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Case Report

Lymphoproliferative disorders of natural killer cells: report of two cases and review of literature

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Abstract: Lymphoproliferative disorders of natural killer cells (LPD-NK) are rare lymphoproliferative diseases involving NK cells. Here, we present two cases of LPD-NK. The first case is a 63-year-old man who presented with high fever, cytopenia, and a history of myelodsyplastic syndrome. He was finally diagnosed with aggressive NK cell leukemia and died due to progression of the disease within 15 days of diagnosis. The second case is a 70-year-old man with granulocytopenia who did not have clinical manifestations; he was diagnosed with chronic lymphoproliferative disorder of NK cells and a watch and wait approach was adopted until six-month follow up. This article describes the clinical features, pathogenesis, diagnosis, treatments, and prognosis of LPD-NK through a literature review of case reports.

Keywords: Lymphoproliferative disorder of natural killer cells, aggressive natural killer cell leukemia, chronic lymphoproliferative disorder of natural killer cells, diagnosis, therapy

Introduction

Lymphoproliferative disorders of natural killer cells (LPD-NK) are rare disorders involving clonal lymphoproliferations arising from natural killer cells. LPD-NK can be further categorized as aggressive natural killer cell leukemia (ANKL) and chronic lymphoproliferative disorders of natural killer cells (CLPD-NK). The 1999 World Health Organization (WHO) classification included T cell and NK cell granular lymphocytic leukemia in the subgroup of mature peripheral T-cell neoplasms [1]. In 2008, a new classification of CLPD-NK was created by the WHO, distinguishing it from the more aggressive form of NK-cell leukemia [2]. According to the 2016 revision of the WHO classification of lymphoid neoplasms, large granular lymphocytes (LGL) can be further categorized into three disorders: T-cell LGL, ANKL, and CLPD-NK [3]. LPD-NK accounts for no more than 5% of all lymphoid neoplasms. The present article describes two cases of LPD-NK.

Case presentation

Case 1

In April 2018, a 63-year-old man presented with pancytopenia and was admitted to the Department of Hematology in our hospital for examination. His hematological values were as follows: white blood cell count 0.87×10^9/L, hemoglobin 76 g/l, platelets 36×10^9/L, lymphocytes 77%, neutrophils 19.6%, and mean corpuscular volume 116.5 fL. A peripheral blood smear revealed 86% lymphocytes, 12% neutrophils, and 2% monocytes. The biochemical examination and routine blood coagulation test results were normal. Cytomorphological bone marrow examination showed myelodysplastic syndrome (MDS) (**Figure 1**). Bone marrow biopsy pathology revealed ALIP. Flow cytometry analysis of bone marrow revealed that 2.2% of myeloid blasts had positive expression of CD34 and CD117. In addition, Fluorescence in situ hybridization (FISH) and chromosome karyotyping was normal. The patient was diagnosed with MDS with multilin-

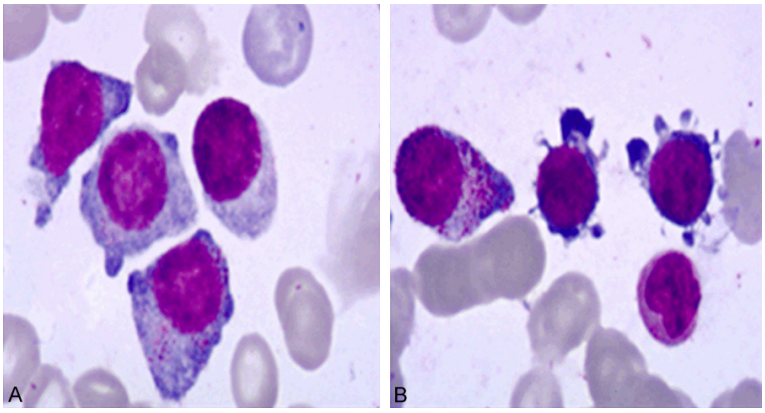

Figure 1. Bone marrow cytology showed MDS. A. This smear displayed a small amount of blast cells. B. The smear showed small megakaryocytes and Pelger-Huet muclear anomaly.

