Hematopathology / Hemophagocytosis and ALCL

Mediastinal Adenopathy, Lung Infiltrates, and Hemophagocytosis

Unusual Manifestation of Pediatric Anaplastic Large Cell Lymphoma: Report of Two Cases

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Abstract

To date, only 1 report describes an anaplastic large cell lymphoma (ALCL) associated with hemophagocytosis in the pediatric population. To better characterize this unusual manifestation of ALCL, we identified 2 additional cases. Both patients had fever, cytopenia, decreased fibrinogen level, mediastinal or hilar adenopathy, minimal to no peripheral adenopathy, and lung infiltrates. Bone marrow biopsies and aspirates revealed striking hemophagocytosis but no ALCL. One patient fulfilled the criteria for hemophagocytic syndrome, but the other lacked 1 criterion. Both patients were initially given a misdiagnosis of infection-associated hemophagocytosis. Definitive diagnosis required lymph node biopsies that showed CD30+, anaplastic lymphoma kinase-1+ ALCL. Both tumors responded to standard lymphoma chemotherapy. One patient achieved complete remission, whereas the other patient died of complications after 2 cycles of therapy. These findings are similar to the first reported case and indicate that pediatric ALCL can manifest with an unusual constellation of symptoms consisting of hemophagocytosis, mediastinal or hilar adenopathy, and lung infiltrates.

Hemophagocytic lymphohistiocytosis (HLH), also known as hemophagocytic syndrome, is a disorder characterized by a proliferation of histiocytes and hemophagocytosis in bone marrow, liver, spleen, and lymph nodes. The Histiocyte Society has established minimal clinicopathologic criteria for the diagnosis of HLH, which include molecular demonstration of a genetic alteration consistent with HLH (eg, PRF or MUNC 13-4) or demonstration of at least 5 of the following 8 criteria: fever, splenomegaly, cytopenia (affecting ≥ 2 cell lines), hypertriglyceridemia or hypofibrinogenemia, hemophagocytosis, low or absent natural killer (NK)-cell cytotoxicity, an increased ferritin level, and an increased level of soluble CD25.1,2

Patients with HLH often have an abrupt onset of symptoms with a fulminant clinical course, and the disease is usually fatal without treatment. However, the prognosis has considerably improved with the current treatment modalities. Results from HLH-94 protocol showed that children who received HLH-94 and stem cell transplantation had a 3-year survival of greater than 60%.1

Two types of HLH have been documented. The primary (familial) form is an autosomal recessive or X-linked disorder that often manifests during infancy, but rare cases have been reported in older children and even in adults.2-4 Various genetic mutations, including perforin and MUNC 13-4 have been described in up to 40% of patients with primary HLH.2 These mutations result in deficient secretion of cytotoxic granules by NK cells. Other rare mutations were reported in the SH2D1A/SAP, RAB27A, and CHS1/LYST genes associated with various congenital syndromes.2 The secondary (sporadic) form is an acquired syndrome caused by a variety of diseases, including viral, bacterial, and protozoan infections; malignancies; and macrophage activation syndrome associated with autoimmune disorders.1,2,5-9
HLH secondary to malignant lymphoma may manifest as an overwhelmingly aggressive disease and mask the underlying lymphoma. Such cases can be a diagnostic challenge, especially in a pediatric population when primary HLH is also a consideration. Secondary HLH is uncommon in adults with anaplastic large cell lymphoma (ALCL), and only 1 case of pediatric HLH secondary to ALCL has been reported. We now report 2 additional pediatric cases of ALCL manifesting as hemophagocytosis. The first case fulfilled the criteria for HLH, whereas the second case fulfilled all but 1 criterion (cytopenia) for HLH.

Materials and Methods

Patient Selection

After approval by the institutional review board, a computer-based search of the pathology report database was conducted to identify all patients diagnosed with ALCL and demonstration of hemophagocytosis in concurrent or additional biopsy specimens. Case 1 was identified from the pathology archives of the Children’s Hospital of Philadelphia, University of Pennsylvania. Case 2 was retrieved from the surgical pathology archives of Duke University Medical Center, Durham, NC.

