Protocol for the Examination of Biopsy Specimens From Pediatric Patients With Ewing Sarcoma

Version: 4.1.0.0
Protocol Posting Date: June 2021
The use of this protocol is recommended for clinical care purposes but is not required for accreditation purposes.

This protocol should be used for the following procedures AND tumor types:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy</td>
<td>Includes specimens designated core needle biopsy, incisional biopsy, excisional biopsy, or other</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewing sarcoma</td>
<td>Includes pediatric patients with osseous and extraosseous Ewing sarcoma family of tumors</td>
</tr>
</tbody>
</table>

The following should NOT be reported using this protocol:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resection (consider Pediatric Ewing Sarcoma Resection protocol)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult Ewing sarcoma*</td>
<td>(consider using bone or soft tissue protocols)</td>
</tr>
<tr>
<td>Round cell sarcoma with EWSR1-non ETS fusions, CIC-rearranged sarcoma, or sarcoma with BCOR-genetic alterations (consider using Bone or Soft Tissue protocols)</td>
<td></td>
</tr>
</tbody>
</table>

*Ewing sarcoma in adults may be treated differently than pediatric Ewing sarcoma and use of the AJCC TNM staging system remains appropriate for adult patients.

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With guidance from the CAP Cancer and CAP Pathology Electronic Reporting Committees.
* Denotes primary author.
Accreditation Requirements
The use of this case summary is recommended for clinical care purposes but is not required for accreditation purposes. The core and conditional data elements are routinely reported. Non-core data elements are indicated with a plus sign (+) to allow for reporting information that may be of clinical value.

Summary of Changes

v 4.1.0.0

- General Reformatting
- Revised Margins Section
- Cytogenic Findings Remodeled
- Elements that are recommended for clinical care purposes are designated as Core and Conditional (indicated by bolded text), while Non-core elements are now indicated with a plus (+) sign
Reporting Template

Protocol Posting Date: June 2021
Select a single response unless otherwise indicated.

CASE SUMMARY: (EWING SARCOMA: Biopsy)
Ewing sarcoma (ES) is a round cell sarcoma showing gene fusions involving one member of the FET family of genes (usually EWSR1) and a member of the ETS family of genes. This malignancy may occur in children and adults in either bone or soft tissue sites, including unusual sites such as skin or leptomeninges. Because ES can occur in bone or soft tissue, AJCC / UICC staging systems for both are included. (Note A)

First priority should always be given to formalin-fixed tissue for histomorphologic evaluation. Special studies (e.g., cytogenetics, fluorescence in situ hybridization [FISH], reverse transcriptase polymerase chain reaction [RT-PCR], and less commonly next-generation sequencing, whole genome and exome analyses) are critical to the molecular workup of ES and may require at least 100 mg of viable, fresh or snap-frozen tissue as the second priority for workup. Although molecular testing for FISH analysis of EWSR1 rearrangement or for RT-PCR analysis of EWSR1-FLI1, EWSR1-ERG, and other ES translocations may be performed on formalin-fixed paraffin-embedded tissue, every attempt should be made to procure fresh/snap-frozen tissue, as this may be a requirement for some treatment protocols. (Note A)

This protocol is based on the experience of the Children’s Oncology Group. For more information, contact The Children’s Oncology Group Biopathology Center. Phone: (614) 722-2890 or (800) 347-2486. (Note A)

SPECIMEN

Procedure (Note B)
___ Core needle biopsy
___ Incisional biopsy
___ Excisional biopsy
___ Other (specify): ______________________
___ Not specified

