Challenge in Diagnosis of CD56+ Lymphoproliferative Disorders

Two Cases of CD56+CD33+ Lymphoma/Leukemia

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• Two cases of CD56+CD33+ leukemia/lymphoma are reported. The patient in case 1 presented with skin rash, diffuse lymphadenopathy, and hepatosplenomegaly. Blasts with monocytoid and lymphoid features were present in the peripheral blood. The tumor cells expressed HLA-DR, CD4, CD33, CD38, and CD56. Cytogenetic analysis revealed del(2)(p13),del(9)(q22),add(6)(q25),add(12)(p12),−13,−18, and −20. The clinicopathologic features were similar to those of blastic natural killer cell leukemia/lymphoma or type 2 dentritic cell leukemia. The patient in case 2 presented with generalized weakness and skin erythema not responding to antibiotics. Circulating blasts with monocytoid features were seen in the peripheral blood. The tumor cells expressed CD7, CD13, CD33, CD38, and CD56, and cytogenetic analysis revealed −5,add(7)(p22),−8, del(10)(p11.2),−12,der(13;14)(p10;p10),+14,−16,−18,−19, and del(20)(q13.1). The clinicopathologic features were consistent with a myeloid/natural killer cell precursor acute leukemia. Both disorders are aggressive hematopoietic malignancies that have similar clinical presentation and morphology but differ in immunophenotype and cytogenetic features.

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CD56+ hematopoietic malignancies are a group of rare and usually highly aggressive malignancies. Based on clinical presentation, immunophenotype, morphology, and cytogenetic analyses, these tumors are classified as malignancies of natural killer (NK) cells, type 2 dentritic cells (DC2), and subsets of T lymphocytes and myeloid cells.[1–3] In general, NK cell neoplasms express CD56 and lack classic T-cell, B-cell, and myeloid markers, whereas subsets of T-cell and myeloid-cell neoplasms express the markers of their own lineages in addition to CD56.[1,4] Recently, DC2 malignancies have been shown to express CD4 and CD56 in the absence of common lymphoid or myeloid lineage markers. DC2 leukemia has the same clinical, immunophenotypic, and cytogenetic features as does blastic NK cell lymphoma/leukemia, as classified by the World Health Organization, and may represent the same entity.[3,5,6] Suzuki et al[4] proposed an NK cell malignancy, which they named myeloid/NK cell precursor acute leukemia, that has clinicopathologic and immunophenotypical features similar to those of blastic NK cell lymphoma/leukemia except that this precursor acute leukemia also expresses the myeloid antigens CD13 and CD33. This entity has been previously described and subsequently recognized by other investigators.[7,8] The differences among blastic NK cell lymphoma/leukemia, myeloid/NK cell precursor acute leukemia, and acute DC2 leukemia are under debate, and their relationships need to be clarified.[1] Here, we report 2 cases of CD56+ CD33+ lymphoma/leukemia, their immunophenotypic and cytogenetic features, and the differences and possible diagnoses.

REPORT OF CASES

Case 1

A 48-year-old Afghan woman without significant past medical history presented in the emergency room complaining of severe right flank pain. Her pain was accompanied by dysuria and urinary urgency and frequency and was relieved after she passed a small renal stone. She had a 3-month history of petechial skin rashes and a 1-week history of generalized weakness. Physical examination revealed diffuse lymphadenopathy and hepatosplenomegaly. There were multiple nonitching petechial rashes present on the chest and back, particularly in areas of pressure. A complete blood count revealed leukocytosis (41 200/μL), anemia (hemoglobin, 8.2 g/dL; hematocrit, 24.1%), and thrombocytopenia (24 200/μL). Blasts with monocytoid and lymphoid features were present in the peripheral blood. A computed tomography scan of the chest and abdomen revealed a small pleural effusion, an enlarged liver, and a spleen with features suggestive of splenic infarcts. Enlarged celiac, portacaval, aortacaval, paraaortic, common iliac, and inguinal lymph nodes were also noted. Serology was negative for human T-cell leukemia virus type 1. The patient subsequently developed headache, new skin rashes, and high fever with signs of pneumonia. A computed tomography scan of the head was unremarkable. Bone marrow and skin biopsies and cytologic examination of cerebrospinal fluid were performed; all revealed malignant cells consistent with acute lymphoma/leukemia. The patient responded to treatment with Ara-C, Idarubicin, and intrathecal Methotrexate. However, the disease relapsed after 6 months of remission, and the patient died 14 months after initial diagnosis.