Histologic Examination and Immunohistochemical Analysis

Formalin-fixed, paraffin-embedded tissue sections were stained with H&E and examined by light microscopy. The histologic diagnosis was made according to the World Health Organization classification.

Immunohistochemical analysis was performed using standard methods. Briefly, 4- to 5-µm sections were prepared from formalin-fixed, paraffin-embedded tissue blocks, air dried, and subjected to deparaffinization with xylene and absolute alcohol. Immunoperoxidase stains were performed using a panel of antibodies that included CD1a (Beckman Coulter, Miami, FL); CD3, CD8, CD20, CD30, CD45, CD45RO, CD68, CD79a, anaplastic lymphoma kinase (ALK)-1, epithelial membrane antigen, granzyme B, perforin, S-100, and T-cell intracytoplasmic antigen (TIA)-1 (DAKO, Carpinteria, CA); and CD2, CD4, CD5, CD7, CD10 (NCL/Vector, Burlingame, CA), with appropriate dilutions and pretreatment as recommended by the manufacturers.

Table 1
Antibodies and Conditions for Immunohistochemical Analysis in Two Cases of Anaplastic Large Cell Lymphoma With Hemophagocytosis

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Dilution</th>
<th>Pretreatment</th>
<th>Heat Source</th>
<th>Vendor</th>
</tr>
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<td>Prediluted</td>
<td>DAKO TR S1699</td>
<td>100°C water bath</td>
<td>Beckman Coulter, Miami, FL</td>
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<td>Pressure cooker</td>
<td>Vector, Burlingame, CA</td>
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<tr>
<td>CD3</td>
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<td>DAKO TR S1699</td>
<td>100°C water bath</td>
<td>Lab Vision, Fremont, CA</td>
</tr>
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<td>Pressure cooker</td>
<td>Vector</td>
</tr>
<tr>
<td>CD5</td>
<td>1:40</td>
<td>10 mmol/L of Tris, pH 9.5</td>
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<tr>
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<tr>
<td>CD8</td>
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</tr>
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<td>DAKO</td>
</tr>
<tr>
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<td>100°C water bath</td>
<td>DAKO</td>
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<tr>
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<td>DAKO TR S1699</td>
<td>Pressure cooker</td>
<td>DAKO</td>
</tr>
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<td>100°C water bath</td>
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<tr>
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</tr>
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<td>None</td>
<td>DAKO</td>
</tr>
<tr>
<td>TIA-1</td>
<td>1:900</td>
<td>None</td>
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ALK, anaplastic lymphoma kinase; EMA, epithelial membrane antigen; LCA, leukocyte common antigen; TIA, T-cell intracytoplasmic antigen; Tris, tris(hydroxymethyl)aminomethane.
commercially available monoclonal antibodies: CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11c, CD13, CD19, CD20, CD22, CD23, CD33, CD45, FMC7, HLA-DR, κ, and λ (BD Biosciences, San Jose, CA). The stained cells were then processed on a bench-top flow cytometer (FACSCalibur, BD Biosciences) and analyzed using Paint-A-Gate or Cell Quest software (BD Biosciences). Cell populations of interest were gated using CD2 and CD7 scatter dot plots. Daily calibration was performed using CaliBRITE Beads (BD Biosciences). Labeled immunoglobulin isotypes (IgG1-fluorescein isothiocyanate and IgG2-phycocerythrin) were used as negative control samples.

**Fluorescence In Situ Hybridization**

Fluorescence in situ hybridization for t(2;5) was performed on the fresh tissue specimen with a dual-color ALK break-apart probe (Vysis, Downers Grove, IL). The tissue was fixed in 3:1 methanol/acetic acid followed by a 20-minute digestion in 10% pepsin/0.001N hydrochloric acid. The ALK break-apart probe was applied to the slides and denatured at 75°C for 1 minute and then incubated at 37°C overnight. The slides were counterstained with 4'-6-diamidino-2-phenylindole. The signals were evaluated in 200 nuclei using a fluorescence microscope equipped with a Chroma 83000 filter set and CytoVision Software (Applied Imaging, Santa Clara, CA).

