Acute Epstein–Barr virus positive cytotoxic T/Nature Killer cell lymphoid hyperplasia (AEBV+CT/NK-LH) mimicking natural killer/T cell lymphoma

Yanlin Zhang (✉ zyl84131421@163.com)
Beijing Friendship Hospital Capital Medical University

Jianlan Xie
Beijing Friendship Hospital Capital Medical University

Yuanyuan Zheng
Beijing Friendship Hospital Capital Medical University

Xiaoge Zhou
Beijing Friendship Hospital Capital Medical University

Research Article

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Abstract

To describe the clinicopathological features of seven patients with acute Epstein–Barr virus (EBV)-positive cytotoxic T cell/natural killer lymphoid hyperplasia (AEBV + CT/NK-LH) in the lymph node, in which initial findings led to a preliminary misdiagnosis of extranodal NK/T cell lymphoma (NKTL). Seven cases in children and young people from China with AEBV + CT/NK-LH were retrospectively analyzed. The patients were healthy prior to illness. Sudden onset occurred in all patients, with high fever as the first symptom followed by lymphadenopathy and hepatosplenomegaly. The disease courses before diagnosis were less than 1.5 months. The majority of lymphocytes in the lesions expressed CD3 and Granzyme B or TIA-1, but not CD5 in all cases. CD56 was expressed in numerous cells in 5 of 7 cases. EBER was detected in medium to large-sized cells (50–100 cells per high-power field) in all cases. TCR gene rearrangement was observed in six cases, among which monoclonal rearrangement was found in four cases. All patients received conservative treatment, but not chemotherapy. Four patients underwent anti-HLH therapy, and the others received anti-inflammatory treatment. All patients survived. All patients underwent long-term clinical observation and follow-up and did not relapse. Therefore, AEBV + CT/NK-LH could elicit malignant features mimicking NK/T cell lymphoma pathologically and benign features mimicking IM clinically. The findings indicated that AEBV + CT/NK-LH should be taken into consideration as a potential diagnosis, thus further clinical information (such as age of onset [whether children and young people], nature of onset [whether sudden], disease course [whether short], symptoms [whether systemic], EBV infection status [whether acute], and lymph node involvement) is needed for accurate diagnosis and prognostic evaluation.

Introduction

Epstein-Barr virus (EBV) is a member of the herpes virus family with double-stranded DNA, which was discovered by Epstein through isolation from Burkitt's lymphoma in 1964 [1]. Generally, EBV infection occurs at an early age in people and is usually asymptomatic. However, if primary EBV infection is delayed in adolescents and young adults, infectious mononucleosis (IM) may develop. IM is a self-limited lymphoid disease with a benign clinical course [2], in which most EBV-infected cells are B cells with few T and NK cells [3]. Moreover, EBV is associated with malignancies, such as classic Hodgkin's lymphoma, Burkitt's lymphoma, and T cell and NK cell lymphoma. In recent years, EBV was found to be associated with chronic active EBV infection (CAEBV), T/NK cell lymphoproliferative diseases (LPDs) [4].

Systemic EBV-positive T-cell lymphoma in children have been defined by the World Health Organization (WHO) [5] as those that develop shortly after primary or acute EBV infection in previously healthy children and young adults or in the setting of CAEBV. Historically, this process has been described under a variety of terms, including: fulminant EBV + T cell LPD of childhood, sporadic fatal IM, fulminant hemophagocytic syndrome in children in Taiwan, fatal EBV-associated hemophagocytic syndrome in Japan, and severe CAEBV. Although it is a systemic disease, it mostly involves the liver and spleen, followed by lymph nodes, bone marrow (BM), skin, and lung. Patients with the disease usually die within days to up to a month after diagnosis [6]. Ohshima et al. [7] proposed a new nomenclature to classify pathological categories of EBV +
T/NK-LPD in 2008, consisting of category A1, A2, A3, and B. Category B was described as monomorphic LPD with clonality and fulminant course. In most papers, it is described as a life-threatening disease.

