Neutrophil-Rich Ki-1–Positive Anaplastic Large Cell Lymphoma

A Study by Fine-Needle Aspiration Biopsy

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Key Words: Cytology; Cytopathology; Fine-needle aspiration; Anaplastic large cell; Non-Hodgkin lymphoma; Cytogenetic; Immunohistochemistry

Abstract

Fine-needle aspiration biopsy (FNAB) is an accurate, cost-effective method of evaluating lymphomas. The neutrophil-rich variant of anaplastic large cell lymphoma (NR-ALCL) is a rare non-Hodgkin lymphoma. To our knowledge, we present the first study of NR-ALCL by FNAB cytology. Histologic confirmation was available for both patients. Both cases were positive for Ki-1 (CD-30) and were either T-cell or null-cell phenotype. FNAB specimens were highly cellular with a single-cell pattern composed of pleomorphic tumor cells, “hallmark” tumor cells, and a background rich in neutrophils that occasionally obscured tumor cells. Diagnosis on FNAB is difficult owing to the rarity of this tumor, its resemblance to Hodgkin lymphoma and other non-Hodgkin lymphomas that express CD30, its similarity to an infectious process, and its occasional confusion with metastatic carcinoma and melanoma. Reproducible cytologic features usually are present, and the diagnosis can be made conclusively by FNAB in conjunction with ancillary studies.

Anaplastic large cell lymphoma (ALCL) is an uncommon non-Hodgkin lymphoma (NHL) with both systemic and cutaneous forms. The cutaneous form is an indolent, progressive, and incurable disease.1 The systemic form may involve lymph nodes or extranodal sites, is aggressive, and is associated with a specific t(2;5)(2p23;5q35) cytogenetic translocation that is much less common in the cutaneous form.1 Systemic ALCL with the t(2;5) translocation is associated with a younger age group and a better prognosis than is ALCL without this translocation.2-5 ALCL may have numerous morphologic types and immunophenotypes and is defined by strong CD30 positivity in both a membranous and paranuclear distribution. However, CD30 positivity in non-Hodgkin lymphoma (NHL) is not a specific marker for ALCL, and rather more defines a heterogeneous group of NHLs.

CD30+ ALCL initially was described as neoplasm of pleomorphic cells, including Reed-Sternberg–like cells, with a tendency to involve lymph node sinuses.6 In addition to this classic variant, ALCL has been subdivided into numerous less common morphologic variants, including the monomorphic, small cell, sarcomatoid, lymphohistiocytic, Hodgkin-related, and neutrophil-rich (NR) subtypes.7-12 NR-ALCL was first described by Mann et al11 in 1995, and subsequently, only a few reports of NR-ALCL have been published. These reports include cutaneous, systemic, and HIV-associated forms 1Table 1.10,11,13-15 It is not known what causes tissue neutrophilia in NR-ALCL, although some authors speculate that interleukin-8 has a role or that chemoattractants are released by tumor cells or endothelial cells.11 NR-ALCL has been associated only rarely with peripheral neutrophilia and is not related to infection.11 It does not seem that NR-ALCL has a different behavior or overall prognosis from other variants of ALCL.
Creager et al / NEUTROPHIL-RICH Ki-1–POSITIVE ANAPLASTIC LARGE CELL LYMPHOMA

Fine-needle aspiration biopsy (FNAB), in conjunction with ancillary studies including flow cytometry and immuno-cytologic analysis, is becoming an accepted, accurate, cost-effective procedure for evaluating both primary and recurrent NHL.16-19 The sparse published cytologic studies of ALCL include both exfoliative and FNAB specimens.20-27 To our knowledge, no reports describing the cytologic features of NR-ALCL have been published. We present the FNAB findings, with tissue and immunohistochemical confirmation, of 2 cases of primary systemic (noncutaneous) NR-ALCL, one a primary diagnosis and the other a recurrent case. The differential diagnosis and immunocytochemical and cytogenetic findings are emphasized.