**Results**

**Clinical Histories**

**Case 1**

A 16-year-old girl was admitted because of a 1-month history of acute onset of fatigue, fever, nonproductive cough, and sore throat that persisted despite antibiotics and antipyretics. An extensive 10-day workup at an outside hospital did not demonstrate leukemia, lymphoma, or infectious organisms. However, mediastinal lymphadenopathy, mild splenomegaly, and lung nodules were noted.

She was transferred to the Children’s Hospital of Philadelphia where admission laboratory tests showed pancytopenia with a WBC count of 1,400/µL (1.4 × 10^9/L; reference range, 3,800-14,000/µL [3.8-14.0 × 10^9/L]); hemoglobin level, 7 g/dL (70 g/L; reference range, 10.5-13.5 g/dL [105-135 g/L]); and a platelet count of 84 × 10^3/µL (84 × 10^9/L; reference range, 150-400 × 10^3/µL [150-400 × 10^9/L]), and the following differential: bands, 15% (0.15); segmented neutrophils, 43% (0.43); lymphocytes, 40% (0.40); monocytes, 2% (0.02); and eosinophils, 0% (0.00). The serum ferritin level was markedly increased, at 5,166 ng/mL (5,166 µg/L; reference range, 36-92 ng/mL [36-92 µg/L]). The findings of coagulation studies were normal as follows: prothrombin time, 13.6 seconds (reference range, 11.5-13.8 seconds); partial thromboplastin time, 32 seconds (reference range, 26-38 seconds); and fibrinogen level, 320 mg/dL (3.2 g/L; reference range, 172-471 mg/dL [1.7-4.7 g/L]). During the next 3 days, a coagulopathy developed with a prothrombin time of 14.2 seconds, a partial thromboplastin time of 53.5 seconds, a decreased fibrinogen level of 124 mg/dL (1.2 g/L), and increased fibrinogen split products, 10 µg/mL (10 mg/L; reference range, <10 µg/mL [<10 mg/L]). A bone marrow biopsy was performed, followed by biopsies of a hilar lymph node and a lung nodule.

**Case 2**

A 5-year-old previously healthy girl was admitted to an outside hospital because of fever for 3 weeks, occasional abdominal pain, and headache. Chest radiographs demonstrated a right middle lobe infiltrate. She was treated for Rocky Mountain spotted fever and pneumonia with antibiotics and antipyretics. Despite these treatments, her fevers and pulmonary infiltrate persisted. She was subsequently transferred to Duke University Medical Center. On admission, laboratory tests disclosed the following values: WBC count, 5,200/µL (5.2 × 10^9/L; reference range 3,800-14,000/µL [3.8-14.0 × 10^9/L]); hemoglobin level, 8.9 g/dL (89 g/L; reference range, 10.5-13.5 g/dL [105-135 g/L]); platelet count, 213 × 10^3/µL (213 × 10^9/L; reference range, 150-400 × 10^3/µL [150-400 × 10^9/L]); and fibrinogen level, 91 mg/dL (0.9 g/L; reference range, 183-484 mg/dL [1.8-4.8 g/L]). A computed tomography scan demonstrated splenomegaly and mediastinal and hilar adenopathy. A bone marrow biopsy was performed, followed by a cervical lymph node biopsy.

**Pathologic and Immunohistochemical Findings**

**Case 1**

A bone marrow aspirate smear and a review of her earlier bone marrow biopsy from 7 days earlier demonstrated increased histiocytes and extensive hemophagocytosis. The bone marrow biopsy did not reveal lymphoid infiltrate, CD30+ cells, or ALK-1+ cells. The patient had fever, splenomegaly, pancytopenia, hyperferritinemia, and hypofibrinogenemia, which together with hemophagocytosis, fulfilled the minimal 5 criteria required by the Histiocytosis Society HLH study group, and, thus, the diagnosis of HLH was made. Although other diagnostic criteria for HLH such as PRF and SAP mutations, NK-cell cytotoxicity, and soluble CD25 level were not further evaluated during the initial workup, an extensive search for an underlying cause began.1

