequently observed in patients with pediatric-onset type CAEBV (P<0.001), hemophagocytic syndrome was observed in bone marrow biopsies in patients with adult-onset CAEBV (P=0.0073).

Indicators for predicting prognosis of patients with adult-onset chronic active Epstein-Barr virus infection

We searched for indicators to predict prognosis at initial diagnosis because at that stage, there is no indication of the severity of CAEBV disease progression.

In log-rank test analysis (Figure 2A-E), thrombocytopenia (platelet count <100×10^9/L), EBNA antibody titer ≥40, and the presence of hemophagocytic syndrome at initial diagnosis were associated with a poor prognosis (i.e. decreased overall survival; P=0.0087, P=0.0236, and P=0.0149, respectively); however, type of infected cell and histological classification were not prognostic factors for overall survival (P=0.587 and P=0.822, respectively). In terms of treatment for CAEBV, although many cases were initially treated with various chemotherapeutic regimens (*Online Supplementary Table S8*), allogeneic hematopoietic stem cell transplantation (HSCT) was found to be the most effective treatment for improving survival (P=0.0289) (Figure 2F and *Online Supplementary Figure S3*).

In both univariate and multivariate analyses for predicting overall survival, log-rank tests yielded similar results (Table 4). Age (> 60 years), high-risk Performance Status (2-4), type of infected cell, elevated lactate dehydrogenase level, number of EBV-DNA copies in peripheral blood, EBER-positive cell counts per high power field, and EBV detected by Southern blot using a terminal repeat probe were not prognostic factors in univariate analysis. Conversely, thrombocytopenia (platelet count <100×10^9/L; hazard ratio=6.157, 95% confidence interval: 2.433–15.58; P<0.001), high EBNA titer (≥40; hazard ratio=2.815, 95% confidence interval: 1.225-2.497, P=0.0148), and not receiving HSCT (hazard ratio=5.410, 95% confidence interval: 1.892–15.47, P=0.0016) were independent poor prognostic factors.

Statistical comparison of overall survival

We compared the overall survival between pediatric-onset CAEBV and ENKTL. Overall survival of patients with adult-onset CAEBV (n=54), pediatric-onset CAEBV (n=75), and ENKTL (n=82) is depicted in Figure 3. Adult-onset CAEBV had a poorer prognosis compared to both pediatric-onset CAEBV and ENKTL (P<0.001 and P=0.0484, respectively). Even when survival rate was stratified by allogeneic HSCT, significant differences in prognosis were observed between adult-onset and pediatric-onset CAEBV (P<0.001) (*Online Supplementary Figure S3*). Furthermore, the prognosis for non-nasal-type ENKTL and adult-onset CAEBV appeared to be comparable (P=0.972) (Figure 4).

Discussion

In the present study, we analyzed 54 patients with adult-onset CAEBV meeting the diagnostic criteria outlined in the Methods section. Non-nasal-type ENKTL, ANKL, and cytotoxic-type and EBV-positive peripheral T-cell lymphomas not otherwise specified (PTCL-NOS) did not meet the diagnostic criteria. As the clinical stage progresses, adult-onset CAEBV may eventually show findings similar to those of malignant lymphomas such as ENKTL, ANKL, and cytotoxic-type and EBV-positive PTCL-NOS. However, it is critical to diagnose CAEBV as early as possible, before fatal complications, such as hemophagocytic syndrome and malignant lymphoma, have developed. The present study showed that adult-onset CAEBV is more weakly associated with some characteristics, such as hypersensitivity to mosquito bites and hydroa vacciniforme, compared to pediatric-onset CAEBV. Although it appears that adult-onset CAEBV overlaps clinically with ENKTL, ANKL, and PTCL-NOS (cytotoxic-type and EBV-positive), many CAEBV cases were diagnosed only after progression to malignant lymphomas. In addition, histopathological analysis alone may make it difficult to differentiate among these diseases; however, CAEBV may be considered symptomatically and clinically completely different because of its unique symptoms.

