Case Report

Chronic active Epstein-Barr virus infection progresses to aggressive NK cell leukemia with a poor prognosis

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Abstract: Epstein-Barr virus (EBV) associated T/NK-cell lymphoproliferative diseases (EBV-T/NK-LPDs) are a cluster of diseases that include chronic active EBV infection (CAEBV) and aggressive NK cell leukemia (ANKL). The pathogenesis of EBV-T/NK-LPDs is largely unclear and the treatment is difficult and in most cases a hematopoietic stem cell transplantation is needed. Hemophagocytic lymphohistiocytosis (HLH) is known to affect the prognosis of patients with EBV-T/NK-LPDs. This study reports a case of a 20-year-old male patient with repeated infectious mononucleosis (IM)-like symptoms such as high fever, splenomegaly, lymphadenopathy for more than two years. The patient had a high EBV-DNA load (NK cells were the main target cells). He was first diagnosed as CAEBV. However, the disease gradually progressed and the patient developed with high ferritin, phagocytosis and monoclonal NK cells in bone marrow, pancytopenia, increased cytokines, and elevated expression of Ki-67. Also, his NK cells had abnormal immunophenotypes and impaired function. The patient had a typical clinical course of progression from CAEBV to ANKL, accompanied by HLH complications and a poor prognosis. Herein, the detailed diagnostic and differential diagnostic process of EBV infection was shown in this report.

Keywords: EBV associated lymphoproliferative disorder, chronic active Epstein-Barr virus infection, aggressive NK-cell leukemia, hemophagocytic lymphohistiocytosis, LYST mutation

Introduction

As per the 2017 World Health Organization (WHO) classification, sixteen types of diseases are defined as Epstein-Barr virus (EBV) associated lymphoproliferative disorders (EBV-LPDs), including chronic active Epstein-Barr virus infection (CAEBV) of T/NK cell type (systemic form) and aggressive NK cell leukemia (ANKL) [1, 2]. EBV-LPDs (T/NK cell type) were categorized into four categories: A1, polymorphic LPD without clonal proliferation; A2, polymorphic LPD with clonality; A3, monomorphic LPD with clonality; and B, monomorphic LPD with clonality and fulminant course. Categories A1, A2, and A3 together form a continuous spectrum of CAEBV disease [3].

CAEBV is a rare disease with high mortality and appears to be more prevalent in East Asian countries. The clinical forms of CAEBV include a systemic form and two cutaneous forms (hydroa vacciniforme-like LPD and severe mosquito bite allergy) [4]. CAEBV disease is characterized by persistent infectious mononucleosis (IM)-like symptoms such as fever, lymphadenopathy, and hepatosplenomegaly. The course of IM patients usually does not exceed one month, and the disease is self-limiting. However, the clinical course of CAEBV is typically longer than three months with histological evidence of organ disease. CAEBV patients have a high load of EBV-DNA in the peripheral blood (blood plasma and peripheral blood mononuclear cells (PBMC)) and positive EBV-encoded small RNAs (EBERs) expression in the tissues [5]. Under the microscope, EBV infected cells with variable morphology and mostly with mild atypia are visible. The bone marrow smear is either normal or has an increased number of lymphocytes. The diagnosis of CAEBV involves clinical presentations, clinical course, EBV load, and histological evidence. EBV infection of B cells, immunodeficiency, malignancy, and autoimmune disorders should also be excluded. The pathogenesis of CAEBV is not that clear, but
CAEBV progresses to ANKL with a poor prognosis

possibly correlated with NF-κB or JAK/STAT pathway [6]. A recent study showed that CAEBV patients have large number of circulating myeloid-derived suppressor cells with the ability to inhibit T-cell growth, which is likely the reason of the proliferation of EBV infected T/NK cells [7]. CAEBV mainly refers to EBV infection of T and NK cells. T-cell CAEBV is more aggressive. NK-cell CAEBV is more prone to malignant transformation to ANKL or extranodal NK/T lymphoma.

Malignant transformation of CAEBV may occur two years later from the onset. ANKL is one of the most common results of clonal evolution of somatic mutations. ANKL, originating from the mature NK cells, is a rare form of malignant lymphoproliferative disorder and is more prevalent in the countries of East Asia and Central and South America [8]. At present, the number of reported cases of ANKL is less than 400 [9-20]. ANKL has a rapid onset and progress. Clinical features, involved sites, and cellular characteristics are three factors that assist the diagnosis of ANKL. The key point of the diagnosis is to identify NK cells with abnormal immunophenotypes: abnormal expression of CD45 and other antigens on the surface of NK cells, clonal abnormalities of NK cells, and increased Ki-67 expression. The prognosis of ANKL is extremely poor, and the survival period of the patients ranges from 2 weeks to 2 months.

