



Educational Case

Educational Case: Ewing sarcoma family of tumors: Clinical presentation, pathologic findings, and differential diagnosis

Oyintoun-emi Ozobokeme, MD^a, Terri E. Jones, MD^{b,*}, Rana Naous, MD^c, Samer N. Khader, MD^d

^a All Saints University School of Medicine, Hillsborough Street, Roseau, Dominica

^b Department of Pathology, Magee-Womens Hospital of the University of Pittsburgh Medical Center, Pittsburgh, PA, USA

^c Department of Pathology, Shadyside Hospital of the University of Pittsburgh Medical Center, Pittsburgh, PA, USA

^d Department of Cytopathology, 5230 Centre Avenue, Shadyside Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see <https://www.journals.elsevier.com/academic-pathology/news/pathology-competencies-for-medical-education-pcme>.¹

Keywords: Pathology competencies, Organ system pathology, Musculoskeletal system, Bone neoplasia, Categories of bone tumors, Ewing sarcoma

Primary objective

Objective MS1.1: Categories of bone tumors. Describe examples of bone-forming, cartilage-forming, and other common bone tumors, including the clinicopathologic features, radiological features, treatment, and prognosis of each.

Competency 2: Organ system pathology; Topic: MS: Musculoskeletal system; Learning Goal 1: Bone neoplasia.

Secondary objectives

Objective N1.1: Genetic mechanisms of neoplasia. Discuss and provide examples of molecular genetic mechanisms that underlie cancers, including germline mutations (including point mutations, deletions, amplifications, and translocations) and epigenetic changes.

Competency 1: Disease mechanisms and processes; Topic: Neoplasia (N); Learning Goal 1: Genetic basis of neoplasia.

Objective SP1.2: Differential diagnosis. List the major differential diagnoses for each type of cytology or surgical pathology specimen derived from a lesion or mass and describe appropriate further studies, both special stains and immunohistochemistry.

Competency 3: Diagnostic medicine and therapeutic pathology. Topic: Surgical pathology (SP). Learning Goal 1: Role in diagnosis.

Patient presentation

A 17-year-old woman presents to the emergency department with a burning pain and swelling of her right shoulder. The patient states that the pain started a month ago, is sporadic, and is related to strain, but is not frequently felt at night. She rates her pain at a 7 on a 1–10 severity of pain scale, with 10 being the highest. She is otherwise healthy and plays on her high school basketball team. She has not had any trauma to her shoulder. The patient states that she has no systemic symptoms, including no recent weight loss or decreased movement. The patient has no personal or family history of cancer or illnesses. Social history is otherwise non-contributory. She is not on any medications.

Diagnostic findings, Part 1

The patient is afebrile, and their vital signs are within normal limits. A 3.0 × 2.5 cm, tender mass is palpable on the anteromedial aspect of her right shoulder. The mass appears to be deep, possibly arising from bone or surrounding soft tissue of her right shoulder with no observed skin discoloration. The mass is soft and feels warm to touch. A cardiac exam reveals a normal S1, S2 with regular rate and rhythm and no murmurs. Lungs are clear on auscultation. The abdomen is soft and non-tender with normal bowel sounds and no masses. There is no hepatosplenomegaly.

* Corresponding author. Department of Pathology University of Pittsburgh Medical Center, Rm 4315, 300 Halket Street, Pittsburgh, PA 15213, USA.

E-mail address: jonest12@upmc.edu (T.E. Jones).

<https://doi.org/10.1016/j.acpath.2022.100051>

Received 3 October 2021; Received in revised form 25 June 2022; Accepted 18 July 2022; Available online xxxx

eISSN2374-2895/© 2022 The Author(s). Published by Elsevier Inc. on behalf of Association of Pathology Chairs. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Question/discussion points, Part 1

How would you summarize your interpretation of the patient's presentation? Discuss the differential diagnosis based on your interpretation

Based on the patient's history and physical exam, the patient has a painful mass arising from the bone or soft tissue of her right shoulder. The differential diagnosis includes inflammatory processes, including reactive and infectious conditions, as well as benign and malignant neoplasms. Osteomyelitis, which may show a prominent periosteal reaction resembling a neoplasm, is a consideration. However, the patient did not describe any history of trauma, drug abuse, and systemic symptoms, and she is afebrile on physical exam. If the mass is neoplastic, it is important to determine whether the tumor arises in or involves bone, soft tissue, or both. Imaging would assist in determining this.

Diagnostic findings, Part 2

Routine blood work is ordered, including a complete blood count (CBC), C-reactive protein, erythrocyte sedimentation rate (ESR), and liver function tests, all of which are within normal limits. An X-ray of her right shoulder is performed and demonstrates a mass involving the right scapula and demonstrating laminar periosteal elevation, which resembles "onion-skinning/peel."

Question/discussion points, Part 2

How would you revise your differential diagnosis given the laboratory and X-ray image findings?

Given that the patient's CBC is within normal limits, it is unlikely that this patient has a joint or bone infection. Patients with infections would typically have an elevated leukocyte count, ESR, and C-reactive protein. This makes a benign, borderline, or malignant neoplasm more likely than an infection in our patient's case.