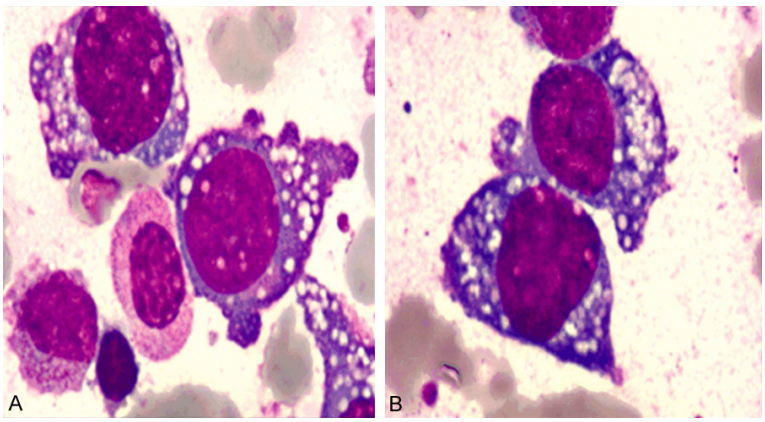

Figure 2. Bone marrow hemocytology showed 10% unclassified abnormal cells. A and B. Bone marrow smears after Wright-Giemsa staining indicated dyshaematopoiesis and some pathological lymphocytes which had a large cell body, visible nucleolus, rich and multi-vacuolated cytoplasm.

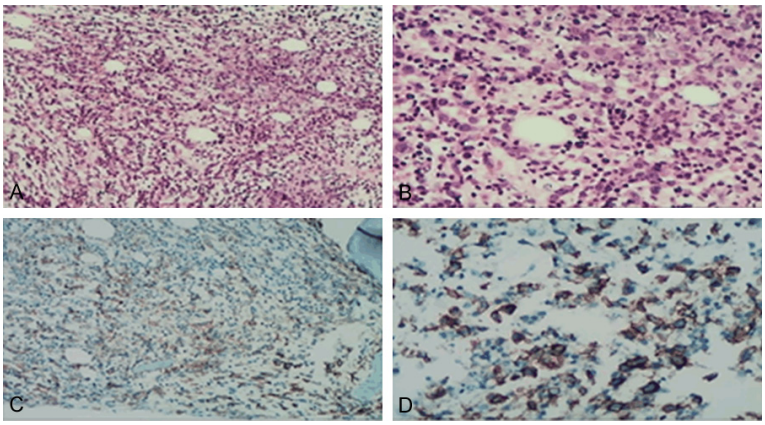

Figure 3. Bone marrow biopsy pathology and immumohistochemical staining revealed ANKL. A, B. Bone marrow pathology after H&E staining indicated that myeloid hyperplasia was markedly active, and atypical cells were distributed in a sheet. C, D. Bone marrow immumohistochemical staining was consistent with the diagnosis of ANKL which showed positive expression of CD56 and negetive expression of CD34, CD3, CD5, CD20, PAX-5, CD30, CD138, BCL-6 and MUM-1.

eage dysplasia (MDS-MLD) and was scored as intermediate-1 risk according to the International Prognostic Scoring System. He received ciclosporin, thalidomide, compound Zaofan pill, and caffeic acid; he refused azacitidine, decitabine, or other therapies.

Four months later, he suffered from high fever. His hematologic parameters values were as follows: white blood cell count 0.35×10^9/L, hemoglobin 59 g/l, platelets 16×10^9/L, neutrophils 0.01×10^9/L. EB-DNA was normal. Bone marrow aspiration showed 10% unclassified abnormal cells with dysplasia in all cell lineages (**Figure 2**). Flow cytometry analysis of bone marrow was negative, indicating that the abnormal cells were too large to be found. Bone marrow biopsy pathology revealed ANKL with positive expression of CD56 and negative expression of CD34, CD3, CD5, CD20, PAX-5, CD30, CD138, BCL-6, and MUM-1 (**Figure 3**). The patient died within 15 days of diagnosis.

