CASE REPORT

Leukemic phase of anaplastic lymphoma kinase positive, anaplastic large cell lymphoma

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ABSTRACT

Anaplastic large cell lymphoma (ALCL) is a distinct type of CD30+ T/null-cell non-Hodgkin's lymphoma that frequently involves nodal and extranodal sites. The presence of leukemic phase in ALCL is extremely rare and occurs exclusively with ALK1-positive ALCL. We describe two patients with ALK1-positive ALCL who developed a leukemic phase with rapid progression of the disease. Immunophenotypic pattern assessed on peripheral blood by flow cytometry revealed CD45, CD30, and CD25 positivity in both cases but NPM-ALK1 was expressed in only one case. Both patients developed leukemic phase as a terminal event of the disease and we share the immunophenotypic features of both cases.

KEY WORDS: Anaplastic large cell lymphoma, flow cytometry, leukemic phase

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INTRODUCTION

Leukemic phase in anaplastic large cell lymphoma (ALCL) is extremely rare with only 20 cases reported in the literature.[1-3] Usually ALK-positive ALCL is associated with favorable a prognosis. However, when ALK-positive ALCL presents in its leukemic phase the prognosis is guarded.[1-4] Traditionally, ALK1 positivity is confirmed using immunohistochemistry (IHC) analysis of lymph node and bone marrow biopsy. We report two cases of leukemic phase of ALK1-positive ALCL with immunophenotypic pattern by flow cytometry.

CASE REPORTS

Case 1

A 16-year-old boy presented with 2-months history of fever, abdominal pain, and generalized lymphadenopathy. Hemogram revealed mild anemia (Hb, 10.2 g/dL) with no abnormal cells on peripheral blood smear (PBS). Imaging studies confirmed generalized lymphadenopathy and hepatosplenomegaly. Cervical lymph node biopsy showed effaced architecture by large atypical cells with nuclear convolutions and intermixed polymorphous infiltrate comprising plasma cells, histiocytes, and eosinophils. Tumor cells expressed LCA, CD30, and ALK1 and were negative for CD3, CD5, and CD20. ALK1-positive ALCL-null-cell type was diagnosed. The bone marrow aspirate/biopsy revealed moderately hypocellular uninvolved marrow (substantiated by IHC). Within 10 days, the patient had persistent fever. Complete blood count (CBC) revealed leukocytosis [total leukocyte count (TLC), 50.5×10^9/L] and thrombocytopenia. PBS [Figure 1] showed large atypical lymphoid cells (40%) having deeply basophilic vacuolated cytoplasm with irregular nuclei.

Peripheral blood (PB) immunophenotypic analysis was performed by standard procedure on BD (Becton-Dickinson: BD Biosciences, San Jose, USA)-FASCantoII analyzer using forward scatter (FSC) vs side-scatter (SSC) gating strategy. Various fluorochrome-conjugated (FITC/PE/PerCP. Cy5.5/APC) monoclonal antibodies, such as CD45, CD3, CD4, CD8, CD5, CD7, CD2, CD25, CD56, TCRαβ, TCRγδ, CD16, CD19, CD30, HLA-DR, CD15, and NPM-ALK1 were used in combinations. The tumor cells had higher FSC vs. SCC. Tumor cells expressed CD45, CD30, CD56, CD25, and cyto-NPM-ALK1 and were negative of rest all markers, including CD3 [Figure 3]. This confirmed the leukemic phase of ALK1-positive ALCL-null-cell type. He succumbed to disease within 24 h.

Case 2

A 10-year-old girl presented with history of fever, weight loss, and right inguinal lymphadenopathy for 2 months, which progressed - as an exophytic growth with skin ulceration. Hemogram showed mild anemia, neutrophilic leucocytosis. Bone marrow aspirate/biopsy revealed normocellular uninvolved marrow.