Case Reports

Case 1

A 36-year-old woman sought care because of a 6-week history of nonproductive cough and was treated for allergic rhinitis. When symptoms failed to resolve, a chest radiograph revealed a mediastinal mass. An 8.3 × 4.1 cm mediastinal mass was confirmed by computed tomography (CT) at Wake Forest University Baptist Medical Center, Winston-Salem, NC. Abdominal and pelvic CT revealed only mild splenomegaly with no reference to focal splenic lesions or exact splenic size. Physical examination was otherwise normal with no adenopathy or splenomegaly. Laboratory studies revealed the following: WBC count, 13,800/µL (13.8 × 10³/L); hemoglobin, 8.7g/dL (87 g/L); hematocrit, 27.7% (0.27); mean corpuscular volume (MCV), 66 µm³ (66 fL); and platelet count, 448 × 10³/µL (448 × 10⁹/L). Blood urea nitrogen, creatinine, and lactate dehydrogenase values were within normal limits.

A diagnostic CT-guided FNAB of the mediastinal mass was performed along with a needle core biopsy. Bilateral bone marrow biopsies and aspirations were negative for malignant lymphoma. The bone marrow confirmed the presence of iron deficiency anemia with no evidence of increased myeloid precursors or increased mature neutrophils. The patient underwent chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisone. She was alive with disease 11 months after initial diagnosis.

Case 2

A 36-year-old woman was diagnosed by excisional biopsy with stage IV ALCL in December 1996 at Wake Forest University Baptist Medical Center. The patient underwent multiple chemotherapy regimens followed by autologous peripheral blood stem cell rescue in January 1998. A recurrence was diagnosed by FNAB from a left cervical lymph node in April 1999. She subsequently received both radiation therapy to her left neck and EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin) chemotherapy. EPOCH was tolerated poorly and discontinued after 1 course. She then was given pulse dexamethasone therapy and had a good response.

Routine follow-up in December 2000 revealed a 6.5-cm firm mass in the upper outer quadrant of the left breast. The remainder of the physical examination revealed a chronically ill appearing white woman in no acute distress. Vital signs

Table 1
Summary of Neutrophil-Rich Variant of Anaplastic Large Cell Lymphoma Cases in the Literature

<table>
<thead>
<tr>
<th>Author</th>
<th>Sex/Age (y)</th>
<th>HIV Status</th>
<th>Anatomic Site</th>
<th>ALK or t(2;5) Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mann et al</td>
<td>F/52</td>
<td>Negative</td>
<td>Skin</td>
<td>ND</td>
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<tr>
<td>F/36</td>
<td>Negative</td>
<td>Lymph node, skin</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>M/48</td>
<td>Positive</td>
<td>Muscle, skin</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>F/24</td>
<td>Negative</td>
<td>Lymph node</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>M/72</td>
<td>Negative</td>
<td>Retroperitoneum</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>M/31</td>
<td>Negative</td>
<td>Lung</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Akhtar et al</td>
<td>M/56</td>
<td>Unknown</td>
<td>Testis</td>
<td>ND</td>
</tr>
<tr>
<td>M/59</td>
<td>Unknown</td>
<td>Lymph node, skin</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Simonart et al</td>
<td>M/74</td>
<td>Unknown</td>
<td>Lymph node</td>
<td>ND</td>
</tr>
<tr>
<td>McCluggage et al</td>
<td>M/39</td>
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<td>ND</td>
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<tr>
<td>F/29</td>
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<td></td>
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<tr>
<td>F/61</td>
<td>Unknown</td>
<td>Lymph node</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Jhala et al</td>
<td>M/44</td>
<td>Positive</td>
<td>Skin</td>
<td>Negative ALK</td>
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<tr>
<td>M/41</td>
<td>Positive</td>
<td>Skin</td>
<td>Negative ALK</td>
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<td>Present study</td>
<td>F/36</td>
<td>Negative</td>
<td>Mediastinum</td>
<td>Negative ALK and t(2;5)</td>
</tr>
<tr>
<td>F/36</td>
<td>Negative</td>
<td>Breast (recurrence)</td>
<td>Negative ALK</td>
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</tr>
</tbody>
</table>

ALK, anaplastic lymphoma kinase; ND, not done.
were normal. There was no palpable adenopathy or splenomegaly, and no masses were found in the right breast. Laboratory studies revealed the following: WBC count, 8,200/µL (8.2 × 10⁹/L); hemoglobin, 12.7 g/dL (127 g/L); hematocrit, 36.9% (0.369); MCV 94.1 µm³ (94.1 fL); and platelet count, 172 × 10³/µL (17.2 × 10⁹/L). The differential count revealed the following: neutrophils, 81% (0.81); lymphocytes, 13% (0.13); monocytes, 5% (0.05); and eosinophils 1% (0.01). Blood urea nitrogen, creatinine, and lactate dehydrogenase values were within normal limits. A diagnostic FNAB of the left breast mass was performed. Bilateral bone marrow biopsies and aspirations demonstrated a normocellular marrow with trilineage hematopoiesis. Neither increased myeloid precursors nor increased mature neutrophils were identified, and no evidence of lymphoma was observed. The patient was alive with recurrent disease 55 months after initial diagnosis.

