Ewing Sarcoma vs Lymphoblastic Lymphoma

A Comparative Immunohistochemical Study

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Abstract

To develop a practical immunohistochemistry panel for distinguishing lymphoblastic lymphoma from Ewing sarcoma (ES), we evaluated 17 ES and 27 lymphoblastic lymphoma and leukemia cases with antibodies to CD99, terminal deoxynucleotidyl transferase (TdT), leukocyte common antigen (LCA), CD43, CD79a, CD20, CD3, vimentin, and neuron-specific enolase (NSE). Three cases were bone lymphomas, 2 initially misdiagnosed as ES. All cases were CD99+. All lymphomas and leukemias were TdT+ compared to none of the ESs. None of the ESs expressed other lymphocytic markers, which were inconsistently expressed in the lymphomas and leukemias: CD43, 33%; LCA, 30%; CD79a, 19%; CD3, 19%; and CD20, 7%. Of the ESs, 88% were vimentin positive compared with 23% of lymphomas and leukemias. Vimentin was stronger and more diffuse in ES. NSE did not reliably stain any cases. When faced with the differential diagnosis of ES vs lymphoblastic lymphoma, an immunohistochemical panel that includes antibodies to CD99 and TdT is useful. Both epitopes are well preserved in fixed and decalcified tissue. A panel composed of antibodies to CD99 and TdT, in conjunction with other lymphocytic markers and vimentin, is highly sensitive and specific.

Diagnosing small round cell bone tumors in children can be problematic, especially in small or crushed biopsy samples limited to fixed tissue. Distinguishing lymphoblastic lymphoma of bone from Ewing sarcoma can be particularly difficult in this regard because these tumors have overlapping morphologic and immunohistochemical features. The distinction, however, is critical because the clinical behaviors and treatments are radically different.

Precursor B-cell lymphoblastic lymphoma is an important mimicker of Ewing sarcoma.1 Like Ewing sarcoma, it often occurs in children and has a propensity to involve bone, often manifesting as a solitary bone tumor. Like Ewing sarcoma, the malignant cells are small and uniform and have a diffusely infiltrative growth pattern. They even can form rosette-like structures.1

Immunohistochemically, lymphoblastic lymphoma also mimics Ewing sarcoma, as it is positive for CD99 (MIC2 gene product) and often nonreactive or only focally positive for conventional lymphoma markers such as leukocyte common antigen (LCA), CD20, and CD3. Thus, the probability of mistaking it for Ewing sarcoma is substantial when a limited immunohistochemical panel is used.

We recently encountered 3 CD99+/LCA-negative lymphoblastic lymphomas of bone. Two initially were misdiagnosed as Ewing sarcoma. The diagnosis in both cases was revised after additional immunohistochemical studies. This experience prompted us to retrospectively evaluate a series of Ewing sarcoma and lymphoblastic lymphoma and acute lymphoblastic leukemia (LL/ALL) cases using a panel of markers with the intent of developing a practical immunohistochemical panel for use in fixed tissue.
Materials and Methods

The materials consisted of formalin-fixed paraffin-embedded tissue blocks from 17 Ewing sarcomas (biopsy specimens from 16 bone and 1 soft tissue primary tumors), 11 lymphoblastic lymphomas (including 3 cases of bone lymphoma), and 16 acute lymphoblastic leukemias (bone marrow core biopsy specimens and/or particles). Many of the specimens had been decalcified.