Benign bone and cartilage tumors in the differential for this patient's age group include enchondroma, osteochondroma, osteoid osteoma, or osteoblastoma.²⁻⁴ However, all of these are unlikely, as they lack a periosteal reaction on imaging, which is seen in the X-ray of our patient's tumor. Benign or borderline soft tissue tumors in the differential diagnosis would include nodular fasciitis, angioleiomyoma, angiomatoid fibrous histiocytoma, and dermatofibrosarcoma protuberans. Nodular fasciitis is a rapidly growing but self-limiting neoplasm that may present as a tender mass. Angioleiomyoma is also a relatively common neoplasm seen in the lower extremities and arises in the dermis or subcutaneous tissue and is occasionally associated with pain. However, this patient presents with burning pain and swelling of the right shoulder. Angiomatoid fibrous histiocytoma is a rare tumor with mostly benign behavior that is typically found in the superficial extremities of young adults and children.² These tumors are usually slowly growing, which would not fit with our patient's month-long history of symptoms. Dermatofibrosarcoma protuberans usually presents as a nodular cutaneous mass with red skin discoloration, which is not present in this patient.²⁻⁴

Malignant diagnostic considerations in this age group and location include bone sarcomas like conventional osteosarcoma, Ewing sarcoma, and soft tissue tumors, such as low-grade fibromyxoid sarcoma, alveolar rhabdomyosarcoma, myxoid liposarcoma, epithelioid hemangioendothelioma, and synovial sarcoma.²⁻⁴ Osteosarcoma is usually seen in children between 13 and 16 years of age; the association with this age group may be related to the adolescent growth spurt. This tumor usually occurs in the metaphyseal growth plate of the knee, proximal tibia, or proximal humerus.² Osteosarcoma has two classic X-ray findings. The first is called "Codman's triangle," which is a lifting of the periosteum by the tumor to form a triangle shape. The second classic finding is called the "sunburst sign," which occurs because of tiny bone fibers that form in

the periosteum making the tumor look like a sunburst.^{3,4} Low-grade fibromyxoid sarcoma, alveolar rhabdomyosarcoma, myxoid liposarcoma, epithelioid hemangioendothelioma, and synovial sarcoma usually involve soft tissue and are uncommon in bone. In our patient's case, the tumor was involving bone thus excluding the aforementioned entities. Additionally, imaging showed characteristic "onion skinning" with an elevated periosteum, a finding that is suggestive of Ewing sarcoma.^{3,4}

What are the most common bone and soft tissue malignancies seen in children?

Sarcomas are malignant tumors that originate in bone or in connective tissues of the body, including dermis, subcutaneous tissue, fascia, muscle, fibrous tissue, tendons, ligaments, nerves, and blood vessels. These tumors can arise from any part of the body but are often seen in the extremities and trunk. The most common sarcomas seen in children are rhabdomyosarcoma, osteosarcoma, and Ewing sarcoma.²⁻⁴

What imaging studies would be useful in narrowing the differential diagnosis?

A computed tomography (CT) scan can help determine the degree of bone destruction and estimate the tumor size using contrast-enhanced CT, which can be used for further evaluation during chemotherapy. On the other hand, magnetic resonance imaging (MRI) is vital to evaluate soft tissue involvement.

How does imaging narrow the differential diagnosis?

On MRI, Ewing sarcoma presents with a low-to-intermediate signal on T1 with gadolinium contrast and a heterogeneously high signal and prominent enhancement with possible "hair on end" low signal striations on T2.^{5,6} However, osteomyelitis demonstrates decreased T1 signal and increased T2 signal due to bone marrow edema; abscesses delineate extraosseous disease spread.⁵⁻⁷ Bone marrow edema is the initial characteristic of acute osteomyelitis seen on MRI, and this can be detected in the first two days of an infection. The clinical presentation and the overall radiologic features seen on MRI make osteomyelitis unlikely.

Diagnostic findings, Part 3

This patient undergoes an MRI of the right shoulder. Representative images from the patient's MRI scan are shown in Fig. 1.

Question/discussion points, Part 3

Interpret the findings in the patient's right shoulder MRI imaging as shown in Fig. 1

Figure 1 demonstrates sagittal (A) and axial (B) MRI of the right shoulder demonstrating a mass (delineated by blue arrows) arising in scapular bone and extending anteriorly and medially into adjacent soft tissue. The lesion measures up to 9 cm.

What is the best approach to obtaining a tissue diagnosis, and how does one ensure that adequate tissue is obtained?

Given the provisional diagnosis and differential diagnosis of this patient, a biopsy of the mass would provide a definitive diagnosis.

The next step to evaluate the patient would include a fine needle aspiration (FNA) or core biopsy of the mass. During FNA or core biopsies, a rapid on-site evaluation (ROSE) may be performed in real time to provide a preliminary interpretation.^{8,9} ROSE can be helpful in determining if there is adequate material in the FNA or biopsy to characterize the mass. ROSE also allows for appropriate triage of a specimen; for

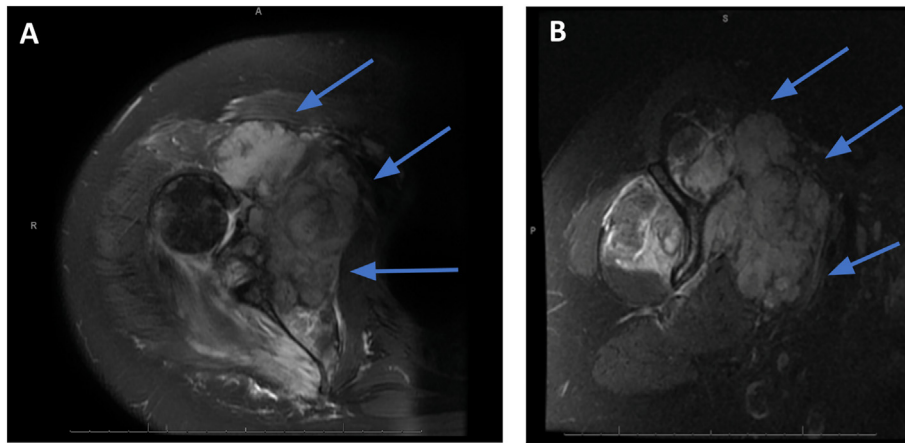


Fig. 1. Sagittal (A) and axial (B) magnetic resonance imaging (MRI) of the right shoulder demonstrating a mass (delineated by blue arrows) arising in scapular bone and extending into soft tissue. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

instance, if a pathologist suspects a particular type of tumor, such as a lymphoma, part of the sample can be collected in special solutions, such as Roswell Park Memorial Institute (RPMI) solution, at the time of procedure, and sent for flow cytometric analysis to identify the hematologic cell types present.^{8,9}