Hemophagocytic lymphohistiocytosis (HLH) is a common life-threatening complication of T/NK-cell hematologic malignancies [21]. It can be part of the initial clinical presentation or arise from disease progression. EBV-associated HLH (EBV-HLH) is caused by excessive macrophage activation and hemophagocytosis, and it is part of EBV-T/NK-LPDs, but it has not included in the classification due to its reactive nature [22]. Patients with HLH have poor prognosis, and present with symptoms such as fever, hepatosplenomegaly, pancytopenia, lymphadenopathy, and a marked increase of cytokines. Lymphohistiocytic infiltrates, hemophagocytosis and EBV-infected cells are visible in histological examinations.

Case presentation

A 20-year-old male patient was admitted to Tongji Hospital (Wuhan, China) due to intermittent fever for over 2 years. The patient had a history of chronic rhinitis, and he was allergic to tetanus antitoxin. He didn’t have specific personal or family history of other diseases.

The patient suffered from repeated fever with temperature up to 40°C, and he felt swelling and pain in the right nose. The results of twice pathological examination of the neof ormation in his nose both indicated chronic mucosal inflammation. He had a high EBV genome load for more than three months. Physical examination showed swelling of the bilateral cervical lymph nodes, no jaundice, no abnormalities in the cardiopulmonary examination, no tenderness of the sternum, and no hepatomegaly. He had splenomegaly (2 cm below the rib cage).

The peripheral blood counts revealed white blood cell (WBC) count was $6.11 \times 10^9$/L, hemoglobin (HGB) concentration was 136 g/L, and platelet (PLT) count was $133 \times 10^9$/L. The serum concentration of ferritin (SF), coagulation test, and the Coombs’ test were normal. Extractable nuclear antigens (ENA) antibody profile and immunoglobulin quantification were normal. Infectologic examinations showed no obvious infection foci. The EBV genome load in PBMC was $>1.0 \times 10^7$ copies/ml and that in the plasma was $6.17 \times 10^5$ copies/ml. With the help of the immunomagnetic beads procedure to sort lymphocytes, we found that the NK cells were the main target cells for EBV ($4.39 \times 10^6$ copies/2$\times 10^5$ cells). Bone marrow smear showed a high proportion of lymphocytes; 22% of the lymphocytes were medium-sized and stained blue with a medium amount of cytoplasm and appeared to have round or sunken nuclei, purple-red particles in the cytoplasm, and false nucleoli (Figure 1A). Flow cytometry analysis revealed that about 34.3% cells (of total karyocytes of bone marrow) expressed CD45, CD2, CD7, CD45R0, CD16dim, CD94, and Perforin (18% positive), without CD3, CD4, CD8, CD5, CD45RA, CD57, CD11b, cCD3, Ki-67 (1.7% positive), CD158a/h, CD158e, and CD158b. The receptor gene rearrangement test of T cells was negative. The karyotype analysis showed 46, XY [20]. Positron emission tomography/computed tomography (PET/CT) examination did not indicate obvious malignant changes.

The patient presented with repeated IM-like symptoms such as high fever, splenomegaly, lymphadenopathy, and high EBV-DNA load for
CAEBV progresses to ANKL with a poor prognosis

more than three months. He had no specific infection foci and no evidence of immunodeficiency, malignancy, and autoimmune disorders. There was no evidence of cytogenetic changes and clonal rearrangement. No characteristic changes were observed in PET/CT scan. Thus, he was diagnosed with CAEBV (NK cell type). After oral antiviral drugs and prednisone treatment, his temperature returned to normal.