EBV infects typically B cells in IM, although a rare case showed EBV infection in T cells \[8\]. Here, we report seven cases of lymphoproliferative disorder caused by EBV-positive cytotoxic T / NK cell proliferation in the lymph nodes; we designated this as acute EBV-positive cytotoxic T/NK cell lymphoid hyperplasia (AEBV + CT/NK-LH). Notably, AEBV + CT/NK-LH is easily misdiagnosed as NK/T cell lymphoma (NKTL), because both share similar features in histology and immunohistochemistry, including a cytotoxic T/NK cell phenotype and EBV positivity. However, based on its clinical characteristics, i.e., spontaneous regression within a short time, AEBV + CT/NK-LH should be recognized as a reactive changes.

**Materials And Methods**

**Case selection and morphologic review**

Information on seven cases with acute EBV infections of T/NK cells was obtained retrospectively from the files of the Department of Pathology, Beijing Friendship Hospital, Capital Medical University (Lymphoma Diagnosis and Research Center, Institute of Beijing Clinical Medicine), a large lymphoma diagnosis and research center, located in Beijing, China. Seven cases were received for consultation during the period of June 2013 to June 2018. The final follow-up was on September 25, 2021. Among them, there were seven cases of AEBV + CT/NK-LH that had the following common features: (1) T/NK cell predominant lymphoproliferation in the lymphnode; (2) EBV infection mainly of cytotoxic T/NK cells; (3) EBV-positive cells of > 50/high-power field;(HPF); (4) no radiotherapy or chemotherapy; and (6) follow-up for a long time.

Clinical information included gender, age, duration of disease, initial symptoms, laboratory test of the blood and EBV detection, imaging examination of the liver and spleen, and follow-up results. Morphological observation included: (1) lymph node structural change and degree of change, (2) complexity of T/NK cell type and composition, (3) presence of necrosis, and (4) lymphocytes atypia.

**Immunohistochemistry And Eber In Situ Hybridization**

Immunohistochemistry (IHC) staining was performed manually on formalin-fixed, paraffin-embedded (FFPE) tissue for immunophenotypic analysis. The detection system of MaxVisionTM 2 kit (Cat. No. KIT-5910/5931) and monoclonal antibodies, including those against CD21, CD20, CD3, CD2, CD5, CD4, CD8, CD56, CD30, Granzyme B, TIA-1, PAX5, LMP1, and Ki-67 provided by Maxin. Bio (Maxin. Bio, Fuzhou, CN) and EBNA2 provided by ABCOM, were used for detection of all relevant antigens. Positive and negative controls were analyzed according to the manufacturer’s instructions. The EBV Probe In Situ Hybridization Kit (Triplex International Biosciences (China) Co. Ltd., Fuzhou, China) was used to detect EBERs. Details of this procedure are described in our previous report \[9\]. Observers counted only definite EBV-positive cells, and the field selection was from an area with a high positive rate. The total number of EBV-positive cells per HPF was recorded.
Double Staining

An immunohistochemical plus EBER in situ hybridization dual-staining technique was performed using the Leica Bond MAX autostainer (Leica, Melbourne, Australia). Sections (2-µm thick) were first stained for EBER with DAB staining (brown), then for CD3 (LN10, RTU; Lecia) and CD20 (L26,RTU; Lecia) followed by visualization of the red staining after application of amino-ethylcarbazole (Bond Polymer Refine Red Detection kit, Leica) as chromogen [9].

T Cell Receptor Gene Clonality Analysis

T cell receptor (TCR) gene rearrangement analysis was performed by PCR using the “Biomed-2” primers (InVivoScribe Technologies, San Diego, CA, USA) [10]. DNA was extracted from FFPE tissue samples using the TIANamp FFPE DNA Kit (DP331) (TIANGEN, Beijing, China). For the gene rearrangement assay, PCR was carried out in a 25-µL volume containing 22.5 µL of master mix, 0.13 µL of AmpliTaq Gold DNA polymerase, and 100 ng of genomic DNA. The cycling profile used for all reactions was as follows: 95°C for 7 min; 35 cycles of 95°C for 45 s, 60°C 45 s, 72°C 90 s; and a 10-min final extension at 72°C. After amplification, PCR products were denatured at 94°C for 5 min followed by a quick chill to reanneal the PCR products at 4°C for at least 60 min. The PCR products were electrophoresed in 6% polyacrylamide gels (BioRad) in 1 × TBE buffer at 120 V for approximately 65 min. The gel was then soaked for 20 min in 100 mL of 0.1 M NaCl solution containing 10 µL of 10 mg/mL Gel Red (Biotium, USA) and photographed using ultraviolet illumination.