After ROSE is complete, the specimen is sent for processing to produce Papanicolaou (Pap) and Diff-Quik-stained (a modified Wright-Giemsa stain) smears, as well as a cell block that is stained with hematoxylin and eosin. The material left in the hub or syringe after each pass can be rinsed in formalin to produce a cell block which would be

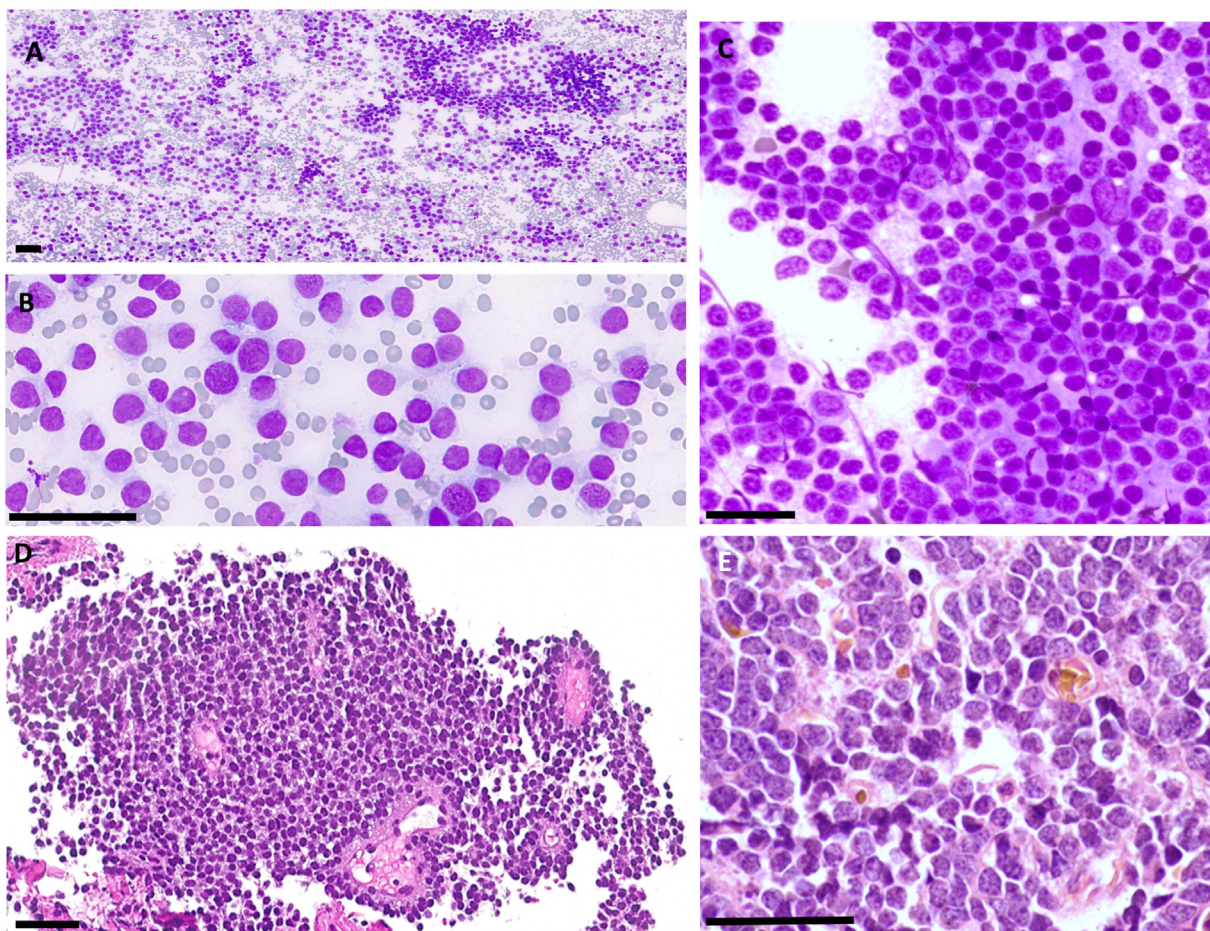


Fig. 2. A-C) Fine needle aspirate smears of the shoulder mass. A cellular aspirate demonstrates isolated and loosely aggregated neoplastic cells. The cells have slightly enlarged nuclei with mildly irregular nuclear borders and pale, finely granular chromatin, and scant to moderate amount of cytoplasm. Few cells show darker chromatin and more irregular nuclear contours. Most cells demonstrate a high nuclear-cytoplasmic ratio and no appreciable nucleoli (touch preparation slides, Diff-Quik stain, bars = 80 μ m). (D-E) Cell block. D: Solid sheet of somewhat monomorphic tumor cells surrounding small vessels "pseudorosettes." E: Higher power demonstrates cells with a high nuclear-cytoplasmic ratio, fine chromatin, and scant cytoplasm (hematoxylin and eosin, bars = 80 μ m).

embedded into paraffin blocks that can be processed like a histopathologic specimen. The cell block histologic sections can demonstrate tissue architecture and can be used for other ancillary studies, such as immunohistochemistry, fluorescence *in situ* hybridization (FISH), and next-generation sequencing (NGS) molecular tests.^{8,9}

What are the cytologic features identified in the aspirate as shown in Fig. 2?