Of the patients diagnosed with adult-onset CAEBV in this study, 18 (33.3%) were diagnosed with malignant lymphoma (ANKL, ENKTL, and EBV + PTCL-NOS) at the time of diagnosis of the CAEBV. When the clinical course of the disease showed the presentation of CAEBV symptoms for diagnosis, the cases were considered to have developed malignant lymphomas during the clinical course of CAEBV. In our analysis, adult-onset CAEBV had a poor prognosis even in cases in which malignant lymphoma has not developed at the time of diagnosis. Simple diagnostic criteria are considered to be necessary

Table 3. Comparison of adult-onset and pediatric-onset chronic active Epstein-Barr virus (EBV) infection patients.

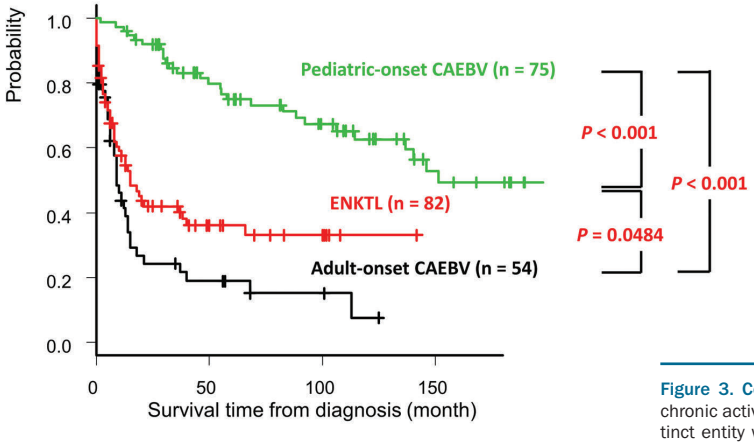
Patients' characteristics	Adult onset (n = 54)	Pediatric onset (n = 75)	P
Sex			
Male, n (%)/female, n (%)	31 (57.4)/23 (42.6)	39 (52.0)/36 (48.0)	0.593
Symptoms and involved sites			
Fever, n (%)	35 (64.8)	65 (86.7)	0.005*
Splenomegaly, n (%)	28 (51.9)	44 (58.7)	0.476
Lymphadenopathy, n (%)	21 (38.9)	30 (40.0)	1
Skin rash, n (%)	21 (38.9)	9 (12.0)	< 0.001*
Lung, n (%)	8 (14.8)	9 (12.0)	0.793
Oral lesion, n (%)	2 (3.7)	4 (5.3)	1
Central nervous system, n (%)	1 (1.9)	4 (5.3)	0.399
Myocarditis, n (%)	1 (1.9)	6 (8.0)	0.238
Past medical history			
Hypersensitivity to mosquito bites, n (%)	4 (7.4)	27 (36.0)	< 0.001*
Hydroa vacciniforme, n (%)	2 (3.7)	13 (17.3)	0.0238*
Laboratory test at initial diagnosis			
Thrombocytopenia, n (%)	26 (48.1)	34 (45.3)	0.481
Transaminase elevation, n (%)	22 (40.7)	54 (72.0)	< 0.001*
Hemophagocytic syndrome, n (%)	25 (46.3)	17 (22.7)	0.0073*
T-cell type/NK-cell type, n (%)	22 (40.7)/32 (59.3)	34 (45.3)/41 (54.7)	0.153

CAEBV: chronic active EBV infection, *Statistically significant difference. Thrombocytopenia: platelet count < 100×10^9 /L.

because adult-onset CAEBV requires active treatments including HSCT.