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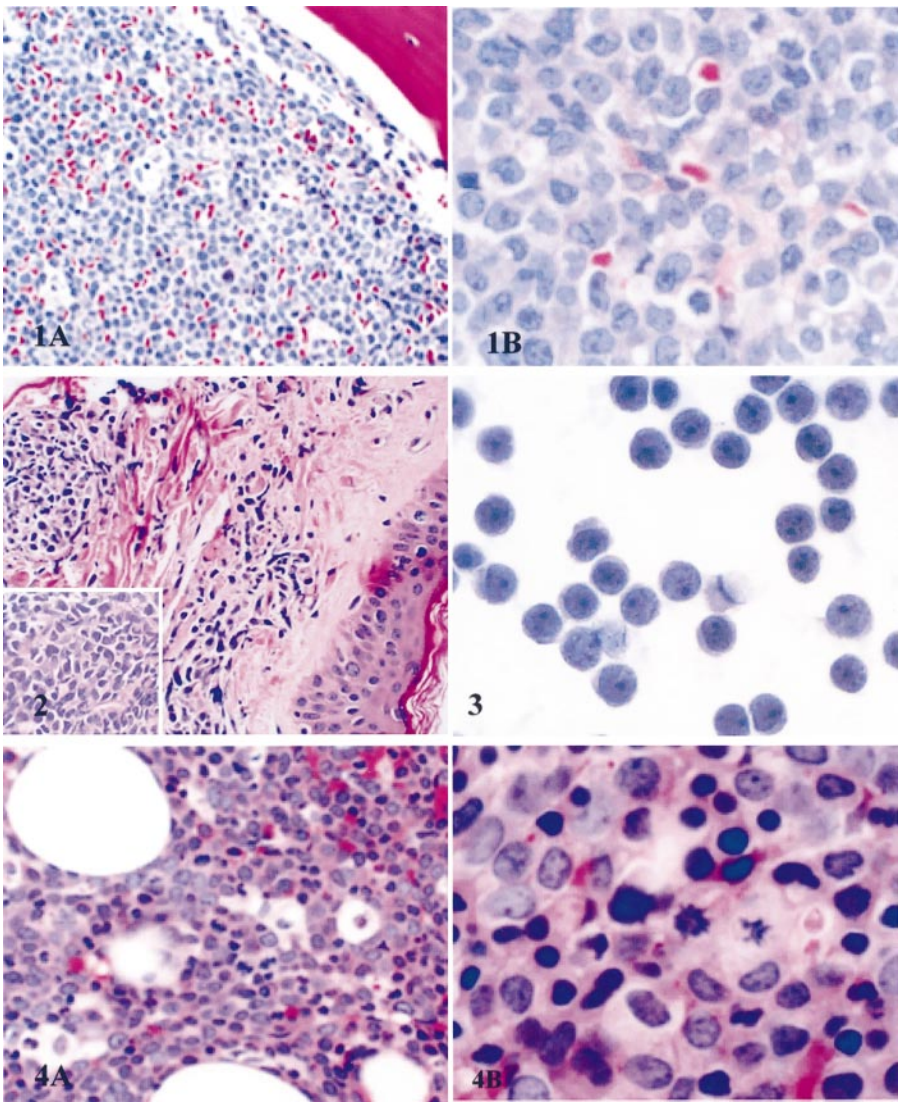

Figure 1. *Bone marrow biopsy from the patient in case 1. A, Bone marrow is completely replaced by blasts. B, Nuclei of tumor cells are irregular with fine chromatin and small nucleoli. Mitoses and intracellular vacuoles are common (hematoxylin-eosin, original magnifications ×200 [A] and ×400 [B]).*