Representative images from the aspirate and cell block are shown in Fig. 2. The aspirate demonstrates neoplastic cells in loose aggregates and as isolated cells. The cells have scant to moderate cytoplasm, with enlarged nuclei, finely granular chromatin, and slightly irregular nuclear borders. The cells have a high nuclear-cytoplasmic ratio without obvious nucleoli. Some cells exhibit increased nuclear contour irregularities and darker chromatin. This cytomorphology is commonly designated as “small round blue cell.” The cell block demonstrates sheets of neoplastic cells with similar nuclear and cytoplasmic features to those seen in the aspirate. Some tumor cells surround small vessels in a “pseudorosette” pattern.

The differential diagnosis in this context includes a small round blue cell tumor, a neuroendocrine tumor, and lymphoma. The specimen was sent for flow cytometry analysis, which did not detect a significant lymphoid population.

What are small round blue cell tumors?

Small round blue cell tumors are a group of childhood tumors that are all characterized by a similar histologic appearance: monotonous populations of small, round cells with a high nuclear-to-cytoplasmic ratio. Since the cytoplasm of these cells is mostly occupied by the hematoxylin-staining nucleus, on lower power the cells appear to be blue. These cells are usually larger in size than a lymphocyte. This appearance of cells may also be called “primitive” or “immature,” as they may resemble immature embryonal or fetal tissue. Tumors that are characterized by small round blue cells on the morphologic exam include neuroblastoma, rhabdomyosarcoma, non-Hodgkin lymphoma, Ewing sarcoma, and nephroblastoma.

How do the aspirate smear and cell block alter the morphologic differential diagnosis?

As mentioned above, the aspirate smear demonstrates loose aggregates of small round blue cells, while the cell block shows a solid sheet of these cells. As seen in Table 1, several morphologic considerations can be helpful in including or excluding an entity in the cytologic differential diagnosis. If the aspirate contains lymphoglandular bodies, which are cytoplasmic fragments of lymphocytes, this might raise the suspicion for lymphoma.⁸ The lack of bone or cartilage matrix in the cell block makes bone and cartilage-forming tumors less likely. Neuroblastoma may form Homer Wright pseudorosettes where the cells form a ring around the central eosinophilic neuropil.⁸ A potential pitfall is that Ewing sarcoma can sometimes show neuroectodermal differentiation and may also form Homer Wright pseudorosettes.² Embryonal rhabdomyosarcoma may show rhabdomyoblasts that have eosinophilic cytoplasm with cross-striations, as one would see in mature skeletal muscle.⁸ These rhabdomyoblasts may form strap cells, which have a bipolar orientation, and tadpole cells, which are unipolar. High-grade carcinomas may have areas of gland formation on the aspirate smear or cell block that would indicate epithelial differentiation.

What special stains could be used to narrow the differential diagnosis further?

The pathologist can perform a panel of IHC stains to further classify the tumor. A classical immunohistochemical profile for Ewing sarcoma

Table 1

Use of cytologic examination and immunohistochemistry in narrowing the differential diagnosis of pediatric and adolescent small round blue cell tumors.

Pediatric and adolescent “small round blue cell” tumors differential diagnosis	Distinguishing morphologic factors on aspirate/cell block	Immunohistochemical panels that may help narrow differential diagnosis ^b
Neuroblastoma	May form Homer Wright pseudorosettes with central neuropil	Synaptophysin, chromogranin
Osteosarcoma	May show evidence of bone formation ^a	IHC rarely helpful. Osteocalcin, ±cytokeratins.
Ewing sarcoma	May also form Homer Wright pseudorosettes	FLI1, CD99, ±synaptophysin, ±neuron-specific enolase, ±NKX2.2
Rhabdomyosarcoma	Embryonal subtype: strap cells, tadpole cells	Desmin, ±myogenin, ±MyoD1
High-grade carcinoma	Gland formation	Cytokeratins
Lymphoma	Lymphoglandular bodies	CD45, ±CD3, ± CD20

^a The presence of bone is more likely to be identified on the cell block preparation.

^b All the stains listed are positive in the applicable tumors, except for those listed with “±,” which designates that the stain may be positive or negative.

includes nuclear staining for FLI1, which is the most common fusion partner for the *EWSR1* gene. Cell membrane expression of CD99 expression is also frequently identified. However, the positive of neither stain is completely specific. Ewing sarcomas are also often positive for one or more neuroendocrine immunohistochemical stains, such as synaptophysin and neuron-specific enolase, which highlights areas of neuroectodermal differentiation present in these tumors. NKX2.2, a stain for neuroendocrine/gliial differentiation, has been demonstrated to have a higher specificity for Ewing sarcoma.

Additional stains were performed to exclude other malignancies, such as lymphoma, carcinomas, and other sarcomas, such as rhabdomyosarcoma. High-grade carcinomas usually retain keratin expression. Lymphomas typically stain positive with CD45 and CD3 or CD20. PHOX2B is sensitive and specific for neuroblastoma when positive. Desmin positivity would be found in rhabdomyosarcoma with diffuse myogenin/MyoD1 positivity in the alveolar subtype and only focal positivity in the embryonal subtype. Figure 3 demonstrates diffuse membranous staining of the tumor cells for CD99 (A) and diffuse nuclear positivity of the tumor cells for FLI-1 (B).

What molecular testing might provide a more specific interpretation?

FISH studies would be helpful as a next step to evaluate the patient's tumor. The FISH study that uses break-apart fluorescent probes specific for the *EWSR1* and *FLI1* genes would be most useful in this case. These probes bind the corresponding genes in cells obtained from a tumor sample.