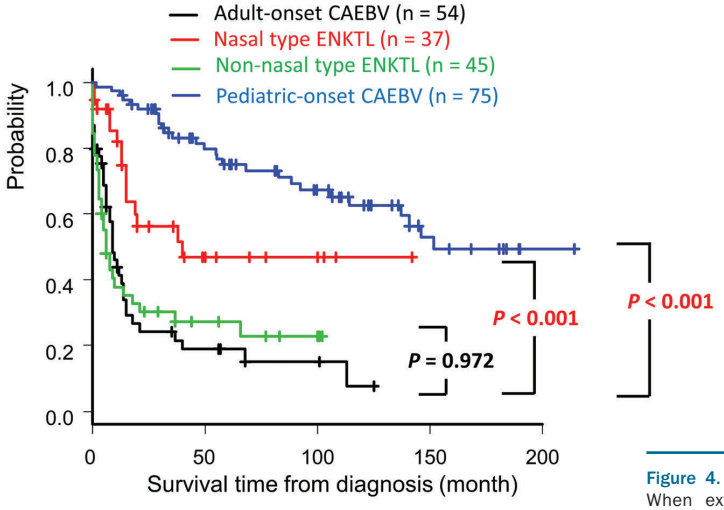
Epstein-Barr virus infection has been considered to be a cause of fever of unknown origin in adults (apart from infectious mononucleosis) and there are some reports describing adult-onset CAEBV not only in Asia, but also in the USA and other regions.[24,25] This suggests that adult-onset CAEBV is an important disease entity to consider in the differential diagnosis of fever of unknown origin, in addition to previously known diseases. Although the clinical features of adult-onset CAEBV were unclear, we found that CAEBV can develop at any age. Although EBV-positive, nodal, and cytotoxic-type PTCL-NOS have been reported to have a poor prognosis,[26] there were no clinicopathological differences between adult-onset CAEBV with nodal lesions and those with extranodal lesions in the present study (*Online Supplementary Table S4*). This result suggests that CAEBV has a different background from PTCL-NOS. Furthermore, although we could not compare the clinical features in detail, we did compare the prognosis between patients with nodular EBV+PTCL-NOS and CAEBV with nodular lesions and found no statistical difference in prognosis (P=0.143) (*Online Supplementary Figure S5*). Patients with CAEBV with nodal lesions tended to have a poor prognosis. In the future, we intend to clarify the concepts in these diseases by accumulating more cases.

In this study, EBV-DNA copy number in peripheral blood plasma was detectable (≥2×10^2 copies/mL) in 97.3% of the cases, and ten EBER-positive cells per high power field were observed in 86.3%, suggesting that these tests may also be useful for diagnosing CAEBV in both adult and pediatric patients.[17] Since many cases with low EBV-related antibody titers were observed, it was suggested



Patient at risk				
Adult-onset CAEBV	54	7	3	0
ENKTL	82	15	7	0
Pediatric-onset CAEBV	75	49	32	15

Figure 3. Comparison of overall survival. Adult-onset chronic active Epstein-Barr virus infection may be a distinct entity with a poorer prognosis compared to pediatric-onset CAEBV and extranodal NK/T-cell lymphoma, nasal type (P<0.001 and P=0.0484, respectively).



Patient at risk					
Adult-onset CAEBV	54	7	3	0	0
Nasal type ENKTL	37	8	4	0	0
Non-nasal type ENKTL	45	7	3	0	0
Pediatric-onset CAEBV	75	49	32	15	6

Figure 4. Comparison of survival for overall survival. When extranodal NK/T-cell lymphoma, nasal type (ENKTL) was divided into "nasal type" and "non-nasal type" according to the anatomical sites of development, there was no statistical difference in prognosis between non-nasal type ENKTL and adult-onset chronic active Epstein-Barr virus infection (P=0.922).

Table 4. Univariate and multivariate analyses for predicting overall survival of adult-onset chronic active Epstein-Barr virus (EBV) infection patients.

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Age ≥ 61 years	1.340 (0.698-2.574)	0.3793		
PS High (2-4)	1.719 (0.885-3.342)	0.1101		
T-cell type	1.190 (0.623-2.274)	0.5989		
Thrombocytopenia (< 100×10^9 /L)	2.277 (1.187-4.368)	0.0133*	6.157 (2.433-15.58)	<0.001*
Elevated LDH	1.610 (0.762-3.402)	0.2125		
EBNA antibody titer ≥ 40	2.341 (0.329-2.497)	0.0351*	2.815 (1.225-8.468)	0.0148*
EBV-DNA in PB ≥ 10^3 copies/mL	0.866 (0.361-2.078)	0.7470		
EBV monoclonality by Southern blot	0.789 (0.229-2.719)	0.7069		
EBER-positive cells ≥30 /HPF	0.637 (0.329-1.235)	0.1821		
Treatment without HSCT	2.524 (1.044-6.104)	0.0398*	5.410 (1.892-15.47)	0.0016*