Hilar lymph node and lung biopsies demonstrated an anaplastic pleomorphic spindle cell proliferation. Higher power revealed that the spindled cells contained a moderate...
amount of pink cytoplasm, pleomorphic nuclei, and prominent nucleoli. By immunohistochemical analysis, these cells were positive for CD8, CD30, CD45, CD45RO, ALK-1, epithelial membrane antigen, granzyme B, perforin, S-100 (focal), and TIA-1. They were negative for CD3, CD4, CD20, and CD79a. These findings were diagnostic of ALCL, sarcomatoid variant. Although ALK-1 positivity in spindle cells can be seen in inflammatory myofibroblast tumor, the positive T-cell markers strongly argue against this diagnosis. Flow cytometry revealed an abnormal T-cell population positive for CD2, CD7, CD8, CD13, CD45, and HLA-DR and negative for CD3, CD4, CD5, and CD33. Cytogenetic analysis identified t(2;5)(p23;q35), confirming the diagnosis of ALCL. **Image 1**.

**Case 2**

The bone marrow biopsy specimen was negative for leukemia but showed a prominent, diffuse increase in histiocytes with many engulfing erythrocytes and their precursors, leukocytes, and platelets. Immunohistochemical analysis for CD68 performed on the bone marrow specimen demonstrated a striking histiocytic infiltrate distributed widely as single cells and in small clusters. CD3 labeled scattered small T cells only. CD30 and ALK-1 were negative in bilateral bone marrow core biopsy specimens. The hemophagocytosis in bone marrow, along with fever, splenomegaly, cytopenia, and hypofibrinogenemia, raised the suspicion of HLH. However, only 4 criteria for the diagnosis of HLH were fulfilled because, although...
the patient was anemic, her platelet and neutrophil counts were normal, thus the criterion for cytopenia, which requires involvement of at least 2 cell lines, was not fulfilled. A diagnosis of hemophagocytosis was made. Because an infectious cause was clinically suspected, further workup for HLH was not performed. The patient was empirically treated for infection with multiple antibiotics.

During her hospital stay, progressive cervical lymphadenopathy rapidly developed. A cervical lymph node biopsy was subsequently performed and showed large atypical cells partially effacing the normal nodal architecture. These cells had abundant pink cytoplasm, eccentric nuclei, and irregular nuclear contours, and some had prominent nucleoli.

These cells were positive for CD2, CD7, CD8, CD30, ALK-1, granzyme B, perforin, and TIA-1. They were negative for CD1a, CD3, CD4, CD5, and CD20. The findings were diagnostic for ALK-1+ ALCL Image 2I.

Clinical Outcome

Case 1

The patient received 2 cycles of methylprednisolone sodium succinate, allopurinol, cyclophosphamide, and vincristine for the lymphoma. Her hospital course was complicated by an acute onset of shortness of breath, hypoxemia, and pulmonary edema during the first round of chemotherapy. She recovered,
with improvement of the HLH. During her second round of chemotherapy, typhilitis and gram-negative sepsis developed. Despite antifungal prophylaxis, Aspergillus pneumonia developed that did not respond to aggressive antifungal medications. Her course worsened with Aspergillus meningitis, acute renal insufficiency, thoracic wound dehiscence, and septic shock. Mechanical ventilation was discontinued 1.5 months after the patient was admitted, and she died shortly thereafter.

**Case 2**

The patient received chemotherapy for lymphoma per POG 9315 protocol (vincristine, doxorubicin, prednisone, and intrathecal cytarabine). Her hospital course was complicated by acute renal failure, acute respiratory failure, and disseminated intravascular coagulation. She recovered from these complications, achieved a complete remission, and remains disease free after 3 years.

**Discussion**

Our cases document several unusual findings regarding pediatric ALCL associated with hemophagocytosis. Both patients had fever, lack of significant peripheral adenopathy or rash, mediastinal adenopathy, hemophagocytosis in bone marrow, and pulmonary infiltrates. The patient in case 1 had multiple bilateral pulmonary nodules, whereas the patient in case 2 had a right middle lobe infiltrate. Of note, the previously documented pediatric case of ALCL associated with HLH, described in an 8-year-old, had similar symptoms: fever, pulmonary nodules, and a lack of peripheral adenopathy or rash. Although the pulmonary infiltrate was proven to be ALCL in 1 case (case 1), the nature of the pulmonary infiltrate in the remaining 2 cases was uncertain. These findings suggest that pulmonary infiltrate may be a common feature in pediatric ALCL–associated HLH.