Diagnostic findings, Part 4

Figure 4 shows the patient's FISH study results. In this study, green probes bind and flank the breakpoint region at the *EWSR1* gene, and red probes bind and flank the breakpoint at the *FLI1* gene. A positive result is seen when there are adjacent red and green fluorescent signals in a cell, which indicates the presence of an *EWSR1-FLI1* gene translocation.

Question/discussion points, Part 4

Given the patient's FISH findings, what is the patient's diagnosis?

Considering the morphology, the intraosseous tumor location, and FISH results, the patient is diagnosed with Ewing sarcoma. If the FISH

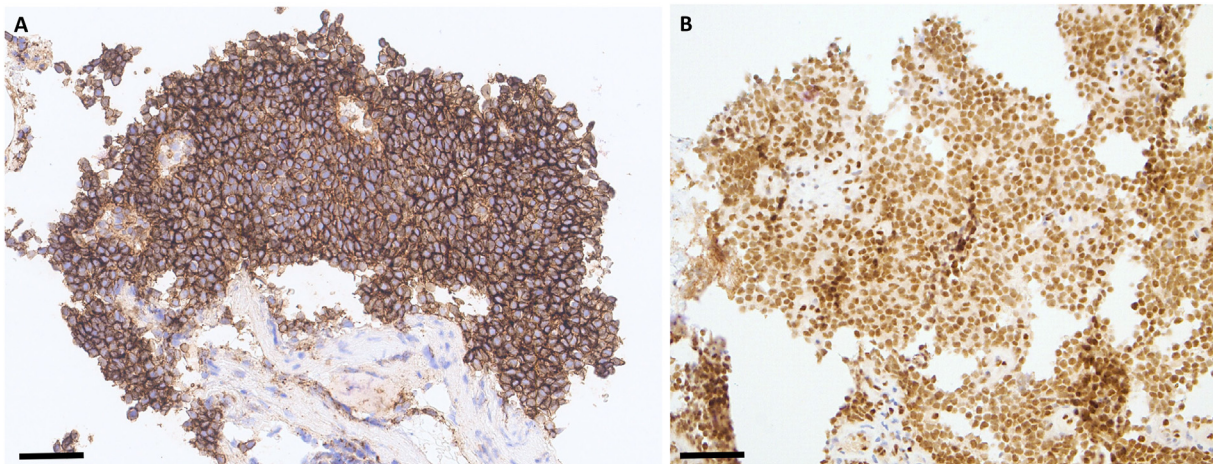


Fig. 3. Immunohistochemical stains performed on the cell block of the shoulder mass. (A) CD99 (immunohistochemical stain; bar = 80 μ m). (B) FLI-1 (immunohistochemical stain; bar = 80 μ m).

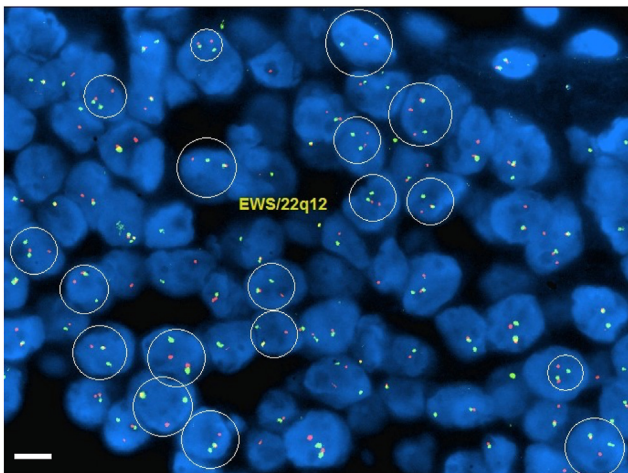


Fig. 4. Fluorescence *in situ* hybridization (FISH) analysis demonstrating a translocation involving the *EWSR1* and *FLI1* genes. The *FLI1/EWSR1* translocation probe consists of green probes (139kb, 151kb) flanking the breakpoint region at the *EWSR1* gene locus and red probes (94kb, 79kb) flanking the breakpoint region at the *FLI1* locus. White circles denote cells with positive results demonstrated by adjacent red and green signals indicating a translocation (bar = 10 μ m). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

results for the *EWSR1-FLI1* gene fusion were negative, an *EWSR1* break-apart FISH study could be performed. If this were to be positive, the differential diagnosis would include round cell sarcomas with *EWSR1-non-ETS* fusion, i.e., an *EWSR1*-rearranged round cell sarcoma with a fusion partner not belonging to the *ETS* family of transcription factors that is usually characteristic of Ewing sarcoma.

What are the molecular/genetic findings in Ewing sarcoma?

The most common genetic alteration that causes Ewing sarcoma is a fusion between the *EWSR1* gene on chromosome 22 and the *FLI1* gene on chromosome 11,^{10,11} as seen in the schematic in Fig. 5. The *EWSR1-FLI1* gene fusion occurs in around 85% of Ewing sarcomas.^{10,11} This type of genetic alteration, which is somatic, is not inherited. Some of the less frequently encountered fusion partners with the *EWSR1* gene in Ewing sarcoma include *ERG*, *FEV*, *ETV1*, and *ETV4*, which all belong to the *ETS* transcription factor family of genes.^{10,11} A novel translocation, t(19; 22)

(q13.4; q12.2), has recently been described in a parapharyngeal extra-skeletal Ewing sarcoma.¹²

Both the *FLI1* and *EWS* proteins, which are produced from the *FLI1* and *EWSR1* genes, respectively, are regulators of transcription. They are important for the growth and development of some cell types by controlling the transcription of specific genes.^{10,11} The *EWSR1-FLI1* fusion gene product, called the EWS-FLI protein, has the functions of both genes.¹⁰ The most specific diagnostic test for Ewing sarcoma is the FISH for the *EWSR1-FLI1* translocation.^{10,11}

What other neoplasms harbor an *EWSR1* rearrangement?