Materials and Methods

Tumors were aspirated by a cytopathologist using 22- to 25-gauge needles attached to a 20-mL syringe using an aluminum syringe holder or were aspirated by a radiologist using CT guidance. FNAB smears were fixed in 95% ethanol and stained using the Papanicolaou technique or air-dried and stained using the rapid Romanowsky method. The remainder of the aspirate material was rinsed into saline, and cytocentrifuged preparations were prepared. Material was fixed in 10% neutral-buffered formalin for cell block preparations and submitted for histologic examination. Aspirate material was obtained specifically in case 1 for cytogenetic evaluation. In both cases, histologic sections of tumor were available for review. Five-micrometer-thick tissue sections from paraffin-embedded tissue and cell-block material were stained with H&E, acid-fast, and methenamine silver stains.

Immunohistochemical studies were performed on needle core biopsy material (case 1) or paraffin-embedded histologic sections from a previous surgical specimen (case 2) using the avidin-biotin-peroxidase complex method described previously. The primary antibodies included CD3, CD20, CD30, CD45RO, CD68, Epstein-Barr virus (EBV) latent membrane protein (LMP), anaplastic lymphoma kinase (ALK)-1 (p80), cytokeratin AE1/AE3, leukocyte common antigen (LCA), epithelial membrane antigen (EMA) (DAKO, Carpinteria, CA), CD5 (Novocastra, Burlingame, CA), and CD15 and CD43 (Becton Dickinson, San Jose, CA). The specificity of the immunoreactions was verified by staining known positive and negative control tissue sections.

Likewise, formalin-fixed, paraffin-embedded sections from both cases were evaluated for the presence of the EBV-encoded RNA-1 (EBER-1) using a 30-base oligonucleotide probe that recognizes a non-poly(A) RNA EBV transcript expressed in latently infected cells (Operon Technologies, San Pablo, CA).

Results

FNAB smears were essentially identical in both cases. Smears were highly cellular and consisted of 2 major components. The first component consisted of a cellular population of malignant discohesive single pleomorphic tumor cells and bizarre wreath-like cells are evident at this magnification. Abundant neutrophils are observed in the background (rapid Romanowsky, ×200).

EBV-encoded RNA-1 (EBER-1) using a 30-base oligonucleotide probe that recognizes a non-poly(A) RNA EBV transcript expressed in latently infected cells (Operon Technologies, San Pablo, CA).29

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Results

FNAB smears were essentially identical in both cases. Smears were highly cellular and consisted of 2 major components. The first component consisted of a cellular population of malignant discohesive single pleomorphic cells in combination with small relatively loosely cohesive clusters of up to 20 similar cells

![Image 1](Case 1) A cellular population of discohesive tumor cells is present in the smears. Pleomorphic tumor cells and bizarre wreath-like cells are evident at this magnification. Abundant neutrophils are observed in the background (rapid Romanowsky, ×200).

![Image 2](Case 2) A population of cells with nuclei ranging from small to large with well-defined nuclear membranes with atypical convolutions were dispersed throughout the smear and were better observed in Papanicolaou-stained smears. The cells were quite variable in morphologic features and size. The nuclei generally were hyperchromatic with irregular chromatin distributions. Nucleoli were conspicuous, and, in rare cells, macronucleoli larger than 7 µm in diameter were present. One to 3 nuclei were identified per cell and were centrally or eccentrically placed. Nuclear morphologic features varied dramatically and included pleomorphic mononucleated giant tumor cells with multiple nuclear lobulations, neoplastic cells with multiple nuclei, wreath-like forms, and horseshoe shapes. In addition, a population of cells with nuclei ranging from small to large with round nuclei and well-defined nuclear membranes with atypical convolutions were dispersed throughout the smear and were better observed in Papanicolaou-stained smears. The
large majority of cells had minimal to moderate amounts of pale-staining basophilic cytoplasm. Erythrophagocytosis was not observed. Numerous benign-appearing lymphocytes and occasional lymphoglandular bodies were scattered throughout the background in both cases. The second component of the smears was a thick population of mature-appearing neutrophils that focally obscured tumor cells. There was no evidence of karyorrhexis or coagulative necrosis.

