ALK-Positive Anaplastic Large Cell Lymphoma With Primary Bone Involvement in Children

Nasir A. Bakshi, MD,1 Charles W. Ross, MD,2 William G. Finn, MD,2 Riccardo Valdez, MD,2 Robert Ruiz, MD,2 Khaldoun Koujok, MD,3 and Bertram Schnitzer, MD2

Key Words: Anaplastic large cell lymphoma; Anaplastic large cell lymphoma kinase; ALK; Primary bone lymphoma; Childhood lymphoma; Neuron-specific enolase

Abstract

We describe the clinical, radiologic, and pathologic features of primary bone anaplastic large cell lymphoma (ALCL) in 3 boys. Radiologic imaging showed lytic lesions involving sacrum, femur, or rib. Bone was the only site of disease in 2 cases; an associated partial lymph node was involved in case 3. Differential diagnoses included osteomyelitis and small round cell tumors of childhood, particularly Ewing sarcoma. Preoperatively, ALCL was not a diagnostic consideration in any case. Two cases showed classic large pleomorphic cells; 1 showed a composite pattern with a distinct small cell component and the more typical large cell type. Neoplastic cells in all cases showed strong CD30 and anaplastic lymphoma kinase expression with relatively weak epithelial membrane antigen positivity. Cytotoxic granule protein was expressed in 2 cases. All cases showed unusually strong expression of neuron-specific enolase (NSE). Two patients were disease-free at last follow-up (15 months and 11 years); 1 patient died of disseminated disease within a year of diagnosis. ALCL should be considered a diagnostic possibility when evaluating neoplastic bone lesions in children. Although expression of NSE in ALCL has not been emphasized in the literature, it is worth noting because it may pose a diagnostic pitfall.

Anaplastic large cell lymphoma (ALCL) is a distinct clinicopathologic entity of non-Hodgkin lymphoma, one that has been included in the World Health Organization (WHO) classification as a T-cell neoplasm.1,2 Most previous studies of childhood ALCL have shown that they constitute 10% to 15% of all childhood lymphomas.3-7 ALCL in children is characterized clinically by a predominance of B symptoms and by frequent extranodal involvement, both primary and secondary, when the disease is disseminated. Because of the tendency to rapid progression and the frequency of B symptoms, most cases of ALCL in children are treated with high-grade B-cell non-Hodgkin lymphoma protocols with CHOP-type (cyclophosphamide, doxorubicin [Adriamycin], vincristine, prednisolone) regimens.7 Although non-Hodgkin lymphomas, both B- and T-cell types, frequently involve the bone marrow, they rarely produce localized bone lesions. Non-Hodgkin lymphomas of bone constitute approximately 5% of malignant bone tumors (in all age groups).8,9 There are few reports in the literature of primary bone presentation of ALCL in children. We report the pathologic and clinical characteristics of ALCL in 3 children with primary bone presentation. The diagnosis of ALCL was based on morphologic and immunologic criteria defined by the WHO classification.

Materials and Methods

Cases

A search of the surgical pathology archives of the Department of Pathology, University of Michigan, Ann Arbor, from July 1991 to June 2003 identified 3 pediatric (age <18
years) patients with primary presentation of ALCL in bone. The cases of children with secondary involvement of bone were excluded.

Clinical Staging

All patients underwent a full staging workup, including physical examination, CBC count, biochemical profile, bone marrow aspirate, and cerebrospinal fluid examination. Imaging was done by x-ray, computed tomography (CT), or magnetic resonance imaging (MRI). Clinical characteristics of the 3 cases are given in Table 1.

Histopathologic Examination

We stained 3- to 5-µm-thick sections from formalin-fixed and paraffin-embedded tissue with H&E. The diagnosis of ALCL was based on the established morphologic and immunohistochemical criteria as defined by the WHO classification of tumors of hematopoietic and lymphoid tissues.2 In all 3 cases, the slides were reviewed concurrently by 4 hematopathologists (N.A.B., C.W.R., W.G.F., and B.S).

Immunohistochemical Analysis

In all cases, immunohistochemical analysis was performed on deparaffinized sections with a panel of monoclonal antibodies using the avidin-biotin complex method on a Ventana ES automated slide stainer (Ventana Medical Systems, Tucson, AZ). The panel of immunohistochemical stains is given in Table 2. Immunohistochemical findings were interpreted by consensus of all authors.