TUMOR

Tumor Site
___ Osseous
   ___ Long bones of upper limb, scapula and associated joints (specify): _________________
   ___ Short bones of upper limb and associated joints (specify): _________________
   ___ Long bones of lower limb and associated joints (specify): _________________
   ___ Short bones of lower limb and associated joints (specify): _________________
   ___ Overlapping lesion of bones, joints and articular cartilage of limbs (specify): _________________
   ___ Bone of limb, NOS (specify): _________________
   ___ Bones of skull and face and associated joints (excluding mandible C41.1) (specify):
      ___ Mandible (specify): ______________________
      ___ Vertebral column (excluding sacrum and coccyx C41.4) (specify): _________________
      ___ Rib, sternum, clavicle and associated joints (specify): _________________
      ___ Pelvic bones, sacrum, coccyx and associated joints (specify): _________________
      ___ Overlapping lesion of bones, joints and articular cartilage (specify): _________________
      ___ Bone, NOS: ______________________
___ Extraosseous
___ Heart / mediastinum
   ___ Heart (specify): _________________
   ___ Anterior mediastinum (specify): _________________
   ___ Posterior mediastinum (specify): _________________
___ Mediastinum, NOS: _________________
___ Overlapping lesion of heart, mediastinum and pleura (specify): _________________
___ Peritoneum and / or retroperitoneum
___ Retroperitoneum: _________________
___ Peritoneum, including omentum and mesentery (specify parts): _________________
___ Peritoneum, NOS: _________________
___ Other soft tissue
___ Head, face, and neck (specify): _________________
___ Upper limb and shoulder (specify): _________________
___ Lower limb and hip (specify): _________________
___ Thorax (specify): _________________
___ Abdomen (specify): _________________
___ Pelvis (specify): _________________
___ Trunk (specify): _________________
___ Overlapping lesion (specify): _________________
___ Other, NOS: _________________
___ Not specified

Tumor Size (for excisional biopsy only)
___ Not applicable
___ Greatest dimension in Centimeters (cm): _________________ cm
+ Additional Dimension in Centimeters (cm): ____ x ____ cm
___ Cannot be determined (explain): __________________

Lymphovascular Invasion (Note C)
___ Not identified
___ Present
___ Cannot be determined: _________________

MARGINS

Margin Status (for excisional biopsy only) (Note D)
___ Not applicable (not an excisional biopsy)
___ All margins negative for tumor

Closest Margin(s) to Tumor
___ Specify closest margin(s): _________________
___ Cannot be determined (explain): __________________

Distance from Tumor to Closest Margin
Specify in Centimeters (cm)
___ Exact distance: _________________ cm
___ Greater than: _________________ cm
___ Other (specify): _________________
___ Cannot be determined: _________________

___ Tumor present at margin

Margin(s) Involved by Tumor
___ Specify involved margin(s): __________________
___ Cannot be determined (explain): __________________
___ Cannot be determined (explain): __________________
+Margin Comment: ____________________

SPECIAL STUDIES (Note E)

Results of these studies may not be available at the time of the final report

+Immunohistochemistry (specify): ____________________

Cytogenetic Findings
___ Not performed
___ Pending
___ EWSR1 rearrangement, fusion partner not known
___ EWSR1-FLI1 gene rearrangement
___ EWSR1-ERG gene rearrangement
___ Other EWSR1 gene rearrangement (specify): ____________________
___ Non-EWSR1 variant translocation (specify): ____________________
___ Other (specify): ____________________
___ No rearrangement identified
___ Not known

Method for Cytogenetic Studies
___ Conventional karyotyping
___ Fluorescent in situ hybridization (FISH)
___ Reverse transcriptase polymerase chain reaction (RT-PCR)
___ Other (specify): ____________________
___ Not known

ADDITIONAL FINDINGS

+Additional Findings (specify): ____________________

COMMENTS

Comment(s): ____________________
Explanatory Notes

A. Tissue Handling
Tissue specimens optimally are received fresh/unfixed because of the importance of ancillary studies, such as cytogenetics and molecular testing, which may prefer fresh tissue. First priority should always be given to formalin-fixed tissues (FFPE) for morphologic evaluation. Ideally, some tissue can be submitted for FFPE without decalcification or following decalcification in EDTA or ETDA+acid decalcification solutions to preserve nucleic acids for molecular testing, including FISH, RT-PCR, and/or next generation sequencing (NGS). Decalcification in pure acid decalcification solutions degrade nucleic acids and limit molecular testing. Following submission of FFPE, submission of fresh tissue for cytogenetics and/or snap freezing a minimum of 100 mg of viable tumor may be needed potential molecular studies and/or COG study purposes. Molecular testing on formalin-fixed paraffin-embedded tissue may be performed for FISH evaluation of EWSR1 rearrangement, for RT-PCR evaluation of EWSR1-FLI1, EWSR1-ERG, and other ES translocations, or NGS. When the amount of tissue is limited, the pathologist can keep the frozen tissue aliquot used for frozen section (usually done to determine sample adequacy and viability) in a frozen state (-70°C is preferable). Translocations may be detected using RT-PCR on frozen or fixed paraffin-embedded tissue, or FISH on touch preparations made from fresh tissue or formalin-fixed paraffin-embedded tissue.