Results

Clinical features

Clinical characteristics of the seven patients are summarized in Table 1. Five patients were male, and two were female. The age of the patients ranged from 10 month to 19 years, with a median age of 5.0 years. As such, all patients were children and young people. They were healthy prior to illness. Sudden onset was seen in all patients, with high fever as the first symptom followed by lymphadenopathy and hepatosplenomegaly based on CT (Computed tomography) scan examination(Fig. 1A, case 5). Course of the disease at diagnosis ranged from 1 to 1.5 months. Two patients (patient 3 and 4,) presented pleural effusion and severe pneumonia. Laboratory examination showed reduced white blood cell counts at different levels in all patients. Thrombocytopenia was seen in 4/7 patients; hemoglobin level decreased in 6/7 patients; LDH level increased in 4/7 patients; FERR(ferritin) level increased in 3/7 patients; and ALT and/or AST levels increased in 5/7 patients. Antibody testing for anti-EBV in the serum and detection of EBV DNA in the blood were available in six (patient 1, 2, 3, 4, 6, and 7). EBV-CA-IgM positivity was observed in three patient (patient 1, 2, and 7), and EBV-VCA-IgG positivity in six patients. Six patients showed increased EBV-DNA. Karyotype was detected in 3 patients and no abnormality was found(patient 1, 5 and 7). None of the cases had a history of hepatitis B or C, and no other immune deficiency diseases were found. Four patients presented with hemophagocytic lymphohistiocytosis (HLH)(patient 2, 4, 6 and 7).
### Table 1
Clinical data of 7 cases with acute Epstein-Barr virus infection of NK/T cells

<table>
<thead>
<tr>
<th>Case no</th>
<th>Sex/Age</th>
<th>Course of disease</th>
<th>Clinical symptoms</th>
<th>Blood test</th>
<th>EBV detected in blood</th>
<th>Follow-up (month)</th>
<th>Alleviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/4 y</td>
<td>1 m</td>
<td>fever, hepatosplenomegaly, lymphadenopathy</td>
<td>WBC 2.3×10⁹/L, HGB 78g/L, PLT 56×10⁹/L, CRP 86.3 mg/L</td>
<td>EBV-CV-IgM+ EBV-CV-IgG+ EB-DNA 3.5×10⁶ copy/mL</td>
<td>36, Alleviation</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M/2 y</td>
<td>1 m</td>
<td>fever, hepatosplenomegaly, lymphadenopathy</td>
<td>WBC 0.7×10⁹/L, HGB 71 g/L, PLT 17×10⁹/L, CRP 133 mg/L, LDH 893 U/L, FERR 10440 ng/mL, ALT 103 U/L, AST 223 U/L</td>
<td>EBV-CV-IgM+ EBV-CV-IgG+ EB-DNA 1.6×10⁴ copy/mL</td>
<td>40, Alleviation</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M/19 y</td>
<td>1.5 m</td>
<td>fever, hepatosplenomegaly, lymphadenopathy, pleural effusion, ascites, pneumonia</td>
<td>WBC 2.7×10⁹/L, HGB 112 g/L, PLT 119×10⁹/L</td>
<td>EBV-CV-IgM- EBV-CV-IgG+ EB-DNA 4.3×10⁵ copy/mL</td>
<td>36, Alleviation</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M/3 y</td>
<td>1 m</td>
<td>fever, hepatosplenomegaly, lymphadenopathy, pleural effusion, pneumonia</td>
<td>WBC 2×10⁹/L, HGB 98 g/L, PLT 140×10⁹/L CRP 111 mg/L, FERR 1179 ng/mL, LDH 695 U/L, AST 51.8 U/L</td>
<td>EBV-CV-IgM- EBV-CV-IgG+ EB-DNA 2.01×10⁴ copy/mL</td>
<td>38, Alleviation</td>
<td></td>
</tr>
</tbody>
</table>

