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

Version: 4.1.1.1
Protocol Posting Date: September 2022

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>
<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.

**Authors**

Jessica L. Davis, MD*; Archana Shenoy, MD; Lea Surrey, MD; Alyaa Al-Ibraheemi, MD; Katherine A. Janeway, MD; Elyssa Rubin, MD; Erin R. Rudzinski, MD.

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 4.1.1.1

- The 'Expert Consultation' question was made optional
- Added Explanatory ‘Note A’ and associated with the ‘Expert Consultation’ question
Reporting Template
Protocol Posting Date: September 2022
Select a single response unless otherwise indicated.

CASE SUMMARY: (EWING SARCOMA: Biopsy)

EXPERT CONSULTATION

+Expert Consultation (Note A)
  ___ Pending - Completion of this CAP Cancer Protocol is awaiting expert consultation
  ___ Completed - This CAP Cancer Protocol or some elements have been performed following expert consultation
  ___ Not applicable

SPECIMEN (Note B)

Procedure (Note C)
  ___ 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 D)
___ Not identified
___ Present
___ Cannot be determined: ______________________

MARGINS

Margin Status (for excisional biopsy only) (Note E)
___ 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 F)
*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
___ Not applicable (Cytogenetic Studies not performed)
___ 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. Expert Consultation
Expert consultation is not required. This question has been added to annotate, if so desired, that the case has been sent out for consultation and thus items of the CAP protocol could not be completed pending expert consultation. Completion of the CAP protocol will then be performed following consultation.

B. 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, to 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

C. 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

D. 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.

E. 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 comment 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

F. Ancillary Studies

Immunohistochemistry

Immunohistochemistry with monoclonal antibodies against the cell surface glycoprotein CD99 is positive in virtually all cases of ES. 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.)

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 NKX2.2, may also be useful for the diagnosis of ES, although NKX2.2 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-FLI 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 EWSR1 rearrangements with specific tumor-defining partners, EWSR1 FISH positivity alone is not diagnostic of ES. Some of these tumors with EWSR1 rearrangement include angiomatoid fibrous histiocytoma, clear cell sarcoma of soft parts, desmoplasic 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 EWSR-ERG fusions can miss rearrangements) or 3) mismatch between the testing approach and the fusion present (eg. EWSR1-ERG present and test is for RT-PCR for EWSR1-FLI1). This underscores the necessity for histologic and immunohistochemical correlation with any molecular testing result.5

Of note, the specific EWSR1 translocation and subtype based upon exon fusion type do not influence treatment, prognosis, or outcome.6

References