Figure 2. *Skin biopsy from the patient in case 1. Atypical lymphoid cells infiltrate the dermis and spare the epidermis. Inset: Lymphoid cells have scanty cytoplasm and irregular nuclei (hematoxylin-eosin, original magnification ×200).*

Figure 3. *Cytology of cerebrospinal fluid from the patient in case 1. Tumor cells have scanty cytoplasm and prominent nucleoli (Papanicolaou stain; original magnification ×400).*

Figure 4. *Bone marrow biopsy from the patient in case 2. A, Bone marrow is infiltrated by medium-sized lymphoblastoid cells. B, Tumor cells with a scanty pale cytoplasm, mostly round nuclei, finely dispersed chromatin, and inconspicuous nucleoli (hematoxylin-eosin; original magnifications ×200 [A] and ×400 [B]).*

Case 2

A 68-year-old white woman with a past medical history of chronic back pain, asthma, and hepatitis C presented with right foot ''cellulitis'' that did not respond to oral antibiotics. Physical examination revealed no lymphadenopathy, hepatosplenomegaly, or skin lesions. A complete blood count revealed a white blood cell count of 8800/μL, hemoglobin of 13.8 g/dL, and a platelet count of 177 000/μL. The differential blood cell count revealed 16% neutrophils, 30% lymphocytes, 41% monocytes, and 10% blasts. The peripheral smear contained blasts with monocytoid features without definite granules present in the cytoplasm. The bone marrow was infiltrated by similar blasts. The patient was treated with Myelotarg (monoclonal antibody to CD33) without response and died of neutropenic sepsis and multiorgan failure soon after admission.

PATHOLOGIC FINDINGS

Histology and Cytology

The bone marrow biopsy from the patient in case 1 revealed a hypercellular marrow (100%) composed almost exclusively of blasts with lymphoid features. The blasts were medium to large and had a moderate amount of pale cytoplasm. The nuclei were irregular with fine chromatin and small nucleoli. Intranuclear vacuoles and mitoses were frequently seen (Figure 1). There were no identifiable azurophilic granules or Auer rods. A skin biopsy revealed atypical lymphoid cells infiltrating the dermis and sparing the epidermis (Figure 2). The same atypical lymphoid cells with prominent nucleoli were identified in the cerebrospinal fluid cytology specimen (Figure 3). The bone marrow biopsy from the patient in case 2 revealed patchy infiltrates of medium to large lymphoblastoid cells. The tumor cells had a small amount of pale to light blue cytoplasm. The nuclei were oval to round with inconspicuous nucleoli. The chromatin was finely dispersed, and mitoses were frequently seen (Figure 4, A). Azurophilic granules were not apparent on the Giemsa-stained bone marrow aspiration slides (Figure 4, B).

Immunophenotype

Flow cytometric analysis of the bone marrow aspiration specimen from the patient in case 1 revealed a blast population that made up 90% of the cells, based on CD45 expression versus side scatter. These blasts expressed HLA-DR, CD2, CD4, CD33, CD38, CD56, and cytoplasmic CD3. Flow cytometric analysis after relapse revealed a similar immunophenotype except for loss of CD33 ex-

Table 1. Immunophenotype of Bone Marrow Biopsy Determined by Flow Cytometry*

Marker	Case 1	Case 2
CD2	+	−
CD3	−	−
CD4	+	−
CD5	−	−
CD7	−	+
CD8	−	−
CD10	−	−
CD11c	−	+
CD13	−	+
CD14	−	−
CD15	−	−
CD16	−	−
CD19	−	−
CD20	−	−
CD25	−	−
CD30	−	−
CD33	+†	+
CD34	−	+
CD38	+	+
CD56	+	+
CD57	−	−
CD61	−	−
CD64	−	−
CD117	−	+
CyCD3	+	+
TdT	−	−
HLA-DR	+	+
Ig κ and λ	−	−
MPO	−	w+

* CyCD3 indicates cytoplasmic CD3; TdT, terminal deoxynucleotidyl transferase; Ig, immunoglobulin; MPO, myeloperoxidase; and w, weak.
† CD33 was negative after relapse.

pression. In the patient in case 2, the tumor cells expressed CD7, CD11c, CD13, CD33, CD34, CD38, and CD56. In both cases, the tumor cells were negative for terminal deoxynucleotidyl transferase, surface CD3, CD5, CD8, CD10, CD16, CD19, CD20, CD25, CD57, and κ and λ light chains (Table 1).