Cytogenetic and Molecular Studies

Conventional cytogenetic studies were performed on the bone biopsy sample in case 1 by Giemsa trypsin G-banding procedures as described previously.10 Karyotypes were recorded according to the International System of Human Cytogenetic Nomenclature.11 In situ hybridization studies to detect Epstein-Barr virus (EBV) RNA were performed using a 30-base oligonucleotide probe for EBV-encoded RNA (EBER-1). The test was performed with the automatic Ventana Benchmark and the Inform EBER probe (Ventana Medical Systems). T-cell receptor gene rearrangement studies by Southern blotting were done in cases 2 and 3. The DNA used for gene rearrangement studies was extracted from frozen tissue specimens according to standard procedures.12 Purified DNA was digested with BamH1, EcoR1, and HindIII restriction enzymes. After digestion, the DNA was size fractionated by agarose gel electrophoresis, transferred to a nylon membrane, and analyzed with radio-labeled probes according to standard methods.12,13 T-cell receptor gene arrangement analysis was carried out with a probe for the constant regions of the T-cell receptor β-chain gene.14 Human placental DNA was used as a germline control sample.

### Table 1

<table>
<thead>
<tr>
<th>Case No./ Sex/Age (y)</th>
<th>Site/ Manifestations</th>
<th>Imaging</th>
<th>Differential Diagnosis</th>
<th>LDH at Diagnosis</th>
<th>Bone Marrow Status (Initial Staging)</th>
<th>Treatment</th>
<th>Survival and Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/3</td>
<td>Sacrum (S1-3)/bone pain and mass</td>
<td>Multiple osteolytic lesions</td>
<td>Ewing sarcoma, sacrococcygeal tumor</td>
<td>High (&gt;1,000 U/L)</td>
<td>+</td>
<td>Chemothry</td>
<td>Alive, 15 mo</td>
</tr>
<tr>
<td>2/M/9</td>
<td>Proximal femur/bone pain</td>
<td>Single osteolytic lesion</td>
<td>Acute osteomyelitis, Ewing sarcoma</td>
<td>NA</td>
<td>–</td>
<td>Chemotherapy and radiation</td>
<td>Died of disease, 1 y</td>
</tr>
<tr>
<td>3/M/14</td>
<td>Rib (8th)/bone and soft tissue mass</td>
<td>Bone mass and osteolytic lesions</td>
<td>Ewing sarcoma, eosinophilic granuloma</td>
<td>Normal (196 U/L)</td>
<td>–</td>
<td>Chemotherapy</td>
<td>Alive, 11 y</td>
</tr>
</tbody>
</table>

LDH, lactate dehydrogenase; NA, not available; +, positive; −, negative.
Results

Case 1

A 3-year-old boy was examined because of a 10-day history of pain in his left buttock and lower extremity. The initial physical examination findings were unremarkable. He did not have lymphadenopathy or hepatosplenomegaly. The serum lactate dehydrogenase (LDH) level was elevated to more than 1,000 IU/L. CT and MRI scans revealed a mass measuring 3.0 × 4.0 × 4.0 cm centered in the left sacrum and invading the sacrospinal canal with compression of the sacral S1 and S2 nerve roots. The CT of the chest, abdomen, and pelvis showed no evidence of mass or lymphadenopathy. A CT-guided biopsy of the sacral mass was performed, and a diagnosis of ALCL was confirmed. A staging bone marrow biopsy was positive for involvement by lymphoma.

The patient was given induction chemotherapy with vincristine, prednisone, cyclophosphamide, intrathecal cytarabine, daunorubicin, and L-asparaginase. Six months after diagnosis, seventh nerve palsy developed. MRI revealed central nervous system involvement by the lymphoma, and he was given additional intrathecal chemotherapy. His condition improved, pain subsided, and the serum LDH level returned to normal. He subsequently was discharged with a follow-up in the clinic for outpatient therapy.

Case 2

A 9-year-old boy had a lytic lesion of the right proximal femur measuring 6.5 × 3.0 × 3.0 cm. The clinical differential diagnosis included osteomyelitis and Ewing sarcoma. There was no evidence of lymphadenopathy or hepatosplenomegaly. Results of the biopsy of the lesion were consistent with ALCL.