Note that classification of many subtypes of sarcoma is not always dependent upon special studies, such as cytogenetics or molecular genetics, but frozen tissue may be required to enter patients into treatment protocols. Discretion should be used in triaging tissue from sarcomas. Adequate tissue should be submitted for conventional light microscopy before tissue has been taken for cytogenetics, electron microscopy, or molecular analysis.

References

B. Procedures

Cytologic Material
Cytological material is usually sufficient to diagnose ES (with supportive immunostains) (Note E). An important limitation of fine-needle aspiration is the limited amount of tissue for additional molecular diagnostic studies and tissue banking (see Note A). Evaluation by a pathologist at the time of the fine-needle biopsy procedure is important to assess the adequacy of the specimen for routine histomorphologic diagnosis and for ancillary studies.

If cytologic material includes fluid, such as pleural effusions or fluid from a liquefactive tumor, the fluid should be centrifuged and the resulting pellet fixed with formalin prior to making a paraffin cell block. The resulting cell block allows for histopathologic examination, immunohistochemical, and/or molecular analysis.

Biopsy (Needle, Incisional, Excisional)
An open incisional biopsy consistently provides a larger sample of tissue and maximizes the opportunity for a specific pathologic diagnosis. However, image guided needle core biopsies are now being performed with greater frequency. Sampling of multiple lesional cores can provide sufficient material for special studies and histomorphologic diagnosis. Excisional biopsy may not include an adequate margin of normal tissue, even with an operative impression of total gross removal.
In cases of non-excisional biopsy (eg, core biopsy, incisional biopsy), the tumor size cannot be determined on pathologic grounds; therefore, imaging data (computed tomography [CT], magnetic resonance imaging [MRI], etc) can be used instead.

References


C. Lymphovascular Invasion (LVI)
Lymphovascular invasion (LVI) indicates whether microscopic lymphovascular invasion is identified in the pathology report. LVI includes lymphatic invasion, vascular invasion, or lymphovascular invasion. Evaluation of LVI may require immunohistochemical staining for endothelial markers (CD31, CD34, D240, etc). By American Joint Committee on Cancer (AJCC) and International Union Against Cancer (UICC) convention, LVI does not affect the T category indicating local extent of tumor unless specifically included in the definition of the T category.

D. Margins
The extent of resection (ie, gross residual disease versus complete resection with negative margins) has the strongest influence on local control of malignancy.1 The definition of what constitutes a sufficiently “wide” margin of normal tissue in the management of ES and the significance of reactive and/or necrotic tissue at the margin are current study questions for the Children's Oncology Group, and may evolve in the future. Currently, any tumor at the margin, whether viable, nonviable, or treated, is considered positive. The significance of treated tumor at the margin when there has been an excellent chemotherapeutic response (ie, greater than 90% tumor necrosis) remains unclear. There is currently no consensus as to whether margins involved by treated tumor require further treatment, and this is considered a negative margin on some studies. The presence of treated tumor at the margin should be reported, however, and can be included in the comments section of the checklist. The following margins are considered adequate:

- Cortical bone margin: 2 to 5 cm
- Fascia, periosteum, and intermuscular septa: 2 mm
- Fat, muscle, and medullary bone: 5 mm

With Ewing sarcoma involving an encapsulated organ, surgical margins are considered to be negative if the organ’s capsule is not surgically violated or breached by the tumor.

References


E. Ancillary Studies

Immunohistochemistry
Immunohistochemistry with monoclonal antibodies against the cell surface glycoprotein CD99 is positive in virtually all cases of ES.1 This glycoprotein is diffusely expressed in the vast majority of cases in a
membranous pattern (Figure 2). The results of staining using monoclonal antibodies O13, HBA71, and 12E7 are similar, but individual tumors may exhibit better staining with one of these antibodies versus other antibodies.