CAEBV: chronic active EBV infection; EBNA: Epstein–Barr virus nuclear antigen 1; EBV-DNA: EBV-deoxyribonucleic acid; PS: Performance Status; EBER: Epstein-Barr virus-encoded RNA; LDH: lactate dehydrogenase; HSCT: hematopoietic stem cell transplantation. *Statistically significant difference; PB: peripheral blood (plasma); HPF: high power field.

that direct verification of increased quantitative EBV values may be important for diagnosis. If EBV infection in T cells or NK cells and an increase in plasma EBV-DNA level are proven, it may be necessary to diagnose CAEBV.

Many differences in the clinical features between adult-onset, and pediatric-onset type CAEBV were elucidated. As for other infectious diseases, there was a lower frequency of fever in adult-onset CAEBV, than in pediatric-onset CAEBV.[27] The frequency of skin lesions was higher in the adult-onset type, while hypersensitivity to mosquito bites and hydroa vacciniforme were significantly much less frequent in adult-onset CAEBV than in pediatric-onset CAEBV. Although there was a difference in the appearance of skin lesions between adult-onset and pediatric-onset CAEBV, it is not known whether this could be explained only by the difference in age at onset. It should be noted that many patients with adult-onset CAEBV do not have a history of hypersensitivity to mosquito bites and hydroa vacciniforme, despite these symptoms having been thought to be clues to the diagnosis of CAEBV.

Adult- and pediatric-onset CAEBV may constitute a continuous spectrum because the diagnostic criteria for adult-onset CAEBV in this analysis were the WHO criteria for "Systemic EBV positive T-cell lymphoproliferative disorders of childhood". Comparing clinical features, CAEBV patients over 50 years old were considered to share the pathogenesis with that of their young and adult-onset counterparts. However, the clinical and molecular details are still unknown. In this analysis, we did not perform molecular biology to investigate the common features. In future, we would like to investigate whether the molecular background of adult- and pediatric-onset CAEBV is the same.

This study suggests that thrombocytopenia, high EBNA antibody titer (≥40), and the presence of hemophagocytic syndrome at the initial diagnosis of CAEBV are prognostic factors in adult patients, as previously reported in children and young adults, and further suggested to be indicators for aggressive therapeutic intervention including allogeneic HSCT.[17,28] It has been reported that patients with clinically aggressive CAEBV have a high level of expression of EBNA-1;[29] this report may support our results. It has also been reported that treatment with EBNA-1-specific T cells may be effective.[30] This may be a treatment option for CAEBV patients with high EBNA antibody levels. Conversely, no difference in prognosis was detected depending on the type of cell infected by EBV in patients with adult-onset CAEBV, which contradicts previous findings in children and young adults.[6,17] Precursor T cells are reported to have the potential to differentiate into NK cells, suggesting that phenotype could be changed.[31] Moreover, it has been proven that a single EBV clonotype can infect multiple NK-cell and T-cell subsets.[32] The identification of infected cells in patients with adult-onset disease may not be very important. In this study, there were 2 patients who did not express cytotoxic molecules such as TIA-1 and granzyme B from their T cells, as determined by immunohistochemistry. Although we could not further characterize the clinical features of these cases because of their small number, further studies are necessary to determine the significance of EBV-infected cells. In addition, there were no differences in prognosis among the three histological classifications (A1, A2, and A3).[9] Regardless of histological category, this study suggests that treatment including allogeneic HSCT is the cure for CAEBV. Although allogeneic HSCT has been reported to be effective,[6,15,17] there is still controversy about when the transplant should be performed and further research is, therefore, needed.