Rearrangements of the *EWSR1* gene first discovered in Ewing sarcoma are also seen in a spectrum of other tumors, which are listed in Table 2. These entities range from Ewing sarcoma, angiomatoid fibrous histiocytoma, myoepithelioma of soft tissue, desmoplastic small round cell tumor, extraskeletal myxoid chondrosarcoma, and clear cell sarcoma of soft tissue.¹¹ Ewing sarcoma and desmoplastic small round cell sarcoma are morphologically similar due to the presence of small, round, and blue cells; and both also occur mostly in young people, which can potentially cause diagnostic confusion.

Recently, the World Health Organization (WHO) described a new entity with *EWSR1* gene rearrangements called round cell sarcoma with *EWSR1-non-ETS* fusions.² These are round or spindle cell sarcomas with *EWSR1* or *FUS* gene fusions involving partners unrelated to the *ETS* gene family that is commonly associated with Ewing sarcoma, hence, referred to by some as Ewing-like sarcomas. They are rare tumors that can occur in children and adults and are often located in bone (particularly the *EWSR1-NFATc2* sarcomas) or deep soft tissue (*EWSR1-PATZ1* sarcomas) and have aggressive radiologic findings with locally destructive or infiltrative properties.²

Characteristic radiologic features like the “onion skinning” seen in Ewing sarcoma are absent in this new entity. *EWSR1* break-apart FISH can be used for detecting the *EWSR1* gene rearrangement in these sarcomas; however, identifying the fusion transcript via NGS is the gold standard for diagnosis and accurate distinction from classic Ewing sarcoma.

This patient has been diagnosed with Ewing sarcoma. How is this tumor staged?

After a diagnosis of Ewing sarcoma is rendered, the next step is the clinical and pathologic staging of the tumor. Unlike other solid tumors that use the American Joint Committee on Cancer (AJCC) and the Union

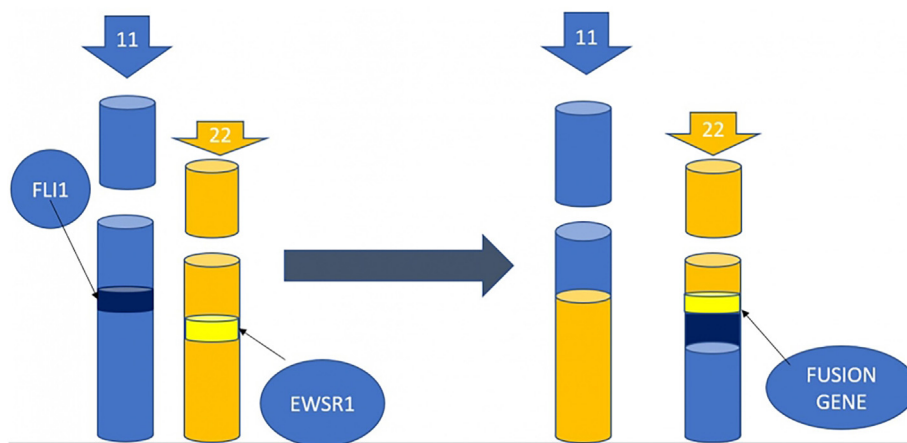


Fig. 5. Schematic of the mechanism of the genetic translocation of Ewing sarcoma. Fusion of *EWSR1* gene (chromosome 22) to the *FLI1* gene (chromosome 11) forms the *FLI1/EWSR1* fusion gene.

Table 2
EWSR1-rearranged tumors.

Neoplasms with <i>EWSR1</i> rearrangement	Fusion partners	Resulting fusion gene(s)
Ewing sarcoma	t(11; 22)(q24; q12)	<i>EWSR1-FLI1</i> <i>EWSR1-ERG</i> <i>EWSR1-FEV</i> <i>EWSR1-ETV1</i> <i>EWSR1-ETV4</i>
Desmoplastic small round cell tumor	t(11; 22)(q12; p13)	<i>EWSR1-WT1</i> <i>EWSR1-ERG</i>
Extraskeletal myxoid chondrosarcoma	t(9; 22)(q22; q12) t(9; 16)(q22; p11) t(9; 17)(q22; q11) t(9; 15)(q22; q21)	<i>EWSR1-NR4A3</i> <i>FUS-NR4A3</i> <i>TAF15-NR4A3</i> <i>TCF12-NR4A3</i>
Clear cell sarcoma of soft tissue	t(3; 9)(q12; q22)	<i>TFG-NR4A3</i>
Angiomatoid fibrous histiocytoma	t(12; 22)(q13; q12)	<i>EWSR1-CREB1</i> <i>EWSR1-ATF1</i> <i>EWSR1-CREB1</i> <i>FUS-ATF1</i> <i>EWSR1-ATF1</i>
Myxoid liposarcoma ^a	t(16; 21)(p11; q22)	<i>FUS-DDIT3</i> <i>EWSR1-DDIT3</i>
Low-grade fibromyxoid sarcoma	t(7; 16) (q32-34; p11)	<i>EWSR1-CREB3L1</i> <i>FUS-CREB3L1</i> <i>FUS-CREB3L2</i>
Myoepithelial tumor of soft tissue	t(6; 22)(p21; q12)	<i>EWSR1-POU5F1</i>
Hyalinizing clear cell carcinoma of salivary gland (HCCC)	t(12; 22)(q13; q12)	<i>EWSR1-ATF1</i>
Primary pulmonary myxoid sarcoma	t(2; 22)(q33; q12)	<i>EWSR1-CREB1</i>
Ewing-like sarcomas		<i>EWSR1-FEV</i> , <i>EWSR1-ETV1</i> , <i>EWSR1-ETV4</i> , <i>EWSR1-SP3</i> , <i>EWSR1-SMARCA5</i> , <i>FUS-NAFTc2</i> , <i>FUS-FEV</i> , <i>FUS-ERG</i> , <i>BCOR-CCNB3</i> , <i>BCOR-MAML3</i> , <i>BCOR ITD</i> , <i>ZC3H7B-BCOR</i> , <i>CIC-FOXO4</i> , <i>CIC-DUX4</i> .