Alleviation: no fever, lymph nodes, liver, and spleen as usual; WBC: white blood cell; HGB: hemoglobin; PLT: platelet; CRP: c-reactive protein; LDH: lactate dehydrogenase; FERR: Ferritin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; MOF: multiple organ failure; NA: not available
<table>
<thead>
<tr>
<th>Case no</th>
<th>Sex/Age</th>
<th>Course of disease</th>
<th>Clinical symptoms</th>
<th>Blood test</th>
<th>EBV detected in blood</th>
<th>Follow-up (month)</th>
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<tr>
<td>5</td>
<td>M/10 m</td>
<td>1 m</td>
<td>fever, hepatosplenomegaly, lymphadenopathy</td>
<td>WBC 3.76×10⁹/L, HGB 106 g/L, PLT 256.4×10⁹/L, CRP 3 mg/L, LDH 754 U/L, AST 61.6 U/L</td>
<td>NA</td>
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<td>6</td>
<td>F/1 y</td>
<td>1 m</td>
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<td>WBC 1.99×10⁹/L, HGB 102 g/L, PLT 63×10⁹/L, ALT 206 U/L, AST 286 U/L, LDH 1670 U/L, CRP 1.43 mg/L</td>
<td>EBV- CV-IgM-EBV- CV-IgG+ EB-DNA 1.09×10⁴ copy/mL</td>
<td>70, Alleviation</td>
</tr>
<tr>
<td>7</td>
<td>M/5 y</td>
<td>1.5 m</td>
<td>fever, hepatosplenomegaly, lymphadenopathy</td>
<td>WBC 2.32×10⁹/L, HGB 94 g/L, PLT 75×10⁹/L, ALT 370 U/L, AST 2392 U/L, FERR 1495.3 ng/mL, CRP 21 mg/L</td>
<td>EBV- CV-IgM+ EBV- CV-IgG+ EB-DNA 4.0×10⁶copy/mL</td>
<td>37, Alleviation</td>
</tr>
</tbody>
</table>

Alleviation: no fever, lymph nodes, liver, and spleen as usual; WBC: white blood cell; HGB: hemoglobin; PLT: platelet; CRP: c-reactive protein; LDH: lactate dehydrogenase; FERR: Ferritin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; MOF: multiple organ failure; NA: not available

**Morphological Features**

Histopathological change of the seven patients are summarized in Table 2. All samples removed for biopsy were lymph nodes. The architectures were mostly destroyed, and part of the lesions involved the peripheral tissue of the node (Fig. 2A). The cortex and medulla of the node were not clear, and few residual follicles and subcapsular sinus existed or were not easy to detect. The cell composition was diverse. Further, neutrophilic granulocyte, eosinophils, and plasma cells were detected at high magnification. In the lesions, there were numerous infiltrating lymphocytes of medium and large size, round and mildly irregular nuclei,
inconspicuous or small nucleoli, and dispersed chromatin (Fig. 2B). Mitotic figures were easily found, and most cells showed high atypia (Fig. 2C, 2D). The lesions were diffuse with focal-to-extensive coagulative necrosis (Fig. 2E). Lymphocyte infiltration in the vascular wall was also seen (Fig. 2F).

Table 2 Morphology, antigen expression, EBER in situ hybridization and TCR gene rearrangements’ results of 7 cases with acute Epstein-Barr virus infections of NK/T cells

<table>
<thead>
<tr>
<th>Case no</th>
<th>Structural destruction</th>
<th>Cell components</th>
<th>Atypia</th>
<th>Coagulative necrosis</th>
<th>CD21</th>
<th>CD3</th>
<th>CD5</th>
<th>CD56</th>
<th>TIA-1</th>
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<tr>
<td>1</td>
<td>Yes</td>
<td>L</td>
<td>H</td>
<td>Yes</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>2</td>
<td>Yes</td>
<td>M</td>
<td>M</td>
<td>No</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>L</td>
<td>H</td>
<td>Yes</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>NA</td>
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<tr>
<td>4</td>
<td>Yes</td>
<td>L</td>
<td>H</td>
<td>Yes</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>L</td>
<td>H</td>
<td>Yes</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>Yes</td>
<td>L</td>
<td>H</td>
<td>Yes</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>H</td>
<td>No</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