Four-micrometer sections from each block were prepared for immunohistochemical study. Staining was performed on a Ventana automated stainer (Ventana, Tucson, AZ) or manually using the avidin-biotin-complex procedure with the following antibodies: (1) CD99 (O13, clone 12E7; dilution 1:25; DAKO, Carpinteria, CA); (2) terminal deoxynucleotidyl transferase (TdT; polyclonal; dilution 1:10, high-temperature citrate buffer pretreatment; Supertech, Rockville, MD); (3) leukocyte common antigen (LCA; CD45RB/CD45, clone PD7/26 and 2B11; dilution 1:80; DAKO); (4) CD79a (clone HM57; dilution 1:50, high-temperature citrate buffer pretreatment; DAKO); (5) CD43 (clone DF-T1; dilution 1:25; DAKO); (6) CD3 (clone PS1; dilution 1:25, high-temperature citrate buffer pretreatment; Vector Laboratories, Burlingame, CA); (7) CD20 (clone L26, dilution 1:80; DAKO); (8) vimentin (clone V9; dilution 1:25, high-temperature citrate buffer pretreatment; DAKO); and (9) neuron specific enolase (NSE; clone BBS/NC/V1/H14; dilution 1:100; DAKO).

Three pathologists (D.R.L., G.B., and M.E.D.) at a multihead microscope examined all slides. Staining intensity and distribution were reported as strongly or weakly positive and as diffuse or focal. Positive vimentin staining of tumor cells and/or stroma was used to control for tissue immunoreactivity. One vimentin-negative case was excluded.

Results

Case Reports of Lymphoblastic Lymphoma of Bone

Case 1

A 29-year-old man sought care because of right ankle pain. Radiographic and magnetic resonance imaging (MRI) studies disclosed 2 bone tumors, one in the talus, the other in the ipsilateral distal tibia. Both tumors were confined to bone with no soft tissue invasion. The MRI revealed that the tibia contained a few smaller satellite nodules within the marrow proximal to the main tumor. Open incisional biopsy disclosed sheets of malignant small cells infiltrating bone and fibroconnective tissue **Image II**. Based on positive CD99 and negative LCA staining, an initial misdiagnosis of Ewing sarcoma was given. After review of the clinical findings and pathology materials 2 weeks later, additional stains were performed, which showed the cells to be strongly TdT-positive and CD20−, CD3−, and CD43−. CD79a staining was not done owing to insufficient remaining tissue in the block. The final diagnosis was lymphoblastic lymphoma of uncertain lineage.

Case 2

An 8-year-old boy with a history of precursor B-cell acute lymphoblastic leukemia, 18 months after allogeneic bone marrow transplantation, was brought for care because of swelling of the left heel. Radiographic and MRI studies disclosed a destructive calcaneal bone tumor with soft tissue invasion. No other tumors were detected. Open incisional biopsy disclosed a small blue cell tumor with extensive crush artifact permeating bone and skeletal muscle **Image 2A** and **Image 2B**. The tumor was positive for CD99 and negative for LCA, CD20, and CD3. It initially was misdiagnosed as Ewing sarcoma at another hospital. This case was seen in consultation a few weeks later. Additional staining showed reactivity for TdT, CD43, and CD79a, establishing the diagnosis of precursor B-cell lymphoblastic lymphoma, consistent with recurrent disease.

Case 3

A 7-year-old girl sustained a pathologic fracture of her left distal radius when she fell on her outstretched hand. Radiographs disclosed a benign-appearing radiolucent bone
tumor in the distal radial metaphysis with pathologic fracture. The clinical differential diagnosis was bone cyst vs fibrous dysplasia. After 6 weeks in a cast, she was taken to surgery. A cystic cavity was discovered, and a small tissue sample was retrieved and sent for frozen section. Uniform small cells with round nuclei Image 3 made up the tumor, and the initial impression before staining was Ewing sarcoma. Immunohistochemical studies showed positive staining for CD99, TdT, and vimentin and negative results for LCA, CD3, CD20, CD43, and CD79a. The final diagnosis was lymphoblastic lymphoma of undetermined lineage.

Microscopic Pathology

In well-preserved specimens, a number of cytologic differences were noted between Ewing sarcoma and LL/ALL Image 4 and Image 5. The LL/ALL cells tended to have less cytoplasm and were less likely to show cytoplasmic clearing from glycogen washout than were the Ewing sarcoma cells. The LL/ALL nuclei tended to be less hyperchromatic than the Ewing sarcoma nuclei, with a more finely dispersed chromatin pattern. However, this was not always the case, since many of the Ewing sarcoma nuclei also had fine chromatin. In many cases, the LL/ALL nuclei had characteristic irregular, convoluted nuclear membranes. Nuclear convolution, however, was not a prominent finding in all cases. Often the nuclei were very round and uniform (Image 3), including those in 2 of the bone tumor cases.