HLH occurs in a variety of settings and often causes diagnostic difficulty in that the clinical findings of fever, hepatosplenomegaly, and cytopenias may suggest acute viral illness. In both of our cases, an initial paucity of peripheral lymphadenopathy at admission shifted the differential diagnosis away from lymphoma. Therefore, both patients were empirically treated for infection. The bone marrow was subsequently examined, which showed hemophagocytosis with no evidence of lymphoma. These cases indicate that pediatric ALCL may manifest initially with hemophagocytosis with fever, pulmonary infiltrate, and isolated mediastinal or hilar adenopathy, with or without other signs of HLH. In pediatric patients with such symptoms, biopsy of any enlarged lymph nodes, including a mediastinal or hilar lymph node, should be considered if the symptoms cannot be attributed to other causes.

Our 2 cases and the reported prior case of ALCL had negative Epstein-Barr virus (EBV) serologic results. Much controversy surrounds the role of EBV in hemophagocytosis secondary to T-cell lymphoma. The presence of EBV in many cases of T- and NK-cell lymphomas with associated HLH has been well documented. Lay et al studied the association of elevated serum cytokine levels with EBV status in lymphoma-associated hemophagocytosis. Transcripts of tumor necrosis factor (TNF)-α were found at a higher level in EBV+ cases than in EBV− cases. Transcripts of interferon (IFN)-γ were consistently detected in lymphoma cases regardless of EBV status. When cultured supernatants from EBV-infected T cells were cocultured with a monocytic cell line, enhanced phagocytosis and secretion of TNF-α, IFN-γ, and interleukin-1α were observed. The authors suggested that EBV-infected T cells up-regulated cytokine secretion and activated macrophages. However, the lack of convincing viral infection in a large proportion of lymphomas with associated HLH allows for further speculation.

In a study of a series of Hong Kong patients, Wong et al proposed that tumor cells themselves from the T/NK-cell lymphoma, independent of viral infection, were responsible for inducing hemophagocytosis by secreting increased IFN-γ and TNF-α. Other studies proposed that an acquired cytotoxic defect in NK cells and cytotoxic T cells contributed increased macrophage activity.

Despite numerous studies, the pathogenesis of secondary HLH is still not completely understood. The recently discovered perforin and MUNC 13-4 gene mutations in primary HLH may have explained the reduced cytotoxic activity observed in patients with HLH. It is interesting that both of our cases showed negative EBV serology but strong expression of granzyme B and perforin in the lymphoma cells. This suggests that the macrophage activation in our cases may be induced by yet another unknown mechanism.

Both patients responded to the chemotherapy treatment targeted on ALCL with complications. One patient (case 2) achieved complete remission, but the other patient (case 1) died of severe infections and end-organ failure after 2 cycles of chemotherapy. The recommended treatment protocol for familial hemophagocytosis is HLH-2004, which includes 8 weeks of dexamethasone, etoposide, cyclosporine, intrathecal methotrexate, and prednisolone in selected cases, followed by stem cell transplantation. In secondary hemophagocytosis, the primary disease can be treated with or without additional HLH therapy.

Recently, high-dose chemotherapy followed by stem cell transplantation has been reported with good outcome in lymphoma-associated hemophagocytosis in an adult population. Targeted therapies such as anti–TNF-α antibody and interleukin-1 receptor antagonist may serve as an effective adjunct to the standard therapy, especially in patients with refractory...
Children with lymphoma-associated hemophagocytosis are usually managed by standard lymphoma protocol. There are insufficient experiences in stem cell transplantation and targeted therapy for children. Both of our cases and the previously reported case received the standard protocol for large cell lymphoma with different outcomes. Additional larger studies are needed to better understand the pathophysiology and clinical behavior and to improve management and, ultimately, survival in affected children.

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