One year later, the patient’s condition worsened. The peripheral blood cell counts progressively decreased, with WBC 1.25×10^9/L, HGB 84 g/L, PLT 22×10^9/L. The SF increased to 696.8 ug/L, lactate dehydrogenase (LDH) increased to 405 U/L, and interleukin-6 (IL-6) increased up to 6.66 pg/ml. The EBV genome load was more than 1.0×10^7 copies/ml in PBMCs and 2.68×10^4 copies/ml in the plasma. Bone marrow smear showed reduced proliferation of granulocytes, 0.5% abnormal lymphocytes with increased cytoplasm and thicker particles (Figure 1B), and phagocytosis could be seen. The flow cytometry analysis of the bone marrow revealed that 10.9% of total karyocytes were NK cells with abnormal phenotypes (accounted for 73.2% of lymphocytes) expressed CD45bri, CD2bri, CD7, CD94, CD159a, perforin (94.7% positive), partially expressed CD161, CD8, Ki-67 (38.6% positive), and were not found to express CD3, CD5, CD4, CD45RA, CD45RO, CD57, CD16, CD11b, TCRαβ, TCRγδ, CD115b, PD-L1, CD158e, PD-1, CD158ah, CD159c (Figure 2). The killing activity of NK cells was significantly reduced to 3.44% (normal activity values ≥15.11%) (Figure 3A). Flow cytometry analysis of the peripheral blood showed that the expression of perforin in CD56+CD3- NK cells was reduced (Figure 4A). The expression of granzyme was roughly normal (Figure 4B). Flow cytometry was also used to detect the CD107a degranulation level in NK cells, and the results indicated that the level of degranulation in the resting state was found to be lowered (Figure 4C) and the degranulation level in the activated state was observed within the normal range (Figure 4D).

The patient had a group of abnormal monoclonal NK cells in the bone marrow, and the expression of Ki-67 was increased. And his NK cells had abnormal immunophenotypes and impaired function (killing activity and degranulation). We considered the CAEBV had malignant transformation to ANKL. At the same time, he had a persistent fever (>38.5°C) for more than 7 days, splenomegaly, pancytopenia, increased SF, reduced NK cell activity, and phagocytosis could be seen in the bone marrow. Thus, the diagnostic criteria of HLH had reached [23]. He was finally diagnosed as CAEBV, ANKL, and HLH with LYST mutation. Then, the “HLH-94” regimen (etoposide (VP-16), dexamethasone, and cyclosporine A) was given and his temperature could be controlled. Later, he received an “L-GEMOX” regimen (L-pegaspargase, gemcitabine, and oxaliplatin) chemotherapy.

At the same time, he was also willing to accept haploidentical hematopoietic stem cell transplantation (HSCT). However, Sanger sequencing verification of his parents showed that the LYST mutation came from his father. And the killing activity of his mother’s NK cells was significantly decreased (1.95%) (the normal values ≥15.11%) (Figure 3B). This suggested that his mother may have other HLH gene mutations. So, his parents were both ineligible to act as HSCT donors. Therefore, we initiated the search for a human leukocyte antigen (HLA)-matched...
CAEBV progresses to ANKL with a poor prognosis

donor in the China Marrow Donor Program for him.

Unfortunately, the patient’s condition deteriorated rapidly (Figure 5). The high fever continued, and the hemophagocytic symptoms progressively worsened, with SF up to >50,000 ug/L and PLT decreased to 8×10^9/L. The patient was treated with basiliximab 20 mg/d for 4 days to control HLH. The SF level once decreased to 2667.2 ug/L, but soon rebounded. The patient died ten days after chemotherapy.

**Discussion**

This study reported a 20-year-old male patient who had a typical clinical course of progression

\[ \text{Figure 2. Flow cytometry analysis of the bone marrow revealed that about 10.9\% NK cells with abnormal phenotypes (of total karyocytes, and about 73.2\% of lymphocytes) expressed CD45bri, CD2bri, CD7, CD94, CD159a, perforin, with partial expression of CD161, CD8, Ki-67 (38.6\% positive).} \]
from CAEBV to ANKL, with HLH complication, and a poor prognosis. Due to the rareness and complexity of the disease, it is difficult to diagnose EBV-LPDs in clinical practice. Based on our experience, immunodeficiency screening, the EBV genome load (PMBC and plasma), the main target cells of EBV, the function and activity of NK cells, and bone marrow related examinations should be combined together to make an accurate diagnosis (Figure 6).

CAEBV is a serious clinical disease, and its prognosis is highly variable. For partial patients who remain stable, “Watch & Wait” treatment and minimum supportive care are the only methods. However, the disease eventually relapses [24]. Since ANKL is rare, there is no defined early treatment regimen. At present, the treatment strategy for ANKL has largely been extrapolated from other lymphoid neoplasms such as extranodal NK/T cell lymphoma. Due to the expression of the multidrug-resistant p-glycoprotein, patients with ANKL are resistant to conventional chemotherapies. The regimen composed of antimetabolites may be the effective chemotherapy-based regimen.
CAEBV progresses to ANKL with a poor prognosis

Figure 4. Flow cytometry analysis of the peripheral blood of the patient showed that the expression of perforin in CD56+CD3- NK cells was reduced (A), the expression of granzyme was roughly normal (B). (C and D) Flow cytometry analysis of the CD107a degranulation level in NK cells of the patient. (C) The level of degranulation was lowered in the resting state; (D) The level of degranulation was within the normal range in the activated state.