L: predominantly composed of large-sized cells; M: predominantly composed of medium-sized cells; S: predominantly composed of small-sized cells; H: high atypia;

M: moderate atypia; NA: not available; P: polyclonal rearrangement

Immunohistochemical And In Situ Hybridization Findings

Immune phenotype and EBV infection characteristics of the seven patients are summarized in Table 3: The majority of lymphocytes in the lesion expressed CD3 and TIA-1 or Granzyme B (Fig. 2G, 2H), but not CD5 (Fig. 2I) in all cases. CD56 expression in the same area was seen in five cases (Fig. 2J). There were only few scattered lymphocytes with CD20 expression. The number of CD8-positive cells (Fig. 2K) was more than that of CD4-positive cells in six cases. One case (case 2) had reduced numbers of CD4- and CD8-positive cells and the degree of reduction was similar for the two cell types. Ki-67 staining was positive in over 50% of cells, except one showing 40% positivity (case 2). The scattered larger cells were CD30-positive in four cases (case 1, 3, 6 and 7). LMP1-positive cells in two cases (case 3 and 5). EBNA2-positive cells in four cases (case 1, 2, 4 and 7, Fig. 2L). EBER in situ hybridization showed positive signal localization in the nucleus. EBER-positive cells were seen in the CD3+/CD56 + areas in five cases; however, the other two were in the CD3+/CD56- areas. EBER-positive cells were not seen in the PAX5- areas in all cases. More than 50 EBER-positive cells per high-power field (> 50/HPF) were observed to be primarily medium- and large-sized (Fig. 3A). EBER/CD3 and EBER/CD20 for Double staining showed EBER-positive cells were in the CD3 + areas in all cases (Fig. 3B and 3C).

Pcr For Tcr Gene Rearrangements
T cell clonality analysis by BIOMED-2 PCR separate protocols revealed that four cases (case 1, 2, 5, and 6) presented a monoclonal rearrangement, but not the other two cases tested (Table 3).

**Follow-up**

Follow-up data were available for all seven patients. Four patients (case 2, 4, 6, and 7) underwent anti-HLH Hemophagocytic lymphohistiocytosis therapy, and the others received anti-inflammatory treatment. All patients survived. The duration of follow-up since diagnosis was from 36 to 84 months with a mean duration of 48.7 months. The follow up CT scan showed no clear mass and enlarged lymph nodes (Fig. 1B, case 5). The symptoms of all patients were relieved, showing no fever, lymph nodes, liver, spleen, and blood tests were within the normal range.

**Discussion**

EBV positive lymphoproliferative diseases are a group of diseases, which represent a broad spectrum of diseases, encompassing various reactive and malignant disorders. In the present study, we described seven patients with AEBV + CT/NK-LH. All of them are alive after long-term clinical observation and follow-up. These cases had the following characteristics: (1) clinically, the patients were young with a median age of 5.0 years. They were as healthy as ordinary people prior to sickness and had a sudden onset with a short disease course (1–1.5 months) and (2) alleviation without the need for radiotherapy or chemotherapy.

NK/T cell lymphoma occurs mostly in adults and is invasive, progressing rapidly in the clinic and almost always shows an extranodal presentation. The nasal cavity, nasopharynx, paranasal sinuses, and palate were most commonly involved, with the nasal cavity being the prototypic site of involvement [6]. Some cases may be accompanied by secondary lymph node involvement [11–13]. The isolated nodal involvement of NK/T-cell lymphoma is extremely rare, and only a few cases have been described in published work [14–18]. Currently, nodal NK/T cell lymphoma is not considered a distinct disease entity in the WHO classification. NK/T cell lymphoma is highly aggressive, with short survival times and poor response to therapy; therefore, clinical outcomes are dismal. Differentiating between AEBV + CT/NK-LH and ENKTL by morphology and immunophenotype is always difficult; however, the self-limiting character of EBV + TLH is an important clue. For conditions with this presentation, anti-inflammatory or antiviral therapies or follow-up only are likely the best options for management of the case.