Inguinal lymph node biopsy showed effacement of architecture by small- to medium-sized tumor cells with irregular nuclei. On IHC, tumor cells expressed CD45, CD30, and ALK1 (nuclear), whereas they were negative for CD20 and CD3. The ALK-positive ALCL-null-cell type was diagnosed. She received MCP842 protocol-based chemotherapy and had normal CBC after completion of therapy. Two weeks later, she presented with recurrence of lymphadenopathy and pleural effusion (involved on cytology). CBC revealed leucocytosis (TLC of 89×10^9/L) with 40% atypical mononuclear cells with thrombocytopenia. Tumor cells [Figure 2] were pleomorphic comprising of large cells with intracytoplasmic vacuolation (25%) and small cells (75%) with nuclear irregularity/convolutions. Small cells morphologically resembled monocytic

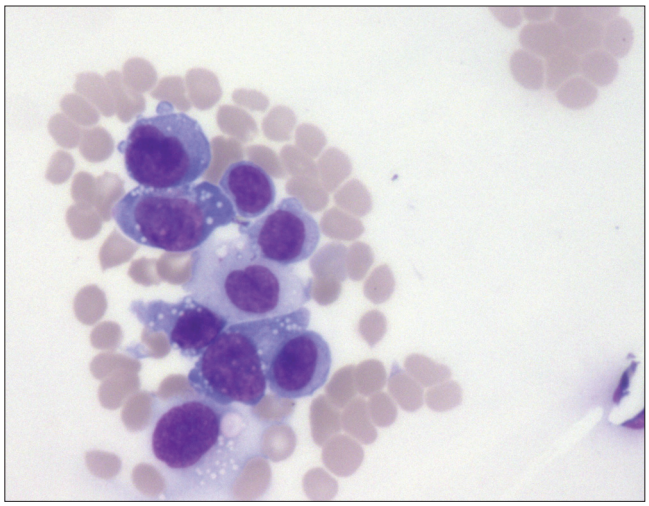

Figure 1: Peripheral blood smear (case 1) showing large atypical lymphoid cells with deep basophilic cytoplasm and cytoplasmic vacuoles (Wright's stain, ×400)

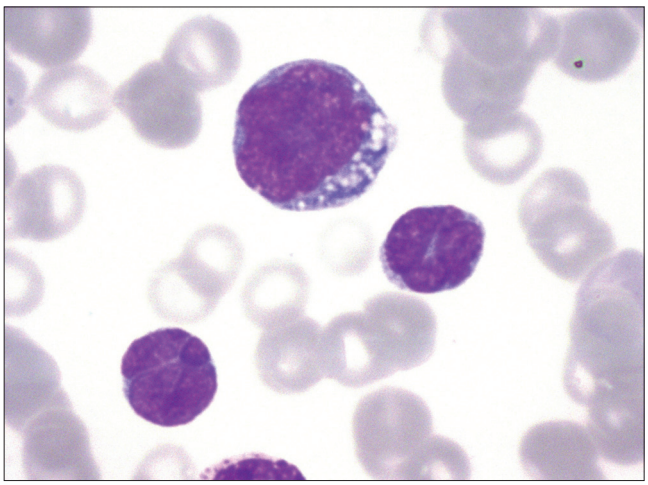

Figure 2: Peripheral blood smear (case 2) showing large atypical lymphoid cell and small monocytoid cells (Wright's stain, ×1000)