In many of the biopsy specimens, the distinction between these 2 tumors was difficult and, in some cases, was impossible. Artifactual distortion and cell crushing, tissue clefthing in biopsy specimens with large amounts of bone (Image 2B), and necrosis hindered optimal evaluation of individual cytologic features in many cases.

Immunohistochemistry

All Ewing sarcoma and LL/ALL cases were strongly and diffusely positive for CD99 with a distinct cytoplasmic membrane pattern Image 6A and Image 6B. All the LL/ALL cases were positive for TdT Image 7A. The staining pattern was nuclear in every case, and the percentage of positive cells ranged from 20% to 100%. Staining intensity was strong in 25 cases and weak in 2. None of the Ewing sarcoma cases stained for TdT Image 7B. The other lymphocytic markers used were expressed inconsistently in the LL/ALL cases: LCA, 30%; CD43, 33% Image 8; CD79a, 19%; CD3, 19%; and CD20, 7%, and all were negative in the Ewing sarcoma cases. Among the LL/ALL cases, 41% stained with none, 33% with 2, 15% with 3, and 11% with 4 of these other lymphocytic markers.

Vimentin staining was seen in 88% of the Ewing sarcoma cases compared with only 23% of the LL/ALL cases. Vimentin tended to be more intensely and diffusely positive in the Ewing sarcoma cases, highlighting a greater amount of filamentous cytoplasm in these cells compared with the LL/ALL cells Image 9A and Image 9B. One of the bone lymphomas (case 3), however, had rather abundant vimentin-positive cytoplasm. NSE was focally and weakly positive in 6 Ewing sarcoma cases and 1 lymphoblastic lymphoma case and was not considered a reliable discriminator.
Discussion

Because the clinical behaviors and treatments are radically different, it is critical not to mistake lymphoblastic lymphoma for Ewing sarcoma. Different and effective chemotherapy regimens are used for these 2 tumors, and in Ewing sarcoma, unlike lymphoma, surgical ablation is an important aspect of treatment. In fact, amputation was contemplated in 2 of our bone lymphoma cases before the diagnosis was revised.

Knowledge about clinical information and results of radiographic and imaging studies often can direct one toward the correct diagnosis. For example, although multiple bone involvement and “skip metastases” are sometimes seen in Ewing sarcoma,2,3 multifocality in general is more compatible with lymphoma or leukemia. In case 1, separate bone tumors were identified in the talus and ipsilateral distal tibia with no evidence of continuity between them, and smaller satellite lesions were present in the tibia. In case 2, by contrast, the patient had a solitary destructive bone tumor of the calcaneus, which would have been compatible with Ewing sarcoma. The history of treated acute lymphoblastic leukemia, however, was a strong clue to the correct diagnosis of recurrent disease. In case 3, the radiographic appearance was that of an indolent tumor, which showed no progression over 6 weeks of observation. This appearance and behavior would be unusual for Ewing sarcoma.

There are a number of cytologic differences between lymphoblastic lymphoma and Ewing sarcoma. Lymphoblastic lymphoma cells tend to have finer chromatin, greater nuclear convolution, and scantier cytoplasm than lymphoblastic lymphoma cells have (H&E, ×100).
distortion and crush artifact. In addition, not every lymphoblastic lymphoma has the characteristic highly convoluted nuclei. The so-called nonconvoluted variant (Image 3) has uniform small round nuclei. Two bone lymphoma cases (cases 1 and 3) had cells with round nuclei, mimicking Ewing sarcoma cells.