Gene Rearrangement Studies

In case 1, possible rearrangements in the T-cell receptor (TCR) and immunoglobulin genes were evaluated using restriction fragment length polymorphism and Southern blot analyses. The genes of TCRβ, TCRδ, immunoglobulin heavy chain, and immunoglobulin light chain were in germline configuration, indicating lack of monoclonality for either the T-cell or B-cell lineages. Gene rearrangement analyses were not done in case 2.

Cytogenetic Analysis

Chromosome analysis was performed using the G-banding technique. In case 1, chromosomes of 14 cells in metaphase from the bone marrow culture were analyzed. Thirteen of these cells had the following chromosome pattern: 43,XX,del(2)(p13),del(9)(q22),add(6)(q25),add(12)(p12),−13,−18, and −20. In case 2, 18 of 20 cells had the following chromosome pattern: 42,XX,−5,add(7)(p22),−8, del(10)(p11.2),−12, der(13;14)(p10;p10),+14,−16,−18,−19, and del(20)(q13.1).

COMMENT

In the absence of the common T-cell, B-cell, and myeloid cell markers, CD56 is usually used to identify NK cells. NK cell neoplasms are a distinctive group of highly aggressive lymphomas/leukemias characterized phenotypically by $CD2^+$, surface $CD3^-$, $CD56^+$, and cytoplasmic $CD3\varepsilon^+$ and etiologically by a strong association with the Epstein-Barr virus.[1,9] Blastic NK cell lymphoma/leukemia, as classified by the World Health Organization, has features similar to those of NK cell lymphoma/leukemia but is more immature, often expresses CD4, sometimes expresses terminal deoxynucleotidyl transferase, and lacks an association with the Epstein-Barr virus.[1,2] Clinically, it presents with systemic involvement, including extensive bone marrow and skin involvement. It does not express the classic T-cell, B-cell, and myeloid cell markers such as CD3, CD13, CD19, CD33, immunoglobulin, and T-cell receptor. Cytogenetically, it has target chromosomes similar to those in other NK cell neoplasms (chromosomes 6, 11, 13, and 17), especially in the region of 6q21–25, which has been recognized as a nonrandom aberration for these tumors.[10,11] However, the diagnosis of blastic NK cell lymphoma/leukemia has recently been challenged by some authors who believe that $CD4^+CD56^+$ malignancies constitute a novel disease entity arising from DC2 instead of NK cells.[3,5,6] Published reports suggest that blastic NK cell lymphoma/leukemia and DC2 lymphoma/leukemia are indistinguishable clinically, immunophenotypically, and cytogenetically and likely represent the same disorder. Cytoplasmic $CD3\varepsilon$, a cellular marker used to exclude DC2, was frequently present in blastic NK cell lymphomas. Another $CD56^+$ lymphoma/leukemia, proposed by Suzuki et al[4] as myeloid/NK cell precursor acute leukemia, also has considerable overlap in clinical presentation, morphology, and immunophenotype with blastic NK cell lymphoma/leukemia. Currently, the relationship of these 2 entities is unclear and the diagnosis relies on subtle differences in immunophenotype: myeloid/NK cell precursor acute leukemia expresses myeloid markers CD13 and CD33 in addition to CD56 and frequently expresses CD7 and CD34. Furthermore, cytogenetic studies have indicated that the most common chromosomal abnormalities in myeloid/NK cell precursor acute leukemia are on chromosomes 7, 3, 10, and 5,[7,12] in contrast to the most common chromosomal abnormalities seen in NK cell neoplasms, including blastic NK cell lymphomas (Table 2). Clinically, both are

Table 2. Immunophenotype and Cytogenetic Abnormalities in Blastic Natural Killer (NK) Cell Lymphoma and Type 2 Dentritic Cell (DC2) Leukemia Versus Myeloid/NK Cell Precursor Acute Leukemia