^a Only a small subset of myxoid liposarcomas demonstrate t(16; 21)(p11; q22) fusions.

for International Cancer Control staging systems, neoplasms of the Ewing sarcoma family of tumors (EFT) in pediatric patients are staged based on the protocol provided by the Children's Oncology Group.^{13,14} Ewing sarcoma in the adult population may be staged using the AJCC TNM staging system.¹⁵

A staging system is a standardized way to describe the extent of cancer, both in terms of tumor size and invasion of local structures, the presence of metastatic foci in lymph nodes, and evidence of distant metastases. Ewing sarcoma is classified as either localized or metastatic. In staging Ewing sarcoma, it is important to note the size of the tumor, the bone or soft tissue which it involves, and whether it has metastasized.¹³⁻¹⁵

While X-ray is the best initial screening imaging modality for bone masses, once an Ewing sarcoma has been diagnosed, additional imaging studies will be performed to stage the cancer. In many cases, positron

emission tomography (PET) with 2-[fluorine-18] fluoro-2-deoxy-D-glucose (FDG) is used to assess for distant, metabolically active metastases.¹³⁻¹⁵ If a suspected metastatic focus is identified on PET, a core biopsy or FNA can be performed to establish a more precise diagnosis.

What is “askin tumor” and what is its relationship to Ewing sarcoma?

“Askin tumor” is a former term for Ewing sarcomas that arise from the chest wall and often involve the lungs, pleura, and ribs. Otherwise, these tumors share identical histologic and immunochemical features and chromosomal translocations with Ewing sarcoma. Other tumors in this spectrum include extraosseous ES, peripheral primitive neuroectodermal tumor, and atypical Ewing sarcoma. A former common term for Ewing sarcoma was “primitive neuroectodermal tumor,” which is now a term

that is not recommended by the WHO classification² in order to form a distinction from this tumor and tumors belonging to the neuroblastoma family and embryonal tumors of the central nervous system. This former term, however, does reflect the fact that these tumors may demonstrate neuroectodermal differentiation.²

What are the risk factors for Ewing sarcoma?

Ewing sarcoma does not show any association with radiation, chemicals, or any other environmental risk factors. Although Ewing sarcoma is rare, it is more common in young, male white persons.²⁻⁴

How is Ewing sarcoma treated?

One of the most important uses of the staging system is to classify patients for treatment purposes based on expected outcomes that rely on imaging and biopsy results. In localized Ewing sarcoma, induction chemotherapy is the first line of treatment. It is commonly a combination of vincristine, doxorubicin (Adriamycin), and cyclophosphamide, alternating with ifosfamide and etoposide. These are also known as the VDC/IE or VAC/IE regimens, respectively.^{13,14} Patients with localized Ewing sarcoma may have micro-metastases that are too small for imaging studies to detect. The treatment of metastatic Ewing sarcoma includes induction chemotherapy and may also include adjunct surgery or radiation.^{13,14}

Imaging studies that can be used to evaluate the metastatic disease include X-rays, CT or MRI scans, bone, or PET scans.^{5,6} Bone marrow biopsies can also be performed to determine if there has been intramedullary spread. If the cancer is confined to a few areas, the main tumor and areas of metastases may be surgically removed. For recurrent tumors, a combination of chemotherapy, radiation therapy, and surgery are used as treatment modalities. Patients considered to have the localized disease may have unfavorable outcomes even with multimodality therapy. This may be related to the persistence of minimal metastatic disease undetected by the traditional method.¹³⁻¹⁵

Teaching points

- Bone tumors are categorized by their neoplastic behavior and metastatic potential as benign or malignant. Some bone tumors are associated with matrix production that can help further classify them as bone-forming tumors – such as osteosarcoma – which produce an immature osteoid matrix or cartilage-forming tumors – such as enchondromas or chondrosarcomas – that are associated with a cartilaginous matrix.
- Many cancers including bone tumors have underlying genetic alterations with driver gene mutations promoting tumorigenesis. Some tumors have simple initiating genetic alteration that leads to tumor formation, such as the t(11; 22)(q24; q12) translocation in Ewing sarcoma, whereas other tumors harbor multiple and complex genetic mutations involved in tumorigenesis, such as leiomyosarcomas.
- Cytology specimens and surgical resection specimens play different roles in diagnosis. A cytologic evaluation may be amenable for primary diagnosis and staging of tumors, due to the small amount of specimen needed for diagnosis. However, depending on the type of tumor and the possibility of sampling bias, definitive diagnosis and subtyping may require surgical resection. Ancillary studies can often be performed on both specimens and can include immunohistochemical stains, fluorescence in situ hybridization studies, and DNA or RNA sequencing.
- Ewing sarcoma has a higher incidence in male white persons.
- The differential diagnosis for Ewing sarcoma includes inflammatory and infectious processes, like osteomyelitis, as well as other benign and malignant bone and soft tissue tumors.