Genetic analysis by next-generation sequencing is a very important issue in order to evaluate subtypes and different genetic abnormalities which result in CAEBV. According to the results of genetic analysis of terminal repeats of EBV, since it was suggested that CAEBV in the early stage has a polyclonal state, genetic analysis by sequencing was not necessarily valid.[9] Although other research groups have detected genetic abnormalities, such as those of the T-cell receptor β repertoire and perforin,[33,34] these genetic abnormalities are not useful for explaining the development and mechanism of progression of CAEBV. In situations in which the actual condition of the disease is unclear, we believe it is important to recognize that there are various types of disease. Next-generation sequencing to analyze the genetic landscape of CAEBV will be necessary and important in the future.

It has been suggested recently that the development of

EBV-related lymphoproliferative diseases could be related to an immune escape mechanism in the programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1) pathway.[35,36] In fact, it has been reported that an anti-PD-1 monoclonal inhibitor is effective in Hodgkin lymphoma and ENKTL.[37-39] The existence of such an immune escape mechanism in CAEBV is presumed. Unfortunately, it was impossible to investigate the immune background of the EBV infection in the present study because of a lack of specimens. With further accumulation of cases, we hope to investigate the immune response against EBV infection in the future, and examine the differences in immunological background between patients with CAEBV and healthy individuals.

Almost all of the Asian cases have a T-cell or NK-cell origin.[17] In contrast, the most common type of CAEBV in the USA originates from B cells.[28] There may be racial differences in susceptibility to EBV infection and host immunity. In the future, it will be necessary to clarify these genetic backgrounds. In the present study we confirmed that adult-onset CAEBV has a poorer prognosis than ENKTL. Although adult EBV-positive T/NK-cell LPD includes various lymphomas, such as ENKTL and EBV-positive PTCL-NOS,[12] we found that CAEBV needs to be distinguished by detailed interview and medical history because of the differences in prognosis and treatment strategies. However, at present, CAEBV and ENKTL can only be distinguished by differences in their clinical courses, given that differences in biological mechanisms of action are not known. Although it is thought that a simple prognostic comparison should not be performed because the treatments are quite different, the present study showed there was no statistical difference in the prognosis between non-nasal type ENKTL and adult-onset CAEBV. In fact, there was a report in which it was difficult to distinguish between the two diseases.[40] In future, further research is necessary to establish novel testing methods to improve the differential diagnosis of these two diseases.

The reasons why adult-onset CAEBV has a poor prognosis may be as follows: (i) recognition of adult-onset CAEBV by physicians is poor, so the condition is often regarded as an unknown fever; (ii) remissions and exacerbations recur for a long period; (iii) treatments are performed after systemic conditions worsen and/or life-threatening complications develop, including hemophagocytic syndrome; (iv) not much is known about the pathobiology; and (v) there is no fundamental treatment.

The accumulation of more cases may help in the recognition of adult-onset CAEBV by revealing the clinical features and elucidating the mechanisms of the molecular pathogenesis.

The following proposed diagnostic criteria for adult-onset CAEBV are very simple and are based on the pediatric-onset disease: (i) several symptoms of infectious mononucleosis are present, such as fever, lymphadenopathies, and hepatosplenomegaly; (ii) exacerbation and remission of symptoms repeat within a certain period; (iii) proven EBV infection in T cells or NK cells in the affected lesions. Hydroa vacciniforme or hypersensitivity to mosquito bites are not essential for the diagnosis, as these symptoms are present in only approximately 30% of cases, even in pediatric-onset CAEBV. The foregoing criteria may help the timely diagnosis of adult-onset CAEBV.

Although there were clinical differences between adult- and pediatric-onset CAEBV, we confirmed that CAEBV is a disease with a varying age of onset. In addition, the prognosis of adult-onset CAEBV appears to be very poor. Therefore, a prescise, early diagnosis and appropriate treatment strategies are critical for adult patients.