Case 2

In June 2019, a 70-year-old man presented with granulocytopenia and was examined by the Department of Hematology in our hospital. His WBC was 3.25×10^9/L with 16% neutrophils and 66% lymphocytes. His HGB was 118 g/l; his PLT were normal (15×10^9/L). A peripheral blood smear revealed that 65% of

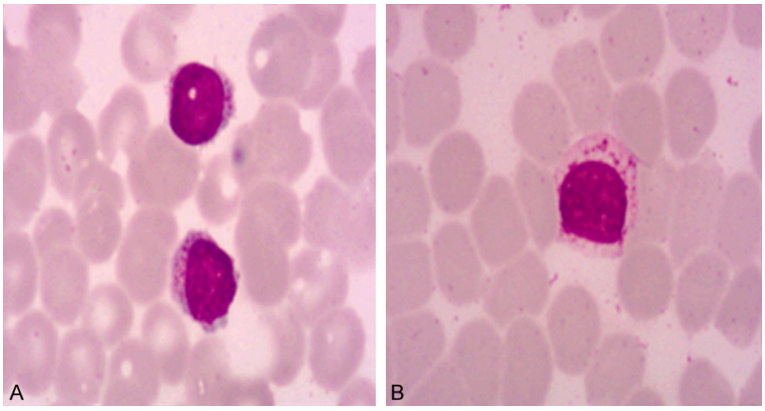

Figure 4. Bone marrow cytology showed large granular lymphocytosis. A, B. Bone marrow smears after Wright-Giemsa staining displayed lymphocytosis and most of these cells had too many particles.

lymphocytes were large granular lymphocytes. The EBV-DNA was normal. Cytomorphologic bone marrow examination and bone marrow biopsy pathology showed lymphocytosis (**Figure 4**). Flow cytometry analysis of bone marrow revealed that 60% of abnormal natural killer cells had positive expression of CD16, CD5, CD7, CD8, and CD94 and negative expression of sCD3, cCD3, CD56, CD4, CD2, NKG2A, NKG2C, CD158a-, CD158b, CD158e, CD57, and CD-161. In addition, PCR direct sequencing assays were performed to identify STAT family genes (STAT1, STAT2, STAT4, STAT5a, STAT5b, and STAT6), JAK/STAT pathway genes (gp130 exon 6, JAK2/V617F, JAK3 exons 13-17, RELA exon 5), and other mutational genes. The results revealed mutation rates for STAT3, ATM, and TCF of 32.1%, 49.9%, and 51.1%, respectively. TCR genes did not exhibit monoclonal rearrangement. A diagnosis of CLPD-NK was made. The patient did not meet the treatment standard and thus, a watch and wait approach was adopted. During the 8-month follow-up period, the patient had no obvious symptoms, and the blood values were not significantly changed.

Discussion

ANKL is a rare malignant lymphoproliferative disorder arising from NK cells. ANKL is highly correlated with Epstein-Barr virus (EBV) and has a very poor prognosis, with a median survival time of less than two months [3]. However, the first case was negative for EBV. A study has shown that there is no difference in the prognosis, morphologic features, and immunophenotypic features of EBV-positive ANKL patients compared to EBV-negative ANKL patients [4].

There is no unified diagnostic standard for ANKL. Diagnosis of ANKL can be made based on eight factors. (1) Patients with ANKL typically present with systemic B symptoms, jaundice, and hepatosplenomegaly. Some patients may also present with lymphadenopathy or pleuroperitoneal fluid, among others. (2) ANKL manifests an extremely aggressive clinical course and has a poor prognosis. (3) Cytomorphologic bone marrow examination and bone marrow pathology show clonal proliferation of large granular lymphocytes arising from NK cells. (4) Immunophenotyping of ANKL usually includes CD3-, CD4-, CD16+/-, CD56+, and CD57-, with a lack of myeloid, T-cell, and B-cell markers. (5) ANKL is highly associated with EBV, although EBV is not necessary for a diagnosis of ANKL. (6) Specific chromosomal abnormalities are lacking. The most common cytogenetic changes in ANKL are long arm deletion of chromosomes 6 and 13. (7) Further, LGL must be excluded for a diagnosis of ANKL [5, 6].

The first case reported here had a history of MDS. This may promote the formation of ANKL. The pathogenesis of ANKL leukemia is unknown. One study analyzed a cytokine array of 39 cases of ANKL. The mutational rates of the JAK/STAT signaling system, TP53, TET2, CREBBP, and MLL2 were 48%, 34%, 28%, 21%, and 21%, respectively. In addition, IL-10 may be able to stimulate the JAK-STAT signaling pathway and the JAK/STAT-MYC-biosynthesis axis may play an important role in the development of ANKL [7].