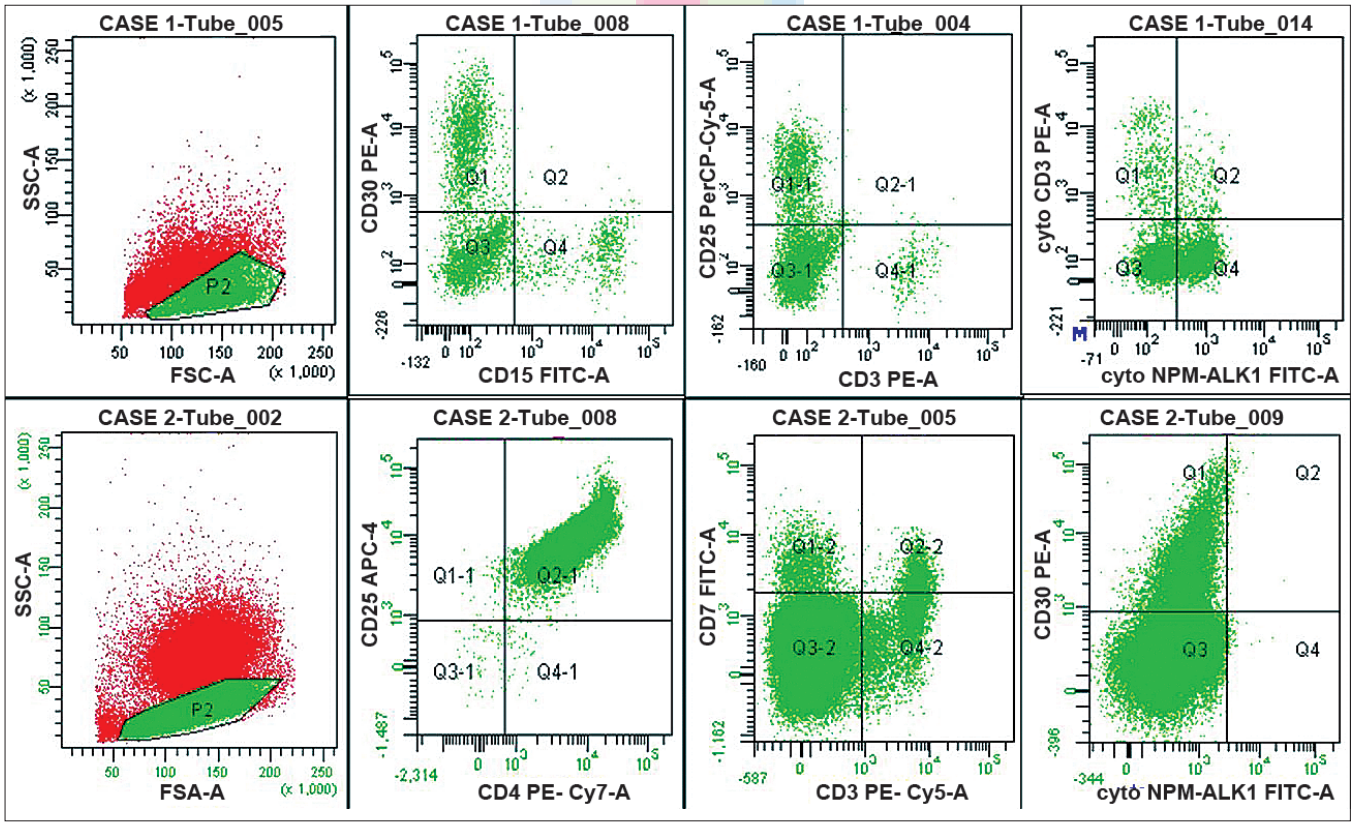

Figure 3: Flow cytometric analysis of peripheral blood. Case 1: Tumor cells gated (green) express CD25, CD30, cytoplasmic NPM-ALK1, and are negative for CD3. Case 2: Tumor cells gated (green) express CD4, CD25, and CD30 but are negative for CD3, CD7, and cytoplasmic NPM-ALK1. *Other positive and negative markers are not shown in this figure

cells. To confirm the nature of these cells, peripheral blood was processed for flow cytometry as per standard protocol. Myeloid markers, such as CD13, CD33, and CD117, were also done. On FSC-SSC, tumor cells were falling in the lymphoid and monocytic region. Tumor cells [Figure 3] expressed CD45, CD30 (21%), CD4, CD2, and CD25 and showed a heterogenous expression of CD117, HLADR, and CD13. Tumor cells were negative for CD3, CD5, CD7, CD8, CD1a, CD56, and CD15. The cytoplasmic expression of NPM-ALK1 could not be demonstrated. This confirmed leukemic phase ALCL-T-cell type. The patient died within 48 h of diagnosis of leukemic-phase ALCL.