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References

1. Cohen JI. Epstein-Barr virus infection. N Engl J Med. 2000;343(7):481-492.
2. Williams H, Crawford DH. Epstein-Barr virus: the impact of scientific advances on clinical practice. Blood. 2006;107(3):862-869.
3. Straus SE. The chronic mononucleosis syndrome. J Infect Dis. 1988;157(3):405-412.
4. Jones JF, Shurin S, Abramowsky C, et al. T-cell lymphomas containing Epstein-Barr viral DNA in patients with chronic Epstein-Barr virus infections. N Engl J Med. 1988;318(12):733-741.
5. Okano M, Kawa K, Kimura H, et al. Proposed guidelines for diagnosing chronic active Epstein-Barr virus infection. Am J Hematol. 2005;80(1):64-69.
6. Kimura H, Hoshino Y, Kanegane H, et al. Clinical and virologic characteristics of chronic active Epstein-Barr virus infection. Blood. 2001;98(2):280-286.
7. Schooley RT, Carey RW, Miller G, et al. Chronic Epstein-Barr virus infection associated with fever and interstitial pneumonitis. Clinical and serologic features and response to antiviral chemotherapy. Ann Intern Med. 1986;104(5):636-643.
8. Tsuge I, Morishima T, Morita M, Kimura H, Kuzushima K, Matsuoka H. Characterization of Epstein-Barr virus (EBV)-infected natural killer (NK) cell proliferation in patients with severe mosquito allergy; establishment of an IL-2-dependent NK-like cell line. Clin Exp Immunol. 1999;115(3):385-392.
9. Ohshima K, Kimura H, Yoshino T, et al. Proposed categorization of pathological states of EBV-associated T/natural killer-cell lymphoproliferative disorder (LPD) in children and young adults: overlap with chronic active EBV infection and infantile fulminant EBV T-LPD. Pathol Int. 2008;58(4):209-217.
10. Kikuta H, Taguchi Y, Tomizawa K, et al. Epstein-Barr virus genome-positive T lymphocytes in a boy with chronic active EBV infection associated with Kawasaki-like disease. Nature. 1988;333(6172):455-457.
11. Okano M, Matsumoto S, Osato T, Sakiyama Y, Thiele GM, Purtilo DT. Severe chronic active Epstein-Barr virus infection syndrome. Clin Microbiol Rev. 1991;4(1):129-135.
12. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375-2390.
13. Hong M, Ko YH, Yoo KH, et al. EBV-Positive T/NK-cell lymphoproliferative disease of childhood. Korean J Pathol. 2013;47(2):137-147.
14. Yamada H, Kohno S, Koga H, et al. An adult case of severe chronic active Epstein-Barr virus infection syndrome. Intern Med. 1992;31(12):1381-1386.
15. Saburi M, Ogata M, Satou T, et al. Successful cord blood stem cell transplantation for an adult case of chronic active Epstein-Barr virus infection. Intern Med. 2016;55(23):3499-3504.
16. Huang Y, Xie J, Ding Y, Zhou X. Extranodal Natural killer/T-cell lymphoma in children and adolescents: a report of 17 cases in China. Am J Clin Pathol. 2016;145(1):46-54.
17. Kimura H, Ito Y, Kawabe S, et al. EBV-associated T/NK-cell lymphoproliferative diseases in nonimmunocompromised hosts: prospective analysis of 108 cases. Blood. 2012;119(3):673-686.
18. Ohga S, Kimura N, Takada H, et al. Restricted diversification of T-cells in chronic active Epstein-Barr virus infection: potential inclination to T-lymphoproliferative disease. Am J Hematol. 1999;61(1):26-33.
19. Henter JI, Horne A, Arico M, et al. HLH-