He was treated with combination chemotherapy including cis-platinum, L-asparaginase, cytarabine, etoposide, and dexamethasone. He also received radiation therapy. His tumor was unresponsive, and later skin lesions developed, which were proven by biopsy to be ALCL. Subsequently, palpable lesions developed on his head, neck, and extremities. These lesions were clinically consistent with disseminated lymphoma. A bone marrow biopsy and aspiration 7 months after initial presentation showed the presence of lymphoma in the bone marrow. Owing to persistent fevers and an immunosuppressed state, further chemotherapy was withheld. Progressive respiratory decline developed, and he died of disease 1 year after initial presentation.

Case 3

A 14-year-old boy was examined because of a 1-month history of a left-sided chest wall mass that had been treated with antibiotics for 2 to 3 weeks. A firm, 3.0 × 3.0 × 2.0-cm, fixed, nodular mass was noted in the left anterior portion of the chest wall, seeming to arise from the fifth rib. There also was a firm, nontender mass in the left axilla. The clinical differential diagnosis included Ewing sarcoma. Radiographs showed a destructive lesion in the left anterior fifth rib. Subsequently, he underwent a complicated excision of the left axillary lymph node and left anterior fifth rib mass. Frozen section biopsy was suggestive of an eosinophilic granuloma. There was no mediastinal mass, and the serum LDH level was within normal limits. A bone marrow biopsy showed no evidence of lymphoma.

After histologic confirmation of ALCL, he was treated with cyclophosphamide, vincristine, methotrexate, and prednisone. Central nervous system prophylaxis was provided with 6 intrathecal doses of methotrexate. He did not receive radiation therapy. Further staging studies showed no evidence of disease elsewhere. At his last follow-up, he was disease-free, 11 years after initial presentation.

Histopathologic and Immunohistochemical Findings

Biopsies in cases 1 and 2 revealed diffuse sheets of large pleomorphic cells. Characteristic hallmark tumor cells were identified in all cases. A biopsy in case 3 revealed a composite pattern with a distinct small cell component along with the more typical anaplastic large cell type. Substantial infiltration of adjacent soft tissues was identified in biopsy specimens from cases 1 and 2. Case 3 also showed...
Bakshi et al / PRIMARY BONE ALCL

partial involvement of an adjacent axillary lymph node. Because of the location and pattern of involvement, it was thought to be secondarily involved by direct spread from the rib.

All cases were positive for CD30 Image 3Al. T-cell markers CD2, CD4 Image 3Bl, and CD45RO were expressed only by the small cell component of ALCL in case 3. The neoplastic cells in all cases expressed anaplastic lymphoma kinase 1 (ALK-1). The ALK-1 staining was nuclear and cytoplasmic in cases 1 Image 3Cl and 3; in case 2, the staining was cytoplasmic Image 3Dl. The small and large cell components in case 3 showed ALK-1 staining, suggesting that the small cells are part of the histologic spectrum of ALCL. Epithelial membrane antigen was expressed strongly in case 2 Image 3El and showed a weak membranous staining pattern in cases 1 and 3. Cytotoxic granule protein (TIA-1) was expressed weakly in 2 of 3 cases Image 3Fl. All cases showed unusually strong cytoplasmic expression of neuron-specific enolase (NSE). Results of immunohistochemical analysis are summarized in Table 3.

Cytogenetic and Molecular Findings

Cytogenetic studies confirmed the presence of t(2;5) (p23;q35) in 17 of 20 cells analyzed in case 1. Monoclonal T-cell receptor β-chain gene rearrangement was identified in cases 2 and 3 by using the Southern blot technique. In situ hybridization for EBV was negative in all 3 cases.

Discussion

Primary lymphomas of bone constitute approximately 5% of all primary malignant bone tumors. They can pose diagnostic difficulties with other more common entities, including Ewing sarcoma, which they simulate radiologically. In adult and pediatric populations, the majority of these intraosseous lymphomas are non-Hodgkin lymphoma of diffuse large B-cell type. ALCL in children sometimes can involve the bone marrow (10%-15%), and, in advanced stages, it can produce destructive bony lesions. It is, however, extremely rare for ALCL to manifest as a primary bone lesion. Few cases, adult or pediatric, have been identified in previous studies.15-21 Only 3 studies included pediatric patients (1 case each) Table 4.15-17 In this report, we describe in detail 3 children with ALCL who had primary bone involvement at presentation.