	Blastic NK Cell Lymphoma, DC2 Leukemia	Myeloid/NK Cell Precursor Acute Leukemia
Immunophenotype	$CD2^+$, $CD4^{+/-}$, $CD11c^-$, $CD33^-$*	$CD7^+$, $CD4^-$, $CD11c^{+/-}$, $CD13^{+/-}$, $CD33^+$, $CD34^+$
Cytogenetic abnormalities†	6q, 13q, 11q, 17p, 9q, 1, 2q, 19p, 20q, 12q, 15p, 18, 21	7p, 3p, 10, 9, 18, 21

* Some tumors with CD33 expression were diagnosed as blastic NK cell lymphoma.
† Chromosome abnormalities listed in the order of frequency reported in the literature.

highly aggressive, although myeloid/NK cell precursor acute leukemia seems to have a worse prognosis than does blastic NK cell lymphoma/leukemia, which has shown some response to the treatment regimens used for lymphomas, especially Ara-C regimens.[13,14]

The patients in the 2 cases presented in this report both had multisystem involvement, including bone marrow, skin, and peripheral blood, although in the patient in case 2 lymphadenopathy and hepatosplenomegaly were not present on physical examination and rapid deterioration of the patient's condition prevented further investigation. Morphologically, the tumor cells from the patient in case 1 were of medium size with pale cytoplasm, irregular nuclei, and conspicuous nucleoli. Immunophenotypically, the tumor cells expressed CD4, CD56, and cytoplasmic CD3, without expressing other T-cell and B-cell antigens. Cytogenetic analysis of these tumor cells revealed chromosome aberrations in the regions (6q25) commonly found in NK cell neoplasms. However, the tumor also expressed CD33, a myeloid antigen disqualifying it as a blastic NK cell lymphoma according to the new World Health Organization classification.[1] The lack of expression of CD7 and the aberrant chromosomal findings make the diagnosis of myeloid/NK cell precursor leukemia less tenable.[4] This cytogenetical abnormality is more suggestive of blastic NK cell lymphoma/leukemia. The immunophenotype of the tumor cells after relapse support this diagnosis. In case 2, the bone marrow biopsy revealed medium to large tumor cells with scanty pale to pink cytoplasm, minimally irregular nuclei, finely dispersed chromatin, and inconspicuous nucleoli. The cells expressed CD7, CD13, CD33, and CD56 and had cytogenetic abnormalities of chromosomes 5, 7p, and 10p, indicative of myeloid/NK cell precursor acute leukemia.

The rarity of $CD56^+$ neoplasms and lack of knowledge of the cell origin renders these lesions difficult to classify, particularly in cases of blastic NK cell lymphoma, myeloid/NK cell precursor acute leukemia, and recently defined DC2 lymphoma. As described here, these 3 entities are all highly aggressive and have identical clinical presentations. Because of a wide spectrum of cytologic features in lymphoma/leukemia as a whole, morphology alone cannot reliably differentiate among these lesions. Whether subtle differences in immunophenotype can be used to separate them is highly debatable. They probably are closely related neoplasms developing at different stages of maturation with expression of different (quantitatively and qualitatively) antigens. It is not uncommon for the immunophenotypic profile of tumor cells to change slightly after relapse, as seen in case 1. Some tumors are difficult to classify into any of the diagnostic entities as currently defined.

The prognostic and therapeutic significance of differentiating among these entities is not clear. Patients with these diseases rarely survive more than 2 years after initial clinical presentation even after intensive chemotherapy or radiation therapy. The patient in case 1 was treated with Ara-C and Idarubicin. Intrathecal Methotrexate was used because of central nervous system involvement. She responded well intially but relapsed in 6 months and died 14 months after the initial diagnosis. The patient in case 2 was treated with Myelotarg, a monoclonal antibody against CD33 used in treating myeloid malignancies,[15] but she did not respond to this treatment.

In summary, diagnosis and classification of $CD56^+$ neoplasms are difficult because of similar clinicopathologic features. Currently, differentiation is achieved by noting subtle differences in immunophenotype. These 2 cases of $CD56^+CD33^+$ neoplasms reported here are consistent with blastic NK cell lymphoma/leukemia and myeloid/NK cell precursor acute leukemia, respectively.

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