Figure 5. Dynamic changes of IL-6, ferritin, LDH, Fibrinogen. After L-GEMOX chemotherapy, the HLH-related indicators gradually deteriorated with IL-6 up to 72.79 pg/ml, ferritin >50,000 ug/L, LDH 1867 U/L, fibrinogen decreased to <1.5 g/L. After given bsiliximab 20 mg/d for 4 days to control HLH, IL-6 and ferritin once declined. No obvious change in LDH and fibrinogen. However, SF and IL-6 quickly rebounded.
CAEBV progresses to ANKL with a poor prognosis

Gene sequencing may be helpful for the risk stratification of EBV-T/NK-LPDs. It has been shown that CAEBV and EBV-HLH patients with gene mutations have a poor prognosis. Mutations of EBV-T/NK-LPDs patients were often related to genes about vesicle trafficking, such as UNC13D and LYST [27]. This patient had a missense mutation in the LYST gene. The protein encoded by the LYST gene is involved in the formation of cytotoxic T cells and cytotoxic vesicles of NK cells. The LYST gene is also involved in the transportation of the effectors in exocytosis, which is required for the terminal maturation of perforin-containing vesicles into the secretory cytotoxic granules [28]. LYST gene mutations cause a prolonged synapse time occurs between cytotoxic lymphocytes and leads to overproduction of inflammatory cytokines, in turn further activates mononuclear phagocytes and promotes hemophagocytosis and release of HLH biomarkers, may also lead to the development of HLH [29].

The early identification of HLH and application of the “HLH-94” regimen would reduce early mortality [23]. The early application of VP-16 is extremely crucial. HLH might recur even with the HLH-94 regimen and become more serious. DEP (doxorubicin, etoposide, and methylprednisolone) regimen and basiliximab are salvage therapies to relapsed/refractory HLH [30]. Supportive therapies such as intravenous immunoglobulin and plasma exchange can also help. Although our patient did not have central nervous system (CNS) associated symptoms, such symptoms are very common in HLH [31, 32]. Once CNS is involved, the condition quickly deteriorates, leading to death very shortly. Early lumbar puncture and intrathecal therapy with methotrexate and corticosteroids are conducive to prevent CNS complications [33].

Due to the urgent condition, most of the ANKL patients can only receive haploidentical HSCT. It is important not only to find an HLA-matched donor but also to detect the specific pathogenic genes and NK cell function of the donor. The father of our patient also had the same LYST mutation. This mutation might be inherited from the father.

Figure 6. Diagnostic flow chart of EBV-LPDs. Patients with EBV genome load (+) in peripheral blood (PB) or EBER (+) in tissue, whether with or without symptoms should be further examined. In the diagnostic process of EBV-LPDs, EBV genome load in plasma and PBMC, EBV target cells, and immunodeficiency screening are important (in yellow). Results of bone marrow related examination should also be considered. If the EBV target cells are NK cells, the function of NK cells should be detected. At the same time, patients need to be monitored for malignant transformation from EBV-T/NK-LPDs to NK/T malignancy.
gene mutation, and the killing activities of his mother’s NK cells were significantly decreased. This suggested that neither his father nor his mother was suitable donors. And achieving complete remission (CR) before HSCT is a prerequisite for successful transplantation outcomes. It was reported that ANKL patients with CR before HSCT showed a significantly better survival after two years [34].

Novel approaches to treat ANKL such as proteasome inhibitor, immune checkpoint inhibitors, demethylated drugs, HDAC inhibitor, JAK inhibitor, BCL-2 inhibitor, and aurora kinase inhibitor are already validated in vitro and may be effective to treat ANKL [35-42]. Autologous EBV-specific cytotoxic T cells and chimeric antigen receptor T cell (CART) immunotherapy are other promising therapeutic approaches for ANKL [43].

In summary, CAEBV and ANKL are more like malignant diseases and progress rapidly. Effective treatment and HSCT need to be adopted as soon as possible.

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Disclosure of conflict of interest

None.

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CAEBV progresses to ANKL with a poor prognosis

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