At initial presentation, staging studies confirmed that all but 1 of our patients had no evidence of disease outside of bone. The only patient (case 3) with extraskeletal disease had accompanying lymphadenopathy, which was more consistent with secondary involvement than with primary nodal disease. Preoperatively, ALCL was not a diagnostic consideration in any case. The initial clinical differential diagnosis of the 3 cases included Ewing sarcoma, eosinophilic granuloma, and acute osteomyelitis. The latter was a strong diagnostic consideration in 1 of the cases that initially presented with only localized bone pain (case 2). Radiologically, all 3 cases presented with osteolytic bone lesions. One patient had multifocal bone involvement (case 1), and one had an adjacent soft tissue mass in addition to lytic bone lesions (case 3). Bone lesions may occur in the axial and appendicular skeleton.

Histologically, the pattern of involvement was similar to ALCL at other sites. In addition to the large pleomorphic lymphoma cells, characteristic hallmark cells were easily identified in all cases. Interestingly, 1 of our cases (case 3) showed a composite pattern with a distinct small cell component.
Image 3 Immunohistochemical analysis. A (Case 1), Strong reactivity in the majority of tumor cells with anti-CD30 (×400). B (Case 3), Small cell component showing positive staining with anti-CD4 (×100). C (Case 1), Anaplastic lymphoma kinase 1 (ALK-1); nuclear and cytoplasmic staining (×400). D (Case 2), ALK-1; only cytoplasmic staining (×200). E (Case 2), Epithelial membrane antigen; membranous staining (×200). F (Case 3), TIA-1 (cytotoxic granule protein); granular cytoplasmic staining (×200).
admixed with the more typical large cell type. In this case, only the large cell component was positive for CD30 by immunohistochemical analysis, whereas the small cell component expressed T-cell antigens (CD2, CD4, and CD45RO), TIA-1, and ALK-1. In case 2, bone marrow involvement was identified histologically 7 months after the initial diagnosis, and this was confirmed by positive immunohistochemical stains for CD30 and ALK-1. Another noteworthy finding in our study was the weak and rather infrequent expression of cytotoxic protein, TIA-1. It had been proposed that most cases of ALCL originate from lymphocytes with cytotoxic potential, and, according to some studies, the expression of cytotoxic proteins correlated with ALK-1 expression. However, our study and similar other studies of bone ALCL in older age groups do not support such a correlation. Our findings also suggest that, unlike nodal ALCL, ALK-1 positivity may not necessarily be a favorable prognostic feature for patients with primary bone ALCL.

Strong expression of NSE was found in tumor cells of all 3 cases. This feature, although reported in the literature, has not been emphasized in the past. It may pose a diagnostic pitfall. For example, a substantial fraction of ALCL specimens may be negative for leukocyte common antigen (CD45). Positive stains for NSE or epithelial membrane antigen may lead to diagnostic confusion, particularly if only a limited screening panel of immunohistochemical markers is used. NSE, therefore, should not be relied on in the differential diagnostic workup of pediatric malignant small round cell tumors, including Ewing sarcoma, neuroblastoma, embryonal rhabdomyosarcoma, and lymphoma. In a series by Lucas et al., 2 of 3 cases of bone lymphoblastic lymphoma initially were misdiagnosed as Ewing sarcoma; NSE did not distinguish these 2 entities. Massarelli et al. studied the expression of NSE in a large series of malignant lymphomas, including Hodgkin lymphoma. NSE showed diffuse cytoplasmic distribution in CD30+ Reed-Sternberg cells, whereas the L&H cells of nodular lymphocyte predominant Hodgkin lymphoma always were negative. Among the non-Hodgkin lymphomas, NSE positivity was found only in lymphomas expressing CD30. No relationship was found between NSE and B or T immunophenotype. In a study of T-cell leukemia/lymphoma, suggested that serum NSE is produced preferentially by the malignant T cells and that it might be a novel marker of disease aggressiveness and a prognostic factor for T-cell lymphomas.

We characterized 3 uncommon pediatric cases of ALCL manifesting primarily with osteolytic bone lesions. All cases were positive for NSE. ALCL should be considered a diagnostic possibility when evaluating neoplastic bone lesions in children.

From the 1Department of Pathology, Oklahoma University Health Sciences Center, Oklahoma City; and Departments of 2Pathology and 3Radiology, University of Michigan, Ann Arbor.

Address reprint requests to Dr Ross: Dept of Pathology, 1301 Catherine St, 5242 Medical Science I, University of Michigan, Ann Arbor, MI 48109-0602.

References