2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;48(2):124-131.
20. Imai S, Sugiura M, Oikawa O, et al. Epstein-Barr virus (EBV) - carrying and - expressing T-cell lines established from severe chronic active EBV infection. Blood. 1996;87(4):1446-1457.
21. Kawamoto K, Miyoshi H, Yanagida E, et al. Comparison of clinicopathological characteristics between T-PLL and PTCL, NOS. Eur J Haematol. 201;98(5):459-466.
22. Kawamoto K, Miyoshi H, Yoshida N, Takizawa J, Sone H, Ohshima K. Clinicopathological, cytogenetic, and prognostic analysis of 131 myeloid sarcoma patients. Am J Surg Pathol. 2016;40(11): 1473-1483.
23. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48(3):452-458.
24. Cunha BA, Petelin A, George S. Fever of unknown origin (FUO) in an elderly adult due to Epstein-Barr virus (EBV) presenting as "typhoidal mononucleosis," mimicking a lymphoma. Heart Lung. 2013;42(1):79-81.
25. Levy L, Nasereddin A, Rav-Acha M, Kedmi M, Rund D, Gatt ME. Prolonged fever, hepatosplenomegaly, and pancytopenia in a 46-year-old woman. PLoS Med. 2009;6(4):e1000053.
26. Kato S, Takahashi E, Asano N, et al. Nodal cytotoxic molecule (CM)-positive Epstein-Barr virus (EBV)-associated peripheral T cell lymphoma (PTCL): a clinicopathological study of 26 cases. Histopathology. 2012;61(2):186-199.
27. Roghmann MC, Warner J, Mackowiak PA. The relationship between age and fever magnitude. Am J Med Sci. 2001;322(2):68-70.
28. Cohen JI, Jaffe ES, Dale JK, et al. Characterization and treatment of chronic active Epstein-Barr virus disease: a 28-year experience in the United States. Blood. 2011;117(22):5835-5849.
29. Iwata S, Wada K, Tobita S, et al. Quantitative analysis of Epstein-Barr virus (EBV)-related gene expression in patients with chronic active EBV infection. J Gen Virol. 2010;91(Pt 1):42-50.
30. Bollard CM. Improving T-cell therapy for Epstein-Barr virus lymphoproliferative disorders. J Clin Oncol. 2013;31(1):5-7.
31. Shen HQ, Lu M, Ikawa T, et al. T/NK bipotent progenitors in the thymus retain the potential to generate dendritic cells. J Immunol. 2003;171(7):3401-3406.
32. Ohga S, Ishimura M, Yoshimoto G, et al. Clonal origin of Epstein-Barr virus (EBV)-infected T/NK-cell subpopulations in EBV-positive T/NK-cell lymphoproliferative disorders of childhood. J Clin Virol. 2011;51(1):31-37.
33. Liu S, Zhang Q, Huang D, et al. Comprehensive assessment of peripheral blood TCRbeta repertoire in infectious mononucleosis and chronic active EBV infection patients. Ann Hematol. 2017;96(4):665-680.
34. Katano H, Ali MA, Patera AC, et al. Chronic active Epstein-Barr virus infection associated with mutations in perforin that impair its maturation. Blood. 2004;103 (4):1244-1252.
35. Kiyasu J, Miyoshi H, Hirata A, et al. Expression of programmed cell death ligand 1 is associated with poor overall survival in patients with diffuse large B-cell lymphoma. Blood. 2015;126(19):2193-2201.
36. Jo JC, Kim M, Choi Y, et al. Expression of programmed cell death 1 and programmed cell death ligand 1 in extranodal NK/T-cell lymphoma, nasal type. Ann Hematol. 2017;96(1):25-31.
37. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med. 2015;372(4):311-319.
38. Younes A, Santoro A, Shipp M, et al. Nivolumab for classical Hodgkin's lymphoma after failure of both autologous stem-cell transplantation and brentuximab vedotin: a multicentre, multicohort, single-arm phase 2 trial. Lancet Oncol. 2016;17(9):1283-1294.
39. Kwong YL, Chan TSY, Tan D, et al. PD1 blockade with pembrolizumab is highly effective in relapsed or refractory NK/T-cell lymphoma failing L-asparaginase. Blood. 2017;129(17):2437-2442.
40. Ohtsuka R, Abe Y, Sada E, et al. Adult patient with Epstein-Barr virus (EBV)-associated lymphoproliferative disorder: chronic active EBV infection or de novo extranodal natural killer (NK)/T-cell lymphoma, nasal type? Intern Med. 2009;48(6):471-474.