DISCUSSION

Subtle bone marrow involvement is seen in 10%-30% ALCL[1-3]; however, it is highly unusual for ALCL to present in the leukemic phase.[1-3] Leukemic phase of ALCL generally affects young males.[1,3-5] and presents with B-type symptoms, particularly high-grade fever.[1,5,6] ALCL may present as leukemic phase at the time of diagnosis, during the course of disease or at relapse.[1,3,6] Patients present with leukocytosis with TLC of $<100 \times 10^9$/L, rarely with TLC of $>100 \times 10^9$/L,[1,3,6] and multiorgan involvement at the time of diagnosis.[1,6]

The leukocytosis with large atypical cells with deep basophilic cytoplasm and vacuolation with thrombocytopenia in both patients were concurrent with reported literature.[1,3,5,6] Lymph node biopsy of case 1 had common pattern and case 2 revealed small cell pattern of ALCL. The leukemic phase of ALCL is commonly seen in small cell variant than other morphological subtypes of ALCL.[2,3,7] The morphology of leukemic cells was comparable with corresponding lymph node histology in both. Also, literature review suggests that circulating lymphoma cells morphologically resemble the tumor cells in the lymph node.[2,4,5] The diagnosis of ALCL with leukemic phase is challenging. Morphological differential diagnoses include conditions with large atypical lymphocytosis, such as infectious mononucleosis, leukemic phase of Burkitt's lymphoma, diffuse large B-cell lymphoma, or peripheral T-cell lymphoma. Diagnosing this requires a high index of suspicion and careful morphological evaluation along with ancillary studies, such as flow cytometry, cytogenetic/molecular techniques.[4]

The T-cell markers, such as CD5, CD7, CD2, CD4, CD8, TCR$\alpha\beta$, and TCR$\gamma\delta$ are efficiently studied by flow cytometry. In ALCL, CD3, commonly used pan T-cell marker, is negative in about 70% cases, whereas CD2, CD4, and CD5 are positive in about 70% cases.[2] With the limited antibody panel used in IHC, understanding of complete immunophenotypic pattern of ALCL cells is not always possible. Morris and colleagues reported the utility of *t*(2;5)/NPM-ALK translocation in ALCL.[6] ALK expression is absent from all postnatal human tissues except rare cells in the brain and its detection indicates pathological expression of tumor cells. Traditionally ALK1 is demonstrated on IHC.[1-3,8,9] ALK1 expression by flow cytometry is described in a few case reports.[1,3,7-10] Juco *et al*.[7] described direct staining for ALK1 expression by flow cytometry on lymph nodes in only 3 cases out of 19 cases of ALCL. Damm- Welk *et al*.[10] evaluated sensitivity and specificity of flow cytometry using antibodies against ALK1 and CD30 for detection of ALCL cells in bone marrow or blood. With FSC vs. SSC gating, tumor cells were mixed with monocytic and even with myeloid cells (confirmed by back gating). Even with the use of CD45 gating with few antibody combinations, a tumor cell separation was not optimal. As ALCL cells appear in the monocytic region of CD45 vs SSC because of their large size and bright CD45-positivity, there is a high chance of missing them or wrong interpretation during analysis.[1,3,6,8,9] As normal hematopoietic cells don't express NPM-ALK1,[2] gating those cells can set a negative cutoff for true positivity. In the first case, in addition to isotypic control, lymphocytes provided the negative cutoff to define the true NPM-ALK1 positivity in tumor cells. However, in case 2, we could not demonstrate ALK positivity due to nonspecific positivity by normal cells. The problem with the demonstration of NPM-ALK1 expression by flow cytometry was mainly related to the fragile nature of neoplastic cells and underrepresented percentage in the cell suspension analyzed. As NPM-ALK1 is intracellular marker, the optimal processing for intracytoplasmic marker may be important. Also, there is a limitation to detect the exact expression of ALK1 by flow cytometry unlike IHC (intracytoplasmic and/or intranuclear).[1,3,6,8,9]

This highlights the role of flow cytometry to study the immunophenotypic pattern in leukemic phase of ALCL using an extensive panel of T-cell markers and other myeloid markers. Thus, flow cytometry has a definite role in the assessment and the confirmation of the diagnosis of leukemic phase of ALCL.

Comment: in our experience we had seen five cases of ALK positive ALCL and all these patients succumbed to disease.

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