H&E-stained sections of paraffin-embedded sections from both cases demonstrated both malignant cells similar to those in the smears and a dense neutrophil infiltrate associated with malignant cells. No acid-fast bacilli or fungal organisms were identified by either acid-fast or Gomori methenamine silver–stained, paraffin-embedded sections of either patient’s tumor.

Immunohistochemical stains performed on needle core biopsy material from case 1 demonstrated positive immunoreactivity in tumor cells for CD30 and CD3. No immunoreactivity was observed for CD15, CD20, CD45 EMA, cytokeratins AE1/AE3, ALK-1, CD-68, or EBV-LMP. In situ hybridization failed to detect EBV EBER-1 RNA sequences. All controls stained appropriately.

In case 2, immunohistochemical stains performed on paraffin-embedded histologic sections demonstrated positive immunoreactivity in tumor cells for CD30 and focal scattered LCA immunoreactivity. No immunoreactivity was observed for CD3, CD5, CD15, CD20, CD43, CD45RO, EMA, ALK-1, CD68, or EBV-LMP. In situ hybridization failed to detect EBV EBER-1 RNA sequences. All controls stained appropriately.

**Discussion**

The understanding of Ki-1–positive ALCL has undergone some change within the last 10 years, owing mostly to the demonstration and significance of the t(2;5) translocation and associated ALK dysregulation. ALCL has a T-cell phenotype in the majority of cases and a null-cell phenotype in the remaining cases, although many immunophenotypically null-cell cases contain T-cell receptor gene rearrangements.7 HIV-related, Hodgkin-related, and other secondary forms of ALCL usually are negative for ALK.7 More than 70% of cases with ALK gene dysregulation are positive for EMA, whereas EMA positivity is much less common in ALCL without ALK gene dysregulation. The presence of t(2;5) is not specific for ALCL and can be seen rarely in large B-cell lymphomas that occasionally also express CD30.7 Large B-cell NHL that demonstrates ALK gene dysregulation tends to have morphologic and clinical features more consistent with diffuse large B-cell lymphoma rather than ALCL and should be classified as such.30

Since CD30+ lymphomas are a heterogeneous group of neoplasms, CD30 expression does not define ALCL; rather, its expression should alert the pathologist to look for ALK expression, as this finding apparently defines an NHL associated with...
a younger age group and a more favorable prognosis when associated with a T-cell or null-cell phenotype.

ALK expression has not been described in NR-ALCL. Only 2 reported cases of NR-ALCL have been examined for the expression of ALK, and both were cutaneous and associated with HIV infection, a group in which ALK gene dysregulation is classically absent. Both tumors in the present series of systemic, non–HIV-associated NR-ALCL demonstrated classic pleomorphic cytology, were either T-cell or null-cell phenotype by immunohistochemical analysis, and were negative for ALK. Cytogenetic evaluation of FNAB material from case 1 failed to detect the presence of the t(2;5) translocation.

The diagnosis of ALCL can be difficult on FNAB material for several reasons: numerous variants of ALCL exist, extranodal sites may be involved, and morphologic overlap with other entities exists. The cytology of ALCL has been defined in studies of both exfoliative and FNAB specimens. Since the first description of NR-ALCL in 1995, only a few reports have been published (Table 1). To our knowledge, we present the first FNAB study of this variant.