There is still no standard treatment for ANKL. Common treatment regimens for ANKL include CHOP (cyclophamide, adriamycin, vincristine, and prednisone), SMILE (dexamethasone, methotrexate, ifosfamide, etoposide, and L-asparaginase), and hematopoietic stem cell transplantation (HSCT). Although some patients demonstrate a response to chemotherapy regimens (containing L-asparaginase), they even-

tually die due to disease progression. To date, HSCT is still the most effective treatment for ANKL patients. One study of 21 ANKL patients in the Center of International Blood and Marrow Transplantation Research database who received allogeneic HSCT reported that the two-year progression-free survival rate and the overall survival rate of patients who underwent allogeneic HSCT and achieved complete remission (CR) was significantly better than patients with active disease (30% vs. 0%, 38% vs. 0%). However, only 24% of patients achieved long-term survival [8]. The identification of new treatment options for ANKL is crucial. A study of the ANKL mutational landscape together with drug profiling reported that suppression of the JAK-STAT pathway may be a new therapeutic target. The study revealed that NK cells of ANKL patients were highly sensitive to the combination of JAK inhibitors (ruxolitinib and tofacitinib) and a BCL2 inhibitor (venetoclax) [9]. Other candidate agents include immune checkpoint inhibitors, HDAC inhibitors, and CRA-T cell immunotherapy.

The first patient died as he was unable to receive chemotherapy due to his critical condition. Thus, it is necessary to improve the early diagnosis rate and take active measures to enable the use of HSCT or new drug combinations in order to provide a better prognosis.

CLPD-NK, is a provisional entity. It accounts for 5% of all LGL cases. CLPD-NK has a median onset of 58 years. There is no correlation between CLPD-NK and EBV. Further, CLPD-NK has an inert clinical course and good prognosis; most patients have no clinical manifestations. One study analyzed the clinical characteristics of an international cohort of 70 CLPD-NK patients and found that the most common symptoms in CLPD-NK patients were B symptoms (30%), organomegaly (26%), associated autoimmune diseases (24%), and autoimmune cytopenias (14%) [10].

A diagnosis of CLPD-NK depends on the following observations. (1) The absolute level of peripheral blood NK cells is greater than 0.5×10^9/L and this level is present for more than six months. (2) NK cell immunophenotyping indicates the presence of CD2+, sCD3-, CD3∈+, TCR-αβ-, CD4-, CD8+, CD16+, and CD56+, and the NK cell ratio is more than 40%. (3) Other NK/T cell lymphomas are excluded [11].

The pathogenesis of CLPD-NK is unknown. Human T-cell leukemia virus may contribute to the formation of CLPD-NK. In addition, Jerez found that 30% of CLPD-NK patients had STAT3 mutations [12].

Most CLPD-NK patients only require follow-up and observation. The treatment indications for CLPD-NK are as follows: (1) an absolute neutrophil count (ANC) of peripheral blood NK cells less than 0.5×10^9/L; (2) no recurrent infections if ANC is more than 0.5×10^9/L; (3) patient is symptomatic or has transfusion-dependent anemia; (4) patient has an associated autoimmune disease [11].

There is no standard treatment for CLPD-NK. General treatment approaches include methotrexate (MTX), cyclophosphamide, cyclosporine (CyA), alemtuzumab, splenectomy, and other drugs. Lamy reported that when CLPD-NK patients have no symptoms, watch and wait approach is appropriate. In addition, when patients have treatment indications, it is preferred that a first-line therapy (MTX or cyclophosphamide) is adopted. If the effect is classified as CR or PR after four weeks of treatment, the first-line program is continued for 12 months. If the treatment is judged to be a failure, a second- or third-line solution (CyA, splenectomy, or clinical trial drug) should be adopted instead [11]. CLPD-NK is easy to miss or ignore due to its inert clinical course and lack of symptoms in most patients. For the CLPD-NK patient described here, we adopted a wait and observe approach because he did not meet the treatment indications. CLPD-NK is easy to miss or ignore due to its inert clinical course and lack of symptoms in most patients.

Conclusion

This article reports two cases of LPD-NK. The first patient presented with high fever, cytopenia, and a history of MDS. He was finally diagnosed with ANKL and died of the disorder. The second patient was characterized by with granulocytopenia; he was diagnosed with CLPD-NK and treatment was watch and wait. LPD-NK are rare and complicated hematologic diseases arising from natural killer cells and have no standard treatment. Therefore, it is important

to raise awareness of this disease and adopt suitable treatment strategies.

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Disclosure of conflict of interest

None.

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