![Figure 2. CD99 staining in Ewing sarcoma shows strong, diffuse, membranous staining. (CD99 antibody O13 with hematoxylin counterstain.)](image)

Lymphoblastic lymphomas/leukemias, rhabdomyosarcomas, synovial sarcomas, solitary fibrous tumors, rhabdoid tumors, neuroendocrine tumors, desmoplastic small round cell tumors, and mesenchymal chondrosarcomas may also demonstrate immunoreactivity to CD99. In some of these tumors, CD99 immunostaining is often weakly granular and intracytoplasmic; in others (lymphoblastic lymphoma/leukemia, occasional cases of poorly differentiated synovial sarcoma, alveolar rhabdomyosarcoma), distinct membrane staining is present, as seen in ES. Because these other tumors with small round cell morphology can exhibit CD99 expression, it is very important to consider including other immunohistochemical stains such as muscle markers (desmin, muscle-specific actin, myoD1, myogenin), S-100, epithelial markers (epithelial membrane antigen, cytokeratin), INI-1, and lymphoid markers (CD45, CD30, TdT, T-cell and/or B-cell markers) when CD99 is performed to properly exclude CD99-expressing tumors. Cytokeratin positivity may be seen in ES and may be diffusely positive in the adamantinoma-like variant of Ewing sarcoma. Newer immunohistochemical antibodies, such as NKLX2.2, may also be useful for the diagnosis of ES, although NKLX2.2 staining may rarely be seen in other small round cell tumors. The value of other immunohistochemical markers for diagnosis, such as Ki-67, p53, and C-kit (CD117), has not been established.

**Chromosomal Translocations**

The 2020 World Health Organization (WHO) classification of bone and soft tissue tumors defines *Ewing sarcoma* as a round cell sarcoma harboring a FET-ETS gene fusion. FET represents a family of genes to include *FUS, EWSR1*, and *TAF15*; whereas the ETS gene family is a large family of transcription factors involved in cell cycle regulation, cellular differentiation, among other functions. In relation to Ewing sarcoma, the characteristic translocations involve the *EWSR1* gene at 22q12, most often either the *FLI1* gene at 11q24 (90-95%) or the *ERG* gene at 21q22 (5-10%). These two fusions account for the vast majority of genetic alterations in ES. It should be emphasized that there are numerous other *EWSR1* or *FUS* gene partners that occur in a minority (5%-10%) of ES. The failure to identify an *EWSR1-FLI1* or *EWSR1-ERG* translocation by RT-PCR or cytogenetics does not exclude ES from the diagnosis. If RT-PCR is negative, in the context of a tumor suspicious for ES, other molecular studies (cytogenetics, NGS) may be important for identification of the less common ES translocations and for discovering novel *EWSR1* translocations in ES. Some of the less common ES translocations involve *FUS* (ch16) rather than *EWSR1*, or involve other ETS partners including *ETV1, ETV4*, or *FEV*. FISH analysis for *EWSR1* (or *FUS*) is helpful as a first step and may confirm the diagnosis in those tumors with histomorphologic
features and immunohistochemical phenotypes of ES. Because other small round cell tumors of childhood can have \textit{EWSR1} rearrangements with specific tumor-defining partners, \textit{EWSR1} FISH positivity alone is not diagnostic of ES. Some of these tumors with \textit{EWSR1} rearrangement include angiomatoid fibrous histiocytoma, clear cell sarcoma of soft parts, desmoplastic round cell tumor, and extraskeletal myxoid chondrosarcoma, as well as a subset of myxoid liposarcomas and myoepithelial carcinoma.

Therefore, considerations when choosing testing methodologies may include, classic versus non-classic histomorphology, immunophenotype, need to confirm translocation partner, turnaround time, cost, and ultimately may be depend on the availability of testing modalities at each institution. While obtaining evidence of a diagnostic fusion is recommended, it should be noted that absence of a fusion can either result from 1) true lack of fusion, 2) test failure (eg. FISH for \textit{EWSR-ERG} fusions can miss rearrangements) or 3) mismatch between the testing approach and the fusion present (eg. \textit{EWSR1-ERG} present and test is for RT-PCR for \textit{EWSR1-FLI1}). This underscores the necessity for histologic and immunohistochemical correlation with any molecular testing result.\(^5\)

Of note, the specific \textit{EWSR1} translocation and subtype based upon exon fusion type do not influence treatment, prognosis, or outcome.\(^6\)

References