From a practical standpoint, the most important factor in evaluating NR-ALCL using FNAB specimens is the initial triage of the specimen. Ancillary studies are of paramount importance in making this diagnosis with aspirate material, and the pathologist must be conscious of this when triaging smears. NR-ALCL has reproducible cytomorphologic features and is identical to classic pleomorphic ALCL, with the exception that tumor cells are admixed within a background rich in neutrophils. The nuclei of “hallmark” cells are characterized as reniform, embryo-like, and horseshoe-like with distinct nuclei that were easily identifiable in cytologic preparations in the present series (Image 2). Initial low-power examination of smears demonstrates a pattern of discohesive tumor cells suggesting the possibility of lymphoma, although a single-cell pattern sometimes is observed in carcinoma. Identifying hallmark cells and dispersed single pleomorphic cells, especially when from nodal material, should alert the pathologist to obtain appropriate material for ancillary studies. At the minimum, tissue should be obtained for direct smears to evaluate the morphologic features, a cell block for immunohistochemical analysis, and possible T-cell receptor gene rearrangement studies and flow cytometric analysis. In addition, cytogenetic analysis for presence of t(2;5) should be considered. Demonstration of anaplastic cytomorphologic features in conjunction with CD30 expression, T-cell phenotype, and/or LCA positivity help secure the diagnosis, especially in conjunction with ALK and/or EMA immunoreactivity. T-cell antigens can be difficult to demonstrate in ALCL, and flow cytometry can be quite helpful, especially since immunoperoxidase techniques are not available or are unreliable for many T-cell markers in paraffin-embedded tissue. The diagnosis of null-cell ALCL should be reserved for tumors that do not demonstrate T-cell and B-cell markers by immunohistochemical analysis and flow cytometry or T-cell gene rearrangements.

The differential diagnosis on aspirated material includes high-grade NHL; Hodgkin lymphoma, especially the suppurative type; carcinoma; malignant melanoma; and, more remotely, an infectious process. Care must be taken to avoid overlooking malignant single cells that may be obscured by a thick background of neutrophils. Infectious possibilities can be easily ruled out by the demonstration of clearly malignant cells. Ancillary studies including negative acid-fast and fungus stains and negative microbiologic cultures support a noninfectious process.

The distinction between Hodgkin lymphoma and NR-ALCL may be difficult, and there is some crossover between the morphologic features and the immunophenotype of these entities. Accordingly, a provisional diagnosis of ALCL, Hodgkin-like, was created by the revised European-American classification of lymphoid neoplasms. In aspirates of Hodgkin lymphoma, the number of neoplastic cells in the smears usually is much lower than what is seen in specimens of ALCL. Neutrophil infiltrates are observed fairly commonly in Hodgkin lymphoma, but usually not to the extent observed in NR-ALCL. EMA is expressed commonly in ALCL and usually is only focally and weakly positive, if at all, in Hodgkin lymphoma. EBV is not observed in ALCL and is frequently present in Hodgkin lymphoma. LCA and T-cell markers are negative in classic
Hodgkin lymphoma by definition, and CD15 is much less commonly positive in ALCL than in Hodgkin lymphoma. To summarize, immunoreactivity for CD45, T-cell antigens, EMA, and CD30 with negativity for CD15 and EBV supports the diagnosis of NR-ALCL, whereas classic Hodgkin lymphoma usually is positive only for CD30, CD15, and EBV. In addition, to our knowledge, ALK-1 immunoreactivity and the t(2;5) translocation have not been reported in Hodgkin lymphoma.

The distinction between carcinoma and NR-ALCL may not always be straightforward on cytologic smears. High-grade carcinomas are frequently necrotic, contain abundant neutrophils, and can have a dispersed, single-cell pattern. EMA positivity in ALCL tumor cells may cause the pathologist to favor a diagnosis of carcinoma. If lymphoid markers CD30 and ALK-1 are performed in conjunction with pan-cytokeratins, the distinction should be clear. Furthermore, hallmark cells are unexpected in carcinoma.

Differentiation between ALCL and large-cell NHL should be routine; however, occasional difficult cases will be encountered. Rare large-cell NHL can express CD30, blurring the line between ALCL and other NHLs. Occasionally, large-cell NHL can manifest with abundant neutrophils, suggesting the diagnosis of NR-ALCL. Immunohistochemical stains for EMA, ALK-1, and B- and T-cell antigens and cytogenetics can be of great help in such cases.

The distinction from malignant melanoma is straightforward when pertinent clinical data and immunohistochemical support are available.

We believe NR-ALCL has reproducible cytologic features, specifically, malignant, discohesive, single pleomorphic cells and small relatively loosely cohesive clusters of up to 15 cells that demonstrate marked pleomorphism. Scattered hallmark cells dispersed among a dense background of neutrophils are present. In the present study, when cytologic smears were used as the primary diagnostic modality (1 case) the diagnosis of NR-ALCL was straightforward and confirmed histologically. Ancillary studies are invaluable adjuncts in the evaluation of these otherwise diagnostically problematic tumors.

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References