Infectious mononucleosis (IM) is an acute disease, usually with EBV infection present predominantly in B cells rather than T cells [19] or rare T cells and NK cells, accompanied by typically high fever, pharyngitis, cervical lymphadenopathy, and resolves spontaneously in a majority of cases. Microscopically, paracortical expansion of the lymph node is obvious with the focal destruction of the lymph node architecture. The infiltrating cells are heterogeneous, including small and large lymphocytes, immunoblasts, histiocytes, and variable numbers of plasma cells and eosinophils [20]. The histological features of IM are so varied that they are sometimes misdiagnosed as malignant lymphoma, especially when numerous large immunoblasts are present. The descriptions of clinical symptoms and prognosis were similar to those of
our present cases, with the exception of the EBV-infected cell types and some morphological features. Although there were two reports describing an IM patient with atypical T-cell proliferation in the nasopharynx, most of the lymphoid cells were also positive for CD2, CD3, CD5, CD7, and without loss of T cell antigen \(^8,^9\). The lesion site, morphological changes, and immune phenotype were different from those in the present cases.

Systemic EBV + T-lymphoma of children in WHO classification \(^5\) and Category B classified by Ohshima et al.\(^7\), develops shortly after primary or acute EBV infection and is accompanied by an aggressive clinical course and atypical lymphoid cell infiltration, and most reported cases showed a monoclonal pattern of T-cell proliferation, progressing toward multiple organ failure, sepsis, and sudden death \(^21–23\). These malignant tumor features overlapped with those of our present cases from the process of clinical manifestations, pathological morphology, to immune phenotype and clone detection, but the present patients achieved remission or recovered without relapse through long-term clinical observation. A similar group of cases has been reported \(^24\), but the patients died quickly, which differs from our study result. The presence of abnormal karyotype will favour a neoplastic condition and is helpful to identify systemic EBV + T-lymphoma of children\(^25\). In present cases, karyotype test of 3 patients showed no abnormality.

In present cases, four patients presented with EBV-associated HLH, which was described as young age and EBV + T/NK cell proliferation with variable clinical findings, including High fever and splenomegaly, cytopenia and liver dysfunction, serological test or the detection of EBV DNA or RNA from the tissues \(^26\).

After anti HLH treatment, 4 cases achieved complete remission. The other 3 cases did not show HLH. In addition to observation and follow-up, they also achieved symptom relief without special treatment. Therefore, AEBV + CT/NK-LH can be shown as the clinical manifestation of HLH, and the prognosis is good after treatment.

Primary EBV-positive nodal T-cell or NK-cell lymphomas have been reported\(^5,^27,^28\). These usually have a monomorphic pattern of infiltration and lack the angiodestruction and necrosis seen in extranodal NK/T-cell lymphoma. They are more common in elderly patients, or in the setting of immune deficiency.

It should be noted that a positive monoclonal population does not necessarily predict malignant behavior because similar populations can be seen in reactive conditions \(^29–32\). We identified a clonal T cell population in four cases (case 1, 2, 5, and 6). Clonality does not necessarily mean malignancy, and our results show that the identification of monoclonal T cell populations is visible in patients with primary EBV infection. Further, it should be noted that prevention of serious complications, such as multiple organ failure, hemophagocytic syndrome, disseminated intravascular coagulation, and sepsis, is very important.