- The X-ray finding of “onion skinning” with an elevated periosteum is highly suggestive of Ewing sarcoma.
- Fine needle aspiration biopsy or core biopsy is required to diagnose Ewing sarcoma. These modalities can also be helpful in staging.
- Ewing sarcoma belongs to a histologic group of tumors called “small round blue cell tumors,” which are characterized by monotonous populations of cells that are usually larger in size than a lymphocyte and have a high nuclear-cytoplasmic ratio, fine chromatin, and scant amounts of cytoplasm.
- The histologic differential diagnosis for small round blue cell tumors includes neuroblastoma, rhabdomyosarcoma, lymphomas, high-grade carcinomas, and Ewing sarcoma.
- Immunohistochemical stains that are positive in Ewing sarcoma include CD99 and FLI-1 (the latter depending on if a FLI-1 fusion is present). Neuroendocrine markers may be focally positive, but keratins are negative.
- Immunohistochemical stains can help narrow the differential diagnosis of a small round blue cell tumor. High-grade carcinomas usually retain keratin expression. Lymphomas typically stain positive with CD45 and CD3 or CD20. PHOX2B is sensitive and specific for neuroblastoma when positive. Desmin positivity would be found in rhabdomyosarcoma with diffuse myogenin/MyoD1 positivity in the alveolar subtype and only focal positivity in the embryonal subtype.
- The most common fusion gene in Ewing sarcoma is t(11; 22)(q24; q12) involving the *EWSR1* gene on chromosome 22 and the *FLI1* gene on chromosome 11. Fusions are commonly tested by using a *EWSR1* break-apart probe fluorescence *in situ* hybridization.
- Ewing sarcoma belongs to a group of neoplasms called the Ewing sarcoma family of tumors. Other members of this group include extrasosseous Ewing sarcoma (EOE) and Askin tumors (Ewing sarcoma involving the chest wall).

Disclaimers

None.

Funding

This educational case did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

None.

References

1. Knollmann-Ritschel BEC, Regula DP, Borowitz MJ, Conran R, Prystowsky MB. Pathology competencies for medical education and educational cases. *Acad Pathol*. 2017;4. doi:10.1177/2374289517715040.
2. De Alava E, Lessnick SL, Sorrensen PH. Ewing sarcoma. In: *WHO Editorial Board. WHO Classification of Tumours of Soft Tissue and Bone*. fourth ed. Geneva: International Agency for Research on Cancer; 2013:271–419.
3. Horvai A. Bones, joints, and soft tissue tumors. In: Kumar V, Abbas AK, Aster JC, eds. *Robbins and Cotran Pathologic Basis of Disease*. tenth ed. Elsevier Saunders; 2021: 1187–1196.
4. Machado I, Llombart-Bosch A. Ewing's sarcoma family of tumors. In: Santini-Araujo E, Kalil RK, Bertoni F, Park Y-K, eds. *Tumors and Tumor-like Lesions of Bone*. Springer; 2015:369–384. doi:10.1007/978-1-4471-6578-1_25.
5. Murphey MD, Andrews CL, Flemming DJ, et al. From the archives of the AFIP. Primary tumors of the spine: radiologic pathologic correlation. *Radiographics*. 1996; 16(5):1131–1158.
6. Revannagowda S, Gangadhar K, Akaike G, Dighe M. Primary intra-abdominal ewing's sarcoma in adults: a multimodality imaging spectrum. *Curr Probl Diagn Radiol*. 2020; 49(2):133–139. doi:10.1067/j.cpradiol.2018.12.009.
7. Bestic JM, Peterson JJ, Bancroft LW. Use of FDG PET in staging, restaging, and assessment of therapy response in Ewing sarcoma. *Radiographics*. 2009;29(5): 1487–1500. doi:10.1148/rg.295095024.
8. Qian X. Soft tissue. In: *Cibas ES and Ducatman BS. Cytology: Diagnostic Principles and Clinical Correlates*. fourth ed. Elsevier; 2014:471–514.

9. Krogerus L, Kholová I. Cell block in cytological diagnostics: review of preparatory techniques. *Acta Cytol.* 2018;62(4):237–243. doi:10.1159/000489769.
10. Noujaim J, Jones RL, Swansbury J, et al. The spectrum of EWSR1-rearranged neoplasms at a tertiary sarcoma centre; assessing 772 tumour specimens and the value of current ancillary molecular diagnostic modalities. *Br J Cancer.* 2017;116(5):669–678. doi:10.1038/bjc.2017.4.
11. Zöllner SK, Amatruda JF, Bauer S, et al. Ewing sarcoma-diagnosis, treatment, clinical challenges and future perspectives. *J Clin Med.* 2021;10(8):1685. doi:10.3390/jcm10081685.
12. Ramos-Rivera G, Adler E, Ramesh KH, Schiff B, Suhrland M, Khader S. Extraskelatal Ewing sarcoma of the parapharyngeal space with a unique translocation, t(19;22)(q13.4;q12.2). *Hum Pathol: Case Reports.* 2016;4:38–41.
13. Treatment of Ewing Tumors by Stage. American Cancer Society. Accessed May 28, 2021. <https://www.cancer.org/cancer/ewing-tumor/treating/by-stage.html>.
14. Hornicek FJ, Baldini EH. Clinical presentation, staging, and prognostic factors of the Ewing sarcoma family of tumors. UpToDate. Updated Jun 03, 2022. Accessed September 22, 2021. <https://www.uptodate.com/contents/clinical-presentation-staging-and-prognostic-factors-of-the-ewing-sarcoma-family-of-tumors>.
15. Amin MB, Greene FL, Edge SB, et al. The Eighth Edition AJCC Cancer Staging Manual: continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA A Cancer J Clin.* 2017;67(2):93–99. doi:10.3322/caac.21388.