Until recently, there had been no specific tissue marker for Ewing sarcoma. By the early 1990s, a monoclonal anti–Ewing sarcoma antibody had been developed. This antibody, marketed as clones O13 and HBA71, marks the MIC2 gene product, a 30- to 32-kd membrane glycoprotein, designated as CD99. The initial excitement about having a Ewing sarcoma–specific marker soon was quenched when it was found to be not entirely specific. A number of other tumors, including other sarcomas and lymphoblastic lymphoma and leukemia, also were found to be CD99+. In fact, this same antibody was actually first discovered in acute lymphoblastic leukemia in 1979, and the antigen has been regarded as a T-cell adhesion factor.

Because LL/ALLs derive from primitive lymphocytes, they frequently do not express markers commonly found in other, more mature forms of lymphoma. They often are negative for LCA, CD20, and CD3. CD99, by contrast, is a useful marker in LL/ALL. It can be demonstrated in the vast

Image 6 Diffuse cytoplasmic membrane staining for CD99 was seen in all Ewing (A) and lymphoblastic lymphoma and leukemia cases (B) (immunoperoxidase, ×100).

Image 7 Nuclear terminal deoxynucleotidyl transferase staining was present in all lymphoblastic lymphoma and leukemia cases (A) and in none of the Ewing sarcoma cases (B) (immunoperoxidase, ×100).
majority of cases and seems to be quite specific for this type of lymphoma.\textsuperscript{11,14,15}

TdT has been used as a marker of LL/ALL for many years. Until recently, however, its use had been limited to direct immunofluorescence technique, and it was not available for fixed tissue specimens. There now exist commercially available polyclonal antibodies that work well in formalin-fixed tissues pretreated with high-temperature epitope-retrieval.\textsuperscript{16} In the present study, we found TdT to be the best discriminator of LL/ALL and Ewing sarcoma. All LL/ALL cases were positive with this marker compared with none of the Ewing sarcoma cases. Similar negative staining results for TdT in Ewing sarcoma have been reported.\textsuperscript{17}

CD43 and CD79a also are useful stains for separating LL/ALL from Ewing sarcoma. CD43 and CD79a can be expressed in both T-cell and precursor B-cell LL/ALLs.\textsuperscript{1,4} CD79a is a good marker for the precursor B-cell phenotype,\textsuperscript{1,4} since it is expressed in early stages of B-cell differentiation and is often positive when other more mature B-cell markers are negative. Ewing sarcoma is virtually always negative with these markers, as we observed.

We also found vimentin to be helpful in the differential diagnosis. It not only is more likely to be positive in Ewing sarcoma but it also highlights the greater amount of filamentous cytoplasm in the cells (Images 9A and 9B).

Cytogenetic and molecular biologic studies also can be useful for distinguishing these 2 tumors. Ewing sarcoma has distinctive cytogenetic and molecular genetic findings (most commonly t[11;22] with EWS/FLI-1 fusion), and LL/ALLs have T-cell receptor or immunoglobulin gene rearrangements. These molecular findings can be detected by fluorescence in situ hybridization\textsuperscript{18} and reverse transcriptase–polymerase chain reaction in fresh-frozen tissue and often in fixed samples as well.\textsuperscript{1,19,20} Such studies, however, are not always feasible, especially in cases limited to small biopsy specimens.

Recently, novel anti–Ewing sarcoma antibodies that recognize the FLI-1 and EWS gene products have been evaluated in experimental immunohistochemical assays.\textsuperscript{21,22} These antibodies, although positive in the majority of Ewing sarcomas, are also positive in a variety of other tumors,
including lymphoblastic lymphoma, and, therefore, do not seem at this time to have significant advantage over CD99.

**Conclusions**

When faced with the differential diagnosis of Ewing sarcoma vs lymphoblastic lymphoma in biopsy material, it is prudent to expand the usual immunohistochemical panel to include CD99, TdT, CD43, CD79a, and vimentin. These markers, CD99 and TdT in particular, are well preserved in fixed tissue and, in combination, reliably differentiate Ewing sarcoma and lymphoblastic lymphoma.

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