It is important to perform a detailed laboratory examination of either EBV load in the serum or expression of EBV in situ aimed to evaluate such cases of sudden onset course. In the present cases, serological studies were incomplete or had not been performed. EBV-CA-IgM positivity was observed in three patient (patient 1, 2, and 7), and EBV-VCA-IgG positivity in six patients, and six patients showed increased EBV-DNA. EBV serology in such cases, therefore, may be clinically misleading, in that it does not show acute
primary or active infection completely. However, the precipitous onset of symptoms in previously healthy young individuals, as well as the biopsy samples showing infiltration of numerous EBER-positive cells, was suggestive of an acute process. In particular, CD8 positive cells increased, suggesting primary EBV infection. EBNA2 (Epstein-Barr virus nuclear antigens 2) is a gene product expressed in the latent infection state of EBV. As a key transcription factor, it regulates the expression of virus and many genes in cells during the process of virus infection of lymphocytes. Furthermore, EBNA-2 was expressed in four cases with scattered positive nuclei, three of which had increased anti-EBV VCA IgM antibodies. EBNA2 is helpful marker which expressed in patients who have a primary immune response or who are immunocompromised, and IM patients usually have EBNA2 positive cell. Additionally, in the present cases, the indexes of liver function and other correlates of illness severity in EBV infection were incomplete or had not been evaluated. Therefore, patients with high viral load in their blood and with a particularly intense expression of EBV in their vital organs seem to require more intense clinical supervision.

If the present cases were not true lymphomas, it is important to identify whether they represent a proliferative disease with low malignancy potential or perhaps a benign monoclonal disease. The histological characteristics are not the only important standard in the diagnosis of malignancy in these individuals. It should be pointed out that these cases of primary or acute EBV infection require high diagnostic alertness, particularly involving children and young adults. Combining clinical and pathological findings, we hypothesize that the present cases are consistent with T/NK cell-IM. However, the diagnosis of IM requires detailed clinical relevant test results. In this group of cases, some clinical data are incomplete because they are consultation cases. Therefore, we designate this disorder as AEBV + CT/NK-LH.

AEBV + CT/NK-LH could elicit pathologically malignant and clinically benign features. Thus, detailed clinical information (such as age of onset [whether children and young people], nature of onset [whether sudden], disease course [whether short], symptoms [whether systemic], EBV infection status [whether acute], and lymph node involvement) is needed for diagnosis and prognostic evaluation of this disease.

Declarations

Compliance with ethical standards

All procedures were conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later versions. Informed consent or a substitute was obtained from all patients included in the study. Ethical approval was obtained from the ethical review board of Beijing Friendship Hospital, Capital Medical University (No: 2019-P2-006-001).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Contributions
Yan-lin Zhang: Data collection and statistics, diagnosis, manuscript writing and follow-up

Jian-Lan Xie: Data collection and follow-up, TCR analysis

Yuan-yuan Zheng: Data collection and diagnosis

Xiao-Ge Zhou: Diagnosis and Writing guidance

References


Table 3

Table 3 is not available with this version

Figures

Figure 1

CT scan images are from the same patient (case 5). CT scan showing enlargement or fusion of lymph nodes, with no clear boundary and uniform density or necrosis (A). The follow up CT scan showed no clear mass and enlarged lymph nodes (B).
Figure 2

Histologic and immunohistochemical presentations of AEBV+CT/NK-LH from case 4.

Lymph node structure damage (H&E, ×100)(A). An angiocentric and angiodestructive growth pattern was present. The lesion was accompanied by a heavy admixture of inflammatory cells (H&E, ×400)(B). Pleomorphic large atypical cells with irregularly folded nuclei (H&E, ×400)(C & D). Coagulative necrosis displaying multifocality (H&E, ×400)(E). Lymphocyte infiltration in vascular wall (H&E, ×400)(F). Atypical cells exhibiting cytoplasmic CD3 (IHC, ×400)(G). Pleomorphic cells were positive for Granzyme B (IHC, ×400)(H). Large cells were negative for CD5 (IHC, ×400)(I). Atypical cells showing strong CD56 staining (IHC, ×400)(J). Almost all cells showed strong granular staining for CD8 (IHC, ×400)(K). EBNA2 showed scattered positive in EBER+ cell (IHC, ×800)(L).

Figure 3
In situ hybridization for EBV-encoded RNA (EBER). In these lesions, almost all of the irregular cells showed nuclear labeling (IHC, ×400)(A). EBER-positive cells (brown) with expression of CD3 (red) (×800)(B). EBER-positive cells (brown) without the expression of CD20 (red) in the membrane and cytoplasm(×800)(C).